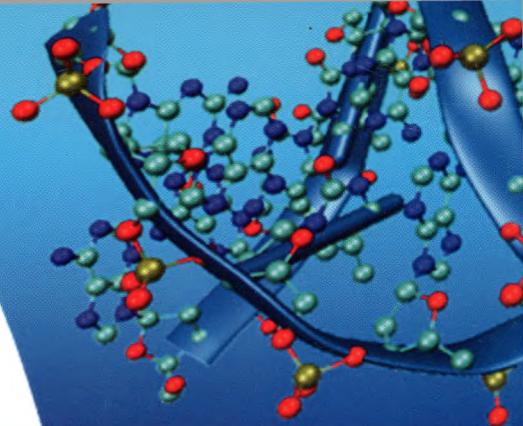




XXXVI Annual Meeting
Sociedad de Bioquímica
y Biología Molecular de Chile

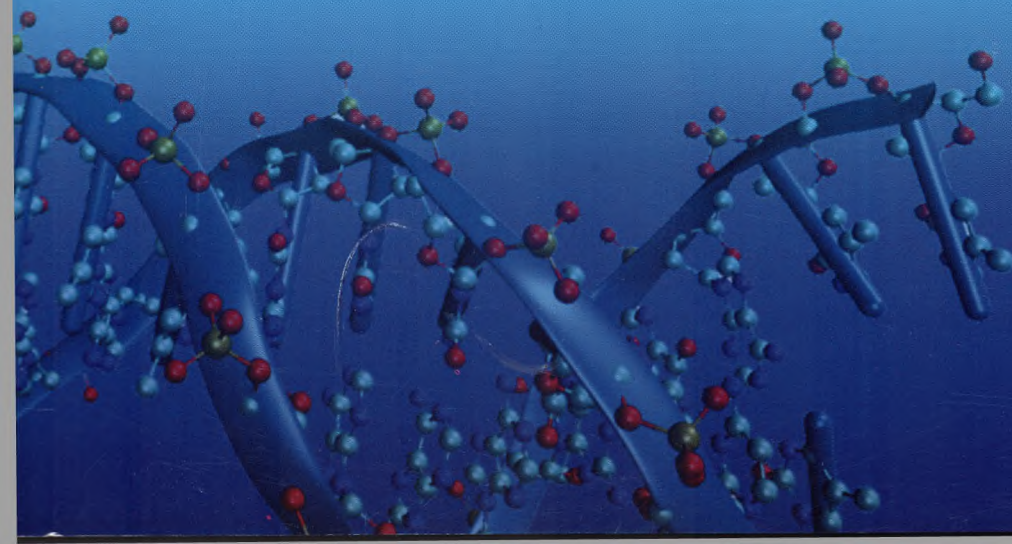
XLIX Annual Meeting
Sociedad Argentina de
Investigación en Bioquímica y
Biología Molecular

LVI Annual Meeting
Sociedad de Biología de Chile

4th Latin American
Protein Society Meeting

November 9 - 14
Puerto Varas

Chile 2013





SOCIEDAD DE BIOQUÍMICA Y BIOLOGÍA MOLECULAR DE CHILE
XLIX ANNUAL MEETING SOCIEDAD ARGENTINA DE
INVESTIGACIÓN BIOQUÍMICA Y BIOLOGÍA MOLECULAR
LVI ANNUAL MEETING SOCIEDAD DE BIOLOGÍA DE CHILE
4th LATIN AMERICAN PROTEIN SOCIETY MEETING

Puerto Varas, Chile, 9 -14 November, 2013



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KEY INFORMATION

Badges

The ID badge included in the registration package is the admission pass to the congress venues. Wearing ID Badge is required for attending to all congress activities.

Internet

Free Wi-Fi Internet Access is available in Dreams Convention Center and Patagónico Convention Center

Abstract Book

A printed booklet with the program information, including speakers and titles of all sessions will be provided with the registration package.

The Congress **Abstract Book** is available online at the PABMB Congress web site (www.pabmbcongress2013.cl)

Language

English is the Congress official language. No English-Spanish translation will be provided.

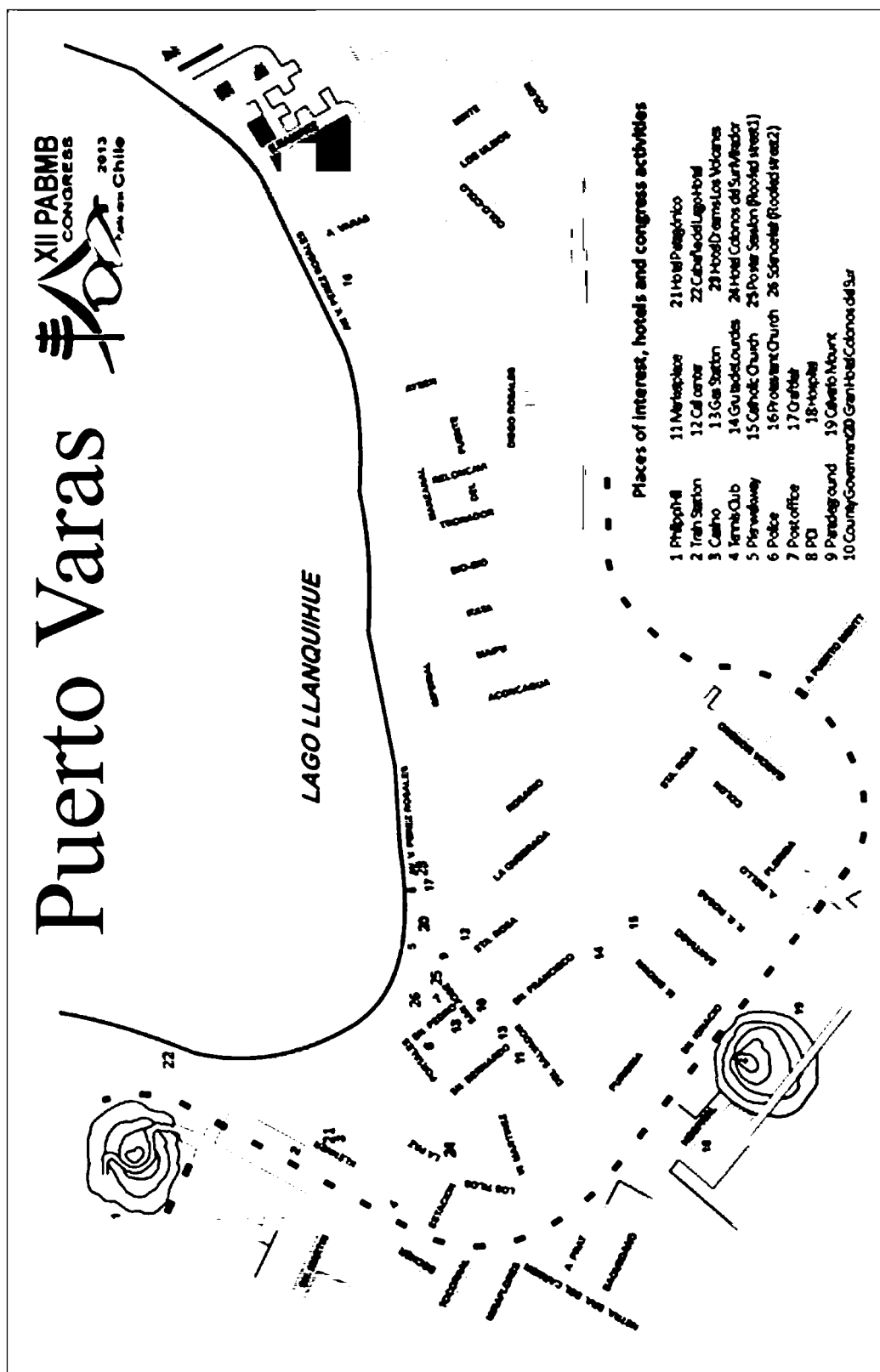
Congress Venue

Dreams Convention Center, Del Salvador 21, Puerto Varas, Chile

Patagónico Convention Center, Klenner 349, Puerto Varas, Chile

Roofed Street Santa Rosa near to Puerto Varas' Main Square

Places of Interest and Congress Activities



Welcome to XII PABMB Meeting

On behalf of the Pan American Association for Biochemistry & Molecular Biology (PABMB), please let me welcome you to the XII PABMB Congress here in Puerto Varas, Chile. It is a great honor for the Chilean Society of Biochemistry and Molecular Biology (SBBM) to have been selected to organize this important Pan-American Congress.

The Pan American Association for Biochemistry & Molecular Biology fosters and supports the growth and advancement of Biochemistry and Molecular Biology within the Americas. To this end, PABMB disseminates information related to education in and practice of the disciplines of Biochemistry and Molecular Biology, by sponsoring and organizing meetings where recent discoveries and developments in these fields are discussed.

This XII PABMB Congress, like all previous PABMB meetings, seeks to advance research of excellence in Biochemistry & Molecular Biology and particularly to promote collaborations between members of the Pan American Societies in these areas of the biological sciences. The meeting will offer the opportunity for fruitful discussion, and hopefully provide a stepping stone to initiate new friendships, collaborations and joint projects.

The Chilean Society of Biochemistry and Molecular Biology fosters both the development of theoretical and experimental research, as well as the opportunity for dissemination of the insights obtained through such activities. The Society also supports all initiatives that seek to optimize scientific development in benefit of the society in general.

The Chilean Society of Biochemistry and Molecular Biology was founded in 1974 and in 1977 the first annual meeting was held. Today this society brings together one of the largest scientific communities within the biological sciences and includes in its ranks scientists from many Chilean universities as well as other countries.

Puerto Varas is located in the south of Chile, in the so-called Lake Region. The town is well known for its German tradition, excellent cuisine, especially fish and seafood, the natural environment, its casino and several 5 star hotels. Puerto Varas is located only 20 km from Puerto Montt on the shore of the Llanquihue Lake, considered one of the largest natural lakes in South America. The perfect cone-shaped peak of the Volcano Osorno, as well as the snow capped peaks of Mt. Calbuco and Mt. Tronador are all clearly visible from the lakefront.

This combination of exceptional natural beauty with comfort and convenience we hope will provide the ideal setting for a highly successful scientific meeting.

Thus, on behalf of the Organizing Committee let me once again welcome you to the XII PABMB Congress in Puerto Varas.

Sergio Lavandero G.
Chair of the Organizing & Program Committee

Jorge Babul C.
Chair of the Scientific Committee

Sponsors

The American Society for Biochemistry and Molecular Biology (ASBMB)

Canadian Society for Molecular Biosciences (CSMB)

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Sociedad Española de Bioquímica y Biología Molecular (SEBBM)

Sociedade Portuguesa de Bioquímica (SBP)

The Federation of European Biochemical Societies (FEBS)

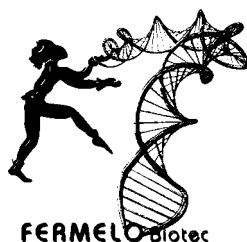
The Protein Society, USA

Universidad Austral de Chile

Exhibitors



eppendorf



SIGMA-ALDRICH™



LECTURES AND SYMPOSIA PROGRAM

Nov 9 10:30-13:00

**Registration
 Dreams Convention Center**

13:00-14:30

Lunch Time

14:30-16:30

**Symposium 1A
 IUBMB EducationSymposium
 Biochemical Education (Part I)
 Dreams Convention Center Salon Puerto Octay**

Chairs: Andrea T. Da Poian
 Raúl Herrera

14:30-15:00 Strategies to improve scientific literacy of school teachersusing hands-on inquiry-based Biochemistry courses. Andrea T. Da Poian, Eliene O. Kozlowski, Luisa A. Ketzer and Wagner S. da Silva. Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro, Brazil.

15:00-15:30 From blackboard to cyberspace: keeping up with changes when teaching Biochemistry and Molecular Biology. Juan Carlos Calvo, Universidad de Buenos Aires, CONICET, Argentina

15:45-16:10 Innovative strategies for teaching Biochemistry Raul Herrera, Universidad de Talca, Chile

15:30-16:00 Teaching concepts of Molecular Biology and Genomics to high school students using portable laboratories in several Latin American countries. Jorge Allende, ICBM, Facultad de Medicina, Universidad de Chile, Chile

16:00-16:30

**Symposium 1B
 SBBM Chile Symposium
 Young Investigators
 Dreams Convention Center Salon Frutillar**

Chair: Alejandra Moya

14:30-15:00 Cell-type specific core transcription machinery regulating neuronal differentiation. Francisco Herrera, University of California, Berkeley, USA

15:00-15:30 The landscape of RNAs bound to the L1 retrotransposon ORF1p protein *in vivo*. Jorge Vera Otárola, IRCAN, University of Nice-Sophia-Antipolis, France

15:30-16:00 Developing tools to follow HIV during T cell virological synapse. María Inés Barría, Mount Sinai School of Medicine, USA

16:00-16:30 HIV-1 Gag protein plays dual role in stress granules assembly. Fernando Valiente-Echeverría, Lady Davis Institute - Jewish General Hospital, McGill University, Montreal, Canada

14:30-16:30

Symposium 1C
SBBM Chile Symposium
Cell Signaling
Patagónico Convention Center Salon Osorno

Chair: Sergio Lavandero

14:30-15:00 **GRK2 as a novel key modulator of insulin signaling.** Federico Mayor. CBM Severo Ochoa, España

15:00-15:30 **Oxidative stress, protein carbonylation and metabolic control.** David Bernlohr, University of Minnesota-Twin Cities, USA

15:30-16:00 **The regulation of macroautophagy.** Daniel J. Klionsky. Life Sciences Institute, University of Michigan, Ann Arbor, MI, USA.

16:00-16:30 **Control of resting free Ca²⁺ in the cytoplasm of skeletal muscles: signal crosstalk from inside to outside and back again.** Paul D. Allen. Department of Molecular Biosciences, SVM, University of California, Davis, USA

14:30-16:30

Symposium 1D
SBCH - LAPSM Symposium
Structure and Function of Proteins
Patagónico Convention Center Salon Calbuco-Tronador

Chair: Juan C. Slebe

14:30-15:00 **Dynameomics.** Valerie Daggett. Department of Bioengineering, University of Washington, Seattle, WA, USA.

15:00-15:30 **The participation of high energy conformational states in catalysis and membrane recognition—plant defensins and thioredoxins.** Fabio C. L. Almeida. Biomolecular NMR Laboratory, National Center of Nuclear Magnetic Resonance, Institute of Medical Biochemistry and Nucleus for Structural Biology and Bioimaging, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.

15:30-16:00 **Fructose-1,6-bisphosphatase: some new developments and old themes.** Joel L. Asenjo¹, Heide C. Ludwig¹, Cristian A. Droppelmann¹, Juan G. Cárcamo¹, Ilona I. Concha¹, Alejandro J. Yáñez¹, María L. Cárdenas², Athel Cornish-Bowden² and Juan C. Slebe¹. ¹Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile. ²Bioénergétique et Ingénierie des Protéines, Institut de Microbiologie de la Méditerranée, CNRS, Aix-Marseille Université, Marseilles, France.

16:00-16:30 **Novel insights into bacterial signaling: the one sequence-multiple structures' perspective.** Felipe Trajtenberg¹, Horacio Botti¹, Daniela Albanesi², Diego de Mendoza², Nicole Larrieux¹ and Alejandro Buschiazzi¹. ¹Unit of Protein Crystallography, Institut Pasteur de Montevideo, Mataojo 2020, Montevideo 11400, Uruguay; ²IBR-CONICET, Universidad Nacional de Rosario, Suipacha 531, (2000) Rosario, Argentina.

16:30-17:00

Coffee Break
Dreams Convention Center / Patagónico Convention Center

17:00-19:00

PABMB Workshop
Looking South:
Collaborations for development of Biochemistry
Patagónico Convention Center Salon Los Volcanes

Chairs: Jorge Babul
Joan Guinovart

Jorge Babul, Universidad de Chile
Joan Guinovart, Institute for Research in Biomedicine, Universitat de Barcelona,
and CIBERDEM, Barcelona, Spain
Manuel Krauskopf, Laureate Education Andean Office
Miguel Ángel De la Rosa, Universidad de Sevilla

19 :00-20:00

Opening Ceremony
Patagónico Convention Center Salon Los Volcanes
Chair: Luis Larrondo

19:20-20:00

Chile and its diverse nature: Geography, ecosystems, flora and fauna. Fabián Jaksic. Pontificia Universidad Católica de Chile.

Opening FEBS Lecture
Patagónico Convention Center Salon Los Volcanes

20:00-21:00

Chair: Victoria Guixé

Beauty and fitness for purpose: architecture of proteins, the building blocks of life. Robert Huber (Nobel Prize). Center for Medical Biotechnology, University of Duisburg-Essen, Germany; Cardiff School of Biosciences; Cardiff University, Cardiff, Wales, UK

21:00

Welcome Reception
Roofed Street Santa Rosa, Puerto Varas Square

Nov 10 09:00-10:00

Lecture 1A
Convention Center Hotel Patagónico Salon Los Volcanes

Chair: Marcelo López-Lastra

Of nature and nurture: connecting RNA biology and metabolism. Matthias Hentze. Director at European Molecular Biology Laboratory (EMBL), Heidelberg, Germany; Co-Director of the "Molecular Medicine Partnership Unit" (MMPU), Heidelberg, Germany

09:00-10:00

IUBMB Lecture 1B
Dreams Convention Center

Chair: Andrew Quest

Caspases, paracaspase, and the space between death and survival. Janna Hachmann, Bran van Raam, Guy Salvesen, Sanford-Burnham Medical Research Institute, La Jolla, California, USA.

10:00-10:30

Coffee Break
Roofed Street Santa Rosa / Convention Center Hotel Patagónico

10:45-12:45

Symposium 2A
IUBMB Education Symposium
Biochemical Education (Part II)
Patagónico Convention Center Salon Osorno

Chair: Athel Cornish-Bowden

10:45-11:15

A century of enzyme kinetic analysis: 1913 to 2013. Kenneth A. Johnson, University of Texas, USA

11:15-11:45

Are virtual enzymes useful for teaching enzyme kinetics? Nadia Krieger, Universidade Federal do Parana, Brasil

11:45-12:15

Understanding the operation and regulation of metabolic pathways requires elementary kinetics: the cases of metabolic control analysis and substrate channeling. Ana Preller, Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Chile

12:15-12:45

Origins and development of enzyme kinetics: Henri, Michaelis, Menten. Athel Cornish-Bowden. CNRS, Marseille, France

10:45-12:45

Symposium 2B
SBBM Chile Symposium
Molecular Biology of Stress in Plants
Patagónico Convention Center Salon Calbuco-Tronador

Chair: Loreto Holuigue

10:45-11:15

Insights from tracking signals and signaling components implicated in plant defense. Jean Greenberg, University of Chicago, USA

11:15-11:45

Role of reactive oxygen species generated in chloroplasts during the interaction of plants with pathogens. Néstor Carrillo, Universidad Nacional de Rosario, Argentina

11:45-12:15

Salicylic acid and reactive oxygen species interplay in the defense response to stress in *Arabidopsis*. Loreto Holuigue. Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Chile

12:15-12:45

Flexible protein structures in plant drought responses. Alejandra Covarrubias, Universidad Nacional Autónoma de México, México

10:45-12:45

Symposium 2C
SAIB Symposium
Lipids: from membrane models to cellular signaling
Dreams Convention Center Salon Puerto Octay

Chair: Gabriela Salvador

10:45-11:15

Membrane protein-lipid binding: insights from mass spectrometry and molecular simulations. Nelson Barrera, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Chile

11:15-11:45 Endocannabinoids and pregnancy loss. Ana María Franchi, Universidad de Buenos Aires, Argentina

11:45-12:15 About the tyrosine phosphatase SHP2 and the membrane receptor oxe1 in the action of arachidonic acid in the regulation of steroid production. Fabiana Cornejo M., Universidad de Buenos Aires, Argentina

**10:45-12:45 Symposium 2D
SBBM Chile Symposium
Biomedicine
Dreams Convention Center Salon Frutillar**

Chair: Andrew Quest

10:45-11:15 Diabetic cardiomyopathy: fat, failure, flux, and Foxo. Joseph A. Hill, Department of Internal Medicine, University of Texas, USA

11:15-11:45 HDL: a new look at an old problem. Jay Heinecke, University of Washington, USA

11:45-12:15 Mechanisms of action and health benefits of flavonoids: is there a role in cardiovascular diseases? Balz Frei, Linus Pauling Institute, Oregon, USA

12:15-12:45 Loss of E-cadherin switches Caveolin-1 function from tumor suppressor to promoter of metastasis. Andrew Quest. Advanced Center for Chronic Diseases (ACCDiS) and ICBM, Facultad de Medicina, Universidad de Chile, Chile

13:00-15:00 Lunch time

**15:00-16:00 Lecture 2A
Alberto Sols Lecture
Patagónico Convention Center Salon Los Volcanes**

Chair: Luis Mayorga

Could we fully understand in a quantitative and predictive manner a living system? Luis Serrano. EMBL/CRG Systems Biology Research Unit, Centre for Genomic Regulation (CRG), Barcelona, Spain; Univarsitat Pompeu Fabra (UPF), Barcelona, Spain; Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain

**15:00-16:00 Lecture 2B
Prof. Eugenio Prodanov Lecture
Dreams Convention Center**

Chair: María Antonieta Valenzuela

Peroxynitrite, a stealthy biological oxidant. Rafael Radi Departamento de Bioquímica. Facultad de Medicina, Universidad de la República, Montevideo, Uruguay

**16:15-16:45 Coffe Break
Roofed Street Santa Rosa, Puerto Varas Square**

16:45-19:15

Posters and Exhibitors
Roofed Street Santa Rosa, Puerto Varas Square

19:30-20:30

Lecture 3A
SEBBM Lecture
Patagónico Convention Center Salon Los Volcanes

Chair: Sergio Lavandero

Role of mitochondrial fusion proteins on metabolism and implications in pathology. Antonio Zorzano. Institute for Research in Biomedicine, Universitat de Barcelona, and CIBERDEM, Barcelona, Spain

19:30-20:30

Lecture 3B
LAPSM Lecture
Dreams Convention Center

Chair: Octavio Monasterio

Molecular origami: protein folding and misfolding in health and disease. Judith Frydman. Biology Department, Stanford University, USA

Nov 11 09:00-10:00

Lecture 4A
Osvaldo Cori Lecture
Patagónico Convention Center Salon Los Volcanes

Chair: Xavier Jordana

The art of war: viral subversion of the host protein synthesis machinery. Marcelo López-Lastra. Laboratorio de Virología Molecular, Instituto Milenio de Inmunología e Inmunoterapia, Centro de Investigaciones Médicas, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile

09:00-10:00

Lecture 4B
Dreams Convention Center

Chair: Martín Montecino

Protein folding stress in neurodegenerative diseases: a look into the ER. Claudio Hetz. Biomedical Neuroscience Institute, Faculty of Medicine, University of Chile. Institute of Biomedical Sciences, Center for Molecular Studies of the Cell, University of Chile, Santiago, Chile. Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, Massachusetts, USA

10:00-10:30

Coffee Break
Roofed Street Santa Rosa / Convention Center Hotel Patagónico

10:45-12:45

Symposium 3A
SBBM Chile Symposium
Calcium signaling in excitable cells
Patagónico Convention Center Salon Osorno

Chair: Cecilia Hidalgo

10:45-11:15

Calcium signaling and calcium sensitization in smooth muscle contraction. Michael Walsh. University of Calgary, Canada

11:15-11:45 RyR-mediated calcium signals induce the expression of genes required for synaptic plasticity. Cecilia Hidalgo. Facultad de Medicina, Universidad de Chile, Chile

11:45-12:15 Transcriptional regulation by DREAM during neurodegeneration. José Naranjo. CSIC, Madrid, España

12:15-12:45 Novel genetic model of the role of the IP3 receptor in familial Alzheimer's disease. Kevin Foskett. University of Pennsylvania, USA

10:45-12:45

Symposium 3B
SBCH - LAPSM Symposium
Membrane Proteins
Patagónico Convention Center Salon Calbuco-Tronador

Chair: Ramón Latorre

10:45-11:15 Temperature-sensitive little machines. Latorre, R¹., Raddatz, N¹., Baez, D¹., González, C¹., Alvarez, O²., ¹Centro Interdisciplinario de Neurociencia, Facultad de Ciencias, Universidad de Valparaíso. ²Departamento de Biología, Facultad de Ciencias, Universidad de Chile.

11:15-11:45 Neurotransmitter-gated ion channels: on how structure relates to function and drug modulation. Cecilia Bouzat. Instituto de Investigaciones Bioquímicas de Bahía Blanca, Argentina.

11:45-12:15 Voltage-driven structural transitions in voltage-gated calcium channels. Riccardo Olcese. Department of Anesthesiology, David Geffen School of Medicine, University of California, Los Angeles, USA.

12:15-12:45 Investigating membrane protein folding. Jim Bowie. Department of Chemistry and Biochemistry, University of California, Los Angeles, USA.

10:45-12:45

Symposium 3C
SBBM Chile-SBBq Brazil
Metabolic Engineering
Dreams Convention Center Salon Puerto Octay

Chair: Ricardo Cabrera

10:45-11:15 Using cofactor engineering and carbon flux perturbations to understand the relationship between pentose-phosphate pathway flux and NADPH production in *Escherichia coli*. Ricardo Cabrera. Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Chile

11:15-11:45 Genome-scale metabolic modeling: a powerful tool for guidance of metabolic engineering. Eduardo Agosín, Chemical and Bioprocess Engineering, Engineering, Pontificia Universidad Católica de Chile, Chile

11:45-12:15 Novel candidate regulators of lipid droplet metabolism revealed by high throughput and chemogenomic analysis. Monica M. Lomeli, Universidade Federal do Rio de Janeiro, Brasil

12:15-12:45 Reengineering the regulation of nitrogen fixation in *Azospirillum brasilense*. A plant-growth promoting bacteria. Fábio de Oliveira Pedrosa, Universidade Federal do Paraná, Brasil

Nov 12 09:00-10:00

**Lecture 6A
 Severo Ochoa Lecture
 Patagónico Convention Center Salon Los Volcanes**

Chair: Claudio Hetz

Coordinated control of gene expression and cell cycle progression by stress-activated protein kinases (SAPKs). Francesc Posas. Cell Signaling Unit, Departament de Ciències Experimentals i de la Salut, Universitat Pompeu Fabra, Barcelona, Spain

09:00-10:00

**Lecture 6B
 Hermann Niemeyer Lecture
 Dreams Convention Center**

Chair: Rosalba Lagos

Dynamics and chemical ecology of microbial interspecies interactions. Roberto Kolter. Department of Microbiology and Molecular Genetics, Harvard Medical School, Harvard University, Boston, Massachusetts. USA

10:00-10:30

**Coffee Break
 Roofed Street Santa Rosa / Patagónico Convention Center**

10:45-12:45

**Symposium 4A
 SBBq-SPB Symposium
 Neurochemistry: discovering new mechanisms and treatments for nervous system diseases
 Patagónico Convention Center Salon Osorno**

Chair: Henning Ulrich

- 10:45-11:15** **Bradykinin in neurogenesis: from neural stem cell differentiation towards preclinical models of Parkinson's disease.** Henning Ulrich. Universidade de São Paulo, Brasil
- 11:15-11:45** **Neurochemical changes in retinal degenerative diseases: targeting neuropeptide Y as a potential therapeutic strategy.** António F. Ambrósio, Universidade de Coimbra, Portugal
- 11:45-12:15** **Modeling schizophrenia with human pluripotent stem cells.** Bruna Paulsen, Universidade Federal do Rio de Janeiro, Brasil
- 12:15-12:45** **Coupling of neuronal activity with local blood flow in normal and Alzheimer's rat brain: the direct role of nitric oxide.** João Laranjinha, Cátia F. Lourenço, Rui M. Barbosa. Center for Neuroscience and Cell Biology and Faculty of Pharmacy, University of Coimbra, Portugal

10:45-12:45

Symposium 4B
SBBM Chile Symposium
Immune Cells: Regulators in Health and Disease
Patagónico Convention Center Salon Calbuco-Tronador

Chairs: Diego Catalán
 Juan Carlos Aguilón

- 10:45-11:15 Dendritic cell targeting for antigen-specific therapy in rheumatoid arthritis.** Ranjeny Thomas. University of Queensland, Australia
- 11:15-11:45 Natural and adaptive Foxp3+ regulatory T cells.** Juan Lafaille. New York University, USA
- 11:45-12:15 The role and treatment potential of natural killer T cells in cancer.** Derek Doherty, Trinity College Dublin, Ireland
- 12:15-12:45 Retinoic acid and IL-33, two new players tuning T cell mediated immunity?** Karina Pino-Lagos, ICBM, Facultad de Medicina, Universidad de Chile, Chile

10:45-12:45

Symposium 4C
SAIB-SBBM Chile Symposium
Extremophiles
Dreams Convention Center Salon Puerto Octay

Chairs: Victoria Guixé
 María Eugenia Farías

- 10:45-11:15 Subsurface geomicrobiology of an extreme acidic environment, Río Tinto.** Ricardo Amils. CBM, Severo Ochoa, España
- 11:15-11:45 Metabolic rearrangement of *Rhodococcus* bacteria during desiccation.** Hector Alvarez. CRIDECIT, UNPATA San Juan Bosco, Argentina
- 11:45-12:15 Archaea in microbial ecosystems associated to minerals in Andean Wetlands: a source of biodiversity and alternative geochemical cycles.** María Eugenia Farías, PROIMI-CONICET, Argentina
- 12:15-12:45 Conserved mechanism for catalysis in hyperthermophilic ADP-dependent kinases from archaea.** Victoria Guixé, Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Chile

10:45-12:45

Symposium 4D
SBCH - LAPSM Symposium
Enzymes and Metabolism
Dreams Convention Center Salon Frutillar

Chair: Michael Handford

- 10:45-11:15 Targeted metabolic engineering of corn enhances oil production.** Ana Alonso. Department of Molecular Genetics, The Ohio State University
- 11:15-11:45 Glycine hydroxymethyltransferase reaction and biosynthesis of glycine: a metabolic relic?** María Luz Cárdenas. Bioénergétique et Ingénierie des Protéines, CNRS Marseille, France.

11:45-12:15 Sorbitol dehydrogenase is a cytosolic protein required for sorbitol metabolism in *Arabidopsis thaliana*. Michael Handford. Departamento de Biología, Facultad de Ciencias, Universidad de Chile.

12:15-12:45 Aspartate metabolism in plants as a model system for studying metabolic regulation. Athel Cornish-Bowden. Centre National de la Recherche Scientifique, Marseilles, France

13:00-15:00 Lunch Time

**15:00-16:00 Lecture 7A
SAIB Lecture
Dreams Convention Center**

Chair: Bernardo González

Why and how bacteria control membrane lipid biosynthesis ? Diego de Mendoza Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET); Departamento de Microbiología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Argentina

**15:00-16:00 Lecture 7B
RELAB Lecture
Patagónico Convention Center Salon Los Volcanes**

Chair: Octavio Monasterio

Cancer and prions: amyloidogenic and prionoid properties of mutant p53. Jerson Silva Institute of Medical Biochemistry at Universidade Federal do Rio de Janeiro, Brasil.

**16:15-17:15 Coffee Break
Roofed Street Santa Rosa / Patagonico Convention Center**

**16:15-17:15 Societies members' meetings
Dreams Convention Center**

**17:30-19:30 Symposium 5A
ASBMB-SBBM Chile Symposium
Single molecule: from proteins to the cell
Patagónico Convention Center Salon Osorno**

Chair: Susan Marqusee

17:30-18:00 Manipulating the protein energy landscape. Susan Marqusee. University of California, Berkeley, USA

18:00-18:30 A single-molecule fluorescence system to study biomolecules under force. Christian A. M. Wilson, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Chile

18:30-19:00 Unraveling and threading: how ATP-dependent proteases apply force to mechanically unfold and translocate protein substrates. Rodrigo Maillard, Georgetown University, USA

19:00-19:30 Single molecule imaging reveals that nuclear architecture controls transcription. Xavier Darzacq. (IBENS) CNRS, France

17:30-19:30

Symposium 5B
SBBM Chile Symposium
Systems Biology
Patagónico Convention Center Salon Calbuco-Tronador

Chair: Rodrigo Gutiérrez

- 17:30-18:00 Post-transcriptional control of gene expression.** Patrick Brown. Department of Biochemistry, Stanford University School of Medicine. Howard Hughes Medical Institute. USA
- 18:00-18:30 Plant leaf development—a systems approach.** Wilhelm Gruissem, Katja Baerenfaller, Lars Hennig, Sean Walsh and members of the AGRON-OMICS Consortium. Department of Biology, ETH Zurich, 8092 Zurich, Switzerland
- 18:30-19:00 Nitrogen regulatory networks controlling plant root growth.** Rodrigo A. Gutiérrez. Department of Molecular Genetics and Microbiology. Pontificia Universidad Católica de Chile, Chile.
- 19:00-19:30 New activities of oxylipins, a class of lipid signals regulating plant immunity.** Kulasekaran, S¹, Aguilera, V¹, Martínez, M¹, Martínez, M¹, Marcos, R¹, Izquierdo, Y¹, Hamberg, M², Castresana, C.¹, ¹Genética Molecular de Plantas CSIC, CNB. ²Department of Medical Biochemistry and Biophysics Karolinska Institutet.

17:30-19:30

Symposium 5C
SAIB-SBBM Chile Symposium
Bacterial Interactions
Dreams Convention Center Salon Puerto Octay

Chairs: Andrea Smania
 Bernardo González

- 17:30-18:00 Mutating for survival: genomic evolution of *Pseudomonas aeruginosa* mutator populations from cystic fibrosis airways.** Andrea Smania. Universidad Nacional de Córdoba, Argentina
- 18:00-18:30 Gene regulation and virulence strategies of the opportunistic pathogen *Serratia marcescens*.** Eleonora García-Vescovi. Universidad Nacional de Rosario, Argentina
- 18:30-19:00 Molecular signaling in interactions among plant growth promoting rhizobacteria and plants.** Bernardo González. Universidad Adolfo Ibáñez, Chile
- 19:00-19:30 Extremely halophilic bacteria, the case of *Salinibacter ruber* as model of study.** Ramón Rosselló-Mora, IMEDEA, CSIC-UIB, España

17:30-19:30

Symposium 5D
SBBM Chile Symposium
Epigenetics
Dreams Convention Center Salon Frutillar

Chair: Alejandra Loyola

- 17:30-18:00 Cancer progenitors and epigenetic contexts.** Rubén Agrelo. Institut Pasteur de Montevideo, Uruguay

18:00-18:30 RNA-directed dynamic histone acetylation. Jim Davie, Manitoba Institute of Child Health, Canada

18:30-19:00 Nucleosomes and transcriptional regulation: factors modulating ATP-dependent chromatin remodeling activity. José Leonardo Gutiérrez, Universidad de Concepción, Chile

19:00-19:30 Histone modification and exchange during gene transcription: signals and mechanisms. Jerry Workman. Stowers Institute, USA

19:45-20:45

Plenary Lecture
Patagónico Convention Center Salon Los Volcanes

Chair: Cecilia Hidalgo

The evolution of the universe: from the Big-Bang to life. María Teresa Ruiz. Departamento de Astronomía, Facultad de Ciencias Físicas y Matemáticas, Centro de Astrofísica y Tecnologías Afines (CATA), Universidad de Chile; Santiago, Chile

Nov 13 09:00-10:00

Lecture 8A
FEBS Lecture
Patagónico Convention Center Salon Los Volcanes

Chair: Miguel Ángel De la Rosa

Biointeractomic scaffold hovering on cytochrome c under programmed cell death. Irene Díaz-Moreno, Jonathan Martínez-Fábregas, Katiuska González-Arzola, Isabel Cruz-Gallardo, Blas Moreno-Beltrán, Alejandra Guerra-Castellano, Carlos A. Elena-Real, José M. García-Heredia, Francisco Rivero-Rodríguez, Antonio Díaz-Quintana and Miguel A. De la Rosa. Instituto de Bioquímica Vegetal y Fotosíntesis, Centro de Investigaciones Científicas Isla de la Cartuja (cicCartuja) Universidad de Sevilla – CSIC, España

09:00-10:00

Lecture 8B
CSMB Lecture
Dreams Convention Center

Chair: Ariel Castro

The mitochondria-associated membrane as a signaling hub: redox meets Rabs. Thomas Simmen. Department of Cell Biology, Faculty of Medicine & Dentistry, University of Alberta

10:00-10:30

Coffee Break
Roofed Street Santa Rosa, Puerto Varas Square

10:30-12:45

Poster and Exhibitors
Roofed Street Santa Rosa, Puerto Varas Square

13:00-15:00

Lunch Time

15:00-17:00

**Symposium 6A
 SBBM Chile Symposium
 Biological Clocks and Light Sensing
 Patagónico Convention Center Salon Osorno**

Chair: Luis F. Larrondo

- 15:00-15:30** **Regulatory networks in fungi governing global responses to changes in light and time.** Jay C. Dunlap. Geisel School of Medicine, Dartmouth, USA
- 15:30-16:00** **Photobiology in model and clinical fungi.** Jennifer Loros. Geisel School of Medicine, Dartmouth, USA
- 16:00-16:30** **Uncovering *Arabidopsis* clock transcriptional circuits by functional genomics.** Jose Pruneda-Paz, University of California, San Diego, USA
- 16:30-17:00** **Light and time, two variables governing transcriptional changes and virulence in fungi: the plant-pathogen *B. cinerea* as a case study.** Luis Larrondo. Facultad de Ciencias Biológicas Pontificia Universidad Católica de Chile, Chile

15:00-17:00

**Symposium 6B
 ASBMB-SBBM Chile Symposium
 Structural Aspects of Signal Transduction
 Patagónico Convention Center Salon Calbuco-Tronador**

Chair: Tracy Handel

- 15:00-15:30** **Functional and structural characterization of the light-activated transduction pathway from *Brucella*.** Gastón Paris. Instituto Leloir, Argentina
- 15:30-16:00** **Atypical function of the typical chemokine receptor, CCR1.** C. Taylor Gilliland¹, Catherina L. Salanga¹, Tetsuya Kawamura¹, JoAnn Trejo², and Tracy M. Handel¹
¹Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, CA 92093; ²Department of Pharmacology, School of Medicine, University of California, San Diego, La Jolla, USA
- 16:00-16:30** **G protein coupling to GPCRs: structural and functional insights into the reciprocal G protein and GPCR interaction.** Roger K. Sunahara, University of Michigan Medical School, USA.
- 16:30-17:00** **Molecules that drive the exocytosis of human sperm dense core granule: second messengers, snares and small G-proteins.** Claudia Tomes, Universidad Nacional de Cuyo, Argentina

15:00-17:00

**Symposium 6C
 SBCH - LAPSM Symposium
 Proteins of bacterial division and inhibitors
 Dreams Convention Center Salon Puerto Octay**

Chair: Octavio Monasterio

- 15:00-15:30** **Intermolecular interactions of FtsZ and ZipA: two essential components of the *E. coli* divisome.** Allen Minton. LBG, Physical Biochemistry Section NIDDK, National Institutes of Health, USA

15:30-16:00 Bacterial division: nucleation and polymerization mechanism of *Escherichia coli* FtsZ. Octavio Monasterio, Departamento de Biología, Facultad de Ciencias, Universidad de Chile

16:00-16:30 Structure-function analysis of MurJ. Natacha Ruiz. Department of Microbiology, The Ohio State University, USA.

16:30-17:00 Morphogenesis of *Escherichia coli*. Tanneke den Blaauwen Faculteit der Natuurwetenschappen, Wiskunde en Informatica, SILS, Amsterdam.

15:00-17:00
Symposium 6D
SBCH - LAPSM Symposium
Cellular response
Dreams Convention Center SalonFrutillar

Chair: Carlos Jerez

15:00-15:30 High metal resistance strategies in extreme acidophilic bacteria. Carlos A. Jerez. Departamento de Biología, Facultad de Ciencias, Universidad de Chile.

15:30-16:00 Mechanisms of signaling and regulation of membrane properties by a bacterial thermosensor. Diego de Mendoza. Departamento de Microbiología de la Facultad de Ciencias Bioquímicas y Farmacéuticas de la Universidad Nacional de Rosario, Argentina

16:00-16:30 Diverse signals lead *Bacillus subtilis* to build a biofilm. Roberto Kolter. Department of Microbiology and Immunobiology, Harvard Medical School, USA.

16:30-17:00 *Salmonella* Typhi: reductive evolution and the process of host specificity. Guido Mora. Departamento de Microbiología, Facultad de Medicina, Universidad Andrés Bello

17:15-17:45
Coffee Break
Dreams Convention Center / Patagónico Convention Center

17:45-19:45
Symposium 7A
Virology
SBBM Chile Symposium
Patagónico Convention Center Salon Osorno

Chair: Rafael A. Medina

17:45-18:15 Entry and exit of alphaviruses and flaviviruses. Margaret Kielian. Albert Einstein, College of Medicine, USA

18:15-18:45 Direct recruitment of the ribosome by the RNA structure: a general feature of viral IRES? Bruno Sargueil. Centre National de la Recherche Scientifique Unité Mixte de Recherche, Paris, France

18:45-19:15 Influenza virus NS1, a master regulator of the antiviral host response. Adolfo García-Sastre. Icahn School of Medicine at Mount Sinai, USA

19:15-19:45 The role of glycosylations in the antigenic properties of Influenza A viruses. Rafael A. Medina. Pontificia Universidad Católica de Chile, Chile

17:45-19:45

Symposium 7B
SBBM Chile Symposium
Cancer translational research
Patagónico Convention Center Salon Calbuco-Tronador

Chairs: Ricardo Armisen
 Catriona Jamieson

- 17:45-18:15 Malignant reprogramming: biologic and therapeutic implications.** Catriona Jamieson, Sanford Consortium for Regenerative Medicine & Moores Cancer Center, University of California San Diego, USA
- 18:15-18:45 Next generation sequencing for improved cancer care.** Ricardo Armisen, Ricardo Verdugo, Katherine Marcelain, Shawn Yost, Kristen Jepsen, Olivier Harismendy & Kelly Frazer. Centro de Investigación y Tratamiento del Cáncer & ICBM, Facultad de Medicina, Universidad de Chile, Chile. Moores Cancer Center & Division of Genome Information Sciences, University of California San Diego, USA
- 18:45-19:15 Biomarkers in cancer management: from scratch to clinical analysis.** Alejandro Corvalán, Maria José Maturana, Wilda Olivares, Andres Rodriguez, Katheleen Saavedra, Alejandra Sandoval & Benjamin Garcia. Center for Advanced Chronic Diseases (ACCDiS), Facultad de Medicina, Pontificia Universidad Católica de Chile, Chile
- 19:15-19:45 Genomic approaches in cancer research.** Kelly Frazer, Moores Cancer Center & Division of Genome Information Sciences, University of California San Diego, USA

17:45-19:45

Symposium 7C
SBCH - LAPSM Symposium
Microorganisms and Molecular Evolution
Dreams Convention Center Salon Frutillar

Chair: David Holmes

- 17:45-18:15 Resolving power of the 16S rRNA for a global classification of uncultured prokaryotes.** Ramón Rosselló-Mora. Departamento de Recursos Naturales, IMEDEA, CSIC-UIB, Crtra. España.
- 18:15-18:45 Protein evolution during the Archean anaerobic/aerobic transition.** David Holmes. Fundación Ciencia y Vida. Universidad Andrés Bello, Chile.
- 18:45-19:15 Dissection of the *in vivo* interplay of molecular features determining protein evolution.** Alejandro Vila, Instituto de Biología Molecular y Celular de Rosario, Universidad Nacional de Rosario
- 19:15-19:45 Gleaning biological insights from Andean microbial community analyses.** Mercedes Zambrano. Corpogen, Bogotá, Colombia

17:45-19:45

Symposium 7D
SBCH - LAPSM Symposium
Supramolecular protein interactions
Dreams Convention Center Salon Puerto Octay

Chair: Rosalba Lagos

17:45-18:15 Amyloid formation modulates the antibacterial activity of microcin E492, a pore-forming bacteriocin. Rosalba Lagos. Departamento de Biología, Facultad de Ciencias, Universidad de Chile.

18:15-18:45 Structural transformation of a transcription factor RfaH. Irina Artsimovitch. Department of Microbiology, The Ohio State University, USA.

18:45-19:15 The protein folding pathway: a coordinated network of molecular chaperones. José María Valpuesta. Centro Nacional de Biotecnología, CSIC, Campus de Cantoblanco, España.

19:15-19:45 Protein Interaction and dynamics using NMR: studies of defensins and allergens. Ana Paula Valente. Institute of Medical Biochemistry at Universidade Federal do Rio de Janeiro, Brasil

20:30-22:00

Closing Dinner
Dreams Convention Center

22:30

Congress Party
Dreams Convention Center

Nov 14 09:30-11:15

Awards Ceremony
Patagónico Convention Center Salon Los Volcanes

Chair: Marcelo López-Lastra

09:30-10:10

Grupo BIOS Award

10:10-10:40

Hermann Niemeyer Medal Award

10:40-10:55

Tito Ureta Award

10:55-11:05

Fermelo-SBBM Chile Awards

11:05-11:15

Biochemical Journal Award

11:15-11:45

Coffee Break
Patagónico Convention Center

11:45-12:45

Closing Lecture
Patagónico Convention Center Salon Los Volcanes

Chair: Marilú Cárdenas

Recent results on mechanochemical conversion in a viral ring ATPase. Carlos Bustamante. Jason L. Choy Laboratory of Single-Molecule Biophysics and Department of Physics; QB3 Institute; Department of Chemistry; Department of Molecular and Cell Biology; Howard Hughes Medical Institute; University of California, Berkeley, California, USA

12:45-13:30

Closing Ceremony
Patagónico Convention Center Salon Los Volcanes



POSTER SESSIONS PROGRAM

November 10, Sunday
Roofed Street Santa Rosa
16:45-19:15
Posters from 1 to 161

1 Area: Biomedicine

Title: Insulin reverses gestational diabetes-increased L-arginine transport involving activation of insulin receptors A and B in human umbilical vein endothelium

Authors: Guzmán-Gutiérrez, E¹, Sáez, T¹, Salsoso, R¹, Arroyo, P¹, Pardo, F¹, Leiva, A¹, Sobrevia, L^{1,2}, ¹Cellular and Molecular Physiology Laboratory (CMPL), Division of Obstetrics and Gynaecology, School of Medicine, Faculty of Medicine, Pontificia Universidad Católica de Chile. ²Centre for Clinical Research (UQCCR), Faculty of Health Sciences, University of Queensland, Australia.

2 Area: Biomedicine

Title: Adaptive responses of mitochondria when under mild copper deprivation, involve changes in morphology, oxphos remodeling, and bioenergetics

Authors: Ruiz, L¹, Jensen, E¹, Buston, R¹, Elorza, A¹, ¹Center for Biomedical Research, Faculty of Biological Sciences and Faculty of Medicine, Universidad Andrés Bello.

3 Area: Biomedicine

Title: Caffeic acid and its alkyl esters: The effect of hydrophobicity in the antioxidant and anti-*Helicobacter pylori* potency

Authors: Chiquetto Paracatu, L¹, Bonacorsi, C², Quinello Gomes De Faria, C², Nazare, A², Segalla Petronio, M², Fonseca, L², Ximenes, V³, ¹Clinical Analysis, School of Pharmaceutical Sciences, Sao Paulo State University. ²Clinical Analysis Sao Paulo State University. ³Department of Chemistry, Faculty of Science, Sao Paulo State University.

4 Area: Biomedicine

Title: Protocatechuic Acid Alkyl Esters as Inhibitors of NADPH Oxidase

Authors: Quinello Gomes De Faria, C¹, Chiquetto Paracatu, L¹, Nazaré, A¹, Zeraik, M², Petrónio, M¹, Silva, D², Fonseca, L¹, Ximenes, V³, ¹ Clinical Analysis, Faculty School of Pharmaceutical Sciences, Sao Paulo State University. ²Department of Organic Chemistry, Faculty School of Pharmaceutical Sciences, Sao Paulo State University. ³Department of Chemistry, Faculty of Science, Sao Paulo State University.

5 Area: Biomedicine

Title: Cytotoxic potential of anthraquinones from *Rhamnus sphaerosperma* var. *pubescens* in uterine tumor cell lines

Authors: MOREIRA T, F¹, Oliveira D M, S², Ocampos F M, M², Souza F, O³, Baviera A, M³, Stuelp-Campelo P, M⁴, Barison, A⁵, Miguel O, G², Soares C, P¹, Brunetti I, L⁶, ¹Clinical Analysis, School of Pharmaceutical Sciences, UNESP/Brazil. ²Pharmacy UFPR/Brazil. ³Clinical Analysis UNESP/Brazil. ⁴Pharmacy PUCPR/Brazil. ⁵Chemistry UFPR/Brazil. ⁶Clinical Analysis, School of Pharmaceutical Sciences, Universidade Estadual Paulista - UNESP.

6 Area: Biomedicine

Title: CK2 regulates ECE-1c by phosphorylation at its N-terminal end

Authors: Niechi, I¹, Díaz, C¹, Nogales, M¹, Armisen, R^{2,3}, Fernández, C^{4,3}, Tapia, J^{1,2,3}, ¹Cell Transformation Lab, Facultad de Medicina, Universidad De Chile. ²ICBM, Facultad de Medicina, Universidad De Chile. ³CITC: Center for Research and Treatment of Cancer, Facultad de Medicina, Universidad De Chile. ⁴Department of Anatomopathology, HCUCH, Universidad de Chile.

7 Area: Biomedicine

Title: Contribution of inflammation and oxidative stress to the cardiorespiratory alterations induced by intermittent hypoxia

Authors: Iturriaga, R¹., Moya, E¹., Del Rio, R¹., ¹Lab Neurobiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.

8 Area: Biomedicine

Title: Association of the *EcoRI* and *XbaI* polymorphisms at the Apolipoprotein B Gene with lipid profile in Colombian Caribbeans

Authors: Mendoza, E^{1,3}., Pereira, N¹., De La Espriella, C²., Villanueva, D³., ¹Medicine Program Universidad Libre de Barranquilla, Colombia. ²Microbiology Program Universidad Libre de Barranquilla, Colombia. ³Pathologic Biochemistry Research Group Universidad Libre de Barranquilla, Colombia.

9 Area: Biomedicine

Title: Biochemical consequences of hyh (M105I) mutation on alpha-SNAP protein during brain development

Authors: Parga, C¹., Ojeda, L¹., Toro, G¹., Muñoz, C¹., Marquez, G¹., Muñoz, R¹., Mardones, G²., Batiz, L¹., ¹Instituto de Anatomía, Histología y Patología, Facultad de Medicina, Universidad Austral De Chile. ²Instituto de Fisiología, Facultad de Medicina, Universidad Austral de Chile.

10 Area: Biomedicine

Title: An expanded and functionally diverse family of Kunitz inhibitors from a metazoan parasite

Authors: Fló, M¹., Soto, E²., Alvarez, B³., Fernandez, C¹., ¹Department of Biosciences, Faculty of Chemistry, Universidad de la Republica. ²Laboratory of Neurophysiology, Institute of Physiology, Benemérita Universidad Autónoma de Puebla. ³Laboratory of Enzymology, Faculty of Science, Universidad de la Republica.

11 Area: Biomedicine

Title: Markers of early endothelial dysfunction in IUGR-derived human umbilical vein endothelial cells revealed by 2d-dige and mass spectrometry analyses

Authors: Caniuguir, A¹., Krause, B¹., Casanello, P¹., ¹Division of Obstetrics and Gynecology & Pediatrics, School of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile.

12 Area: Biomedicine

Title: Ryanodine receptor-mediated Ca²⁺ release underlies iron-induced mitochondrial fission and stimulates mitochondrial Ca²⁺ uptake in primary hippocampal neurons

Authors: Paula-Lima, A^{1,2}., SanMartin, C²., García, A³., Hartell, S^{3,4}., Núñez, M⁵., Hidalgo, C⁶., ¹Department of Basic and Communitarian Sciences, Faculty of Dentistry, Universidad De Chile. ²BNI and CEMC Universidad De Chile. ³BNI, Faculty of Medicine, Universidad de Chile. ⁴Anatomy and Developmental Biology Program, ICBM, Faculty of Medicine, Universidad de Chile. ⁵Department of Biology, Research Ring on Oxidative Stress in the Nervous System, Faculty of Sciences, Universidad de Chile. ⁶Physiology & Biophysics Program, ICBM, Faculty of Medicine, Universidad de Chile.

13 Area: Biomedicine

Title: Assessment of the antioxidant capacity of the uremic solutes and their mixtures through *in vitro* model systems

Authors: Assis R, P¹., Castro J F, A²., Gutierrez V, O¹., Arcaro C, A¹., OLIVEIRA O M M, F²., BAVIERA A, M¹., BRUNETTI I, L¹., ¹Department of Clinical Analysis, School of Pharmaceutical Sciences, UNESP/Brazil. ²Department of Biochemistry and Technological Chemistry, Institute of Chemistry, UNESP/Brazil.

14 Area: Biomedicine

Title: Preparation and characterization of ACE-inhibitor peptides, employing plant peptidases from *Bromelia hieronymi* on bovine whey proteins.

Authors: Bruno, M¹., Liggieri, C¹., Bertucci, J¹., ¹Ciencias Biológicas, Ciencias Exactas, Universidad Nacional de La Plata, Argentina.

15 Area: Biomedicine

Title: Development of porous chitosan-gelatin composite scaffolds for bone tissue engineering applications

Authors: Forero, J¹., Acevedo, C²., Roa, E¹., Osses, N¹., ¹Química, Ciencias, Pontificia Universidad Católica De Valparaíso. ²Centro de BIOTECNOLOGÍA, Centro de BIOTECNOLOGÍA, Universidad Técnica Federico Santa María.

16 Area: Biomedicine

Title: Characterization of HTLV-1 Tax protein secreted from peripheral blood mononuclear cells of patients with paraparesis

Authors: Medina, F¹., Quintremil, S¹., Alberti, C¹., Godoy, F¹., Puente, J¹., Ramírez, E^{2,3}., Cartier, L⁴., Valenzuela, M¹., ¹Bioquímica y Biología Molecular, Ciencias Químicas y Farmaceuticas, Universidad De Chile. ²Programa de Virología, ICBM, Medicina, Universidad de Chile. ³Virología ISP. ⁴Dept Ciencias Neurológicas, Medicina, Universidad De Chile.

17 Area: Biomedicine

Title: Biological characterization and chemical interference of trypanothione biosynthesis in trypanosomatids

Authors: Medeiros, A¹., Fiestas, L²., Benítez, D²., Charquero, D²., Comini, M²., ¹Departamento de Bioquímica, Facultad de Medicina, Universidad de la República. ²Laboratory Redox Biology of Trypanosomes, Institut Pasteur of Montevideo, Montevideo, Uruguay.

18 Area: Biomedicine

Title: Discovery of 5-HT₆ receptor ligands: Design, synthesis and biological evaluation of novel N-Arylsulfonylindole derivatives

Authors: Lagos, C^{1,2}., Vera, G²., Almendras, S²., Recabarren-Gajardo, G²., ¹Department of Endocrinology, School of Medicine, Pontificia Universidad Católica de Chile. ²Department of Pharmacy, School of Chemistry, Pontificia Universidad Católica de Chile.

19 Area: Biomedicine

Title: Environmental contaminants (Tributyltin) affect the estrogen and progesterone receptors binding capacity in the uterus of ovariectomized rats.

Authors: Rodríguez Piñón, M¹., Bernardes Graceli, J²., Tasende, C¹., ¹Biochemistry, Department of Molecular and Cellular Biology, Veterinary Faculty, Universidad de la República, Montevideo, Uruguay. ²Department of Morphology, Ciencias da Saude, Federal University of Espírito Santo-UFES, Espírito Santo, Brazil.

20 Area: Biomedicine

Title: Prevention of myocardial ischemia-reperfusion injury by inhibition of the proteasome

Authors: Montecinos, L¹., Pezoa, J¹., Olmedo, I¹., Pedrozo, Z¹., Said, M²., Donoso, P¹., Sánchez, G³., ¹Departamento de Fisiología y Biofísica, Facultad de Medicina, Universidad de Chile. ²Centro de Investigaciones Cardiovasculares, Facultad de Ciencias Médicas, Universidad Nacional de La Plata, Argentina. ³Departamento de Fisiopatología, Facultad de Medicina, Universidad de Chile.

21 Area: Biomedicine

Title: Determination of similarities between monoaminergic proteins and nicotinic receptors: an approach to rational design of poly-pharmacological drug

Authors: Moller, P^{1,2}, Reyes, M¹, Alzate, J², Rojas, C², Contreras, J², ¹Programa Doctorado en Biotecnología, Química y Biología, Univesidad de Santiago de Chile. ²Escuela de Bioinformática, Facultad de Ingeniería, Universidad de Talca.

22 Area: Biomedicine

Title: Arbovirus Oropouche: Expression of structural proteins in insect cells for structural and serological diagnosis research

Authors: Uehara, M¹, Juncansen, C¹, Garcia, F., B¹, Ferreira, M., U², Nogueira, M., L³, Sperança, M., A¹, ¹CCNH UFABC Universidade Federal do ABC. ²Departamento de Parasitologia, Instituto de Ciências Biomédicas, Universidade de São Paulo. ³Laboratório de Virologia Faculdade de Medicina de São José do Rio Preto.

23 Area: Biomedicine

Title: Rapamycin-conditioned dendritic cells activated with MPLA show a tolerogenic profile

Authors: Narvaez, E¹, Pérez, F¹, Gajardo, T¹, Soto, L¹, Catalán, D¹, Aguilón, J¹, Pino-Lagos, K¹, ¹Facultad de Medicina, Immune Regulation and Tolerance Research Group (www.irtgroup.cl), Programa de Inmunología, Universidad de Chile.

24 Area: Biomedicine

Title: Nitric oxide production mediates dexmedetomidine-induced cardiac preconditioning

Authors: Riquelme, J^{1,2}, Montecinos, L², Hassi, M², Fernandez, C¹, Ibacache, M³, Pedrozo, Z², Sanchez, G², Lavandero, S^{1,2}, ¹Centro de Estudios Moleculares de la Célula, Facultad de Ciencias Químicas y Farmacéuticas, Universidad De Chile. ²Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad De Chile. ³División de Anestesiología, Facultad de Medicina, Pontificia Universidad Católica de Chile.

25 Area: Biomedicine

Title: The apoptosis/necrosis ratio of H₂O₂-induced death of lymphocytes as a possible biomarker for early detection of Alzheimer's disease

Authors: Ponce, D¹, Salech, F¹, Henríquez, M¹, Quest, A^{1,2}, Behrens, M^{1,2,3,4}, ¹Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad De Chile. ²Centro de Estudios Moleculares de la Célula, Facultad de Medicina, Universidad De Chile. ³Neurología y Neurocirugía, Hospital Clínico, Universidad De Chile. ⁴Neurología Clínica Alemana de Santiago, Chile.

26 Area: Biomedicine

Title: Electrophilic signaling of fatty acid nitroalkenes in Amyotrophic Lateral Sclerosis

Authors: Rubbo, H¹, Diaz-Amarilla, P², Trostchansky, A¹, Freeman, B³, Cassina, P¹, Barbeito, L², ¹Biochemistry, Medicine, Universidad de la República. ²Neurobiología Instituto de Investigaciones Biológicas Clemente Estable and Instituto Pasteur Montevideo. ³Department of Pharmacology University of Pittsburgh.

27 Area: Biomedicine

Title: The treatment based on ISAV or hRSV fusion protein containing apoptotic bodies induces a greater maturation of dendritic cells derived from C57BL/6 mice, than in the absence of viral fusion proteins

Authors: Barrientos C., Mena J., López X., Cortez M, Montoya M, Spencer E, Acuña-Castillo C Center of Aquaculture Biotechnology, Faculty of Chemistry and Biology, University of Santiago de Chile

28 Area: Biomedicine

Title: Wnt signaling modulates transcription of translocation-prone genes in hematopoietic cells

Authors: Ugarte, G¹, Vargas, M¹, Medina, M¹, Leon, P¹, Roman, F¹, Caracci, M¹, Elorza, A¹, Gutierrez, S², Loyola, A³, De Ferrari, G¹, ¹Centro de Investigaciones Biomédicas y Centro FONDAP de Regulación del Genoma, Facultad de Ciencias Biológicas, Universidad Andrés Bello. ²Dept. Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción. ³Lab. Epigenética y Cromatina Fundación Ciencia & Vida.

29 Area: Biomedicine

Title: Insulin reversal of gestational diabetes mellitus reduced adenosine transport requires downregulation of insulin receptor A and p42/44^{mapk} in human umbilical vein endothelium

Authors: Westermeier, F^{1,2}, Salomón, C^{3,1}, Arroyo, P¹, Guzmán-Gutiérrez, E¹, Pardo, F¹, Leiva, A¹, Farías, M¹, Sobrevia, L^{1,3}, ¹Departamento de Ginecología y Obstetricia, Facultad de Medicina, Pontificia Universidad Católica De Chile. ²Escuela de Química y Farmacia, Facultad de Ciencia, Universidad San Sebastián. ³UQ Centre for Clinical Research, Faculty of Health Sciences, University of Queensland.

30 Area: Computational Biology and Bioinformatics

Title: Molecular modeling and structural analysis of TASK-1 potassium channel interacting with the blocker A1899

Authors: Martínez, G^{1,2}, Poblete, T², González, W³, ¹Vicerrectoría de Investigación y Estudios Avanzados Pontificia Universidad Católica de Valparaíso. ²Escuela de Ingeniería en Bioinformática, Facultad de Ingeniería, Universidad de Talca. ³Centro de Bioinformática y Simulación Molecular Universidad de Talca.

31 Area: Computational Biology and Bioinformatics

Title: Allosteric communication pathways and vibrational energy flow in proteins: PDZ-2 case

Authors: Miño, G¹, Martínez, L², ¹Group of NanoMaterials, Departamento de Física, Facultad de Ciencias, Universidad de Chile. ²Department of Physical Chemistry, Institute of Chemistry, State University of Campinas - UNICAMP.

32 Area: Computational Biology and Bioinformatics

Title: Genome assembly of the lignocellulolytic fungus *Penicillium purpurogenum* using third-generation sequencing technology

Authors: Eyzaguirre, J¹, Mardones, W¹, Di Genova, A², Maass, A³, ¹Ciencias Biológicas, Ciencias Biológicas, Universidad Andrés Bello. ²Centro de Modelamiento Matemático, Facultad de Ciencias Físicas y Matemáticas, Universidad de Chile. ³Centro de Modelamiento Matemático, Facultad de Ciencias Físicas y Matemáticas, Universidad de Chile.

33 Area: Computational Biology and Bioinformatics

Title: An Ontology of *C. elegans* resources and experiments to study extended lifespan phenotype

Authors: Cifuentes, J^{1,2}, Callahan, A³, Melo, F^{1,2}, Dumontier, M^{3,4}, ¹Laboratorio de Bioinformática Molecular. Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica De Chile. ²Laboratorio de Bioinformática Molecular Instituto Milenio en Inmunología e Inmunoterapia. ³Dumontier Laboratory, Department of Biology, Faculty of Science, Carleton University. ⁴Stanford Center for Biomedical Informatics Research, School of Medicine, Stanford University.

34 Area: Computational Biology and Bioinformatics

Title: A trade-off between amino acid metabolic cost and protein sequence diversity in proteome composition

Authors: Krick, T¹, Shub, D², Verstraete, N⁵, Ferreiro, D., Alonso, L³, Shub, M⁴, **Sanchez, I⁶**, ¹Departamento de Matematica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires and IMAS-CONICET. ²Department of Biological Sciences SUNY Albany. ³Protein Structure-Function and Engineering Laboratory Fundacion Instituto Leloir and IIBBA-CONICET. ⁴IMAS-CONICET Universidad de Buenos Aires. ⁵Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires and IQUIBICEN-CONICET.

35 Area: Computational Biology and Bioinformatics

Title: Putative binding site for the positive allosteric modulator SB-206553, in 5-HT_{2B} and 5-HT₃ receptors

Authors: **Rojas, C¹**, Contreras, J¹, Möller, P², Reyes, M³, Alzate-Morales, J⁴, ¹Escuela de Ingeniería en Bioinformática, Facultad de Ingeniería, Universidad de Talca. ²Programa Doctorado en Biotecnología, Facultad de Química y Biología, Universidad de Santiago de Chile. ³Unidad de Metodología de la Investigación, Facultad de Ciencias Médicas, Universidad de Santiago de Chile. ⁴Centro de Bioinformática y Simulación Molecular, Facultad de Ingeniería, Universidad de Talca.

36 Area: Computational Biology and Bioinformatics

Title: Ligand prediction of orphan pockets by three-dimensional comparison of protein binding sites

Authors: **Schüller, A^{1,2}**, Almonacid, L^{1,2}, Melo, F^{1,2}, ¹Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile. ²Molecular Bioinformatics Laboratory Millennium Institute on Immunology and Immunotherapy.

37 Area: Computational Biology and Bioinformatics

Title: Assessing the molecular impact of mutations in Protein Superfamilies identified in whole-exome sequences of tumors

Authors: **Almonacid, D¹**, Soto, J¹, Pizarro, D¹, Gonzalez-Nilo, F¹, Peterson, T², Kann, M², ¹Department of Biological Sciences Universidad Andres Bello. ²Department of Biological Sciences University of Maryland, Baltimore County.

38 Area: Computational Biology and Bioinformatics

Title: Modulation of P2X4 receptors by Alfaxalone, studied via molecular dynamic simulations

Authors: **Alveal, N¹**, Navarrete, C², Coddou, C³, Escalona, Y⁴, Perez-Acle, T⁴, Stojilkovic, S⁵, Huidobro-Toro, J², Barrera, N², ¹Fisiología Pontificia Universidad Católica de Chile. ²Physiology, Pontificia Universidad Católica de Chile. ³Section on Cellular Signaling NICHD. ⁴Fundación Ciencia para la Vida Fundación Ciencia para la Vida. ⁵Section on Cellular Signaling NIH.

39 Area: Computational Biology and Bioinformatics

Title: Structural characterization of the internal cavity of the pore in K⁺ channel shaker: contribution of Pro475 residue to the ion current at atomic-level perspective

Authors: **Varas, I¹**, Marabolí Boutaud, V¹, Márquez-Miranda, V^{1,2}, Sepúlveda, R^{1,2}, González-Nilo, F^{1,3}, ¹Center for Bioinformatics and Integrative Biology, Facultad de Ciencias Biológicas, Universidad Andrés Bello. ²Doctorado en Biotecnología Universidad Andrés Bello. ³Centro Interdisciplinario de Neurociencias de Valparaíso (CINV) Universidad de Valparaíso.

40 Area: Computational Biology and Bioinformatics

Title: Identification of novel transient receptor potential (TRP) superfamily members and structural characterization of their activation by PIP₂

Authors: Alegría-Arcos, M¹., Almonacid, D^{1,2}., Poblete, H²., Latorre, R³., González-Nilo, F¹., ¹Center for Bioinformatics and Integrative Biology (CBIB), Facultad de Ciencias Biológicas, Universidad Andrés Bello. ²Centro de Bioinformática y Simulación Molecular Universidad de Talca. ³Centro Interdisciplinario de Neurociencias de Valparaíso (CINV) Universidad de Valparaíso.

41 Area: Computational Biology and Bioinformatics

Title: Computational methods to analyse the release of membrane proteins from detergent micelles in the gas phase

Authors: Montenegro, F¹., Barrera, M¹., Robinson C., V²., Barrera N., P¹., ¹Department of Physiology, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile. ² Department of Chemistry University of Oxford.

42 Area: Enzymes and Metabolism

Title: Increased glucose tolerance and insulin sensitivity in streptozotocin-diabetic rats treated with curcumin incorporated in yoghurt

Authors: Gutierrez V, O¹., Assis R, P¹., Arcaro C, A¹., Costa P, I¹., Baviera A, M¹., Brunetti I, L¹., ¹Clinical Analysis, School of Pharmaceutical Sciences, UNESP/Brazil.

43 Area: Enzymes and Metabolism

Title: Characterization of phenoloxidase activity from spider *Polybetes pythagoricus* hemocyanin

Authors: Cunningham, M¹., Laino, A¹., Suarez, G¹., Lino, A¹., Lavarias, S²., ¹Bioquímica, Facultad de Ciencias Médicas, Universidad Nacional de La Plata. ²Instituto de Limnología, Facultad de Ciencias Médicas, Universidad Nacional de La Plata.

44 Area: Enzymes and Metabolism

Title: Oxidative metabolism of polyamines and embryonic development in *Rhinella arenarum* embryos exposed to azinphos methyl

Authors: Lascano, C³., Del Brío, D³., Ferrari, A^{1,2}., Venturino, A^{3,2}., ¹Química Biológica, Ciencias Médicas, Universidad Nacional del Comahue. ²LIBIQUIMA, IDEPA, CONICET. ³Biología Aplicada, Ciencias Agrarias, Universidad Nacional del Comahue.

45 Area: Enzymes and Metabolism

Title: Bag3 controls skeletal muscle cell metabolism

Authors: Peña-Oyarzún, D¹., Rodríguez, A¹., Lavandero, S¹., ¹Departamento de Bioquímica y Biología Molecular, CEMC, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.

46 Area: Enzymes and Metabolism

Title: Kinetics and thermostability studies of a hyperthermophilic bifunctional ADP-dependent glucokinase/phosphofructokinase from *Methanocaldococcus jannaschii*

Authors: Herrera-Morande, A^{1,2}., Castro-Fernandez, C¹., Rivas-Pardo, J¹., Vega, C²., Guixé, V¹., ¹Departamento de Biología, Facultad de Ciencias, de la Universidad de Chile. ²CSIC, Madrid Centro de Investigaciones Biológicas.

47 Area: Enzymes and Metabolism

Title: Temporo-spatial analysis of glucose transporter glut8 expression in mammary gland during pregnancy, lactation and weaning

Authors: Muñoz, M¹., Villagrán, M³., Venegas, C³., Ruminot, I³., Parra, E³., Faundez, V²., Mardones, L³., ¹Departamento de Ciencias Básicas, Facultad de Medicina, Universidad Católica de La Santísima Concepción. ²Departamento de Ingeniería Ambiental y Recursos Naturales, Facultad de Ingeniería, Universidad Católica de La Santísima Concepción. ³Departamento de Ciencias Básicas, Facultad de Medicina, Universidad Católica de La Santísima Concepción.

48 Area: Enzymes and Metabolism

Title: Identification, characterization and functional expression of a histidine ammonia-lyase from *Rhodobacter sphaeroides* (Rs)

Authors: Escalona, E¹., Gentina, J²., Guzmán, L³., Aguilar, F⁴., ¹Laboratorio de Química-Biológica, Ciencias, Pontificia Universidad Católica de Valparaíso. ²Laboratorio de Cultivos Microbianos Pontificia Universidad Católica de Valparaíso. ³Laboratorio de Química-Biológica Pontificia Universidad Católica de Valparaíso. ⁴Laboratorio de Espectroscopía Molecular. Pontificia Universidad Católica de Valparaíso.

49 Area: Enzymes and Metabolism

Title: GRK2 as a novel target in insulin resistance and obesity

Authors: Vila-Bedmar, R²., Lucas, E²., Cruces, M²., Heijnen, C¹., Kavelaars, A¹., Mayor Jr., F²., Murga, C²., ¹Department of Symptom Research, Division of Internal Medicine, The University of Texas MD Anderson Cancer Center, Houston, USA. ²Biología Molecular, Ciencias, Autónoma de Madrid.

50 Area: Enzymes and Metabolism

Title: Cloning and expression of mouse liver agmatinase

Authors: Romero, N¹., García, D¹., Quiñones, M¹., Benítez, J¹., Carvajal, N¹., Uribe, E¹., Departamento de ¹Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.

51 Area: Enzymes and Metabolism

Title: Study of cruzipain expression in virulent and avirulent forms of *Trypanosoma cruzi* clone H510

Authors: San Francisco, J., Muñoz, C., Astudillo, C., Gutiérrez, B., González, J. Molecular Parasitology Unit, Faculty of Health Sciences, University of Antofagasta, Antofagasta, CHILE. ¹Unidad de Parasitología Molecular, Facultad de la Salud, Universidad de Antofagasta.

52 Area: Enzymes and Metabolism

Title: Learning metabolism by PBL-based analysis of web articles in Biochemistry

Authors: Bunster, M¹., Valenzuela, N¹., Márquez, C²., Bruna, C³., ¹Bioquímica y Biología Molecular, Ciencias Biológicas, Universidad de Concepción. ²Biología Celular, Ciencias Biológicas, Universidad de Concepción. ³Bioquímica y Biología molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.

53 Area: Gene expression

Title: The establishment of the cytosolic H3K9me1 modification is regulated by a cross-talk between SetDB1 and JMJD1B

Authors: Alvarez, F¹., Saavedra, F¹., Gurard-Levin, Z²., Diaz Celis, C¹., Almouzni, G²., Loyola, A^{1,3}., ¹Laboratory of Epigenetics & Chromatin Fundación Ciencia & Vida. ²Nuclear Dynamics and Genome Plasticity Institut Curie. ³Facultad de Medicina Universidad San Sebastián.

54 Area: Gene expression

Title: Identification and expression patterns of ABA perception genes (PYR family) in *Fragaria vesca* and *Fragaria chiloensis*

Authors: Lizana, R¹, Stappung, Y¹, Herrera, R¹, Moya-Leon, A¹, ¹Laboratorio de Fisiología Vegetal, Instituto de Biología Vegetal y Biotecnología, Universidad de Talca.

55 Area: Gene expression

Title: Evaluating light's effect on differentiation and virulence of the plant pathogen *Botrytis cinerea*: characterization of the White Collar complex

Authors: Canessa, P¹, Hevia, M¹, Larrondo, L¹, ¹Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.

56 Area: Gene expression

Title: Structural domains within the 5'untranslated region of the HIV-1 full length mRNA influence IRES activity

Authors: Carvajal, F¹, Vallejos, M¹, Pino, K¹, Slater, A^{2,3}, Melo, F^{2,3}, López-Lastra, M¹, ¹Laboratorio de Virología Molecular, Instituto Milenio de Inmunología e Inmunoterapia, Centro de Investigaciones Médicas, Escuela de Medicina, Pontificia Universidad Católica de Chile, Marcoleta 391, Santiago, Chile. ²Molecular Bioinformatics Laboratory, Millennium Institute on Immunology and Immunotherapy Pontificia Universidad Católica de Chile, Portugal 49, Santiago, Chile. ³Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas Pontificia Universidad Católica De Chile, Alameda 340, Santiago, Chile.

57 Area: Gene expression

Title: The residence of the yeast SWI/SNF complex on several gene regulatory regions is dependent on the HMGB protein Hmo1

Authors: Hepp, M¹, Workman, J², Gutierrez, J¹, ¹Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile. ²Stowers Institute for Medical Research, Kansas City, Missouri, USA.

58 Area: Gene expression

Title: SCL and RUNX1 regulate P1 promoter RUNX1 expression gene by association to RUNX sites binding

Authors: Martinez, M¹, Gutierrez, S¹, ¹Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad De Concepción.

59 Area: Gene expression

Title: Effect of CK2 α overexpression on ribosomal protein transcription in *Schizosaccharomyces pombe*

Authors: Moreira-Ramos, S¹, Montes, M¹, Barraza, V², Arias, L², Maldonado, E², ¹Programa de Biología Celular y Molecular, Facultad de Medicina Norte, Universidad de Chile. ²Programa de Biología Molecular y Celular, Facultad de Medicina Norte, Universidad De Chile.

60 Area: Gene expression

Title: Heterologous expression of *Pneumocystis carinii* TFIIB in the fission yeast *Schizosaccharomyces pombe*: An evidence of functional conservation on basal transcription mechanism

Authors: Rojas, D², Moreira-Ramos, S¹, Montes, M¹, Ponce, C², Vargas, S², Maldonado, E¹, ¹Programa Biología Celular y Molecular, ICBM, Medicina, Universidad de Chile. ²Programa Microbiología y Micología, ICBM, Medicina, Universidad de Chile.

61 Area: Gene expression

Title: Effects of vaccination and challenge against *Caligus rogercresseyi* on IgT mRNA expression in mucus of rainbow trout (*Onchoryncus mykiss*)

Authors: Nualart, D¹., Silva, H¹., Cortés, M¹., Troncoso, J²., Gonzalez, J²., Yañez, A¹., ¹Instituto de Bioquímica y Microbiología, Ciencias, Universidad Austral de Chile. ²innovation EWOS.

62 Area: Gene expression

Title: Relative Expression of genes involved in iron metabolism of *P. salmonis* in different growth conditions

Authors: Machuca, A²., Godoy, M¹., Martinez, V²., ¹ETECMA CIBA. ²FAVET-INBIOGEN. Animal Science, Faculty of Veterinary Sciences, University of Chile.

63 Area: Gene expression

Title: Effect of polar auxin transport changes on ripening and the proposed role of sugar

Authors: Serrano, A¹., Kuhn, N¹., Arce, P¹., ¹Genética Molecular y Microbiología, Ciencias Biológicas, Pontificia Universidad Católica de Chile.

64 Area: Gene expression

Title: A promoter element located in t(8:21) break point region is controlled by RUNX1

Authors: Hinojosa, M¹., Gutiérrez, S¹., ¹Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.

65 Area: Gene expression

Title: Characterization of tREST as a transcriptional regulator of the non-neuronal gene CDH1 (E-cadherin) in the H69 cell line of SCLC. Iris Aurora Nava Jimenez¹*, Carlos Ortuño Pineda², Jesús Valdés

Authors: Iris Aurora Nava Jimenez¹*, Carlos Ortuño Pineda², Jesús Valdés Flores¹ Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV), México, D.F., ¹Bioquímica Centro de Investigación y de Estudios Avanzados del Instituto Politecnico Nacional (CINVESTAV).

66 Area: Gene expression

Title: Functional studies of PcACE1 transcription factor from *P. chrysosporium* with copper

Authors: Silva, M¹., Bull, P¹., Valdivia, M¹., Rizzo, M¹., Villegas, C¹., ¹Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.

67 Area: Gene expression

Title: The unfolded protein response is required for growth under complex carbon sources in *Neurospora*

Authors: Goity, A¹., Montenegro-Montero, A¹., Larrondo, L¹., ¹Departamento Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.

68 Area: Immunology

Title: IL-33-and retinoic acid-signaling control CD4+ T cell biology

Authors: Morales, R¹., Campos-Mora, M¹., Gajardo, T¹., Narváez, E¹., Catalán, D¹., Aguillón, J¹., Pino-Lagos, K¹., ¹Immune Regulation and Tolerance Group, Medicina, Universidad de Chile.

69 Area: Immunology

Title: Study of nrp-1 expression in a murine skin transplant model

Authors: Campos-Mora, M¹., Morales, R¹., Narvaez, E¹., Catalán, D¹., Aguillón, J¹., Pino-Lagos, K¹., ¹Immune Regulation and Tolerance Research Group (www.irtgroup.cl), Programa de Inmunología, ICBM, Facultad de Medicina, Universidad De Chile.

70 Area: Immunology

Title: Murine macrophages stimulated *in vitro* with mollusk hemocyanins (KLH, CCH and FLH) present a different expression profile of pro-inflammatory cytokines and chemokines

Authors: Zhong, T¹., Arancibia, S¹., Del Campo, M¹., Tampe, R¹., Manubens, A²., Becker, M^{2,1}., ¹Investigación Fundación Ciencia y Tecnología para el Desarrollo (FUCITED). ²Investigación y Desarrollo Biosonda S.A.

71 Area: Immunology

Title: Priming of the kinin B1 receptor improves human neutrophil responses to fMet-Leu-Phe (fMLP)

Authors: Figueroa, C¹., Matus, C¹., Barría, F¹., Nualart, D¹., Pavicic, F¹., Andrade, Y¹., Bhoola, K¹., Ehrenfeld, P¹., ¹Anatomy, Histology and Pathology, Medicine, Universidad Austral de Chile.

72 Area: Immunology

Title: Contribution of the IL-33 Receptor to the pathogenesis of inflammatory bowel disease

Authors: Díaz-Jiménez, D¹., Dubois, K¹., De La Fuente, M¹., Quera, R²., Hermoso, M¹., ¹Laboratorio de Inmunidad Innata, Programa de Inmunología, Medicina, Universidad de Chile. ²Departamento de Gastroenterología Clínica Las Condes.

73 Area: Immunology

Title: Effect of cell envelope of different *Porphyromonas gingivalis* clinical isolates on viability of Gingival Epithelial Cells

Authors: Bugueño, I¹., Venegas, D¹., Soto, C¹., Dutzan, N¹., Salinas, D¹., Díaz, L¹., Bravo, D¹., Hoare, A¹., ¹Oral Microbiology, Dentistry, Universidad de Chile.

74 Area: Immunology

Title: Soluble MICA in the serum promotes decreased NKG2D receptor expression on NK cells in gastric cancer patients

Authors: Kramm, K¹., Ribeiro, C¹., Bustamante, M²., Garrido, M¹., Hernández, C¹., Molina, M¹., ¹Laboratorio Evasión Inmune, Programa Disciplinario de Inmunología, ICBM, Facultad de Medicina, Universidad de Chile. ²Departamento de Cirugía Digestiva, Hospital del Salvador Universidad de Chile.

75 Area: Immunology

Title: Interferon-I activity reporter cell lines to explore natural and synthetic libraries-derived compounds which can modulate the cytokine's potency

Authors: Bürgi, M¹., Prieto, C¹., Etcheverrigaray, M¹., Kratje, R¹., Bollati-Fogolín, M²., Oggero, M¹., ¹Laboratorio de Cultivos Celulares, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral. ²Unidad de Biología Celular Instituto Pasteur de Montevideo.

76 Area: Immunology

Title: Effect of *Drimys winteri* whole extract and its active principle poligodial on the expression of immune genes in the ASK-1 cell line infected with *Saprolegnia parasitica* oomycetes

Authors: Pereira, D¹., Carrasco, H²., Astuya, A³., ¹Cell Culture and Marine Genomics Laboratory, Marine Biotechnology Unit, Ciencias Naturales y Oceanográficas, Universidad de Concepción. ²Chemical Sciences, Faculty of Sciences, Universidad Andrés Bello. ³Marine Biotechnology Unit, Ciencias Naturales y Oceanográficas, Universidad de Concepción.

77 Area: Microbiology

Title: Dissecting the Multifunctional Nucleoprotein of Arenaviruses

Authors: Ortiz Riaño, E¹., Cheng, B¹., De La Torre, J²., Martínez-Sobrido, L¹., ¹Microbiology and Immunology University of Rochester. ²Immunology and Microbial Science Scripps Research Institute.

78 Area: Microbiology

Title: Clinical and virological characterization of hospitalized influenza A patients between 2011-2012

Authors: Barrera, A^{1,2}., Budnik, I^{3,1}., Marco, C¹., Aravena, M³., Gutierrez, C⁴., Lopez, T⁴., Perez, R³., Vizcaya, C³., Ferres, M^{1,3}., Medina, R^{1,3,2,5}., ¹Laboratorio de Virología Molecular, Escuela de Medicina, Pontificia Universidad Católica de Chile. ²Instituto Millenio en Inmunología e Inmunoterapia PUC. ³Division de Pediatría, Escuela de Medicina, Pontificia Universidad Católica de Chile. ⁴Depto. Enfermedades Infecciosas, Escuela de Medicina, Pontificia Universidad Católica de Chile. ⁵Department of Microbiology, Global Health and Emerging Pathogens Institute Icahn School of Medicine at Mount Sinai, New York, USA.

79 Area: Microbiology

Title: Polysaccharides isolated from *Aloe barbadensis* Miller (*Aloe vera*) are excellent prebiotic molecules

Authors: Quezada, M¹., Salinas, C¹., Gotteland, M²., Cardemil, L¹., ¹Departamento de Biología, Facultad de Ciencias, Universidad de Chile. ²Departamento de Nutrición, Facultad de Medicina, Universidad de Chile.

80 Area: Microbiology

Title: Standardizing a method of purifying bovine viruses: BLV from milk sample and BVDV from cell culture

Authors: Fredericksen, F¹., Valdivia, S¹., Blaña, C¹., Villalba, M¹., Ramírez, F¹., Olavarria, V¹., ¹Instituto de bioquímica y microbiología, Ciencias, Universidad Austral de Chile.

81 Area: Microbiology

Title: Microcin E492 amyloid formation is retarded by posttranslational modification

Authors: Marcoleta, A¹., Aguilera, P¹., Marín, M¹., Mercado, G¹., Valpuesta, J²., Monasterio, O¹., Lagos, R¹., ¹Departamento de Biología, Facultad de Ciencias, Universidad de Chile. ²Centro Nacional de Biotecnología, Consejo Superior de Investigaciones Científicas, Campus Cantoblanco, Universidad Autónoma de Madrid, España.

82 Area: Microbiology

Title: Metabolic diversity of bacterial communities from soils invaded by *Eschscholzia californica*

Authors: De Armas, M¹., Leiva, D¹., Orlando, J¹., Carú, M¹., ¹Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile.

83 Area: Microbiology

Title: Involvement of poly(ADP-ribose) glycohydrolase in *T. cruzi* proliferation and host cell infection

Authors: Vilchez Larrea, S¹.,Schlesinger, M¹.,Kevorkian, L¹.,Flawia, M¹.,Alonso, G¹.,Fernandez Villamil, S¹.,¹Laboratorio Regulación Metabólica en Tripanosomatidos INGEBI-CONICET-UBA.

84 Area: Microbiology

Title: The plant growth promoting rhizobacteria *Burkholderia phytofirmans* PsJN protects *Arabidopsis thaliana* against the virulent pathogen *Pseudomonas syringae* DC3000

Authors: Timmermann, T¹.,Armijo, G².,Poupin, M¹.,Holuigüe, L².,Gonzalez, B¹.,¹Laboratorio de Bioingeniería, Facultad de Ingeniería y Ciencias, Universidad Adolfo Ibáñez.²Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.

85 Area: Microbiology

Title: Genetic redundancy of acetyl-CoA transferases in key steps of aromatic compounds metabolism in *Cupriavidus pinatubonensis* JMP134

Authors: Leiva, P¹.,Donoso, R¹.,Pérez-Pantoja, D¹.,Gonzalez, B^{1,2}.,¹Laboratorio de Bioingeniería, Ingeniería y Ciencias, Universidad Adolfo Ibáñez.²Millennium Nucleus on Plant Functional Genomics P. Universidad Católica de Chile.

86 Area: Microbiology

Title: qPCR for Specific Detection of *Piscirickettsia salmonis*: New Genomic Insights

Authors: Calquín, P¹.,Haro, R¹.,Yáñez, A¹.,¹Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile.

87 Area: Microbiology

Title: Identification of essential genes for *in vitro* surviving of *Salmonella* Typhimurium to a non-lethal cefotaxime concentration and their putative relationship with virulence

Authors: Molina-Quiroz, R¹.,Silva V., C¹.,Santiviago C., C¹.,¹Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.

88 Area: Microbiology

Title: Stress-triggered signaling for survival or suicide of *Streptococcus pneumoniae* in pneumocytes

Authors: Cian, M.,Cortes, P.,Piñas, G.,Yandar Barahona, N.,Echenique, J¹.,¹Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba.

89 Area: Microbiology

Title: Low temperature biosynthesis of fluorescent nanoparticles by a *Shewanella* sp. isolated from Antarctica: Potential of a multiresistant genus to produce intracellular nanostructures from heavy metals

Authors: Plaza, D^{1,2}.,Gallardo, C².,Straub, Y².,Perez-Donoso, J².,¹Bioquímica, Ciencias Químicas y Farmacéuticas, Universidad de Chile.²CBIB, Biological Sciences, Andres Bello.

90 Area: Microbiology

Title: Immunolocalization of SxtA, SxtF and SxtM proteins in PSTs producing strains of Cyanobacteria

Authors: Riquelme, B¹.,Soto-Liebe, K¹.,Spoonner, D¹.,Vásquez, M¹.,¹Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.

91 Area: Miscellaneous

Title: 2-Bromo-1,4-naphthoquinone and ascorbic acid: A promising drug combination for tumor treatment

Authors: Graciani, F¹, Camargo, M², Varanda, E², Ximenes, V¹, ¹Biochemistry, Farmacy, Universidade Estadual Paulista - UNESP, ²Mutagenesis, Farmacy, Universidade Estadual Paulista - UNESP.

92 Area: Miscellaneous

Title: Effect of reboxetine treatment on auditory attention and memory in stress rats

Authors: Pérez-Valenzuela, C¹, Peñalosa, V¹, Rojas-Thomas, F², Dagnino-Subiabre, A², ¹Fisiología, Ciencias, Universidad de Valparaíso, ²Fisiología, Ciencias, Universidad De Valparaíso.

93 Area: Miscellaneous

Title: Quantifying elastic changes in DNA molecules after interacting with Mitomycin C using magnetic tweezers at single molecule level

Authors: Muñoz, R¹, Wilson, C.A.M.², Leachman, S³, Bustamante, C^{4,5}, Melo, F¹, ¹Departamento de Física, Facultad de Ciencias, Universidad de Santiago de Chile, ²Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, ³Department of Chemistry, QB3 Institute University of California, ⁴Department of Chemistry, QB3 Institute University of California, ⁵Howard Hughes Medical Institute University of California.

94 Area: Miscellaneous

Title: Development of a novel CYTB gene marker for fish species genotyping using PCR-RFLP technique as a tool for the experiential teaching of molecular biology: Is that really salmon in my sushi?

Authors: Fuentes, A^{1,2}, Morán, A¹, Gutiérrez, M¹, ¹Science Education Lab Bioquímica.cl, ²Laboratorio de Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.

95 Area: Miscellaneous

Title: Hantavirus glycoproteins mediate viral self-assembly and entry into the cell

Authors: Acuña, R¹, Cifuentes-Muñoz, N¹, Marquez, C¹, Bulling, M¹, Mancini, R², Lozach, P³, Tischler, N¹, ¹Molecular Virology Laboratory Fundación Ciencia & Vida, ²Eidgenössische Technische Hochschule Institute of Biochemistry, ³Laboratory for Cell Biology of Arboviral Infections, INRS Institut Armand Frappier, Université du Québec.

96 Area: Molecular Cell Biology

Title: Remodelling of the TAR RNA motif mediated by the RNA helicase DDX3 is required for the HIV-1 genomic RNA to enter in cap-dependent translation initiation

Authors: Soto Rifo, R¹, Ohlmann, T², ¹ICBM, Programa de Virología, Facultad de Medicina, Universidad de Chile, ²Human Virology Department CIRI, INSERM U 1111, Ecole Normale Supérieure de Lyon.

97 Area: Molecular Cell Biology

Title: Astrocyte $\alpha v \beta 3$ -integrin engages neuronal Thy-1 and induces neurite retraction via CBP-mediated Src inactivation and ROCK pathway activation

Authors: Maldonado, H^{3,1}, Herrera-Molina, R², Quest, A³, Leyton, L^{1,3}, ¹Biomedical Neuroscience Institute, Medicina, Universidad de Chile, ²Neurochemistry and Molecular Biology Leibniz Institute for Neurobiology, Magdeburg, Germany, ³Laboratorio de Comunicaciones Celulares, Centro de Estudios Moleculares de la Célula, Medicina, Universidad de Chile.

98 Area: Molecular Cell Biology

Title: Internal ribosome entry site within the gag coding region: toward a new therapeutic target ?

Authors: Deforges, J¹, Ameur, M¹, Chamond, N¹, Ulryck, N¹, Sargueil, B¹, ¹UMR 8015, Faculté de Pharmacie, Université Paris Descartes.

99 Area: Molecular Cell Biology

Title: Endothelin-Converting Enzyme-1 isoform c is regulated by the Wnt/ β -catenin pathway and modulates invasion in colon cancer cells

Authors: Cabello, P¹, Silva, E¹, Niechi, I¹, Huerta, H¹, Armisen, R^{2,3}, Fernandez, C^{4,5}, Tapia, J⁶, ¹Cell Transformation Laboratory, Faculty of Medicine, University of Chile. ²CITC: Center for Research and Treatment of Cancer, ICBM, Faculty of Medicine, University of Chile. ³CITC: Center for Research and Treatment of Cancer, ICBM, Faculty of Medicine, University of Chile. ⁴Department of Anatomopathology HCUCH, CITC: Center for Research and Treatment of Cancer, Faculty of Medicine, University of Chile. ⁵Department of Anatomopathology HCUCH, Faculty of Medicine, University of Chile. ⁶Cell Transformation Laboratory, ICBM, CITC: Center for Research and Treatment of Cancer, Faculty of Medicine, University of Chile.

100 Area: Molecular Cell Biology

Title: Contribution of LOX-1 activation by oxLDL to tumor angiogenesis in prostate cancer.

Authors: Gonzalez, I¹, Cerro, R¹, Parra, N¹, Toledo, J¹, ¹Fisiopatología, Ciencias Biológicas, Universidad de Concepción.

101 Area: Molecular Cell Biology

Title: Protein kinase A regulates ER-mitochondria communication during stress conditions in HeLa cells

Authors: Bravo-Sagua, R¹, Rodríguez, A¹, Quiroga, C¹, Paredes, F¹, Parra, V², Ortiz-Sandoval, C³, Simmen, T³, Quest, A⁴, Lavandero, S^{1,2,4}, ¹Centro de Estudios Moleculares de la Célula, Facultad de Ciencias Químicas y Farmacéuticas/Facultad de Medicina, Universidad de Chile. ²Department of Internal Medicine, Southwestern Medical Center, University of Texas. ³Department of Cell Biology, Faculty of Medicine and Dentistry, University of Alberta. ⁴Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile.

102 Area: Molecular Cell Biology

Title: Distribution of matrix metalloproteinase-2 (MMP-2) in sheep cervix during the oestrous cycle

Authors: Casuriaga, D¹, Gonzalez, R¹, Rodríguez Piñón, M¹, ¹Biochemistry, Department of Molecular and Cellular Biology, Veterinarian Faculty, Udelar, Montevideo, Uruguay.

103 Area: Molecular Cell Biology

Title: Transcriptional factor FoxO1 modulates mitochondrial metabolism and morphology in cardiomyocytes

Authors: Quiroga, C¹, Riveros, C¹, Acuña, F¹, Lavandero, S^{1,2,3}, ¹Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences, University of Chile. ²Center for Molecular Studies of the Cell, Faculty of Medicine, University of Chile. ³Department of Internal Medicine University of Texas Southwestern Medical Center, USA.

104 Area: Molecular Cell Biology

Title: Tanycytes MCT1 inhibition *in vivo* affects feeding behavior

Authors: Barahona, M¹, Cortes-Campos, C¹, Martínez, F¹, Ordenes, P¹, Villagra, M¹, Elizondo, R¹, Garcia, M¹, ¹Biología Celular, Ciencias Biológicas, Universidad de Concepción.

105 Area: Molecular Cell Biology

Title: Interaction between non-coding mitochondrial RNAs and double-stranded-RNA binding proteins

Authors: Briones, M^{2,1}, Oliveira-Cruz, L¹, Burzio, V^{2,1}, Burzio, L^{1,2}, ¹Fundación Ciencia & Vida Andes Biotechnologies S.A. ²Departamento de Ciencias Biológicas Universidad Andrés Bello.

106 Area: Molecular Cell Biology

Title: Astrocytes inhibit nitric oxide-dependent Ca²⁺ dynamics in activated microglia: Involvement of ATP released via pannexin 1 channels

Authors: Montero, T¹, ¹Neurociencias, Medicina, Pontificia Universidad Católica de Chile.

107 Area: Molecular Cell Biology

Title: Functional study of the Aurora kinase protein family in *Trypanosoma cruzi*

Authors: Fassolari, M¹, Flawiá, M¹, Alonso, G¹, ¹Laboratorio de Regulación Metabólica en Tripanosomatidos Instituto de Investigaciones en Ingeniería Genética y Biología Molecular (INGEBI-CONICET).

108 Area: Molecular Cell Biology

Title: ESCRT complexes are involved in acrosomal membrane deformations required for regulated exocytosis in human spermatozoa

Authors: Pocognoni, C¹, Belmonte, S¹, Mayorga, L¹, ¹IHEM (CONICET-UNCuyo) Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Mendoza, Argentina..

109 Area: Molecular Cell Biology

Title: Effect of acute stress on FMRP phosphorylation by Akt-mTOR and MAPK pathways in rat hippocampus

Authors: Aguayo, F¹, Rojas, P¹, Pacheco, A¹, García, G¹, Muñoz, M¹, Márquez, R¹, Fiedler, J¹, ¹Departamento de Bioquímica y Biología Molecular, Laboratorio de Neuroplasticidad y Neurogenética, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.

110 Area: Molecular Cell Biology

Title: Identification of a promoter region in the intron 5 of RUNX1 gene

Authors: Ortiz, F¹, Hinojosa, M¹, Pihan, C¹, Gutiérrez, S¹, Departamento de ¹Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.

111 Area: Molecular Cell Biology

Title: Rheb interacts with LKB1 kinase; implications on LKB1 functions

Authors: Armijo, M¹, Castro, A¹, ¹Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.

112 Area: Molecular Cell Biology

Title: Polyglucosan molecules induce apoptosis via the *intrinsic pathway* in male germ cells

Authors: Villarroel-Espindola, F¹, Gutierrez, N¹, Concha, I¹, Slebe, J¹, ¹Bioquímica y Microbiología, Ciencias, Universidad Austral de Chile.

113 Area: Molecular Cell Biology

Title: Association of VDR with the nuclear matrix: Identification of specific domains

Authors: Ruiz-Tagle, C¹, Montecino, M¹, ¹Center for Biomedical Research and FONDAP Center for Genome Regulation, Faculty of Biological Sciences and Faculty of Medicine Universidad Andres Bello, Santiago, Chile.

114 Area: Molecular Cell Biology

Title: Inhibition of Andes hantavirus entry into the cell

Authors: Barriga, G, P^{1,4}, Acuña, R¹, Villalón, F¹, Mardones, G², Monasterio, O³, Tischler, N, D^{1,4}, ¹Molecular Virology Laboratory Fundación Ciencia & Vida. ²Instituto de Fisiología, Universidad Austral de Chile. ³Laboratorio de Biología Estructural y Fundamental, Universidad de Chile. ⁴Facultad de Ciencias Biológicas Universidad Andrés Bello.

115 Area: Molecular Cell Biology

Title: Decrease in NAD⁺/NADH ratio produces mitochondrial dysfunction and prevents development of cardiomyocyte hypertrophy

Authors: Oyarzun, A¹, Troncoso, R¹, Pennanen, C¹, Lavandero, S^{1,2}, ¹Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences/ Faculty of Medicine, University of Chile. ²Department of Internal Medicine University of Texas, Southwestern Medical Center, Dallas, Texas, USA.

116 Area: Molecular Cell Biology

Title: Vitamin C recycling and the protective function of the astrocyte against neuronal death induced by oxidative stress and dehydroascorbic acid

Authors: Ferrada, L¹, Salazar, K¹, Martínez, F¹, Nualart, F¹, ¹Biología Celular, Ciencias Biológicas, Universidad de Concepción.

117 Area: Molecular Cell Biology

Title: Biochemical and cellular characterization of the Golgi apparatus protein GOLPH3 in human breast cancer cells

Authors: Ross, B¹, Tenorio, M¹, Acuña, D¹, Arriagada, C¹, Cavieres, V¹, Burgos, P¹, Mardones, G¹, ¹Physiology, Medicine, Universidad Austral de Chile.

118 Area: Molecular Cell Biology

Title: ATP induces altered gene expression in adult fibers from C57 mice with cancer

Authors: Neira, J¹, Espinosa, A^{1,3}, Buvinic, S^{2,3}, Quest, A^{1,3}, Jaimovich, E^{1,3}, ¹ICBM, Facultad de Medicina, Universidad de Chile. ²ICBM, Facultad de Odontología, Universidad de Chile. ³CEMC, Medicina, Universidad de Chile.

119 Area: Molecular Cell Biology

Title: Galectin-1, a protein involved in the polymerization of the Reissner Fiber?

Authors: Muñoz, R¹, Kaehne, T², Guerra, M³, Rodríguez, S¹, Jara, M¹, Salazar, P¹, Rodríguez, E¹, ¹Instituto de Anatomía, Histología y Patología, Medicina, Universidad Austral de Chile. ²Institute of Experimental Internal Medicine, Otto-von-Guericke, Medicine, University Magdeburg, Magdeburg, Germany. ³Instituto de Anatomía, Histología y Patología, Facultad de Medicina, Universidad Austral de Chile, Valdivia, Chile.

120 Area: Molecular Cell Biology

Title: *Trypanosoma cruzi* induces cellular proliferation and differentiation in the human trophoblast

Authors: Droguett, D^{3,1}, Castillo, C³, Liempi, A³, Duaso, J³, Barahona, K², Maya, J³, Galanti, N³, Kemmerling, U³, ¹Estomatología, Ciencias de la Salud, Universidad De Talca. ²ICBM, Programa de Anatomía y Biología del Desarrollo, Facultad de Medicina, Universidad de Chile. ³ICBM, Programa de Anatomía y Biología del Desarrollo, Facultad de Medicina, Universidad de Chile.

121 Area: Plant Biology

Title: Arabidopsis Sirtuin 1 is a Histone Deacetylase and Participates in the Regulation of Flowering Time

Authors: Torres, E¹, Holzmänn, C¹, Jordana, X¹, ¹Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.

122 Area: Plant Biology

Title: MEF26, a PPR protein necessary for efficient RNA Editing at two sites in two mitochondrial transcripts in *Arabidopsis thaliana*

Authors: Moreno, S¹, Arenas, A¹, Takenaka, M², Jordana, X², ¹Genética Molecular y Microbiología, Ciencias Biológicas, Pontificia Universidad Católica de Chile. ²Molekulare Botanik Universität Ulm.

123 Area: Plant Biology

Title: A key enzyme the bug?: Characterization of *Candidatus phytoplasma AYWB* malic enzyme

Authors: Saigo, M², Mussi, M², Alvarez, C², MacLean, A¹, Andreo, C², Hogenhout, S¹, Drincovich, M², ¹Cell and Developmental Biology John Innes Centre, Norwich, United Kingdom. ²CEFOBI Universidad Nacional de Rosario, Argentina.

124 Area: Plant Biology

Title: Root architecture and gene expression in response to salt stress in chilean quinoa genotypes

Authors: Ruiz, K., Silva, H¹, ¹Producción Agrícola, Laboratorio de Genómica Funcional & Bioinformática, Ciencias Agronómicas, Universidad de Chile.

125 Area: Plant Biology

Title: Cloning and characterization of a xyloglucan endo-transglycosylase/hydrolase gene of radiata pine seedlings

Authors: Valenzuela, C¹, Ramos, P¹, Moya-León, M¹, Herrera, R¹, ¹Instituto de Biología Vegetal y Biotecnología Universidad de Talca.

126 Area: Plant Biology

Title: Characterization of *Solanum lycopersicum* defense response to pathogen attack under the influence of nitrogen availability

Authors: Retamal, I^{1,2}, Hoppe, G^{1,2}, Canessa, P¹, Vega, A², ¹Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile. ²Departamento de Ciencias Vegetales, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile.

127 Area: Plant Biology

Title: *ERF115* gene codes for a transcription factor involved in tolerance to salinity stress in *Arabidopsis thaliana*

Authors: Salinas, P¹, León, L¹, Del Río, V¹, Holuigue, L¹, ¹Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.

128 Area: Plant Biology

Title: Functional promoter analysis of *GRXC9*, a gene activated by a non-canonical salicylic acid-dependent pathway in *Arabidopsis*

Authors: Herrera, A¹, Carvallo, L², Holuigue, L², ¹Genética Molecular y Microbiología, Ciencias Biológicas, Pontificia Universidad Católica de Chile. ²Departamento de Genética Molecular y Microbiología, Núcleo Milenio en Genómica Funcional de Plantas, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.

129 Area: Plant Biology

Title: Identification of pollen specific promoters from *Arabidopsis thaliana* and their use to generate male gametophyte ablation

Authors: Muñoz-Strale, D¹, León, G¹, ¹Centro de Biotecnología Vegetal, Ciencias Biológicas, Universidad Andrés Bello.

130 Area: Protein Structure and Function

Title: Modular hyperthermostable bacterial endo- β -1,4-mannanase: molecular shape, flexibility and new structural insights

Authors: Silva, V¹, Colussi, F¹, Neto, M², Braz, A¹, Squina, F³, Garcia, W¹, ¹CCNH Universidade Federal do ABC - UFABC, ²Física e Biofísica Universidade Estadual Paulista, ³Laboratório Nacional de ciência e tecnologia do Bioetanol - CTBE Centro Nacional de Pesquisa em Energia e Materiais.

131 Area: Protein Structure and Function

Title: Dissection of the Mechanism of Protein Unfolding and Translocation by the AAA+ Protease Machine ClpXP

Authors: Rodriguez, P¹, Maillard, R², Bustamante, C³, ¹Biophysics Graduate Group, Carlos Bustamante Lab, University of California at Berkeley, ²Jason L. Choy Laboratory of Single-Molecule Biophysics University of California-Berkeley, ³Physics, Chemistry, and Molecular and Cell Biology University of California - Berkeley.

132 Area: Protein Structure and Function

Title: Biochemical characterization of metal-nucleotide binding in an ADP-dependent phosphofructokinase from *Pyrococcus horikoshii*.

Authors: Abarca, M¹, Ramirez-Sarmiento, C¹, Castro-Fernandez, V¹, Merino, F², Guixé, V², ¹Departamento de Biología, Facultad de Ciencias, Universidad de Chile, ²Department of Cell and Developmental Biology Max Planck Institute for Molecular Biomedicine.

133 Area: Protein Structure and Function

Title: Structure of the dimerization domain of Bcy1, the regulatory subunit of the cAMP dependent protein kinase from *Saccharomyces cerevisiae*, and interaction with a peptide derived from IRA2, a GTPase activating protein involved in the cAMP signalling pathway

Authors: González Bardeci, N¹, Caramelo, J², Rossi, S¹, Larrieux, N³, Trajtenberg, F³, Buschiazzi, A³, Moreno, S¹, ¹Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, ²Laboratorio de Biología Estructural y Celular Fundación Instituto Leloir, ³Unidad de Cristalografía de Proteínas Institut Pasteur.

134 Area: Protein Structure and Function

Title: Identification of a pro-amyloidogenic region in microcin E492, a pore-forming bacteriocin

Authors: Lobos, P¹, Bignon, E¹, Monasterio, O¹, Lagos, R¹, ¹Laboratorio de Biología Estructural y Molecular, Facultad de Ciencias, Universidad de Chile.

135 Area: Protein Structure and Function

Title: Characterization of the microcin E492 pore-forming activity using liposomes

Authors: Bignon, E¹, Monasterio, O¹, Lagos, R¹, ¹Departamento de Biología, Facultad de Ciencias, Universidad de Chile.

136 Area: Protein Structure and Function

Title: On the tertiary-quaternary coupling of the allosteric activation of glucosamine-6-P deaminase from *Escherichia coli*

Authors: Vazquez, R¹., Zonszein-Strauss, S¹., Álvarez-Añorve, L¹., Díaz-Jiménez, D¹., Calcagno, M¹., ¹Biochemistry, Medicine, National Autonomous University of Mexico.

137 Area: Protein Structure and Function

Title: Insights into cross-reactivity of allergens: structure, dynamics and stability of allergen Gad m 1 free and in complex with single chain variable fragment by solution NMR Spectroscopy

Authors: Moraes, A¹., Ackerbauer, D²., Kostadinova, M²., Bublin, M²., De Oliveira, G¹., Ferreira, F³., Almeida, F¹., Breiteneder, H²., Valente, A¹., ¹Centro Nacional de Ressonância Magnética, Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro, Rio de Janeiro. ²Department of Pathophysiology and Allergy Research Medical University of Vienna, Vienna, Austria. ³Christian Doppler Laboratory for Allergy Diagnosis and Therapy University of Salzburg, Salzburg, Austria.

138 Area: Protein Structure and Function

Title: Effects of chaotropic agents on the activity and aggregation of the bacteriocin gicin A

Authors: Fernández-Oyarzún, P¹., Cortés-Cortés, P¹., Baez, M²., Corsini, G¹., ¹Laboratorio de Bacteriología Molecular, Centro de Investigación Biomédica (CIB), Facultad de Medicina, Universidad Diego Portales. ²Laboratorio de Bioquímica, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.

139 Area: Protein Structure and Function

Title: Structural changes in *E. coli* phosphofructokinase-2 upon subunit dissociation induced by chemical, temperature and mutational perturbations

Authors: Ramirez-Sarmiento, C¹., Baez, M²., Villalobos, P¹., Babul, J¹., Komives, E³., Guixe, V¹., ¹Departamento de Biología, Facultad de Ciencias, Universidad de Chile. ²Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile. ³Department of Chemistry and Biochemistry University of California San Diego.

140 Area: Protein Structure and Function

Title: Structural Basis of CCR2 Sulfotyrosine Recognition by the human beta-defensin 6

Authors: S. De Paula, V¹., Monteiro, R¹., L.C. Almeida, F¹., P. Valente, A¹., ¹Instituto de Bioquímica Médica Universidade Federal do Rio de Janeiro.

141 Area: Protein Structure and Function

Title: Structural and kinetic studies of an uncommon allosteric Glucosamine 6-P deaminase

Authors: Marcos, J¹., Álvarez-Añorve, L¹., Zonszein, S¹., Dominguez-Ramírez, L²., Calcagno, M¹., ¹LFQIP, Bioquímica, Medicina, Universidad Nacional Autónoma de México. ²Div. de Ciencias Biol. y de la Salud Universidad Autónoma Metropolitana.

142 Area: Protein Structure and Function

Title: The energetic cost of knotting a polypeptide chain in its unfolded state determined by optical tweezers

Authors: Bustamante, A¹., Reyes, J¹., Obando, P²., Guerra, D²., Wilson, C.A.M.¹., Bustamante, C^{3,4}., Baez, M¹., ¹Laboratorio de Bioquímica, departamento de Bioquímica y Biología molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile. ²Laboratorio de Visualización y Manipulación de Moléculas individuales, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia. ³Jason L. Choy Laboratory of Single-Molecule Biophysics University of California, Berkeley. ⁴Institute for Quantitative Biosciences (QB3) University of California, Berkeley.

143 Area: Protein Structure and Function

Title: Insights into the role of Pro475 in the ion permeation process of Shaker K-channel using non-equilibrium molecular dynamics

Authors: Sepúlveda, R¹., Aguayo, D¹., Araya, I¹., Varas, I¹., Naranjo, D²., Gonzalez-Nilo, F^{1,2}., ¹Center for Bioinformatics and Integrative Biology (CBIB) , Facultad de Ciencias Biológicas, Universidad Andrés Bello. ²Centro Interdisciplinario de Neurociencia de Valparaíso (CINV) Universidad de Valparaíso.

144 Area: Protein Structure and Function

Title: Corner spider's Phospholipase D antibodies in subjects without loxoscelism and its relation with bacterial phospholipases D phosphodiesterases

Authors: Subiabre, M¹., Rojas, J¹., Arán, T¹., Ordenes, K¹., Orrego, P¹., Catalán, A¹., Araya, J¹., ¹Departamento de Tecnología Médica, Facultad de Ciencias de la Salud, Universidad de Antofagasta.

145 Area: Protein Structure and Function

Title: Insights into the mechanism for DNA capture and transport by a type II topoisomerase is provided by a high-resolution structure of an 'open' topoisomerase II-DNA complex

Authors: Laponogov, I^{2,1}., Veselkov, D²., Crevel, I¹., Pan, X¹., Fisher, M¹., Sanderson, M²., ¹Biomedical Sciences, St. Georges, University of London. ²Randall Division of Cell and Molecular Biophysics, Medicine and Life Sciences, Kings College London.

146 Area: Protein Structure and Function

Title: Biochemical mechanisms of protein oxidative modifications in membranes: Connecting lipid peroxidation with amino acid oxidation

Authors: Bartesaghi, S¹., Herrera, D¹., Radi, R¹., ¹Departamento de Bioquímica, Facultad de Medicina, Universidad de la República, Uruguay.

147 Area: Protein Structure and Function

Title: Enzymes stepping on land mines

Authors: Riedel, C¹., Wilson, C.A.M.²., Hamadani, K¹., Marqusee, S¹., Presse, S³., Bustamante, C¹., ¹Biophysics UC Berkeley. ²Ciencias Químicas y Farmacéuticas Universidad de Chile, Santiago. ³Physics Department Indiana Univ.-Purdue Univ. Indianapolis.

148 Area: Protein Structure and Function

Title: Unraveling the function of thioredoxin-interacting protein (TXNIP) through structural studies

Authors: Amorim, G¹., Aguiar, R¹., Valente, A¹., Almeida, F¹., ¹National Center for Nuclear Magnetic Resonance, BioNMR Laboratory, Federal University of Rio de Janeiro.

149 Area: Signaling

Title: Changes in kinase and phosphatase activities in PC12 cells cultured with HTLV-1 infected cell conditioning medium

Authors: Quintremil, S¹., Medina, F²., Rivera, M²., Puente, J²., Ramirez, E^{3,4}., Cartier, L⁵., Valenzuela, M²., ¹Biochemistry Universidad de Chile. ²Bioquímica y Biología Molecular, Ciencias Químicas y Farmaceuticas, Universidad de Chile. ³Programa de Virología, ICBM, Medicina, Universidad De Chile. ⁴Virología ISP. ⁵Ciencias Neurológicas, Medicina, Universidad de Chile.

150 Area: Signaling

Title: Electrical stimulation induces interleukin 6 expression in skeletal muscle through extracellular ATP, by activating Ca²⁺ signals and an interleukin 6 autocrine loop

Authors: Bustamante, M¹., Fernández-Verdejo, R¹., Vásquez, J²., Jaimovich, E¹., **Buvinic, S^{2,1}**., ¹Centro de Estudios Moleculares de la Célula, ICBM, Facultad de Medicina, Universidad de Chile. ²Departamento de Ciencias Básicas y Comunitarias, Facultad de Odontología, Universidad de Chile.

151 Area: Signaling

Title: Human RIC-8B overexpression increases the intracellular levels of cAMP in HEK293T cells in response to isoproterenol

Authors: **Sánchez, R¹**., Maureira, A¹., Castro, A¹., Hinrichs, M¹., Olate, J¹., Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.

152 Area: Signaling

Title: Glucagon-Like Peptide-1 changes mitochondrial dynamics and metabolism in vascular smooth muscle cells (VSMC) by a PKA/Drp-1 signaling pathway

Authors: **Torres, G¹**., Morales, P¹., Norambuena, I¹., Michea, L²., Lavandero, S^{1,2}., Chiong, M¹., ¹CEMC. Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile. ²Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile.

153 Area: Signaling

Title: Insulin promotes cardiomyocyte mitochondrial fusion by increasing Opa-1 expression through the Akt-mTOR-NFκB pathway

Authors: **Lopez-Crisosto, C²**., Parra, V^{2,1}., Chiong, M²., Lavandero, S^{2,1}., ¹Department Internal Medicine University of Texas Southwestern Medical Center. ²Centro de Estudios Moleculares de la Célula, Facultad de Ciencias Químicas y Farmacéuticas & Facultad de Medicina, Universidad de Chile.

154 Area: Signaling

Title: Angiotensin-(1-9) regulates cardiomyocyte mitochondrial calcium by AT2 receptor

Authors: **Sotomayor Flores, C²**., Rivera Mejías, P²., Morales, P²., López Crisosto, C²., Jalil, J¹., Ocaranza, M¹., Chiong, M²., Parra, V²., Lavandero, S²., ¹Cardiovascular Diseases Division, School of Medicine, Pontifical Catholic University of Chile. ²Center for molecular studies of the cell, Faculty of Chemical and Pharmaceutical sciences/Faculty of Medicine, University of Chile.

155 Area: Signaling

Title: Reducing membrane cholesterol induces glucose transport through GLUT4 and generates calcium transients in skeletal muscle: a possible treatment for insulin resistance?

Authors: **Llanos, P^{1,2}**., Contreras-Ferrat, A²., Osorio-Fuentealba, C²., Espinosa, A²., Hidalgo, J²., Hidalgo, C^{2,3}., Jaimovich, E²., ¹Department of Basic and Communitarian Sciences, Faculty of Dentistry, Universidad de Chile. ²CMEC, Faculty of Medicine, Universidad de Chile. ³ICBM and BNI, Faculty of Medicine, Universidad de Chile.

156 Area: Signaling

Title: Deciphering Arabidopsis Calmodulin-binding Protein IQ67-Domain (IQD) roles

Authors: Triassi, A¹., Drincovich, M¹., **Lara, M¹**., ¹CEFOBI-CONICET, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario.

157 Area: Signaling

Title: Study of the Ca²⁺ independent spontaneous postsynaptic currents at *Drosophila*'s neuromuscular junction

Authors: Sepúlveda, M¹., López, F¹., Delgado, R¹., **Roth, A¹**., ¹Departamento de Biología, Facultad de Ciencias, Universidad de Chile.

158 Area: Signaling

Title: Novel candidate regulators of lipid droplet metabolism revealed by high throughput and chemogenomic analyses

Authors: Montero-Lomeli, M¹., Bozaquel-Morais, B¹., Masuda, C¹., Madeira, J¹., ¹Instituto de Bioquímica Médica Universidade Federal do Rio de Janeiro.

159 Area: Signaling

Title: Crosstalk between Toll-like receptor 4 and autophagy in L6 myotubes

Authors: Espinoza, S¹., Navarro, M¹., Díaz, A¹., Troncoso, R¹., García, L¹., ¹Departamento de Bioquímica y Biología Celular, Ciencias Químicas y Farmacéuticas, Universidad de Chile.

160 Area: Biomedicine

Title: Expression of structural proteins of a Mayaro Virus in insect cells: application in serological diagnosis and molecular studies

Authors: Sperança M., A¹., Cabral A., D¹., Garcia F., B¹., Uehara, M¹., Momo L H, S¹., Nogueira M., L²., Ferreira, M., U³., ¹Center for natural and human sciences Universidade Federal do ABC. ²Virologia Faculdade de Medicina de São José do Rio Preto. ³Instituto de Ciências Biomédicas, Parasitologia, Universidade de São Paulo.

161 Area: Biomedicine

Title: Metabolic remodeling in breast cancer: implications in the design of potential anti-cancer agents

Authors: Urra, F^{1,2}., Ramirez-Rodriguez, O²., Ferreira, J³., Araya-Maturana, R²., Cárdenas, C¹., ¹Cellular Metabolism and Bioenergetic Laboratory, Faculty of Medicine, Universidad de Chile. ²Department of Organic and Physical Chemistry, Faculty of Chemical and Pharmaceutical Sciences, Universidad de Chile. ³Laboratory of Bioenergetics and Cancer, Faculty of Medicine, Universidad de Chile.

November 11, Monday
Roofed Street Santa Rosa
16:45-19:15
Posters from 162 to 313

162 Area: Biomedicine

Title: The differential expression of Non-Coding Mitochondrial RNAs can be used as a diagnostic tool for Cervical Cancer

Authors: Dadlani, K^{1,2}, Villota, C^{1,3}, Ávila, R¹, Gabler, F⁴, López, C¹, Socías, M⁵, Zapata, L⁶, Burzio, L^{1,3}, Villegas, J^{1,3}, ¹Fundación Ciencia & Vida Andes Biotechnologies S.A. ²Facultad de Ciencias Químicas y Farmacéuticas Universidad de Chile. ³Facultad de Ciencias Biológicas Universidad Andrés Bello. ⁴Anatomía y Patología Hospital Clínico San Borja-Arriarán. ⁵Dept. Ginecología y Obstetricia Clínica Alemana. ⁶Departamento de Anatomía y Patología Hospital Barros Luco Trudeau.

163 Area: Biomedicine

Title: Cx43 hemichannel activity induced by cytokines and high glucose inhibit the glucose transport sensitive to insulin in human endothelial cells

Authors: Avendaño, B¹, ¹Neurociencias, Facultad de Medicina, Pontificia Universidad Católica de Chile.

164 Area: Biomedicine

Title: Establishment of primary cultures of advanced cervical cancer as a model for selective antisense therapy

Authors: Ávila, R¹, Farfán, N^{2,1}, Dadlani, K^{1,3}, Villota, C^{1,2}, Lobos-González, L¹, Burzio, L^{1,2}, Villegas, J^{1,2}, ¹Fundación Ciencia & Vida Andes Biotechnologies S.A. ²Fac. Ciencias Biológicas Universidad Andrés Bello. ³Fac. Ciencias Químicas y Farmacéuticas Universidad de Chile.

165 Area: Biomedicine

Title: Exome-capture sequencing strategy for the identification of genetic risk factors of cholesterol gallstone disease in Chilean families with early onset disease

Authors: Bustos, B^{1,4}, Pérez-Palma, E^{1,4}, Azócar, L^{2,4}, Moraga, C^{3,4}, Vidal, M^{3,4}, Gutiérrez, R^{3,4}, De Ferrari, G^{1,4}, Miquel, J^{2,4}, ¹Centro de Investigaciones Biomédicas, Facultad de Ciencias Biológicas, Universidad Andrés Bello. ²Dept. Gastroenterología, Facultad de Medicina, Pontificia Universidad Católica de Chile. ³Dept. Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile. ⁴Centro FONDAP de Regulación del Genoma Santiago, Chile.

166 Area: Biomedicine

Title: Identification of Copy Number Variants (CNV) from exome-capture sequencing in Chilean families with early-onset cholesterol gallstone disease

Authors: Pérez-Palma, E^{1,4}, Bustos, B^{1,4}, Moraga, C^{3,4}, Azócar, L^{2,4}, Vidal, M^{3,4}, Gutierrez, R^{3,4}, Miquel, J^{2,4}, De Ferrari, G^{1,4}, ¹Centro de Investigaciones Biomédicas, Facultad de Ciencias Biológicas, Universidad Andrés Bello. ²Dept. de Gastroenterología, Facultad de Medicina, Pontificia Universidad Católica de Chile. ³Dept. Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica De Chile. ⁴Centro FONDAP de Regulación del Genoma Santiago, Chile.

167 Area: Biomedicine

Title: Wnt/ β -catenin signaling regulates Nlgn3 expression in hippocampal neurons

Authors: Medina, M¹., Necuñir, D¹., Caracci, C¹., De Ferrari, G¹.,¹Centro de Investigaciones Biomédicas y Centro FONDAP de Regulación del Genoma, Facultad de Ciencias Biológicas, Universidad Andrés Bello.

168 Area: Biomedicine

Title: Interplay between cholinergic and glutamatergic transmission are essential to induce long-lasting synaptic enhancement of gabaergic synapses

Authors: Moreno, M¹., Ahumada, J¹., Morales, K¹., Bonansco, C¹., Fuenzalida, M¹.,¹Centro de Neurobiología y Plasticidad Cerebral, Departamento Fisiología, Ciencias, Universidad de Valparaíso.

169 Area: Biomedicine

Title: Differentiation of humans adipose-derived mesenchymal stem cell (hASC) into insulin producing cells (IPC)

Authors: Jara, C¹., Caviedes, R¹., Caviedes, P¹.,¹Programa de Farmacología Molecular y Clínica. ICBM, Facultad de Medicina, Universidad de Chile.

170 Area: Biomedicine

Title: Multipotent mesenchymal stromal cells are renoprotective in both type 1 and type 2 diabetes mellitus mice

Authors: Cabezas, F^{4,1}., Ezquer, F⁴., Giraud-Billoud, M^{4,2}., Carpio, D³., Conget, P⁴., Ezquer, M⁴.,¹Escuela de Ingeniería en Biotecnología, Facultad de Ciencias Biológicas, Universidad Andrés Bello.²Instituto de Fisiología, Medicina, Universidad Nacional de Cuyo IHEM-CONICET.³Instituto de Histología y Patología, Facultad de Medicina, Universidad Austral de Chile.⁴Instituto de Ciencias, Facultad de Medicina, Universidad del Desarrollo.

171 Area: Biomedicine

Title: Inhibition of IP3R causes hyperpolarization and decreases mitochondrial fusion

Authors: Lovy, A¹., Cardenas, J².,¹Center for Neuroscience Research, School of Medicine, Tufts University.²Anatomy and Developmental Biology, Medicine, University of Chile.

172 Area: Biomedicine

Title: Decreasing Ski levels is a key event in the oncogenic mechanism of Aurora A kinase

Authors: Rivas, S¹., Mosquera, J¹., Rojas, D¹., Armisen, R^{1,3}., Maldonado, E¹., Colombo, A¹., Tapia, J^{1,3}., Michea, L^{1,3}., Hayman, M²., Marcelain, K^{1,3}.,¹Instituto de Ciencias Biomédicas (ICBM), Facultad de Medicina, Universidad de Chile.²Microbiology and Molecular Genetics Department Stony Brook University.³Centro de Investigación y Tratamiento del Cáncer, Facultad de Medicina, Universidad de Chile.

173 Area: Biomedicine

Title: Protective effects of trimetazidine from palmitate-induced cardiomyocyte lipotoxicity

Authors: Kuzmich, J¹., Parra, V^{1,2}., Verdejo, H^{1,3}., Chiong, M¹., García, L¹., Castro, P., Jensen, M⁴., Bernlohr, D⁵., Lavandero, S^{1,2}.,¹Bioquímica y Biología Molecular, Ciencias Químicas y Farmacéuticas, Universidad de Chile.²Department of Internal Medicine, TX University of Texas Southwestern Medical Center.³Departamento de Cardiología, Facultad de Medicina, Pontificia Universidad Católica de Chile.⁴Endocrine Research Unit, MN Mayo Clinic.⁵Department of Biochemistry, Molecular Biology and Biophysics, MN University of Minnesota.

174 Area: Biomedicine

Title: ApoA-I natural variant G26R triggers a pro-inflammatory response in murine macrophages

Authors: Ramella, N¹, Schinella, G², Ríos, J³, Rosu, S¹, Andujar, I³, Tricerri, A¹, Rimoldi, O¹, ¹INIBIOLP - CONICET, Facultad de Ciencias Médicas, Universidad Nacional de La Plata. ²Cátedra de Farmacología Básica, Facultad de Ciencias Médicas, Universidad Nacional de La Plata. ³Departament de Farmacologia Universitat de Valencia.

175 Area: Biomedicine

Title: Increased activity and expression of ecto-5'-nucleotidase (CD73) in glioblastoma stem-like cells

Authors: Dellarossa, A¹, Garrido, W¹, Rocha, J¹, Oyarzún, C¹, San Martín, R¹, Quezada, C¹, ¹Laboratorio de Patología Molecular, Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile.

176 Area: Biomedicine

Title: Neuroprotective effects of the local delivery of XBP1s into the *Substantia Nigra* of adult mice

Authors: Mercado, M¹, Valdés, P², Molina, C², Vidal, R^{2,3}, Parsons, G⁴, Armentano, D⁴, Schneider, B⁵, Hetz, C^{1,3}, ¹Institute of Biomedical Sciences, Facultad de Medicina, Universidad de Chile. ²Biomedical Neuroscience Institute, Facultad de Medicina, Universidad de Chile. ³Santiago, Chile, Neurounion Biomedical Foundation. ⁴MA, USA, Genzyme Corporation. ⁵Lausanne, Switzerland, Brain Mind Institute.

177 Area: Biomedicine

Title: Long-term ω -3 fatty acids supplementation restore both GABAergic synaptic transmission and memory impaired of chronically stressed rats

Authors: Pérez, M¹, Dagnino-Subiabre, A², Fuenzalida, M¹, ¹Centro de Neurobiología y Plasticidad Cerebral, Facultad de Ciencias, Universidad de Valparaíso. ²Laboratorio de Neurobiología y Conducta, Facultad de Ciencias, Universidad de Valparaíso.

178 Area: Biomedicine

Title: POLYCYSTIN-1, a new mechanosensor to regulate cardiac hypertrophy via I-type calcium channel

Authors: Pedrozo, Z¹, Criollo, A², Gillette, T², Lavandero, S¹, Hill, J^{2,3}, ¹Departamento de Bioquímica y Biología Molecular Universidad de Chile. ²Department of Internal Medicine (Cardiology) University of Texas Southwestern Medical Center. ³Department of Molecular Biology University of Texas Southwestern Medical Center.

179 Area: Biomedicine

Title: Characterization of glioblastoma stem-like cells in relation to the multiple drug resistance phenotype

Authors: Rocha, D¹, Garrido, W², Rocha, A², Uribe, D², Gonzalez, C², Hueicha, K², San Martín, R², Quezada, C², ¹Laboratorio de Patología Molecular, Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile. ²Laboratorio de Patología Molecular, Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile.

180 Area: Biomedicine

Title: The Inhibition of NF-KappaB activation by sodium tungstate modulates the inflammation in diabetic kidney rats

Authors: Jaramillo, K¹, Cumian, A¹, Mansilla, D¹, Silva, P¹, Carpio, D², Burgos, R³, Yáñez, A³, ¹Instituto de Bioquímica y Microbiología, Ciencias, Universidad Austral de Chile. ²Instituto de Histología y Patología, Medicina, Universidad Austral de Chile. ³Instituto de Farmacología, Ciencias Veterinarias, Universidad Austral de Chile.

181 Area: Biomedicine

Title: Optimal IFN- γ secretion in human NK cell is dependent on PGC1 α mitochondrial biogenesis induced by IL-2

Authors: Ibañez, J²., Martin, A^{2,1}., Cordova, A²., Acuña, C²., Miranda, M¹., Montoya, M²., ¹Bioquímica y Biología Molecular, Ciencias Químicas y Farmacéuticas, Universidad de Chile. ²Biología, Química y Biología, Universidad de Santiago de Chile.

182 Area: Computational Biology and Bioinformatics

Title: Docking and MM-GB/SA calculations in predicting the binding modes and binding free energies of biphenyl amide derivatives as P38 kinase inhibitors

Authors: Alzate-Morales, J¹., De La Torre Marquez, P¹., Poblete, H¹., ¹Centro de Bioinformática y Simulación Molecular, Facultad de Ingeniería, Universidad de Talca.

183 Area: Computational Biology and Bioinformatics

Title: Molecular simulations suggest an allosteric contribution to the DNA-binding cooperativity of Oct4 and Sox2

Authors: Merino, F¹., Cojocar, V¹., ¹Department of Cell and Developmental Biology Max Planck Institute for Molecular Biomedicine, Münster, Germany.

184 Area: Computational Biology and Bioinformatics

Title: A new model of TPK4 potassium channel

Authors: Caballero, J¹., Gonzalez, W¹., ¹Centro de Bioinformática y Simulación Molecular, Facultad de Ingeniería, Universidad de Talca.

185 Area: Computational Biology and Bioinformatics

Title: Two chromophorylated linkers from the phycobilisome of Gracilaria chilensis

Authors: Lobos, F¹., Becerra, P¹., Bruna, C¹., Bunster, M¹., ¹Laboratorio de Biofísica Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.

186 Area: Computational Biology and Bioinformatics

Title: Homology Modeling of Calcium Channel CA_v1.2 in Open Conformation: Structural characterization of the selectivity filter from atomic-level perspective

Authors: Marabolí-Boutaud, V¹., Márquez-Miranda, V²., Neely, A³., González-Nilo, F^{1,3}., ¹Center for Bioinformatics and Integrative Biology, Facultad de Ciencias Biológicas, Universidad Andrés Bello. ²Doctorado en Biotecnología Universidad Andrés Bello. ³Centro Interdisciplinario de Neurociencia de Valparaíso (CINV) Universidad de Valparaíso.

187 Area: Computational Biology and Bioinformatics

Title: Use of BLAST as a tool for mapping *Xenopus laevis* scaffolds onto *Xenopus tropicalis* chromosomes: Is it the right tool? How well it performs?

Authors: Almonacid, L^{1,2}., Riadi, G¹., Larraín, J³., Melo, F^{1,2}., ¹Departamento de Genética Molecular y Microbiología Pontificia Universidad Católica de Chile. ²Molecular Bioinformatics Laboratory, Millennium Institute on Immunology and Immunotherapy Pontificia Universidad Católica de Chile. ³Center for Aging and Regeneration and Millennium Nucleus in Regenerative Biology Pontificia Universidad Católica de Chile.

188 Area: Computational Biology and Bioinformatics

Title: Addition of non-native electrostatic interactions in coarse-grained and all-atom structure-based models for protein folding and binding

Authors: Ramirez-Sarmiento, C²., Merino, F¹., Babul, J²., ¹Department of Cell and Developmental Biology Max Planck Institute for Molecular Biomedicine. ²Departamento de Biología, Facultad de Ciencias, Universidad de Chile.

189 Area: Computational Biology and Bioinformatics

Title: Characterization of NAD(P) binding sites using distance dependent knowledge-based potentials

Authors: Fuentealba, M¹., Cabrera, R¹., ¹Departamento de Biología, Facultad de Ciencias, Universidad de Chile.

190 Area: Computational Biology and Bioinformatics

Title: Comparisons between *Xenopus tropicalis* and *Xenopus laevis* genome and protein sequences: large repetitions, inversions and evolutionary implications

Authors: Riadi, G¹., Larraín, J²., Faunes, F²., Lee, D²., Melo, F³., ¹Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile. ²Center for Aging and Regeneration and Millennium Nucleus in Regenerative Biology, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile. ³Molecular Bioinformatics Laboratory, Millennium Institute on Immunology and Immunotherapy, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.

191 Area: Computational Biology and Bioinformatics

Title: Adsorption of multi-protein complexes characterized by atomic force microscopy imaging and nanomechanical simulations

Authors: Santander, E¹., Barrera, N¹., ¹Department of Physiology, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile.

192 Area: Computational Biology and Bioinformatics

Title: Structure-based prediction of transcription factor binding sites using a numerical optimization technique

Authors: Norambuena, T¹., Melo, F¹., ¹Genética Molecular y Microbiología, Ciencias Biológicas, Pontificia Universidad Católica de Chile.

193 Area: Computational Biology and Bioinformatics

Title: Rational Design of Dendrimer-Based Nanoparticles as Transfection Reagents: from atomic-scale perspective to transfection efficiency

Authors: Marquez-Miranda, V¹., Vidaurre, S¹., Otero, C³., Camarada, M²., Comer, J²., AMALRAJ, J²., Velasquez, L³., Santos, L²., Gonzalez-Nilo, F¹., ¹Center for Bioinformatics and Integrative Biology, Facultad de Ciencias Biológicas, Universidad Andrés Bello. ²Nanobiotechnology Division Fraunhofer Chile Research. ³Center for Integrative Medicine and Innovative Science, Facultad de Medicina, Universidad Andrés Bello.

194 Area: Computational Biology and Bioinformatics

Title: Identification and *in silico* analysis of membrane proteins of different isolates of *Piscirickettsia salmonis*: Insights in to survival strategies and pathogenicity

Authors: Millar, A^{1,2}., Tapia, P²., Guzmán, D²., Méndez, T³., Gómez, F⁴., Henríquez, V⁴., Marshall, S^{4,5}., Valdés, J²., ¹Programa de Doctorado en Biotecnología, Ciencias Biológicas, Universidad Andrés Bello. ²División Bio-Cómputo, Centro para la Biotecnología de Sistemas, Fraunhofer Chile Research. ³Ciencias Biológicas Universidad Andrés Bello. ⁴Laboratorio de Genética e Inmunología Molecular Pontificia Universidad Católica de Valparaíso. ⁵División Acuicultura, Centro para la Biotecnología de Sistemas, Fraunhofer Chile Research.

195 Area: Computational Biology and Bioinformatics

Title: Characterization of binding affinity, transport and control release of Angiotensin 1-7 using dendrimer technology

Authors: Pacheco C, N²., Cabello-Verrugio, C¹., Varas, I²., González-Nilo, F^{2,3}., ¹Laboratorio de Biología y Fisiopatología Molecular (LBFM), Facultad de Ciencias Biológicas, Universidad Andrés Bello. ²Center for Bioinformatics and Integrative Biology, Facultad de Ciencias Biológicas, Universidad Andrés Bello. ³Fundación Fraunhofer Chile Research Fundación Fraunhofer Chile Research.

196 Area: Computational Biology and Bioinformatics

Title: The genetic canonical code enhances the stability of the primary structure of proteins subject to punctual random mutations

Authors: Osorio, M¹., Gallardo, M²., ¹Laboratorio de Biofísica, Facultad de Medicina, Universidad Diego Portales. ²Laboratorio de Biofísica, Escuela de Medicina, Universidad Diego Portales.

197 Area: Enzymes and Metabolism

Title: Expression and characterization of a GH 62 alpha-L-arabinofuranosidase from *Aspergillus fumigatus* WMO

Authors: Pérez, R¹., Eyzaguirre, J¹., ¹Ciencias Biológicas, Ciencias Biológicas, Universidad Andrés Bello.

198 Area: Enzymes and Metabolism

Title: Properties of the electron transport chain of *Burkholderia tropica*

Authors: JARAMILLO, R¹., Bolívar, H¹., Contreras, M²., ¹Centro de Investigación, Ciencias de la Salud, Universidad Libre Seccional Barranquilla. ²Bioquímica y Biología Estructural, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México (UNAM).

199 Area: Enzymes and Metabolism

Title: Beyond glycogen synthesis: functional characterization of glycogen synthase in Sertoli cells

Authors: Maldonado, R¹., Lopez, C¹., Mancilla, H¹., Villarroel-Espíndola, F¹., Cereceda, K¹., Vander Stelt, K¹., Covarrubias, A¹., Slebe, J¹., Méndez, R²., Concha, I¹., ¹Instituto de Bioquímica y Microbiología, Ciencias, Universidad Austral de Chile. ²IRB Barcelona Institut for Research in Biomedicine.

200 Area: Enzymes and Metabolism

Title: Dynamic Localization of Glucokinase and its Regulatory Protein in Hypothalamic Tanycytes

Authors: Salgado, M^{1,2}., Tarifeño, E²., Ordenes, P²., Yañez, M²., Elizondo, R²., Uribe, E¹., García, M²., ¹Bioquímica y Biología Molecular, Ciencias Biológicas, Universidad de Concepción. ²Biología Celular, Ciencias Biológicas, Universidad de Concepción.

201 Area: Enzymes and Metabolism

Title: FoxO transcription factors in a diabetic cardiomyopathy model

Authors: Vásquez-Trincado, C¹., Campos, C²., Espinosa, A²., Navarro-Márquez, M³., Lavandero, S¹., ¹Centro de Estudios Moleculares de la Célula (CEMC), Facultad de Ciencias Químicas y Farmacéuticas. Facultad de Medicina, Universidad de Chile. ²Escuela de Tecnología Médica, Facultad de Medicina, Universidad de Chile. ³Centro de Estudios Moleculares de la Célula (CEMC), Facultad de Ciencias Químicas y Farmacéuticas., Universidad de Chile.

202 Area: Enzymes and Metabolism

Title: Activity of AMP-activated kinase (AMPK) and glycogenolytic enzymes in the final pH of *Longissimus thoracis* muscle in steer carcasses

Authors: Galaz, A¹., Strobel, P¹., Apaoblaza, A²., Jerez, N³., Ramírez, A¹., Gallo, C¹., ¹Instituto de Ciencia Animal, Ciencias Veterinarias, Universidad Austral De Chile. ²Programa de Doctorado en Ciencias Veterinarias-Escuela de Graduados, Ciencias Veterinarias, Universidad Austral De Chile. ³Ciencia Animal, Ciencias Veterinarias, Universidad de Zulia, Venezuela.

203 Area: Enzymes and Metabolism

Title: Different effects of progesterone and estradiol on native and its chimeric isoform aldosterone synthase that cause Familial Hyperaldosteronism type 1 (FH-1): Studies *in vitro*

Authors: Fuentes, C¹., Vecchiola, A¹., Lagos, C¹., Allende, F²., Valdivia, C^{3,1}., Campino, C^{1,3}., Tapia-Castillo, A¹., Solari, S²., Carvajal, C^{3,1}., Fardella, C^{3,1}., ¹Department of Endocrinology, School of Medicine, Pontificia Universidad Católica de Chile. ²Department of Clinical Laboratories, School of Medicine, Pontificia Universidad Católica de Chile. ³Millennium Institute of Immunology and Immunotherapy Pontificia Universidad Católica de Chile.

204 Area: Enzymes and Metabolism

Title: Biocompatible microcapsules as carriers of L-asparaginase, an approved enzyme drug in therapy of leukemia

Authors: Konrad, M¹., ¹Enzyme Biochemistry Max Planck Institute for Biophysical Chemistry.

205 Area: Evolution

Title: Reconstruction of last common ancestor of arginases and agmatinases

Authors: Quiñones, M¹., Castro-Fernandez, V²., Guzmán, B³., Montes, P¹., García, D¹., Benítez, J¹., Romero, N¹., Guixé, V²., Carvajal, N¹., Uribe, E¹., ¹Depto. Bioquímica y Biología Molecular, Facultad Ciencias Biológicas, Universidad de Concepción. ²Depto. de Biología, Facultad de Ciencias, Universidad de Chile. ³Depto. de Zoología, Facultad Ciencias Naturales y Oceanografías, Universidad de Concepción.

206 Area: Evolution

Title: All paths lead to Rome: Evolutionary convergence and divergence of K⁺ channel blocking toxins

Authors: Rodríguez De La Vega, R¹., Sunagar, K^{2,3}., Giraud, T¹., ¹Laboratoire d'Ecologie, Systématique et Evolution Université Paris-Sud. ²Centro Interdisciplinar de Investigação Marinha e Ambiental Universidade do Porto. ³Departamento de Biologia, Faculdade de Ciências, Universidade do Porto.

207 Area: Evolution

Title: Local properties of the genetic code could determine global stability conditions on transcription or translation errors

Authors: Salinas, D¹., Gallardo, M¹., Osorio, M¹., ¹Centro de Investigación Biomédica, Facultad de Medicina, Universidad Diego Portales.

208 Area: Gene expression

Title: The inhibition of protein degradation affects the dynamics of cytosolic histones H3 and H4

Authors: Púas, G¹., Loyola, A^{1,2}., ¹Cromatina y Epigenética Fundación Ciencia&Vida. ²Facultad de Medicina Universidad San Sebastián.

209 Area: Gene expression

Title: The full-length mRNA of human T-cell leukemia virus type 1 (HTLV-1) harbors and IRES element that is dependent on the ribosomal protein S25

Authors: Olivares, E^{3,1}, Landry, D², Pino, K³, Rossi, F³, Navarrete, C⁴, Huidobro-Toro, J⁴, Thompson, S², López-Lastra, M³, ¹Programa de Doctorado en Microbiología Universidad de Santiago de Chile. ²Department of Microbiology University of Alabama at Birmingham, Birmingham AL, USA. ³Escuela de Medicina Pontificia Universidad Católica de Chile. ⁴Departamento de Fisiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.

210 Area: Gene expression

Title: Role of RCO-1 in circadian transcriptional regulation in *Neurospora crassa*

Authors: Olivares-Yañez, C¹, Larrondo, L¹, ¹Departamento Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.

211 Area: Gene expression

Title: Unveiling Novel Transcriptional Networks behind the Circadian Clock of *Neurospora crassa*

Authors: Munoz-Guzman, F¹, Caballero, V¹, Larrondo, L², ¹Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile. ²Departamento Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.

212 Area: Gene expression

Title: Circadian clock and carbon catabolite repression in *Neurospora crassa*: insights into the role of the CRE-1 transcription factor in the crosstalk of two regulatory systems

Authors: Díaz, R¹, Larrondo, L¹, ¹Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.

213 Area: Gene expression

Title: Phytoene synthase genes of *Malus domestica* codify for functional enzymes that are expressed differentially during fruit ripening

Authors: Acosta, D¹, Cerda, A¹, Arias, D¹, Stange, C¹, ¹Departamento de Biología, Facultad de Ciencias, Universidad de Chile.

214 Area: Gene expression

Title: Characterization of the BcFRQ protein and its role in the *Botrytis cinerea* circadian clock

Authors: Müller, H¹, Hevia, M¹, Canessa, P¹, Larrondo, L¹, ¹Departamento Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.

215 Area: Gene expression

Title: Potential use of the β actin promoter of chinese hamster cells in the expression system of recombinant proteins

Authors: Zúñiga, R¹, Gutiérrez, M¹, Collazo, N¹, Valenzuela, L¹, Leiva, L¹, Aguillón, J¹, Molina, M¹, ¹Centro de InmunoBioTecnología (<http://www.centroibt.cl>), Programa Disciplinario de Inmunología, Instituto de Ciencias Biomédicas (ICBM), Medicina, Universidad de Chile.

216 Area: Gene expression

Title: The *Piscirickettsia salmonis* infection *in vitro* on salmon cell line induced changes in the gene expression of type 4b Secretion System

Authors: Cortes, M¹, Oliver, C¹, Pontigo, J¹, Nualart, D¹, Yañez, A¹, ¹Instituto de Bioquímica y Microbiología, Ciencias, Universidad Austral de Chile.

217 Area: Gene expression

Title: Sumoylation modulates vitamin D-mediated transcription in osteoblasts

Authors: Merino, P¹, Salinas, K¹, Montecino, M¹, ¹Center for Biomedical Research and FONDA Center for Genome Regulation, Faculty of Biological Sciences and Faculty of Medicine, Universidad Andrés Bello.

218 Area: Gene expression

Title: Biochemical and Functional Analysis of CREB and C/EBP β binding sites in the human RIC-8B gene promoter

Authors: Maureira, A¹, Sánchez, R¹, Hinrichs, M¹, Olate, J¹, Gutiérrez, J², ¹Laboratorio de Genética Molecular, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción. ²Laboratorio de Regulación Transcripcional, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.

219 Area: Gene expression

Title: Characterization of a circadian clock in *Botrytis cinerea* and its role in pathogenesis using *Arabidopsis thaliana* as a plant model

Authors: Hevia, M¹, Canessa, P¹, Müller, H¹, Larrondo, L¹, ¹Depto. Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.

220 Area: Gene expression

Title: Characterization of transcriptional regulation of *MYBA1*, mediated by glucose, during fruit ripening of *Vitis vinifera*

Authors: Serrano, A¹, Nuñez, C¹, Delrot, S², Arce-Johnson, P¹, ¹Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile. ²Institut des Sciences de la Vigne et du Vin University Bordeaux.

221 Area: Gene expression

Title: SRS transcriptome: a useful resource for understanding host-pathogen interaction

Authors: Martinez, V¹, Dettleff, P¹, ¹FAVET-INBIOGEN. Animal Science, Faculty of Veterinary Sciences, University of Chile.

222 Area: Gene expression

Title: Post-transcriptional control of gene expression

Authors: Brown, P¹, ¹Department of Biochemistry Stanford University School of Medicine.

223 Area: Gene expression

Title: Histone modifications at the MLH1 promoter respond differentially to 5-aza-dC treatment

Authors: Sepúlveda, J¹, Riquelme, A¹, Gutiérrez, S¹, ¹Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.

224 Area: Immunology

Title: Development of a sandwich Enzyme-Linked Fluorescence Assay (ELFA) for detecting cathelicidin from Rainbow trout fry

Authors: Santana, P¹., Guzmán, F²., Mercado, L^{3,2}., ¹Grupo de Marcadores Inmunológicos en Organismos Acuáticos. Instituto de Biología, Facultad de Ciencias, Pontificia Universidad Católica de Valparaíso. Universidad Técnica Federico Santa María. ²Núcleo Biotecnológico Curauma (NBC) Pontificia Universidad Católica de Valparaíso. ³Grupo de Marcadores Inmunológicos en Organismos Acuáticos. Instituto de Biología, Facultad de Ciencias, Pontificia Universidad Católica de Valparaíso.

225 Area: Immunology

Title: Selection of scFv constructs that bind to human MICA using multiple antigenic presentation systems and Phage Display library

Authors: Leiva, L¹., Zúñiga, R¹., Lohs-Rojas, A¹., Sotelo, P²., Gutiérrez-González, M¹., Collazo, N¹., Ribeiro, C¹., Aguillón, J¹., Molina, M¹., ¹Centro de InmunoBioTecnología (<http://www.centroibt.cl>), Programa Disciplinario de Inmunología, Instituto de Ciencias Biomédicas (ICBM), Facultad de Medicina, Universidad de Chile. ²Departamento de Biotecnología, Facultad de Ciencias Químicas, Universidad Nacional de Asunción.

226 Area: Immunology

Title: Chromatographic analysis and functional characterization of synovial fluid from rheumatoid arthritis patients

Authors: Campos, J^{1,2}., Maggi, J^{1,2}., Tobar, P^{1,2}., Schäfer, C^{1,2}., Beltrán, A^{1,2}., Catalán, D^{1,2}., Aguillón, J^{1,2}., ¹Immune Regulation and Tolerance Group, Programa Disciplinario de Inmunología, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile. ²Millenium Institute on Immunology and Immunotherapy Universidad de Chile.

227 Area: Immunology

Title: IL-10 regulates mica expression on the surface of gastric adenocarcinoma cells

Authors: Garrido, M¹., Hernández, C¹., Kramm, K¹., Ribeiro, C¹., Molina, M¹., ¹Programa Disciplinario de Inmunología, Facultad de Medicina, Universidad de Chile.

228 Area: Immunology

Title: Rab14 regulates antigen presentation by major histocompatibility complex type I (MHC-I) in dendritic cells infected with *Chlamydia trachomatis*

Authors: Capmany, A¹., Cebrián, I¹., Damiani, T¹., ¹IHEM-CONICET, FCM, UNCuyo.

229 Area: Immunology

Title: The CD1d-natural killer T cell axis in the immune response against human gastric adenocarcinoma

Authors: Ribeiro, C¹., Galvez, F¹., Perez, G¹., Cristi, F¹., Kramm, K¹., Bustamante, M²., Hernández, C¹., Garrido-Tapia, M¹., Aguillón, J¹., Molina, M¹., ¹Programa Disciplinario de Inmunología (ICBM), Facultad de Medicina, Universidad de Chile. ²Departamento de Cirugía Digestiva - H. Salvador, Facultad de Medicina, Universidad de Chile.

230 Area: Immunology

Title: Differential expression of cell-surface MICA/B and CD44 in the tumor tissue and gastric mucosa of patients with gastric adenocarcinoma

Authors: Galvez, F²., Bustamante, M¹., Contreras, H²., Morales, C²., Kramm, K²., Garrido-Tapia, M²., Hernandez, C²., Aguillón, J²., Molina, M²., Ribeiro, C²., ¹Departamento de Cirugía Digestiva - H. Salvador, Facultad de Medicina, Universidad de Chile. ²Programa Disciplinario de Inmunología (ICBM), Facultad de Medicina, Universidad de Chile.

231 Area: Immunology

Title: *In vitro* evaluation of the cellular immune response of *Mytilus chilensis* hemocytes in primary culture exposed to Saxitoxin

Authors: Carrera, C¹, Aballay, A¹, Ulloa, V¹, Gallardo-Escárate, C², Astuya, A³, ¹Cell Culture and Marine Genomics Laboratory, Marine Biotechnology Unit, Faculty of Natural and Oceanographic Sciences, Universidad De Concepción. ²Laboratory of Biotechnology and Aquatic Genomics, Interdisciplinary Center for Aquaculture Research (INCAR), Universidad De Concepción. ³Marine Biotechnology Unit, Ciencias Naturales y Oceanográficas, Universidad De Concepción.

232 Area: Immunology

Title: Increased Reactive Oxygen Species is associated with an altered NFκB-dependent IL-6 gene expression

Authors: Henríquez-Olguin, C¹, Altamirano, F², Valladares, D¹, Jaimovich, E¹, ¹Centro de Estudios Moleculares de la Célula, ICBM, Facultad de Medicina, Universidad de Chile. ²Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis.

233 Area: Microbiology

Title: Glucose effect on expression of carotenogenic genes and pigment synthesis in a *Xanthophyllomyces dendrorhous mig1* mutant.

Authors: Bravo, N¹, Alcaíno, J¹, Barahona, S¹, Marcoleta, A², Baeza, M¹, Cifuentes, V¹, ¹Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile. ²Departamento de Biología, Facultad de Ciencias, Universidad de Chile.

234 Area: Microbiology

Title: The cytoplasmic dynein complex is important for Murine Leukemia Virus Infection

Authors: Garces, A¹, Arriagada, G¹, ¹Ciencias Biológicas, Ciencias Biológicas, Universidad Andrés Bello.

235 Area: Microbiology

Title: Genetic and Structural analysis of *Porphyromonas gingivalis* Lipopolysaccharide and its role in antimicrobial compounds resistance

Authors: Soto, C¹, Díaz, L¹, Fuentes, A¹, Salinas, D¹, Venegas, D¹, Dutzan, N¹, Hoare, A¹, Bravo, D¹, ¹Laboratorio de Microbiología Oral, Facultad de Odontología, Universidad de Chile.

236 Area: Microbiology

Title: Development of an enzyme-linked immunosorbent assay for evaluating the efficacy of vaccines against SRS

Authors: Sandoval, R¹, Espinoza, C², Valenzuela, K¹, Cárcamo, J¹, Yañez, A¹, ¹Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile. ²Ciencias Veterinarias Universidad de Concepción.

237 Area: Microbiology

Title: Characterization of gene clusters involved in the biosynthesis of new compounds in freshwater cyanobacteria

Authors: Soto-Liebe, K¹, Fuentes-Valdés, J^{1,2}, Vásquez, M¹, Arce-Johnson, P¹, ¹Genética Molecular y Microbiología, Ciencias Biológicas, Pontificia Universidad Católica De Chile. ²Ingeniería Química y Bioprocesos, Facultad de Ingeniería, Pontificia Universidad Católica de Chile.

238 Area: Microbiology

Title: Phenoloxidase activity and its relationship with the specificity and selectivity in bipartite cyanolichens of the genus *Peltigera*

Authors: Leiva, D¹., De Armas, M¹., Carú, M¹., Orlando, J¹., ¹Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile.

239 Area: Microbiology

Title: Cell division proteins interact with PBP2b and contribute to morphogenesis in *Streptococcus pneumoniae*

Authors: Yandar Barahona, N., Albarracin Orio, A., Piñas, G., Cian, M., Cortes, P., Echenique, J¹., ¹Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba.

240 Area: Microbiology

Title: Preliminary characterization of a bacteriocin-like compound produced by an Antarctic strain of *Pseudomonas*

Authors: Pavlov, M^{1,2}., Cárdenas, C⁴., González, M³., Marshall, S^{1,4}., ¹Departamento de Biología, Facultad de Ciencias, Pontificia Universidad Católica de Valparaíso. ²Doctorado en Biotecnología Universidad Técnica Federico Santa María. ³Laboratorio de Biorrecursos Instituto Antártico Chileno. ⁴Núcleo de Biotecnología de Curauma Pontificia Universidad Católica de Valparaíso.

241 Area: Microbiology

Title: Antibacterial activity determination of cobalt (III) and copper (II) complexes with carbohydrate ligand

Authors: Corsini, G¹., Wiese, G¹., Rivas, E¹., Atria, A²., Parada, J²., ¹Centro Investigación Biomédica, Medicina, Universidad Diego Portales. ²Departamento de Química Inorgánica y Analítica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.

242 Area: Microbiology

Title: Analysis of different DNA mismatches on RecA-catalyzed recombination *in vitro*

Authors: Borgogno, V¹., Monti, M¹., Pezza, R²., Argaraña, C¹., ¹CIQUIBIC-CONICET, Dpto Química Biológica, Fac. Ciencias Químicas, Universidad Nacional de Córdoba- República Argentina. ²Oklahoma Medical Research Foundation Oklahoma City, OK, USA

243 Area: Microbiology

Title: Complete nucleotide sequence of a widely dispersed lineage of a Mayaro Virus isolated from a febrile illness patient resident in a farming settlement in the Brazilian State of Acre

Authors: Speranca M, A¹., Juncansen, C¹., Suzuki R, B¹., Nogueira M, L²., Ferreira M, U³., ¹Center for natural and human sciences Universidade Federal do ABC. ²Virologia Faculdade de Medicina de São José do Rio Preto. ³Instituto de Ciências Biomédicas, Parasitologia, Universidade de São Paulo.

244 Area: Miscellaneous

Title: Vascular effect of the proanthocyanidins (PAs) from seed and skin *País* grape extracts in human chorionic vein

Authors: Fernández, K¹., Grandon, R²., González, M²., ¹Chemical Engineering, Faculty of Engineering, University of Concepcion. ²Laboratory of Vascular Physiology, Department of Physiology, Faculty of Biological Sciences, University of Concepción.

245 Area: Miscellaneous

Title: Role and Regulation of Ryanodine Receptor Expression in Synaptic Plasticity (LTP)

Authors: Valdés, I¹, Barrientos, G², More, J², San Martín, C², Muñoz, P³, Hidalgo, C⁴, ¹BNI and CEMC, Faculty of Medicine, Universidad de Chile. ²Physiology and Biophysics Program, ICBM, Faculty of Medicine, Universidad de Chile. ³CINV, Faculty of Sciences, Universidad de Valparaíso. ⁴BNI and CEMC, Physiology and Biophysics Program, ICBM, Faculty of Medicine, Universidad de Chile.

246 Area: Miscellaneous

Title: Identification and characterization of interleucine-like growth factors in the human pathogen *Trichomonas vaginalis*

Authors: Muñoz, C¹, Catalan, A¹, Astudillo, C¹, San Francisco, J¹, Araya, J¹, Gutierrez, B¹, González, J¹, ¹Departamento Tecnología Médica, Facultad Cs. de la Salud, Universidad de Antofagasta.

247 Area: Miscellaneous

Title: Different roles of the Andes hantavirus Gc cytoplasmic tail and transmembrane region in virus-cell fusion

Authors: Márquez C., L¹, Sarno, V¹, Tischler N., D¹, ¹Molecular Virology Laboratory Fundación Ciencia & Vida.

248 Area: Miscellaneous

Title: Cell bioassay as a toxicological tool for detection of paralytic shellfish poison

Authors: Aballay, A¹, Diogenes, J², Ulloa, V³, Astuya, A³, ¹Cell Culture and Marine Genomics Laboratory, Marine Biotechnology Unit, Faculty of Natural and Oceanographic Sciences, Universidad de Concepción. ²Ctra. Poble Nou, Km 5,5, 43540 Sant Carles de la Ràpita, Spain., IRTA. ³Cell Culture and Marine Genomics Laboratory, Marine Biotechnology Unit, Faculty of Natural and Oceanographic Sciences, Universidad de Concepción.

249 Area: Miscellaneous

Title: Conjugation of CdTe-GSH Quantum Dots to IgG and IgY for immunoassays

Authors: Barra, P¹, González, V¹, Valderrama, I¹, Loto, I¹, Pérez-Donoso, J², Gutiérrez, M¹, ¹Biolmaging Lab NEON - Creative Labware. ²Nanobiotechnology Lab, School of Biological Sciences, UNAB.

250 Area: Miscellaneous

Title: Improved method for the biomimetic synthesis of biocompatible fluorescent nanoparticles for multiplex analytical assays

Authors: Valderrama, I¹, Barra, P¹, Acuña-Rougier, C¹, Pérez-Donoso, J², Gutiérrez, M¹, ¹Biolmaging Lab NEON - Creative Labware. ²Nanobiotechnology Lab, School of Biological Sciences, UNAB.

251 Area: Miscellaneous

Title: Spectrophotometric mobile technologies for science and education

Authors: Brescia, I^{1,2}, Toledo, I³, Lagos, I^{1,2}, Dadlani, K^{1,2}, Peralta, A^{2,4}, Oliva, M², Nemerich, H⁵, Cabrera, R¹, ¹Biochemistry and Molecular Biology, Sciences Faculty, Universidad de Chile. ²Product Development Lab4U SPA. ³Mobile Development Birdie LTDA. ⁴Product Development Lab4U SPA. ⁵Development & Platform Evangelism Microsoft Chile.

252 Area: Miscellaneous

Title: Development of an inexpensive imaging platform and consumables for multiplex western blotting

Authors: Gutierrez, M¹, Valderrama, I¹, Barra, P¹, Perez, J², ¹Biolmaging Lab NEON - Creative Labware. ²Centro de Bioinformática y Biología Integrativa Universidad Andres Bello.

253 Area: Molecular Cell Biology

Title: E-cadherin-dependent regulation of Caveolin-1-enhanced Rac1 activation and migration in metastatic cancer cells

Authors: Díaz-Valdivia, N¹., Díaz, J¹., Torres, V²., Leyton, L¹., Quest, A¹., ¹ Cell Communication Laboratory, Center for Molecular Studies of the Cell, Institute of Biomedical Sciences, Faculty of Medicine, Universidad de Chile. Universidad De Chile. ²Laboratory of Cellular and Molecular Biology, Department of Basic and Communitarian Sciences, Faculty of Dentistry, Universidad de Chile. Universidad de Chile.

254 Area: Molecular Cell Biology

Title: Mechanism of *Trypanosoma gambiense* resistance to human serum

Authors: Pérez-Morga, D¹., Uzureau, P²., Uzureau, S²., Lecordier, L¹., Fontaine, F¹., Tebabi, P¹., Homblé, F¹., Grélard, A³., Zhendre, V⁴., Nolan, D⁵., Lins, L⁶., Crowet, J⁶., Pays, A¹., Felu, C¹., Poelvoorde, P¹., Vanhollebeke, B¹., Moestrup, S⁷., Lyngsø, J⁸., Pedersen, J⁹., Mottram, J¹⁰., Dufourc, E¹¹., Perez-Morga, D¹., Pays, E¹., ¹Molecular Biology, Sciences, Université Libre de Bruxelles. ²Molecular Biology, Sciences, Université Libre de Bruxelles. ³Institut Polytechnique Bordeaux, CNRS, Université de Bordeaux. ⁴Institut Polytechnique Bordeaux, CNRS, Université Libre de Bordeaux. ⁵School of Biochemistry and Immunology Trinity College. ⁶Center of Numerical Molecular Biophysics Université de Liège. ⁷Biomedicine, University of Aarhus. ⁸Chemistry University of Aarhus. ⁹Chemistry University of Aarhus. ¹⁰Institute of Infection, Immunity and Inflammation University of Glasgow. ¹¹Institut Polytechnique Bordeaux Université Bordeaux.

255 Area: Molecular Cell Biology

Title: Caveolin-1 promotes migration and invasion of metastatic cancer cells via a novel Rab5-Rac1 signalling axis

Authors: Díaz, J¹., Mendoza, P²., Leyton, L³., Quest, A³., Torres, V⁴., ¹Center for Molecular Studies of the Cell (CEMC). Institute of Biomedical Sciences, Faculty of Medicine, Universidad de Chile. ²Laboratory of Cellular and Molecular Biology. Department of Basic and communitarian Sciences, Faculty of Dentistry, Universidad de Chile. ³CEMC. Institute of Biomedical Sciences, Faculty of Medicine, Universidad de Chile. ⁴Laboratory of Cellular and Molecular Biology, Department of Basic and communitarian Sciences, Faculty of Dentistry, Universidad de Chile.

256 Area: Molecular Cell Biology

Title: Mitochondria-ER communication is disrupted in cardiomyocyte hypertrophy

Authors: Pennanen, C¹., Gutierrez, T¹., Troncoso, R¹., Jaimovich, E²., Lavandero, S¹., ¹Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile. ²Laboratorio de Fisiología del Músculo, Instituto de Ciencias Biomédicas, Programa de Biología Celular y molecular, Facultad de Medicina, Campus Norte, Universidad de Chile.

257 Area: Molecular Cell Biology

Title: Estrogen and progesterone receptors in normal canine mammary tissue during the reproductive cycle

Authors: Tasende, C¹., Della Cella, C²., Sauto, R²., López, C²., ¹Area Bioquímica, Departamento de Biología Molecular y Celular, Facultad de Veterinaria, Universidad de la República, Uruguay. ²Departamento de Patología y Clínica de Pequeños Animales, Facultad de Veterinaria, Universidad de la República, Uruguay.

258 Area: Molecular Cell Biology

Title: Activation of the kinin B1 receptor induces cellular migration of estrogen-sensitive breast cancer cells

Authors: Budinich, E¹., Figueroa, C¹., Andrade, Y¹., Pavicic, M¹., Matus, C¹., Ehrenfeld, P¹., ¹Instituto de Anatomía, Histología y Patología, Facultad de Medicina, Universidad Austral de Chile.

259 Area: Molecular Cell Biology

Title: Generation of stable breast cancer cell lines with silenced or augmented vitamin C transporter expression

Authors: Roa, F¹., Muñoz, C¹., Salvatori, O¹., Gonzalez, M¹., Sotomayor, K¹., Peña, E¹., Vera, J¹., Rivas, C¹., ¹Fisiopatología, Ciencias Biológicas, Universidad de Concepción.

260 Area: Molecular Cell Biology

Title: Effect of LDL-Ox on genes related to inflammatory process in macrophages cells

Authors: Gonzalez, M¹., Ledda, A²., Grasa, M²., Mansilla, L³., Toledo, J⁴., Garda, H⁴., Esteve Rafols, M⁵., ¹La Plata, Ciencias Médicas, Universidad Nacional de La Plata. ²Nutrición y Bromatología, Biología, Universidad de Barcelona. ³Ciencias Básicas y Fisiológicas, Ciencias Médicas, Universidad Nacional de La Plata. ⁴Ciencias Básicas y Fisiológicas, Medicina, Universidad Nacional de La Plata. ⁵Nutrición y Bromatología, Biología, Universidad de Barcelona.

261 Area: Molecular Cell Biology

Title: The Antisense Non Coding Mitochondrial RNAs modulates expression of Cell Cycle and Survival Genes

Authors: Fitzpatrick, C^{1,2}., Bendek, M^{1,2}., Oliveira-Cruz, L²., Burzio, L^{1,2}., Burzio, V^{1,2}., ¹Facultad de Ciencias Biológicas Universidad Andrés Bello. ²Fundación Ciencia & Vida Andes Biotechnologies S.A.

262 Area: Molecular Cell Biology

Title: DNA double strand breaks on *RUNX1* gene are induced by etoposide treatment of differentiated blood cells

Authors: Schnake, N¹., Stuardo, M¹., Hinojosa, M¹., Gutiérrez, S¹., ¹Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.

263 Area: Molecular Cell Biology

Title: Doxorubicin inhibits autophagy in cultured rat cardiomyocytes

Authors: Pizarro, M., Chiong, M., Lavandero, S. Laboratorio de Transducción de Señales Moleculares de la Célula, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.

264 Area: Molecular Cell Biology

Title: Physiological effects of adenosine in podocytes and in diabetic glomerulopathy

Authors: Jaramillo, C¹., Alarcón, S¹., Quezada, C¹., San Martín, R¹., ¹Bioquímica y Microbiología, Ciencias, Universidad Austral de Chile.

265 Area: Molecular Cell Biology

Title: 5-aza-2'-deoxycytidine treatment induced changes in histone acetylation pattern at the p16 and MLH1 promoters in HL-60 and HeLa cells

Authors: Riquelme, A¹., Sepúlveda, J¹., Gutiérrez, S¹., ¹Bioquímica y Biología Molecular, Ciencias Biológicas, Universidad de Concepción.

266 Area: Molecular Cell Biology

Title: Antioxidant properties of the *Pinus radiata* bark extract in salmonid cell lines *in vitro*

Authors: Ortiz, S²., Roa, J¹., Ulloa, V¹., Roeckel, M²., Aspé, E²., Astuya, A¹., Fernández, K²., ¹Cell Culture and Marine Genomics Laboratory, Marine Biotechnology Unit, Faculty of Natural and Oceanographic Sciences, University of Concepción. ²Chemical Engineering, Faculty of Engineering, University of Concepción.

267 Area: Molecular Cell Biology

Title: P27Kip1 is involved in autophagy-dependent survival of Tsc2-null cells during rapamycin treatment

Authors: Ziehe, J¹.Campos, T¹.,Castro, A¹.,Departamento de ¹Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.

268 Area: Molecular Cell Biology

Title: Emotional stress increases cFOS and Arc early gene products: correlation with mRNAs and protein levels of Matrix Metalloproteinase (MMP) 9 in Rat hippocampus

Authors: Pacheco, A¹.Andrés, S¹.,Muñoz, M¹.,Pizarro, J¹.,Aguayo, F¹.,Peralta, F².,Aliaga, E².,Fiedler, J¹.,¹Laboratorio de Neuroplasticidad y Neurogenética, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.²Escuela de Kinesiología, Facultad de Ciencias, Pontificia Universidad Católica de Valparaíso.

269 Area: Molecular Cell Biology

Title: Analysis of SNAP/NSF complex in the mouse oocyte physiology

Authors: De Paola, M¹.Arcos, A².,Bello, O¹.,Cappa, A¹.,Rinaldini, E³.,Mayorga, L¹.,Bátiz, F².,Michaut, M^{1,3}.,¹IHEM, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo.²IAHP, Facultad de Medicina, Universidad Austral de Chile.³ICB, Instituto de Ciencias Básicas, Universidad Nacional de Cuyo.

270 Area: Molecular Cell Biology

Title: Sall2 - NUA1 relationship in cell survival under metabolic stress

Authors: Riffo, E¹.Palma, M¹.,Castro, A¹.,Pincheira, R¹.,¹Bioquímica y Biología Molecular, Ciencias Biológicas, Universidad de Concepción.

271 Area: Molecular Cell Biology

Title: Chronic stress - inadaptability and its relationship with imbalance in mirnas levels in rat hippocampus

Authors: Muñoz, M¹.Gutierrez, R².,Vidal, E².,Fiedler, J¹.,¹Laboratorio de Neuroplasticidad y Neurogenética, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.²Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.

272 Area: Molecular Cell Biology

Title: Electrical stimuli release atp to increase ros production via p2y1-nox2 in skeletal muscle fibers

Authors: Díaz, A¹.Campos, C².,Jaimovich, E¹.,Espinosa, A².,¹Centro de Estudios Moleculares de la Célula (CEMC), Medicina, Universidad de Chile.²Escuela de Tecnología Médica, Medicina, Universidad de Chile.

273 Area: Molecular Cell Biology

Title: Sortin2 effect analysis in the development of lateral roots in different mutants of *A. thaliana*

Authors: Cruz, M¹.Norambuena, L¹.,Departamento de Biología, Facultad de Ciencias, Universidad de Chile.

274 Area: Molecular Cell Biology

Title: Differential effect of restraint stress on dorsal and ventral rat hippocampus: changes in mRNA levels of plasticity markers and glutamate receptors subunits

Authors: García-Rojo, G¹.Muñoz, M¹.,Fiedler, J¹.,¹Facultad de Ciencias Químicas y Farmacéuticas, Laboratorio de Neuroplasticidad y Neurogenética Universidad de Chile.

275 Area: Molecular Cell Biology

Title: Understanding the role of Sall2 in the regulation of cell cycle

Authors: Salgado, G¹., Farkas, C²., Pincheira, R²., ¹Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad De Concepción. ²Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.

276 Area: Plant Biology

Title: Metabolomic profiling during ripening of peach fruits: common and distinct metabolic processes among peach varieties

Authors: Monti, L³., Gabilondo, J¹., Budde, C¹., Valentini, G¹., Osorio, S²., Fernie, A²., Andreo, C³., Lara, M³., Drincovich, M³., ¹EEA San Pedro INTA, Argentina. ²Molecular Plant Physiology Max Planck Institute, Germany. ³CEFOBI Universidad Nacional de Rosario, Argentina.

277 Area: Plant Biology

Title: Phenylpropanoids biosynthesis pathway are activated in response to stem inclination in seedlings of radiata pine

Authors: Ramos, P¹., Moya-León, A¹., Herrera, R¹., ¹Laboratorio de Fisiología Vegetal y Genética Molecular, Instituto de Biología Vegetal y Biotecnología, Universidad de Talca.

278 Area: Plant Biology

Title: Phenylpropanoid metabolites are required in the Antarctic ecotype of *Colobanthus quitensis* (Kunth) Bartl. to tolerate UV-B radiation

Authors: Contreras, R¹., Pizarro, M¹., Parada, R¹., Zúñiga, G¹., ¹Departamento de Biología, Laboratorio de Fisiología y Biotecnología Vegetal, Facultad de Química y Biología, Universidad de Santiago de Chile.

279 Area: Plant Biology

Title: Ectopic application of EXLX1 increases grain weight in *Triticum aestivum*

Authors: Delgado, F¹., Calderini, D²., Claude, A¹., ¹Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile. ²Instituto de Producción y Sanidad Vegetal, Facultad de Ciencias Agrarias, Universidad Austral de Chile.

280 Area: Plant Biology

Title: Development of an *in vitro* culture protocol for micropropagation of Chilean strawberry (*Fragaria chiloensis*)

Authors: Concha, C¹., Zúñiga, P¹., Ríos, D¹., Figueroa, C¹., ¹Manejo de Bosques y Medio Ambiente, Facultad de Ciencias Forestales, Universidad de Concepción.

281 Area: Plant Biology

Title: The effect of tetracycline in the natural antioxidant properties of chilean unifloral honeys

Authors: Mejias, E¹., Montenegro, G¹., ¹Departamento de Ciencias Vegetales, Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile.

282 Area: Plant Biology

Title: Analysis of VvNAC-1: a NAC transcriptional activator found induced in grapevine's compatible viral interactions

Authors: Arce, A¹., Godoy, F¹., Arce-Johnson, P¹., ¹Genética Molecular y Microbiología, Cs. Biológicas, Pontificia Universidad Católica de Chile.

283 Area: Plant Biology

Title: Functional Characterization of Pollen-Specific Kinases Trough the Generation of Knock-down Transgenic Plants Expressing Artificial microRNAs (amiRNAs)

Authors: Ibeas, M¹., Peralta, C¹., León, G¹.,¹Centro de Biotecnología Vegetal, Ciencias Biológicas, Universidad Andrés Bello.

284 Area: Plant Biology

Title: Transcriptional and post-transcriptional regulation of four pollen-specific genes coding for protein kinases in *Arabidopsis thaliana*

Authors: Parra, S¹., León, G¹.,¹Centro de Biotecnología Vegetal, Ciencias Biológicas, Universidad Andrés Bello.

285 Area: Plant Biology

Title: Effects of the plant growth-promoting bacteria *Burkholderia phytofirmans* PsJN in the auxin-signalling pathway gene expression in *Arabidopsis thaliana* plants

Authors: Greve, M¹., Timmermann, T¹., Poupin, M¹.,¹Laboratorio de Bioingeniería, Facultad de Ingeniería y Ciencias, Universidad Adolfo Ibáñez.

286 Area: Plant Biology

Title: Functional analysis of the grapevine *VvMYB24* gene reveals its possible role in anther development

Authors: Meyer, C¹., Espinoza, C¹., Matus, J²., Aquea, F¹., Arce-Johnson, P¹.,¹Departamento de Genética Molecular y Microbiología, Ciencias Biológicas, Pontificia Universidad Católica de Chile.²Centre for Research in Agricultural Genomics (CRAG)- CSIC Universitat Autònoma de Barcelona.

287 Area: Plant Biology

Title: Identification of salicylic acid-inducible genes coding for Glutathione S-transferases with antioxidant properties in the defense response to stress in *Arabidopsis thaliana*

Authors: Ugalde, J¹., Salinas, P¹., Holuigue, L¹.,¹Genética Molecular y Microbiología, Ciencias Biológicas, Pontificia Universidad Católica de Chile.

288 Area: Protein Structure and Function

Title: Biochemical and structural characterization of a α -D-galactopyranosidase from the pectinolytic fungus *Penicillium purpurogenum*

Authors: Faúndez, C¹., Morales-Quintana, L²., Herrera, R²., Moya-León, M²., Eyzaguirre, J¹.,¹Ciencias Biológicas, Ciencias Biológicas, Universidad Andrés Bello.²Instituto de Biología Vegetal y Biotecnología Universidad de Talca.

289 Area: Protein Structure and Function

Title: Effects of oxidation of lysozyme by hypohalous acids and haloamines on enzymatic activity and aggregation

Authors: Petrônio, M¹., Ximenes, V².,¹Análises Clínicas, Faculdade de Ciências Farmacêuticas, Universidade Estadual "Júlio de Mesquita Filho" UNESP.²Química, Química, Unesp.

290 Area: Protein Structure and Function

Title: Study of lipid transport system in South American tarantula *Grammostola rosea*

Authors: Cunningham, M¹., Laino, A¹., Suarez, G¹., Lino, A¹., Garcia, F¹.,¹Bioquímica, Facultad de Ciencias Médicas, Universidad Nacional de La Plata.

291 Area: Protein Structure and Function

Title: Cysteine residues are important determinants of functional and folding properties of the ascorbic acid transporter-2, SVCT2

Authors: Aylwin, C¹, Sweet, K¹, Rivas, C¹, Vera, J¹, ¹Departamento de Fisiopatología, Facultad de Ciencias Biológicas, Universidad de Concepción.

292 Area: Protein Structure and Function

Title: Point mutations in GTP hydrolysis sites of both bacterial tubulin monomers (BtubA/B) causes loss of their function due to a low filament formation

Authors: Hurtado, F¹, Lagos, R¹, Monasterio, O¹, ¹Departamento de Biología, Facultad de Ciencias, Universidad de Chile.

293 Area: Protein Structure and Function

Title: Sequence evolution of the intrinsically disordered and globular domains of the adenovirus E1A oncoprotein

Authors: Glavina, J¹, Sanchez, I¹, ¹Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires and IQUIBICEN-CONICET.

294 Area: Protein Structure and Function

Title: FtsEX interaction Network in the Bacterial Divisome

Authors: Maturana, D¹, Órdenes, A¹, Lagos, R¹, Monasterio, O¹, ¹Laboratorio de Biología Estructural y Molecular, Facultad de Ciencias, Universidad de Chile.

295 Area: Protein Structure and Function

Title: A cysteine-less form of the GLUT2 glucose transporter

Authors: Elgueta, C², Cuevas, A², Oyarzún, R², Ojeda, L², Salas, M², Rivas, C¹, Vera, J¹, Reyes, A², ¹Departamento de Fisiopatología, Facultad de Ciencias Biológicas, Universidad de Concepción. ²Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile.

296 Area: Protein Structure and Function

Title: γ -tubulin-CCT interaction. A theoretical and experimental structural approach

Authors: Pouchucq, L¹, Escalona, Y¹, Araya, G¹, Maturana, J¹, Valpuesta, J², Lagos, R¹, Monasterio, O¹, ¹Departamento de Biología, Facultad de Ciencias, Universidad de Chile. ²CSIC-España Centro Nacional de Biotecnología.

297 Area: Protein Structure and Function

Title: Structural and Functional Characterization of Cargo-Binding Sites on the μ 4-Subunit of Adaptor Protein Complex 4

Authors: Ross, B¹, Lin, Y¹, Corales, E¹, Burgos, P¹, Mardones, G¹, ¹Physiology, Medicine, Universidad Austral de Chile.

298 Area: Protein Structure and Function

Title: Structural and thermodynamics characterization of variants from cardiac troponin C present in cardiomyopathic patients

Authors: Marques, M¹, De Oliveira, G¹, Pinto, J², Sorenson, M¹, Silva, J¹, ¹Medical Biochemistry Institute Federal University of Rio de Janeiro. ²Department of Molecular and Cellular Pharmacology University of Miami Miller School of Medicine.

299 Area: Protein Structure and Function

Title: Structural features of potassium channel blocking by AcalI1425, a novel toxin from sea anemone

Authors: Amorim, G^{1,2}, Madio, B³, DePaula, V², Almeida, F², ¹Instituto de Bioquímica Médica, Centro Nacional de Ressonância Magnética Nuclear, Universidade Federal do Rio de Janeiro. ²National Center for Nuclear Magnetic Resonance, BioNMR Laboratory, Federal University of Rio de Janeiro. ³Department of Physiology, Institute of Biosciences, University of Sao Paulo.

300 Area: Protein Structure and Function

Title: NMR mapping of Human β -defensin 6 interaction with glycosaminoglycan: a model for extracellular matrix complex

Authors: S. De Paula, V¹, H. Pomin, V¹, C.L. Almeida, F¹, P. Valente, A¹, ¹Instituto de Bioquímica Médica Universidade Federal do Rio de Janeiro.

301 Area: Protein Structure and Function

Title: Prion Protein Aggregation Triggered By Neutrophil Extracellular Traps (NETs)

Authors: Anjos, D¹, Azevedo, E¹, Vieira, T¹, Foguel, D¹, Saraiva, E², Silva, J¹, ¹Medical Biochemistry Institute Federal University of Rio de Janeiro. ²Microbiology Institute - IMPPG Federal University of Rio de Janeiro.

302 Area: Protein Structure and Function

Title: Substrate specificity and fold evolution in the 6-phosphogluconate dehydrogenase family

Authors: Maturana, P¹, Cabrera, R¹, ¹Laboratory of Biochemistry and Molecular Biology, Department of Biology, Faculty of Sciences, Universidad de Chile.

303 Area: Protein Structure and Function

Title: identification of residues conforming an allosteric regulatory site on the GLUT1 transporter

Authors: Lagos, I¹, Arce, R¹, Oyarzún, R¹, Pérez, A¹, Salas, M¹, Salas-Burgos, A², Vera, J², Rivas, C², Reyes, A¹, ¹Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile. ²Departamento de Fisiopatología, Facultad de Ciencias Biológicas, Universidad de Concepción.

304 Area: Protein Structure and Function

Title: Physicochemical study of the transcriptional repressor NagC from *Escherichia coli*

Authors: Díaz-Jiménez, D¹, Zonszein, S¹, Alvarez-Añorve, L¹, Marcos-Viquez, J¹, Calcagno, M¹, ¹Biochemistry, Medicine, National Autonomous University of Mexico.

305 Area: Protein Structure and Function

Title: A novel method to characterize interactions among biomolecules in living cells

Authors: Stortz, M¹, Presman, D², Pooley, J^{2,3}, Hager, G², Pecci, A¹, Levi, V⁴, ¹Química Biológica, Ciencias Exactas y Naturales, Universidad de Buenos Aires / IFIByNE- Conicet. ²Laboratory of Receptor Biology & Gene Expression National Cancer Institute, NIH, Bethesda, USA. ³Laboratories for Integrated Neuroscience and Endocrinology University of Bristol, UK. ⁴Química Biológica, Ciencias Exactas y Naturales, Universidad de Buenos Aires / IQUIBICEN-Conicet.

306 Area: Signaling

Title: Combined inhibition of protein kinase CK2 and cyclooxygenase-2 is highly detrimental for viability of colon cancer cells. Potential pharmacological targets for diagnosis and treatment of colon cancer

Authors: Silva, E¹, Yefi, R¹, Nogales, M¹, Fernandez, C², Tapia, J³, ¹Cell Transformation Lab, ICBM, Faculty of Medicine, University of Chile. ²Department of Anatomopathology, HCUCH; CITC: Center for Research and Treatment of Cancer, Faculty of Medicine, University of Chile. ³Cell Transformation Lab, ICBM; CITC: Center for Research and Treatment of Cancer, Faculty of Medicine, University of Chile.

307 Area: Signaling

Title: Thy-1-induced migration requires $\beta 3$ integrin expression and activation of the PI3K/Rac1 pathway in primary astrocytes

Authors: Kong, M^{1,3,2}, Alvarez, A^{3,1}, Rojas-Mancilla, E^{4,3}, Cárdenas, A^{1,3}, Morales, P^{4,3}, Quest, A¹, Leyton, L^{1,3}, ¹Laboratorio de Comunicaciones Celulares, Centro de Estudios Moleculares de la Célula, Medicina, Universidad de Chile. ²Departamento Biomédico, Ciencias de la Salud, Universidad de Antofagasta. ³Instituto de Neurociencia Biomédica, Medicina, Universidad de Chile. ⁴Programa de Farmacología Molecular y Clínica, ICBM, Medicina, Universidad de Chile.

308 Area: Signaling

Title: Light-dependent activation of Transient Receptor Potential (TRP) channels C5, A1 and V1 in *Ulva compressa* (Chlorophyta) exposed to copper excess

Authors: González, A¹, Gómez, M¹, Moenne, A¹, ¹Laboratorio de Biotecnología Marina, Facultad de Química y Biología, Universidad de Santiago de Chile.

309 Area: Signaling

Title: Characterization of a multiprotein complex containing PANNEXIN-1 in a muscle cell line

Authors: Arias-Calderon, M¹, Jaimovich, E¹, Buvinic, S^{1,2}, ¹Laboratorio de Fisiología Celular del Músculo, Centro de Estudios Moleculares de la Célula, ICBM, Facultad de Medicina, Universidad de Chile. ²Departamento de Ciencias Básicas y Comunitarias, Facultad de Odontología, Universidad de Chile.

310 Area: Signaling

Title: Key roles of sPLA₂ in the inflammatory response during retinal injury

Authors: Rodríguez Diez, G¹, Sánchez Campos, S¹, Salvador, G¹, ¹Biochemistry, INIBIBB-CONICET, Universidad Nacional del Sur.

311 Area: Signaling

Title: Interleukin-6 induces ErbBs receptor phosphorylation, ERK1/2 pathway activation and utrophin expression in dystrophic myotubes

Authors: Juretic, N¹, Díaz, J¹, Romero, F¹, Jaimovich, E¹, Riveros, N¹, ¹Programa de Biología Celular y Molecular, ICBM, Facultad de Medicina, Universidad de Chile.

312 Area: Signaling

Title: Incretin GLP-1 promotes endoplasmic reticulum – mitochondria communication in vascular smooth muscle cells by activating a PKA/Mitofusin-2 pathway

Authors: Morales, P¹, Torres, G¹, Sotomayor-Flores, C¹, Rivera-Mejías, P¹, Núñez, C¹, Cartes, B¹, Lavandero, S^{1,2}, Chiong, M¹, ¹CEMC, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile. ²Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile.

313 Area: Signaling

Title: Insulin/NF- κ B/VCAM-1 signaling pathway in the protection to cardiac ischemia

Authors: Díaz, A¹, Humeres, C¹, González, V¹, Lavandero, S¹, García, L¹, ¹Laboratorio Transducción de Señales, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.

314 Area: Signaling

Title: Herp regulates insulin response in skeletal muscle cells

Authors: Navarro-Marquez, M¹., Paredes, F¹., Troncoso, R¹., Vasquez-Trincado, C¹., Jaimovich, E¹., Lavandero, S^{1,2}., ¹Centro de Estudios Moleculares de la Célula, Facultad de Ciencias Químicas y Farmacéuticas & Facultad de Medicina, Universidad de Chile. ²Department of Internal Medicine (Cardiology Division) University of Texas Southwestern Medical Center, Dallas, Texas, USA.

315 Area: Signaling

Title: Effect of transcriptional factor FoxO1 on gene expression of metabolic enzymes mediated by testosterone in cardiomyocytes

Authors: Pavez, M¹., Valladares, D¹., Duran, J¹., Venegas, N¹., Varela, D¹., Lavandero, S²., Estrada, M¹., ¹Fisiología y Biofísica, Facultad de Medicina, Universidad de Chile. ²Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.

316 Area: Signaling

Title: CaMKII increases MEF2 activity to induce cardiomyocyte hypertrophy by testosterone

Authors: Duran, J¹., Valladares, D²., Pavez, M³., Venegas, N³., Basualto-Alarcon, C³., Lavandero, S⁴., Estrada, M⁵., ¹Programa de Fisiología y Biofísica y Programa de Biología Celular y Molecular, Instituto de Ciencias Biomédicas, Facultad de Medicina., Universidad de Chile. ² Programa de Fisiología y Biofísica, Instituto de Ciencias Biomédicas, Facultad de Medicina., Universidad de Chile. ³Programa de Fisiología y Biofísica, Instituto de Ciencias Biomédicas, Facultad de Medicina., Universidad de Chile. ⁴Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile. ⁵ Programa de Fisiología y Biofísica, Instituto de Ciencias Biomédicas, Facultad de Medicina., Universidad de Chile.

317 Area: Biomedicine

Title: Amyloid- β peptide oligomers impair spatial learning and decrease c-fos and RyR2 hippocampal protein levels

Authors: More, J¹., Valdez, J^{2,3}., Paula-Lima, A⁴., Hidalgo, C²., ¹CEMC & BNI Universidad de Chile. ²ICBM, Faculty of Medicine, Universidad De Chile. ³CENEM Universidad de Chile. ⁴Basic Sciences, Dentistry, Universidad de Chile.

November 13, Wednesday
Roofed Street Santa Rosa
10:30-12:45
Posters from 318 to 477

318 Area: Biomedicine

Title: Unveiling the death mechanism induce by InsP3R inhibition in a cancer model

Authors: Bustos, G¹., Lovy, A²., Molgo, J³., Foskett, K⁴., Cardenas, C⁵., ¹Cellular Metabolism and Bioenergetic Lab, Facultad de Medicina, Universidad de Chile. ²Center for Neuroscience Research, School of Medicine, Tufts University. ³Institut de Neurobiologie Alfred Fessard CNRS. ⁴Physiology, Perelman School of Medicine, University of Pennsylvania. ⁵Anatomy and Developmental Biology, School of Medicine, Universidad de Chile.

319 Area: Biomedicine

Title: Role of mitochondria in neuronal Ca²⁺ and ROS signals induced by amyloid- β peptide oligomers

Authors: Barattini, P¹., SanMartín, C¹., Hidalgo, C^{2,1}., Paula-Lima, A^{3,1}., ¹CEMC, BNI Universidad de Chile. ²ICBM, Faculty of Medicine, Universidad de Chile. ³Basic Sciences, Faculty of Dentistry, Universidad de Chile

320 Area: Biomedicine

Title: Maternal obesity-induced endoplasmic reticulum stress reduces migration in human umbilical vein endothelial cells

Authors: Villalobos-Labra, R¹., Sáez, P¹., Westermeier, F¹., Kusanovic, J²., Poblete, J²., Mardones, F³., Sobrevia, L¹., Fariás-Jofré, M¹., ¹Cellular and Molecular Physiology Laboratory (CMPL), Medical Research Centre (CIM), Division of Obstetrics and Gynaecology, School of Medicine, Medicine, Pontificia Universidad Católica de Chile. ²Division of Obstetrics and Gynecology, School of Medicine, Medicine, Pontificia Universidad Católica de Chile. ³Division of Public Health, School of Medicine, Medicine, Pontificia Universidad Católica de Chile.

321 Area: Biomedicine

Title: Short-term high-fat diet induces mitochondrial Ca²⁺ mishandling and insulin resistance in skeletal muscle prior to mitochondrial damage

Authors: Contreras-Ferrat, A¹., ¹Biología Celular y Molecular, Facultad de Medicina, Universidad de Chile.

322 Area: Biomedicine

Title: Effect of statins on microRNA expression signature in HepG2 cells

Authors: Zambrano, T¹., Hirata, R²., Hirata, M²., Cerda, A²., Salazar, L¹., ¹Center of Molecular Biology and Pharmacogenetics, Faculty of Medicine, Universidad de La Frontera. ²Department of Clinical Analysis, Faculty of Pharmaceutical Sciences, Universidade de Sao Paulo.

323 Area: Biomedicine

Title: Grafting of subcommissural organ (SCO) explants to diminish or repair abnormalities in brain development associated to congenital hydrocephalus

Authors: Salazar, P¹., Vio, K²., Guerra, M²., Rodríguez, S²., Jara, C²., Jara, M²., McAllister, P³., Johanson, C⁴., Rodríguez, E²., ¹Instituto de Histología y Anatomía, Medicina, Universidad Austral de Chile. ²Instituto de Anatomía, Histología y Patología, Medicina, Universidad Austral de Chile. ³Pediatric Neurosurgery Division, Department of Neurosurgery, University of Utah, Utah, USA. ⁴Department of Neurosurgery, Alpert Medical School, Brown University, NY, USA.

324 Area: Biomedicine

Title: Identification of Novel 11 β -HSD1 Inhibitors with Combined Ligand- and Structure-Based Virtual Screening

Authors: Lagos, C¹., Vecchiola, A¹., Fuentes, C¹., Allende, F²., Solari, S²., Cifuentes, M³., Campino, C^{1,4}., Owen, G⁵., Carvajal, C^{1,4}., Fardella, C^{1,4}., ¹Department of Endocrinology, School of Medicine, Pontificia Universidad Catolica de Chile. ²Department of Clinical Laboratories, School of Medicine, Pontificia Universidad Catolica de Chile. ³Institute of Nutrition and Food Technology (INTA) Universidad de Chile. ⁴Millennium Institute of Immunology and Immunotherapy Pontificia Universidad Catolica de Chile. ⁵Department of Physiology, Faculty of Biological Sciences, Pontificia Universidad Catolica de Chile.

325 Area: Biomedicine

Title: PGC1 α and mitochondria biogenesis in activated human NK cells from elderly donors

Authors: Godoi, B³., Ahumada, V³., Ibañez, J³., Acuña, C³., Hirsch, S¹., Miranda, D²., Montoya, M³., ¹INTA Universidad de Chile. ²Bioquímica y Biología Molecular, Ciencias Químicas y Farmacéuticas, Universidad de Chile. ³Biología, Química y Biología, Universidad de Santiago de Chile.

326 Area: Biomedicine

Title: Use of biomimetic CdTe-GSH Quantum Dots in photodynamic therapy (PDT): understanding the molecular mechanism of phototoxicity

Authors: Straub Y, D^{1,2}., Muñoz, C³., Plaza, D¹., Pérez-Donoso J, M¹., ¹BioNanotechnology and Microbiology Lab, Center for Bioinformatics and Integrative Biology CBIB, Biological Sciences Department, Universidad Andrés Bello. ²Faculty of Ciencias Universidad De Chile. ³Faculty of Chemical and Biological Sciences Universidad de Santiago de Chile.

327 Area: Biomedicine

Title: Changes in proteins related with calcium homeostasis in skeletal muscle from HFD fed mice

Authors: Campos, C¹., Jaimovich, E²., Espinosa, A²., ¹Escuela de Tecnología Médica, Facultad de Medicina, Universidad de Chile. ²Centro de Estudios Moleculares de la Célula, Facultad de Medicina, Universidad de Chile.

328 Area: Biomedicine

Title: Photophysical study of the interaction between CdTe-GSH fluorescent nanoparticles synthesized by green chemistry and chlorine e6 for potential applications on Photo Dynamic Therapy (PDT)

Authors: Straub Y, D^{1,2}., Pires, L³., Pratavieira, S⁴., Bagnato, V⁴., Kurachi, C⁴., Pérez-Donoso J, M., ¹BioNanotechnology and Microbiology Lab, Center for Bioinformatics and Integrative Biology CBIB, Biological Sciences Department, Andres Bello University. ²Faculty of Sciences University of Chile. ³Institute of Physics of São Carlos Universidad de Sao Paulo. ⁴Institute of Physics of São Carlos University of Sao Paulo.

329 Area: Biomedicine

Title: Sodium tungstate mediated ERK phosphorylation in HK-2 cells and rat kidney

Authors: Rañinao, C¹., Silva, P¹., Perez, M¹., Jaramillo, K¹., Bertinat, R¹., Yañez, A¹., ¹Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile.

330 Area: Biomedicine

Title: Angiotensin-(1–9) decreases blood pressure and cardiac damage induced by deoxycorticosterone by Mas and AT2 receptor independent mechanism

Authors: Morales, C^{7,1}., Moya, J⁷., Novoa, U⁷., Michea, L²., Chiong, M³., Lavandero, S^{4,5}., Jalil, J⁶., Ocaranza, M⁷., ¹2Center of Molecular Studies of the Cell, , Faculty of Chemical and Pharmaceutical SciencesMedicine, Universidad de Chile.²Center of Molecular Studies of the Cell, Medicine, Universidad de Chile.³Center of Molecular Studies of the Cell, Chemical and Pharmaceutical Sciences , Universidad de Chile.⁴Center of Molecular Studies of the Cell, Chemical and Pharmaceutical Sciences and Medicine, Universidad de Chile.⁵ Internal Medicine, Medicine, University of Texas, Southwestern Medical Center.⁶Cardivascular Diseases, Medicine, Pontificia Universidad Católica de Chile.⁷Cardiovascular Diseases, Medicine, Pontificia Universidad Católica de Chile.

331 Area: Biomedicine

Title: A role for the Ca²⁺-dependent repressor DREAM in the onset and progression of Huntington disease

Authors: Naranjo, J¹., Mellström, B¹., ¹CSIC, Madrid Centro Nacional de Biotecnología.

332 Area: Biomedicine

Title: Chimeric Protein GLUT4myc-eGFP *in vivo* electroporated in *Flexor digitorum brevis* muscle allows to quantify real time translocation from vesicles to sarcolemma

Authors: Utreras-Mendoza, Y⁴., Casas, M¹., Llanos, P²., Espinosa, A³., Jaimovich, E⁴., Contreras-Ferrat, A⁴., ¹ICBM, Facultad de Medicina, Universidad de Chile.²Ciencias Básicas y Comunitarias, Facultad de Odontología, Universidad de Chile.³Escuela de Tecnología Médica, Facultad de Medicina, Universidad De Chile.⁴Biología Celular y Molecular, CEMC, Facultad de Medicina, Universidad de Chile.

333 Area: Biomedicine

Title: Sodium tungstate decreases the progression of renal damage through inhibition of fibrosis and apoptosis in diabetic rat kidney

Authors: Silva, P¹., Jaramillo, K¹., Perez, M¹., Carpio, D²., Yañez, A¹., ¹Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile.²Instituto de Histología y Patología, Facultad de Medicina, Universidad Austral de Chile.

334 Area: Biomedicine

Title: Diabetes modifies extracellular metabolism and physiological effects of adenosine in renal proximal epithelial tubule cells

Authors: Kretschmar, C¹., Villablanca, C¹., Oyarzún, C¹., Salinas, C¹., San Martín, R¹., ¹Bioquímica y Microbiología, Ciencias, Universidad Austral de Chile.

335 Area: Computational Biology and Bioinformatics

Title: Molecular dynamics simulation and site-directed mutagenesis suggest a ternary complex mechanism for the catalysis of alcohol acyltransferase of mountain papaya fruit

Authors: Morales-Quintana, L¹., Nuñez-Tobar, M¹., Moya-León, M¹., Herrera, R¹., ¹Laboratorio de Fisiología Vegetal y Genética Molecular, Instituto de Biología Vegetal y Biotecnología, Universidad de Talca.

336 Area: Computational Biology and Bioinformatics

Title: Towards the analysis of key specificity determinants in DNA recognition by the transcription factors MarA, SoxS and Rob in Enterobacteria

Authors: Melis, F^{1,3,2}, Rodriguez, F^{1,3}, Melo, F^{1,3}, ¹Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile. ²Instituto de Química, Facultad de Ciencias, Pontificia Universidad Católica de Valparaíso. ³Laboratorio de Bioinformática Molecular Instituto Milenio en Inmunología e Inmunoterapia.

337 Area: Computational Biology and Bioinformatics

Title: Determination of putative binding sites on human $\alpha 7$ nicotinic acetylcholine receptor to positive allosteric modulators type I and II through computational studies

Authors: Contreras, J¹, Moller, P^{1,2}, Reyes, M², Rojas, C¹, Alzate, J¹, ¹Escuela de Bioinformática, Facultad de Ingeniería, Universidad de Talca. ²Programa Doctorado en Biotecnología, Química y Biología, Universidad de Santiago de Chile.

338 Area: Computational Biology and Bioinformatics

Title: *Acidithiobacillus* phylogenomics: understanding ecophysiology in extreme acidic environments by whole genome evolutionary reconstruction

Authors: González, C^{1,2}, Guzmán, D², Tapia, P², Valdés, J², Holmes, D¹, ¹Center for Bioinformatics and Genome Biology, Facultad de Ciencias Biológicas, Fundación Ciencia & Vida, Universidad Andrés Bello, ²Bio-Computing Division, Center for System Biotechnology, Fraunhofer Chile Research Foundation.

339 Area: Computational Biology and Bioinformatics

Title: Study of sequence/structure/function relationships of catalytic domain of DNA polymerases using multiple sources of structural comparison

Authors: Slater, A^{1,2}, Melo, F^{1,2}, ¹Molecular Bioinformatics Laboratory, Departamento Genética Molecular y Microbiología, Facultad Ciencias Biológicas, Pontificia Universidad Católica de Chile. ²Millennium Institute on Immunology and Immunotherapy, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.

340 Area: Computational Biology and Bioinformatics

Title: Assessing the importance of cooperativity of hydrogen bonding through Density Functional Theory calculations

Authors: Alzate-Morales, J¹, Adasme-Carreño, F¹, Muñoz-Gutierrez, C¹, Navarro-Retamal, C¹, Fritz, R¹, Ireta, J², ¹Centro de Bioinformática y Simulación Molecular, Facultad de Ingeniería, Universidad de Talca. ²Departamento de Química, Fisicoquímica Teórica, División de Ciencias Básicas e Ingeniería, Universidad Autónoma Metropolitana-Iztapalapa.

341 Area: Computational Biology and Bioinformatics

Title: Transcriptomic analysis of TRPM4 gene signatures in prostate cancer patients and their potential role in the progression of the disease

Authors: Sagredo, E⁴, Sagredo, A⁴, Baez, P⁴, Tapia, J^{1,5}, Marcelain, K^{2,5}, Stutzin, A^{4,3}, Armisen, R^{4,5}, ¹Programa de Biología Celular y Molecular, ICBM, Facultad de Medicina, Universidad de Chile. ²Programa de Genética Humana, ICBM, Facultad de Medicina, Universidad de Chile. ³Centro de Estudios Moleculares de la Celula, Facultad de Medicina, Universidad de Chile. ⁴Programa de Fisiopatología, ICBM, Facultad de Medicina, Universidad de Chile. ⁵U-CANCER: Red de Medicina Traslacional en Cáncer, Facultad de Medicina, Universidad de Chile.

342 Area: Computational Biology and Bioinformatics

Title: *In silico* studies to understand structure/function of exopolyphosphatase from *Escherichia coli*

Authors: Boetsch, C¹., Lisa, A¹., Beassoni, P¹.,¹Dto. Biología Molecular, Ciencias ExFQyN, Universidad Nacional de Río Cuarto.

343 Area: Computational Biology and Bioinformatics

Title: Increased local flexibility of the forkhead domain of FOXP2 transcription factor is required for domain swapping

Authors: Villalobos, P¹., Ramirez-Sarmiento, C¹., Babul, J¹.,¹Departamento de Biología, Facultad de Ciencias, Universidad de Chile.

344 Area: Computational Biology and Bioinformatics

Title: Distribution modeling for Chilean Amphibian species: a MAXENT approach

Authors: Moreno, R¹., Ortiz, J²., Camus, P³., Bacigalupe, L⁴., Labra, F¹.,¹Centro de Ciencias Ambientales CIENCIA-UST, Facultad de Ciencias, Universidad Santo Tomás.²Departamento de Zoología, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción.³Departamento de Ecología, Facultad de Ciencias, Universidad Católica de la Santísima Concepción.⁴Instituto de Ciencias Ambientales & Evolutivas, Facultad de Ciencias, Universidad Austral de Chile.

345 Area: Computational Biology and Bioinformatics

Title: Molecular Dynamics Simulations of Current-Voltage in BK and Shaker K⁺ Channels

Authors: Araya, I¹., Sepúlveda, R¹., Márquez, V¹., Aguayo, D^{1,2}., Bravo, F¹., Gonzalez-Nilo, F^{1,2}.,¹Center for Bioinformatics and Integrative Biology (CBIB), Facultad de Ciencias Biológicas, Universidad Andrés Bello.²Centro Interdisciplinario de Neurociencias de Valparaíso (CINV) Universidad de Valparaíso.

346 Area: Computational Biology and Bioinformatics

Title: Towards an accurate identification of DNA mobile elements in eukaryote genomes

Authors: Valdebenito, B¹., Riadi, G¹.,¹Centro de Bioinformática y Simulación Molecular - CBSM, Facultad de Ingeniería, Universidad de Talca.

347 Area: Computational Biology and Bioinformatics

Title: A beta-complex statistical four body contact potential combined with a hydrogen bond statistical potential recognizes the correct native structure from protein decoy sets

Authors: Sánchez-González, G¹., Kim, J²., Kim, D²., Garduño-Juárez, R^{3,4}.,¹Biofísica, Facultad de Ciencias, Universidad Autónoma del Estado de Morelos, México.²Department of Industrial Engineering, Voronoi Diagram Research Center, Hanyang University, Seoul, Korea.³Instituto de Ciencias Físicas Universidad Nacional Autónoma de México.⁴Centro de Ciencias de la Complejidad Universidad Nacional Autónoma de México.

348 Area: Computational Biology and Bioinformatics

Title: Carbon use in cyanobacteria from genus *prochlorococcus* in the anoxic marine zone from the eastern-south pacific: a metagenomic analysis

Authors: Astorga, M¹., Ulloa, O².,¹Departamento de Ciencias Biológicas, Facultad de Ciencias Biológicas, Universidad de Concepción.²Departamento de Oceanografía, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción.

349 Area: Computational Biology and Bioinformatics

Title: Comparative three-dimensional structure modeling of protein-DNA complexes with MODELLER: application to the prediction of transcription factor DNA binding specificity

Authors: Ibarra, I¹., Cares, J¹., Melo, F¹.,¹Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.

350 Area: Computational Biology and Bioinformatics

Title: Prediction and structural characterization of copper (Cu 2 +) binding sites in the Kunitz trypsin inhibitor 3 (KT13) protein expressed in poplars

Authors: Reyes, L¹., Vergara, A²., Guerra, F³., ¹Escuela de Ingeniería en Bioinformática, Facultad de Ingeniería, Universidad de Talca. ²Centro de Bioinformática y Simulación Molecular, Facultad de Ingeniería, Universidad de Talca. ³Instituto de Biología Vegetal y Biotecnología Universidad de Talca.

351 Area: Computational Biology and Bioinformatics

Title: Synthetic Toxins Based on Dendrimers as Ion Channel Blocker

Authors: Navas, C^{1,2}., Márquez Miranda, V¹., González-Nilo, F^{1,2}., ¹Center for Bioinformatic and Integrative Biology, Facultad de ciencias Biológicas, Universidad Andrés Bello. ²Centro Interdisciplinario de Neurociencias de Valparaíso (CINV) Universidad de Valparaíso.

352 Area: Enzymes and Metabolism

Title: Differential properties of four α -L-arabinofuranosidases secreted by the fungus *Penicillium purpurogenum*

Authors: Ravanal, M¹., Eyzaguirre, J¹., ¹Ciencias Biológicas Universidad Andrés Bello.

353 Area: Enzymes and Metabolism

Title: Characterization of extracellular amylase, cellulase, xylanase and chitinase activities, from cultures of different Antarctic yeast species

Authors: Carrasco, M¹., Barahona, S¹., Alcaíno, J¹., Cifuentes, V¹., Baeza, M¹., Departamento de ¹Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile.

354 Area: Enzymes and Metabolism

Title: Autophagy contributes to germ cell survival during a glutathione depletion condition

Authors: Mancilla, H¹., Cereceda, K¹., Maldonado, R¹., Villarroel-Espíndola, F¹., Montes De Oca, M¹., Angulo, A¹., Castro, M¹., Slebe, J¹., Lavandero, S²., Concha, I¹., ¹Instituto de Bioquímica y Microbiología, Ciencias, Universidad Austral de Chile. ²Centro Estudios Moleculares de la Célula (CEMC) Universidad de Chile.

355 Area: Enzymes and Metabolism

Title: Mitochondrial complex I inhibition as inducer of cell death in human acute lymphoblastic leukemia

Authors: Jaña, F¹., Urra, F¹., Faini, F²., Ríos, M¹., Ferreira, J¹., Cárdenas, C¹., ¹Institute of Biomedical Sciences, Faculty of Medicine, Universidad de Chile. ²Department of Chemistry, Faculty of Sciences, Universidad de Chile.

356 Area: Enzymes and Metabolism

Title: Interaction of agmatinase-like protein with Mn²⁺ and Zn²⁺ ions

Authors: Benítez, J¹., Cofré, J¹., Montes, P¹., Vallejos, A¹., García, D¹., Martínez-Oyanedel, J¹., Carvajal, N¹., Uribe, E¹., ¹Bioquímica y Biología Molecular, Ciencias Biológicas, Universidad de Concepción.

357 Area: Enzymes and Metabolism

Title: The Limch1 protein has agmatinase activity

Authors: García, D¹., Benítez, J¹., Romero, N¹., Gonzalez, A¹., Quiñonez, M¹., Carvajal, N¹., Uribe, E¹., ¹Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.

358 Area: Enzymes and Metabolism

Title: A novel fungal phytase active at low temperatures

Authors: Castillo, B²., Palma, F¹., Alvarez, A²., Pozo, P¹., Reyes, A²., Costa, M¹., ¹Instituto de Ciencias y Tecnología de los Alimentos, Facultad de Ciencias Agrarias, Universidad Austral de Chile. ²Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile.

359 Area: Enzymes and Metabolism

Title: Bioinformatics and Functional Analysis of 2-aminophenol Metabolic Pathway in *Burkholderia xenovorans* LB400

Authors: Strahsburger, E¹., Chirino, B¹., Agulló, L¹., González, M¹., Seeger, M¹., ¹Laboratorio de Microbiología Molecular y Biotecnología Ambiental Universidad Técnica Federico Santa María.

360 Area: Enzymes and Metabolism

Title: Preliminary purification and kinetic characterization of different isoforms of *Trametes versicolor* laccases

Authors: Brescia, I¹., Lopéz, E¹., Cabrera, R¹., Facultad de Ciencias, Universidad de Chile.

361 Area: Enzymes and Metabolism

Title: possible role of WNT3A ligand in the regulation of muscle glycogen synthase (MGS) in germ line GC-1 (spermatogonia type b) and GC-2 (spermatocytes)

Authors: Covarrubias, A¹., Mancilla, H¹., Villarroel, F¹., Maldonado, R¹., López, C¹., Cereceda, K¹., Vander Stelt, K¹., Castro, M¹., Slebe, J¹., Concha, I¹., ¹Instituto Bioquímica y Microbiología, Ciencias, Universidad Austral de Chile.

362 Area: Evolution

Title: Complex interactions of evolutionary models explain the actual diversity of relaxin and hemoglobin gene families

Authors: Opazo, J¹., ¹Instituto de Ciencias Ambientales y Evolutivas, Facultad de Ciencias, Universidad Austral de Chile.

363 Area: Evolution

Title: Functional novelty in enzyme evolution: Paleomolecular study of the ADP-dependent sugar kinases family

Authors: Castro-Fernandez, V¹., Guixé, V¹., ¹Laboratorio de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad de Chile.

364 Area: Gene expression

Title: Effect of histone deacetylase– and demethylase-inhibitors over replication and transcriptional activity of the Hepatitis B Virus

Authors: Alarcón, V¹., Muñoz, F¹., Flores, Y¹., Villanueva, R²., Loyola, A^{3,1}., ¹Epigenetics & Chromatin Fundación Ciencia & Vida. ²Ciencias Biológicas Universidad Andrés Bello. ³Facultad de Medicina Universidad San Sebastián.

365 Area: Gene expression

Title: Genome-wide screen implicates lipid metabolism in transcriptional regulation of Protein Kinase A subunits

Authors: Pautasso, C²., Cañonero, L²., Chua, G¹., Zaremberg, V¹., Rossi, S²., ¹Biological Chemistry University of Calgary. ²Química Biológica - IQUIBICEN, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires.

366 Area: Gene expression

Title: Epigenetic regulation of the ribosomal cistron seasonally modulates enrichment of H2A.Z- and H2A.Zub- in response to different environmental inputs in carp (*Cyprinus carpio*)

Authors: Simonet, N¹., Rudolffi, P¹., Nardocci, G¹., Molina, A¹., Alvarez, M²., ¹Ciencias Biológicas, Ciencias Biológicas, Universidad Andrés Bello. ²Departamento de Ciencias Biológicas, Facultad de Ciencias Biológicas, Universidad Andrés Bello.

367 Area: Gene expression

Title: The hepatitis C virus (HCV) IRES Domain III participates in the recruitment and positioning of the eukaryotic Ribosome

Authors: Angulo, J^{1,3}., Deforges, J²., Chamond, N²., Ulryck, N²., López-Lastra, M³., Sargueil, B²., ¹Programa de Doctorado en Microbiología Universidad de Santiago De Chile, Santiago, Chile. ²Laboratoire de Cristallographie et RMN Biologiques - CNRS UMR8015 Université Paris Descartes, Paris, France. ³Laboratorio de Virología Molecular, Instituto Milenio de Inmunología e Inmunoterapia (IMI), Centro de Investigaciones Médicas, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile.

368 Area: Gene expression

Title: Analysis of Expression Profiles of Cell Cycle and DNA Repair Genes upon Knockdown of the Antisense Noncoding Mitochondrial RNA

Authors: Oliveira-Cruz, L^{1,2}., Araya, M¹., Fitzpatrick, C^{1,3}., Briones, M^{1,3}., Burzio, L^{1,4}., Burzio, V^{1,4}., ¹Andes Biotechnologies Fundación Ciencia y Vida. ²Natural Science Institute Universidad de las Américas. ³Cellular Biology, Biological Science, Universidad Andres Bello. ⁴Cellular Biology Universidad Andres Bello.

369 Area: Gene expression

Title: Role of ASH2L and NCOA6 in glucocorticoid-mediated apoptosis in leukemic cells

Authors: Silbermins, M¹., Pecci, A²., Rocha Viegas, L³., ¹Fisiología, Biología Molecular y Celular (FBMC), Facultad de Ciencias Exactas y Naturales (FCEN), Universidad de Buenos Aires (UBA). ²Química Biológica, FCEN, UBA-IFIBYNE-CONICET. ³FBMC, FCEN, UBA-IFIBYNE-CONICET.

370 Area: Gene expression

Title: The eukaryotic RNA binding protein Mex3A stimulates translation of the Andes virus (ANDV) SmRNA

Authors: Castillo, E¹., Barriga De Vicente, F²., Astudillo, P¹., Batlle, E²., López-Lastra, M¹., ¹Escuela de Medicina Pontificia Universidad Católica de Chile. ²Colorectal Cancer Lab Institute for Research in Biomedicine (IRB).

371 Area: Gene expression

Title: Characterization of Iron Metabolism Genes of *Piscirickettsia salmonis*

Authors: Calquín, P¹., Haro, R¹., Yáñez, A¹., ¹Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile.

372 Area: Gene expression

Title: Florfenicol-resistant *P.salmónis*: characterization of ABC and RND family efflux pump involved in resistance

Authors: Valdivia, S¹., Sandoval, R¹., Olavarría, V¹., Valenzuela, P¹., Cárcamo, J¹., Yáñez, A¹., ¹Instituto de Bioquímica y Microbiología, Ciencias, Universidad Austral de Chile.

373 Area: Gene expression

Title: Expression of small RNAs in response to oxidative stress in *Acidithiobacillus ferrooxidans*

Authors: Alamos, P¹, Shmaryahu, A², Farah, C¹, Moya, F³, Levicán, G³, Orellana, O¹, ¹Molecular and Cellular Biology, Medicine, Universidad de Chile. ²Bioinformatic Science and Life Foundation. ³Biology, Chemistry and Biology, Universidad de Santiago de Chile.

374 Area: Gene expression

Title: The transcriptional co-repressor Ski regulates the expression of a cohort of pericentromeric genes at the early G₁ phase of the cell cycle

Authors: Cappelli, C³, Urzúa, U^{2,4}, Toro, J³, Cassanova, E³, Donoso, G³, Rivas, S³, Orostica, K³, Verdugo, R^{3,4}, Armisen, R^{4,1}, Tapia, J^{2,4}, Marcelain, K^{3,4}, ¹Programa de Fisiopatología, ICBM, Medicina, Universidad de Chile. ²Programa de Biología Celular y Molecular, ICBM, Medicina, Universidad de Chile. ³Programa de Genética Humana, ICBM, Medicina, Universidad de Chile. ⁴Centro de Investigación y Tratamiento del Cáncer, Medicina, Universidad de Chile.

375 Area: Gene expression

Title: P53 and SP1 transcriptionally regulate the SALL2 transcription factor

Authors: Farkas, C¹, Sanhueza, D¹, Hepp, M¹, Gutiérrez, J¹, Castro, A¹, Pincheira, R¹, ¹Bioquímica y Biología Molecular, Ciencias Biológicas, Universidad de Concepción.

376 Area: Gene expression

Title: Experimental validation of mirrorRNAs in human transcriptome

Authors: Munita, R., Gysling, K¹, ¹Departamento de Biología Celular y Molecular, Ciencias Biológicas, Pontificia Universidad Católica de Chile.

377 Area: Gene expression

Title: Regulatory role of C/EBPB isoforms and chromatin remodeling complexes in transcriptional regulation of the human albumin gene

Authors: Valenzuela, N¹, Gutiérrez, J¹, ¹Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.

378 Area: Gene expression

Title: Generation of expression vectors containing core promoters with TATA box and 5'top motifs, initiator, motif ten element and downstream promoter element

Authors: Valenzuela, L¹, Zúñiga, R¹, Gutiérrez, M¹, Collazo, N¹, Molina, M¹, Aguillón, J¹, ¹Centro de InmunoBioTecnología (<http://www.centroibt.cl>), Programa Disciplinario de Inmunología, Instituto de Ciencias Biomédicas (ICBM), Facultad de Medicina, Universidad de Chile.

379 Area: Gene expression

Title: Epigenetic regulation complexes including WDR5, EZH2, PRMT5, NO66 and UTX control transcription of Runx2

Authors: Rojas, A¹, Allende, M², Montecino, M¹. ¹Faculty of Biological Sciences, Universidad Andres Bello and ²Faculty of Sciences, Universidad de Chile.

380 Area: Gene expression

Title: Characterization of histone H3 translocation complexes

Authors: Saavedra, F¹., Alvarez, F¹., Díaz, C¹., Imhof, A²., Loyola, A^{1,3}., ¹Laboratory of Epigenetics & Chromatin Fundación Ciencia & Vida. ²Department of Molecular Biology, Adolf Butenandt Institute Ludwig-Maximilians-University. ³Facultad de Medicina Universidad San Sebastián.

381 Area: Immunology

Title: TIM-1: A candidate marker of human regulatory B cells

Authors: Aravena, O¹., Ferrier, A¹., Vega, P¹., Pino-Lagos, K¹., Soto, L^{1,2}., Aguillón, J¹., Catalán, D¹., ¹Programa Disciplinario de Inmunología, Medicina, Universidad de Chile. ²Hospital Clínico, Sección de Reumatología, Universidad de Chile.

382 Area: Immunology

Title: FcγRIIB modulates IL-10 secretion by regulatory B cells: Implications in systemic sclerosis

Authors: Ferrier, A¹., Aravena, O¹., Fonseca, E¹., Berendsen, J¹., Pino-Lagos, K¹., Aguillón, J¹., Soto, L^{1,2}., Catalán, D¹., ¹Immune Regulation and Tolerance Research Group, Programa Disciplinario de Inmunología, ICBM, Facultad de Medicina, Universidad de Chile. ²Hospital Clínico, Reumatología, Universidad de Chile.

383 Area: Immunology

Title: Galectins are involved in infection caused by the intracellular pathogen *Chlamydia trachomatis*

Authors: Leiva, N²., Rabinovich, G¹., Damiani, T²., ¹CONICET IBYME. ²IHEM-CONICET, FCM, UNCuyo.

384 Area: Immunology

Title: Tolerogenic dendritic cells generated with dexamethasone and monophosphoryl lipid a modulate antigen-specific memory CD4+ T cell responses in a pro-tolerogenic direction

Authors: Maggi, J^{1,2}., Pesce, B^{1,2}., Campos, J^{1,2}., Tobar, P¹., Pino-Lagos, K¹., Soto, L^{1,2}., Catalán, D^{1,2}., Aguillón, J^{1,2}., ¹Immune Regulation and Tolerance Research Group, Programa Disciplinario de Inmunología, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile. ²Millennium Institute on Immunology and Immunotherapy Universidad de Chile.

385 Area: Immunology

Title: Phenotypic characterization of murine tolerogenic dendritic cells generated with dexamethasone and monophosphoryl lipid a and preliminary evaluation of their therapeutical properties in a mice model of arthritis

Authors: Schäfer, C¹., Vergara, D¹., Aravena, O^{1,2}., Pesce, B²., Maggi, J²., Narváez, E¹., Pino-Lagos, K¹., Aguillón, J¹., ¹Immune Regulation and Tolerance Group, Programa Disciplinario de Inmunología, ICBM, Facultad de Medicina, Universidad de Chile. ²Millennium Institute on Immunology and Immunotherapy (MIII) Universidad de Chile.

386 Area: Immunology

Title: Role of Complement resistance Protein in *Trypanosoma cruzi* virulence

Authors: ASTUDILLO, C¹., San Francisco, J²., Muñoz, C²., Gutierrez, B²., González, J²., ¹DEPARTAMENTO DE TECNOLOGÍA MÉDICA, CIENCIAS DE LA SALUD, UNIVERSIDAD DE ANTOFAGASTA. ²Tecnología Médica, Ciencias de la Salud, Universidad de Antofagasta.

387 Area: Immunology

Title: *Helicobacter pylori* increases NK cell response by induction of NKG2DL surface expression in gastric adenocarcinoma cells

Authors: Hernández, C^{1,2}, Garrido-Tapia, M¹, Kramm, K¹, Ribeiro, C¹, Molina, M¹, ¹Inmunología, ICBM, Medicina, Universidad de Chile. ²Escuela de Tecnología Médica, Medicina, Universidad de Chile.

388 Area: Immunology

Title: Contribution of a conserved metal motif to the antimicrobial activity of trout hepcidin

Authors: Alvarez, C^{3,2,1}, Guzman, F³, Marshall, S^{2,3}, Cardenas, C³, Mercado, L^{2,3}, ¹Doctorado Biotecnología Universidad Técnica Federico Santa María. ²Laboratorio de Genética e Inmunología Molecular, Ciencias, Pontificia Universidad Católica de Valparaíso. ³Núcleo Biotecnología Curauma Pontificia Universidad Católica de Valparaíso.

389 Area: Immunology

Title: Generation of tolerogenic monocyte-derived dendritic cells from rheumatoid arthritis (RA) patients aimed at providing a treatment for RA

Authors: Tobar, P¹, Garcia, P^{1,2}, Hoyos, L^{1,2}, Morales, R^{1,2}, Maggi, J^{1,2}, Pesce, B^{1,2}, Pino-Lagos, K¹, Soto, L^{1,3}, Catalán, D^{1,2}, Aguillón, J^{1,2}, ¹Immune Regulation and Tolerance Research Group, Programa Disciplinario de Inmunología, ICBM, Medicina, Universidad de Chile. ²Millennium Institute on Immunology and Immunotherapy Millennium Institute on Immunology and Immunotherapy. ³Hospital Clínico Universidad de Chile Universidad de Chile.

390 Area: Microbiology

Title: Alternative electron donor in P450s systems of the carotenogenic yeast *Xanthophyllomyces dendrorhous*

Authors: Gutiérrez, M¹, Rojas, M², Baeza, M¹, Cifuentes, V¹, Alcaíno, J¹, ¹Departamento de Ciencias Ecológicas y Centro de Biotecnología, Ciencias, Universidad de Chile. ²Departamento de Química, Ciencias, Universidad de Chile.

391 Area: Microbiology

Title: Role of a general transcription repressor involved in catabolite repression in the regulation of carotenogenesis in *Xanthophyllomyces dendrorhous*

Authors: Córdova, P¹, Barahona, S¹, Alcaíno, J¹, Baeza, M¹, Cifuentes, V¹, ¹Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile.

392 Area: Microbiology

Title: The ICY1 gene of *Saccharomyces cerevisiae* is involved in ammonium uptake during alcoholic fermentation

Authors: García, V¹, Aguilera, O¹, Herrera, J¹, Ganga, M¹, Martínez, C¹, ¹Ciencia y Tecnología de los Alimentos, Tecnológica, Universidad de Santiago de Chile.

393 Area: Microbiology

Title: Single- and dual-species biofilms of *Klebsiella pneumoniae* and *Escherichia coli* clinical isolates from patients with catheter-associated urinary tract infections

Authors: Galván, E¹, Ielpi, L¹, ¹Laboratorio Genética Bacteriana Fundación Instituto Leloir, IIBBA-CONICET.

394 Area: Microbiology

Title: Interaction between biofilm matrix components in *Rhizobium leguminosarum*

Authors: Abdian, P¹., Caramelo, J²., Voza, N¹., Von Bilderling, C³., Russo, D¹., Pietrasanta, L³., Zorreguieta, A¹., ¹Laboratorio de Microbiología Molecular y Celular Fundación Instituto Leloir. ²Laboratorio de Biología Estructural y Celular Fundación Instituto Leloir. ³Centro de Microscopías Avanzadas Universidad de Buenos Aires - Argentina .

395 Area: Microbiology

Title: Structural-functional analysis of *Helicobacter pylori* the HU protein at acidic pH

Authors: Toledo, H¹., Almarza, O²., ¹Biología Celular y Molecular, ICBM, Facultad de Medicina, University of Chile. ²Biología Celular y Molecular, ICBM, Facultad de Medicina, Universidad de Chile.

396 Area: Microbiology

Title: Assessment of the protein interactions of Acy, a new membrane protein involved in Z ring orientation during cell division of the filamentous cyanobacterium *Anabaena* sp. PCC 7120

Authors: Geoffroy, C¹., Mandakovic, D¹., Vásquez, M¹., ¹Genética Molecular y Microbiología, Ciencias Biológicas, Pontificia Universidad Católica de Chile.

397 Area: Microbiology

Title: Involvement of some *Cupriavidus pinatubonensis* JMP134 aromatic catabolism pathways in degradation of root exudates and rhizosphere colonization of *Arabidopsis thaliana*

Authors: Ruiz, D¹., Dasencich, P¹., González, B¹., ¹Laboratorio de Bioingeniería, Facultad de Ingeniería y Ciencias, Universidad Adolfo Ibáñez.

398 Area: Microbiology

Title: Immune response and invasiveness ability of intracellular *Escherichia coli* isolated from Inflammatory bowel diseases patients: invasiveness in eukaryotic cells

Authors: De La Fuente, M¹., Díaz-Jiménez, D¹., Araya, D²., Álvarez-Lobos, M³., López-Köstner, F⁴., Vidal, R²., Quera, R⁵., Hermoso, M¹., ¹Laboratorio de Inmunidad Innata, Programa de Inmunología, Medicina, Universidad de Chile. ²Programa de Microbiología, Medicina, Universidad de Chile. ³Departamento de Gastroenterología, Medicina, Pontificia Universidad Católica de Chile. ⁴Unidad de Coloproctología Clínica Las Condes. ⁵Departamento de Gastroenterología Clínica Las Condes.

399 Area: Microbiology

Title: Root colonization and long-range signaling by *Burkholderia phytofirmans* PsJN contribute differentially to saline stress tolerance in *Arabidopsis thaliana*

Authors: Rojas, S¹., Poupin, M¹., Tamayo, J¹., Ledger, T¹., ¹Laboratorio de Bioingeniería, Facultad de Ingeniería y Ciencias, Universidad Adolfo Ibáñez.

400 Area: Microbiology

Title: *Burkholderia phytofirmans* PsJN improves growth of *Arabidopsis thaliana* under drought stress conditions

Authors: Tamayo, J¹., Rojas, S¹., Ledger, T¹., ¹Laboratorio de Bioingeniería, Facultad de Ingeniería y Ciencias, Universidad Adolfo Ibáñez.

401 Area: Microbiology

Title: MutS controls the action of DNA Polymerase IV

Authors: Margara, L¹., Argaraña, C¹., Monti, M¹., ¹CIQUIBIC-CONICET, Dpto Química Biológica, Fac. Ciencias Químicas, Universidad Nacional de Córdoba- República Argentina.

402 Area: Microbiology

Title: Assembly and arrangement of the DNA translocase SpoIIIE for the chromosome segregation during the sporulation of *Bacillus subtilis*

Authors: Diaz-Celis, C¹., Shin, J²., Liu, N³., Joh, J³., Araya-Secchi, R⁴., Onoa, B¹., Bustamante, C^{2,1,5,6,3}., ¹California Institute for Quantitative Biosciences University of California, Berkeley. ²Howard Hughes Medical Institute University of California, Berkeley. ³Department of Molecular and Cell Biology University of California, Berkeley. ⁴Computational Biology Lab (DLab) Fundación Ciencia & Vida, Ñuñoa, Santiago, Chile. ⁵Department of Physics University of California, Berkeley. ⁶Department of Chemistry University of California, Berkeley.

403 Area: Microbiology

Title: *In vitro* characterization of cytotoxicity of the antimicrobial polypeptide, gicin A on normal and tumor cells

Authors: Rivas-Yañez, E¹., Fernández-Oyarzún, P¹., Corsini, G¹., ¹Departamento de Bacteriología Molecular, Centro de Investigación Biomédica (CIB), Facultad de Medicina, Universidad Diego Portales.

404 Area: Miscellaneous

Title: Effect of Enriched Environment on the Auditory Attention of Stressed Rats

Authors: Gárate-Pérez, M¹., Rojas-Thomas, F¹., Dagnino-Subiabre, A¹., ¹Fisiología, Ciencias, Universidad de Valparaíso.

405 Area: Miscellaneous

Title: Characterization of proteases produced for *Pseudoalteromonas issachenkonii* and *Flavobacterium* sp., obtained from rey jorge island, antarctica

Authors: Peralta-Figueroa, C¹., Muñoz, G²., Martínez-Oyanedel, J¹., González-Rocha, G²., Bunster, M¹., ¹Laboratorio de Biofísica Molecular, Ciencias Biológicas, Universidad de Concepción. ²Laboratorio de Investigación en Agentes Antibacterianos, Ciencias Biológicas, Universidad de Concepción.

406 Area: Miscellaneous

Title: Kinetic studies of the hydrolysis of pectin by commercial pectinases

Authors: Farias, F³., Biz, A³., De Paula, D³., Motter, F³., Nosedá, M³., Richard, P¹., Krieger, N²., Mitchell, D³., ¹Synthetic Biology Division VTT Technical Research Centre of Finland. ²Departamento de Química, Setor de Ciências Exatas, Universidade Federal do Paraná. ³Departamento de Bioquímica e Biologia Molecular, Setor de Ciências Biológicas, Universidade Federal do Paraná.

407 Area: Miscellaneous

Title: Evaluation of the antimicrobial and antiproliferative effect of venom of Chilean tarantulas and Chilean Scorpions

Authors: Rojas, J¹., Arán, T¹., Subiabre, M¹., Araya, P¹., Quitral, P¹., Rivera, D¹., Herrera, D¹., Ordenes, K¹., Orrego, P¹., Catalán, A¹., Araya, J¹., ¹Tecnología Médica, Facultad Ciencias de la Salud, Universidad de Antofagasta.

408 Area: Molecular Cell Biology

Title: ECM-specific caveolin-1 phosphorylation on tyrosine 14 promotes melanoma migration

Authors: Ortiz, R²., Díaz, N²., Díaz, J^{1,2}., Torres, V^{1,2}., Cárdenas, A²., Mendoza, P^{1,2}., Leyton, L²., Quest, A²., ¹Department of Basic and Communitarian Sciences, Faculty of Dentistry, Universidad de Chile. ²Laboratory of Cellular Communication, Center for Molecular Studies of the Cell, ICBM, Faculty of Medicine, Universidad de Chile.

409 Area: Molecular Cell Biology

Title: Functional study of *Toxoplasma gondii* small heat shock protein 20

Authors: De Napoli, M^{4,1}., Coceres, V^{4,1}., Arrizabalaga, G²., Moreno, S³., Angel, S^{4,1}., Corvi, M^{4,1}., ¹Laboratorio de Parasitología Molecular Universidad de San Martín. ²School of Medicine Indiana University. ³CTEGD and Department of Cellular Biology University of Georgia. ⁴N/A IIB-INTECH.

410 Area: Molecular Cell Biology

Title: Estrogen receptors concentrations in canine mammary tumors

Authors: Tasende, C¹., Della Cella, C²., Sauto, R²., López, C²., Dutra, F³., ¹Area Bioquímica, Departamento de Biología Molecular y Celular, Facultad de Veterinaria, Universidad de la República, Uruguay. ²Departamento de Patología y Clínica de Pequeños Animales, Facultad de Veterinaria, Universidad de la República, Uruguay. ³Departamento de Patología, Laboratorio Regional Este DILAVE, MGAP, Uruguay.

411 Area: Molecular Cell Biology

Title: Distribution of matrix metalloproteinase-2 (MMP-2) in sheep cervix during the oestrous cycle

Authors: Casuriaga, D¹., Gonzalez, R¹., Rodríguez Piñón, M¹., ¹Area Bioquímica, Departamento de Biología Molecular y Celular, Facultad de Veterinaria, Universidad de la República, Uruguay.

412 Area: Molecular Cell Biology

Title: Stimulation of the kinin B1 receptor activates specific signaling pathways and transactivates the epidermal growth factor receptor (EGFR) in normal and neoplastic human keratinocytes

Authors: Matus, C¹., Mejia, A¹., Ehrenfeld, P²., Pavicic, M²., Hidalgo, M²., Burgos, R¹., Figueroa, C²., ¹Instituto de Farmacología y Morfología Veterinaria, Ciencias Veterinarias, Universidad Austral de Chile. ²Instituto de Anatomía, Histología y Patología, Medicina, Universidad Austral de Chile.

413 Area: Molecular Cell Biology

Title: Stimulation of kinin B1 receptor induces expression/release of vascular endothelial growth factor (VEGF) and metalloproteases (MMPs) in tumor microenvironment cells

Authors: Pavicic, M¹., Matus, C¹., Budinich, E¹., Carmona, P²., Figueroa, C¹., Ehrenfeld, P¹., ¹Instituto de Anatomía, histología y patología, Facultad de Medicina, Universidad Austral de Chile. ²Instituto de Fisiología, Facultad de Medicina, Universidad Austral de Chile.

414 Area: Molecular Cell Biology

Title: HERP: a novel regulator of cardiomyocyte hypertrophy

Authors: Torrealba, N¹., Fernandez, C¹., Paredes, F¹., Pedrozo, Z¹., Lavandero, S^{1,2}., ¹Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences/Faculty of Medicine, Universidad de Chile. ²Department of Internal Medicine, Southwestern Medical Center, University of Texas, USA.

415 Area: Molecular Cell Biology

Title: Rab39-vesicular transport is usurped by *Chlamydia trachomatis* to acquire host cell lipids

Authors: Gambarte Tudela, J¹., Capmany, A¹., Quintero, C¹., Goud, B²., Damiani, T¹., ¹IHEM-CONICET, FCM, UNCuyo. ²Curie Institute Paris.

416 Area: Molecular Cell Biology

Title: Purinergic and glutamatergic interplay in the upregulation of astrocyte-neurons signaling in the epileptic hippocampus

Authors: Alvarez, C¹., Morales, J²., Wellman, M²., Fuenzalida, M²., Roncagliolo, M²., Bonansco, C²., ¹Fisiología Universidad de Valparaíso. ²Fisiología Universidad de Valparaíso.

417 Area: Molecular Cell Biology

Title: *In vivo* distribution of candidate receptors for Andes Hantavirus entry into susceptible cells

Authors: García M, P^{1,2}, Salamin, M³, Padula, P⁴, Otth, C³, Tischler N, D^{1,2}, ¹Molecular Virology Laboratory Fundación Ciencia & Vida. ²Facultad de Ciencias Biológicas Universidad Andrés Bello. ³Instituto de Microbiología Clínica Universidad Austral de Chile. ⁴Departamento de Virología Instituto Nacional de Enfermedades Infecciosas, A.N.L.I.S. "Dr. Carlos G. Malbrán" .

418 Area: Molecular Cell Biology

Title: Obesity in pregnancy alters human equilibrative nucleoside transporters 1 expression and intracellular distribution in human umbilical vein endothelial cells

Authors: Sáez, T¹, Pardo, F¹, Salsoso, R¹, Fuenzalida, B^{1,2}, Guzmán-Gutiérrez, E¹, Arroyo, P¹, Leiva, A¹, Sobrevia, L¹, ¹Departamento de Ginecología y Obstetricia, Medicina, Pontificia Universidad Católica de Chile. ²Departamento Biomédico, Ciencias de la Salud, Universidad de Antofagasta.

419 Area: Molecular Cell Biology

Title: Silencing effect of Cathepsin L variant on HCT116 cell proliferation

Authors: Riquelme, O¹, Rivas, F¹, Hinojosa, M¹, Bustamante, S¹, Reyes, C¹, Gutiérrez, S¹, Morin, V¹, ¹Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.

420 Area: Molecular Cell Biology

Title: Prostaglandin E2 disrupts association of caveolin-1 to β -catenin/E-cadherin complex, allowing β -catenin release and promoting malignant progression of colon cancer cells

Authors: Yefi, R¹, Silva, E¹, Niechi, I¹, Cabello, P¹, Huerta, H¹, Quest, A², Tapia, J³, ¹Cell Transformation Lab, Faculty of Medicine, University of Chile. ²ICBM, Cellular Communication Laboratories, CITC, Faculty of Medicine, University of Chile. ³ICBM, Cell Transformation Lab, CITC, Faculty of Medicine, University of Chile.

421 Area: Molecular Cell Biology

Title: Obesity in pregnancy decreases NO synthesis due to inactivation of e-NOs in human umbilical vein endothelium

Authors: Pardo, F¹, Guzman-Gutierrez, E¹, Arroyo, P¹, Saez, T¹, Salsoso, R¹, Leiva, A¹, Sobrevia, L¹, ¹Ginecología y Obstetricia, Facultad de Medicina, Pontificia Universidad Católica de Chile.

422 Area: Molecular Cell Biology

Title: Rheb regulation of p27KIP promotes metabolic stress-induced autophagy in colon cancer cells

Authors: Campos, T¹, Ziehe, J¹, Palma, M¹, Picheira, R¹, Castro, A¹, ¹Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.

423 Area: Molecular Cell Biology

Title: Effects of the blockade of astrocyte to astrocyte signaling on synaptic transmission in hippocampal slices

Authors: Wellmann, M¹, Álvarez-Ferradas, C¹, Morales, J¹, Fuenzalida, M¹, Roncagliolo, M¹, Sáez, J², Bonansco, C¹, ¹Fisiología, Ciencias, Universidad de Valparaíso. ²Ciencias Fisiológicas, Ciencias Biológicas, Pontificia Universidad Católica de Chile.

424 Area: Molecular Cell Biology

Title: Herp protects Hela cells from oxidative stress-induced cell death

Authors: Paredes, F¹., Gatica, D¹., Quiroga, C¹., Parra, V¹., Bravo, R¹., Torrealba, N¹., Navarro, M¹., Pennanen, C¹., Troncoso, R¹., Jaimovich, E²., Lavandero, S^{1,2}., ¹Biología Molecular y Bioquímica, Ciencias Químicas y Farmacéuticas, Universidad de Chile. ²Fisiología y Biofísica, Medicina, Universidad de Chile.

425 Area: Molecular Cell Biology

Title: Insulin and Testosterone regulate glucose uptake by GRP78 in human endometrial stromal cells

Authors: Rosas, C¹., Poblete, C¹., Vera, J²., Romero, C^{1,3}., Lavandero, S⁴., Vega, M^{1,3,4}., ¹Endocrinology and Reproductive Biology Lab, University of Chile Clinical Hospital, University of Chile. ²Department of Physiopathology, Faculty of Biology Ciencias, University of Concepción. ³Obstetric/Gynecology, University of Chile Clinical Hospital, University of Chile. ⁴Center for Molecular Studies of the Cell, Chemical and Pharmaceutical Sciences and Faculty of Medicine, University of Chile.

426 Area: Molecular Cell Biology

Title: Sall2 transcription factor deficiency is associated with decreased p53- dependent apoptosis induced by genotoxic insults

Authors: Escobar, D¹., Morales-Gedda, M¹., Sánchez, M¹., Sanhueza, D¹., Castro, A¹., Pincheira, R¹., ¹Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.

427 Area: Molecular Cell Biology

Title: Nifedipine treatment reduces atp release, cell death and damage in dystrophic MDX muscles

Authors: Valladares, D¹., Altamirano, F¹., Henríquez, C¹., Buvinic, S²., Jaimovich, E¹., ¹Centro de Estudios Moleculares de la Célula (CEMC), Laboratorio de Fisiología Celular del Músculo, Facultad de Medicina, Universidad de Chile. ²Departamento de Ciencias Básicas y Comunitarias, Facultad de Odontología, Universidad de Chile.

428 Area: Molecular Cell Biology

Title: Differential regulation of somatolactin genes in response to endocrine disruptors in pituitary of *Cyprinus carpio*

Authors: Valenzuela, G¹., Perez, A¹., Stolzenbach, M¹., Romero, A²., Figueroa, J¹., Laengst, G³., Kausel, G¹., ¹Instituto de Bioquímica y Microbiología, Ciencias, Universidad Austral de Chile. ²Instituto de Patología Animal, Veterinaria, Universidad Austral de Chile. ³Biochemistry III, Biochemistry and Preclinic, University Regensburg, Germany.

429 Area: Plant Biology

Title: Peach Leaf Curl Disease: study of *Taphrina deformans*-*Prunus persica* interaction

Authors: Svetaz, L²., Godly, C²., Valentini, G¹., Daorden, M¹., Cervigni, G²., Drincovich, M²., Lara, M²., ¹EEA - San Pedro INTA, Argentina. ²CEFOBI-CONICET, FCByF, UNR, Argentina.

430 Area: Plant Biology

Title: *Burkholderia phytofirmans* PsJN primes *Arabidopsis thaliana* plants enhancing saline stress tolerance

Authors: Pinedo, I¹., Ledger, T¹., Timmermann, T¹., Zuñiga, A¹., Donoso, R¹., Poupin, M¹., ¹Laboratorio de Bioingeniería, Facultad de Ingeniería y Ciencias, Universidad Adolfo Ibáñez.

431 Area: Plant Biology

Title: Characterization of the defense response given by the *loci* Run1 and/or Ren1 in *Vitis vinifera* against *Erysiphe necator*

Authors: Schlechter, R¹., Agurto, M¹., Armijo, G¹., Serrano, C¹., Almendra, C¹., Hoffmann, S²., Kozma, P²., Arce-Johnson, P¹., ¹Genética Molecular y Microbiología, Ciencias Biológicas, Pontificia Universidad Católica De Chile. ²Research Institute for Viticulture and Enology University of Pécs.

432 Area: Plant Biology

Title: The transcription factor *ZINC FINGER PROTEIN 7* is a direct target of the histone acetyltransferase *Gcn5* in *Arabidopsis thaliana*

Authors: Aquea, F^{1,2}., Long, J²., ¹Genética Molecular y Microbiología, Ciencias Biológicas, Pontificia Universidad Católica De Chile. ²Molecular, Cell and Developmental Biology Department University of California Los Angeles.

433 Area: Plant Biology

Title: Family affairs: interactions among protein phosphatases with Kelch-like domains in *Arabidopsis thaliana*

Authors: Maselli, G¹., Wolosiuk, R¹., Mora-García, S¹., ¹Fundación Instituto Leloir Fundación Instituto Leloir.

434 Area: Plant Biology

Title: Hormonal control of polar auxin transport during initial grapevine berry development

Authors: Kuhn, N¹., Abello, C¹., Arce, A¹., Arce, P¹., Deluc, L²., ¹Genética Molecular y Microbiología, Ciencias Biológicas, Pontificia Universidad Católica de Chile. ²Horticulture, Agriculture and Life Sciences, Oregon State University.

435 Area: Plant Biology

Title: Loss of function of NADP-malic enzyme 1 results in lower sensitivity to aluminum

Authors: Badia, M¹., Gerrard Wheeler, M¹., Andreo, C¹., Drincovich, M¹., ¹CEFOBI Universidad Nacional de Rosario.

436 Area: Plant Biology

Title: Methyl jasmonate treatment induces changes in fruit ripening by modifying the expression of several ripening genes in *Fragaria chiloensis* fruit

Authors: Concha, C¹., Figueroa, N¹., Poblete, L¹., Oñate, F¹., Schwab, W²., Figueroa, C¹., ¹Manejo de Bosques y Medio Ambiente, Facultad de Ciencias Forestales, Universidad de Concepción. ²Biotechnology of Natural Products Technische Universität München.

437 Area: Plant Biology

Title: Bioactive phenolic compounds of chilean unifloral honeys inherited from floral specific source

Authors: Mejias, E¹., Montenegro, G¹., ¹Departamento de Ciencias Vegetales, Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile.

438 Area: Plant Biology

Title: Role of the E3 sumoligase *SlZ1* in the regulation of salicylic acid biosynthesis in *Arabidopsis*

Authors: Farias, D¹., Salinas, P¹., Holuigue, L¹., ¹Genética Molecular y Microbiología, Ciencias Biológicas, Pontificia Universidad Católica de Chile.

439 Area: Plant Biology

Title: Saline stress induces activation of the early jasmonate responses through a COI1-dependent signaling pathway in *Arabidopsis thaliana* roots

Authors: Miranda, G¹, Pavicic, M¹, Díaz, F¹, Figueroa, P¹, ¹Escuela de Biotecnología, Facultad de Ciencias, Universidad Santo Tomás.

440 Area: Plant Biology

Title: Gene expression of the Homeobox and GRF transcription factor families on early stages of fruit development in *Vitis vinifera*

Authors: Andrade, D¹, Godoy, P¹, Muñoz, M¹, Arce-Johnson, P¹, ¹Genetica Molecular y Microbiología, Ciencias Biológicas, Pontificia Universidad Católica de Chile.

441 Area: Plant Biology

Title: Effect of oxidative stress on the vernalization response using several *Arabidopsis* ecotypes that express the *FRI/FLC* module

Authors: Moraga, F¹, León, G¹, ¹Laboratorio de Desarrollo y Reproducción de Plantas, Centro de Biotecnología Vegetal, Ciencias Biológicas, Universidad Andrés Bello.

442 Area: Plant Biology

Title: Molecular and functional characterization of 4 Pollen Specific Kinase coding genes (*PSK1-4*) from *Arabidopsis thaliana*

Authors: Lucca, N¹, Ibeas, M¹, León, G¹, ¹Centro de Biotecnología Vegetal, Ciencias Biológicas, Universidad Andrés Bello.

443 Area: Plant Biology

Title: High throughput screening of small bioactive molecules that alter polarized root hair growth

Authors: Rodríguez-Furlán, C¹, Raikhel, N², Hicks, G², Norambuena, L¹, ¹Plant Molecular Biology Laboratory, Department of Biology, Faculty of Sciences, Universidad de Chile, Santiago, Chile. ²Institute for Integrative Genome Biology, Center for Plant Cell Biology and Department of Botany and Plant Sciences University of California, Riverside, USA.

444 Area: Plant Biology

Title: The transcription factors WRKY7, 11 and 17 have a role in the transcriptional repression of UPR responding genes during the establishment of immune responses in *Arabidopsis thaliana*

Authors: Blanco, F¹, Arraño, P¹, Galilea, B¹, Moreno, A¹, ¹FONDAP Center for Genome Regulation, Nucleo Milenio en Genómica Funcional de Plantas, Centro de Biotecnología Vegetal, Facultad de Ciencias Biológicas, Universidad Andres Bello.

445 Area: Plant Biology

Title: Endocytosis dynamics synthetically induced leads lateral root initiation most likely changing cell fate in *Arabidopsis thaliana*

Authors: Morales, S¹, Pérez-Henríquez, P¹, Norambuena, L¹, ¹Departamento de Biología, Facultad de Ciencias, Universidad de Chile.

446 Area: Plant Biology

Title: Quorum Sensing of *Burkholderia phytofirmans* (PsJN) plays a role in colonization and plant growth promotion of *Arabidopsis thaliana*

Authors: Zuñiga, A¹, González, B^{2,1}, ¹Bioingeniería, Ingeniería y Ciencias, Universidad Adolfo Ibáñez. ²Ingeniería y Ciencias Universidad Adolfo Ibáñez.

447 Area: Plant Biology

Title: Synthesis and degradation of the phytohormone indole-3-acetic acid in *burkholderia phytofirmans* PsJN, a plantgrowth-promoting β -proteobacterium

Authors: Donoso, R^{1,2}, Zúñiga, A^{2,1}, González, B^{2,1}, ¹Núcleo Milenio de Genómica Funcional de Plantas Pontificia Universidad Católica de Chile. ²Laboratorio de Bioingeniería, Ingeniería y Ciencias, Universidad Adolfo Ibáñez.

448 Area: Protein Structure and Function

Title: Nitration of Peroxiredoxin 2. Structural changes and potential consequences on redox signaling.

Authors: Denicola, A¹, Randall, L¹, Manta, B^{1,2}, Hugo, M¹, Gil, M^{1,2}, Nelson, K³, Batthyány, C², Trujillo, M¹, Poole, L³, ¹Facultad de Medicina, Facultad de Ciencias, Universidad de la República, Uruguay. ²Uruguay Institut Pasteur de Montevideo. ³Department of Biochemistry Wake Forest School of Medicine, NC, USA.

449 Area: Protein Structure and Function

Title: Influence of pH on the conformational changes of hyperthermostable bacterial endo- β -1,4-mannanase and relevance for enzymatic activity

Authors: Moura Da Silva, V¹, Colussi, F², Oliveira Neto, M³, Braz, A¹, Squina, F⁴, **Garcia, W⁵**, ¹CCNH Universidade Federal do ABC. ²UFABC Universidade Federal do ABC. ³Física e Biofísica Universidade Estadual Paulista. ⁴CTBE Laboratório Nacional de Ciência e Tecnologia do Bioetanol. ⁵Centro de Ciencias Naturais e Humanas (CCNH) Universidade Federal do ABC (UFABC).

450 Area: Protein Structure and Function

Title: Crystal structure of GumB, an outer membrane lipoprotein essential for secretion of xanthan

Authors: Jacobs, M¹, Bianco, M¹, Salinas, S¹, Ielpi, L¹, ¹Laboratorio de Genética Bacteriana Fundación Instituto Leloir.

451 Area: Protein Structure and Function

Title: Structural bases of hexacoordination in globins

Authors: Borón, I⁶, Capece, L⁴, Pennacchietti, F², Wetzler, D⁶, Bruno, S¹, Chisari, L⁶, Abbruzzetti, S², Luque, J³, Viappiani, C², Martí, M^{4,5}, Estrín, D⁵, Nadra, A⁶, ¹Department of Biochemistry and Molecular Biology University of Parma. ²Department of Physics, NEST Istituto Nanoscienze-CNR, University of Parma. ³Departament de Fisicoquímica and Institut de Biomedicina (IBUB), Facultat de Farmàcia, Universitat de Barcelona. ⁴Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. ⁵Departamento de Química Inorgánica, Analítica y Química Física, INQUIMAE-CONICET, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. ⁶Departamento de Química Biológica, IQUBICEN-CONICET, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires.

452 Area: Protein Structure and Function

Title: Protein structure of C24 gene from *Bizionia argentinensis* resembles the phage T4 long tail fiber receptor-binding tip: a new role for a recent insertion or a novel marine bacteriophage?

Authors: Pellizza, L¹., Aran, M¹., Klinke, S²., Rinaldi, J²., Sycz, G²., Lanzarotti, E³., Vazquez, S⁴., Lopez, J⁵., Smal, C¹., Paris, G²., Guimaraes, B⁶., Mac Cormack, W⁷., Turjanski, A³., Goldbaum, F²., Cicero, D¹., ¹NMR Laboratory Fundacion Instituto Leloir. ²Molecular Immunology and Microbiology Laboratory Fundacion Instituto Leloir. ³Department of Inorganic, Analytical and Physical Chemistry Universidad de Buenos Aires. ⁴Industrial Microbiology and Biotechnology, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires. ⁵Virology, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires. ⁶SOLEIL Synchrotron France. ⁷IAA Instituto Antartico Argentino.

453 Area: Protein Structure and Function

Title: Intramolecular Dynamics within the N-Cap-SH3-SH2 Regulatory Unit of the c-Abl Tyrosine Kinase Reveal Targeting to the Cellular Membrane

Authors: De Oliveira, G¹., Pereira, E¹., Ferretti, G¹., Valente, A¹., Cordeiro, Y²., Silva, J¹., ¹Medical Biochemistry Institute Federal University of Rio de Janeiro. ²Facultade de Farmácia Federal University of Rio de Janeiro.

454 Area: Protein Structure and Function

Title: Tubulin-Colchicine binding inhibition by Phenstatin derivatives: 1,4-Dihydroxy-2-aroynaphthalenefuran-2-carbaldehyde and 1,4-Dihydroxy-2-aroynaphthalene 1H-pyrrole-2-carbaldehyde

Authors: Nova, E¹., Gutiérrez, E^{2,3}., Benites, J⁴., Villanelo, F¹., Araya, G., Maturana, D⁵., Lagos, R., Monasterio, O., ¹Departamento de Biología, Facultad de Ciencias, Universidad de Chile. ²Departamento de Ciencias Químicas y Farmacéuticas Universidad Arturo Prat. ³Departamento de Ciencias Químicas y Farmacéuticas, Facultad de Farmacia, Universidad Arturo Prat. ⁴Instituto de Etnofarmacología (IDE) Universidad Arturo Prat. ⁵Cono Sur Nanotemper Technologies GmbH.

455 Area: Protein Structure and Function

Title: Identification of amino acid residues conforming an exofacial allosteric site on the human hexose transporter GLUT1

Authors: Lagos, I²., Arce, R²., Oyarzún, R²., Pérez, A²., Ojeda, L²., Salas, M²., Salas-Burgos, A¹., Rivas, C¹., Vera, J¹., Reyes, A²., ¹Departamento de Fisiopatología, Facultad de Ciencias Biológicas, Universidad de Concepción. ²Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile.

456 Area: Protein Structure and Function

Title: All1765, All1769 and All1770 proteins; a putative protein complex in membranes of *Anabaena* sp. PCC7120

Authors: Trigo, C¹., Mandakovic, D²., Gómez, G¹., Adell, M³., Fita, I³., Vázquez, M¹., ¹Departamento de Genética Molecular y Microbiología, Ciencias Biológicas, Pontificia Universidad Católica de Chile. ²Departamento de Genética Molecular y Microbiología, Ciencias Biológicas, Pontificia Universidad Católica de Chile. ³Laboratorio de Cristalografía de proteínas, Instituto de Biología Molecular de Barcelona (IBMB-CSIC), Universidad de Barcelona.

457 Area: Protein Structure and Function

Title: Functional multimeric complexes of the svct2 ascorbic acid transporter

Authors: Gatica, M¹., Sweet, K¹., Muñoz, A¹., Aylwin, C¹., Mendoza, P¹., Peña, E¹., Rivas, C¹., Vera, J¹., ¹Fisiopatología, Ciencias Biológicas, Universidad de Concepción.

458 Area: Protein Structure and Function

Title: Mutations in residue F380 in the S6 transmembrane helix uncouples voltage sensor activation to BK channel gating

Authors: Carrasquel-Ursulaez, W^{1,2}, Contreras, G², Sepúlveda, R^{3,4}, Aguayo, D³, González-Nilo, F³, González, C¹, Latorre, R¹, ¹Centro Interdisciplinario de Neurociencias de Valparaíso Universidad de Valparaíso. ²Doctorado en Ciencias mención Neurociencias Universidad de Valparaíso. ³Center for Bioinformatics and Integrative Biology Universidad Andrés Bello. ⁴Doctorado en Biotecnología Universidad Andrés Bello.

459 Area: Protein Structure and Function

Title: Cold-adapted enzyme from the ADP-dependent sugar kinase family: characterization and comparison with a hiperthermophilic homologue

Authors: Zamora, R¹, Castro-Fernandez, V¹, Rivas-Pardo, J¹, Guixé, V¹, ¹Departamento de Biología, Facultad de Ciencias, Universidad de Chile.

460 Area: Protein Structure and Function

Title: Determination of the stoichiometry of interacting 5-HT_{3A} and P2X₂ receptors by atomic force microscopy

Authors: Soto, P¹, Naulin, P¹, Barrera, N¹, ¹Ciencias Fisiológicas, Ciencias Biológicas, Pontificia Universidad Católica de Chile.

461 Area: Protein Structure and Function

Title: Structural studies of the connexin 26/30 hemichannels via atomic force microscopy imaging

Authors: Naulin P, A¹, Liu, Y², Harris A, L², Contreras J, E², Barrera N, P¹, ¹Department of Physiology, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile. ²Department of Pharmacology & Physiology, New Jersey Medical School, Rutgers University.

462 Area: Protein Structure and Function

Title: Molecular determinants of the functional and structural properties of the ascorbic acid transporter-2, SVCT2

Authors: Sweet, K¹, Gatica, M¹, Escobar, E¹, Peña, E¹, Mendoza, P¹, Medina, M¹, Salas, A², Rivas, C¹, Vera, J¹, ¹Fisiopatología, Ciencias Biológicas, Universidad de Concepción. ²Farmacología, Ciencias Biológicas, Universidad de Concepción.

463 Area: Protein Structure and Function

Title: *In vivo* counting of single flagellar motor protein, FliM, using photoactivated localization microscopy

Authors: Shin, J¹, Lee, S^{3,2}, Lee, A², Diaz-Celis, C³, Bustamante, C^{3,4,5,2,1}, ¹Howard Hughes Medical Institute University of California, Berkeley. ²Department of Physics University of California, Berkeley. ³California Institute for Quantitative Biosciences University of California, Berkeley. ⁴Department of Molecular and Cell Biology University of California, Berkeley. ⁵Department of Chemistry University of California, Berkeley.

464 Area: Signaling

Title: Thy-1-induced migration requires β 3 Integrin expression, ATP release and P2X7R activation in TNF α - treated primary astrocytes

Authors: Alvarez, A^{1,2}, Kong, M^{1,2}, Cardenas, A^{2,1}, Rojas-Mancilla, E^{2,3}, Herrera-Marschitz, M^{2,3}, Quest, A¹, Leyton, L^{1,2}, ¹Laboratorio de Comunicaciones Celulares, Centro de Estudios Moleculares de la Célula, Facultad de Medicina, Universidad de Chile. ²Instituto de Neurociencia Biomédica (BNI) Universidad de Chile. ³Programa de Farmacología Molecular y Clínica, ICBM-Facultad de Medicina, Universidad de Chile.

465 Area: Signaling

Title: Liver X receptor: effect of two cholestenoic acid analogues on mouse mammary epithelial cells

Authors: Grinman, D¹., Dansey, M¹., Navalesi, D²., Samaja, G^{3,4}., Alvarez, L^{3,4}., Veleiro, A^{3,4}., Burton, G^{3,4}., Pecci, A^{2,1}., ¹IFIByNE, UBA, CONICET. ²Dpto. de Química Biológica, Ciencias Exactas y Naturales, Universidad de Buenos Aires. ³Dpto. de Química Orgánica, Ciencias Exactas y Naturales, Universidad de Buenos Aires. ⁴UMYFOR, UBA, CONICET.

466 Area: Signaling

Title: Role of phospholipase D pathway in the inflammatory response of the retinal pigment epithelium cells

Authors: Mateos, M¹., Kamerbeek, C¹., Giusto, N¹., **Salvador, G¹**., ¹Biochemistry, INIBIBB-CONICET, Universidad Nacional del Sur.

467 Area: Signaling

Title: A novel auto-phosphorylation mechanism of PKA activity regulation in *Saccharomyces cerevisiae*

Authors: Solari, C^{1,2}., Camarano, A^{1,2}., Moreno, S^{1,2}., **Portela, P²**., ¹Química Biológica, Facultad de Ciencias Exactas y Naturales, Buenos Aires. ²Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Buenos Aires.

468 Area: Signaling

Title: Bag3 regulates LC3 expression to increase basal autophagy in HeLa cells

Authors: **Rodríguez, A¹**., Peña, D¹., Troncoso, R¹., Chiong, M¹., Behl, C²., Lavandero, S^{1,3}., ¹Centro Estudios Moleculares de la Célula, Facultad de Química y Farmacia, Universidad de Chile. ²Institut für Pathobiochemie Johannes Gutenberg Universität, Mainz, Germany. ³Department of Internal Medicine University of Texas Southwestern Medical Center, Dallas, Texas, USA..

469 Area: Signaling

Title: Ryanodine receptor-mediated Ca²⁺ signals and ros are key components of synaptic plasticity promoted by the neurotrophin BDNF

Authors: Adasme, T¹., Paula-Lima, A²., Hidalgo, C³., ¹BNI & CEMC, Departamento de Fisiología y Biofísica, Facultad de Medicina, Universidad de Chile. ²Departamento de Ciencias Básicas y Comunitarias, Facultad de Odontología, Universidad de Chile. ³CEMC, BNI, ICBM, Departamento de Fisiología y Biofísica, Facultad de Medicina, Universidad de Chile.

470 Area: Signaling

Title: Role of intercellular adhesion molecule-1 (ICAM-1) in cardioprotective actions of insulin

Authors: **González, V¹**., Díaz, A¹., Gómez, M¹., Vivar, R¹., García, L¹., ¹Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.

471 Area: Signaling

Title: The TRPM4 channel regulates the Epithelial-Mesenchymal transition in prostate cancer cells

Authors: **Sagredo, A⁵**., Sagredo, E⁵., Baez, P⁵., Luco, C⁵., Simon, F¹., Tapia, J^{2,6}., Marcelain, K^{3,6}., Stutzin, A^{5,4}., Armisen, R^{5,6}., ¹Departamento de Ciencias Biológicas, Facultad de Ciencias Biológicas, Universidad Andres Bello. ²Programa de Biología Celular y Molecular, ICBM, Facultad de Medicina, Universidad de Chile. ³Programa de Genética Humana, ICBM, Facultad de Medicina, Universidad de Chile. ⁴Centro de Estudios Moleculares de la Célula, Facultad de Medicina, Universidad de Chile. ⁵Programa de Fisiopatología, ICBM, Facultad de Medicina, Universidad de Chile. ⁶U-CANCER: Red de Medicina Traslacional en Cáncer, Facultad de Medicina, Universidad de Chile.

472 Area: Signaling

Title: Angiotensin-(1-9)/AT2 receptor signaling pathway a novel regulator of cardiomyocyte mitochondrial dynamics

Authors: Rivera Mejías, P¹, Sotomayor Flores, C¹, Morales, P¹, Lopez Crisosto, C¹, Jalil, J², Ocaranza, M², Chiong, M¹, Parra, V^{1,3}, Lavandero, S^{1,3}, ¹Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences & Faculty of Medicine, University of Chile. ²Cardiovascular Division, School of Medicine, Pontifical Catholic University of Chile. ³Department of Internal Medicine University of Texas Southwestern Medical Center.

473 Area: Signaling

Title: Testosterone increases PGC-1 α activity in rat cardiomyocytes inducing a PPAR- α mediated response

Authors: Basualto-Alarcon, C¹, Duran, J¹, Venegas, N¹, Silva, P¹, Estrada, M¹, Programa de ¹Fisiología y Biofísica, Facultad de Medicina, Universidad de Chile.

474 Area: Signaling

Title: Angiotensin-(1-9) modulates Akt via the angiotensin II receptor type 2 (AT2R)

Authors: Barrientos, V¹, Alzamora, R¹, Chiong, M², Ocaranza, M³, Michea, L^{1,4}, ¹Fisiología y Biofísica/ICBM, Medicina, Universidad de Chile. ²Center for Molecular Studies of the Cell, Ciencias Químicas y Farmacéuticas, Universidad de Chile. ³Departamento de Enfermedades Cardiovasculares, Medicina, Pontificia Universidad Católica de Chile. ⁴Millennium Institute on Immunology and Immunotherapy, Facultad de Medicina, Universidad de Chile.

475 Area: Protein Structure and Function

Title: DNA glycosylase and AP endonucleases. presence, activity and involvement in *T. cruzi* DNA repair and infection persistence **Authors:** Sepúlveda, S., Valenzuela, L., Ponce, I., Bahamondes, P., Sierra, S., Kemmerling, U., Cabrera, G., Galanti, G. Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Santiago, Chile.

476 Area: Protein Structure and Function

Title: FLAP endonuclease. presence and activity in cell proliferation and DNA repair in *Trypanosoma cruzi*

Authors: Ponce, I., Sepúlveda, S., Valenzuela, L., Kemmerling, U., Cabrera, G., Galanti, N. Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Santiago, Chile.

477 Area: Microbiology

Title: Gene polymorphism of Mb1951c ORF, in *Mycobacterium bovis* field isolates

Authors: Díaz, C¹, Marcos Mancilla¹, Alvaro Figueroa¹ and Ana María Zárraga¹, ¹Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile.

XII PABMB CONGRESS
XXXVI ANNUAL MEETING SOCIEDAD DE BIOQUÍMICA Y BIOLOGÍA MOLECULAR D CHILE
XLIX ANNUAL MEETING SOCIEDAD ARGENTINA DE INVESTIGACIÓN BIOQUÍMICA Y BIOLOGÍA MOLECULAR
LVI ANNUAL MEETING SOCIEDAD DE BIOLOGÍA DE CHILE
4th LATIN AMERICAN PROTEIN SOCIETY MEETING

Puerto Varas • Chile • Noviembre 9 - 14 • 2013

LECTURES

1. Caspases, Paracaspase, and the Space Between Death and Survival

Hachmann, J¹, VanRaam, B¹, Salvensen, G¹, ¹Institute, La Jolla, California, USA Sanford-Burnham Medical Research.

Apoptosis is said to be dependent on caspases. But it has become pretty clear now that supposedly pro-apoptotic caspases can, paradoxically, also trigger non-apoptotic events. Moreover, the universe of caspase homologs includes proteases with distant relationship to caspases, which program entirely different cell signaling pathways. This talk will focus on the biochemical mechanisms that allow caspase-8 and its distant homolog (paracaspase) to program apoptotic cell death *versus* an opposing pathway to cell survival and clonal expansion.

2. The Evolution of the Universe: From the Big-Bang to Life

Ruiz, M¹, ¹Departamento de Astronomía Universidad de Chile.

Our generation is the first, in human evolution, to know our history, the history of the Universe. Thanks to new instrumentation dedicated to astronomical observations we have gathered the necessary data to describe the "geography" and "history" of the Universe starting with the Big-Bang, followed by the creation of the all other chemical elements leading to the existence of life. I will describe the evolution of the Universe in a sequence of events; star formation, star death, formation of planetary systems, the building blocks of life, search for extraterrestrial life.

3. Chile and its diverse nature: Geography, ecosystems, flora and fauna

Jaksic, F¹, ¹Departamento de Ecología Pontificia Universidad Católica de Chile.

I provide an overview of the geographic uniqueness of Chile, a long (4200 km) and narrow (177 km on average) country on the western fringe of South America. I describe the latitudinal (18° to 56° South), and altitudinal (0 to 6,960 m elevation) gradients and their consequences for the generation of diverse ecosystems, flora, and fauna. From North to South I cover the Atacama Desert, the High Andean plateau, the Norte Chico Desert, the Mediterranean Scrub, Coastal and Andean ranges, the Valdivian forest, the Nothofagus forest, the Patagonian Steppe and glaciers, and the Marine Coastal areas. For each of these ecosystems I present the more characteristic elements of their flora and fauna.

4. The mitochondria-associated membrane as a signaling hub: redox meets Rabs

Simmen, T¹, ¹Department of Cell Biology University of Alberta.

Our laboratory focuses on proteins that regulate the calcium-mediated crosstalk between the endoplasmic reticulum (ER) and mitochondria. ER-mitochondria calcium flux is critical for mitochondria respiration and ATP production, but also determines the speed of apoptosis during oxidative stress. Therefore, ER-mitochondria calcium flux is a major determinant of cellular growth and survival. The bidirectional, intracellular calcium flux occurs on the mitochondria-associated membrane (MAM). Here, a group of ER chaperones and cytosolic trafficking molecules dictates the readout of ER-mitochondria calcium flux. For instance, calnexin confers cells with the ability to signal ER stress to mitochondria. Another example is Rab32, whose expression is tied to ER stress. This small GTPase not only determines MAM composition, but also mitochondrial structure via the interaction with the mitochondrial fission machinery on the MAM.

5. Coordinated control of gene expression and cell cycle progression by stress-activated protein kinases (SAPKs).

Posas, F¹,¹Cell Signaling Unit, Departament de Ciències Experimentals i de la Salut Universitat Pompeu Fabra (UPF).

Exposure of cells to increases in extracellular osmolarity results in the activation of the Hog1/p38 family of stress-activated protein kinases (SAPKs). Activation of these SAPKs is required to generate a set of osmoadaptive responses essential to survive under high osmolarity highly conserved among eukaryotic cells. Osmostress induces a large number of genes, which indicates the necessity to regulate several aspects of the cell physiology for proper cellular adaptation. In yeast, the Hog1 SAPK controls several steps in mRNA biogenesis from transcription initiation to mRNA export and translation. In response to stress, the SAPK also modulates cell cycle progression. Both in yeast and mammals, p38/Hog1 control cell cycle by delaying different phases of cell cycle by acting on several components of the cell cycle core machinery. In fact, the processes of transcription and cell cycle are strongly coordinated to maximize cell survival upon stress.

6. The Art of War; Viral subversion of the host protein synthesis machinery.

López-Lastra, M¹,¹Laboratorio de Virología Molecular, Instituto Milenio de Inmunología e Inmunoterapia, Centro de Investigaciones Médicas, Escuela de Medicina, Pontificia Universidad Católica De Chile.

Viruses are obligate intracellular parasites and depend on cells for their replication. They have however evolved mechanisms to ensure that their replication can be achieved in an efficient manner. The expression of viral proteins is frequently subject to regulation at the level of protein synthesis. The dominant target of such control is the initiation step of protein synthesis. Indeed, during the early stages of infection, viral mRNAs must compete with their host counterparts for the protein synthetic machinery that mediates translation of both viral and cellular mRNAs. To circumvent this competition viruses use diverse strategies so that ribosomes can be recruited selectively to their mRNAs. In some instances these unorthodox strategies allow the viral mRNAs to preferentially recruit the host translational machinery even though outnumbered by cellular mRNAs. Some of the strategies used by viruses to ensure efficient translation initiation of their mRNAs will be explored and discussed in the context of pathogens that are relevant for human health.

7. Dynamics and Chemical Ecology of Microbial Interspecies Interactions

Kolter, R¹,¹Microbiology&Immunobiology, Medical School, Harvard University. (Sponsored by Rosalba Lagos)

In nature microbes almost invariably live in the context of multi-species communities. While microbial ecologists have studied the composition of such communities, molecular geneticists have largely studied microbial processes in the context of pure cultures in the laboratory. To bridge the gap between these two general approaches, we have been studying artificial communities in terms of how microbial development is affected and how secreted products mediate these effects. We have begun to apply the latest technological advances of mass spectrometry, including nano-scale desorption/ionization mass spectrometry and imaging mass spectrometry, to open new windows in order view the chemical interactions taking place between different species of bacteria. Our latest findings suggest that interspecies interactions trigger massive system-wide changes in the secreted metabolome of bacteria and that this secreted metabolome does indeed affect the community. Our results illustrate that the interactions underlying bacterial communities is surprisingly vast and dynamic.

8. Protein folding stress in neurodegenerative diseases: a look into the ER

Hetz, C¹,¹Programa de Biología Celular, Facultad de Medicina, Universidad De Chile. (Sponsored by Support From FONDECYT No. 1100176, 15010006, FONDEF D1111007 Millennium Institute No. P09-015-F, Muscular Dystrophy Association, Alzheimer Association, And ALS Therapy Alliance.)

Most neurodegenerative diseases share a common neuropathology, primarily featuring the presence of abnormal protein inclusions containing specific misfolded proteins. Recent evidence indicates that alteration in organelle function is a common pathological feature of protein misfolding disorders. The endoplasmic reticulum (ER) is an essential compartment for protein folding, maturation, and secretion. Signs of ER stress have been extensively described in most experimental models of neurological disorders. To cope with ER stress, cells activate an integrated signaling response termed the Unfolded Protein Response (UPR), which aims to reestablish homeostasis through transcriptional upregulation of genes involved in protein folding, quality control and degradation pathways. Here I overview our efforts to assess the role of ER stress in protein misfolding disorders, and discuss possible strategies to target the UPR with therapeutic benefits. In addition I will summarize recent evidence from our group indicating a possible contribution of ER stress to the etiology of ALS, where specific mutations in ER chaperones may underlay the pathology.

9. Brain GLYCOGEN METABOLISM and neurodegeneration

Duran, J^{1,2}, Vallès-Ortega, J^{1,4}, Sinadinos, C¹, Saez, I^{1,4}, Tevy, F¹, Milán, M^{1,3}, Guinovart, J^{4,1,2,1} - Institute for Research in Biomedicine (IRB Barcelona).²- Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM).³- Institució Catalana de Recerca i Estudis Avançats (ICREA).⁴Department of Biochemistry and Molecular Biology University of Barcelona.

The metabolic activity of the brain is largely supported by externally provided glucose. However, some glucose is stored locally in the form of glycogen, mainly in astrocytes, which can degrade this polymer to provide neurons with energy-rich substrates. Strikingly, neurons accumulate large glycogen-like deposits in a variety of diseases, such as Lafora disease (LD) and Adult polyglucosan body disease (APBD), but also during normal aging in the form of *corpora amylacea* (CA). We have identified the molecular mechanism that links mutations found in LD patients with glycogen accumulation and disease. Furthermore, we modeled LD in mice to observe the deleterious effects of polyglucosan accumulation. Since LD mutations impinge on pathways other than glycogen metabolism (i.e. autophagy and ER-stress), we used mice and flies to test whether enhanced accumulation of glycogen causes neuronal death *per se*. Finally, we studied the impact of reduced glycogen synthesis in model organisms. Our results confirm the hypothesis that abnormal accumulation of glycogen in neurons contributes to disease- and age-related neurodegeneration.

10. Mechanisms of genomic stability during cell division

Sunkel, C¹,¹IBMC, Instituto de Biologia Molecular e Celular Universidade do Porto.

Maintenance of genomic stability during cell division relies to a large extent on the spindle assembly checkpoint (SAC). The SAC monitors kinetochore-microtubule attachment, preventing APC/C-mediated mitotic exit until proper microtubule-kinetochore attachment is achieved. In the absence of microtubule attachment proteins involved in SAC signalling localize to kinetochores and generate a "wait anaphase" signal that prevents mitotic exit. Recently we have shown that a cascade of protein kinases including Polo, Aurora B, Mps1 and BubR1 play a major role in the production of an effective APC/C inhibitor. Interestingly, SAC genes also appear to be involved in other aspects of genomic stability when analysed in specific tissues within a developing organism. Down-regulating Bub3, BubR1 or Mad2 within the developing wing imaginal cells causes cell death. However, when apoptosis is prevented these mutant cells transform and effectively become tumorigenic with high levels of aneuploidy, cell autonomous growth and extensive proliferative potential. Importantly, SAC genes prevent tumorigenesis in these cells independently of their kinetochore localization suggesting that they might function as classical tumour suppressors. Our recent work in these two areas will be presented.

11. Beauty and Fitness for Purpose: Architecture of proteins, the Building Blocks of life

Huber, R^{1,2,3},¹Cardiff School of Biosciences Cardiff University.² Max-Planck-Institut für Biochemie.³Zentrum für Medizinische Biotechnologie Universität Duisburg-Essen. Atomic views of protein structures are determined with increasing pace in the last twenty years by a rapid development of methods and instrumentation of protein crystallography, electron microscopy, and nuclear magnetic resonance, allowing the determination of very large and complex protein structures. These structures document the beauty and unlimited versatility of the proteins' architecture, but reveal also unexpected relationships, allowing views on biological evolution far back in time. The structures are a basis for understanding the proteins' binding specificities and catalytic properties (chemistry), their spectral and electron transfer properties (physics), and their roles in physiological systems (biology and medicine). They allow design and development of specific ligands of target proteins opening novel strategies for therapeutic intervention and development of new medicaments and for plant protection. The basis of the studies described and of molecular biology in general is X-ray diffraction of crystals, discovered by Laue in 1912 in München. We celebrated the Laue Centennial in the year 2012. With a few slides I shall commemorate history and development of Laue's discovery, which revolutionized many fields of science.

12. Molecular Origami: Protein folding and misfolding in the eukaryotic cytosol

Frydman, J¹,¹Biology Department Stanford University.

Achieving correct protein folding and quality control is essential for normal cellular function. The accumulation of misfolded proteins is emerging as central to a wide range of disease states, including many neurodegenerative disorders such as Huntington's and Prion Disease. Molecular chaperones are a diverse family of enzymes that monitor and maintain all aspects of protein homeostasis. The role of chaperones in these diverse functions will be discussed. Chaperones assist the folding of newly translated and stress-denatured proteins, as well as affects protein quality control. Systems approaches identified a chaperone network linked to the protein synthesis apparatus assists protein biogenesis. The emergence of this translation-linked chaperone network likely underlies the elaborate co-translational folding process necessary for the evolution of larger multidomain proteins characteristic of eukaryotic cells. A stress-inducible chaperone network protects cells from environmental stress and assists quality control. These chaperones also communicate with the ubiquitin-proteasome pathway to clear misfolded proteins from the cell. Protein quality control in the eukaryotic cytosol relies on the sequestration of misfolded cytosolic proteins in specific quality control compartments. Our studies of chaperone function provide a framework to understand the link between protein misfolding and human disease.

13. Why and How Bacteria Control Membrane Lipid Biosynthesis

De Mendoza, D^{1,1},¹Institute of Molecular and Cellular Biology of Rosario (CONICET) and Department of Microbiology University of Rosario.

A crucial but often overlooked aspect of cell biogenesis and survival is the need for precise control of the biosynthesis of membrane lipids. Fatty acids and their derivatives play essential roles in all living organisms as components of membranes and source of metabolic energy. Biosynthesis of these compounds involve repeated cycles of condensation, reduction and dehydration of carbon-carbon linkages, which are carried out by a single multifunctional polypeptide (type I systems) in higher eukaryotes and by dissociable enzymes (type II systems) in bacterial cells, plant chloroplasts and malaria parasites. Yet despite the complexity of these biosynthetic pathways, biological membranes maintain stable compositions that are characteristic for different organisms, tissues and intracellular organelles. Nevertheless, the precise homeostatic mechanisms maintaining the concentration of lipids at particular levels are largely unknown.

In the lecture an overview will be presented of our research on the control that Gram-positive bacteria cells exert over the biosynthesis of their membrane lipids. This will be followed by a discussion of the role of malonyl-CoA, a universal intermediate in fatty acid biosynthesis, as a ligand of the global transcriptional repressor FapR that controls membrane lipid homeostasis in a wide range of Gram-positive bacteria.

14. Could we fully understand in a quantitative and predictive manner a living system?

Serrano, L^{1,2,3,1},¹Centre for Genomic Regulation (CRG) EMBL/CRG Systems Biology Research Unit.²- Universitat Pompeu Fabra (UPF).³- Institució Catalana de Recerca i Estudis Avançats (ICREA).

One of the main aims in Systems Biology is the full quantitative understanding of a living system. Because of its small genome size (689 coding genes) and a large repertoire of existing resources, *M. pneumoniae* M129, the causative agent of walking pneumonia and other extra-pulmonary pathologies in humans is an ideal model this purpose. Over the past few years we have assembled what is probably the most complete data set acquired for an organism: comprehensive datasets exist for its transcriptome, metabolome, proteome, phospho- and lys-acetyl-proteome, and DNA methylome as well as a full analysis of gene essentiality. Moreover we have derived 1,619 condition-dependent and consistently generated "omics" datasets for the model bacterium *M. pneumoniae*. Nevertheless and despite this extensive -omics dataset we still don't understand all regulatory layers that determine the abundance of proteins and their complex interplay which characterize the physiological state of a cell. The reason being that dissecting putative interactions among these regulatory layers and quantifying their contributions has been proven to be extremely difficult. Here we will present a global vision of the data acquired, our efforts for integrating it in to an unified model and the hurdles that we have encountered in achieving our final goal of getting the first full understanding of a cell.

15. Role of mitochondrial fusion proteins on metabolism and implications in pathology

Zorzano, A¹,¹IRB Barcelona, CIBERDEM Universitat de Barcelona.

Mitochondrial dynamics includes the movement of mitochondria along the cytoskeleton, the regulation of mitochondrial architecture (morphology and distribution), and connectivity mediated by tethering and fusion/fission events. Mitochondrial fusion is catalyzed by Mfn1, Mfn2 and OPA1 in mammalian cells whereas mitochondrial fission is operated by Drp1, Fis1 and Mff proteins. The relevance of these events in mitochondrial and cell physiology has been partially unraveled after the identification of the genes responsible for mitochondrial fusion and fission, and after the identification that mutations in two mitochondrial fusion genes (Mfn2 and OPA1) cause prevalent neurodegenerative diseases (Charcot-Marie Tooth type 2A and Kjer disease). Recent data from our laboratory indicate that alterations in the activity of the mitochondrial fusion protein Mfn2, the metalloprotease OMA1 or the mitochondrial fission protein Drp1 have a deep impact on metabolism in cells and tissues. In addition, our data support the view that at least Mfn2 regulates insulin signaling in tissues, and that pathophysiological conditions such as human type 2 diabetes or obesity are characterized by deficient Mfn2 expression. During the talk, the implications of mitochondrial fusion proteins on metabolic regulation, and on the pathophysiology of major metabolic disorders will be discussed. In addition, the mechanisms that link mitochondrial dynamics proteins and metabolism will also be analyzed.

16. Of nature and nurture: connecting RNA biology and metabolism

Hentze, M¹,¹Heidelberg, Germany European Molecular Biology Laboratory (EMBL).

RNA-binding proteins (RBPs) orchestrate virtually all aspects of RNA biology. I plan to speak about the identification of "all" mRNA binding proteins (the "mRNA interactomes") of mammalian cells and yeast using a new system-wide approach that we refer to as "Interactome capture" (1,2). We have identified hundreds of new RBPs that are significantly enriched over negative controls shown by analysis of independent repeat experiments. The described method is broadly applicable to study mRNA interactome composition and dynamics in varied biological settings. These data and unpublished work from hepatocytic Huh-7 cells and yeast shed new light on diverse aspects of RNA biology, including RBPs in disease and novel RNA-binding architectures (3). We also identify enzymes of intermediary metabolism that moonlight as RBPs in vivo, implicating these in the recently proposed REM (RNA/enzyme/metabolite) networks for the coordination of cell metabolism and gene expression (4). (1) Castello et al., Cell 149, 1393-1406, 2012 (2) Castello et al., Nature Protoc. 8, 491-500, 2013 (3) Castello et al., Trends i Genet. 29, 318-327, 2013 (4) Hentze and Preiss, TiBS 35, 423-426, 2010

17. Cancer and Prions: Amyloidogenic and Prionoid Properties of Mutant p53

Silva, J¹, ¹National Institute of Science and Technology for Structural Biology and Bioimaging, Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro.

p53, the tumor suppressor protein, loses its function in more than 50% of all human cancers. To evaluate the role of aggregation in cancer, we investigated whether wild-type (WT) p53 and the *hot-spot* mutant R248Q could aggregate as amyloids. The central domains (p53C) of both constructs aggregated into a mixture of oligomers and fibrils. R248Q had a greater tendency to aggregate than WT p53. Full-length p53 aggregated into amyloid-like species that bound thioflavin T. The amyloid nature of the aggregates was evaluated using X-ray diffraction, electron microscopy, FTIR, cell viability assay, ThT binding and anti-amyloid immunoassay. The X-ray diffraction pattern of the fibrillar aggregates was consistent with the typical conformation of cross β -sheet amyloid fibers. A seed of R248Q p53C amyloid oligomers and fibrils resulted in acceleration of the aggregation of WT p53C, a behavior typical of a prion. The R248Q mutant co-localized with amyloid-like species in breast cancer samples, which further supported its prion-like effect. A tumor cell line containing mutant p53 also revealed massive aggregation of p53 in the nucleus. We conclude that aggregation of p53 into a mixture of oligomers and fibrils sequesters the native protein into an inactive conformation that is typical of a prionoid. This prion-like behavior of oncogenic p53 mutants provides an explanation for the negative dominance effect observed in cancer.

18. Biointeractomic Scaffold Hovering on Cytochrome c under Programmed Cell Death

Díaz-Moreno, I¹, Martínez-Fabregas, J¹, González-Arzola, K¹, Cruz-Gallardo, I¹, Moreno-Beltrán, B¹, Guerra-Castellano, A¹, Elena-Real, C¹, García-Heredia, J¹, Rivero-Rodríguez, F¹, Díaz-Quintana, A¹, De La Rosa, M¹, ¹Institute of Plant Biochemistry and Photosynthesis, CSIC, University of Seville - CSIC.

Cytochrome *c* (Cc), a small soluble hemeprotein, is highly conserved along evolution. In mammals, Cc plays a dual role in cell life and death: Under homeostatic conditions, Cc is retained inside the mitochondria and acts as an electron shuttle in the electron transfer respiratory chain. Upon apoptotic stimuli, however, Cc is released into the cytoplasm so as to serve as an essential key factor by binding to Apaf-1 and further assembling the apoptosome, the machinery responsible for activation of caspases. The mitochondria-to-cytoplasm Cc translocation has been long considered as a random event, although it is an evolutionarily conserved process among organisms. This finding, along with the fact that apoptosis remains active in Apaf-1 knockout mutants but not in Cc knockout mutants, lead one to wonder if cytoplasmic Cc could play other possible signaling functions.

To better understand the role of Cc in the onset of apoptosis and to harmonize the different phenotypes of Apaf-1 and Cc knockout mutants, we have recently proposed that Cc interacts, in apoptotic cells, with an ample set of pro-survival and anti-apoptotic proteins, thereby interfering with cell survival signaling and unlocking programmed cell death. Within a complex regulatory network, Cc would avoid the spatial and temporal co-existence of pro- and anti-apoptotic signals, so letting the apoptotic program progress rapidly and efficiently. Furthermore, the post-transcriptional regulation of the mRNA encoding for Cc, along with the post-translational modifications of the hemeprotein by phosphorylation or nitration, open a new way to understand the Cc-dependent activation and progression of programmed cell death.

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19. Peroxynitrite, a stealthy biological oxidant

Radi, R^{1,2,1}, Bioquímica Universidad de la República.²Center for Free Radical and Biomedical Research Universidad de la República.

Peroxynitrite is a short-lived peroxide formed from the diffusion-controlled reaction of nitric oxide ($\cdot\text{NO}$) and superoxide radicals ($\text{O}_2^{\cdot-}$). With a $\text{pK}_a \sim 6.8$, both peroxynitrite anion (ONOO^-) and peroxynitrous acid (ONOOH) coexist biologically. Peroxynitrite can directly promote oxidations in biomolecules and also evolve to oxidizing free radical intermediates such as nitrogen dioxide ($\cdot\text{NO}_2$) and carbonate radicals ($\text{CO}_3^{\cdot-}$) after reaction of ONOO^- with CO_2 . While thiol oxidation occurs by a bimolecular reaction involving a two-electron redox process, protein tyrosine nitration depends on peroxynitrite-derived radicals and the intermediacy of tyrosyl radical. Excess peroxynitrite disrupts mitochondrial function and triggers cell death *via* oxidation and nitration reactions. Peroxynitrite usually represents an endogenous toxicant in a variety of disease states but it can also act as a cytotoxic effector against invading pathogens. The oxidizing capacity of peroxynitrite is modulated by potent endogenous antioxidant mechanisms, most notably the enzymes of peroxiredoxin family that decompose ONOOH *via* a fast reaction with active site thiols. The toxicity of peroxynitrite can be neutralized pharmacologically by a series of synthetic compounds, including the Mn-porphyrins. In the presentation, I will analyze central aspects on the biological chemistry of peroxynitrite and how its actions can be modulated *in vitro* and *in vivo*.

20. Recent Results on Mechanochemical Conversion in a Viral Ring ATPase

Bustamante, C^{1,1}, Physics, Chemistry, Molecular and Cell Biology University of California.

As part of their infection cycle, many viruses must package their newly replicated genomes inside a protein capsid. Bacteriophage phi29 packages its 6.6 mm long double-stranded DNA using a pentameric ring nano 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 bacteriophage phi29 is ideal to investigate these questions and is a remarkable machine that must overcome entropic, electrostatic, and DNA bending energies to package its genome to near-crystalline density inside the capsid. Using optical tweezers, we find that this motor can work against loads of up to ~ 55 piconewtons on average, making it one of the strongest molecular motors ever reported. We establish the force-velocity relationship of the motor. Interestingly, the packaging rate decreases as the prohead fills, indicating that an internal pressure builds up due to DNA compression attaining the value of ~ 6 MegaPascals at the end of the packaging. This pressure, we show, is used as part of the mechanism of DNA injection in the next infection cycle. We have used high-resolution optical tweezers to characterize the steps and intersubunit coordination of the pentameric ring ATPase responsible for DNA packaging in bacteriophage Phi29. By using non-hydrolyzable ATP analogs and stabilizers of the ADP bound to the motor, we establish where DNA binding, hydrolysis, and phosphate and ADP release occur relative to translocation. We show that while only 4 of the subunits translocate DNA, all 5 bind and hydrolyze ATP, suggesting that the fifth subunit fulfills a regulatory function. Finally, we show that the motor not only can generate force but also torque. We characterize the role played by the special subunit in this process and identify this the symmetry-breaking mechanism. These results represent the most complete studies done to date on these widely distributed class of ring nano motors. We have also recently dissected the throttle control mechanism of the motor at high capsid filling.

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Puerto Varas • Chile • Noviembre 9 - 14 • 2013

SYMPOSIA

21. Krieger, N¹., Mitchell, D².,¹Departamento de Química, Setor de Ciências Exatas, Universidade Federal do Paraná.²Departamento de Bioquímica e Biologia Molecular, Setor de Ciências Biológicas, Universidade Federal do Paraná. (Sponsored by Athel Cornish-Bowden)

22. Origins and development of enzyme kinetics: Henri, Michaelis, Menten Cornish-Bowden, A¹.,¹Bioénergétique et Ingénierie des Protéines CNRS - Aix-Marseille Université. The foundations of enzyme kinetics were laid by Leonor Michaelis and Maud Menten 100 years ago. Although they built on studies of various earlier authors, most notably Adrian Brown and Victor Henri, they were the first to construct a theory on the basis of properly designed and executed experiments, the first to appreciate the importance of proper attention to pH, and the first to use an analytical approach that remains the standard for experiments today.

23. *Manipulating the protein energy landscape* Marqusee, S¹.,¹Department of Molecular & Cell Biology, Institute for Quantitative Biosciences QB3, University of California, Berkeley. Decoding the information in the primary sequence of a protein is one of the most fundamental challenges in modern biology. A protein's sequence encodes more than just its three-dimensional native structure; it encodes the entire energy landscape – an ensemble of conformations whose energetics and dynamics are finely tuned. I will present recent results in my lab aimed at probing the high-energy fluctuations on the energy landscape using a combination of single molecule and ensemble-based studies.

24. Investigating Membrane Protein Folding Bowie, J¹.,¹Department of Chemistry and Biochemistry University of California. Understanding protein folding is essential for many of the central quests of structural biology including structure prediction, design, disease mechanisms and evolutionary pathways. Nevertheless, we still have a rudimentary understanding of membrane protein folding. I will describe tools we have developed for studying folding and some of what we have learned about how they fold. I will particularly focus on the role of hydrogen bonds in folding and dynamics.

27. Natural and Adaptive Foxp3+ regulatory T cells.

25. Histone modification and exchange during gene transcription: Signals and Mechanisms

Venkatesh, S., Smolle, M., **Workman, J¹**, ¹Workman lab Stowers Institute for Medical Research.

Maintenance of ordered chromatin structure over the body of genes is vital for the regulation of transcription. Increased access to the underlying DNA sequence results in the recruitment of RNA polymerase II to inappropriate, promoter-like sites within genes, resulting in unfettered transcription. Our recent studies show how Set2-mediated methylation of histone H3 on Lys36 (H3K36me) maintains chromatin structure by limiting histone dynamics over gene bodies, either by recruiting chromatin remodelers that preserve ordered nucleosomal distribution or by lowering the binding affinity of histone chaperones for histones, preventing their removal.

Silva, H¹, Weiss, J¹, Bilate, A¹, Gobert, M¹, **Lafaille, J¹**, ¹Kimmel Center for Biology and Medicine at the Skirball Institute New York University.

Foxp3⁺ regulatory T cells (Treg cells) are major lymphocytes involved in immune tolerance. They can be generated in the thymus (tTregs) or in the peripheral tissues (pTregs); the latter cells are often referred to as adaptive or inducible Treg cells. tTreg and pTreg cells are believed to have complementary functions, at least in part due to their poorly overlapping T cell receptor repertoires. They also have different stability, with tTregs displaying more stable Foxp3 expression. We will discuss a series of differences and similarities in tTreg and pTreg development as well the implications of these differences to the development of Treg-mediated cell therapies.

28. Modeling schizophrenia with human pluripotent stem cells

Paulsen, B¹, ¹Laboratório Nacional de Células-Tronco Embrionárias, Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro.

26. Post-transcriptional control of gene expression.

Brown, P¹, ¹Department of Biochemistry Stanford University School of Medicine.

The post-transcriptional lives of RNA transcripts are remarkably diverse, play a central role in biological regulation and still rich with opportunities for discovery. I will focus on two recent findings from my research group. First, I will present evidence for abundant, developmentally regulated circular RNA isoforms in animals, plants, fungi and protozoa, and describe some of their features. Second, I will present some surprising new insights into the process of translation elongation from a new analysis of data from deep sequencing of ribosome-protected mRNA fragments, which allows us to separately measure the kinetics of decoding and translocation at each codon.

Schizophrenia has been considered a clinical syndrome rather than a single disease. Nevertheless, the mechanisms behind the onset of the disorder have been only partially elucidated. Human induced pluripotent stem cells (hiPSCs) emerged as a new platform for the study of cellular and molecular basis of schizophrenia, by complementing other experimental models with exclusive advantages such as the recapitulation of brain development. Our data show evidence of altered gene expression, as well as changes in metabolic and atomic profiles, during neurogenesis of hiPSCs derived from schizophrenic patients compared to controls. These results expand our understanding about the disease and may provide new insights for potential targets aiming personalized treatments and drug screening.

29. Insights from tracking signals and signaling components implicated in plant defense

Greenberg, J¹, Cecchini, N², Davern, S³, Fowler, J⁴, Gifford, A⁵, Jelenksa, J⁶, McKnight, T⁷, Mirzadeh, S⁸, Shrestha, J², Standaert, R³, Tateda, C², Zhang, Z², ¹Molecular Genetics and Cell Biology, Professor, University of Chicago. ²Molecular Genetics and Cell Biology University of Chicago. ³Biosciences Oakridge National Labs. ⁴Radiochemistry Brookhaven National Lab. ⁵Biosciences Brookhaven National Lab. ⁶MGCB University of Chicago. ⁷Biomedical Science and Engineering Oakridge National Labs. ⁸Nuclear Science and Technology Oakridge National Labs. (Sponsored by National Science Foundation And Department Of Energy)

During pathogen infection, plants respond to both pathogen- and self-derived molecules, which can affect local and systemic signaling. We are working with the bacterial pathogen *Pseudomonas syringae* (and molecules derived from *P. syringae*) and several hosts, including Arabidopsis, tomato, *Nicotiana benthamiana* and poplar. I will discuss our recent efforts to efficiently deliver and/or track both pathogen- and plant-derived molecules as well as the plant signaling components important for responding to them. The emerging picture suggests that signals and response components are often highly dynamic and subject to multiple layers of regulation. The work highlights the utility of using sensitive assays and tracking processes over time.

30. Protein Interaction and dynamics using NMR: studies of defensins and allergens

Valente, A¹, ¹Structural Biology, Associate Professor, Federal University of Rio de Janeiro. (Sponsored by FAPERJ, CAPES, CNPq, INCT- INBEB.)

In the talk I will show the importance of protein conformation exchange process both in defensins-membrane, defensin-receptor and antibody-epitope recognition. We have used chemical shift perturbation and relaxation parameters measurements to evaluate the importance of motions in the ps-ns and μs-ms timescales. In the case of defensins, multiple motions seem to be correlated with the stages of membrane and receptor recognition over a large timescale range. I will also discuss the modulation observed in the antibody-epitope affinity due to changes in dynamic. We observed that the decrease in conformational exchange and no change in the structure of epitope, lead to the higher affinity, inducing higher cross-linking to fce receptors. ACKNOWLEDGEMENTS: FAPERJ, CAPES, CNPq, INCT- INBEB.

31. Influenza virus NS1, a master regulator of the antiviral host response

García-Sastre, A¹, ¹Department of Microbiology Icahn School of Medicine at Mount Sinai, NY .

The NS1 protein of influenza virus is a multifunctional protein that interacts with multiple biological pathways in virus-infected cells. It inhibits both cytoplasmic and nuclear processes involved in activation of the antiviral response. It also induces PI3kinase promoting viral replication. In many instances, these functions are strain and host specific, and might require adaptation and fine tuning to the new host when influenza virus jumps species. We have evaluated the impact of different NS1 functions in pathogenesis and virus replication in human and animal strains of influenza of pandemic concern. Strain specific effects might offer clues on the ability of this viral protein to become adapted to different host environments.

32. Diverse Signals Lead *Bacillus subtilis* to Build a Biofilm

Kolter, R¹, ¹Microbiology& Immunobiology, Medical School, Harvard University.

For nearly two decades, we have been studying the molecular genetics of biofilm formation by *Bacillus subtilis*. As a consequence, we now know in remarkable details the regulatory networks that lead from environmental stimuli to the cellular response of producing an extracellular matrix, the defining feature of the biofilm. We are now in a position to study the details of the environmental signals and cues that the bacterium recognizes as propitious conditions for matrix production. There are at least four protein kinases whose activities feed into the master regulator Spo0A and we have identified signals and cues that are differentially recognized by these kinases. The emerging view is that *B. subtilis* has evolved a remarkably complex network to respond to extremely diverse signals and cues with matrix production.

33. Malignant reprogramming: biologic and therapeutic implications

Jamieson, C¹,¹Sanford Consortium for Regenerative Medicine & Moores Cancer Center University of California San Diego.

We specialize in myeloproliferative disorders (MPDs) and leukemia. Myeloproliferative neoplasms are a family of uncommon but not rare degenerative disorders in which the body overproduces blood cells. Myeloproliferative neoplasms can cause many forms of blood clotting including heart attack, stroke, deep venous thrombosis, and pulmonary emboli and can develop into acute myelogenous leukemia. Although some effective treatments are available, they are laden with serious side effects. In addition, individuals can become resistant to the treatments. Our laboratory studies the mutant stem cells and progenitor cells in myeloproliferative neoplasms. These cells can give rise to cancer stem cells. Cancer stem cells may lie low to evade chemotherapy and then activate again later, causing disease progression and resistance to treatment. Our goal is to find more selective, less toxic therapies.

34. Membrane Protein-Lipid Binding: Insights from Mass Spectrometry and Molecular Simulations

Barrera, N¹,¹Physiology, Biological Sciences, Pontificia Universidad Católica De Chile.

Membrane proteins play essential roles for maintaining homeostasis, sensing and reactivity of living cells. The lipid membrane environment and specific lipid binding usually regulate their cellular role. However structural studies regarding the identification of the nature and stoichiometry of the membrane protein-lipid bindings are still very challenging and new methodologies are constantly required. Recently it has been discovered a new method based on mass spectrometry (MS) to characterize the lipid stoichiometry and nature directly on intact membrane proteins. In this symposium recent insights from the MS application on membrane protein-lipid complexes will be discussed, in particular, in combination with molecular dynamics simulations of the binding events. Funded by Millennium Science Initiative P10-035F, Fondecyt 1120169, Wellcome Trust and Anillo ACT 1108 grants.

35. Single molecule imaging reveals that nuclear architecture controls transcription.

Darzacq, X¹,¹Functional Imaging of Transcription Ecole Normale Supérieure.

While membranes do not compartmentalise the nucleus, it shows a complex organisation at many scales. Spatial organisation of chromatin and transcription factors can modulate nuclear functions and in order to study this relation, we have developed methods to localise proteins and mRNAs at the single molecule level and with spatial resolutions in the range of a few nanometers (modifications and improvements of PALM, sptPALM and STORM using adaptive optics). Moreover, proteins move throughout the nucleus by diffusion, transiently and repetitively contacting their target sites. While DNA has been reported as a guide facilitating target search in the cell by restricting 3 dimensional explorations to a 1 dimensional search, such exploration modes were not envisioned mediated by protein-protein interactions. I will discuss chromatin and RNA polymerase II organisation in the nucleus as well as mechanisms guiding proteins to their targets in the nucleoplasm.

36. The participation of high energy conformational states in catalysis and membrane recognition – plant defensins and thioredoxins.

Almeida, F¹,¹Structural Biology, Institute of Medical Biochemistry, Federal University of Rio de Janeiro. (Sponsored by FAPERJ, CAPES, CNPq, INBEB-CNPq,

Program Science Without Borders-CNPq) Proteins are dynamic entities that move in a hierarchy of timescales that goes from picoseconds to seconds. Motions that occur in microseconds to seconds define biologically relevant events that are frequently involved in binding, allostery and catalysis. We measured relaxation parameter, relaxation dispersion experiments and molecular dynamic simulation in order to correlate hydration of and slow dynamics. We will show how conformational dynamics of yeast thioredoxin 1 is correlated with the hydration equilibrium of a water cavity near the active site. The residue Asp24 that is well-recognized as a proton acceptor in the catalytic mechanism also controls dynamic events of the protein that are important for recognition and catalysis. We also probed the dynamics of plant defensins and correlated to its ability to bind to membrane targets. Using relaxation dispersion methods we characterized the structural property of the excited state conformation of sugar cane defensin 5.

37. New insights of IGF-1 signaling in the heart

Lavandero, S¹,¹Bioquímica y Biología Molecular, Facultad Ciencias Químicas y Farmacéuticas, Universidad De Chile. (Sponsored by FONDECYT 1120212, Anillo De Ciencia Y Tecnología ACT 1111)

Insulin-like growth factor-1 (IGF-1) is a peptide hormone with pleiotropic effects. In the heart, IGF-1 signaling regulates contractility, metabolism, hypertrophy, autophagy, senescence and apoptosis. Deficiency of IGF-1 is associated with an increased risk of cardiovascular disease, whereas cardiac activation of IGF-1 receptor (IGF-1R) protects from the detrimental cardiac effects of high-fat diet and myocardial infarction. The IGF-1R activates multiple pathways through its intrinsic tyrosine kinase activity and also through coupling to heterotrimeric G protein. These pathways involve classic second messengers, phosphorylation cascades, lipid signaling, Ca²⁺ transients and gene expression. In addition, IGF-1R is able to trigger signaling in different subcellular locations including the plasma membrane, perinuclear T-tubules and also in internalized vesicles. An updated view of the complex scenario of IGF-1 signaling in the heart will be shown in this presentation

38. RNA-Directed Dynamic Histone Acetylation

Davie, J¹,¹Khan, D¹,¹Gonzalez, C¹,¹Winnipeg, Manitoba Manitoba Institute of Child Health.

Histone deacetylases (HDACs) and lysine acetyltransferases (KATs) catalyze dynamic acetylation of histones, at regulatory and coding regions of transcribed genes. Highly phosphorylated HDAC2 is recruited within corepressor complexes to regulatory regions, while the nonphosphorylated form is associated with the gene body. HDAC1 and 2 are in complex with splicing factors. The co-recruitment of HDAC1/2 and splicing factors is transcription dependent. HDAC1/2 and KAT2B (PCAF) are associated with nascent pre-mRNA. Inhibition of HDAC activity and knockdown of HDAC1 and/or HDAC2 demonstrated that these enzymes were involved in alternative splicing of *MCL1*. Inhibition of HDAC activity increased the occupancy of KAT2B and level of H3 and H4 acetylation over the *MCL1* alternative exon 2. Thus, nonphosphorylated HDAC1/2 is recruited to pre-mRNA by splicing factors to act at the RNA level with KAT2B to catalyze dynamic histone acetylation and alter the nucleosomal structure of the *MCL1* alternative exon and splicing of *MCL1* pre-mRNA.

39. Genomic approaches in cancer research.

Frazer, K¹,¹Moore's Cancer center & Division of Genome Information Sciences University of California San Diego.

An important research focus of our lab is to use genomic information to understand disease predisposition, progression and patients' response to drugs. For this, we develop genomic and bioinformatics approaches to identify new biomarkers or candidate drug targets. Our expertise is diverse, spanning from human genetics and functional genomics, to cancer genomics and clinical sequencing. Our main mission is to accelerate the translation of genomic discoveries into the clinic. Members of the division collaborate closely with clinical investigators. In particular, in cancer we look at the molecular profile of tumors of a variety of cancer types to identify novel therapeutic targets, improve disease classification, and understand carcinogenesis and cancer progression. In particular, we develop approaches to integrate both genetic, epigenetic and expression information.

40. Voltage-driven structural transitions in voltage-gated calcium channels

Pantazis, A¹,¹Savalli, N¹,¹Sigg, D²,²Neely, A³,³**Olcese, R⁴**,⁴Anesthesiology University of California, Los Angeles.²Spokane, WA dPET.³Centro Interdisciplinario de Neurociencias, Facultad de Ciencias Universidad de Valparaíso, Valparaíso, Chile.⁴Anesthesiology and Physiology University of California, Los Angeles.

Excitation-evoked Ca²⁺ influx is the fastest and most ubiquitous chemical trigger for a multitude of cellular processes including muscle contraction, hormone and neurotransmitter release, and gene expression. The magnitude and timing of Ca²⁺ influx relies on the as-yet-unknown operation of four homologous, but non-identical, voltage-sensing domains (VSD I-IV) that control the pore in voltage-gated calcium channels (Ca_v). We optically tracked the voltage-dependent movements of individual VSDs in human L-type channels (Ca_v1.2) and discovered that each exhibits distinct voltage-sensing and kinetic properties. The pore-VSD interaction energies, determined from a structure-based allosteric model of gating and ionic currents and VSD movements, revealed that pore opening is coupled to the activation of VSDs II and III, with minor contributions by VSDs I and IV. These results imply that the mechanism of activation of Ca_v channels diverges from that of voltage-dependent sodium and potassium channels, which are controlled by three and four VSDs, respectively.

41. HIV-1 Gag protein plays a dual role in Stress Granules assembly

Valiente-Echeverria, F^{1,2}, Mouland, A^{1,3,2},¹HIV-1 RNA Trafficking Laboratory, Lady Davis Institute, Jewish General Hospital.²Department of Experimental Medicine, Faculty of Medicine, McGill University.³Department of Microbiology and Immunology McGill University. (Sponsored by Travel Award From The Canadian Institutes Of Health Research)

In response to viral infection, cells mount a robust, anti-viral immune response to create an inhospitable environment for viral replication. One such host response leads to the assembly of stress granules (SG), translationally silent sites of RNA triage. Here we demonstrate that the structural HIV-1 Gag protein blocks the assembly of SGs in several cell types and even when SG assembly is forced by supraphysiological expression of G3BP1 and TIA-1. Our analyses reveal that an interaction between Gag and the translation factor eEF2 contributes to the blockade imposed by HIV-1. Depleting eEF2 relieved the blockade but also resulted in impaired virus production and infectivity. Moreover, Gag disassembled pre-formed stress granules via interaction with G3BP1. Understanding how HIV-1 counters anti-viral stress responses lays the groundwork for new strategies to bolster host cell immune defences against invading pathogens. This work is supported by a grant from the Canadian Institutes of Health Research (MOP-38111) and F.V.-E. was a recipient of a Postdoctoral Fellowship from Becas Chile

42. The protein folding pathway: a coordinated network of molecular chaperones

Valpuesta, J¹,¹Estructura de Macromoléculas Centro Nacional de Biotecnología (CNB-CSIC).

Molecular chaperones are a group of proteins devoted to either assisting the folding of other proteins or to their degradation. The former they generally do it by protecting the aggregation-prone regions of these proteins, thus allowing them to reach their native conformation using the information encoded in their own amino acid sequences. As for the later, this is achieved by the interaction of the chaperone:substrate complex with certain factors (cochaperones) which direct the complex to the proteasome degradation pathway. In both processes, chaperones can work by themselves but in most cases their function is performed by the coordinated concourse of different chaperones, which form transient complexes, thus acting like "assembly lines" that make more efficient the protein folding and degradation processes. The talk will be devoted to analyze some of these "assembly lines", a work that has been mainly carried out by electron microscopy, a technique suited for the study of transient protein complexes generated during the folding and degradation processes.

43. Biomarkers in cancer management: from scratch to clinical analysis

Corvalan, A¹, Maturana, M¹, Olivares, W¹, Rodriguez, A¹, Saavedra, K¹, Sandoval, A¹, Garcia, B¹,¹Hematology and Oncology, Medicine, Pontificia Universidad Católica De Chile.

We are involved in translational cancer research at basic, clinical and population levels. Our research is primarily focused on cancer epigenetics with an emphasis on the discovery and validation of tumor suppressor genes inactivated by DNA methylation and/or microRNAs. These discoveries act as potential biomarkers for the early diagnosis of cancer, monitoring treatment response and developing new treatments. Through this research, we discovery and now we are currently developing a novel screening and triage test for the early detection of gastric cancer based on cell free DNA in plasma (NCT01774266). We are also involved in the role of two p53-dependant tumor suppressor genes, Reprimo (RPRM) and p73, in the pathogenesis of gastric cancer by experimental and clinical approaches. In addition we have a strong interest in cancer systems biology, specifically exploring cancer heterogeneity through a data-mining approach by using existing databases and open-source / in-house bioinformatic softwares.

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44. Novel genetic model of the role of the IP₃ receptor in Familial Alzheimer's disease

Foskett, K¹,¹Department of Physiology U. Pennsylvania .

Exaggerated intracellular Ca²⁺ signaling is a robust proximal phenotype observed in cells expressing familial Alzheimer's disease (FAD) mutant presenilins (PSs). The mechanisms that underlie this phenotype are controversial, and their relevance for AD pathogenesis is unknown. Here, we used a genetic approach to identify the mechanisms involved and to evaluate their role in the etiology of AD in two FAD mouse models. Genetic reduction of the type 1 inositol trisphosphate receptor (InsP₃R1) by 50% normalized exaggerated Ca²⁺ signaling in cortical and hippocampal neurons. In PS1M146V knock-in mice, reduced InsP₃R1 expression restored normal ryanodine receptor and CREB-dependent gene expression and rescued aberrant hippocampal long-term potentiation (LTP). In 3xTg mice, reduced InsP₃R1 expression profoundly attenuated amyloid beta accumulation and tau hyper-phosphorylation, and rescued hippocampal LTP and memory deficits. These results indicate that exaggerated Ca²⁺ signaling, associated with FAD PS, is mediated by the InsP₃R and contributes to pathogenesis *in vivo*.

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45. Targeted Metabolic Engineering of Corn Enhances Oil Production

Alonso, A¹, Cocuron, J², Ross, Z²,¹Molecular Genetics The Ohio State University.²CAPS The Ohio State University.

In view of our rapidly growing demands of vegetable oil, enhancing fatty acid synthesis in maize (*Zea mays*) represents tremendous nutritional and economic benefits. In corn kernels, the endosperm and the embryo are the main site for synthesis and accumulation of starch and oil, respectively. So far, breeding efforts to achieve elevated oil content in maize resulted in smaller endosperm and therefore lower yield. We hypothesize that the key to increasing oil content in maize kernels without affecting the yield lies on directly changing their carbon metabolism, not their size. To test our hypothesis, we measured the intracellular metabolite levels (metabolomics) in maize embryos and performed ¹³C-based metabolic flux analysis (fluxomics) to identified metabolic bottlenecks in fatty acid synthesis. Then we specifically overexpressed a target gene involved in central metabolism of maize embryos, which resulted in 35% increase in embryo oil content without affecting the size of the kernels. This study hence describes the combination of innovative tools that will pave the way for controlling seed composition in important food crops.

46. Diabetic Cardiomyopathy; Fat, Failure, Flux, and FoxO

Hill, J¹,¹Cardiology UT Southwestern Medical Center.

The incidence and prevalence of diabetes mellitus are each increasing rapidly in societies around the globe. The majority of patients with diabetes succumb ultimately to heart disease, much of which stems from atherosclerotic disease and hypertension. However, the diabetic milieu is itself intrinsically noxious to the heart, and cardiomyopathy can develop independent of elevated blood pressure or coronary artery disease. This process, termed diabetic cardiomyopathy, is characterized by significant changes in the physiology, structure, and mechanical function of the heart. Presently, therapy for patients with diabetes focuses largely on glucose control, and attention to the heart commences with the onset of symptoms. When the latter develops, standard therapy for heart failure is applied. However, recent studies reveal that specific elements of the pathogenesis of diabetic heart disease are unique, including a prominent role for the transcription factor FoxO. These discoveries raise the prospect of diabetes-specific therapeutic intervention.

47. Gleaning biological insights from Andean microbial community analyses

Zambrano, M¹,¹Molecular Genetics Corporación Corpogen.

The Andean mountain range in Colombia is part of a volcanic belt with geothermal activity that is manifested by the presence of several fumaroles and hot springs. Several of these hot springs were analyzed to assess populations present and to do bioprospecting. Although different in terms of water chemistry and community structure, all sampled springs shared characteristics, indicative of a similar origin. An in-depth analysis of one of these springs revealed a microbial community dominated by bacteria, with organisms potentially involved in cycling of ferrous and sulfur-containing minerals and phototrophic organisms, most of which were eukaryotic micro-algae. A functional description of this same spring was inferred by analyzing whole metagenome reads, identifying genes indicative of a community geared towards survival in acidic and oligotrophic waters with high incidence of ultraviolet radiation. Proteorhodopsin genes, which code for bacterial transmembrane light-driven proton pumps, were also present and suggest that these photosystems could be involved in harvesting energy and perhaps enhancing survival in these remote and relatively isolated natural ecosystems.

48. Reengineering the regulation of nitrogen fixation in *Azospirillum brasilense*, a plant-growth promoting bacteria.

Pedrosa, F¹, Huergo, L¹, Yates, M¹, Rigo, L¹, Wassem, R², Monteiro, R¹, Steffens, M¹, Cruz, L¹, Chubatsu, L¹, Souza, E¹,¹Biochemistry and Molecular Biology, Federal University of Paraná, Universidade Federal do Paraná.²Genetics, Federal University of Paraná, Universidade Federal do Paraná. (Sponsored by National Institutes Of Science And Technology/Brazilian Research Council/Ministry Of Science, Technology And Innovation And Araucaria Foundation Of Paraná, Brazil)

Biological nitrogen fixation is the process of reduction of dinitrogen (N₂) to ammonia catalyzed by nitrogenase, an enzyme complex present only in the prokaryotes. Nitrogenase is composed by the metalloenzymes dinitrogenase reductase and dinitrogenase, both highly sensitive to oxygen. Regulation of *nif* genes expression in *A. brasilense* involves primarily the expression and control of the transcription regulator, the NifA protein by ammonium and oxygen. Control of NifA activity by ammonium ions involves the nitrogen regulation system (Ntr). Nitrogenase activity in *A. brasilense* is reversibly inactivated *in vivo* by NH₄⁺ and oxygen through ADP-ribosylation of dinitrogenase reductase. This complex system links carbon/energy and nitrogen metabolisms and offers opportunities for specific modification in the regulation of *nif* genes expression, nitrogenase activity control and ammonium assimilation/secretion. New strategies targeting the regulatory cascade of nitrogenase expression and activity control aiming at increased ammonium secretion are under development.

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49. HDL: A new look at an old problem

Heinecke, J¹,¹Department of Medicine University of Washington.

Clinical studies have shown a robust inverse association between the levels of HDL cholesterol (HDL-C) and the risk of cardiovascular disease (CAD). These observations, together with the large residual disease risk among CAD subjects who are treated with statins, have triggered intense interest in therapies that raise levels of HDL-C. However, the beneficial increases in HDL-C levels have been recently questioned. Trials of certain drugs that elevated HDL-C levels have been terminated prematurely because of predicted lack of clinical benefit. These studies point toward a lack of uniform relationship among HDL-C levels, HDL function, and cardiovascular risk. Moreover, inferences about the function of HDL cannot necessarily be drawn from changes in HDL cholesterol concentration. Because HDL-C levels provide limited information about HDL's cardioprotective effects, quantifying HDL-C may be a poor way to explore HDL's functions. We have developed and validated a new method to measure HDL-P concentration. This approach demonstrated that HDL-P concentration is reduced 30% in CAD patients when compared with controls. Importantly, HDL-P strongly and independently predicts CAD status in subjects after adjusting for HDL-C levels, raising the possibility that HDL-P is a better approach to assessing CAD risk than is HDL-C.

50. The regulation of macroautophagy

Klionsky, D¹,¹Life Sciences Institute University of Michigan.

Over thirty genes have been identified in yeast that are specific to macroautophagy, and homologs have been identified for many of these genes in higher eukaryotes. Although some of the detailed mechanism of macroautophagy has been elucidated, many questions concerning the molecular basis of the pathway remain unanswered. For example, how is the initial sequestering compartment, the phagophore, nucleated? What is the origin of the membrane used for expansion of the phagophore to form the autophagosome? What are the roles of the various autophagy-related proteins in the process of autophagosome biogenesis? We have been analyzing the regulation of macroautophagy in *Saccharomyces cerevisiae*. One of the central autophagy-related (Atg) proteins is Atg8, a ubiquitin-like protein that is conjugated to phosphatidylethanolamine. The amount of Atg8 determines the size of autophagosomes; however, the factors controlling the expression of ATG8 have not been determined. Recently, we have been analyzing the regulation of ATG8 transcription. Because of the conserved nature of this process the insight we gain from understanding regulation in the genetically tractable yeast system will provide additional direction for analyses in higher eukaryotes.

51. Retinoic acid and IL-33, two new players tuning T cell mediated immunity?

Pino-Lagos, K¹,¹Morales, R¹,¹Perez, F¹,¹Campos-Mora, M¹,¹Gajardo, T¹,¹Narvaez, E¹,¹Soto, L¹,¹Catalan, D¹,¹Aguillon, J¹,¹Immune Regulation and Tolerance group (www.irtgroup.com), Programa Disciplinario de Inmunología, Instituto de Ciencias Biomédicas, Faculty of Medicine, University of Chile.

During the last years several groups have reported that T cell polarization and differentiation to a certain subset of T helper cell is a dynamic and plastic phenomenon. In this regard, different molecules have been identified as modulators of this process, including retinoic acid (RA) and IL-33. Currently, it is established that RA synthesis can be triggered under inflammatory conditions, that RA is produced by dendritic and stromal cells from peripheral tissues, and that RA's function provokes not only differential migration to gut mucosa, but it also acts as a pivotal factor for CD4⁺ T cell differentiation (Regulatory T cells, Th1- and Th17-type cells). Similarly, IL-33 has been also implicated with T cell polarization. Our recent work is focused in addressing the question of whether RA and IL-33 activities somehow relate during the development of immunity. Or in other words, can RA-producing cells (i.e. RALDH⁺) get triggered by IL-33 and determine the outcome of the immune response? For this purpose we are using a vaccination and skin allograft transplantation murine *in vivo* models. With our studies, we hope to contribute on RA and IL-33 knowledge in cellular immunology, which may have a relevant impact on the development of new therapeutics. Funded by CONICYT 791100001 and FONDECYT 11121309.

52. A role for the Ca²⁺-dependent repressor DREAM in the onset and progression of Huntington disease

Naranjo, J¹,¹Mellström, B¹,¹CSIC, Madrid Centro Nacional de Biotecnología.

Deregulated intracellular Ca²⁺ homeostasis underlies synaptic dysfunction and is a common feature in neurodegenerative processes. DREAM/calnenilin/ KChIP-3 is a multifunctional Ca²⁺ binding protein with specific functions in different subcellular compartments. In the nucleus, the Ca²⁺-free form of DREAM binds tightly to DRE sequences in the DNA and controls the expression of several genes related to Ca²⁺ homeostasis, neuronal excitability and neuronal survival. DREAM mutants unable to respond to Ca²⁺ and/or cAMP will disturb gene regulation leading to changes in the physiology of the synapses that might be determinant for or predispose to neuronal damage and death. We have used transgenic mice over expressing dominant active DREAM mutants, i.e. insensitive to Ca²⁺, and DREAM deficient mice to assess the role of DREAM in the onset of unbalanced motor coordination and neurodegenerative processes found in chemically- or genetically-induced mouse models of Huntington disease (HD). In addition, we have tested drugs able to bind to DREAM for an effect on the onset and progression of motor dysfunction in the R6/2 mouse model of HD. Funded by grants from Fundacion LaCaixa, CEE (LSHM-CT-2004-512039), Era-Net-SAF2008-04753-E y CIBERNED.

53. Uncovering Arabidopsis clock transcriptional circuits by functional genomics

Pruneda-Paz, J¹,¹Division of Biology University of California San Diego.

Extensive clock-regulated transcriptional networks control almost every biological process in plants. Clock controlled physiological responses are coupled with daily oscillations in environmental conditions resulting in enhanced fitness and growth vigor. The molecular architecture of plant clocks is based on multiple interlocked transcriptional regulatory loops, however the redundant nature of clock circuits limits the discovery of the underlying molecular mechanisms. To uncover direct regulators of a key clock component, CCA1, we recently established a functional genomics approach in which the yeast-one hybrid system is used to screen a collection of Arabidopsis transcription factors (TFs) for their binding to its promoter region. Here, we present the construction of the most comprehensive fully sequence-validated array of Arabidopsis TFs. This 1956-clone collection includes ~80% of all TFs predicted by most TF-specific databases and provides a significant clone number increase compared to previous publicly available resources. Following this strategy and an improved procedure to perform high-throughput Y1H automated screens we identified novel regulators of CCA1. Furthermore, the resource presented here provides a comprehensive toolset for the discovery and validation of direct transcriptional regulators for any Arabidopsis gene.

54. Aspartate metabolism in plants as a model system for studying metabolic regulation

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Although allosteric feedback regulation of enzymes has been known for more than fifty years, and many examples of isoenzymes have also been discovered, the roles of these and other characteristics of metabolic pathways in metabolic regulation have mainly been studied at a theoretical level: this has been useful, but it has lacked confirmation from experiments or computer simulation of how feedback regulation and isoenzymes actually affect metabolic systems. The metabolism of aspartate provides an ideal system for understanding them in detail, not only because its regulation in *Escherichia coli* has been a classic example of metabolic regulation for many years, but also because in chloroplasts of *Arabidopsis thaliana* this pathway offers many examples of all of the relevant characteristics: allosteric inhibition and activation, branches leading to several different products, lysine, S-adenosyl methionine, threonine and lysine, all of which need to be produced in regulated amounts, and numerous cases of isoenzymes. Moreover, a considerable amount of experimental information about the kinetic properties of the enzymes concerned is available. Thus there is now evidence from computer simulation and experiments that feedback regulation does act in branched pathways in the way theoretical studies have suggested, and we now have a better idea of the role of isoenzymes.

55. Oxidative Stress, Protein Carbonylation and Metabolic Control

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Obesity-induced insulin resistance is accompanied by increased adipose tissue macrophage infiltration leading to ER stress, mitochondrial dysfunction and the accumulation of increased reactive oxygen species. Using adipose tissue from high fat fed, insulin resistant C57Bl/6J mice, or cultured 3T3-L1 adipocytes, we have identified that the expression of several anti-oxidant enzymes are down regulated by inflammatory cytokines. Down regulation of Prdx3, GPx4, GSTA4, and ALDH2 (but not SOD2), leads to increased hydroxyl radical production and the formation of reactive aldehydes that covalently modify proteins in a reaction termed protein carbonylation. iTRAQ based proteomics has identified that the major carbonylated proteins in the mitochondrion are two subunits of Complex I (NDUFA2 and NDUFA3) and silencing of either Complex I subunit leads to markedly reduced NADH oxidase activity, attenuated respiration, and a potentiation of reactive oxygen species production. Carbonylation of Complex I subunits leads to accumulation an increase in NADH/NAD, elevated TCA cycle intermediates and a corresponding increase in protein acetylation. Overall, these results suggest that protein carbonylation may underlie mitochondrial dysfunction and ER stress. Supported by NIH DK084669 to DAB and the Minnesota Obesity Center (NIH DK050456).

56. Screening bacterial cell growth and cell division inhibitors by FRET (Förster Resonance Energy Transfer)

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The increase in resistant bacteria demands the development of new antibiotics against preferably new targets to enlarge the number of different antibiotic classes. that can be of choice to save lives. The common approach is to test compounds for their ability to kill bacteria or to design compounds that inhibit protein activities *in vitro*. In the former case the mode of action is not known and in the latter case, it is not known whether the compound will pass the very impermeable barrier of the bacterial envelope. To reduce the cost of antibiotic development, it would be much more efficient to have assays available that detect the target of the compound and its membrane permeability simultaneously. The bacterial cytoskeleton proteins MreB and FtsZ are essential for the recruitment of the protein complexes involved in length growth and in cell division, respectively. An assay that measures their polymerization in living bacteria would be a very useful and cost effective tool for the screening of new antibiotics that target these proteins. Förster Resonance Energy Transfer or FRET is one of the ideal tools to measure protein interactions because it seldom provides false positives. We have developed an *in vivo* FRET assay for MreB and FtsZ polymerization and show that the depolymerization of MreB by A22 can be effectively measured by this assay.

57. Amyloid formation modulates the antibacterial activity of microcin E492, a pore-forming bacteriocin.

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Microcin E492 (MccE492) is a low-molecular-mass channel-forming bacteriocin that generates amyloid structures. The formation of amyloid fibers modulates the antibacterial activity by sequestering the toxic species. MccE492 can be modified at the C-terminus with a salmochelin-like molecule, which is essential for the recognition of the siderophore outer membrane receptors. Afterwards, MccE492 is translocated to the periplasm and inserted into the inner membrane of the target cell. The modification however is not essential for the pore-formation activity, because both unmodified and modified MccE492 can form pores in membranes. The fiber morphology of both forms is the same, but the aggregation kinetics of the modified form presents a considerable delay compared to unmodified MccE492. This can be explained by a different conformation of the precursors, which present a different content of secondary structure, that affects the nucleation process. An amyloidogenic region was identified through mutagenesis, and this region turned out to be different to that required for pore formation. MccE492 appears to be a helpful model for studying the toxic structure of an amyloid, because a conserved folding pathway with oligomeric intermediates characterizes most amyloid fibers. Supported by FONDECYT grant 1100141

58. Dynameomics

Daggett, V¹, ¹Bioengineering University of Washington.

The goal of Dynameomics is to perform atomistic molecular dynamics (MD) simulations of representative proteins from all known folds in explicit water in their native state and along their thermal unfolding pathways. We began by creating a Consensus Domain Dictionary—a list of meta-folds for systematic coverage of fold space. From this analysis there are 1695 unique protein fold families and a target, or representative, was chosen from each. Only 807 of these targets represent ‘simulatable’ protein, autonomously structured, folds, which has implications for the use of domain dictionaries in bioinformatics. Simulation of these targets has been completed and will be discussed here. The behavior of several specific targets is highlighted to illustrate some of the general properties in the full data set and to demonstrate the role of MD in understanding protein function and stability. To encourage understanding of the relationships between protein dynamics, function and disease, we have constructed a web site, complementary to the PDB, which allows access to a novel hybrid relational/multidimensional database to view and interrogate simulations of the Top 100 targets: <http://www.dynameomics.org>. We, and others, are now mining the Dynameomics database in an attempt to determine the general rules governing protein folding and function.

59. Structure-function analysis of MurJ

Ruiz, N¹, ¹Microbiology The Ohio State University.

The peptidoglycan (PG) cell wall is a glycopeptide matrix that surrounds the cytoplasmic membrane of most bacteria, conferring cell shape and osmoprotection. Gram-negative bacteria build the PG sacculus in their periplasm using the precursor known as lipid II, a large amphipathic molecule composed of undecaprenyl-diphosphate and a disaccharide-pentapeptide. After its synthesis in the cytoplasmic leaflet of the inner membrane, lipid II is translocated across the membrane by flippases that are thought to shield its hydrophilic moiety as it crosses the hydrophobic core of the membrane. The integral membrane protein MurJ has been proposed to function as the lipid II flippase in *Escherichia coli* because it is essential for PG biogenesis and homologous to known flippases that translocate undecaprenyl-diphosphate-linked oligosaccharides across the cytoplasmic membranes of bacteria. Here, we present a 3-D model suggesting that the structure of MurJ resembles that of related exporters. Using *in vivo* topological studies, we have generated data that support key features of this model including that MurJ has 14 transmembrane domains and a central solvent-exposed cavity. Furthermore, we have demonstrated that specific, charged residues localized in this cavity are essential for function. Together, our studies suggest that MurJ functions as an essential transporter in PG biosynthesis.

60. Dendritic cell targeting for antigen-specific therapy in rheumatoid arthritis

Thomas, R¹, ¹Diamantina Institute The University of Queensland. (Sponsored by The University Of Queensland Diamantina Institute)

Disease modifying strategies are available for treatment of rheumatoid arthritis (RA), and good response rates are achieved. However, limitations include toxicity, a response rate ceiling, cost and rationing of biologic therapies, inability to cure or permanently reverse RA pathology, and inability to prevent disease. Recent evidence suggests that treatment of very early RA with immunomodulatory drugs can delay or attenuate disease onset. Approximately 80% of RA patients develop anti-CCP autoantibodies targeted against citrullinated self antigens. This subset is more likely to have RA-associated HLA-DR risk alleles. We developed citrullinated antigen-specific immunotherapy for RA, targeting dendritic cells (DC). Antigen-loaded autologous DC, generated *ex vivo* in the presence of an inhibitor of NF- κ B, transferred antigen-specific tolerance and suppressed inflammatory arthritis in mice. In a proof-of-concept phase I clinical trial of modified autologous DC therapy for induction of citrullinated autoantigen-specific tolerance, safety, efficacy and metabolic data suggest that further clinical intervention studies are justified. To simplify this therapeutic strategy for future trials, we developed cell free immunotherapy to target DC *in situ* with liposomes encapsulating citrullinated peptide and NF- κ B inhibitor. A clinical trial of this product is planned in RA patients, with antigen-specific T cell monitoring.

61. *Salmonella* Typhi: Reductive evolution and the process of host specificity

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Reductive evolution refers to the process of gene decay that occur in most bacterial genomes characterized by the presence of disrupted and eroded genes. These are considered as pseudogenes, which are the loss of genetic information by deletion or insertion. Comparative genomics has shown that host-specific bacteria contain more inactive genes than closely related bacteria that are not host specific. This correlation is evident in the case of *Salmonella* Typhi, a human specific pathogen, which contain 5% of its genes as pseudogenes and *Salmonella* Typhimurium, a broad host range pathogen, containing only 1% of genes as pseudogenes. Our approach to the study of host specificity mediated by pseudogenes has lead us to trans-complement functional genes from *S. Typhimurium* into *S. Typhi*. As an example, we trans-complemented *sseJ* gene resulting in evident changes in the behaviour of bacteria in contact with eukaryotic cells, suggesting that the loss of *sseJ* contributed to the *S. Typhi* adaptation to the systemic infection in humans. We have also found a gene, *shdA*, annotated as pseudogene, which is in fact an eroded gene, still encoding a product that mediates adhesión to eukaryotic cells. This is suggesting that the host adaptation process is going on in our days and still continue in *S. Typhi*. Proyecto FONDECYT: 1110120

62. MOLECULES THAT DRIVE THE EXOCYTOSIS OF HUMAN SPERM DENSE CORE GRANULE: SECOND MESSENGERS, SNAREs and SMALL G-PROTEINS

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Exocytosis is used by eukaryotic cells to release biological compounds and to insert specific lipids and proteins in the plasma membrane. Our lab is interested in unveiling the molecular mechanisms that drive exocytosis of the mammalian sperm's acrosome, a very large dense-core secretory granule that is released near the egg at fertilization. We have established that exocytosis of the acrosome depends on members of a highly conserved proteinaceous fusion machinery that includes small GTPases (Rab3, Rab27, and Rap), SNAREs, proteins that interact with them, and the calcium sensor synaptotagmin. Sperm exocytosis also requires calcium, cAMP and its target Epac, a GEF for Rap. Sperm's Rabs and SNAREs do not undergo constitutive activation/inactivation or assembly/disassembly cycles; rather, these reactions are unidirectional and coupled to the progress of exocytosis. Exposure to exocytotic inducers first increases and later decreases the population of cells exhibiting endogenous GTP-bound Rabs and Rap in the acrosomal region of the sperm head. The three small G proteins are activated in the sequence Rab27→Rab3→Rap during the exocytotic cascade. By means of chimaeric proteins, we have discovered that Rab3A-GTP is a molecule with positive and negative properties segregated in different domains of the polypeptide. These seemingly opposite attributes are expressed at different times during the secretory cascade.

63. Bradykinin in neurogenesis: From neural stem cell differentiation towards preclinical models of Parkinson's disease

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Bradykinin(BK)and related kinins are released into the plasma or interstitial fluid after proteolytic cleavage of kininogens.BK actions mediated through kinin-B2 receptors do not only participate in inflammation and blood pressure regulation, but have also been described for neurotransmission and neuromodulation. Novel functions for BK and its receptor were studied using neural stem and progenitor cells isolated from fetal rat or mouse brain (E14 or E12.5,respectively) as in vitro models whose differentiation into neural and glial phenotypes closely resembles events occurring during cortex development.The presence of the kinin-B2 receptor antagonist HOE-140resulted in reduced neurogenesis and enhanced gliogenesis of neurospheres obtained from embryonic telencephalon.we found that migration of neural progenitors was largely restricted in the inhibitor.These results were confirmed in migration and differentiation assays with neurospheres obtained from kinin-B2 receptor knockout mice. Neurogenic and neuroprotective features of BK in neurodegenerative diseases were further characterized in vitro and in vivo.Effects exerted by 6 OH-dopamine, such as cell death and inhibition of migration and neurogenesis of neurospheres induced to differentiation into dopaminergic neurons, were abolished by BK.Moreover,degeneration of dopaminergic neurons and clinical symptoms in a rat model of Parkinson's disease were mostly reversed following a single bradykinin injection

64. About the tyrosine phosphatase SHP2 and the membrane receptor OXER1 in the action of arachidonic acid in steroidogenic cells

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AcsI4, a long chain fatty acyl-CoA synthase, has a critical role in the intracellular mobilization and metabolization of arachidonic acid (AA). In steroidogenic tissues, the lipoxygenated metabolites of AA prompt the expression of StAR, a mitochondrial protein that regulates the rate-limiting step of the steroidogenic process. Previously we showed the participation of protein tyrosine phosphatases (PTP) in AcsI4 induction in steroidogenic systems. A pharmacological approach indicated that SHP2, a non-receptor PTP, could be the enzyme involved in the process. Using plasmid mediated gene transfer and RNAi-mediated gene silencing, we demonstrate that SHP2 determines the expression of the AA-metabolizing enzyme AcsI4 and that SHP2 has an obligatory role in steroidogenesis by MA-10 cells, a mouse Leydig cell line. Once induced, AcsI4 mobilizes AA and directs its metabolization. We hypothesized that the lipoxygenated metabolites of AA act through OxeR1, a membrane receptor which main agonists are the 5-lipoxygenated products of AA metabolism. Indeed, the use of pharmacological antagonists or agonists of the OxeR1 decreases or increases, respectively, the cAMP-dependent StAR expression and steroid production by steroidogenic cells. In summary, we describe the AcsI4-dependent involvement of the PTP SHP2 in the regulation of the intracellular mobilization of AA and the participation of OxeR1, an oxoeicosanoid receptor, in the stimulation of steroidogenesis.

65. Next generation sequencing for improved cancer care

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Improved genomic and next generation sequencing technologies have yielded a vast amount of information and provided numerous insights into cancer pathogenesis. However, translating these discoveries into patient care remains a major challenge. In the last years, advances of targeted therapies have improved the quality of life and treatment success in cancer patients. These developments have been possible since the availability of technologies, such as modified versions of the PCR technique, that allow the diagnosis of mutations in specific genes that are responsible for promoting or modulating cancer progression. In order to improve these assays, we are developing and validating a methodology based on ultra deep targeted sequencing of tumor genomes. We have piloted this approach using the Illumina TruSeq technology to develop a streamlined sample preparation to facilitate clinical implementation. The amplified targeted exons (~200bp long) from cancer genes are directly sequenced on the Illumina MiSeq platform at a very high coverage depth (~5,000x). We have interrogated 30 cancer samples from breast cancer patients and were able to detect low-prevalence somatic mutations in all samples. This methodology will allow the adequate diagnosis and the rational indication of targeted therapies in cancer patients, avoiding the use of oncologic treatments with low chances of success. Funding: Fondef D1111029.

66. Glycine hydroxymethyltransferase reaction and biosynthesis of glycine: a metabolic relic?

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Enzymes and metabolism are two inseparable concepts, as metabolism is the consequence of enzyme action. Therefore enzymes appear as very suitable therapeutic targets for metabolic problems. However the probability that an enzyme will be a good target *in vivo* depends on the metabolic network and its constraints, a point that is often forgotten in drug development. Glycine hydroxymethyltransferase, catalyst for the biosynthesis of glycine from serine, illustrates the complexity of the problem. Clinical and nutritional studies suggest that glycine should be classified as a conditionally essential aminoacid, as there appears not to be enough for physiological needs. This is puzzling, because biosynthetic pathways are normally regulated to satisfy metabolic demands. As glycine is required for the synthesis of several metabolites, and in particular for collagen, the most abundant protein in the human body (one-third of collagen residues are glycine), a glycine deficit could have repercussions for collagen-related illnesses. If this deficiency were due to insufficient activity of glycine hydroxymethyltransferase it could be a good drug target, but it is not because the analysis of the metabolic network indicates that capacity for synthesising glycine is constrained by the demand for tetrahydrofolate-C1 and not by the demand for glycine. The biosynthesis of glycine is adequate for unicellular organisms, but not for large animals, with their high production of collagen.

67. A Century of Enzyme Kinetic Analysis, 1913 to 2013

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In their 1913 paper Leonor Michaelis and Maud Menten sought to achieve ?the final aim of kinetic research, namely to obtain knowledge on the nature of the rea_ction from a study of its progress?. The challenge of the day was to account for the full time course of product formation in testing the postulate that the rate of an enzyme-catalyzed reaction was proportional to the concentration of enzyme-substrate complex. They did so without knowing the concentration or even the chemical nature of enzymes?a tribute to the power of quantitative kinetic analysis. Today, the important questions have advanced to asking how enzymes achieve such extraordinary efficiency and specificity, and structural and spectroscopic studies have provided a powerful complement to kinetic analysis to greatly expand our understanding of enzyme catalysis. While the techniques for data collection and analysis have advanced to meet the sophistication of the questions that are being addressed, kinetic analysis has remained as a cornerstone of enzymology because studies of the rate of reaction allow alternative pathways to be distinguished. Here, I will briefly review the methods of kinetic analysis developed by Michaelis and Menten that go beyond the simple initial velocity methods for which they are known, and contrast their analysis with modern computer-based global data fitting methods and state-of-the-art methods to study the progress of reactions occurring at the active sites of enzymes.

68. LOSS OF E-CADHERIN SWITCHES CAVEOLIN-1 FUNCTION FROM TUMOR SUPPRESSOR TO PROMOTER OF METASTASIS

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Cancer is a leading cause of human suffering and death worldwide, whereby most patient deaths are caused by metastatic dissemination rather than primary tumor growth. Understanding the mechanisms that favor such development is currently an area of great interest. Caveolin-1 (CAV1), a member of the caveolin family of scaffolding proteins, is paradoxically implicated in cancer development and progression both as a tumor suppressor and promoter of metastasis. In recent years, research from this laboratory has focused on shedding light on this enigma. Tumor suppression by CAV1 was linked to E-cadherin-dependent suppression of genes, including *survivin* and *COX2*, and augmented apoptosis. However, in the absence of E-cadherin, CAV1 promotes metastasis. More recent studies implicate CAV1 tyrosine phosphorylation and activation of a novel Rab5-Rac1 signaling axis in enhanced tumor cell migration, invasion and metastasis. In the presence of E-cadherin, signaling via this pathway and metastasis are suppressed. Thus, CAV1 switches roles in an E-cadherin-dependent manner. Understanding such context-dependent variations in protein function has important ramifications for our basic understanding of signal transduction processes and cancer, as well as for the development of successful strategies to treat the disease. *Acknowledgements:* FONDECYT-FONDAP15010006, FONDECYT1090071 AND 1130250, ACT1111

69. Obtaining insights into structure, dynamics and function of glycoproteins

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Glycosylation is one of the main post-translational modifications observed to occur in nature. Upon covalent linking to polypeptide sequences, glycan chains promote a dramatic enhancement of proteins functional diversity. Such linkage, until now characterized to occur through N-, O-, C- or S- atoms from amino acid residues or membrane anchored GPI, has been observed in all domains of life. However, in spite of the huge biological significance of carbohydrates, a more complete understanding of their functional role is impaired by the limitation of currently available experimental techniques to offer precise, atomic level models of glycan structure and dynamics. As a promising strategy to circumvent difficulties in carbohydrates crystallization and in obtaining an adequate number of NOE signals, our group has been applying a series of low cost computational strategies in order to build biologically meaningful, structural models of several complete glycoproteins. Such models, as obtained for extensin, antithrombin, thrombin, PglB, arylsulfatase A and NETNES, among others, have been able to offer new insights on protein dynamics and biological function. From the transmission of allosteric events to the glycan modulation of proteins activation, including the rationing of extensin glycosylation on root hair phenotype 3D atomistic models have been shown to possess a great potential to contribute in the understanding of the carbohydrate role upon attachment to proteins.

70. Extreme Acidophilic Bacteria Use Different Strategies to Resist High Metal Concentrations

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Acidithiobacillus ferrooxidans contains canonical copper resistance determinants that do not explain the extremely high copper concentrations (over 100 mM) this bacterium used in industrial recovery of metals is able to stand. This suggests the existence of additional efficient metal resistance mechanisms. Besides having an intrinsic positive inside to retard entrance of protons and other cations, *A. ferrooxidans* seems to have a wide repertoire of strategies to confront metal stress: presence of genomic islands, a polyP-based Cu-resistance system and an oxidative stress defence system. Furthermore, the analysis of cells grown in the presence of copper by means of quantitative proteomics showed a downregulation of porins and the overexpression of canonical efflux ATPases, new Cu-resistance determinants such as Cu chaperones and RND efflux pumps. Other cellular responses seen were an increased expression of the enzymes involved in biosynthesis of amino acids with high affinity for copper, a putative periplasmic disulfide isomerase and proteins involved in peptidoglycan synthesis, suggesting a group of proteins to repair the damage caused by copper stress on the cell envelope. These knowledge will not only help to understand the mechanism of copper resistance in this extreme acidophile but it could be used also to select the best fit members of the bioleaching community to attain more efficient industrial biomining processes. Grant FONDECYT 1110214

71. Nucleosomes and Transcriptional Regulation: Factors Modulating ATP-dependent Chromatin Remodeling Activity.

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ATP-dependent chromatin remodeling activity is involved in several aspects of DNA metabolism, including transcriptional regulation. Among the protein complexes harboring this activity, the yeast SWI/SNF complex is the founding member. Elements affecting this activity have been described, such as certain histone modifications, transcription factors and High Mobility Group (HMG) proteins. Little is known about the latter two aspects. In order to gain insight into the influence of yeast HMG proteins (Nhp6A, Nhp6B and Hmo1) and transcription factors on SWI/SNF activity, we performed several *in vitro* nucleosome remodeling assays. Studies addressing the impact of these HMG proteins on nucleosome remodeling also included *in vivo* assays. Our results show that the HMG proteins studied stimulate SWI/SNF activity and that the physical association of SWI/SNF to several gene promoters depends on the presence of these proteins. Nucleosome remodeling assays using reconstituted mononucleosomes harboring a binding site for a transcription factor which physically interacts with the SWI/SNF complex, shown that nucleosome disassembling catalyzed by SWI/SNF is negatively affected by the distance between the nucleosome and the transcription factor binding site. Taken together, our results contribute to the understanding of how ATP-dependent chromatin remodeling activity can be enhanced and modulated in the cell nucleus. FONDECYT 1130818, DIUC 211.037.014-1.0.

72. The role of glycosylations in the virulence and antigenic properties of Influenza A viruses

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Influenza A viruses circulate in multiple host in nature, including seasonally in humans resulting in a costly and yearly health problem worldwide. In humans these viruses evolve, adapt and undergo immune selection pressure resulting in the modulation of viral fitness and antigenic properties. Glycosylations of the influenza Hemagglutinin (HA) protein play a crucial role in modulating virulence and have a drastic effect in the humoral responses induced by these viruses. We demonstrated that glycosylations in the globular head of HA efficiently mask the polyclonal antibody response of previously circulating strains. Glycosylation at residue 144 of HA not only evades the pre-existing antibodies, but also induces a broad-spectrum humoral response aimed at the globular head, possibly by redirecting the antibodies to other antigenic sites. Deleting glycosylations from an HA containing 3 glycosylations, made this virus similar to an antigenically distant unglycosylated virus. Of interest, the 144 glycosylated virus had increased avidity to the human α -2,6 sialic acid receptor as compared to a non-glycosylated virus or viruses with 2 or more glycosylations, indicating that these modifications can also affect the receptor binding activity of influenza viruses. Our results shed light into the fine balance between fitness, tropism and the immune-modulatory effects of glycosylations in the HA and its potential use to study the breadth of immune responses to infection and vaccination.

73. Teaching concepts of Molecular Biology and Genomics to high school students using portable laboratories in several Latin American Countries

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Hands-on performance of scientific experiments is essential for understanding both fundamental scientific concepts and the essence of scientific knowledge generation, yet most secondary schools in Latin America lack scientific equipment necessary to perform experiments in modern biology. To circumvent this difficulty the Latin American Network of Biological Sciences (RELAB) has implemented a pilot project with the UNAM of Mexico, the University of Costa Rica and the University of Chile. These institutions organized one week intensive theoretical and practical courses on fundamental concepts of molecular biology for biology teachers of secondary school level. The teachers that approved these courses could request a visit of a portable laboratory to their respective schools. Two doctoral students accompanied the equipment to provide support in the setting up the experiments by secondary school students. During 2012-2013, this scheme was tested in the three countries with a very enthusiastic responses from secondary school students who performed experiments using PCR, bacterial transformation with foreign genes and gel electrophoresis of DNA and proteins. This approach is now being implemented in Uruguay at the Universidad de la Republica and a grant proposal has been presented to Chilean CONICYT to use this scheme in teaching other experimental sciences. This work was funded by the Wellcome Trust, UNESCO, RELAB, IANAS and the Fundación Allende Connelly.

74. Unraveling and Threading: How ATP-Dependent Proteases Apply Force to Mechanically Unfold and Translocate Protein Substrates

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ATP-dependent proteases are vital for the maintenance of protein homeostasis in the cell, but the mechanisms by which these machines couple ATP binding and hydrolysis into mechanical force to unfold and translocate protein substrates remain unclear. Here we use optical tweezers to study the mechanisms of force generation and intersubunit coordination in the ClpXP protease from *Escherichia coli*. ClpX is a homohexameric motor of the AAA+ family that uses ATP to bind, unfold and translocate protein substrates into the degradation chamber of the ClpP peptidase. We determine that phosphate release is the force-generating step in the ATPase cycle of ClpX. We find that highly coordinated conformational changes in two, three or four ATPase subunits drive a rapid translocation burst. Regardless of the number of translocating subunits, the timing between bursts is constant and independent of ATP concentration. ClpX must use its maximum successive firing capacity of four subunits to unfold stable substrates like GFP. Unraveling of GFP is cooperative and proceeds via two intermediate states. The first GFP unfolding intermediate results from the extraction of beta-strand 11 and is the rate-limiting step in the GFP unfolding reaction. The mechanisms described here provide important new insights into the operating principles of ATP-dependent proteases and may have critical implications for the understanding of other ring-shaped ATPases of the AAA+ family.

75. Fructose-1,6-bisphosphatase: some new developments and old themes

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Liver and kidney are able to synthesize and degrade glucose simultaneously, which requires a very sensitive control system for preventing the formation of futile cycles. Fructose-1,6-bisphosphatase (FBPase), controls a tightly regulated step of gluconeogenesis: AMP and fructose-2,6-bisphosphate (Fru-2,6-P₂) bind to allosteric and active sites, respectively, and inhibit FBPase while activating phosphofructokinase, its counterpart in glycolysis. In addition, FBPase is inhibited by its substrate Fru-1,6-P₂ at high concentrations (>30 μM). Here, we shall be concerned with the mechanism that accounts for the quaternary transition and cooperativity of FBPase in the inhibition of catalysis by AMP. On the other hand, although the inhibition of FBPase by excess substrate has been known for many years its mechanism has never been resolved. Now, we shall present kinetic and fluorescence data, as well as studies of hybrid tetramers with mixed subunits, to clarify the cause of the inhibition. We shall propose that binding of the first two substrate molecules, in the same dimer of the enzyme, produces a conformational change at the other dimer, reducing the substrate affinity and catalytic activity of its subunits. (This work was supported by grants 1090740 from FONDECYT (Chile) and S-2013-45 from Dirección de Investigación de la Universidad Austral de Chile).

76. Neurotransmitter-gated ion channels: On how structure relates to function and drug modulation

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Neuronal alpha7 receptors participate in cognition, synaptic plasticity and neuroprotection. They belong to the family of pentameric neurotransmitter-gated ion channels, which contain a large extracellular domain that carries the agonist binding sites and a transmembrane domain that forms the ion channel. We combined mutagenesis, cell-expression and patch-clamp recordings to decipher how agonist binding is coupled to channel opening. We identified a network of loops that relays structural changes from the binding site towards the channel, and also contributes to open-channel lifetime and desensitization. Thus, this coupling region controls the beginning and duration of a synaptic response. Because alpha7 contains five identical agonist binding sites, we explored how occupancy relates to open-channel stability. We found that alpha7 can elicit full biological responses with only one occupied binding site. This may adapt alpha7 to its physiological role, since it often localizes distal to sites of nerve-released ACh, binds ACh with low affinity, and thus it may operate with low agonist occupancy. Decreased alpha7 activity is associated with neurological and neurodegenerative disorders, and positive allosteric modulators (PAMs), which do not disrupt the temporal control provided by the neurotransmitter, are emerging as novel therapeutic tools. We have also deciphered mechanisms and sites of action of PAMs with the aim of contributing to rational drug design.

77. Cell-type specific core transcription machinery regulating neuronal differentiation.

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The role of transcriptional activators and repressors in cellular differentiation and reprogramming is widely recognized. Much less is known about the specific roles that core promoter recognition complexes play during these processes. These core promoter recognition factors are part of the pre-initiation complex (PIC) and required for Pol II recruitment and transcriptional activation. Originally assumed to be invariant, the PIC is now known to have cell-type promoter and potentially enhancer specific compositions. Despite the critical role that these factors play in transcriptional activation, relatively little is known about the PIC composition at the promoters of genes expressed in terminally differentiated neurons. In this project we focused on the changes that occur in the TAFs (TBP-associated factors) upon motor neuron (MN) differentiation and in particular in an orphan TAF paralog (TAF9b). We find that this TAF paralog is specifically required for MN differentiation of mES cells while largely dispensable for global gene expression in undifferentiated conditions. ChIP-seq reveals that TAF9b binds preferentially to a subset of enhancers and promoters of active genes in *in vitro* differentiated EB/MN. Consistent with our *in vitro* results, RNA-seq analyses of dissected TAF9b KO spinal columns reveal specific deregulation of neuronal genes, confirming that TAF9b *in vivo* is required for the efficient expression of neuronal genes.

78. A single-molecule fluorescence system to study biomolecules under force

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Proteins exert force in many biological processes and single-molecule manipulation has increasingly become an essential methodology for the study of macromolecular dynamics. We have built a novel instrument for force spectroscopy that combines the capabilities of magnetic tweezers and single molecule Förster resonance energy transfer (smFRET). A supermagnet exerts force on 2.1 µm antidigoxigenin-coated magnetic beads tethered to specific proteins under study. These fluorescently labeled proteins are functionalized with DNA handles containing biotin and immobilized on the surface of a flow chamber in a total internal reflection fluorescent microscope via streptavidin interactions. 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. The model system that we are currently studying in our instrument is adenylate kinase. This enzyme was successfully labeled with fluorescent dyes via unnatural amino acid and click chemistry and was attached to DNA via cysteine chemistry. AK was first characterized in optical tweezers and was found to unfold around 25 pN during force-extension experiments with a fast 4 nm intermediate at 15 pN. Data from our instrument confirms the existence of this intermediate under force and could correspond to the unfolding of the ATP binding domain (HHMI-CB, NIH-SM).

79. Intermolecular interactions of FtsZ and ZipA: two essential components of the E. coli divisome

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The self-association of FtsZ and the hetero-association of FtsZ and ZipA have been studied under a variety of conditions using a combination of biophysical techniques, including measurement of ideal and nonideal sedimentation equilibrium, sedimentation velocity, composition gradient single angle and multi-angle light scattering, and fluorescence correlation spectroscopy. The combination of techniques and precision of the data permit rather firm quantitative conclusions to be drawn regarding the mechanisms of self-assembly under the conditions of our experiments. In the presence of GDP, Mg-linked self-association of FtsZ leads to the non-cooperative formation of a polydisperse distribution of linear fibrils, while in the presence of GTP or the GTP analog GMPCPP, Mg-linked self-association of FtsZ leads to the highly cooperative formation of a paucidisperse distribution of large cyclic oligomers. The interaction between both a soluble truncated form of ZipA and FtsZ, and between a full length ZipA embedded in a lipid-containing nanodisk and FtsZ was studied in the presence of GDP. In both cases, a single molecule of either soluble ZipA or nanodisk-embedded ZipA can bind with approximately equal affinity to a monomer or any linear oligomer of FtsZ ($n < \sim 6$). The addition of a high concentration of inert proteins was found to significantly enhance the tendency of FtsZ to self-associate in accordance with predictions of excluded volume theory.

80. Archaea in microbial ecosystems associated to minerals in Andean Wetlands: a source of biodiversity and alternative geochemical cycles

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Atacama's lakes are extreme environments where microbial diversity develops associated to minerals, (biofilms, microbial mats, microbialites and endoevaporites). All of them are highly productive systems developing under extreme conditions. Diversity is dominated by Archaea and bacteria which rubisco fijation of C would be precluded. The arising question is how do they obtain energy? How do they fix C? The answer could be in alternative pthways to fix C different than Calvin cycle and in the relationship among As and bacteria. In that way, As(III) has been proposed as an ancient source of energy used by the last universal common ancestor (LUCA) based on As(III) oxidase phylogenetic analysis. In the multi-extreme Lake at Volcano Galan (4650 m), a red biofilm was found. It was composed mainly by Haloarchaea (93%). Metagenomic analysis indicated a high abundance of As(III) oxidases (Aio) and respiratory As(V) reductases (ArrA) coded by Haloarchaea. Phylogenetic analysis supported a pre-LUCA hypothesis. A pure culture of an *Halorubrum* strain obtained from this biofilm, showed the presence of *aio* and *arrA* genes and grew better in the presence of As (III) transforming it to As(V) indicating its functionality. Our results demonstrate, for the first time, that Haloarchaea, respire As through As(III) oxidation and give a strong support to the use of As(III) as a primary source of energy in early forms of life.

81. Nitrogen Regulatory Networks Controlling Plant Root Growth

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

82. Using cofactor engineering and carbon flux perturbations to understand the relationship between pentose-phosphate pathway flux and NADPH production in *Escherichia coli*

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At the steady state, the central metabolic pathways produce intermediates and cofactors at rates matching the stoichiometry of biomass production. When *Escherichia coli* (*E. coli*) feeds on glucose as the sole carbon source, c.a. 75% of the NADPH pool is contributed by the highly NADP-specific dehydrogenases of the oxidative branch of the pentose-phosphate pathway (oxPPP) and the transhydrogenase PntAB. Through the non-oxidative branch, the biosynthetic intermediates not used at the same rate as NADPH are enroued to glycolysis. In this work, we investigate the properties of oxPPP as contributor of the NADPH pool, by using two strategies: changing from NADPH to NADH the cofactor produced by the glucose-6-phosphate dehydrogenase (G6PDH) and altering carbon flux distribution by deletion of the phosphoglucisomerase (glycolysis) and transketolase (non-oxidative branch) reactions. The rates of glucose and O₂ consumption, as well as biomass, acetate and CO₂ production were measured for the different mutant strains in the exponential phase of growth in batch cultures, and used to analyze their metabolic phenotype by Flux Balance Analysis. Our results indicate that in *E. coli* NADPH production by G6PDH is detrimental for growth when the upper part of glycolysis is operative but beneficial when it is blocked, and suggest that the activity of PntAB enables the maintenance of the NADPH pool in spite of carbon flux redistribution between glycolysis and oxPPP. FONDECYT 1121170

83. TEACHING BIOCHEMISTRY USING AN ACTIVE LEARNING APPROACH

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Teaching biochemistry in the current state of science and society requires a special motivation for learning, especially for students of degrees other than Biochemistry or scientific careers. 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, allowing 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 undergraduates who take biochemistry courses as complementary subject on their careers should be seen as an opportunity to complement theoretical aspects on the current courses. Three different approaches could be used: (I) a description of the basic concepts, like in a book but using dynamic figures; (II) Modelled proteins indicating key motifs at their three-dimensional structure and residues where inhibitors can be bound; and (III) elaborating active quizzes where students can followed their learning. Building knowledge based on practical experience can improve student's competence on basic science and the learning process can be complemented by the use of dynamic models. All the information has been compiled in a CD which is distributed to the students.

84. Structural transformation of a transcription factor RfaH

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RfaH, a member of the universally conserved family of NusG-like transcription factors, activates expression of horizontally acquired genes that have a sequence called *ops* in their leader regions. After recruitment to the elongating RNA polymerase at the *ops* site, RfaH reduces transcriptional pausing and termination and is thought to recruit a ribosome to the nascent RNA to initiate translation, thereby eliminating Rho-mediated polarity. RfaH binding to, and anti-pausing modification of, the RNA polymerase are mediated by the N-terminal domain (NTD), whereas the C-terminal domain (CTD) interacts with the ribosome. The CTD undergoes a complete refolding from an α -helical hairpin to a β -barrel in the course of RfaH action. In the α -helical state, the CTD masks the RNA polymerase-binding site on the NTD, preventing unwarranted recruitment to genes lacking *ops*. Upon binding to *ops*, RfaH domains dissociate and the CTD refolds into a β -barrel, allowing binding to the ribosomal protein S10. Our findings reveal that the CTD fold is determined by the NTD availability and suggest that the CTD may refold back into the α -helices when RNA polymerase dissociates from RNA and RfaH, thereby completing the cycle. We propose that similar transformations between two alternative structural states may occur in other NusG homologs, enabling these universally conserved regulators to play distinct regulatory roles by interacting with diverse partners.

85. The landscape of RNAs bound to the L1 retrotransposon ORF1p protein *in vivo*

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LINE-1 (L1) sequences comprise 17% of human DNA. They are the only active and autonomous retrotransposons in our genome. L1 retrotransposition requires the formation of a ribonucleoprotein particle containing at least the L1 RNA and the two L1-encoded proteins, ORF1p and ORF2p. ORF1p is an RNA-binding protein and ORF2p exhibits endonuclease and reverse transcriptase activities essential for L1 replication. The function of ORF1p in this process is poorly understood. It is hypothesized to assemble preferentially *in cis* on the L1 RNA from which it is translated *in vivo*, and accumulates in cytoplasmic stress granules. To get insight into the function of ORF1p in retrotransposition and in other potential cellular processes, we performed high-throughput sequencing of RNA isolated by crosslinking immunoprecipitation of endogenously expressed ORF1p in human cells. We also analyzed gene expression upon shRNA-mediated reduction of ORF1p levels by RNA-seq. As expected, we identified the L1 RNA as a partner of the ORF1p protein. Nevertheless, we also identified many other cellular RNAs bound to ORF1p *in vivo*. Knocking-down ORF1p expression does not impact the steady-state levels of these bound RNAs. The binding *in vivo* of ORF1p protein of L1 retrotransposon to many cellular RNAs suggests a role for ORF1p in regulating gene expression at post-transcriptional levels. G.C. Lab is supported by Avenir (INSERM-INCa) and European Research Council Starting Grants.

86. Light and Time, two variables governing transcriptional changes and virulence in fungi: the plant-pathogen *B. cinerea* as a case study.

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The non-pathogenic fungus *Neurospora crassa* has been a premier model for the study of circadian clocks, but it is the only fungus where these subcellular pacemakers have been molecularly characterized. In *N. crassa* the circadian clock is based on a transcriptional-translational feedback loop that impinges rhythmicity to at least 10% of the transcriptome. This implies the existence of transcriptional cascades through which temporal and light information travel. We are using several high-throughput strategies, as bioluminescence-based screens and Protein Binding Microarrays, to understand this process. In addition, we have also focused on the impact that circadian clocks have on quantifiable traits, as virulence. Thus, we are dissecting the mechanisms by which the plant-pathogenic fungus *Botrytis cinerea* senses light and coordinates daily gene expression. We have found that *B. cinerea* mutants in clock components exhibit impaired pathogenicity. Moreover, using *Arabidopsis thaliana* as a model, we have observed that the time of the day at which the fungus interacts with the vegetable tissue is key in determining its virulence. These results provide the first evidence of functional clock and light-sensing machinery in *B. cinerea*, which affect the plant-pathogen interaction. Currently, through comparative genomics and transcriptomics we are exploring how the clock feeds into the existing transcriptional networks and modulate physiology and virulence. FONDECYT1131030

87. Role of reactive oxygen species generated in chloroplasts during the interaction of plants with pathogens

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Plants react to invading microorganisms by eliciting a hypersensitive response involving expression of pathogen-associated genes, production of reactive oxygen species (ROS) and localised cell death (LCD) at the site of accession. Virulent pathogens usually overcome this response, leading to infection and disease. ROS are generated in different compartments (chloroplasts, mitochondria, apoplast), and the role played by these various sources in the overall response is largely unknown. Plants expressing cyanobacterial flavodoxin (Fld) display increased tolerance to multiple sources of abiotic stress. Fld acts as a generalised antioxidant, preventing ROS build-up in plastids without affecting their accumulation in other compartments, providing a tool to probe the role played by plastid ROS in plant-pathogen interactions. We exposed Fld tobacco lines to *Pseudomonas* strains displaying different interactions (non-host, avirulent, virulent). In non-virulent interactions, LCD and ROS production were light-dependent and protected by Fld. Death was also evident in virulent interactions but was light-independent and differed from the non-virulent ones phenotypically and biochemically. Non-virulent lesions were dry and chlorotic whereas virulent ones were moist and necrotic. Lipid oxidations were mediated by ROS and enzymes, respectively. These differences are discussed in the context of the mechanisms by which plants and attacking parasites sense and contain each other.

88. PLANT LEAF DEVELOPMENT—A SYSTEMS APPROACH

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The development of a young leaf still engaged in cell division into a fully expanded, photosynthetically active leaf is characterized by distinct phenotypic profiles and molecular profiles of the individual cellular components. To reveal the systems behavior of leaf development we combined an integrated analysis of quantitative transcript and large-scale protein measurements at four different stages of Arabidopsis leaf number 6 development with phenotypic information. This established how dynamic RNA and protein patterns relate to phenotypic changes during leaf development. We found that an unexpectedly large number of transcripts show strong stage and condition dependent diurnal fluctuations, but are not matched by protein level fluctuations. Across the growth stages the protein and transcript data displayed a similar trend with some notable exceptions that could be related to protein localization. Comparing the molecular profiles of leaf growth in continuous water deficit revealed an unexpected adaptation response at transcript and protein levels that is different from the typical and well-established drought stress response. Together, this unprecedented dataset establishes the basis for dissection of gene networks that control leaf development and quantitative outputs at the tissue-scale level. Baerenfaller et al. (2012) Systems-based analysis of Arabidopsis leaf growth reveals adaptation to water deficit. **Mol Systems Biol** 8: 606 Funded by EU FP6 (LSHG-CT-2006-037704)

89. Functional and structural characterization of the light-activated transduction pathway from *Brucella*.

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Bacteria use sensor histidine kinases (HK) to detect environmental signals and to induce the corresponding metabolic adaptation. Upon detection of the environmental signal, HK autophosphorylates in a conserved histidine residue. The phosphoryl group is transferred to the second component, a response regulator (RR), that typically binds DNA and activates the transcription of target genes. In previous work we have identified a LOV domain containing HK protein from *B. abortus* (LOVHK) that is important for the virulence of the bacteria. This protein contains an N-terminal photosensory LOV domain, a PAS domain and a C-terminal histidine kinase domain. The LOV domain binds FMN as cofactor and upon illumination with blue-light performs a self-contained photocycle that activates the autophosphorylation of the HK domain. Structural analysis of LOV domain shown that a beta sheet and a C-terminal alpha helix from the LOV domain are the key structural elements involved in the mechanism of signal transduction to other protein domains. The crystal structure of HK domain suggest that this domain suffer large conformational changes involving the N-terminal alpha helices. Our analysis points to the alpha helices connecting the different domains as the key elements for intramolecular signal transduction. This mechanism increase the autophosphorylation which initiates a signal transduction pathway that controls the expression of relevant genes for *Brucella* virulence.

90. The role and treatment potential of natural killer T cells in cancer

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Invariant natural killer T (iNKT) cells are cytotoxic T lymphocytes with semi-invariant antigen receptors that recognize glycolipids presented by CD1d. Upon activation with glycolipids, iNKT cells can kill tumor cells, secrete cytokines and activate other cells of the adaptive immune system. Therapeutic activation of iNKT cells in mice can prevent tumor growth, but clinical trials in humans have to date been disappointing. To translate findings from murine studies into cancer therapies for humans, we have characterised the antitumor activities of human iNKT cells. Human iNKT cells are found at 100-fold lower frequencies in blood but are enriched in certain body locations and depleted in cancer patients. They have distinct cytokine profiles and adjuvant effects for dendritic cells and B cells compared to murine iNKT cells. Human iNKT cells comprise 3 distinct subsets and while CD8⁺ and CD4⁺CD8⁻ iNKT cells promote antitumor immunity, CD4⁺ iNKT cells may inhibit antitumor responses. Synthetic glycolipids can be used to optimise the antitumour potential of iNKT cells. Thus, tumour immunotherapy involving human iNKT cells may require the use of sorted iNKT cell subsets and selected glycolipid cocktails. Alternatively, our studies have led to the discovery of other novel CD1d-restricted T cells with antitumor potential. This work was supported by Science Foundation Ireland, Irish Health Research Board and the Irish Research Council.

91. Salicylic acid and reactive oxygen species interplay in the defense response to stress in *Arabidopsis*

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Salicylic acid (SA) and reactive oxygen species (ROS) are key signals involved in defense responses to biotic and abiotic stress in plants. Evidence supports the idea that SA is crucial to control cellular redox homeostasis in stressed plants. Looking for clues to understand this role of SA, we identify the subset of SA-induced genes that mediate early defense responses in *Arabidopsis*. A subset of these genes code for antioxidant and detoxifying enzymes, such as glutaredoxins (GRX) and glutathione S-transferases (GST). We studied the transcriptional activation mechanism and the role of GRX and GST genes in the defense response to stress. These genes are early and transiently induced by SA and stress, by a mechanism independent or partially dependent of NPR1 co-activator. Using GRXC9 gene as a model, we show that SA transiently recruits RNA polymerase II to GRXC9 promoter, requiring the constitutive binding of TGA2 to *as-1-like* elements from its promoter. A critical role for TGA2 in activate the expression of antioxidant SA-responsive genes is revealed. On the other hand, gain and loss of function assays performed with GRXS13 allowed to identify it as a member of the ROS-scavenging/antioxidant network in *Arabidopsis*. The interplay between SA and ROS in controlling the expression of antioxidant genes and redox homeostasis in the defense response is discussed. FONDECYT (1100656) and Millennium Nucleus for Plant Functional Genomics (P10-062-F).

92. Resolving power of the 16S rRNA for a global classification of uncultured prokaryotes

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Since the beginnings of the use of the 16S rRNA gene sequence as molecular clock for prokaryotes has revealed a tremendous hidden diversity for which over 99% seem to be uncultivable. Since then, molecular microbial ecology studies have generated millions of environmental sequences that are deposited in public repositories currently exceeding 3.5 million, and contrasts with about 11,000 classified species. For taxonomic purposes our group has created the LTP database (www.arb-silva.de/projects/living-tree) that harbors the 16S rRNA gene sequence of all classified species. With it we have created rational taxonomic boundaries for the higher taxa of *Bacteria* and *Archaea*. We have applied boundaries to check the resolution of different 16S rRNA gene stretches to evaluate the use of partial sequences for the circumscription of taxa. On the other hand, we also evaluated the use of the taxonomic boundaries to construct a taxonomy of environmental sequences equivalent to the taxonomic hierarchy used for cultured organisms. Altogether, the results indicate that only almost full sequences guarantee the circumscription of taxa, but that the complete catalogue of environmental sequences can be used for the construction of a stable hierarchical classification for both the cultured and uncultured microorganisms. Finally, and given the current rate of sequencing, most of the taxa present in common habitats will be discovered by the end of the decade

93. Metabolic rearrangement of *Rhodococcus* bacteria during desiccation

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Rhodococci are non-sporulating bacteria frequently found in desert environments. Desiccation is one of the most critical factors occurring in such extreme places, including several challenges such as carbon starvation, osmotic, oxidative and hydric stresses. In order to understand the metabolic adaptation of *Rhodococcus* to desiccation, we have been performing diverse genomic, transcriptomic and physiological studies. Our results indicated that rhodococcal cells maintain active their metabolism, and exert a metabolic rearrangement to cope desiccation. The mobilization of stored triacylglycerols was essential for cell survival under these conditions. A wide set of genes encoding lipases, enzymes for β -oxidation and glyoxylate pathways, and a respiratory NADH dehydrogenase complex, was induced under desiccation. The carbon flux through the glyoxylate shunt may reduce the amount of NADH in cells, contributing to a decrease of oxidative radicals under such conditions. Lypolysis in desiccated cells may provide precursors for compatible solutes, components of the extracellular polymeric substance (EPS) and energy production. The induction of a (Ni-Fe)-hydrogenase system may provide additional energy to cells under such conditions. The biosynthesis of diverse osmolites (trehalose, ectoine, and glycine), carotenoids and an EPS, was also induced. The reorganization of metabolism seems to be a relevant component of the adaptation of rhodococcal cells to desiccation.

94. Extremely halophilic bacteria, the case of *Salinibacter ruber* as model of study

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Extremely halophilic prokaryotes thrive in environments in where the salt concentrations exceed nearly 10 times that of the seawater. The most relevant extremophilic prokaryotes are members of the *Halobacteriaceae* family within the domain *Archaea* as they thrive in high abundance and diversity in extreme haline habitats. On the other hand, members of the domain *Bacteria* had been thought for many years to be unable to colonize such hypersaline habitats. However, about one decade ago, *Salinibacter* sp. was discovered as being the first extremely halophilic bacterium with ecological relevance by using molecular microbial ecology tools. Since then, hundreds of representatives had been isolated and studied from different point of views as genomics, microdiversity and biogeography. The only cultured species of the genus, *S. ruber*, is a member of the *Rhodothermaceae* family, which appears very loosely affiliated with *Bacteroidetes*. The lifestyle and metabolism resembles very much that of the co-occurring *Archaea*, a fact that explains its late discovery. Since its discovery, hundreds of strains had been brought to pure culture and its taxonomy, genomics, microdiversity and biogeography had been studied by means of the pioneering techniques as genomics and metabolomics, and most probably may be one of the environmentally occurring bacteria that had been most exhaustively studied in the last decade being an excellent model of environmental and autoecological research

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95. Genomic evolution of *Pseudomonas aeruginosa* mutator populations in cystic fibrosis chronic airway infections

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Hypermutability due to deficiency in the DNA mismatch repair system (MRS) is highly selected in *P. aeruginosa* (PA) chronic airway infection (CAI) in cystic fibrosis (CF) patients and it may be an adaptive strategy used by PA for diversification and persistence. However, to date the evolution and dynamics of PA mutators has not been explored at a population level. This work studied the impact of hypermutability on PA genome diversity during its evolution in CAI. Whole genome sequencing was conducted in cross-sectional and longitudinal analyses of 27 isolates from 2 CF patients infected by distinct PA clones. We found that once emerged, MRS-mutator isolates reach high frequencies and dominate the population. Phylogenetic analysis revealed high diversity among contemporary clones due to a high accumulation of mutations skewed towards transitions and towards small indels in simple sequence repeats (SSR). Moreover, we established that SSR together with MRS deficiency can bias mutagenic pathways during CAI. Notably, a *mutS*-carrying-mutation subpopulation showed low mutation frequency evidencing a reversion phenomenon. This is the first recording of such phenomenon in CAI. Even though the evolution of mutators in CAI was marked by negative selection and genetic drift, evidence of parallel evolution in pathoadaptive genes, resulting in the alteration of antibiotic resistance, virulence, motility and attachment functions, was observed.

96. ENDOCANNABINOIDS AND PREGNANCY LOSS

FRANCHI, A¹, ¹CEFYO CONICET.

uman reproduction is rather an inefficient process, with about half of all conceptuses lost before the expected term. Endocannabinoids are unsaturated fatty acid derivatives which act as endogenous ligands for cannabinoid receptors and mimic the effects of natural existing cannabinoids. It has been clearly demonstrated that endocannabinoid signaling influences female reproduction during early pregnancy. Anandamide (AEA), the major endocannabinoid studied so far, has a role in implantation and embryo development. However there is now compelling evidence that aberrant expression of factors involved in the endocannabinoid pathway in the placenta and circulating lymphocytes results in spontaneous miscarriage and poor pregnancy outcomes. We studied the participation of AEA in a murine model of early pregnancy loss in order to develop useful tools to prevent miscarriage. We observed that AEA is involved in the synthesis of the pro-inflammatory molecules such as nitric oxide and prostaglandins that contribute to the embryonic resorption. Our results also demonstrated that AEA induces cell death which is associated with morphological alterations, such as nuclear condensation, DNA fragmentation and upregulation of caspase-3/7 activities.

Also we observed that the metabolism of AEA and the nitric oxide synthesis are regulated by progesterone.

97. Developing tools to follow HIV during T cell virological synapses

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HIV-1 infection can spread from infected to uninfected T cells through adhesive contacts called virological synapses (VS). In this process, cell-surface envelope (Env) initiates adhesion and viral transfer into an uninfected cell. Previous studies from our laboratory using an infectious, fluorescent clone of HIV, allow us to track the movement of Gag in live CD4 T cells and captured the direct translocation of HIV across the virological synapse through video microscopy. We had gain insights in the role of Gag during VS however our understanding of Env is limited. We decide to expand our findings and study the recruitment of Env during VS formation. To track Env we have developed live cell Env tagging methods that are fully compatible with VS mediated infection. Infectivity of cell-free tagged virus was maintained at WT HIV-1 levels. Cell-to-cell transfer of tagged virus through the VS is also similar in efficiency to WT virus. We were able of irreversible binding of Env by co-culturing cells with anti-biotin fluorophore conjugates being fully compatible with cell-to-cell transfer through the VS. Moreover, to follow the relationship between Env and Gag recruitment, we have created a virus with tagged Env and mCherry tag in Gag. With pre-incubation of anti-biotin we observe labeling of endocytosed Env, which cycles between the TGN and the cell surface. This approach may discern whether Env that accumulates at the VS comes predominantly from surface or internalized recycling pools. This work was supported by grants NIAID AI074420, the BWF and the Irma T Hirsch Award to B.K.C.

98. GRK2 as a novel key modulator of insulin signaling

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G protein-coupled receptor kinase 2 (GRK2), first identified as regulator of GPCR, is emerging as a key player in the tuning of insulin response, body weight gain and metabolic rate. GRK2 acts as a negative modulator of insulin signaling in adipocytes/myocytes, and its expression is enhanced in experimental models of insulin resistance (IR), and in blood cells from metabolic-syndrome patients. Global GRK2^{+/-} mice maintain glucose tolerance and insulin signaling in the major insulin-responsive tissues in TNF α -, age- or high fat diet (HFD)-induced IR models, also displaying a leaner phenotype. These mice also show greater energy expenditure and a better thermogenic capacity. Importantly, using a tamoxifen-inducible global GRK2 homozygous (Cre^{+/+}-GRK2^{-/-}) mice model, we find that a decrease in GRK2 levels is able to reverse an already established IR phenotype, triggering protection against HFD-induced obesity, improved glucose tolerance and enhanced insulin signaling in target tissues. Interestingly, GRK2 also modulates cardiac insulin signaling. 9-month-old GRK2^{+/-} mice display enhanced heart insulin sensitivity, increased expression of physiological hypertrophy-related genes, and repression of genes linked to pathological hypertrophy. Moreover, cardiac GRK2 levels increase in IR contexts, such as after HFD. Thus, the enhanced GRK2 expression reported in cardiovascular pathologies would promote a heart-IR state and lead to the impairment of key metabolic and cardioprotective pathways. Overall, our data support GRK2 as a target in the treatment of IR-related pathologies.

99. From the blackboard to cyberspace: keeping up to date with the changes when teaching Biochemistry and Molecular Biology.

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Our world is three-dimensional. Everything around us has depth, volume, and we are used to it. When teaching Biochemistry, we use the blackboard and draw flat cells, shapeless organelles and even worse when correlating function with conformation, using even flatter molecules somehow interacting with each other. As teachers we need to make students understand the importance of shape, motion and change in cellular responses. And here technology comes in handy. Internet is filled with examples of rotating molecules; simple and complex structures are shown from different viewpoints, allowing us to recognize motifs, domains and active sites. Enzymes come to life converting substrates into products. Computers and video can be combined in a presentation filled with information as well as entertainment, making the lecture more amiable without losing content. I started teaching Biochemistry in 1979, getting hold of any technology available, starting with super-8 mm films, recording enzyme kinetics from a black and white monitor showing a hyperbolic saturation curve, to the more sophisticated video and computer resources showing molecular modelling. Even more, why not teach the importance of primary structure when dealing with proteins and DNA using music as an example? These are times of information and we need to use it to convey the message to the students: life depends on conformation, interaction, shape and motion and there is no better way than showing it.

100. Hippocampal synaptic plasticity, which requires Ca²⁺ signals generated by ryanodine receptor (RyR)-mediated Ca²⁺ release, increases RyR expression

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Hippocampal neurons (rat) express the three mammalian isoforms of ryanodine receptor (RyR) channels, which together with the inositol 1,4,5 receptors mediate Ca²⁺ release from the endoplasmic reticulum. We have investigated, via selective RyR inhibition, the role of RyR-mediated Ca²⁺ release in hippocampal synaptic plasticity, which entails changes in synaptic efficacy (LTP, LTD) and dendritic spine morphology. Results will be presented showing that the spine elongation and growth produced by treatment of primary hippocampal neurons with brain-derived neurotrophic factor (BDNF) requires RyR2-mediated Ca²⁺ signals. Moreover, theta burst stimulation (TBS) of hippocampal slices produced sustained LTP, which lasted up to 3 h without decay. Inhibition of RyR-mediated Ca²⁺ release by pre-incubation of hippocampal slices with inhibitory concentrations of ryanodine prevented LTP induction by TBS but did not affect paired-pulse responses, suggesting postsynaptic targets. Analysis of RyR mRNA (q-PCR) and protein (Western blot) levels showed that slices subjected to 1 h of sustained LTP had significantly increased RyR1 and RyR2 mRNA levels and RyR2 protein content relative to slices not stimulated, but did not display changes in RyR3 mRNA and protein levels. We interpret these results as an indication that RyR2-mediated Ca²⁺ release plays a central role in hippocampal synaptic plasticity. Supported by FONDECYT (1100052, 11110322, 3120093) and MILLENNIUM BNI P-09-015F.

101. The sweet growth of plant cells: new players on the O-glycosylation pathway

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Root hairs are single cells specialized in the absorption of water and nutrients. Growing root hairs require intensive cell-wall changes to accommodate cell expansion at the apical end by a process known as **tip growth**. The cell wall of plants is a very rigid structure comprised largely of polysaccharides and hydroxyproline-rich glycoproteins (HRGPs) that include extensins (**EXTs**). However, during plant growth these cells increase 200 times their original size. It has been assumed that chemical changes of wall constituents and wall networks orchestrated by enzymes and cell-wall modifying genes are implicated in the process. Proline hydroxylation, an early posttranslational modification of cell wall HRGPs that is catalyzed by prolyl 4-hydroxylases (**P4Hs**), defines the subsequent O-glycosylation sites in **EXTs** (which are mainly arabinosylated) and AGPs (which are mainly arabinogalactosylated). We explored the biological function of **P4Hs**, arabinosyltransferases, and **EXTs** in root hair cell growth. Biochemical inhibition or genetic disruption resulted in the blockage of polarized growth in root hairs and reduced arabinosylation of **EXTs**. Our results demonstrate that correct **O-glycosylation** on **EXTs** is essential for cell-wall self-assembly and, hence, root hair elongation. The changes that O-glycosylated cell-wall proteins undergo during growth represent a starting point to unravel the entire biochemical pathway involved in plant growth and development.

102. New activities of oxylipins, a class of lipid signals regulating plant immunity.

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Oxylipins are a big family of lipid derivatives produced from fatty acids through complex enzymatic pathways initiated by the action of lipoxygenases (9-LOX and 13-LOX) and alpha-dioxygenases (alpha-DOX). The production of oxylipins from polyunsaturated fatty acids can also occur non-enzymatically in the presence of singlet oxygen or free radicals. Studies with oxylipin deficient mutants showed the participation of the 9-LOX and alpha-DOX pathways in the three layers of defense - **pre-invasion, apoplastic and systemic defense**- triggered by *Arabidopsis* to prevent *Pseudomonas syringae* pvtomato DC3000 infection. In addition we showed that the application of specific oxylipins exerted a defensive activity by triggering stomata closure and reducing bacterial growth and tissue damage. Further knowledge on the mode of action of oxylipins was obtained by characterization of *noxy* mutants (*non-responding to oxylipins*), which are deficient in signaling the response to the 9-LOX derivative, 9-HOT and 9-KOT. In accordance with the role of 9-LOX in plant defense, we found that a high percentage of *noxy* mutants were altered in their response to virulent *Pseudomonas*. Moreover, we found that the *noxy* mutations affected to the signaling pathway activated after cellular damage to maintain cell wall integrity. The location of *noxy* mutations at loci encoding mitochondrial proteins indicated the participation of this organelle in the establishment of robust immunity.

103. Molecular signaling in interactions among plant growth promoting rhizobacteria and plants

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Plant growth promoting rhizobacteria (PGPR) increase growth or reduce stress susceptibility (biotic and abiotic) in plants. Little is known about the molecular aspects of PGPR-plant interactions. We have studied the interaction of *Arabidopsis thaliana* with two closely related β -proteobacteria: the PGPR *Burkholderia phytofirmans* PsJN, and the non-PGPR *Cupriavidus pinatubonensis* JMP134. Both bacteria colonize *A. thaliana* rhizospherically as well as endophytically; use *A. thaliana* root exudates as growth sources and have specific transcriptional responses to the presence of these plant components. Conversely, the plant has specific transcriptional responses to the presence of bacteria, which are markedly different whether bacteria are alive or dead. Only strain PsJN promote plant growth of *A. thaliana*, its effects being observed throughout the whole plant life cycle. Protection to infection by a phytopathogen, or saline conditions is also triggered by strain PsJN. The specific bacterial function is dependent on the promoted plant trait. Among them, production of volatile organic compounds, modulation of ethylene phytohormone levels, isynthesis and degradation of the phytohormone 3-indoleacetic acid; and bacterial quorum sensing, which in turn control motility and exopolysaccharide production genes. These results suggest a complex array of molecular signaling and transcriptional responses leading plant-bacteria interactions. Funded by FONDECYT 1110850. NM-PFG (P10-062-F)

104. Bacterial division: Nucleation and polymerization mechanism of *Escherichia coli* FtsZ.

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FtsZ is a major protein in bacterial cytokinesis that polymerizes to form the Z-ring. The best model to explain FtsZ polymerization is a cooperative mechanism with a critical concentration, where the first step is the formation of a dimer nucleus through a conformational change allowing straight polymer elongation. Subsequently, nucleotide hydrolysis drives depolymerization through a transition between straight and curved states of FtsZ that weakens the interaction between subunits in the polymer. This work assessed experimentally the hypothesis that FtsZ self-assembles in the presence of GDP and GTP and that a conformational change occurs in FtsZ structure when the nucleotides exchange at the active site. The monomer-to-dimer equilibrium indicates a significant fraction of stable dimers at the critical concentration for polymerization, supporting a role of the dimeric species in the first steps of FtsZ polymerization. To study the conformational changes induced by exchange between GDP and GTP nucleotides, i.e., in the presence of straight and curved polymers, respectively, we used time-resolved intrinsic fluorescence of site-directed tryptophan mutants. Changes at the interdomain interface were detected upon nucleotide exchange. These conformational changes were related to a hinge-like movement between domains that might be responsible for the transition between straight and curved polymers.

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105. Direct recruitment of the ribosome by the RNA structure: a general feature of viral IRESes?

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As obligate intracellular parasites, viruses require their host's molecular machinery for most of the steps of their replicative cycle. Many different mechanisms that promote viral mRNA translation over cellular mRNA's have been explored. Among them, the internal entry of the ribosome is a phenomenon during which the translation initiation complex is directly recruited in close proximity of the initiation codon. This translation initiation pathway which requires only a subset of cellular initiation factors depends on the 5'UTR sequence and structure. To date, four main molecular mechanisms relying on four different RNA structures have been characterized. In addition, we have identified an atypical Internal Ribosome Entry Site (IRES) present in the HIV gag coding region. It relies on the ability of the HIV gag RNA to specifically interact with the small ribosomal subunit with a high affinity. Such a property is characteristic of two classes of IRESes (classes III and IV), while the two others (classes I and II) are believed to recruit the initiation complex through direct interactions with cellular proteins. In this work we show that the RNA of class II IRESes also specifically bind to the 40S ribosomal subunit, and using biochemical reconstitution *in vitro*, we propose a new model for the molecular mechanism of class II IRESes. The implication of these results for the evolution of viral translation will be discussed. This work is supported by the CNRS and ANRS.

106. Gene regulation and virulence strategies of the opportunistic pathogen *Serratia marcescens*

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Serratia marcescens is a wide host range enterobacterium that can be isolated from plants, insects and nematodes, also being an opportunistic pathogen of mammals. In recent years, *S. marcescens* has become a growing problem for public health, particularly in hospitalized or immunocompromised patients. However, little is known about factors and mechanisms that contribute to *S. marcescens* pathogenesis within its host. We demonstrate that once internalized in non-professional phagocytic cells, *Serratia* is able not only to persist but also to multiply inside a large membrane-bound compartment with autophagic-like features. Our findings reveal that this pathogen maneuvers the canonical progression of autophagosome maturation, altering the vesicular traffic in order to tailor a suitable niche for survival and proliferation within the host cell. This ability of bacteria to interact, colonize and invade live hosts or abiotic niches relies on their capacity to cope with life-threatening conditions posed by the environment. Two-component systems are one of the most ubiquitous signal transduction mechanisms by which bacteria generate adaptive responses to environmental challenges. We will discuss our recent findings, disclosing the role of the PhoP/PhoQ and RcsCDB systems as master regulators of the expression of determinants required for the interaction of *Serratia* with the host.

107. Regulatory Networks in Fungi Governing Global Responses to Changes in Light and Time

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Most fungi are highly responsive to their immediate environment, having developed sophisticated means to respond acutely to changes in their photic environment as well as circadian clocks to anticipate repeating environmental changes. *Neurospora* has proven to be a tractable model for understanding the networks underlying these responses. In this organism, blue light is detected by FAD stably bound by the transcription factor WC-1, eliciting photochemistry that drives a conformational change in the complex of WC-1 and WC-2 (WCC) resulting in activation of gene expression from promoters bound by the WCC. Changes in light intensity are detected by a second photoreceptive protein, VVD, that again uses FAD as a chromophore (Chen, Dunlap & Loros, FGB 47, 922-9, 2010). The circadian system allows anticipation of recurring environmental changes, and comprises a negative feedback loop wherein the WCC, in the dark, drives expression of *frq*. FRQ stably interacts with casein kinase 1 and with FRH (a putative RNA helicase that does not function enzymatically in the clock), and after phosphorylation-mediated delays, the complex downregulates the WCC (Baker, Loros, & Dunlap, FEMS Microbiol. Rev.36: 95-106, 2012). Using the tools of next generation sequencing, recombineering and luciferase reporters, the molecular details of the clock itself as well as network ramifying from the clock out to primary, secondary, and tertiary targets of light and clock control can now be described.

108. Calcium signaling and calcium sensitization in smooth muscle contraction

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Smooth muscle contraction is activated primarily by an increase in cytosolic free Ca^{2+} concentration due to stimulus-induced Ca^{2+} entry from the extracellular space and Ca^{2+} release from the sarcoplasmic reticulum. Ca^{2+} binds to calmodulin, which activates myosin light chain kinase to phosphorylate the regulatory light chains (LC₂₀) of myosin at S19; this activates cross-bridge cycling and contraction. Many contractile stimuli also induce Ca^{2+} sensitization (increased force without a change in Ca^{2+} concentration) by activation of the RhoA/Rho-associated kinase pathway, leading to phosphorylation of the myosin targeting subunit (MYPT1) of myosin light chain phosphatase (MLCP) at T855, or of the phosphatase inhibitory phosphoprotein CPI-17 at T38. Both phosphorylations result in a decrease in MLCP activity and, therefore, an increase in LC₂₀ phosphorylation. Certain contractile stimuli, e.g. endothelin-1 acting on renal afferent arterioles, induce LC₂₀ diphosphorylation at T18 and S19, which slows the rate of LC₂₀ dephosphorylation and relaxation, resulting in a prolonged contractile response. Two kinases, integrin-linked kinase and zipper-interacting protein kinase, are responsible for LC₂₀ diphosphorylation and are, therefore, potential therapeutic targets for the treatment of pathological conditions, such as cerebral vasospasm following subarachnoid hemorrhage, which are associated with hypercontractility. *Supported by the Canadian Institutes of Health Research.*

109. Neurochemical changes in retinal degenerative diseases: targeting neuropeptide Y as a potential therapeutic strategy

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Diabetic retinopathy (DR) and glaucoma are leading causes of vision loss worldwide and treatments available are not effective. Both diseases are characterized by retinal neural degeneration, being retinal ganglion cells (RGCs) mainly affected, and inflammation has a key role in their pathogenesis. We showed that exposure to chronic elevated glucose and diabetes induce several neurochemical alterations in retinal cells, such as alterations in glutamate release and on the expression and function of ionotropic glutamate receptors, impairment of calcium homeostasis, etc.. Neuropeptide Y (NPY) is a 36 aa peptide, very abundant in the brain, being also expressed in the retina, mainly in inner retinal layers. However, the physiological role of NPY in the retina is unknown. We have found that NPY and NPY receptors can be expressed in different retinal cell types. NPY is able to modulate calcium changes and the spiking activity of RGCs. Moreover, NPY exerts potent neuroprotective effects against retinal cell death and is able to inhibit microglia activation and neuroinflammation. In conclusion, NPY appears to have important modulatory roles in the retina and NPY receptors might be considered potential therapeutic targets for the treatment of retinal degenerative diseases characterized by a neuroinflammatory component. Support: FCT, Portugal (PTDC/SAU-NEU/099075/2008, PTDC/NEU-OSD/1113/2012, PEst-C/SAU/UI3282/2011 and 2013 and PEst/SAU/LA0001/2011), and COMPETE/FEDER.

110. Subsurface geomicrobiology of an extreme acidic environment, Río Tinto

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The microbial ecology characterization of Río Tinto, Iberian Pyrite Belt (IPB), has proven the importance of the iron cycle, not only in generating the extreme conditions of the environment but also in maintaining the high level of microbial diversity, mainly eukaryotic, detected in the water column. The extreme conditions of the Tinto basin are the consequence of a very active subsurface bioreactor sustained by the high concentration of metal sulfides existing in the IPB. A preliminary drilling project (Mars Analogue Research and Technology Experiment) was carried out to intersect ground waters interacting with the mineral ore to provide evidence of subsurface microbial activities. Currently we are developing the IPBSL drilling project to further characterize the microbial diversity existing in the subsurface of the IPB. The geological analysis of the retrieved cores showed an important alteration of mineral structures associated to the presence of water. Ion chromatography of soluble compounds showed the existence of putative electron donors, electron acceptors and a variable concentration of organic acids, which suggest the presence of an active subsurface ecosystem. Enrichment cultures from selected samples showed evidences of active iron and sulfur cycles, together with methanogenic, methanotrophic, denitrification and acetogenic activities. Our current knowledge of the subsurface geomicrobiology of the IPB will be presented.

The IPBSL is an advanced ERC grant

111. Mechanisms of Action and Health Benefits of Flavonoids: Is there a Role in Cardiovascular Diseases?

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Atherosclerosis, the underlying cause of ischemic heart disease and stroke, is an inflammatory disease of arteries in a hyperlipidemic milieu. In addition, oxidative stress and redox imbalances in cell signaling have been implicated. Increased fruit and vegetable consumption is associated with a decreased incidence of cardiovascular diseases, including heart disease, stroke, and hypertension. Most of these health benefits of fruits and vegetables are derived from their high content of micronutrients and fiber. In addition, fruits and vegetables contain a large number of flavonoids, which also may exert beneficial health effects. Flavonoids have antioxidant properties *in vitro*, but cannot make significant contributions to "free-radical scavenging" *in vivo*. Acting as xenobiotics rather than nutrients, flavonoids elicit Phase I and II responses and, via Nrf2 activation, increase endogenous GSH synthesis. This may "indirectly" increase antioxidant protection *in vivo*. In contrast, certain flavonoids activate cell signaling pathways resulting in increased endothelial nitric oxide synthase activity, which in turn improves vasodilation and inhibits platelet aggregation. Another non-antioxidant mechanism may be decreased vascular inflammation, as indicated by inhibition of endothelial adhesion molecule and chemokine expression. Together, these mechanisms may contribute to the lower risk of cardiovascular diseases observed with increased fruit and vegetable intake.

112. Conserved mechanism for catalysis in hyperthermophilic ADP-dependent kinases from archaea

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The Embden-Meyerhof pathway is highly conserved between different organisms. However, several archaea have evolved major modifications, being one of the most striking the presence of ADP-dependent glucokinases (GKs) and phosphofructokinases (PFKs), which represent a unique family of kinases that belong to the ribokinase superfamily. For these enzymes there is no agreement about the magnitude of the structural transitions associated with ligand binding and whether they are meaningful to the enzyme function. We used the ADP-dependent glucokinase from *Thermococcus litoralis* as a model to investigate the conformational changes observed in X-ray crystallographic structures upon substrate binding and to compare them with those determined in solution in order to understand their interplay with the glucokinase function. We employed a multidisciplinary approach combining the determination of the kinetic mechanism, small angle X-ray scattering (SAXS) and X-ray crystal structure determinations of the enzyme in the apo form and as a ternary complex form; this afforded the direct comparison of the structures of the same enzyme in all ligand states.

The combined data allowed us to resolve the open-closed conformational transition that accounts for the complete reaction cycle and to identify the corresponding clusters of amino acids residues responsible for it. These results provide molecular bases for a general mechanism conserved across the ADP-dependent kinase family. Fondecyt 1110137.

113. Strategies to improve scientific literacy of school teachers using hands-on inquiry-based Biochemistry courses

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In the last decades, Brazil has reached a prominent position in the world rank of scientific production. Despite this progress, the establishment of a scientific culture in Brazilian society is still challenging. Our group has been offering hands-on inquiry-based courses to primary and secondary students, which aim to introduce them to the scientific method and improve their interest in science. More recently, we started new initiatives focused on the improvement of the scientific literacy of school science teachers. Here we describe two intensive short-term courses offered to school teachers or to last-year students of Biological Sciences courses. One of them was developed in a small city in Amazonia region, which presents great flora diversity and unique popular culture. There, we trained science teachers to act as tutors in an inquiry-based course offered to primary and secondary students in the city. The other initiative consists in a discipline offered to a Master Program to school science teachers, in which the main objective was to work with core disciplinary concepts through an active teachers engagement in "doing science". The discipline, named "Energy transformation in the living organisms", intends to deal with the main Biochemistry subjects that take part of the high-school science curriculum, namely, fermentation, photosynthesis and cellular respiration processes. In all editions of those courses, it was notable the improvement on teachers understanding of the concepts worked together with a differentiated vision about science.

114. Photobiology in Model and Clinical Fungi

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Light is an indispensable energy source for life and also serves as influential environmental information of daily and seasonal time to organisms across kingdoms. Within the fungi, molecular mechanisms of light responsiveness are best understood in the model system *Neurospora crassa*. 6% of the *Neurospora* genome is light inducible at the level of gene expression, controlled largely by the LOV domain transcriptional activator White Collar-1 (WC-1) in complex with White Collar-2 (WC-2). This acute response to light results in cascades of transcriptional activators and repressors that eventually control biological outputs including development and general metabolism. An additional LOV containing photoreceptor, Vivid (VVD) permits the organism to repress the initial light response and then respond to increasing amounts of light, a process called photoadaptation, and also facilitates accurate entrainment of circadian rhythms. We have begun the molecular characterization underpinning a complex response to light in the clinically important fungus *Aspergillus fumigatus*. It responds to red and blue light, with melanin pigmentation, conidial germination and stress responses under control of multiple photoreceptors including the WC-1 homolog LreA and a phytochrome, FphA playing unique and overlapping roles. Both the light responsive physiology of this organism and its photobiology are complex and involves input from additional, as yet uncharacterized, photosensory pathways.

115. Novel insights into bacterial signaling : the 'one sequence - multiple structures' perspective

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We have focused on the study of two-component systems (TCS), key players in bacterial signaling, to better understand signal-transmission with molecular detail. The TCS DesK/DesR controls fatty acid desaturation in *Bacillus subtilis* in response to cold shock and other membrane-altering effectors. We had previously put forward a model of signal-dependent allosteric control of the sensor kinase catalytic activity [1,2]. We have now turned our attention to the response regulator DesR. A canonical activation pathway has been widely accepted to explain phosphorylation-mediated control of response regulator function, allosterically coupling the phosphorylation site to the $\alpha 4\beta 5\alpha 5$ surface. Here, using a combination of crystallography, SAXS and *in vitro/in vivo* functional analyses, we unveil DesR activation control by phosphorylation [3]. A novel, non-canonical route was identified, that couples phosphorylation to dimerization and cognate kinase binding. Bacterial signaling has become a good example of the 'new view' in protein structure/function principles, with 'old roots' in the MWC model of allostery and Weber's contributions to proteins' conformational equilibria. One sequence allows proteins to adopt more than one structure, and these alternate structures are frequently the basis of switching and transmission of information. [1] Albanesi *et al.* (2009) *PNAS* **106**:16185 [2] Trajtenberg *et al.* (2010) *JBC* **285**:24892 [3] Trajtenberg *et al.* (2013) *submitted*

116. Control of Resting Free Ca²⁺ in the Cytoplasm of Skeletal Muscles: Signal Crosstalk from Inside to Outside and Back Again

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Ca²⁺ is a key signal in almost all cells, and is of critical importance in excitable tissues such as skeletal muscle, heart and the nervous system. In most cells the resting free Ca²⁺ concentration ([Ca²⁺]_i) in the cytoplasm is approximately 100nM. The boundary theorem proposes that this is the result of an equilibrium of Ca²⁺ in and out through the sarcolemma. Whereas there is no question that the vast majority of Ca²⁺ in any tissue is in the extracellular space where the concentration is 10,000 fold higher than inside the cell, as it is usually written this ignores the equilibrium inside the cell between Ca²⁺ leak and uptake across the ER/SR membrane. In addition it also ignores the signals from the ER to the sarcolemma to maintain ER Ca²⁺ stores and in excitable cells the cross talk between ER/SR Ca²⁺ release channels (RyRs) and sarcolemmal proteins, among them the slow voltage gated Ca²⁺ channel, which in addition to its role as the voltage sensor in skeletal muscle also acts as a negative regulator of both the active gating and leak state of the RyR. In addition both through classical ICRAC interactions and other less understood mechanisms, signals are passed from the SR to the surface membrane Ca²⁺ entry pathways. These equilibria are frequently altered in both clinical (e.g. DMD) and silent (e.g. MH) myopathies resulting in elevated [Ca²⁺]_i and can ultimately lead to cell death.

117. Understanding the operation and regulation of metabolic pathways requires elementary kinetics: the cases of Metabolic Control Analysis and Substrate Channeling.

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Enzyme kinetics is essential for understanding how enzymes work, and also for the comprehension of the operation and regulation of metabolic pathways. Examples illustrating the importance of elementary kinetics in understanding Metabolic Control Analysis (MCA) and Substrate Channeling will be discussed, showing that these subjects may be addressed in biochemistry courses for undergraduate students.

The development of MCA provided a theoretical framework for the quantitative estimation of the degree of control exerted by a particular enzyme over the flux and on the concentration of intermediates on a given pathway. Relevant in this context are the estimations of the control and elasticity coefficients for each enzyme involved in the pathway, in replace of the qualitative misleading concept of rate limiting enzyme.

From a structural point of view, strong evidence suggests that sequential enzymes in a pathway are organized in supramolecular complexes in which substrate channeling may occur. One advantage is the reduction of the transit time for the movement of intermediates from one active site to the next. Substantial kinetic evidence supports this statement, including measurement of the transit time of reaction intermediates, isotope dilution of endogenous reaction intermediates, competing reactions, and transient-state kinetics. In addition, the existence of allosteric communication between active sites has been shown to be essential for efficient channeling.

118. Entry and Exit of Alphaviruses and Flaviviruses

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Our research focuses on the molecular mechanisms of virus-membrane fusion and virus budding using alphaviruses and the closely related virus Rubella virus (RuV), and flaviviruses such as dengue virus (DENV). Alphaviruses, RuV and flaviviruses enter cells by endocytic uptake and then fuse their membrane with the endosome membrane in a reaction triggered by the low pH of the endocytic vesicle. During alphavirus and flavivirus biogenesis, a companion protein forms a closely-associated dimer with the fusion protein, and is believed to protect it from low pH and premature fusion during exocytic transport. We have defined key residues that mediate these dimeric interactions in the alphaviruses and DENV and demonstrated that they are required for pH protection and virus production. The DENV companion protein must be proteolytically matured to permit virus fusion, and we have identified a critical residue in that promotes the low pH-dependent maturation step, allowing capture of a DENV maturation intermediate. Our studies of RuV fusion have defined a novel and critical role for calcium in the virus fusion mechanism. Calcium binds conserved residues in the two fusion loops of the fusion protein, and is required for the interaction of the fusion protein with the target membrane and for virus fusion and infection.

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119. From sugars to protein folding in the secretory pathway: the role of Glucosidase II beta subunit

Orsi, R², Stigliano, I², Peterson, F¹, Olson, L¹, Alculumbre, S², Dahms, N¹, Parodi, A², D'Alessio, C^{2,3}, Department of Biochemistry Medical College of Wisconsin, Milwaukee, WI, USA.²Fundacion Instituto Leloir IIBBA CONICET, Buenos Aires, Argentina.³School of Sciences University of Buenos Aires.

Glucosidase II (GII) is a key regulator of the quality control of glycoprotein folding mechanism in the endoplasmic reticulum (ER). GII is responsible for sequentially removing the two innermost glucoses from the Glc₃Man₉GlcNAc₂ glycan transferred upon protein *N*-glycosylation and of the glucose residue added to folding intermediates by the ER glycoprotein conformational sensor, the UDP-Glc:glycoprotein glucosyltransferase. GII is a heterodimer composed of a catalytic GII α subunit and a regulatory GII β subunit. We demonstrated that GII β is involved in GII α 's ER localization and mediates *in vivo* enhancement of *N*-glycan trimming by GII through its C-terminal mannose 6-phosphate receptor homology (MRH) domain. Moreover, GII regulates the permanence of slow folding proteins in the ER as it shows a decreased activity toward demannosylated *N*-glycans present in such species, thus prolonging the half-lives of monoglucosylated glycans able to interact with ER lectin/chaperones calreticulin and calnexin. We determined the structure of a functional GII β MRH domain by NMR spectroscopy. We identified a conserved residue (W409) present only in GII β MRH domains that influences GII glucose trimming activity *in vivo*. A model of the GII β MRH domain bound to Man₉ provides insight into how GII β enhances the catalytic activity of GII and gives an explanation for the reduced GII activity toward slow-folding demannosylated glycoproteins. Grants: ANPCyT, CONICET, Mizutani, NIH, HHMI

120. Protein Evolution During the Archean Anaerobic/Aerobic Transition

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The atmosphere in the Archean, about 3.5 x 10⁹ years ago, is considered to have been reducing consisting of CO₂-N₂ and a little H₂. Life in this period would have used principally sulfur, iron, perchlorate and nitrate as electron sinks. Oxygen did not become a major electron sink until the rise of oxygenic photosynthesis starting about 2.7 x 10⁹ years ago, known as the great oxidation event (GOE). Some anaerobes survived the GOE in residual anoxygenic niches, but most had to adapt to rising oxygen levels that challenged them to evolve mechanisms to combat oxidative stress but also provided them with a new, abundant and energetically favorable electron sink. This presentation will discuss models for the evolution of electron transport proteins that allowed the exploitation of oxygen. It is our contention that these proteins arose mainly by coopting previously existing folds and domains used for the anaerobic reduction of sulfur, iron, perchlorate and nitrate rather than by the generation of *de novo* folds. We further hypothesize that their origin preceded the GOE by many millions of years and first evolved in acidophilic microorganisms living in "oasis of oxygen". This is the most parsimonious hypothesis consistent with the phylogenetic data, defining a unique chronology of structural innovation and shows the power of molecular archaeology to uncover genomic repositories of evolutionary history.

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121. Protein N-glycosylation precursors: novel functional role in lateral root development in *Arabidopsis thaliana*

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Protein N-glycosylation has an important role in protein trafficking and functionality. Dolichol biosynthesis is the first and limiting step for protein N-glycosylation. Dolichol is the N-glycan acceptor at the endoplasmic reticulum synthesized by the reduction of polyprenol. In *S. cerevisiae* and human the enzymes responsible for dolichol biosynthesis have been highlighted as key component in protein N-glycosylation. The loss of their function causes impairment of protein N-glycosylation and protein trafficking as well as severe defects at whole organism. In *Arabidopsis* we have found three ortholog genes to the known polyprenol reductases: the *AtDFG10-1* to *-3*. All of these gene products share the functional domain at their C-terminal which has been directly related to N-glycosylation. Reverse genetic has revealed that *AtDFG10-1* and *-2* have role in postembryonic lateral root (LR) development. We have shown that LR formation stimulated by the chemical Sortin2 is linked to its effect over endocytic trafficking. Interestingly, the loss of function of *AtDFG10* leads to resistance to Sortin2 cellular effect in the endocytosis as in terms of LR induction. Therefore, a link between *AtDFG10s* function and LR formation via endocytic trafficking can be established. Overall, the evidences indicate *AtDFG10-1* and *-2* are new functional endomembrane system components with a promising role in protein N-glycosylation and critical impact in LR development in *Arabidopsis*. FONDECYT 11080240, 1120289

122. Atypical Function of the Typical Chemokine Receptor, CCR1

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Increasing structural information about the mechanisms underlying ligand-dependent receptor activation is beginning to emerge with the recent expansion in GPCR crystal structures. However, some GPCRs are also able to adopt active conformations in the absence of agonist that result in the initiation of signal transduction and receptor down-modulation. In this presentation, we show that the chemokine receptor, CCR1, exhibits significant constitutive activity leading to a variety of cellular responses. CCR1 expression is sufficient to induce inhibition of cAMP formation, increased F-actin content, and basal migration of leukocytes. The constitutive activity leads to basal phosphorylation of the receptor, recruitment of β -arrestin2 and subsequent receptor internalization. CCR1 concurrently engages $G\alpha_i$ and β -arrestin2 in a multi-protein complex, which may be accommodated by homo-oligomerization. The data suggests the presence of two functional states for CCR1; whereas receptor coupled to $G\alpha_i$ functions as a canonical GPCR albeit with high constitutive activity, the CCR1: β -arrestin2 complex is required for G protein independent constitutive receptor internalization. The pertussis toxin-insensitive uptake of chemokine by the receptor suggests that the CCR1: β -arrestin2 complex may be related to a potential scavenging function of the receptor, which may be important for maintenance of chemokine gradients and receptor responsiveness in complex fields of chemokines during inflammation. This work was funded by NIH U01 GM094612 and R01 GM081763 to TMH and RO1 GM090689 to JT

123. Novel candidate regulators of lipid droplet metabolism revealed by high throughput and chemogenomic analyses

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Lipid droplets are dynamic intracellular organelles that store the neutral lipids. Manipulation of their content is important to attain high levels of triacylglycerol for biotechnological purposes. Acetyl-CoA carboxylase (ACCase) is a key enzyme in fatty acid synthesis and its activity is correlated with the amount of TAG synthesized. The signaling pathways that govern lipid droplets dynamics are not well understood. To discover novel ACCase regulators we screened a ~4860 knockout yeast library for differential content of lipid droplets and for tolerance to an ACCase specific inhibitor. By a chemiogenomic approach we were able to build an hybrid network by combining genetic/physical gene-gene interactions and drug-gene interactions which allowed us to map new genes that interact with ACCase. By overlapping these results to those obtained in the lipid droplet screen we identified 12 novel genes whose deletion led to a positive correlation between ACCase activity and intracellular lipid content. Among the novel regulators we found that the Ser-Thr phosphatase Sit4 modulates an important regulator of ACCase, the Snf1/AMPK kinase. We also describe the ubiquitin proteasome-dependent degradation pathway as novel regulator of acetyl CoA carboxylase activity. Our results provide a network that can now be manipulated to regulate TAG content in yeast.

124. Temperature-sensitive little machines

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TRPM8 channels are a polymodal receptors activated by voltage, low temperatures and agonists. The structural data suggests that the distal C-terminus (1064–1104 amino acid residues) form a coiled-coil motif. Our aim was to investigate the importance of this structure in the thermo-sensitivity of TRPM8. TRPM8 wild type and deletion mutants of the coiled-coil region of this channel were cloned and expressed in *Xenopus* oocytes. Macroscopic currents were recorded by patch-clamp technique in cell-attached configuration at different temperatures (10°C–40°C). An analysis of the conductance-voltage curves determined from tail currents showed that values of the voltage at which the open probability is 0,5 ($V_{1/2}$) saturate at high and low temperatures. These results and limiting slope analysis of the open probability–voltage curves demonstrated that TRPM8 channel gating is best explained using an allosteric gating model for both voltage and temperature dependence. On the other hand, gradual deletions of the coiled-coil region (from 1064 to 1104 residues) promoted a gradual decrease in the temperature sensitivity of TRPM8 channel, without affecting the voltage dependence. The decrease in the enthalpy (ΔH) promoted by gradual deletions of the distal C-terminus suggests that coiled-coil region is a structural determinant of the thermo sensitivity in TRPM8 channels. Our results also indicate that the voltage and temperature sensors are localized in different structural domains. Supported by FONDECYT grants 1110430 to R.L., CONICYT AT-24100020 to N.R. CINV is a Millennium Scientific Institute.

125. Cancer progenitors and epigenetic contexts.

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In mammals, silencing of one of the two X chromosomes is necessary to achieve dosage compensation. The non coding RNA *Xist* triggers X inactivation. Gene silencing by *Xist* is only possible in certain developmental contexts that exist in cells of the early embryo and specific hematopoietic progenitors. A potential reason for restricting the activity of this silencing pathway might be to safeguard against illegitimate changes of cell identity which could lead to malignant transformation. Critical silencing factors may only be present in these contexts giving an explanation of why *Xist* is not operative outside these contexts. It has been demonstrated that *Xist* is functional in lymphoma tumor cells, where SATB1 was identified as a silencing factor for *Xist* mediated chromosome silencing. Importantly new results suggest that loss of p16 is *aprrerequisite* for SATB1 to *exert its function*. Analyzing *Xist* and SATB1 function in different tumor models could have potential implication for diagnosis and therapy.

Acknowledgments: Prof Anton Wutz, Institute of Molecular Health Sciences, ETH, Zürich, SWITZERLAND. Transgenic and Experimental Animal Unit. Institut Pasteur de Montevideo, Montevideo, URUGUAY.

126. Dissection of the *in vivo* interplay of molecular features determining protein evolution

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Protein evolution can be described as a random walk on a sequence space where mutations are fixed based on their organismal fitness impact. Fitness is a measure of the host organism's ability to reproduce faster at the environment conditions. Two of the major challenges in the field of protein evolution are to understand: how the structural, functional and biophysical features of proteins determine molecular evolution, and how mutations accumulate and impact in these molecular features. The order in which mutations appear is not trivial, since the effect of each mutation depends on the genetic background, a phenomenon known as epistasis.

We have analyzed the fitness effects evinced by the accumulation of mutations in the evolutionary pathways of a Metallo- β -lactamase, a Zn(II)-dependent enzyme able to provide antibiotic resistance to pathogenic and opportunistic bacteria. We have assessed the impact of four different mutations and their combinations in the protein function in the periplasmic space. This approach allows us to fill the gap between the biophysical features determined *in vitro* in purified proteins, and the resistance conferred to bacteria, which ultimately represents organismal fitness. We found that epistatic interactions in the evolutionary pathways can be accounted for by three molecular properties evaluated *in periplasma*: catalytic efficiency, protein stability and enzyme activation by binding of the essential Zn(II) cofactor *in vivo*.

127. G protein coupling to GPCRs: structural and functional insights into the reciprocal G protein and GPCR interaction

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Recent advances in the structural biology of G protein-coupled receptors have unraveled many intricacies of ligand binding. Likewise, structural and biochemical properties of heterotrimeric G proteins have enhanced our understanding of the mechanism underlying effector interactions and GTPase activity. Our recent efforts to elucidate the crystal structure of the β_2 -adrenergic receptor (β_2 AR), in a complex with the stimulatory G protein, Gs, have provided insight into the mechanism of activation of G proteins by GPCRs. These data have helped to delineate how hormone binding to GPCRs leads to GDP release on G proteins, the principle step that precedes GTP binding and G protein activation. The crystal structure, complemented by analyses by electron microscopy and deuterium exchange mass spectrometry, reveal dramatic changes in the G protein α -subunit. Some of these changes reveal how G proteins in turn allosterically regulate the receptor itself by stabilizing a 'closed' conformation on the extracellular face of the receptor. The 'closed' conformation is supported by ligand binding studies, the kinetics of which suggests that nucleotide-free G proteins slows ligand dissociation. Moreover, that coupling of a nucleotide-free G protein to a ligand-free, or empty receptor, will also stabilize the 'closed' conformation and physically prevent a ligand's access to the hormone binding site. Such structural changes and the slower observed ligand dissociation rates account for the G protein-dependent high affinity binding properties of agonists. Together these data support a plausible model for the mechanism for receptor-mediated nucleotide exchange, G protein activation and agonist binding.

128. Flexible Protein Structures in Plant Drought Responses

Covarrubias, A¹,¹- U. Nacional Autónoma de México.

The dogma that relates to the function of a protein with a defined three-dimensional structure has been challenged over the last years by the discovery and characterization of proteins known as intrinsically unstructured or disordered proteins (IUPs or IDPs). These proteins have a high structural flexibility, which enables them to adopt different structures and recognize various ligands while preserving their specificity. Examples of these, among many others, are certain transcriptional factors as p53 or GCN4, cyclins, or the cycline-dependent kinase inhibitors as Sic1 and p27, or α -synuclein involved in Parkinson's disease. Interestingly, the structural versatility in these proteins has been associated in some cases with multifunctionality. Proteins that have similar properties to those found in unstructured proteins have been described in plants. These proteins are highly hydrophilic and have a high content of glycine and other small amino acids and have been called 'hydrophilins'. LEA (Late Embryogenesis Abundant) proteins are plant hydrophilins, whose accumulation is closely linked to water deficit conditions, imposed during development (e.g. seed or pollen) or by changes in the environment such as drought, salinity or freezing. The functional characterization of these proteins has shown that they are required for an optimal plant response against water deficit. Moreover, *in vitro* partial dehydration assays indicate that they are able to protect other proteins of the deleterious effects caused by low water availability.

I will present and discuss some of the findings regarding their role of LEA proteins in plant adaptation to water deficit, as well as those concerning to their function, and how they relate to their intrinsic structural flexibility.

129. SORBITOL DEHYDROGENASE is a cytosolic protein required for sorbitol metabolism in *Arabidopsis thaliana*

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In *Rosaceae* species, sorbitol is produced in source organs and phloem-transported to sink organs where, via sorbitol dehydrogenase (SDH) it is oxidised to fructose, which is then stored or metabolised. However, in almost all other families, including *Brassicaceae*, sucrose is the main sugar transported. Nevertheless, sorbitol and/or SDH activity have been detected in some of these families, but their physiological roles are poorly understood. To determine the roles of sorbitol and related enzymes in non-sorbitol translocating species, we identified AtSDH in *Arabidopsis thaliana*. Using a GFP-tagged version and anti-SDH antisera, we determined that SDH is cytosolically localised, consistent with bioinformatic predictions. SDH accumulates in both source and sink organs, and in the presence of NAD⁺, recombinant SDH exhibits greatest oxidative activity with sorbitol, ribitol and xylitol as substrates. Under normal growth conditions, *sdh*- mutants develop as wild-type; however, they exhibit reduced dry weight and primary root length compared to wild-type when grown in the presence of sorbitol. Additionally, under short-day conditions, the mutants are more resistant to dehydration stress, with a reduced loss of leaf water content when watering is withheld, and a greater survival rate on re-watering. This evidence suggests that limitations in the metabolism of sugar alcohols alter the growth of *Arabidopsis* and its response to drought. Funding: Fondecyt 1100129.

130. Coupling of neuronal activity with local blood flow in normal and Alzheimer's rat brain: the direct role of nitric oxide

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Traditionally, the understanding of communication in the brain has been based on the idea that neurons communicate serially across chains of synapses. This notion has been reformulated as new mediators support that neurons broadcast their signals widely, targeting different cell types. This is critical for the neurovascular coupling process and, ultimately, for brain health.

Following neuronal activation the increased need for O₂ and glucose require a tight controlled mechanism by which a signal is sent from neurons (producer site) to pericytes/endothelial/smooth muscle cells (transducer site) in order to achieve an increase in cerebral blood flow (CBF). Any impairment in this process, the neurovascular coupling (NVC), may be translated into dysfunction associated with toxic phenomena and disease.

We here provide the proof of concept that nitric oxide (NO) originating from the neuronal isoform of nitric oxide synthase (nNOS) upon glutamatergic stimulation diffuses towards vessels, inducing a localized increase of blood flow with consequent provision of energetic substrates to neurons. We have done this in vivo and in real-time by simultaneously recording NO production, blood flow and oxygen tension by using a tri-component microsensor array consisting of ¹⁵N-selective microelectrode, an ejection pipette and a laser Doppler sensor (for blood flow measurements) inserted stereotaxically in the brain of anesthetized rat (Wistar). The pathophysiological implications of NO-mediated neurovascular coupling were further assessed in a triple-transgenic model of Alzheimer's disease, AD, (3xTg-AD).

Data supports that the impairment in neurovascular coupling in AD likely results from a dysfunction at the transducer site (vasculature), rather than at the signal producer site (NO-producing neurons).

Thus, neuronal-derived NO, via diffusion, is a direct mediator of neurovascular coupling and the coupling is impaired in the later stages of AD at transducer vascular sites.

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POSTERS

1. Insulin reverses gestational diabetes-increased L-arginine transport involving activation of insulin receptors A and B in human umbilical vein endothelium.

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Gestational diabetes (GD) associates with higher expression and activity of cationic amino acid transporters 1 (hCAT-1) in HUVEC. Insulin receptor A (IR-A) and B (IR-B) isoforms are expressed in HUVEC. Insulin reduces L-arginine transport in HUVEC from GD, but the role of IR-A and IR-B is unknown. We evaluated whether insulin reverses GD-increased L-arginine transport and hCAT-1 expression involving IR-A and IR-B in HUVEC. L-Arginine transport (31-1000 μ M, 3 μ Ci/ml, 37°C, 1 minute), hCAT-1 expression and transcriptional activity were measured in cells from normal (Nc) or GD (GDc) pregnancies. Cells knockdown for IR-A (Ad-IR-A) or IR-B (Ad-IR-B) were preincubated (8 hours) with insulin (1 nM). GD associates with higher maximal velocity (V_{max}) without changes in apparent K_m for L-arginine transport, increased hCAT-1 protein abundance, mRNA levels and transcriptional activity compared Nc. Similar results were found in Nc exposed to insulin. Insulin reverses GD-increased L-arginine transport, hCAT-1 expression and transcriptional activity. Insulin effects in Nc and GDc were abolished in Ad-IR-A and Ad-IR-B cells. In conclusion, insulin reverses GD-increased hCAT-1-mediated L-arginine transport and expression requiring expression of both insulin receptor isoforms. Support: CONICYT ACT-73 PIA, AT-24120944, FONDECYT (1110977/1080534/3130583). EG-G and TS hold CONICYT-PhD fellowships. RS holds Faculty of Medicine, Pontificia Universidad Católica of Chile fellowship.

2. Adaptive responses of mitochondria when under mild copper deprivation, involve changes in morphology, oxphos remodeling, and bioenergetics.

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If it breathes, then it needs copper. Copper is essential for cytochrome c oxidase and involved in the generation of mitochondrial membrane potential ($mt\Delta\Psi$). OXPHOS remodeling, mitochondrial biogenesis and fusion and fission events (MtDy) are dynamic processes involved in keeping $mt\Delta\Psi$ steady. Under stressful conditions they must rearrange/adjust to keep mitochondrial health. These processes are defined as mitochondrial adaptive responses. Copper deficiency is associated with anemia and cardiac and neurobiological disorders. Furthermore, giant/large mitochondria have been described. There is a connection between copper, bioenergetics and MtDy which has not been explored. Mice were IP-injected with the copper chelator bathocuproine disulfonate to induce a mild copper deficiency. Under these conditions, the fusion proteins MFN2 and OPA-1 were up-regulated; OXPHOS was remodeled for higher respiration. A new mitochondrial giant morphology was found and called "butternut squash" mitochondria which seem to be an intermediate state between normal and swollen mitochondria. Interestingly, OXPHOS remodels toward individual complexes, supporting higher respiratory rates and ATP synthesis. In addition, we have recently shown that up-regulation of MFN2 and OPA-1 stimulates fusion events in erythroid K562 cells. COCHILCO-FONDECYT 1100995; FONDECYT 3110171; UNAB DI-10-10R; Nucleo-UNAB DI-209-12/N

3. Caffeic acid and its alkyl esters: The effect of hydrophobicity in the antioxidant and anti-*Helicobacter pylori* potency.

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Helicobacter pylori pathogenic action involves the colonization of the gastrointestinal tract and a large production of reactive oxygen species (ROS) by the neutrophils attracted to the site of infection. The aim of this study was to evaluate caffeic acid and its alkyl esters as inhibitors of the release of ROS by *Helicobacter pylori* activated neutrophils and their bactericidal effect. The increased hydrophobicity caused by esterification had direct consequence in their efficiency as bactericidal agents against *H. pylori* and inhibitors of the production of ROS by neutrophils. The minimum inhibitory concentration (MIC) decreased from higher than 1000 µg/mL (caffeic acid) to 250 µg/mL to butyl and heptyl caffeate. The release of total ROS, superoxide anion and hypochlorous acid by activated neutrophils was also significantly decreased and the esters were more efficient than the acid precursor. In conclusion, the alkyl esters of caffeic acid have two properties that are complementary for the treatment of *H. pylori* infections: bactericidal activity and inhibitory effect upon generation of ROS by neutrophils. Hence, we propose that these easily synthesized and non-expensive substances should be applied to *in vivo* experimental models of *H. pylori* induced gastric infections.

4. Protocatechuic Acid Alkyl Esters as Inhibitors of NADPH Oxidase

Quinello Gomes De Faria, C¹, Chiquetto Paracatu, L¹, Nazaré, A¹, Zeraik, M², Petrólio, M¹, Silva, D², Fonseca, L¹, Ximenes, V³, ¹Clinical Analysis, Faculty School of Pharmaceutical Sciences, Sao Paulo State University.²Department of Organic Chemistry, Faculty School of Pharmaceutical Sciences, Sao Paulo State University.³Department of Chemistry, Faculty of Science, Sao Paulo State University. (Sponsored by Fundação De Amparo A Pesquisa Do Estado De São Paulo (FAPESP))

There is mounting evidence of the deleterious effects of reactive oxygen species produced by activated leukocytes. The objective of this study was to evaluate the activity of protocatechuic acid and its alkyl esters as potent inhibitors of NADPH oxidase. The esters, methyl (P1), ethyl (P2), heptyl (P7) and decyl (P10) protocatechuates were synthesized from protocatechuic acid (P0). Their oxidation potential, hydrophobicity, inhibition of superoxide anion (O₂^{•-}), and the abilities to affect hypochlorous acid (HOCl) production by leukocytes were studied. The increased hydrophobicity (logP, 0.81-4.82) of the esters was not correlated with a significant alteration in their oxidation potential (0.222-0.298 V). The esters were strong inhibitors of O₂^{•-} released by activated neutrophils (P7, 10µM, 97.4%; 1µM, 68.2%), (P0, 10µM, 39.1%; 1µM, 16.5%) and peripheral blood mononuclear cells (P7, 10µM, 98.3%; 1µM, 68.7%), (P0, 10µM, 38.6%; 1µM, 30.5%) than its acid precursor. This tendency was also found for the inhibition of the release of HOCl (P7, 10µM, 83.5%; 1µM 52.5%), (P0, 10µM, 0.0%, 1µM 0.0%). A correlation was found between the carbon chain length and the relative inhibitory potency. These results show that, besides the redox potential, the hydrophobicity can also be a determinant factor for the design of NADPH oxidase inhibitors. The esters of protocatechuic acid are promising drugs for treatment of chronic inflammatory diseases. Supported by: FAPESP

5. Cytotoxic potential of anthraquinones from *Rhamnus sphaerosperma* var. *pubescens* in uterine tumor cell lines

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The genus *Rhamnus* includes species with great potential pharmacological, which are widely used in folk medicine. *Rhamnus sphaerosperma* var. *pubescens* is a species native in Brazil, and it is popularly known as "Fruto-de-pombo". Moreover there are still no studies that describe their biological properties. Thus, the aim of this study was to evaluate the potential cytotoxic of anthraquinones from this species. The stems of *Rhamnus sphaerosperma* var. *pubescens* were collected in Curitiba-PR, Brazil, in May 2011. The dried plant was submitted to ethanol extraction and liquid-liquid partitioning in Soxhlet apparatus modified. After column chromatography of fractions, two crystalline samples were obtained, and were analyzed by Nuclear Magnetic Resonance spectroscopy of ¹H and ¹³C. These compounds were identified as anthraquinones chrysophanol (1,8-dihydroxy-3-methylantraquinone) and physcion (1,8-dihydroxy-3-methoxy-6-methylantraquinone). The anthraquinones were evaluated in cytotoxicity assay through sulforhodamine B, at concentrations of 3.125 to 50.0 µg/mL, in SiHa (ATCC® HTB35™) and C33-A (ATCC® HTB31™) cell lines. DMSO (0.05%) was used as a vehicle to the anthraquinones and as vehicle control and doxorubicin (50.0 µg/mL) was used as a positive control. Physcion showed better results than chrysophanol, with inhibition of cell viability in 74.1±4.6%, in SiHa, and 53.6±8.7% in C33-A, at a concentration of 50.0 µg/mL. Financial support: CAPES, UNESP, UFPR.

6. CK2 regulates ECE-1c by phosphorylation at its N-terminal end.

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Protein kinase CK2 phosphorylates over 300 proteins regulating essential cellular processes, most of them of key importance in cancer. Endothelin Converting Enzyme-1 (ECE-1) is a membrane metalloprotease involved in Endothelin-1 synthesis, and also has been related to cancer progression. There are four different ECE-1 isoforms, which have the same transmembrane sequence and extracellular C-terminal domain, but differ in the N-terminal cytoplasmatic portion. It has been described that isoform ECE-1c is the most related with cancer progression. Interestingly, its N-terminal end displays three putative phosphorylation sites for CK2. Thus, we studied whether CK2 phosphorylates ECE-1c on this region, thereby modulating its function in an *in vitro* cell model.

GST-fused N-terminal end of ECE-1c (GST-NT-ECE1c) and recombinant CK2 were expressed, purified, and incubated in presence of [³²P]-γ-ATP. Radioactive bands were visualized by autoradiography. To evaluate CK2-dependent ECE-1c stability, NT-ECE1c fused to GFP was expressed in HEK-293T cells which were incubated with CHX and then treated with either a CK2 specific inhibitor (TBB) or a proteasome inhibitor (MG-132). Protein levels were assessed by western blotting using an anti-GFP antibody.

Our results show that indeed CK2 phosphorylated *in vitro* to NT-ECE1c, as well as this CK2-dependent phosphorylation promoted ECE-1c stabilization.

7. Contribution of inflammation and oxidative stress to the cardiorespiratory alterations induced by intermittent hypoxia

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Chronic intermittent hypoxia (CIH), a main feature of obstructive sleep apnea, potentiates carotid body (CB) chemosensory responses to hypoxia, which in turn enhanced hypoxic sympathetic and ventilatory responses and led to hypertension. Since CIH produces oxidative stress and increased inflammatory cytokines in the CB, we hypothesized if CIH-induced carotid chemosensory potentiation may depend on CB oxidative stress and inflammation. Carotid chemosensory responses to hypoxia were recorded from male Sprague-Dawley rats exposed to CIH (5%O₂, 12 times/h for 8 hs) for 21 days. To separate the effects of oxidative stress and inflammation on CB chemosensory function, rats exposed to CIH were treated with ascorbic acid or ibuprofen. 3-nitrotyrosine (3-NT) formation was used as an index of CB oxidative stress. We found that CIH-induced 3-NT formation was prevented by ascorbic acid treatment, but not by ibuprofen. Similarly, ascorbic acid prevented the enhanced CB chemosensory responses, but ibuprofen failed to prevent the enhanced hypoxic CB chemosensory responses. Thus, our results showed that the CIH-induced CB chemosensory alterations depend mainly on the oxidative stress rather than of the inflammatory processes.

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8. Association of the *EcoRI* and *XbaI* polymorphisms at the Apolipoprotein B Gene with lipid profile in Colombian Caribbeans.

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Numerous studies have shown that single nucleotide polymorphisms (SNPs) in apolipoprotein B (ApoB) gene play an important role in lipid metabolism. Insufficient information exists about the distribution and influence of the ApoB SNPs on plasma lipid concentration in Colombian Caribbeans. The aim of this study was to determine the association of ApoB *XbaI* and *EcoRI* polymorphisms with lipid profile in Colombian Caribbeans. Genomic DNA was extracted from 109 healthy adult subjects. ApoB polymorphisms were determined by PCR-Restriction Fragment Length Polymorphisms (RFLP) and plasma lipid including total cholesterol (TC), triglycerides (TG), LDL cholesterol (LDL-C) and HDL cholesterol (HDL-C) were measured. Genotype frequencies of *XbaI* were X+X+ (16,5%), X+X- (38,5%) and X-X- (45%) and *EcoRI* were E+E+ (77%), E+E- (23%) and E-E- (0%). *XbaI* polymorphism was only associated with LDL-C levels ($p < 0.05$). Most of the subjects with X-X- (61%) and X+X- (76%) genotypes showed higher plasma levels of LDL-C than those with X+X+ genotype. *EcoRI* polymorphism was associated with high LDL-C and low HDL-C levels ($p < 0.05$). The SNPs were in Hardy-Weinberg equilibrium ($p > 0.05$) and no linkage disequilibrium was found between the two polymorphisms ($p > 0.05$). ApoB *XbaI* and *EcoRI* polymorphisms seem to be associated with dyslipidemia in the Colombian Caribbean population. This study was financed by Universidad Libre de Barranquilla and COLCIENCIAS, Colombia.

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9. Biochemical consequences of hyh (M105I) mutation on alpha-SNAP protein during brain development

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α -SNAP is a key component of the intracellular membrane fusion machinery that mediates recruitment of the ATPase NSF to SNARE membrane protein complexes. Recently, it has been shown that α -SNAP also interacts directly with membrane lipids and that this interaction is relevant to the protein efficiency. The naturally occurring M105I or hyh mutation in α -SNAP causes a neurodevelopmental disorder in mice characterized by a distinct disruption of N-cadherin-based adherens junctions in radial glial cells; however, the biochemical consequences of M105I mutation on α -SNAP protein function are poorly understood. *Does the M105I mutation alter α -SNAP membrane binding properties?* Here we performed immunofluorescence, subcellular fractionation, detergent phase separation, flotation and membrane sheets assays to evaluate membrane-binding and hydrophobic properties of α -SNAP-WT and M105I. On the other hand, co-immunoprecipitation and GST-pulldown assays were performed to evaluate protein-protein interactions. Our results showed that α -SNAP-M105I is less associated to membranes than WT. Furthermore, we observed that hydrophobic and membrane lipid-binding properties of α -SNAP-M105I are reduced. Protein-protein interactions experiments showed that α -SNAP interacts with N-cadherin and other proteins of adherens junctional complexes. Altogether our results indicate that M105I mutation alters key biochemical properties of α -SNAP which may further impact on its functionality

10. An expanded and functionally diverse family of Kunitz inhibitors from a metazoan parasite

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We are characterising a family of secreted monodomain Kunitz proteins from the flatworm *Echinococcus granulosus* (EgKU-1–EgKU-8) acting at the interface with the dog definitive host. Typically, Kunitz inhibitors are serine protease inhibitors that tightly bind to the active site through an antiprotease loop mimicking a substrate, yet they are cleaved extremely slowly; in addition, some members of Kunitz families from animal venoms act as channel blockers. Our studies show that the parasite family contains molecules with either activity. On the one hand, the paralogs EgKU-3 and EgKU-8 behaved as typical inhibitors of chymotrypsins and trypsins, respectively (K_i 10^{-11} M). In turn, EgKU-7, which carries an extension at the C-terminus of the domain, inhibited trypsins with similar K_i , but differed in enzyme interaction: i) it resisted proteolysis by canine trypsins but not by the cationic bovine enzyme; and ii) activity assays with a form lacking the extension indicated that the latter would participate in inhibition. On the other hand, the paralogs EgKU-1 and EgKU-4, which do not inhibit proteases, blocked voltage-dependent potassium channels (Kv; IC50 10^{-7} M); and also acid-sensing ion channels (ASIC; IC50 10^{-9} M), an activity as yet not described for Kunitz proteins. Taken together, our results suggest that Kunitz families in animal venoms and parasite secretions expand and diversify through similar mechanisms.

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11. Markers of early endothelial dysfunction in IUGR-derived human umbilical vein endothelial cells revealed by 2d-dige and mass spectrometry analyses

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Intrauterine growth restriction (IUGR), a clinical condition in which the fetus does not reach the expected growth for a determined gestational age, associates with short- and long-term endothelial dysfunction. We hypothesize that this dysfunction is reflected as changes in the proteome of IUGR-derived Human Umbilical Vein Endothelial Cells (HUVEC). For this purpose the proteome of HUVEC isolated from IUGR and control subjects were analyzed. **Methods.** Protein extracts from HUVEC were cleaned and labeled using CyDye fluor dyes. Isoelectric focusing was done in 3-7 and 6-11 pH strips and later separated by SDS-PAGE. 2D-gels were scanned and analyzed using Delta-2D software, and spots with significant changes analyzed using MALDI-TOF MS. Finally, the peptide mass fingerprint was identified using MASCOT program. **Results.** The identified proteins were assessed by univariate, multivariate and hierarchical clustering to distinguish changes in IUGR and control samples. Proteins that showed major changes included cytoskeleton, chaperones, proteasome and others associated with different cell functions, including protein sub-cellular location, and stability. **Conclusions.** These results show that IUGR-derived endothelial cells present important changes at the proteome level revealing new functions that would be altered in the IUGR-endothelium. The highly restrictive analysis performed suggests that these proteins represent molecular markers of this condition. **FONDECYT-1120928**

12. Ryanodine receptor-mediated Ca²⁺ release underlies iron-induced mitochondrial fission and stimulates mitochondrial Ca²⁺ uptake in primary hippocampal neurons

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We have reported previously that addition of Aβ oligomers to primary hippocampal neurons stimulates Ca²⁺ release from the endoplasmic reticulum via the redox-sensitive ryanodine receptor (RyR) channels, generating persistent Ca²⁺ signals that promote the disruption of the mitochondrial network. Moreover, addition of sub-lethal iron levels to these neurons generates reactive oxygen species and also elicits cytoplasmic Ca²⁺-signals, which arise mostly from RyR-mediated Ca²⁺ release. Here, we describe that 24 h incubation of hippocampal neurons with iron enhanced agonist-induced RyR-mediated Ca²⁺ release and caused mitochondrial network fragmentation in 43% of neurons, a response significantly prevented by RyR inhibition. Agonist-induced activation of RyR-mediated Ca²⁺ release promoted mitochondrial Ca²⁺ uptake in control neurons and in neurons that displayed non-fragmented mitochondria after iron treatment but not in neurons with fragmented mitochondria. Yet, after increasing cytoplasmic Ca²⁺ by ionomycin addition neurons with fragmented mitochondria displayed significant Ca²⁺ uptake. Taken together, our results indicate that stimulation of redox-sensitive RyR-mediated Ca²⁺-release by iron causes significant neuronal mitochondrial fragmentation, which presumably contributes to the impairment of neuronal function produced by iron accumulation. *Support: FONDECYT (11110322-1100052-1100599-1130068) FONDECYT-FONDAP 1501006; MILLENNIUM BNI P-09-015F, PIA-CONICYT ACT1114*

13. Assessment of the antioxidant capacity of the uremic solutes and their mixtures through *in vitro* model systems

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Chronic Kidney Disease (CKD) is characterized by progressive and irreversible loss of kidney function, which may lead patients to hemodialysis (HD). In view of the prevalence of oxidative stress in CKD patients, made worse by the fact that antioxidant low-molecular-weight biomolecules are washed out of circulation by HD, and given the knowledge about the antioxidant action of determined uremic solutes (US) that accumulate in CKD, we used *in vitro* model systems to test the antioxidant potential of nine US. In model systems for O₂⁻ and H₂O₂, none of these US showed scavenging activity. Four of these US (*p*-cresol, phenol, L-tyrosine and uric acid) were effective antioxidants and their IC₅₀ were found in model systems: ABTS⁺, HOCl/OCl⁻ and crocin bleaching assay (ROO⁻). In the analysis of the mixture of these four US, each one of the US captured 12.5% for the IC₅₀ of the mixture to ABTS⁺ or HOCl/OCl⁻, exhibiting a practically exact additive effect. For ROO⁻ capture, mixtures of pairs of the four US were tested; these showed the same mass proportions in the IC₅₀ of each mixture as in the IC₅₀ of each US alone, demonstrating again an additive effect. The use of the IC₅₀ as an analytical approach to prepare US mixtures allows the determination of their individual scavenging capacities and may be useful for the assessment of the oxidative status of CKD and HD patients, as well as for the effectiveness of antioxidant interventions. Financial support: CNPq, FAPESP.

14. Preparation and characterization of ACE-inhibitor peptides, employing plant peptidases from *Bromelia hieronymi* on bovine whey proteins.

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Some dietary proteins contain bioactive peptides encrypted in their sequences that can be released by specific enzymatic hydrolysis, transforming food that contains them into functional food. Milk proteins are precursors of bioactive peptides with potential antihypertensive activity, which exert their action by inhibiting a key enzyme in the regulation of blood pressure: angiotensin-converting enzyme (ACE). The aim of this work was to obtain bovine whey protein hydrolyzates using peptidases from *Bromelia hieronymi*, a native species of Argentina, to determine ACE inhibitory activity in hydrolyzates and to realize a preliminary characterization of obtained peptides. Crude extracts were obtained by grinding unripe fruits with 0.1 M sodium phosphate buffer, pH 6.0. The homogenate was centrifuged and supernatant was treated with four volumes of cold ethanol. Precipitated proteins were suspended in the same buffer and the partially purified extract obtained was used to hydrolyze whey proteins at 45 °C. SDS-PAGE peptide profiles showed growing degradation of main whey protein fractions with the reaction time, and the hydrolysis degree reached was 11% at 180 min. Ultrafiltered hydrolyzates (3 kDa cut off) containing 1 mg/ml of peptides were analyzed by reverse phase chromatography and mass spectrometry. Maximum ACE inhibitory activity was detected in hydrolyzate of 180 min, presenting an IC₅₀ of 0.6 mg/ml; therefore it could be used in functional food manufacturing.

15. Development of porous chitosan-gelatin composite scaffolds for bone tissue engineering applications.

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The search for strategies to replace or restore bone tissue lost by trauma or pathologies remains a major clinical challenge to biomedical researchers. In this context, bone tissue engineering has emerged as an effective alternative therapy to repair bone defects. Among the many materials that have been evaluated for the production of scaffolds in regenerative medicine, chitosan with its antimicrobial properties and gelatin with cell adhesion property offer a unique combination that has significant potential as polymeric matrix in tissue engineering. In this study, a 3D porous chitosan/gelatin scaffold was prepared via polyelectrolyte complex formation, freeze drying and crosslinking with glutaraldehyde. Assays were performed at different freezing temperatures to determine the effect on the morphological and physical characteristics of the material. Micrographs were obtained by Scanning Electron Microscopy to quantify the pore size (110-140µM) and to visualize interconnected pores. The porosity of composite was determined by gas picnometry, obtaining a porosity between 97-99%. Scaffold physical characterization was assessed by Differential Scanning Calorimetry to obtain a glass transition temperature between 37-40°C. The generated scaffold supports the attachment, spreading and proliferation of mouse embryonic fibroblast (MEF) cells, suggesting that is potentially useful for bone tissue engineering purposes.

16. Characterization of HTLV-1 Tax protein secreted from Peripheral Blood Mononuclear Cells of patients with paraparesis.

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The human retrovirus HTLV-1 infects CD4⁺ lymphocytes triggering a disease characterized by axonal degeneration, called HTLV-1 Associated Myelopathy/Tropical Spastic Paraparesis (HAM/TSP). Despite the neurological damage, the virus does not infect neurons, being the viral secretable protein Tax associated with the pathogenesis. We have detected higher levels of Tax in HAM/TSP patients than in asymptomatic carriers. This investigation focused on the secretory pathway of Tax and its post-translational modifications. Using an inhibitor and activators of the RE-Golgi secretory pathway we observed in Peripheral Blood Mononuclear Cells (PBMCs) culture a concomitant secretion of Tax with the chaperone calreticulin indicating Tax secretion through this classical pathway. The characterization of secreted Tax showed to be polyubiquitinated. Either plasmatic soluble Tax or infected lymphocytes that cross the CNS blood barrier could account for the presence of Tax in the cerebrospinal fluid. Therefore, Tax may extracellularly induce axonal degeneration of corticospinal motor neurons. An *in vitro* model with differentiated human neuroblastoma cells, SH-SY5Y cells, cultured with MT-2 cells secreted products (HTLV-1 infected cell line) showed that Tax produces neurite retraction. These findings suggest a pathological relevance of Tax secreted from HTLV-I infected T-cells, the main viral reservoir *in vivo*, in paraparesis.

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17. Biological characterization and chemical interference of trypanothione biosynthesis in trypanosomatids

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Therapeutic options for the treatment of human trypanosomiasis and leishmaniasis are still limited. The thiol redox metabolism of trypanosomatids relies on a small molecule absent in mammals, the trypanothione (T(SH)₂). Our work is focused on targeting T(SH)₂ biosynthesis for the development of safer and more effective chemotherapies against trypanosomiasis. Active expression, compartmentalization and intracellular concentration of trypanothione synthetase (TryS) was investigated in three life stages of *T. cruzi* and in infective *T. brucei* parasites. *In vitro* metabolic complementation studies with a *T. brucei* cell line defective in TryS (RNAi) revealed the exclusive dependence of the parasites on T(SH)₂ despite being able to take up glutathione from the extracellular medium. *In vivo* down-regulation of TryS by RNAi demonstrated the physiological relevance of T(SH)₂ for parasite survival in mice. A high-through-put screening assay was developed with the aim to identify enzyme's inhibitors. From a chemical library of more than 350 compounds, several inhibitors of *TcTryS*, *TbTryS* and *Leishmania infantum* TryS have been identified. For *TbTryS* we found two series of active compounds: 6-arylpyrido(2,3-d) pyrimidines and 1-(benzo[d]thiazol-2-yl)-4-benzoyl-3-hydroxy-5-phenyl-1H-pyrrol-2(5H)-one derivatives, displaying low μM IC₅₀ values and reasonable trypanocidal activity against infective *T. brucei*. (*The three first authors contributed equally to this work*)

18. Discovery of 5-HT₆ receptor ligands: Design, synthesis and biological evaluation of novel N-Arylsulfonylindole derivatives

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Obesity epidemic is one of the top 10 global health problems, for which no efficacious pharmacotherapy exists. In Chile, 25.1% of the adult population is obese and 39.3% overweight. The 5-HT₆ receptor has emerged as target for the development of anti-obesity drugs, due its implications in the regulation of food intake and body weight. **Aim** The primary goal of this work was focused on the generation of novel heterocyclic compounds, in particular N-arylsulfonyl indole derivatives targeting the 5-HT₆ receptor. **Methods** The design of the molecules was based on pharmacophore approaches, chemical criteria, receptor modeling and docking studies. The synthesized molecules were evaluated in their binding affinity for 5-HT₆ receptors by radioligand competition assays in HEK293 cells. **Results** A library of 1728 compounds was docked against the 5-HT₆ receptor model to prioritize the synthesis, and for further biological assays. All tested compounds displayed inhibition of [¹²⁵I]-SB258585 binding to 5-HT₆ receptors. The two most potent compounds displayed a K_i of 18.4 and 14.6 nM respectively. **Conclusions** We have designed and evaluated a novel series of N-Arylsulfonylindoles, all of which exhibited inhibitory activity on the radioligand binding for the 5-HT₆ receptor. Two of these compounds exhibited high inhibitory activity in the nanomolar range and represent lead compounds for this new series of compounds. **Funding** FONDECYT N° 11121418 grant.

19. Environmental contaminants (Tributyltin) affect the estrogen and progesterone receptors binding capacity in the uterus of ovariectomized rats.

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Triorganotins as Tributyltin (TBT) are environmental contaminants and endocrine disruptors that produce reproductive toxicity in different animal models. The aim was to evaluate the effect of TBT administration on the uterine binding capacity to the ovarian steroid hormones. Wistar female rats (12 wk old, weighing ± 230 g) were ovariectomized 21 days before the treatment with TBT (100 ng/kg/day for 15 day by gavage, n=6) or vehicle (ethanol 0.4%, n=6). The estrogen receptor (ER) and progesterone receptor (PR) binding capacity by ligand-binding assay were determined. The ER and PR apparent dissociation constant (Kd, nM) values (means $\pm$ s.e.m) were not affected by the treatment (0.93 ± 0.23 and 1.32 ± 0.40 , respectively). Tributyltin treatment significantly increased the ER concentration (fmol/mg protein) as compared with controls (47.8 ± 5.6 vs. 30.6 ± 2.5 , respectively, $p<0.05$). A tendency to increased the PR concentrations (fmol/mg protein) by TBT treatment also was found (47.2 ± 10.0 vs 24.5 ± 2.7 , for TBT and controls respectively, $p=0.09$). The uterine toxicity of TBT may be exerted by changes in the uterine capacity to response to the ovarian steroid hormones.

20. Prevention of myocardial ischemia-reperfusion injury by inhibition of the proteasome.

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Deregulation of the proteasome pathway may contribute to the contractile dysfunction and cell death (infarct size) observed after cardiac ischemia-reperfusion (IR). The sarcoplasmic reticulum (SR) calcium release channel (RyR) and mitofusin 2, a protein involved in the attachment of SR and mitochondria to tune mitochondrial activity with the energetic needs, are two crucial proteins degraded by the proteasome. The aim of this work was to investigate the effect of proteasome inhibition on the content of RyR and mitofusin 2, and on the recovery of the contractile parameters and infarct size following 30 minutes of ischemia and 60 minutes of reperfusion in a Langendorff perfused rat heart model. We found that inhibition of the chymotrypsin-like activity of the proteasome (with MG-132, $0.5 \mu\text{M}$) significantly prevented the decrease in RyR (47 % of the control after IR vs 70 % of the control after IR with MG-132) and mitofusin 2 (50 % of the control after IR vs 80 % of the control after IR with MG-132). MG-132 also produced a significant 42 % recovery of the left ventricular developed pressure compared to 12 % recovery of the basal value after IR in the absence of the inhibitor. Likewise, the infarct size was reduced from 41 % in controls after IR to 16 % after IR in the presence of MG-132. In conclusion inhibition of the chymotrypsin-like activity of the proteasome protects the heart from the harmful effects of IR. FONDECYT grants 1110257 and 1130407

21. Determination of similarities between monoaminergic proteins and nicotinic receptors: an approach to rational design of poly-pharmacological drug

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Psychiatric disorders have been growing steadily, however due to the complexity of the neural circuits that regulate them have been limited their physiopathological understanding, because it involves changes in neuronal connection network where the monoaminergic system and the neuronal nicotinic acetylcholine receptors (nAChRs) play an important role. Considering that the psychiatric disorders are the result of a complex network of molecular events, it is essential to develop drugs that act in a polyselective way. In this work we built molecular models of 5-HT_{2C} and $\alpha 7$, which are directly implicated in neuronal disorders. We performed molecular dynamics to determinate its physicochemical properties at structural level. Then we searched cavities into the models aiming to find new binding sites. These cavities were structurally compared looking for similarities. More similar cavities between both systems were used to perform a virtual screening to narrow the range of known compounds that bind in these cavities. Our results show a high structural similarity between the cavities, showing conserved residues in their active sites. In addition, docking analysis show that the bond between the ligand with the cavities are mediated by hydrogen bonds, electrostatic and phi-phi interactions which determinate their selectivity. This information is envisioned to prove useful for the design of novel neuronal inhibitors.

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22. Arbovirus Oropouche: Expression of Structural Proteins in Insect Cells for Structural and Serological Diagnosis Research

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Oropouche fever (OF), caused by the Oropouche virus (OROV), is one of the most important arboviruses in tropical areas of South and Central Americas, being responsible for more than 500 thousands human cases in the last years. Due to its unspecific clinical signs, OF is usually mistaken with dengue or others arboviruses. Thus, a specific and sensitive diagnostic method is necessary to detect OROV. Accordingly, the aim of this project is to obtain the recombinant structural proteins (Nucleocapsid-Ns, Glycoproteins G1 and G2) to perform structural studies and specific and sensitive serological tests. From OROV BeAn19991 strain all three structural OROV proteins were cloned into HBM-TOPO insect expression system purchased from Invitrogen. Recombinant constructs were used to generate baculoviruses in Sf-9 exponential growing insect cells by transfection. The recombinant Ns and G2 proteins were obtained from supernatant and pellet of infected insect cells, respectively, after purification on Ni-NTA affinity chromatography. The expression of the structural OROV proteins was verified by Western blotting with anti-histidine antibody. Biochemical analysis is being performed to verify the glycosylation patterns of OROV G2 protein. Afterwards, the sensitivity and specificity of the recombinant OROV antigens will be determined by using serum samples from OROV infected patients. Acknowledgement: FAPESP (2009/11347-6) and CAPES

23. Rapamycin-conditioned dendritic cells activated with MPLA show a tolerogenic profile

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The development of cellular therapies using immune cells with tolerogenic properties is a current research topic, opening promising interventions in transplantology. Dendritic cells (DC) are professional antigen presenting cells (APC) with the unique ability to both start and regulate the immune response. Rapamycin is an antibiotic with immunosuppressive properties widely used in organ transplantation. The aim of this work was to generate tolerogenic murine bone marrow-derived CD11c+ DCs, which at day 7 were purified using magnetic beads. These cells were treated with Rapa and stimulated with MPLA (Rapa-mDCs) for 48 hours to then characterize them phenotypically by flow cytometry. Our results show that Rapa-DCs exhibit low expression of the co-stimulatory molecules CD40 and CD86, but display high levels of the migration molecule CCR7 and the inhibitory molecule PD-L1. In regard to cytokine production, quantified by ELISA, our data indicate that Rapa-mDCs produce significantly lower levels of IL-6 and to a lesser extent IL-12. In preliminary transplantation experiments, Rapa-mDCs-treated mice show high survival of skin allograft, mainly by accumulating Foxp3+ T cells in the graft-draining lymph nodes. Considering that MPLA is an analogue of LPS to activate human cells with no toxic effect, our results suggest that Rapa-mDCs could be considered as a potential cell therapy to control unwanted inflammatory responses. Funded by CONICYT 791100001, FONDECYT 11121309.

24. Nitric oxide production mediates dexmedetomidine-induced cardiac preconditioning

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Myocardial infarction, a major cause of death worldwide, is generated as a consequence of prolonged ischemia-reperfusion, resulting in cardiomyocyte death. The myocardium possesses innate physiologic adaptive processes like cardiac preconditioning that render it more resistant to potentially lethal ischemic injury. The eNOS-nitric oxide (NO) signaling pathway has been associated with cardiac preconditioning. The alpha 2 adrenoreceptor agonist Dexmedetomidine (DEX) used in anesthesiology also exerts preconditioning effects in cardiac ischemia-reperfusion. However, the molecular mechanism by which DEX renders cardioprotection remains unknown. Our group has recently shown that DEX attenuates myocardial infarction and promotes the phosphorylation of eNOS. Therefore, we evaluated here the role of the eNOS-NO pathway in the protective effect of DEX. The results showed that a 25 min infusion of DEX 10 nM protected adult rat hearts subjected to ischemia-reperfusion using the Langendorff technique. We also found that DEX promotes the activation of eNOS and stimulates NO production. Furthermore, the preconditioning effect of DEX after 30 min ischemia and 120 min reperfusion is lost by the co-administration of the NOS inhibitor L-NAME or the NO scavenger PTIO. We concluded that preconditioning effect of DEX in myocardial infarction depends on the eNOS-NO pathway.

25. The apoptosis/necrosis ratio of H₂O₂-induced death of lymphocytes as a possible biomarker for early detection of Alzheimer's disease.

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Introduction. We have previously reported on an age-dependent increases in apoptosis/necrosis ratios in lymphocytes exposed to hydrogen peroxide (H₂O₂)-induced oxidative stress, which was not accompanied by a change in overall death. We also reported on an increased susceptibility to cell death of lymphocytes from Alzheimer's disease (AD) patients that was related to the degree of dementia severity. **Objective.** To explore the apoptosis/necrosis ratios induced by oxidative stress in lymphocytes from AD and Mild Cognitive Impairment (MCI) or very early AD patients. **Methods.** Lymphocytes were extracted from peripheral blood and exposed to H₂O₂ for 20 h. Cell death was evaluated by flow cytometry and propidium iodide (PI) staining, whereby viable (PI-negative), apoptotic (PI-positive, hypodiploid) and necrotic (PI-positive diploid) cells were distinguished. **Results.** AD patients showed significantly increased apoptosis/necrosis ratios related to dementia severity. Interestingly, also in MCI patients apoptosis/necrosis ratios were increased without a change in overall death. **Conclusions.** These observations suggest that the increase in apoptosis in response to oxidative stress is detectable at very early stages of AD (MCI). Importantly, early changes in apoptosis/necrosis ratios in response to oxidative stress may serve as a non-invasive biomarker for early detection of Alzheimer's disease. **Acknowledgements—**Fondecyt1110189 (MIB), Fondecyt 1130250, ACT1111 (AFGQ).

26. Electrophilic signaling of fatty acid nitroalkenes in Amyotrophic Lateral Sclerosis

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Nitro-fatty acids (NO₂-FA) are endogenously-produced electrophilic signaling mediators of anti-inflammatory responses. This work addresses the potential protective role of NO₂-FA in Amyotrophic Lateral Sclerosis (ALS). Dominant mutations in Cu/Zn superoxide dismutase (SOD1) cause familial forms of ALS, a neurodegenerative disease characterized by the progressive loss of motor neurons. It has been demonstrated that activation of the transcription factor Nrf2 in astrocytes confers protection to neighboring neurons, improving motor neuron survival. We have assayed the effects of NO₂-FA on a model of motor neuron degeneration induced by astrocytes expressing the ALS-linked SOD1^{G93A} mutation. Addition of nitroalkenes (nitroarachidonic acid or nitrooleic acid) to spinal cord astrocytes significantly induce Nrf2 activation as well as antioxidant phase II enzymes expression in astrocytes, concomitant with increasing intracellular glutathione levels. Moreover, a transient exposure of SOD1^{G93A} astrocytes to NO₂-FA prevented their toxicity to motor neurons. In vivo data further supports a novel neuroprotective role of NO₂-FA administration on SOD^{G93A} mice, due to their ability to cross the brain blood barrier and improve grip strength and survival.

27. The treatment based on ISAV or hRSV fusion protein containing apoptotic bodies induces a greater maturation of dendritic cells derived from C57BL/6 mice, than in the absence of viral fusion proteins.

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The treatment of cancer includes conventional and non-conventional therapies, such as the immunotherapy. In our laboratory, we developed an immunotherapy based on immunization with apoptotic bodies (AB). This treatment was able to protect the 20% of mice, which did not develop any tumors using the B16 melanoma model. The aim of this work is to determine the mechanism behind this protection; therefore, we suggest that our AB cause the maturation and activation of dendritic cells (DCs), and that this could be improved using infectious salmon anemia virus (ISAV) fusion protein or human respiratory syncytial virus (hRSV) fusion protein containing apobodies.

We generated DCs derived from bone marrow of C57BL/6 mice, under standard conditions. After 6 days, cells were treated for 24 h with LPS 1:1 and 2:1 AB:DCs, where AB may contain fusion proteins (ISAV or hRSV) or not. Subsequently, we determined the presence of DCs maturation markers (CD86, MHC-II), by flow cytometry. Unstimulated DCs were positive for MHC class II (48.7%) and CD86 (17.1%). LPS-stimulated DCs, showed increased levels of MHC class II (59.7%) and CD86 (58.7%), relative to negative control. 1:1 and 2:1 AB-treated DCs, showed a similar increase in MHC class II and CD86. Viral fusion proteins containing AB appear to increase even more the activation markers in DCs. This approach could be an attractive therapeutic strategy for cancer treatment. FONDECYT 1110734, FIA PYT-2012-0022, VIU 110049.

28. Wnt signaling modulates transcription of translocation-prone genes in hematopoietic cells

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Wnt/ β -catenin signaling has a crucial role in the self-renewal, proliferation and differentiation of hematopoietic lineages and its deregulation is thought to be involved in the onset/development of leukemia. Likewise, the transcription factor RUNX1 plays essential roles in determining the fate of diverse cell types and is required for definitive hematopoiesis. Here we identify a novel T-cell factor/lymphoid enhancer factor (TCF/LEF)-response element in the promoter of the human RUNX1 gene that responds to pharmacological or molecular activation of Wnt/ β -catenin signaling in leukemia cell lines HL-60 and Jurkat and in CD34+ primary human hematopoietic progenitor cells. Since the structure of the RUNX1 gene is rearranged in various types of leukemia, we examined the transcriptional activity of Runx1-translocation partners MECOM (MDS1 and EVI1 complex locus) and ETO (RUNX1T1) and found that the expression of these genes was readily induced in CD34+ cells in response to a purified Wnt3a protein. RUNX1 and MECOM transcriptional events were specifically co-localized within the nucleus in higher order spatial genome organization centers. We propose that Wnt/ β -catenin signaling induces a coordinated expression of RUNX1 and RUNX1-translocation partners, increasing the potential for a chromosomal translocation event, thus promoting the onset of myeloid leukemia. Funding: FONDECYT 3130509 to GDU; and Anillo ACT-1119 and FONDAP-CRG 15090007 to GVD.

29. Insulin reversal of gestational diabetes mellitus reduced adenosine transport requires downregulation of insulin receptor A and p42/44^{mapk} in human umbilical vein endothelium

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Gestational diabetes mellitus (GDM) associates with placental endothelial dysfunction. Human umbilical vein endothelial cells (HUVEC) from GDM exhibit reduced adenosine uptake via human equilibrative nucleoside transporters 1 (hENT1), and insulin reverses this phenomenon by restoring hENT1 expression. Insulin receptor isoforms A (IR-A) and B (IR-B) are expressed in HUVEC and GDM results in higher IR-A mRNA expression. However, IR-A potential involvement on insulin reversal of hENT1-adenosine transport in HUVEC is unknown. Objective: We hypothesized that insulin reversal of GDM-reduced hENT1 expression and activity is dependent on restoring IR-A expression and its associated cell signaling. Design: Umbilical cords were collected after delivery from 33 full-term normal or 33 full-term GDM pregnancies. GDM effect on hENT1 expression and activity, and the role of IR-A/IR-B expression and signalling (p42/44mapk and Akt) in HUVEC was assayed. Results: GDM associates with higher IR-A/IR-B mRNA expression and p42/44mapk/Akt activity ratios. Insulin reversed GDM-reduced hENT1 expression/activity, IR-A/IR-B mRNA expression and p42/44mapk/Akt ratios to normal pregnancies, effects abolished only in cells knockdown for IR-A. Conclusions: Insulin requires IR-A expression leading to modulation of p42/44mapk-associated signalling to restores GDM-reduced hENT1-adenosine transport and hENT1 expression to values in normal pregnancies. Insulin beneficial effects on GDM-reduced adenosine transport in HUVEC could be a protective mechanism for placental macrovascular endothelial dysfunction in GDM. Support: CONICYT (ACT-73 PIA, AT-24120944), FONDECYT (3130583, 1110977, 11110059, 1121145). CONICYT-PhD fellowship (EG-G, PA).

30. Molecular Modeling and Structural Analysis of TASK-1 Potassium Channel Interacting with the Blocker A1899

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Two-pore domain potassium (K_{2P}) channels are expressed as functional dimers in the central nervous system, cardiovascular system, genitourinary system and gastrointestinal system. They are related with several pathologies in humans. Thus, members of this family have emerged as molecular candidates for the action of pharmacological agents. The K_{2P} channel TASK-1 is an important modulator of multiple sclerosis and in 2011 a highly-selective blocker of TASK-1, named A1899, was discovered. It was suggested that A1899 acts as an open-channel blocker and binds to residues forming the wall of the central cavity. In 2012 the first crystal structures of K_{2P} channels were published. Electron density maps revealed two open lipid cavities or fenestrations, one on each side of the dimer, that expose the central cavity to the membrane. We constructed homology models of TASK-1, based on the crystal structures of the recently crystallized K_{2P} channels and studied the specific binding site of A1899. Our results suggest that: - A1899 could enter to TASK-1 through the pore driven by the residues N240, V243, M247. - A1899 binds to residues of the selectivity filter through hydrogen bonds, blocking the ion flux. - A1899 is stabilized mainly by hydrophobic interactions of the fenestration residues (which is in agreement with its LogP value = 4.738).

31. Allosteric communication pathways and vibrational energy flow in proteins: PDZ-2 case.

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Allosteric communication in proteins is an open and central problem in structural biochemistry. A significant body of evidence have correlated the path of signal propagation in proteins with the path of energy flow in proteins, with velocities of propagation in the order of 10 Å ps⁻¹. A particular technique to evaluate transmission of vibrational energy in proteins is anisotropic thermal diffusion (ATD). ATD is a molecular-dynamics pump-probe method that has been used to study of allosteric communication pathways in proteins. Here a specific residue is heated by the increase in its kinetic energy (pump) and the flow and the instantaneous increase in vibrational energy is measured in different sites of the protein (probe). As protein structure have a network of contacts, the vibrations will propagate anisotropically through them and the communication pathway of the system can be recovered. The PDZ-2 was selected because its specific characteristic of transferring allosteric information without significant conformational change. The results of the scanning of PDZ-2 domain using ATD shows an agreement with the experimental and computational reports of literature, that is, the heat flows anisotropically and through the connectivity network of the PDZ-2 protein, e.g., mainly along pathways I and II.

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32. Genome assembly of the lignocellulolytic fungus *Penicillium purpurogenum* using third-generation sequencing technology.

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The lignocellulolytic fungi produce and secrete a variety of enzymes to degrade the lignocellulosic biomass. This is a renewable energy source, and the enzymes that are involved in its degradation are of interest for biotechnological applications. The filamentous fungus *P. purpurogenum* grows on a variety of natural and artificial lignocellulosic substrates. Our goal is to identify genes from *P. purpurogenum* potentially related to lignocellulose degradation. For this purpose, we have sequenced the genome using a combination of second (Illumina HiSeq 2000) and third-generation (PacBio RS II) sequencing technologies. The PacBio reads were corrected using the Illumina reads and assembled together with the Illumina reads using Cellera 7.0 assembler. The estimated genome size is 39 Mb and the assembly includes 99.7% of this size. Over 50% of the assembly is represented in scaffolds larger than 1,052 kb and 89% of the genome is contained in 60 scaffolds. The GC content of genome is 39%. We annotated 8984 genes of which 347 are putative Carbohydrate Active Enzymes (CAZymes). The CAZymes were manual curated to obtain a gold standard database. This genome includes a "novel set" of putative lignocellulolytic enzyme genes. To our knowledge, this is the first fungal genome sequenced using this "hybrid sequencing" approach. Grant support: FONDECYT 1130180; UNAB DI-31-12/I and DI-61-12/R; MECESUP UAB0802; BASAL-CMM; FONDAP-GENOME REGULATION CENTER.

33. An Ontology of *C. elegans* resources and experiments to study extended lifespan phenotype.

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At present, dozens of treatment that promote lifespan in *C. elegans* have been identified. One of the most studied is the mutation of insulin/IGF-1 pathway. This pathway act through the genes regulated by the daf-16 transcription factor. Despite the fact that daf-16 regulated genes have been identified, is not clear which ones plays major roles in promoting lifespan. Over the past 13 years, the scientific community involved in the study of *C. elegans* has been collecting accurate, current, accessible information concerning the genetics, genomics and biology nematodes, this data is stored by WormBase consortium. Here, we describe our work to identify genes of interest to extended lifespans in worm. First, we converted the WormBase database and insulin/IGF-1 expression RNA-Seq experiments using the Resource Description Framework (RDF), a machine interpretable format that facilitates large scale data integration and query answering. We then compared the down-regulated genes from RNA-Seq experiments with RNAi knockdown experiments, and identified five genes that specifically promote lifespan and are under control of daf-16. Finally we expanded our search to find other phenotypes associated with genes that promote lifespan and we found decreases in the brood size, increase protein expression and change the activity of the worms as common phenotypes. This results agree with the soma disposable theory of aging. Acknowledgments: Government of Canada, Conicyt.

34. A trade-off between amino acid metabolic cost and protein sequence diversity in proteome composition

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The twenty protein coding amino acids are found in proteomes with different relative abundances. The most abundant amino acid, leucine, is nearly an order of magnitude more prevalent than the least abundant amino acid, cysteine. In order to explain this widespread phenomenon, we develop a theoretical model that postulates that (1) protein synthesis takes place with a limited supply of energy and (2) there is a trade-off between the metabolic cost of protein synthesis and the ability of the cell to synthesize as many different protein sequences as possible, as measured by entropy. We consider two contributions to amino acid metabolic cost, the number of ATP molecules required for synthesis and amino acid decay due to chemical reactions. These simple considerations are able to reproduce the relative abundances of amino acids in more than one hundred proteomes with better accuracy than previous models.

35. Putative binding site for the positive allosteric modulator SB-206553, in 5-HT_{2B} and 5-HT₃ receptors.

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At present, the pathophysiological understanding and searching of solutions for the central nervous system's related diseases (CNS) such as depression, Parkinson, reflex vomiting, anxiety and personal appreciation problems, where the serotonergic receptor family, plays an important role, appear as new challenges in the area of pharmacology. Based on that information, it is important to find and understand the molecular mechanisms of action of the drugs that possess the ability to interact with this receptors; this knowledge will help us to obtain more effective drugs with fewer side effects.

In this work, we perform a comparison of two proteins of the serotonin receptors family; the crystallographic structure of the 5-HT_{2B} receptor, and 5-HT₃ receptor which described a structural approximation in homomeric stoichiometry, which were obtained by homology modeling techniques. we investigated whether novel binding sites were formed or not and then it was performed molecular docking simulations to shed light on the mechanisms involved in the binding process for different ligands. We initially consider the serotonin and a new compound SB-206553, a positive allosteric modulator. Then, by means of molecular dynamics (MD) simulations, we establish which of the ligand-protein interactions was more stable over time, by obtaining relevant information at the atomic level, as well as molecular details of its thermodynamic stability and function.

36. Ligand prediction of orphan pockets by three-dimensional comparison of protein binding sites

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Predicting ligands of protein binding sites without known binders (orphan pockets) is a complex problem in structure-based drug design. Here, we present a new method for ligand prediction of orphan pockets by three-dimensional comparison with binding sites of known protein-ligand complexes. Pockets are represented by the Cartesian coordinates of Ca and hydrogen bond donor/acceptor atoms of residues within 6.0 Å of bound ligands. Next, pockets are compared by a clique matching algorithm. Finally, different similarity measures are calculated (root mean squared distance of matched atoms, (relative) structural overlap) to rank analyzed pockets. We devised a benchmark based on 201 high-resolution protein-ligand complexes with known binding affinity, employed our method to retrieve related pockets, and analyzed the results by means of receiver operator characteristic (ROC). Best results were obtained with the structurally conserved binding site of serine proteases (area under the ROC curve, AUC=0.99), intermediate results for the ATP-binding site of protein kinases (AUC=0.86) and moderate results for the structurally diverse binding site of glycosylases (AUC=0.79). Analysis of the respiratory syncytial virus matrix protein, a potential drug target, revealed similarity to nucleoside ribosyltransferases. We conclude that clique-based comparison of protein pockets is a promising tool in structure-based drug design. Acknowledgments: FONDECYT 1131065 and ICM P09-016-F.

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37. Assessing the Molecular Impact of Mutations in Protein Superfamilies Identified in Whole-Exome Sequences of Tumors

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With the emergence of ever cheaper technologies for DNA sequencing, today there are thousands of tumor samples that have been subjected to whole-exome sequencing. We downloaded the whole-exome somatic mutations for 3332 patients affected by 16 different types of cancer from The Cancer Genome Atlas initiative of the National Cancer Institute of the USA. We then normalized the data taking into account mutational heterogeneity of the samples by applying the MutSigCV method, which uses patient-specific mutation frequency, and gene-specific background mutation rates to determine statistically the genes that are significantly mutated in each sample. Of these significantly mutated genes, we identified 107 genes that are mutated in at least two different cancer types, with 10 superfamilies accounting for a large proportion of these mutated genes. We are characterizing each of these 10 superfamilies in terms of the conservation of the amino acid positions which are mutated, quantifying the effects of the mutations on the free-energy landscapes of the mutated proteins, and rationalizing these mutations in the context of the molecular function of each protein. Our method also allows us to evaluate the likelihood of mutations at equivalent positions in homologous proteins to generate cancer phenotypes, which is helping us define novel molecular markers for cancer.

38. Modulation of P2X4 receptors by Alfaxalone, studied via Molecular Dynamic Simulations

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P2X receptors are expressed throughout the human body including the nervous, cardiovascular and immune systems. Neuromodulation by extracellular ATP activates P2X4 receptors (P2X4R) allowing the pass to Na⁺, K⁺ and Ca²⁺ ions. The zebrafish P2X4R was recently crystallized in a closed-apo and ATP-bound open states, and although it has been proposed how ATP binding triggers activation of a P2X4R; this model is still incomplete because their cytosolic domains are absent. Neurosteroids such as alfaxalone potentiates the ATP response evoked by the P2X4R, where binding sites should be located in the transmembrane region of each subunit. Here a full P2X4R from rat was modeled and molecular dynamics (MD) simulations were carried out in the absence (closed-apo) or presence (open-holo) of ATP plus alfaxalone (one or three molecules). The MD trajectories of each system provided us with detailed information about the conformational changes triggered by alfaxalone and the interaction network within these systems. Funded by Millennium Science Initiative P10-035F, Fondecyt 1120169 and Anillo ACT 1108 grants.

39. Structural Characterization of the Internal Cavity of the Pore in K⁺ Channel Shaker: Contribution of Pro475 Residue to the Ion Current at Atomic-Level Perspective.

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Potassium channels allow K⁺ flux outside and inside of the cell. They are essential for the electric current generation that controls membrane excitability. K⁺ channels constitute one of the most diverse membrane protein families and show a large diversity of single-channel conductance. K⁺ channels contain a highly conserved ion-selective pore with a K⁺ selectivity filter composed of 6 residues. In the case of high-conductance channels, they have a couple of conserved glutamate residues at the internal entrance of the pore, which are not present in low-conductance K⁺ channels. Charge neutralization of these residues results in reduced outward currents. Performing an alignment between Shaker, a low-conductance K⁺ channel, and channels with high-conductance, we identified that residue P475 aligns with one of the conserved glutamate residues, suggesting a crucial role of this residue in the conductance of the channel. Previous studies showed that single mutations of P475 located at the entrance of the inner cavity of the pore of the Shaker channel increase the conductivity 6 to 8 fold. In order to understand the importance of residue P475, we performed free-energy calculations with molecular dynamics simulations for P475(WT), P475D and P475Q mutants, using Adaptive Biasing Force method based on thermodynamics integration. M.M.V. and S.R. thank CONICYT doctoral fellowship. All authors thank FONDECYT Project 1131003 and CINV (Millennium Science Initiative 09-022-F).

40. Identification of novel transient receptor potential (TRP) superfamily members and structural characterization of their activation by PIP₂

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Transient receptor potential (TRP) channels are a superfamily of calcium channels that play a role in sensory transduction of different somatosensory modalities, including thermosensitivity, and pain, among others. We performed iterative BLAST searches of a set of representative TRP channels against the NR database to identify novel superfamily members. Sequence relationships among the identified members were presented as sequence similarity networks (SSN), where nodes represent proteins and edges represent BLAST E-values, percent identities or alignment lengths between proteins. The nodes in the SSNs are then *colored* according to the associated functional properties of each channel to allow identification of structure-function relationships within the superfamily. We investigated the activation of TRPV1 and TRPM8 channels by phosphatidylinositol-4,5-bisphosphate (PIP₂). As no channels of this superfamily have been crystallized, our laboratory generated a model for a TRPV1 channel a few years ago. For this work, we generated a model for a TRPM8 representative member. We performed docking and molecular dynamics studies allowing us to characterize the binding sites of PIP₂ in both channels. It turns out these binding sites are analogous and not homologous as it would be expected. We provide some structural insight as to why this is the case. HP thanks to CONICYT doctoral fellowship. This work was supported by FONDECYT 1131003 and CINV (Millennium Initiative, 09-022-F)

41. Computational methods to analyse the release of membrane proteins from detergent micelles in the gas phase

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Purified membrane proteins in solution are usually complexed to detergent micelles to maintain their solubility. We have discovered a new method based on Mass Spectrometry to analyze the structure of intact membrane proteins in the gas phase. This process involves first the transfer of membrane protein-micelle complexes (MPMCs) from solution into gas phase and second the release of the protein after MPMC are collided with gas molecules at high kinetic velocity. However the precise molecular mechanism of this process is unknown. We present here three computational methods to analyse different MPMCs in gas phase: EmrE-DM, BtuCD-DDM and KirBac3.1-TriDM. The first method uses the increased temperature on molecular dynamics (MD) in gas phase, which is analogous to the internal energy increase occurred within the mass spectrometer. The second method is a probabilistic model based on MPMC geometrical properties. Using the solvent accessible surface area (SASA) calculated for each residue and detergent molecule in the complex, it provides a higher probability of collision at larger SASA values. The third method uses nano-mechanical simulations of the MPMC collided to gas molecules. All three methods have shown that KirBac3.1-TDM is the most stable MPMC and also provided a molecular mechanism of the release of intact membrane proteins in the gas phase. Funded by Millennium Science Initiative P10-035F, Fondecyt 1120169 and Wellcome Trust grants.

42. Increased glucose tolerance and insulin sensitivity in streptozotocin-diabetic rats treated with curcumin incorporated in yoghurt

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Previous results showed that the treatment of diabetic rats with curcumin incorporated in yoghurt improved various physiological and biochemical markers of diabetes, including plasma and urine glucose levels. The objective of this study was to evaluate the changes in glucose tolerance and insulin sensitivity of diabetic rats treated with curcumin-supplemented yoghurt. Male Wistar rats (150±10g) were injected with streptozotocin (40mg.kg⁻¹) to induce diabetes and they were divided into 2 groups, diabetic rats treated with yoghurt (DYO) and with 90mg.kg⁻¹ curcumin incorporated in yoghurt (DC90). After 1 and 15 days of treatment, the following assays were performed: oral glucose tolerance test (OGTT), insulin tolerance test (ITT) and fasting glycemia. Insulin serum levels were also determined. Data were expressed as mean±SEM and considered significant at p<0.05 (ANOVA). Both glucose tolerance (DYO: 39,080±2859.5; DC90: 30,595±2018.6, mg.dL⁻¹.120min⁻¹) and glucose disappearance rate (DYO: 0.0123±0.002; DC90: 0.0251±0.003, %.min⁻¹) were higher in DC90 after 15 days of treatment. Insulin levels (ng.mL⁻¹.120min⁻¹) were not different between DYO (26±6.8) and DC90 (43±19.7). There were no differences in the AUC values of fasting glycemia between the groups, after 1 or 15 days of treatment. The curcumin incorporated in yoghurt increased both insulin sensitivity and glucose tolerance in diabetic rats explaining, at least in part, the benefits of this treatment to diabetes.

43. Characterization of phenoloxidase activity from spider *Polybetes pythagoricus* hemocyanin

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Hemocyanin (Hc) is generally accepted as an oxygen transporter, although this pigment is functionally converted into a phenoloxidase-like enzyme (PO) by some reagents including SDS. PO is present in almost all organisms, functioning as an initiator of melanin synthesis. The aim of this work is to characterize PO enzymatic activity in spider *Polybetes pythagoricus*. Out of the three lipoproteic fractions isolated by hemolymph ultracentrifugation of *P. pythagoricus*, VHDL was separated, which contains hemocyanin as the major apoprotein and transports most of the circulating lipids. PO activity was assayed using dopamine as substrate by spectrophotometrical method at 475 nm. After testing several incubation times with SDS at a concentration of 10 mM, PO activity developed only after 20-min incubation. The kinetics parameters, *Km* and *Vmax*, were 0.407 mmol.l⁻¹ and 0.081 μ mol.min⁻¹.mg protein⁻¹, respectively. Hill coefficient was approximately 1 indicating Michaelis-Menten behavior. Great temperature dependence was observed with an optimum value at 42°C. It was performed a delipidation control of VHDL by SDS-PAGE-analysis, using the same SDS concentration as in enzymatic assays. From the observed results we can conclude that Hc is a very stable protein, which needs an exhaustive treatment to develop PO activity in comparison with Hc of other arachnids.

This work was supported by grants from: CONICET-PIP no. 075 and PICT no. 2010-1270, Argentina.

44. Oxidative metabolism of polyamines and embryonic development in *Rhinella arenarum* embryos exposed to azinphos methyl.

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Polyamines (PA) are aliphatic polycations essential for the normal progression of embryonic development. We have shown that the pesticide azinphos methyl (AM) alters PA metabolism, resulting in an increase in putrescine and a decrease in spermidine and spermine. The oxidative degradation of PAs generates reactive species. Our objectives are to characterize the oxidative degradation of PAs and to determine if there is a link between this process and the alteration of embryonic development. Embryos of the toad *R. arenarum* were employed and the activities of diamine oxidase (DAO) and acetyl polyamine oxidase (APAO) were determined. Also, documentation of morphological alterations and a correlation analysis between enzyme activities and malformations were carried out. We observed a significant increase in DAO activity in embryos exposed to 2 mg/L AM at the end of embryonic development and a significant decrease in APAO activity at intermediate stages in embryos exposed to 0,5-, 2- and 9 mg/L AM. The number of malformed individuals increased in embryos exposed to 9 mg/L AM. However, no correlation between DAO, APAO and malformations was observed. More studies are needed to characterize the oxidative metabolism of polyamines and to determine which of the degrading enzymes, if any, contributes to the alteration of *R. arenarum* embryonic development. This work was supported by grants from: Univ. Nac. Comahue 04 I 004; ANPCyT PICT 214/07 and CONICET PIP 2010-2012 00655.

45. Bag3 controls skeletal muscle cell metabolism

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The co-chaperone Bag3 is a key protein controlling degradative processes in response to stress such as autophagy. Recent reports point out that impaired autophagy leads to insulin resistance. On the other hand, previous evidences showed that Bag3 knockout mice die 3 weeks after birth with progressive muscle deterioration. Furthermore, serum profile from Bag3^{-/-} mice showed diminished circulating levels of glucose and insulin. However, it is still unknown whether Bag3 regulates cell energy metabolism. The aim of this study was to study the role of Bag3 on glucose metabolism in differentiated rat L6 myotubes. To achieve this, L6 myotubes were treated with small interference RNA targeting Bag3 mRNA (siBag3). Bag3 protein levels decreased near 50% compared to the scramble siRNA. Glucose uptake was assessed using ³H-2-deoxyglucose assay. The results showed that siBag3 abrogated insulin response but also increased glucose basal incorporation. Surprisingly, no changes were observed in Ser-473 Akt phosphorylation under 100 nM insulin. Taken together, these results suggest that siBag3 abrogates insulin response in L6 myotubes by altering insulin signaling pathway downstream Akt and/or by involving other glucose transporters like Glut-1. Proyecto Anillo ACT 1111 (SL)

46. Kinetics and thermostability studies of a hyperthermophilic bifunctional ADP-dependent glucokinase/phosphofructokinase from *Methanocaldococcus jannaschii*.

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In several archaea, the glycolytic flux proceeds through a modified version of the Embden-Meyerhof pathway where the phosphofructokinase and glucokinase reactions use ADP instead of ATP as phosphoryl donor. In the hyperthermophilic archaeon *M. jannaschii* a homologue enzyme that shows both ADP-dependent phosphofructokinase and glucokinase activities (ADP-*Mj*PFK/GK) has been identified. In this work we analyzed the protein stability of ADP-*Mj*PFK/GK compared to other members of the ADP-dependent family. Heat denaturation assays were monitored by circular dichroism, which shows that ADP-*Mj*PFK/GK unfolds at 90°C only in the presence of denaturing agents (guanidine hydrochloride), thus being more stable than *Thermococcus litoralis* glucokinase and *Pyrococcus horikoshii* phosphofructokinase. We studied the use of different sugars as phosphate acceptor substrates showing that ADP-*Mj*PFK/GK phosphorylates mainly glucose (Glu) and fructose-6-phosphate (F6P). Also, we determined the kinetic parameters for both substrates and its kinetic mechanism. The enzyme has a high affinity for F6P (K_m 3 μ M) compared to Glu (K_m 476 μ M) and similar ADP affinity using either Glu (K_m 32 μ M) or F6P (K_m 66 μ M) whereas the k_{cat} for glucokinase activity is 40 s⁻¹ and for phosphofructokinase 14 s⁻¹. Kinetic analysis at different co-substrate concentrations showed evidence for a sequential kinetic mechanism. Fondecyt1110137.

47. Temporo-spatial analysis of glucose transporter glut8 expression in mammary gland during pregnancy, lactation and weaning

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Glucose uptake in mammary gland is essential as energy supply needed for high proliferation rate during pregnancy. Moreover, in lactation, glucose supports the high metabolic activity necessary for synthesis of milk nutrients such lactose and fatty acids, processes which take place in different intracellular compartments of epithelial mammary cells. The glucose transporter 8 (GLUT8) is a class III facilitative glucose transporter and although it has been found in mammary gland, its physiological role remained unknown. Due to the importance of intake and intracellular compartmentalization of glucose in pregnancy and lactation, we studied the expression and the cellular localization of GLUT8 in mouse mammary gland during pregnancy, lactation and weaning by immunohistochemistry, RT-PCR and western blot. Our results revealed that GLUT8 is expressed constitutively in luminal epithelial cells of the mammary glands and in adipocytes of the stroma, but is absent in mioepithelial and ductal cells. Interestingly, GLUT8 expression showed an intracellular granular pattern in luminal epithelial cells which progressively increment through the pregnancy and the lactation, increasing considerably in early lactation (2 days) and reaching its highest expression at late lactation (10 days). This particular pattern of expression suggests that the GLUT8 transporter is involved in the glucose compartmentalization necessary for lactose synthesis. Support FONDECYT 11121367

48. Identification, characterization and functional expression of a hystidine ammonia-lyase from *Rhodobacter sphaeroides* (Rs)

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It has already been described that *Rs* has an tyrosine ammonia-lyase enzyme (*RsTALCII*) codified in its chromosome 2. The *RsTALCII* catalyzes the conversion of tyrosine to p-hydroxycinnamic acid (pHCA) a compound with attractive biological activities as: anti-tumoral, lipid-lowering and anti-inflammatory. Also it has been described that *RsTALCII* enzyme accepts tyrosine and phenylalanine as substrate. A second putative ammonia-lyase enzyme was found in the chromosome 1 of *Rs* (*RsHALCI*) which was identified, cloned and functionally expressed using the pRSET-B vector in *Escherichia coli*. The aim of this research was to characterize the *RsTALCII* and *RsHALCI* at biochemical and molecular level. From bioinformatic analysis it was found that the amino acid sequence comparison between both enzymes showed an 87% of structural homology. The *hal* and *tal* genes were amplified by PCR from the genomic DNA from *Rs* and cloned in the pRSET-B vector. The protein expression was induced and enzymatic assays were carried on using cell extract. Our results showed that *RsTALCII* show tyrosine or phenylalanine ammonia-lyase activity. The production of pHCA at flask level was measured by HPLC. Our results illustrate that *Rs* contains an hystidine ammonia-lyase enzyme in chromosome 1. *RsHALCI* activity was measured with hystidine as substrate to produce urocánico acid validating bioinformatic results. Acknowledge: CONICYT-PCHA/DOCTORADO-NACIONAL/2013-21130474. PUCV, project 037.430-2012.

49. GRK2 as a novel target in insulin resistance and obesity.

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We have reported that mice hemizygous for G protein-coupled receptor kinase 2 (GRK2), a kinase of G protein coupled-receptors, are protected towards the development of insulin resistance (IR) and obesity under different conditions.

Given the lack of selective and effective GRK2 inhibitors, we analysed whether a reduction in GRK2 could revert an already consolidated obese and IR phenotype in vivo by using tamoxifen (Tx)-inducible global GRK2 homozygous (Cre^{-/-}GRK2^{-/-}) mice and Floxed/Floxed wild-type littermates (Cre^{-/-}GRK2^{F/F}) as controls. A global reduction in GRK2 was achieved by Tx injection in the middle of the HFD feeding once the mice had reached an obese and IR phenotype.

Tx-induced GRK2^{-/-} mice are protected against diet-induced obesity. We detected no differences in food intake between genotypes, and GRK2^{-/-} mice showed no signs of fasted hyperglycemia or hyperinsulinemia after a HFD. Improved glucose tolerance and a more potent activation of insulin signaling was found in Tx-induced GRK2^{-/-} tissues, and these animals were resistant to the development of liver steatosis and to macrophage infiltration.

These results prove for the first time that GRK2 downregulation during a HFD is able to prevent body weight gain, reduce adiposity and revert glucose intolerance and IR, and serve as a first proof of concept that decreasing GRK2 levels can not only prevent but also revert an already established obese and IR phenotype in vivo.

50. Cloning and expression of mouse liver agmatinase.

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Agmatine is a polyamine whose biological importance lies in its action as a neurotransmitter, neuromodulator, hypoglycemic, among others. The only enzyme specific for the hydrolysis of agmatine to putrescine and urea is agmatinase, therefore play a crucial role in the regulation of physiological levels of agmatine. The sequence alignment of agmatinases from different species revealed that the mouse sequence catalogued as agmatinase, differs in four of seven key residues for the interaction of the enzyme with the divalent ion Mn²⁺, required for the hydrolysis of the guanidino group in members of ureohydrolase superfamily, to which it belongs, so that some authors have proposed that this enzyme would have little or no ability to catalyze the hydrolysis of agmatine. In this work we cloned a mouse liver cDNA for agmatinase and its expression in *Escherichia coli* JW2904, knock out on *E. coli* agmatinase gene, generated an enzyme with undetectable agmatinase activity in vitro, at now, our conclusion support the hypothesis that this enzyme not has ability to hydrolysis of agmatine.

51. Study of cruzipain expresssion in virulent and avirulent forms of *Trypanosoma cruzi* clone H510

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Trypanosoma cruzi virulence depends among others of the parasite strains or clone and several virulence factors have already described. Cruzipain, the major *T.cruzi* cysteine proteinase its a virulence factor involved on cell invasion and host immune resistance. Here, we use a H510 clone of *T.cruzi* in order to demonstrate the role of cruzipain on virulence. A virulent form of the H510 clone have been cultured on mice during 25 years, named H510*vir*, and avirulent form, was cultured by on axenic médium named H510*avir*. Then, we evaluated the invasion of both variants in presence or not of anti-cruzipain antibodies. In the same way, the protein expression of cruzipain in different *T.cruzi* stages of the both variants forms were evaluated by western blot and immunofluorescence. The expression of mRNA was evaluated by real time PCR. Finally the secretome of both variants was evaluated by immunoblot. H510*vir* was five times more infective to Vero Cells than H510*avir*. However, cell invasion of H510*vir* was reduced by 60% when parasites were incubated in presence of anti cruzipain antibodies. Western blot revealed that cruzipain was more expressed on H510*vir*. Immunofluorescent reveled that cruzipain was poorly expressed on trypomastigote surface. However the enzyme was secreted in higher ammount by H510*vir* trypomastigotes. Finally, the expression of cruzipain mRNA was also higher in H510*vir*. These results, confirm that cruzipain is associated with the *T.cruzi* virulence.

52. Learning metabolism by PBL-based analysis of web articles in Biochemistry

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PBL promotes learning and skills such as collaborative work and critical thinking, presenting advantages compared to traditional lectures. On the other hand, assessment has to be contextualised in real life to achieve authentic learning. Consequently, PBL-based analysis of web articles was used to teach metabolism in Biochemistry.

Students analysed articles regarding health or nutrition issues which were debatable from a metabolic point of view. Teams worked on 2 problems, each one during 4 sessions distributed in 2 weeks. Finally, they presented their conclusions, focusing on the process of resolution and difficulties. Students agreed to assist and commit to the activity through a Learning Contract.

Co, auto and hetero-evaluation was performed using a progress assessment chart. Students' opinion was assessed using a checklist. The majority indicated that the cases helped them to apply knowledge to real life. The perception that PBL promotes the ability to solve problems, critical thinking and collaborative work is noteworthy. Students agree that they learnt from the topic analysed by their group. Tutors valued that teams identified the problem and concluded correctly, specially mentioning students' enthusiasm and motivation, including those with low class attendance and grades. This intervention suggests that PBL-based analysis of web articles in place of traditional lectures is a valuable alternative to promote learning of biochemistry

53. The establishment of the cytosolic H3K9me1 modification is regulated by a cross-talk between SetDB1 and JMJD1B

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The processing of the cytosolic histones includes the translation of the mRNA by ribosomes and the translocation to the nucleus of the synthesized protein. This processing occurs in a cascade of maturation, which includes the correct folding and the association with different chaperones and enzymes, in four cytosolic complexes. In contrast to the highly modified nucleosomal histone H3, the cytosolic histone H3 only has few modifications. It is acetylated in the lysines 14 and 18 (H3K14K18ac) and monomethylated in the lysine 9 (H3K9me1). Our previous results suggested that the modification H3K9me1 occurs during the translation of the histone, while associated to the ribosome. With the goal of investigating this, we isolated ribosomes, observing that the histone H3 associated with the ribosome is H3K9me1. In addition, western blot analyses showed that the enzymes SetDB1 and JMJD1B, the H3K9me1 methyltransferase and demethylase, respectively, associate with the ribosome. Knock down of these enzymes affected the levels of the methylation mark in the ribosome as well as in the cytosolic fraction. Other marks and the level of the different chaperones are affected too. Our results suggest the existence of a cross-talk between the enzymes SetDB1 and JMJD1B at the ribosomes to regulate the level of the H3K9me1 mark present in the cytosolic histone H3. **Funded by FONDECYT 1120170, Basal Project PFB16.**

54. Identification and expression patterns of ABA perception genes (PYR family) in *Fragaria vesca* and *Fragaria chiloensis*

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The diploid woodland strawberry, *Fragaria vesca*, is recognized as a model for the more complex octoploid species such as *F. × ananassa* (the commercial strawberry) and *F. chiloensis* (the native Chilean strawberry). *F. vesca* exhibits a short seed to seed cycle, its draft genome sequence has been published, and therefore, it becomes a good model for studying fruit ripening. Control of ripening in non climacteric fruit such as strawberry remains unclear, although auxin and abscisic acid (ABA) may have a role on it. During ripening of strawberry fruit auxin levels decrease while ABA increases. ABA is synthesized in the achenes of the fruit and it is perceived in the receptacle by different ABA receptors, like the PYR/PYL family. In this work, we identified 8 PYR genes in *F. vesca*'s genome, and they belong to different phylogenetic groups. The transcription level of the 8 PYR genes was determined by qPCR in different fruit ripening stages and vegetative tissues (stem, roots, flowers, leaves and runners). Different transcriptional patterns were recorded for the PYR genes, and among them, PYR7 is mainly expressed in fruit tissues and its transcripts increases during ripening of *F. vesca* and *F. chiloensis*. This suggests that PYR7 could be involved in ABA perception and ripening regulation in both fruit species. R. Lizana acknowledges Universidad de Talca for his doctoral fellowship. Research supported by Fondecyt 1110792 and Anillo ACT-1110 projects.

55. Evaluating light's effect on differentiation and virulence of the plant pathogen *Botrytis cinerea*: characterization of the White Collar complex

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Light can affect several aspects of an individual's physiology, including pathogenesis. While several fungal models have been used to understand light perception, other agricultural-relevant fungi still remain, in terms of their responsiveness to light, in the dark. *B. cinerea* is a fungal pathogen causing disease on a wide range of plants. Natural *B. cinerea* isolates exhibit high diversity in their predominant mode of reproduction. Thus, the majority of naturally occurring strains reproduce asexually via conidia and sclerotia, and sexually via apothecia. Early studies reported on specific developmental responses to treatments with near-UV, blue, red and far-red light. To unravel the signaling machinery triggering development, and possibly also affecting virulence, we initiated the characterization of the transcription factor/photoreceptor BcWCL1 and its partner BcWCL2, that form the White CollarComplex (WCC) in *B. cinerea*. Using mutants either abolished in or exhibiting enhanced WCC signaling, we show that the WCC mediates transcriptional responses (TRs) to white light, inhibiting conidiation in response to this stimulus. WCC is also required for coping with excessive light, oxidative stress and to achieve full virulence. Although several TRs are abolished in the KO of *bcwcl1*, the expression of some genes is still induced by light while a distinct conidiation pattern in response to daily light oscillations is enhanced. Fondecyt postdoc 3110127, Fondecyt 1131030.

56. Structural domains within the 5'untranslated region of the HIV-1 full length mRNA influence IRES activity

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The 5' leader of the human immunodeficiency virus type 1 (HIV-1) genomic RNA is a multifunctional region that can fold into a conserved secondary structure which regulates multiple activities during viral replication including translation. The functional RNA elements include the *trans*-activating region (TAR), the polyadenylation (polyA) signal, the primer binding site (PBS), the dimerization initiation site (DIS), the splice donor (SD), and the packaging signal (Psi). The HIV-1 5'leader also harbors an internal ribosome entry site, the HIV-1 IRES, which drives translation initiation under conditions that hinder cap-dependent translation initiation. The structural components of the HIV-1 IRES remain unknown. In this study we explore the impact of a series of mutations identified in natural viral isolates on the activity the HIV-1 IRES. Results show that the introduction of mutations within the sequence of the reference pNL4.3 5'leader has very little impact on its IRES activity. Then we evaluate the influence of the conserved RNA structural motifs of the HIV-1 5'leader on the activity of the viral IRES. Results suggest that the HIV-1 IRES has a modular nature and that the PBS, DIS, and SD structures contribute in different degrees to its activity. FONDECYT 1130270 and ICM Project P09/016-F.

57. The residence of the yeast SWI/SNF complex on several gene regulatory regions is dependent on the HMGB protein Hmo1.

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Nucleosomes generally restrict the access of DNA-binding proteins to their sites. ATP-dependent chromatin remodeling machines, such as the SWI/SNF complex, facilitate this access. SWI/SNF is involved in transcriptional regulation of several genes in yeast. HMGB proteins also play a significant role in chromatin dynamics. In this context, we observed that yHMGB proteins, including Hmo1, stimulate remodeling activity. It is currently unknown whether the presence of SWI/SNF on gene regulatory regions is dependent on these proteins. By carrying-out assays using in vitro reconstituted nucleosomes, we studied the effect of yHMGB proteins on SWI/SNF activities. The effect of these proteins on SWI/SNF association to gene promoters was ascertained by ChIP-chip assays. Additionally, we analyzed the effect of yHMGB proteins on nucleosome positioning for gene promoters under direct control of SWI/SNF. Our results indicate that yHmo1 stimulates binding of SWI/SNF to the nucleosome and also co-localize with this complex on several gene promoters. Importantly, the association of SWI/SNF to the promoter region of several genes is diminished in the absence of Hmo1. Consistently, the analysis of nucleosome positioning in the promoter region of some of these genes reveals an altered pattern in the absence of this protein. Taken together, our results demonstrate that the residence and activity of SWI/SNF on several gene promoters is dependent on yHMGB proteins. FONDECYT 1130818.

58. SCL and RUNX1 regulate P1 promoter RUNX1 expression gene by association to RUNX sites binding

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RUNX1 is a transcription factor essential to hematopoiesis process. Its gene is located in chromosome 21 and two promoters, P1 and P2, regulate its transcription. Analysis of P1 promoter's sequence identified several consensus sites for different hematopoietic transcription factors include SCL, RUNX, and others, suggesting a possible role in transcriptional regulation of RUNX1 gene. Also several report have shown that these two transcription factors are associated to regulate target genes. To test if P1 promoter is regulated by SCL and RUNX1 we cloned P1 in a luciferase--reporter vector, and we measured reporter activity to SCL response. We observed increased reporter activity in a doses-dependent manner. To determinate association of SCL to P1 promoter we performed chromatin immunoprecipitation (ChIP) in hematopoietic cells (HL-60 and Jurkat cells), our results show that SCL binds to SCL site. Interestingly, we also detect SCL enrichment in RUNX sites present in P1 promoter. Using ReChIP assays we demonstrate that RUNX1 and SCL simultaneously associate to P1 promoter through RUNX binding sites. Taken together our results demonstrate that in HL-60 and Jurkat cells SCL regulate P1 promoter activity and associates to RUNX1 protein. FONDECYT 11306970

59. Effect of CK2 α overexpression on ribosomal protein transcription in *Schizosaccharomyces pombe*.

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In *Schizosaccharomyces pombe*, ribosomal protein (rp) gene promoters contain a TATA analogue element called HomolD box. Rrn7 is the HomolD-binding protein, which forms a complex with RNAPII machinery. Rrn7 is a protein kinase CK2 substrate, and its phosphorylation affects the HomolD-binding activity, thereby affecting the HomolD-box directed transcription. So far, it has been well characterized *in vitro* regulation by CK2, which is also found in HomolD-containing promoters, but it is unknown if CK2 is able to regulate the transcription of rp genes. In this work we overexpressed the catalytic subunit of CK2 (CK2 α) in a *Schizosaccharomyces pombe* strain, which contains Rrn7-tagged protein. Levels of ribosomal protein mRNA were measured, and the interaction between Rrn7 with both CK2 α and β subunits were studied. We obtained that CK2 α overexpression affected ribosomal protein mRNA level. We found an *in vivo* association between both CK2 subunits and Rrn7. We concluded that CK2 regulates transcription of ribosomal protein genes via modulation of Rrn7.

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60. Heterologous expression of *Pneumocystis carinii* TFIIIB in the fission yeast *Schizosaccharomyces pombe*: An evidence of functional conservation on basal transcription mechanism.

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The fungus *Pneumocystis*, an opportunist pathogen, is the major etiologic agent of pneumonia in immunocompromised persons as HIV-infected people. Surprisingly, studies on immunocompetent people showed the presence of this pathogen without signs of illness. The study of biochemical features of *Pneumocystis* is crucial to generate adequate therapies to prevent infections, however this pathogen cannot be grown in laboratory so it is necessary other sources of information. One of them is the genomic sequence of *Pneumocystis carinii*, the rat pathogen. Since fission yeast and *Pneumocystis* are genetically related, we hypothesized that TFIIIB from *P. carinii* can replace the function of the yeast TFIIIB in a heterologous expression system. The coding sequence of TFIIIB from *P. carinii* (Pneumo-IIB) was cloned and expressed in BL21(DE3) cells. Purified recombinant Pneumo-IIB was able to recognize a TATA-containing probe in a complex with TBP from *S. pombe*. Heterologous expression in *S. pombe* was performed using an expression vector repressible with thiamine. Pneumo-IIB was overexpressed in cells grown in minimal medium. By immunoprecipitation assays we observed interaction of TBP and Pneumo-IIB. In chromatin immunoprecipitation (ChIP) assays we found association of Pneumo-IIB and nmt1 promoter, a TATA-box containing sequence. These results show the first approach of functional conservation of basal transcription mechanism between *Pneumocystis* and fission yeast.

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61. Effects of vaccination and challenge against *Caligus rogercresseyi* on IgT mRNA expression in mucus of rainbow trout (*Onchoryncus mykiss*).

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Recently, has been described a new type of immunoglobulin Tau (IgT) in teleost fish, mainly in rainbow trout, which is expressed in gills, head kidney, spleen and intestinal mucus in fish that have been challenged with intestinal parasite. This immunoglobulin T has a similar role to that described in mammalian IgA, whose function is to defend the organism against several pathogens that cross the barrier defenses of the fish and prevent the establishment of the mucosal membranes. However, no studies on the effect of vaccination and subsequent challenge against *Caligus rogercresseyi* on IgT expression in tissues of rainbow trout. Using specific primers for conventional PCR and Real Time was achieved to demonstrate the presence of this immunoglobulin in both cases with differential expression, where its expression increases in mucus with kidney in vaccinated fish. Likewise shows that IgM has a similar expression pattern. IgT presence in fish mucus vaccinated and challenged against this ectoparasite, opens discussion about the relevance of an acquired generation antibody immune response to fight the infestation of fish with *Caligus*.

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62. Relative Expression of genes involved in iron metabolism of *P. salmonis* in different growth conditions.

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P. salmonis, the most important bacterial pathogen that affect salmon production in Chile. Its growth can be either facultative or in cell culture media. The goal of this work was to study through RT-qPCR the relative expression of some genes involved in iron metabolism in other bacteria (*Fur*, *FeoA*, *FeoB*, *Bfr*, *FhuC*, *FhuF*) that were annotated in a *P. salmonis* sequence. We grew a local strain of the bacteria in different growth conditions; *in vivo* (head kidney of moribund fish) or in solid media supplemented with either with sheep blood (EBA) or FeCl₃ media. Our results indicate that *Fur* is up-regulated under abundant quantities of iron (media with FeCl₃) in compare to salmon kidney and enriched blood agar. *FeoB* did not any changes in expression between all media and *FeoA* is down-regulated in media with FeCl₃, when compared with EBA media. On the other hand, *FhuC* and *FhuF* are up-regulated in abundant iron condition. Finally, *Bfr* is overexpressed in FeCl₃ media when compare to EBA, but down-regulated when compare to the *in vivo* samples. In this work we show that many genes involved in iron metabolism in other bacteria, play a significant role in the iron metabolism of *Piscirickettsia salmonis*, thus leading to a further understanding of the host pathogen interaction. Acknowledgements: Conicyt scholarship and Fondecyt 1120608

63. Effect of polar auxin transport changes on ripening and the proposed role of sugar

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Grapevine fruit development is divided in three stages: the formation stage (stage I), the lag phase (stage II) and the ripening stage (stage III). Ripening involves sugar accumulation, softening and size increase. It has been proposed that auxin delays ripening. In plants, auxin levels are fine tuned, being changes in polar auxin transport one of the mechanisms for auxin homeostasis control. On the other hand, has been demonstrated that sugar can be a signaling molecule able to control gene expression. In *Arabidopsis* genes related to polar auxin transport are induced under sugar treatments. The aim of this work is to determine whether polar auxin transport affects ripening and whether sugar regulates genes encoding for putative polar auxin transporters, named *VvPINs*. Blocking polar auxin transport resulted in ripening advance, similar to sugar treatments. On the other hand, we found that sugar treatments of berries modified the expression of *VvPINs*. These results suggest that there is a link between auxin homeostasis and sugar accumulation starting at the onset of ripening. To our knowledge, this is the first work that studies sugar mediated control of ripening through auxin regulation.

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64. A promoter element located in t(8:21) break point region is controlled by RUNX1

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t(8:21) is one of the most frequent chromosomal translocation found in leukemia. To date all the break points mapped are located in intron 5 of *RUNX1* gene. Interestingly, this intron exhibits DNaseI hypersensitive sites, which has been associated to *cis* regulatory elements. Therefore, we hypothesize that regulatory elements are harbored in intron5 of the *RUNX1* gene. In order to test this hypothesis, we performed bioinformatics analysis of ChIPseq data available from the server ENCODE to identify regions of intron 5 enriched in epigenetics marks associated with regulatory elements. We show that in hematopoietic cells one region is enriched in K4H3me, K4H3me3 and K27H3ac that colocalizes with DNaseI hypersensitive sites. These marks have been associated with promoter modules. Indeed, when we cloned this region (PPR=putative promoter region) in a reporter vector, we found that it activates expression of the reporter gene in an orientation dependent manner. Moreover, when we analyze putative transcription factors binding sites, we found one *RUNX* binding motif. Indeed when we transfected the promoter with *RUNX1* expression vector we found that *RUNX1* modulates the PPR expression. Using ETS database, we have assembled a putative messenger for this promoter. Moreover, by RT-PCR we have shown that this RNA is expressed differentially in several cell lines. Taken together our results shown a novel gene encoded in intron 5 of *RUNX1* gene controlled by *RUNX1*.

65. Characterization of tREST as a transcriptional regulator of the non-neuronal gene CDH1 (E-cadherin) in the H69 cell line of SCLC.

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The Small Cell Lung Cancer (SCLC) is characterized by the expression of neuronal genes and by the silencing of non-neuronal genes. SCLC has been related with the silencing activity of REST (RE-1 Silencing Transcription factor), which represses the transcription of several neuronal genes in non-neuronal cells via its binding to a Regulatory Element (RE-1). In SCLC, REST expression is decreased but the tREST isoform expresses by alternative splicing. Currently, through *in silico* analysis, several RE-1's have been identified in non-neuronal genes, like CDH1, who plays important roles in cell-cell adhesion and had been related with the initiating of metastatic process in SCLC. In the present work, to investigate if tREST participates in the regulation expression of CDH1 gene, which had been identified a RE1 consensus sequence in its promoter region. We observed a decrease in CDH1 mRNA when tREST was transfected in MRC5 cells (which do not express endogenous tREST), these data suggest a relation between tREST and CDH1. Next, electrophoretic mobility shift assays (EMSA) showed the interaction between tREST and RE1^{CDH1} element. Additionally, H69 cells were transfected with a plasmid containing RE1^{CDH1} element coupled to the luciferase gene, cloned upstream of the SV40 promoter, this experiment showed a marked inhibition of Luciferase reporter gene, suggesting that the RE1^{CDH1} element might play a role in the negative regulation of the CDH1 non-neuronal gene in H69 cells of SCLC.

66. Functional studies of PcACE1 transcription factor from *P. chrysosporium* with copper

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Copper is a key element in living organisms. Transcription factors in *S. cerevisiae* regulate its intracellular concentration; ACE1 is activated at abundant copper and MAC1 when scarce. ACE1 contains three Cys motifs like CXXCXnCXCH in the amino terminal, and MAC1, one at the amino and two at the carboxy terminal. We have isolated the ortholog of ACE1, PcACE1, from *Phanerochaete chrysosporium*, a white rot fungus. It contains three Cys motifs at the amino terminal as ACE1, and two at the carboxy terminal as MAC1. In this work we present results about the relevance of Cys motifs in PcACE1. Cys PcACE1 mutants were obtained by site directed mutagenesis, transformed into *Saccharomyces cerevisiae* ace1D and analyzed for PcACE1 functional activity at high and low copper. Carboxy terminal truncations lacking Cys motifs indicate that the last one is fundamental for PcACE1 transcriptional activity. Furthermore, PcACE1 contains an inhibitory region for transcriptional activity localized to the C-terminal. Single mutations C134S, C300S, C304S, C306S, C432S and C452S, as wild type, are resistant to 50 µM CuSO₄. C304S and C302S replacement mutants have about 70% transcriptional activity in the presence of copper, compared to 100% WT PcACE1. Currently we are working with double and multiple Cys mutations to ascertain the function of a group of Cys residues.

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67. The unfolded protein response is required for growth under complex carbon sources in *Neurospora*

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Accumulation of misfolded proteins in the endoplasmic reticulum (ER) triggers the unfolded protein response (UPR), a conserved signaling pathway that maintains protein-folding homeostasis in the ER. The protein secretion pathway of filamentous fungi is of special interest due to their high secretion capacity and therefore its molecular dissection has important biotechnological implications. Recently, the ascomycete *N. crassa* has emerged as a model system for the study of plant cell wall deconstruction, a process with valuable industrial applications. *Neurospora*, not only proficiently degrades plant cell walls but it also presents a variety of molecular tools that makes it an ideal model for the study of this process. Degradation of plant biomass requires the secretion of large amounts of hydrolytic enzymes, providing an ideal natural system in which to study UPR. In this work, we identified the *Neurospora* homolog of the yeast HAC1 protein, a key bZIP transcription factor involved in UPR. To assess its role in *Neurospora*, we generated a *hac1* KO strain and evaluated the expression of typical UPR targets under ER stress conditions and studied its growth under carbon sources that would require secretion of large amounts of enzymes. Our results suggest that a proper protein-folding capacity is required for normal growth under demanding conditions, reinforcing the biotechnological importance of this pathway. Fondecyt 1131030.

68. IL-33-and retinoic acid-signaling control CD4⁺ T cell biology

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Interleukin (IL)-33 participates in murine inflammatory disease models, including organ transplantation. Evidence suggests that IL-33 is implicated in T helper and regulatory T cell function. Similarly, Retinoic acid (RA) gets triggered during inflammation, playing a role in dendritic cells (DCs) and T cell function. Based on this, we decided to study the role of IL-33 in RA-mediated immunity. First, bone marrow (BM)-DCs were generated *in vitro*, treated with GM-CSF/IL-4 to induce Raldh activity, and with IL-33, evaluating IL-33 receptor (ST2) expression and maturation status on Raldh⁺ DCs. Spleen-isolated CD4⁺ T cells were co-cultured with these differentially-treated BM-DCs for 4 days to analyze cytokine production by ELISA. Additionally, we used a murine skin transplantation setting as an inflammatory model to study both ST2 expression and T cell-derived cytokine production. Our *in vitro* results indicate that CD11c⁺Raldh⁺ cells down-regulate ST2 expression, displaying a mature phenotype. IL-33 treatment did not affect DCs phenotype, but CD4⁺ T cells co-cultured with IL-33-treated CD11c⁺Raldh⁺ cells secreted higher levels of IFN γ . *In vivo*, skin allograft-draining lymph nodes contained higher proportion of CD11c⁺Raldh⁺ cells expressing low levels of ST2, and secreted high levels of IFN γ with respect to syngeneic controls. These results indicate that IL-33 and RA-signaling cross-talk modulate CD4⁺ T cell biology. (Funded by CONICYT 791100001, FONDECYT 11121309).

69. Study of nrp-1 expression in a murine skin transplant model

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Regulatory T cells (Tregs) modulate the immune system, suppressing harmful immune responses for the body, thus they are essential for immunological self-tolerance and homeostasis. Searching for Tregs surface markers remains a priority in the field of cellular therapies. It has been described that Neuropilin-1 (Nrp1) is preferentially expressed by thymus-derived Treg cells (tTregs) which exhibit the surface phenotype CD4⁺CD25^{hi}Nrp1^{hi} and express Foxp3. In this work, we analyzed the *ex vivo* expression of Nrp1 in different tissues from Foxp3/GFP reporter mice. Using a model of skin allograft rejection, we analyzed the expression of Nrp1 on CD4⁺ T cells present in graft-draining lymph nodes (GDLN), spleen and skin grafts from both, syngenic- and allogenic-grafted mice. Our *ex vivo* data indicate that 50-80% of CD4⁺CD25^{hi}Nrp1^{hi} T cells express Foxp3, which allow us to consider this population as Tregs. Ten days post-surgery, we found an increase in number and proportion of GDLN-resident Nrp1⁺CD4⁺ T cells in allogenic-grafted mice versus controls (syngenic-grafted mice). However, there is a significant decrease in number and proportion of GDLN-resident CD4⁺CD25^{hi}Nrp1^{hi} T cells from allogenic-grafted mice. Our findings corroborate Nrp1 expression on Tregs. Regarding cellular dynamics during rejection, a decrease in Nrp1⁺ Tregs suggest that these cells might be migrating to the graft, losing phenotypic stability or both. (Funded by CONICYT 791100001, FONDECYT 11121309).

70. Murine macrophages stimulated *in vitro* with mollusk hemocyanins (KLH, CCH and FLH) present a different expression profile of pro-inflammatory cytokines and chemokines

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Hemocyanins, the oxygen-transporting glycoproteins of mollusks, when are inoculated in mammals, they are capable of initiate a potent activation of the immune system, reason why they have been used as a biotechnological tool in the development of antibodies, vaccines and as an immunostimulant in superficial bladder cancer. The hemocyanin from *Megathura crenulata* (KLH) has traditionally been used for these purposes. However, biodiversity of hemocyanins has promoted interest in finding new candidates with better immunological properties. Therefore, emerge the hemocyanins from *Concholepas concholepas* (CCH) and *Fissurella latimarginata* (FLH), the latter with superior immunostimulatory properties than KLH and CCH. Hemocyanins promote Th1 responses, however the early immune mechanisms involved are not well understood. Since antigen immunogenicity is associated with its processing by antigen presenting cells, we have studied the interaction of hemocyanins with dendritic cells and macrophages and found that they are endocytosed and slowly processed. With aims of understanding further effects of hemocyanins on macrophages and how triggers the Th1 respond, we present here RT-qPCR and ELISA results from an array of cytokines and chemokines of macrophages stimulated within 24 hours. The main results showed that FLH exerts the greatest effects on the expression of pro-inflammatory cytokines, although all Th2 genes were down-regulated at the same rate. Project FONDECYT 1110651.

71. Priming of the kinin B1 receptor improves human neutrophil responses to fMet-Leu-Phe (fMLP)

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After a first contact with low concentrations of a specific mediator, human neutrophils acquire a state of preactivation known as priming. A new contact with an appropriated mediator produces an amplification of neutrophil response. Our aim was to determine whether the B1 receptor (B1R) agonist, Lysdes[Arg⁹]bradykinin (LDBK) acts as a priming agent. Stimulation of neutrophils with LDBK did not increase [Ca²⁺]_i but, priming for 10 min, prior to stimulation with 100 nM fMLP produced a concentration-dependent increase when compared to unprimed cells. [Ca²⁺]_i increased 18% and 30% in neutrophils primed with 0.1 nM and 100 nM LDBK, respectively. LDBK-primed neutrophils, stimulated with 10⁻⁵ M fMLP, increased the release of myeloperoxidase, elastase and cathepsin G when compared to unprimed cells. Phagocytosis of FITC-latex particles, by neutrophils stimulated with 1 nM fMLP, increased two-fold in LDBK-primed neutrophils (1 nM LDBK for 30 min). Stimulation of neutrophils with 0.1 to 1000 nM LDBK did not result in ROS production but, when cells were primed with 1 nM LDBK for 120 min, ROS levels in response to 100 nM fMLP increased by 1.7 ± 0.1 times. Our experiments show the ability of the B1R agonist to modulate neutrophil responses to fMLP suggesting that B1R activation may be crucial for an efficient functional activity of the human neutrophil. Supported by FONDECYT 1110464, Chile

72. Contribution of the IL-33 Receptor to the pathogenesis of inflammatory bowel disease.

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Background: The signaling through IL-33/IL-33R axis has been associated with gut mucosal immunity. We have suggested that IL-33 and its receptor variants, IL-33R and soluble IL-33R (sIL-33R) contributes to inflammatory bowel disease (IBD) pathogenesis. Furthermore, our *in vitro* studies have demonstrated that corticoids induce sIL-33R through the participation of the glucocorticoid receptor (GR) and a mechanism that involves a glucocorticoid response element (GRE) present in the distal promoter. The aim of this work was to evaluate molecular mechanisms by which pharmacological therapy used in IBD patients affects IL-33R. Method: Blood samples from IBD patients were included in the study. Serum sIL-33R levels and genetic changes were determined by ELISA and PCR-sequencing. Results: Serum levels of sIL-33R significantly increased in patients receiving corticoids compared to those using other therapy (p < 0.05). The presence of a polymorphic variant (rs6543115) located in a functional GRE in IL-33R gene was evaluated in 123 IBD patients and 113 healthy subjects. The polymorphic genotype was associated with IBD, with higher sIL-33R serum levels, use of steroids and clinical risk factors (p < 0.05). Conclusions: Our findings indicate that corticoids induce sIL-33R production and the presence of the rs6543115 SNP in IBD patients is associated with adverse phenotype and higher sIL-33R levels, which might be related to an increased gene expression.

73. Effect of cell envelope of different *Porphyromonas gingivalis* clinical isolates on viability of Gingival Epithelial Cells

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Chronic periodontitis is an infectious disease that causes inflammation and loss in the teeth supporting tissues. Infection by *Porphyromonas gingivalis* plays a central role in the etiology of disease and the high variability in virulence factors between different strains, can induce distinct responses in infected tissues. In this work, we evaluated some aspects of cellular envelope in several isolates of *P.gingivalis* from healthy and periodontitis individuals. Specifically, we characterized macromorphology, biofilm formation, surface charge, presence of capsule and the effect in the viability of Gingival Epithelial cells (GECs). Our results showed that differences in growth and macromorphologic characteristics were found between healthy and ill isolates. Moreover, biofilm formation ability was higher in all clinical isolates, compared to reference strains. However, no significant differences between healthy and periodontitis isolates were found. In addition, clinical isolates showed higher polarity than reference strains and capsule was present in almost all of them. Finally, an increase in the viability of infected GECs at 24 hours post-infection was observed, being higher in the isolated of ill donors. Interestingly, the isolate showing the lowest surface polarity and the higher ability to form biofilm induced a high increase in cell viability, suggesting that these features could be correlated with a more virulent strain.

74. Soluble MICA in the serum promotes decreased NKG2D receptor expression on NK cells in gastric cancer patients

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Gastric cancer (GC) is the second most common cause of cancer death worldwide. Natural killer (NK) cells play an important role in the immune defense against transformed cells. They express the activating receptor NKG2D, whose ligands, such as MICA, are generally overexpressed on tumors. The aim of this work was to analyze MICA and NKG2D receptor expression in patients with GC. Flow cytometry was used to measure cell surface MICA and NK cell-associated NKG2D expression in gastric primary tumor and adjacent mucosa, as well as NKG2D expression on peripheral NK cells. Serum levels of soluble MICA (sMICA) were determined by ELISA. We observed that cells from the gastric tumor and adjacent mucosa showed low levels of cell surface MICA, while significantly higher levels of sMICA in the serum were detected in GC patients, as compared to healthy individuals. Interestingly, expression of NKG2D was significantly reduced on tumor-infiltrating and peripheral NK cells from GC patients, as compared with the corresponding gastric mucosa and peripheral NK cells from healthy donors, respectively. MICA+ serum from GC patients downregulated surface NKG2D on control lymphocytes. Our results provide evidence that sMICA may contribute to gastric tumor evasion of the immune response mediated by NK cells. Financial support: Fondecyt 1130330 and 11110456 and Beca CONICYT para Estudios de Magíster en Chile, 2011.

75. Interferon-I activity reporter cell lines to explore natural and synthetic libraries-derived compounds which can modulate the cytokine's potency

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The clinical effects of IFNs depend on their use as biotherapeutics or their self-production in autoimmune diseases. The development of new IFN-I reporter assays (RA) could be of a high clinical value to search for compounds that could increase the IFNs therapeutic efficiency or decrease their side effect in the mentioned illnesses. With the above-mentioned aim, simple, fast and robust RA were developed to analyze lots of compounds simultaneously giving reliable and reproducible results. Four human cell lines: WISH, A549, HEp-2 and HeLa were used to develop new reporter gene assays based on the expression of the eGFP gene under the control of type I IFN-inducible Mx2 promoter. Thus, the eGFP expression was directly correlated with the hIFN-I concentration in the sample. We analyzed a natural library of 176 compounds and 288 from a synthetic library provided by *HelmholtzZentrum für Infektionsforschung*, Braunschweig, Germany. We isolated 21 inhibitory and 1 enhancer compounds. Their cytotoxicity, effective doses, antiviral and anti-proliferative activity, residual and reversible effect, and their action on cell cycle and apoptosis were evaluated. We could easily discriminate positive from negative compounds. The Z factor, was always higher than 0.8, reflecting the excellent quality of the bioassay. This search for compounds which can improve or block type I IFN's activity shows a big potential in view of their therapeutic implications.

76. Effect of *Drimys winteri* whole extract and its active principle poligodial on the expression of immune genes in the ASK-1 cell line infected with *Saprolegnia parasitica* oomycetes

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Fungal infections, also called mycosis are one of the pathologies that generate important economical losses in the aquaculture industry. *Saprolegnia parasitica* has been described as the main pathological species of aquatic organisms. Present treatments for this pathogen have not been successful due to the lack of knowledge about the mechanism of infection, this has incited much interest in the study of these mechanisms and encouraged the search for vegetal bioactive compounds for more specific treatments. The aim of this study was to evaluate the protecting activity of *D. winteri* extract and its active compound poligodial on an *in vitro* cellular model infected with *S. parasitica* by RT-QPCR. Treatment with poligodial and *D. winteri* extract, both showed an immunomodulatory effect on the expression of immunorelevant genes like IL-1 β , IFN α and MHCII, further upon the challenge with *S. parasitica*, IL-1 β presented an up-regulation at short times, while IFN α and MHCII showed a down regulation during the same time intervals studied. On the other hand, under our experimental conditions, after *D. winteri* or poligodial pretreatment and *S. parasitica* infection, the cells showed a compensation on the observed downregulation of IFN α and MHCII upon pathogen incubation, suggesting a potential immunomodulation of these natural extracts that could be used as a phytodrug against this type of pathogen in the aquaculture industry.

77. Dissecting the Multifunctional Nucleoprotein of Arenaviruses

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Arenaviruses are negative-sense bi-segmented RNA viruses that can cause severe hemorrhagic fever in humans. Potential person-to-person transmission, and the likelihood of infections through aerosolized forms of these viruses, have led to their inclusion on the list of select agents by the United States HHS Department. These concerns are aggravated by the lack of FDA-licensed vaccines or specific treatment against arenaviral infections. Arenavirus genome encodes only four proteins: the polymerase (L), the matrix-like protein (Z), the glycoprotein (GP), and the nucleoprotein (NP). NP encapsidates the viral genome, and together with L, is involved in viral replication and transcription. Recently, the amino acid regions and individual residues of NP required for counteracting the type I interferon response (IFN-I) have been described. However, interactions of NP with other viral components were poorly understood. We have characterized domains in the prototypic Lymphocytic Choriomeningitis Virus (LCMV)-NP, that are involved in NP-NP and NP-Z interactions. We identified the NP-NP interaction domain at the N-terminal region, while the NP-Z interaction domain is restricted to the C-terminal region, overlapping with the previously described anti-IFN-I domain. However, specific amino acid mutations affecting the NP anti-IFN-I function did not affect its interaction with Z, suggesting that different residues contribute to these two distinct and separable NP functions.

78. Clinical and virological characterization of hospitalized influenza A patients between 2011-2012

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Disease outcome caused by influenza A virus is modulated by both viral and host factors. The efficient transmissibility shown by the 2009 H1N1 pandemic strain during its emergence, emphasized the value of monitoring the clinical evolution of infected individuals to establish efficient clinical treatment strategies. No in-depth longitudinal studies have been conducted with influenza virus patients integrating pathogenesis, clinical findings, analysis of immune responses of the host and a characterization of the virological properties. In this study for the years 2011-2012, serial of nasopharyngeal swabs and blood samples were collected from a total of 61 patients hospitalized with influenza A. The majority of patients had risk factors by either pre-existing comorbidities or because they belonged to high-risk age groups. A large proportion (56,3 %) of severe individuals had some level of immunosuppression. We identified 5 individuals who showed a decrease in seroconversion titers (day 21) against the homologous virus strain, in comparison to the level of antibodies in pre-infection sera (day 1). To investigate genomic variation of the virus during the acute phase of infection, we are currently sequencing 86 samples collected from 23 individuals during the first 7 days of infection.

CONICYT 79100014 y FONDECYT 1121172. Instituto Milenio en Inmunología e Inmunoterapia. Center for Research in Influenza Pathogenesis (CRIP) del NIAID-NIH

79. Polysaccharides isolated from *Aloe barbadensis* Miller (*Aloe vera*) are excellent prebiotic molecules.

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Aloe vera plants have medicinal properties. We isolated two polysaccharides (PS): PS1 from green leaf tissue and PS2 from leaf gel. Both molecules have glycosidic linkages resistant to enzymatic hydrolysis by the intestinal tract and by enterobacteria present in the intestine. By *in vitro* bacterial growth analysis, the prebiotic potential of these isolated and purified polysaccharides was tested. For this, the growth of 8 *Lactobacillus* and *Bifidobacterium* strains was evaluated in a culture medium containing these polysaccharides as the sole carbon source. Bacterial growth was compared with that induced by glucose (Glc) or a commercial prebiotic polysaccharide, Nutra Food. The results show that PS1 induces growth similar to that induced by Glc and higher than that induced by Nutra Food. PS2 increases growth to levels similar to Nutra Food while a mix of PS1 and PS2 induces growth lower than that induced by Glc but higher than that of Nutra Food. By quantitative RT-PCR, the benefit of each polysaccharide in the specific intestinal *Lactobacillus* and *Bifidobacterium* populations present in human stools was evaluated. Stools were cultured in a bioreactor and population growth was compared to that induced by Glc. After 48 h in the bioreactor, PS2 was a better carbon source than PS1 for *Lactobacillus* and comparable to Glc for *Bifidobacterium* populations. These results indicate that both polysaccharides have prebiotic properties. Project VIU 110038, FONDECYT 1130025.

80. STANDARDIZING A METHOD OF PURIFYING BOVINE VIRUSES: BLV FROM MILK SAMPLE AND BVDV FROM CELL CULTURE

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BLV and BVDV are spherical virus with ssRNA (+) which diameter is between 80-100 nm and 50 nm respectively, both of them causing the major infectious diseases with great economic impact on global livestock. Therefore, is necessary to design a detection system that allows an early and effective diagnosis. In order to develop an effective detection system against cattle viruses is important to design primers against viral genome sequences from viral local isolates. However, one of the main issues in sequencing viral genome is its contamination with the host genome. For this purpose, we have designed a purification system using discontinuous gradient OptiPrep™ (iodixanol), to purify Chilean viral isolates from milk and serum samples from infected animals. To check the purification of both viruses, qPCR assays were performed with different markers for both viruses and the host. The results, based on Ct differences suggest that both viruses have a high purity degree. The difference between the BLV Ct and the host gene (β -actin) Ct is 9 Ct (25 and 34 respectively). While BVDV and the host gene Ct have a difference of 15 Ct (22 and 37 respectively). Therefore, these results hint that both viruses have high purity degree. Based on the results, we can say that OptiPrep™ is an effective method for the viral purification. INNOVA12IDL2-16212.

81. Microcin E492 amyloid formation is retarded by posttranslational modification

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Microcin E492(MccE492), an antibacterial peptide with the ability to form amyloid fibers, is exported as a mixture of two forms: unmodified (inactive) and posttranslationally modified at the C terminus with a salmochelin-like molecule (active). During the stationary phase, the unmodified form accumulates, and MccE492 is rapidly inactivated. The aim of this work was to demonstrate that the increase in the proportion of unmodified MccE492 augments the ability of this bacteriocin to form amyloid fibers, which in turn decreases antibacterial activity. To this end, strains with altered proportions of the two forms were constructed. The increase in the expression of the maturation genes augmented the antibacterial activity during all growth phases and delayed the loss of activity in the stationary phase, while the ability to form amyloid fibers was markedly reduced. Conversely, a higher expression of MccE492 protein produced concomitant decreases in the levels of the modified form and in antibacterial activity, and a substantial increase in the ability to form amyloid fibers. Moreover, seeds formed using exclusively the nonmodified form were more efficient in amyloid formation with a shorter lag phase, indicating that the nucleation process is improved. Unmodified MccE492 incorporation into amyloid fibers was kinetically more efficient than the modified form, probably due to the existence of a conformation that favors this process. Supported by FONDECYT grant 1100141.

82. Metabolic diversity of bacterial communities from soils invaded by *Eschscholzia californica*.

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Eschscholzia californica is an invasive plant widely distributed in Chile. Results from our lab suggest that it may alter the metabolic activity of soils. However, some questions related to the influence of the plant phenological stages on the structure of the soil microbial communities are still unanswered. The aim of this study was to explore the impact of *E. californica* invasion on the metabolic diversity of soils through a common garden experiment. *E. californica* seeds were germinated in pots and unplanted pots were used as controls of non-invaded soils. The first sampling was performed at the flowering stage, when the half of the plants was extracted and the rest was left to continue their life cycle. Later, two samplings were conducted matching with the plant fruiting and senescence, respectively. The functional diversity of the soil microbial communities was assessed using a system containing 31 different carbon sources. The estimation of the kinetic parameters A (maximum absorbance), mm (maximal rate of color development) and I (lag time) was performed by fitting the data to a Gompertz equation. The higher values of A and mm were detected during the fruiting stage in invaded soils. Moreover, the metabolic diversity indexes of invaded soils were significantly higher than those of the non-invaded soils. These results suggest a direct relationship between the plant phenological stages and the functional structure of the soil microbiota. FONDECYT 3110140.

83. Involvement of poly(ADP-ribose) glycohydrolase in *T. cruzi* proliferation and host cell infection

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Trypanosoma cruzi, etiological agent of Chagas' disease, has a complex life cycle which involves the invasion of mammalian host cells, differentiation and intracellular replication. Poly(ADP-ribosylation) is an early cellular response to DNA damage and is a concerted and dynamic process: poly(ADP-ribose) polymerases catalyze the transfer of ADP-ribose (ADPr) to specific target proteins, whereas poly(ADP-ribose) glycohydrolase (PARG) represents the main pADPr hydrolyzing activity in the cell. Scarce information is available on the role of pADPr metabolism in *T. cruzi*, here we report the possible role for *T. cruzi* PARG in the cell cycle of these parasites. PARG inhibitors ADP-HPD or DEA (1 µM), reduce *in vitro* *T. cruzi* epimastigote growth by ~35% when compared to control cultures and lead to a delay in the progression of the cell cycle in HU synchronized cultures, particularly affecting the S phase. More importantly, we demonstrate that the lack of PARG activity in different host cells by chemical inhibition or iRNA, reduces the percentage of infected cells as well as the number of amastigotes per cell and trypomastigotes in the supernatant, leading to a nearly complete abrogation of the infection process. PARG has proven to be an important player in the life cycle of *T. cruzi* and has emerged as a promising potential therapeutic for the treatment of Chagas' disease.

84. The plant growth promoting rhizobacteria *Burkholderia phytofirmans* PsJN protects *Arabidopsis thaliana* against the virulent pathogen *Pseudomonas syringae* DC3000.

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Soil bacteria referred as plant growth promoting rhizobacteria (PGPR), can promote plant growth and protect plants from several stress and pathogen diseases. Some PGPR induce a systemic resistance (ISR), which primes plants to an accelerated defense response against fungi, bacteria, viruses and even insects. Several studies demonstrate that the expression of ISR in plants is dependent on the PGPR species. Little is known about the molecular mechanisms that trigger the ISR response. Here, we report for the first time that a β -proteobacterium (*Burkholderia phytofirmans* PsJN) protects *Arabidopsis thaliana* against the virulent pathogen *Pseudomonas syringae* DC3000. *Arabidopsis* plants were grown with PsJN and after 2 weeks they were infected with strain DC3000. Disease progression was measured by the proliferation of pathogenic bacteria. Our results indicated that PsJN strain was able to induce resistance in *A. thaliana* to this pathogen. This response was not triggered by the inactivated strain, *E. coli* or *Cupriavidus pinatubonensis*. The plant defense responses were studied with three PsJN mutants. Also, the responses of *A. thaliana* mutants in different defense related-hormone pathways and the transcription profiles of key defense related genes in plants were determined. Our results indicate that an active and specific mechanism of strain PsJN is required to induce this defense response in the plants.

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85. Genetic redundancy of acetyl-CoA transferases in key steps of aromatic compounds metabolism in *Cupriavidus pinatubonensis* JMP134.

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Several enzymes responsible for degradation of aromatic compounds (AC) in catabolic bacteria are encoded by more than one gene. In catabolism of AC like benzoate, 4-hydroxybenzoate, 4-hydroxyphenylacetate, among others, the transference of acetyl-CoA to different intermediates is required. The genome of *Cupriavidus pinatubonensis* JMP134, a well-known AC degrader, encodes three acetyl-CoA transferases involved in catabolism of the above-mentioned AC substrates. We studied the role of these genes. Mutants of genes *pcaJ* (acetyl-CoA transferase of the beta-ketoadipate pathway), *mmfG* (acetyl-CoA transferase of the methylmuconolactone pathway) and *dcaJ* (hypothetically encoding a acetyl-CoA transferase involved in tricarboxylic acids degradation) were obtained, as well as double mutants, and the triple mutant. Growth tests showed that all three single and the double mutants grew on tested AC, whereas the triple mutant grew on some of the tested AC, indicating true functional redundancy. Single and double mutants still converted β -ketoadipate and acetoacetate to the expected products; however, the triple mutant did not produce them. Heterologous studies with *E. coli* and *P. putida* mutants, devoid of transferase activity, and complemented with each of the three *C. pinatubonensis* genes, showed full recovery of the ability to transform substrates into the respective products (*E. coli*) and growth on AC (*P. putida*).

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86. qPCR for Specific Detection of *Piscirickettsia salmonis*: New Genomic Insights

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Piscirickettsia salmonis, the etiological agent of the Salmonid Rickettsial Septicaemia, is responsible for up to 96% of the mortalities and represents over 56% of diagnoses in Chilean aquaculture. To control the disease dissemination is urgently needed a precise, fast, sensitive and specific method that allows comparison of results from different diagnostic laboratories to detect and prevent the spreading of *Piscirickettsia salmonis*. Primers and TaqMan probes (FAM, HEX) were designed to amplify and detect two different genes of *P. salmonis* and the 18S rRNA gene of *Salmo salar*. The qPCR experiments were done as mono and multiplex using SYBR Green and TaqMan probes. The specificity of primers and probes was tested with genomic DNA from different isolates of *P. salmonis* and different bacteria. The sensitivity was tested with salmon farm tissues and plasmidial DNA. The amplification patterns from qPCR for every gene showed amplification of one fragment with the expected size. The melting curves/fluorescent signals from FAM/HEX hasn't interference or background. Specific genes of *P. salmonis* showed amplification only in samples containing *P. salmonis* DNA, confirming that the kit has high specificity and also high sensitivity. This single-reaction triplex qPCR kit allows rapid, specific, reliable and sensitive detection of *P. salmonis* in salmon tissues infected with these bacteria.

87. Identification of essential genes for *in vitro* surviving of *Salmonella* Typhimurium to a non-lethal cefotaxime concentration and their putative relationship with virulence

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A dose-dependent dual role for antibiotics has been proposed, acting as toxic and also as signal molecules affecting bacterial fitness. In this context, the induction of motility and virulence genes in cultures exposed to non-lethal antibiotic concentrations has been observed. We evaluated the effect of sub-inhibitory concentrations of cefotaxime (CTX), in the virulence of *S. Typhimurium* in BALB/c mice. We performed competition assays by intraperitoneal injection of a 1:1 mixture of treated/untreated bacteria. An increased colonization of internal organs was observed for CTX-exposed cultures. To identify essential genes for surviving to CTX, a *S. Typhimurium* transposon library (~60.000 mutants) was grown in the presence and absence of CTX. Using a high-throughput screening we identified by negative selection 50 mutants that were selected after CTX exposition. Caught our attention *bcfF*, *stbB*, *sinI* and *pefB* (fimbrial biosynthesis), *ttrS* and *ttrB* (tetrathionate reduction, associated with survival in the gut), *baeS* / (kinase in a two-components system associated to ciprofloxacin response), *rcaA* (capsule biosynthesis) and *nei* (remotion of oxidized pyrimidines, suggesting DNA damage) mutants, which may explain the observed phenotype *in vivo*. In addition, the enrichment of *ybhQ*, *stbD*, *yfhH*, *yjeL*, *yecN*, *ratB* and *ybbL* mutants (membrane proteins) in CTX-treated cultures indicates a bacterial growth advantage to antibiotic exposure.

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88. Stress-triggered Signaling for Survival or Suicide of *Streptococcus pneumoniae* in Pneumocytes

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S. pneumoniae is a main human pathogen that survives to acidic environment through the Acid Tolerance Response (ATR) mechanism. We previously described the acidic-stress induced autolysis (ASIL) that release virulence factors favoring pathogenesis and that F₀F₁-ATPase is involved in the acidic stress response. The *atpC* mutants (*atpC* encodes the subunit c of this enzyme) showed a repressed ATR, but an increased ASIL. Here, we found that this pathogen was able to survive several hours inside A549 pneumocytes or macrophages RAW 267.4. We showed that F₀F₁-ATPase, and consequently ATR, are necessary for intracellular life of the pneumococcus in macrophages. In addition, we described that ATR induction and intracellular life of pneumococci in pneumocytes are controlled by ComDE and CiaRH, two-component systems (TCS) that had been involved in the ASIL mechanism, showing opposite roles. Briefly, CiaRH is essential for ATR (ComE represses activation) whereas ComE is necessary for ASIL (CiaRH protects induction). These results suggested that both TCSs control these antagonistic processes, either *in vitro* or in a cellular model. This is the first report of TCSs that acts like checkpoints to control a stress response and to decide between a survival and a suicide mechanism in a cell model. This biological model contributes to the knowledge of bacterial pathogenesis under stress conditions in host tissues, where pathogens need to survive to establish infections.

89. Low temperature biosynthesis of fluorescent nanoparticles by a *Shewanella* sp. isolated from Antarctica: Potential of a multiresistant genus to produce intracellular nanostructures from heavy metals.

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Fluorescent nanoparticles or quantum dots (QDs) are structures highly organized at nanoscale, composed mainly by elements as Cd, Se, Te, In or As. They possess unique properties (chemical, physical, optical) in comparison to larger scale materials. Production of QDs by bacteria could represent a greener alternative than classic methods. Our group previously determined the importance of thiols involved on microbial oxidative stress defenses on biosynthesis of QDs. In this context, Antarctica presents extreme environmental conditions (low temperature, high UV radiation, high salinity, etc.) to find stress-resistant bacteria capable to biosynthesize QDs. We isolated a psychrotolerant *Shewanella* sp. able to grow in LB medium with high concentrations of CdCl₂ and K₂TeO₃ at 15° C. Strain was exposed to these salts under QDs biosynthesis conditions at three temperatures (10, 15 and 28° C). Also, they were exposed to increasing concentrations of metal according to their metal-resistance concentrations. Total thiol content was determined after treatments with metals in order to relate thiols content with QDs production. *Shewanella* sp. displayed minimal Inhibitory concentrations of 500 and 125 µg/mL for CdCl₂ and K₂TeO₃, respectively. Bacteria were capable to produce intracellular QDs, even at high metal concentrations. QDs showed size variation depending on time and temperature exposition.

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90. Immunolocalization of SxtA, SxtF and SxtM proteins in PSTs producing strains of Cyanobacteria.

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Paralytic shellfish poisoning toxins (PSTs) are a group of naturally occurring neurotoxic alkaloids produced among several genera of freshwater cyanobacteria and marine dinoflagellates. The *sxt* cluster encodes the proteins responsible for the synthesis saxitoxin (STX) and its analogues. Bioinformatic analyses show that at least six proteins encoded by *sxt* genes could be located or anchored to the membrane (SxtA, SxtD, SxtF, SxtJ, SxtM and SxtU). The aim of this work was to determine the sub-cellular location of SxtA (initiator enzyme of STX biosynthesis), SxtF and SxtM (MATE transporter family proteins) and to determine if these proteins are in fact part of a membrane anchored protein complex. We confirmed the presence of SxtA, SxtF and SxtM, associated to the cytoplasmic and thylakoids membranes in *C. raciborskii* strains: MVCC14, T3, ITEPA3, PMC0.01 and *R. brookii* D9 by immunolocalization. We observed different patterns in the sub-cellular location of SxtA, SxtF and SxtM between the toxic cyanobacterial strains analyzed. Nevertheless, these three Sxt proteins showed the same localization pattern in each strain. As well, we detected the SxtF protein as part of a protein complex by BN-PAGE / Western blot analysis. Our results reinforce that Sxt proteins are probably forming a "complex" anchored to the membrane.

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91. 2-Bromo-1,4-naphthoquinone and ascorbic acid: A promising drug combination for tumor treatment

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Recently, we demonstrated that 2-bromo-1,4-naphthoquinone (BrQ) in combination with ascorbic acid (AA) was more efficient than Apatone™, a combination of vitamin K₃ (2-methyl-1,4-naphthoquinone, VK₃) and AA, as a source of hydrogen peroxide (H₂O₂), which is involved in its antitumor activity. Here we compare these combinations as cytotoxic agents to breast cancer cells (MCF-7 BUS) and Chinese hamster ovary (CHO) normal cells. The cytotoxicity was evaluated by the sulforhodamine assay. The combinations were evaluated in the following concentration ratios (μM/μM) (BrQ or VK₃/AA): 10/1000 **(1)**, 5/500 **(2)**, 2.5/250 **(3)**. Alone, VK₃, BrQ and AA were not cytotoxic, provoking less than 10% of death for MCF-7 and CHO cells. VK₃/AA and BrQ/AA were more cytotoxicity to MCF-7 than to CHO (MCF-7 60% and CHO 20%). At ratio **(1)** there was not statistically significant difference the cytotoxic effect of these combinations. However, at ratio **(2)** and **(3)**, BrQ/AA was more cytotoxic than VK₃/AA. For instance, at ratio **(3)** BrQ/AA was two-fold more effective than VK₃/AA. In conclusion, both drug combinations were more cytotoxic to the tumor cells compared to the normal cells, which is an indication of their selectivities and potential applicability for cancer treatment. Moreover, the drug combination BrQ/AA was two-fold more potent than VK₃/AA. This is evidence that this combination could be also tested for cancer treatment as have been done for VK₃/AA. Supported by: FAPESP and CAPES

92. Effect of reboxetine treatment on auditory attention and memory in stress rats

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Chronic stress affects several nuclei of limbic system inducing alterations in both memory and emotion processing. Moreover, produces dendritic atrophy in several nuclei of auditory system affecting the auditory learning. Depression is a stress-related pathology and reboxetine is used to treat this disease. We analyze the reboxetine effect on auditory attention in chronic stressed rats. Male Sprague-Dawley rats were trained in a two-alternative choice task (2-ACT), a behavioral paradigm to study auditory attention in rats. Trained animals that reached a performance over 70% of correct trials in the 2-ACT were randomly assigned to control, stress, control-vehicle, stress-vehicle, control-reboxetine and stress-reboxetine experimental groups. Stress group animals were subject to movement restriction stress protocol. To analyze the effects of restraint stress and reboxetine treatment on the auditory attention, trained rats of all groups were subjected to 50 2-ACT trials one day before and one day after of the stress period. Chronic stress significantly impaired the auditory attention, and the reboxetine treatment prevents this effect. In addition, reboxetine had an anxiolytic effect and improves the spatial memory measured through Y-maze. These results provide important information about the stress and reboxetine effects on complex cognitive behaviors like attention and memory.

93. Quantifying elastic changes in DNA molecules after interacting with Mitomycin C using Magnetic tweezers at single molecule level.

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Changes in elastic properties of biopolymers are important, since they are commonly associated with the control of biological function. Several authors propose ways to modify DNA elasticity and one is through interaction with small molecules. In this work we explore by magnetic tweezers, the changes in elastic properties of the DNA, such as the end to end distance, contour length and the persistence length of lambda DNA once interacts with Mitomycin C (MMC), a small molecule that forms a crosslink with DNA and whose effects are been used as an anticancer therapy. Previous works reported that MMC cross-linking the DNA, only after a reductive process. Here MMC is used unreduced and reduced in vitro by Dithiothreitol (DTT). We found changes in persistence length from 44 nm to 34 nm after added the unreduced MMC, while the reduced MMC by DTT keep the persistence length at the same concentration. The contour length and the end to end distance of DNA with unreduced and reduced MMC decrease in both cases. In Conclusion the MMC interacts with DNA not only when it is reduced, and in the case of unreduced MMC the value in the three parameters are considerable different, while with activated MMC the big change is in the contour length, that suggest a big efficiency in the link of MMC to DNA.

Acknowledgments : Susan Marqusee for the use of the instrumentation. Funding : CONICYT Beca de Doctorado 2010 , Vicerectoria Académica Universidad de Santiago.

94. Development of a novel CYTB gene marker for fish species genotyping using PCR-RFLP technique as a tool for the experiential teaching of molecular biology: Is that really salmon in my sushi?

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In the process of evolution synonymous or nonsynonymous substitutions generate great genetic variability among orthologous genes. The knowledge of the nucleotide sequence of a particular gene and its orthologs in various species allow us to selectively identify these species using molecular biology techniques. The concepts that underlie this kind of molecular diagnosis are not easily understood by most undergraduate students given the lack of practical teaching tools. We have developed such a teaching tool by using PCR-RFLP (polymerase chain reaction restriction fragment length polymorphism) to identify the highly polymorphic mitochondrial CYTB gene (cytochrome B gene). With this marker we can genotype and identify fish species present in the Chilean sea and commonly sold in supermarkets and farmer markets. Degenerated PCR primers are used to amplify a region of CYTB which is then restriction enzyme digested. The polymorphisms present in this region of the gene allow for specie-specific restriction fragment length differentiation using agarose electrophoresis. Our genotyping assay allow us identify several fish species (*Brotula clarkae*, *Merluccius gayi*, *Liza macrolepis*, *Brama australis*, *Salmo salar*, *Centropomus undecimalis*) collected from marketplaces, and it presents a great tool for citizen science and teaching laboratories in high schools and undergraduate programs. Funded by InnovaChile-CORFO StartUp Chile grant 12SUP-16986 and Bioquímica.cl S.A.

95. Hantavirus glycoproteins mediate viral self-assembly and entry into the cell

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Hantavirus are enveloped viruses composed of four structural proteins. Yet, it remains largely unknown how these viruses enter and exit from cells. Here we show that the plasmid-driven expression of Andes (ANDV) and Puumala (PUUV) hantavirus Gn and Gc envelope glycoproteins leads to their self-assembly into virus-like particles (VLPs) released into cell supernatants. The viral nucleoprotein was not required for particle formation. VLPs were pleomorphic and reacted with patient sera. Further, a Gc endodomain deletion mutant did not abrogate VLP formation. In absence of a reverse-genetic system for hantaviruses, the VLPs were further used to study specific steps of hantavirus entry into cells. Specifically, VLPs bound to cells and low pH was enough to trigger the virus-cell membrane fusion mechanism including VLP-membrane interaction, glycoprotein multimerization changes and fusion.

96. Remodelling of the TAR RNA motif mediated by the RNA helicase DDX3 is required for the HIV-1 genomic RNA to enter in cap-dependent translation initiation.

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It was thought for many years that translation initiation by the cap-dependent mechanism on the HIV-1 genomic RNA (gRNA) and other viral transcripts was impaired by the presence of a highly structured 5'-untranslated region (5'-UTR). As such, it was proposed that the TAR RNA motif, which is located at the very 5' end of all viral transcripts acts as a structural barrier that interferes with the recognition of the cap structure by the host translation initiation machinery. However, despite this complex architecture of the viral 5'-UTR, translation initiation on the gRNA occurs by a highly efficient cap-dependent mechanism. Here, we demonstrate that this is due to the recruitment of the DEAD-box RNA helicase DDX3 during the early events of HIV-1 gRNA translation initiation. As such, we show that DDX3 binds to the viral 5'-UTR to catalyse the ATP-dependent unwinding of the TAR RNA motif resulting in the cap moiety being accessible to the translation initiation machinery. Interestingly, this preparative process required for the gRNA to enter in cap-dependent translation initiation is compartmentalized in large cytoplasmic granules, which are assembled by DDX3 in the presence of a subset of translation initiation factors but in the absence of any canonical cap-binding protein. **Acknowledgements** Fondecyt Initiation grant N°11121339 and ANRS NM/N°1616/CSS 2/AO 2010

97. Astrocyte $\alpha\beta 3$ -integrin engages neuronal Thy-1 and induces neurite retraction via CBP-mediated Src inactivation and ROCK pathway activation.

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The interaction between astrocyte $\alpha\beta 3$ Integrin and neuronal Thy-1 induces neurite retraction; however, the associated signaling pathways in neurons and how Thy-1, a glycosylphosphatidylinositol-anchored protein, conveys signals to the cell interior are unknown. Here, we evaluated whether Csk-Binding-Protein (CBP) acts as a transmembrane transducer in Thy-1-induced Src and ROCK-mediated neurite retraction.

CAD cells and rat cortical neurons were stimulated with $\alpha\beta 3$ -Fc integrin. Phosphorylation of Src (Y527), MLCII and Cofilin (ROCK effectors) were analyzed by western blotting. CBP, Thy-1 and Src interactions were evaluated by co-immunoprecipitation and in co-localization studies (STED microscopy). ROCK participation was evaluated using a Y276232 inhibitor and neurite retraction was used as a read-out. For cells treated with $\alpha\beta 3$ Integrin, increased Src, MLCII and Cofilin phosphorylation were detected; also, enhanced co-localization of CBP-Thy-1 and Thy-1-pSrcY527 was observed, as well as a decrease in co-localization of CBP with Src. CBP, Thy-1 and Src co-precipitated in primary cultures. Y27632 prevented neuronal retraction caused by $\alpha\beta 3$ Integrin.

These results indicate that CBP, Thy-1 and Src form a membrane complex that transduces $\alpha\beta 3$ -Integrin-mediated signals to the cell interior to increase ROCK activity and trigger neurite retraction. FONDECYT 1110149(LL) and 1130250(AFGQ); BNI P09-015-F(LL) and Anillo ACT1111(AFGQ); Conicyt 24121665, Becas Chile(HM).

98. Internal ribosome entry site within the gag coding region : toward a new therapeutic target ?

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Primate lentiviruses genomic RNA can serve both as an mRNA that encodes for Gag and Gag-Pol polyproteins and as a propagated genome. We previously reported the presence of an IRES activity embedded within the Gag coding region itself that drives the production of several isoforms of the Gag polyprotein. In addition, *in vitro* reconstitution experiments revealed that the initial step of initiation complex formation is the recruitment of the 40S ribosomal subunit and eIF3. This mechanism is conserved amongst HIV-1, HIV-2 and SIV_{mac}, indicating that those properties are important for the virus cycle.

In order to define the RNA structural determinants responsible for the formation of the IRES/eIF3/40S ternary complex, we have been following functional and biochemical approaches in parallel. Interestingly, we found that there is not one, but two ribosome binding sites that correspond to the previously described INS sequences.

To determine the functional role of the IRES activity, we assayed *in vitro* the translation efficiency of mutants unable to recruit the ribosome. Interestingly, we were able to establish a link between dimerization and translation. In parallel, we have been following a drug screening strategy to identify small molecules that would inhibit the ribosome recruitment. Such molecules would allow us to confirm the role of the IRES on gag translation, and pave the way to the definition of the IRESgag as a new therapeutic target.

99. Endothelin-Converting Enzyme-1 isoform c is regulated by the Wnt/ β -catenin pathway and modulates invasion in colon cancer cells.

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Endothelin Converting Enzyme-1 (ECE-1) is an integral membrane Zn- metalloprotease that mediates the key step of activation of Big-Endothelin-1 to the active peptide Endothelin-1 (ET-1; 21aa) through its catalytic extracellular domain. In many cancers, the levels of ET-1 are up-regulated, promoting malignant characteristics like augmented proliferation and angiogenesis. Furthermore, recent studies of the 4 isoforms of ECE-1 (a, b, c and d) propose to ECE-1c as a mediator of cell invasiveness, giving to this enzyme an renewed role in cancer metastasis in a way dependent of ET-1. In order to investigate the functional relationship between the Wnt/ β -catenin pathway and ECE-1c expression in colon cancer, we analyzed the putative positioning of β -catenin and members of the Tcf/Lef transcription factor family in the ECE-1c promoter region (ChIP assay). Additionally, inhibition of CK2 with TBB, a positive regulator of β -catenin, reduced the ECE-1c promoter-dependent luciferase reporter assay. Finally, modulation of ECE-1c expression regulated the invasive capacity of human colon cancer cells in a matrigel assay. These results suggest to ECE-1c as a novel target of the Wnt/ β -catenin pathway, which is involved in the acquisition of malignant progression of colon cancer cells. Supported by CONICYT Ph.D. fellowships (P.C., E.S., I.N., H.H.), FONDECYT 1120132 and ICGEB CRP/CHI10-01 grants (J.C.T.).

100. Contribution of LOX-1 activation by oxLDL to tumor angiogenesis in prostate cancer.

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Expression and function of lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) has been associated with several diseases such as endothelial dysfunction, atherosclerosis and obesity. In these pathologies oxLDL/LOX-1 activates signaling pathways that promote cell proliferation, cell motility and angiogenesis. Recent studies have demonstrated the expression of LOX-1 in prostate cancer cells (CaP) and in human prostatic adenocarcinomas with clinicopathological stages III and IV, characterized by local invasion and metastasis. However, its function in prostate cancer has not been unraveled to date. Our goal was to analyze the contribution of oxLDL/LOX-1 to tumor angiogenesis through the evaluation of expression of pro-angiogenic molecules and angiogenesis in CaP models, with overexpression and silencing of LOX-1 receptor. Our results demonstrated that the activation of LOX-1 by oxLDL increases the expression of pro-angiogenic molecules as VEGF, MMP-2 and MMP-9 in a dose dependent manner. In this respect, we determined that expression of these molecules is enhanced in cells overexpressing CaP LOX-1 and prevented with the use of shRNAs against LOX-1. Noticeably, the use of cell lines CaP/LOX1(+), CAP/LOX1(-) xenografts on chorioallantoic membranes in chicken embryo models, showed that oxLDL/LOX-1 generates an increase in the angiogenesis of tumor-associated stroma.

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101. Protein kinase A regulates ER-mitochondria communication during stress conditions in HeLa cells

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The endoplasmic reticulum (ER) is crucial for protein and calcium homeostasis. In response to stress, the ER increases its contacts with mitochondria in order to facilitate calcium transfer between both organelles and thus boost metabolism. Protein kinase A (PKA) regulates mitochondrial dynamics; however, whether this kinase regulates ER-mitochondria cross-talk remains unknown. We addressed this question in HeLa cells subjected either to ER stress or to mTOR inhibition.

Both types of stress increased interorganelle proximity, as assessed by fluorescence microscopy. Also, increased calcium transfer, mitochondrial fusion and oxygen consumption were observed, suggesting an increase in mitochondrial bioenergetics. These changes correlated with PKA relocation to mitochondria, and inhibition of the small GTPase DRP1 implicated in mitochondrial fragmentation. Addition of PKA inhibitor H89 prevented both mitochondrial fusion and augmented oxygen consumption, highlighting the dependence of these changes on PKA. Surprisingly, however, ER-mitochondrial proximity was further increased by H89 treatment.

Our results suggest a dual role for PKA in the control of mitochondria: on the one hand, PKA is necessary to enhance metabolism, but on the other, its activity seems to restrict ER-mitochondria proximity. To determine whether different pools of PKA control these facets of mitochondrial dynamics or whether these effects are compensatory responses requires further research.

102. Distribution of matrix metalloproteinase-2 (MMP-2) in sheep cervix during the oestrous cycle.

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The aim of this work was determine the histological distribution of matrix metalloproteinase-2 (MMP-2) isozyme in the ovine cervix during the oestrous cycle. Cranial (Cr) and Caudal (Ca) cervical zones were obtained from Corriedale adult ewes at Days 1 (n=7), 6 (n=5) or 13 (n=7) after oestrus detection (Day 0). The distribution of MMP-2 was assayed by immunohistochemistry using a polyclonal antibody (hMMP-2, cs-8835, Sta. Cruz Biotech., Inc. CA-USA). Positive and negative MMP-2 nuclei were counted (400X) in 1000 cells in each stromal compartment: superficial and deep fold stroma and superficial and deep wall stroma. The percentage of MMP-2 positive cells (%MMP-2) was calculated and analyzed by ANOVA (Mixed Proc, SAS). The MMP-2 immunostaining was associated mainly to active fibroblasts, some to inactive fibroblasts and occasionally to leucocytes. The %MMP-2 (mean±sem) was lower on Cr (0.97±0.27) than on Ca (2.83±0.43), (P<0.0004). The %MMP-2 trend to be higher on Day 1 than on Day 13 in Cr (1.56±0.40 and 0.38±0.50, respectively, P=0.080), whereas was lower on Days 1 and 6 than on Day 13 in Ca (2.76±0.61, 1.43±1.05 and 4.30±0.43), (P<0.006). The results suggest that the active fibroblasts may be involved in cervical collagenolysis, with a greater potential in the Ca zone. The percentage of MMP-2 positive fibroblasts could be differentially hormone-modulated during the oestrous cycle depending on the cervical zone.

103. Transcriptional factor FoxO1 modulates mitochondrial metabolism and morphology in cardiomyocytes

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Our lab has recently established cardiomyocyte mitochondrial morphology and metabolism is regulated by insulin (Parra et al. Diabetes in press, 2013). The transcriptional factor FoxO1 is negatively regulated by insulin and over-activated in pathological conditions like diabetic cardiomyopathy. FoxO1 is also a key regulator of glucose metabolism in hepatic, skeletal muscle, adipose tissue and heart, but it is unknown whether FoxO1 regulates mitochondrial dynamics and metabolism. We evaluate here the role of FoxO1 in cardiomyocyte mitochondrial morphology and metabolism. To this end, FoxO1-GFP or the constitutive active form (FoxO1CA-GFP) were over-expressed and silenced with siRNA for FoxO1 in cultured rat cardiomyocytes. Our data showed that expression of FoxO1CA stimulated a fragmented mitochondrial phenotype among a decrease in mitochondrial metabolism assessed by mitochondrial membrane potential and oxygen consumption. The expression of FoxO1CA also led to an increase and decrease in Drp-1 and Opa-1 expression, GTPases involved in mitochondrial fission and fusion, respectively. We concluded that FoxO1 stimulates mitochondrial fission and alters cellular metabolism.

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104. Tanycytes MCT1 inhibition *in vivo* affects feeding behavior

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We previously described the expression of MCT1 in the hypothalamus, particularly in β 1 tanycytes, which are in close connection with ventromedial hypothalamic neurons controlling food intake. We propose that inhibition of MCT1 expression in tanycytes affects the regulation of food intake. An adenovirus encoding EGFP reporter gene and a shRNA to knock-down MCT1 expression (Ad-shMCT1) was generated and its inhibitory capacity was confirmed in primary tanycyte cultures. MCT1 expression was assessed by qRT-PCR and Western blot analyses. Subsequently, MCT1 expression was inhibited in rats through injection of Ad-shMCT1 into the third ventricle using stereotactic surgery. Feeding behavior was measured in two fast-refed cycles (24h-24h) by assessing the grams of food eaten, body weight, frequency of intake and the period of satiety during the refed period. We found that Ad-shMCT1 produces an effective and specific knock down of the target gene *in vivo* without altering the expression of MCT4. Animals injected with Ad-shMCT1 showed differences in the frequency of intake and the development of satiety compared to the control group (Ad-sh β gal). However, there was not differences in the amount of food consumed or body weight. These results suggest that MCT1 located in tanycytes are involved in the control of food intake in rats, affecting satiety response and the frequency of food intake. Acknowledgement: Funded by FONDECYT 1100705.

105. Interaction between non-coding mitochondrial RNAs and double-stranded-RNA binding proteins.

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Our group described a family of human non-coding mitochondrial RNAs (ncmtRNAs) consisting of Sense (SncmtRNA) and Antisense (ASncmtRNA) transcripts, which contain inverted repeats joined to the 5' end of the sense or antisense 16S mitochondrial rRNA, respectively, forming stem-loop structures. Although the function of these transcripts is unknown, there is a strong relationship between their expression and proliferative status, observed by *in situ* hybridization (ISH). Analysis of the double-stranded RNA region of these transcripts led us to consider an interaction with proteins that possess dsRNA binding domains (DRBPs). DRBPs have specificity for stem-loop structures of many RNAs, in which we find proteins involved in diverse cellular mechanisms. One candidate group of DRBPs that could interact with the ncmtRNAs are proteins involved in the microRNA (miRNA) pathway such as Dicer, Drosha, Argonaute-2 and ADAR1, since by bioinformatic analysis we came up with putative microRNAs originated from ASncmtRNA with complementarity to the 3'UTR of cyclinD1 and survivin. In this work we carried out RNA immunoprecipitation assays on lysates from SK-MEL-2 human melanoma cells, using antibodies against the different proteins studied. We determined that all of the aforementioned proteins are coimmunoprecipitated with ncmtRNAs. This interaction indicates that the ncmtRNAs may be involved in the miRNA pathway. FONDECYT 1110835

106. Astrocytes inhibit nitric oxide-dependent Ca²⁺ dynamics in activated microglia: Involvement of ATP released via pannexin 1 channels

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During inflammation, microglia exhibit increased levels of free intracellular Ca²⁺ ([Ca²⁺]_i) and nitric oxide (NO) production. However, whether NO, Ca²⁺ dynamics and gliotransmitter release are reciprocally modulated is not fully understood. More importantly, the effect of astrocytes is unknown. Our aim was to address if astrocytes could regulate NO-dependent Ca²⁺ dynamics and ATP release in LPS-stimulated microglia. Griess and Fura-2AM time-lapse assays of microglia revealed that LPS produced an increase in basal [Ca²⁺]_i that depended on the sequential activation of iNOS, COXs and EP₁ receptor. TGFβ1 released by astrocytes inhibited the abovementioned responses. Luciferin/luciferase and dye uptake experiments showed that ATP release from LPS-stimulated microglia occurred via pannexin 1 (Pannx1). Interestingly, TGFβ1 released by astrocytes inhibited ATP-induced calcium response in LPS-stimulated microglia. Finally, COX/EP₁ receptor signaling and activation of P2 receptors via ATP released through Pannx1 channels were critical for the increased NO production in LPS-stimulated microglia. Thus, calcium dynamics depend on the inflammatory profile of microglia and can be modulated by astrocytes. The understanding of mechanisms underlying glial cell regulatory crosstalk could contribute to the development of new treatments to reduce inflammatory cytotoxicity in several brain pathologies.

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107. Functional study of the Aurora kinase protein family in *Trypanosoma cruzi*

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Aurora kinase protein family plays a key role during cell division. These enzymes were described in most of the studied organisms showing differences in the number of members among different species. Among the processes where they are involved, it is worth to mention, centrosome maturation and division, chromatin condensation, mitotic spindle assembly, control of kinetochore-microtubule attachments and initiation of cytokinesis. In this work, our aim was to characterize three Aurora kinases (TcAUK1, -2 and -3) that we have previously found in *T. cruzi* and determine their role in the parasite biology. We have initiated functional studies for these enzymes by subcellular localization, protein overexpression and cell cycle analysis. Here we show that TcAUKs are regulated through variation of their subcellular localization during the cell cycle progression. In addition, recently we found evidence for SUMO posttranslational modification of TcAUKs. The overexpression of each of these enzymes leads to growth curve alterations and cell cycle disturbances. Taking together our results, we concluded that TcAUK1 could be considered as the canonical Aurora kinase showing the dynamic subcellular localization described for human Aurora B. Moreover, this enzyme is involved in kinetoplast division cycle. On the other hand, TcAUK2 and TcAUK3 are involved in processes related to microtubule dynamics.

108. ESCRT complexes are involved in acrosomal membrane deformations required for regulated exocytosis in human spermatozoa.

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The acrosome reaction of human spermatozoa is a calcium-dependent regulated exocytosis. Fusion between the outer acrosomal and plasma membranes causes the release of the acrosomal contents. The exocytic process can be arrested at a stage where SNARE proteins are assembled in trans complexes by preventing the release of calcium from the acrosome. At this stage, acrosomes are swollen, with deep invaginations of the outer acrosomal membrane. We have proposed that these membrane deformations are part of the mechanism of vesiculation. Invaginations of the acrosomal membrane are topologically equivalent to the formation of internal vesicles in endosomes, a process that depends on the assembly of the ESCRT complexes. We are exploring the possibility that the same mechanism is involved in acrosomal exocytosis. A dominant-negative mutant of VPS4, the ATPase responsible for the disassembly of the membrane attached ESCRT proteins, and the anti-VPS4 antibody inhibited acrosomal exocytosis of permeabilized human spermatozoa. Moreover TSG101 UEV, the ESCRT I domain that bind ubiquitin, and Snf7, the most important ESCRT III protein, were also inhibitory. TEM images show abnormal bending of the acrosomal membrane when sperm were stimulated in the presence of the dominant negative VPS4 or with anti-VPS4 antibodies. These observations suggest that the deformations of the acrosomal membrane necessary for acrosomal exocytosis are shaped by an ESCRT-dependent mechanism.

109. Effect of acute stress on FMRP phosphorylation by Akt-mTOR and MAPK pathways in rat hippocampus

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Glucocorticoids (GCs) are steroid hormones whose secretion increases in response to stress. The GCs target different brain areas by genomic and non-genomic mechanism. Acute stressor exposure to adult rats, including restraint stress decreased neurogenesis and enhances hippocampus-dependent associative learning.

Different mechanisms of synaptic plasticity require *de-novo* protein synthesis. In neurons, some mRNAs are transported to dendrites and translated by highly regulated process. In this context, the Fragile X Mental Retardation Protein (FMRP) is a RNA-binding protein which transports mRNAs associated to protein complexes. Also, phosphorylated FMRP acts as translational repressor. FMRP phosphorylation is modulated by PP2A and S6K1 activities related to Akt-mTOR and MAPK pathways.

We evaluated the effect of acute restraint stress on p-FMRP protein levels and the activation of Akt-mTOR and MAPK pathways in hippocampi extracts. Adult male rats were stressed during 0.5 or 2.5 h and sacrificed immediately after restraint or 6 and 24 h post stress. Stress procedure increased p-Akt/Akt levels twice; and p-Erk1/Erk1, p-Erk2/Erk2 increased after 6 h post stress, suggesting that both pathways are activated in different temporal pattern. Also the p-FMRP/FMRP levels did not change during and after restraint procedure, nevertheless could be important to evaluate changes in FMRP considering that this protein is regulated by itself at translational levels. FONDECYT1120528

110. Identification of a promoter region in the intron 5 of RUNX1 gene

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Translocations are one of the most frequent genetic alterations associated with leukemia. Among the acute myeloid leukemia (AML) one of the most frequent is the t(8;21) that involves the *RUNX1* and *ETO* genes. To date, all the break point for t(8;21) have been mapped in three regions in the intron 5 of the *RUNX1* gene designated as breakpoint cluster regions 1-3 (BCR1-3). One of these BCRs, BCR3, exhibits hypersensitivity to DNaseI and low nucleosomal occupancy. These characteristics have also been found in other translocations (like the MLL cluster region) and usually have been associated with presence of regulatory regions. Therefore, we hypothesize that BCR3 may harbor a transcriptional regulatory region. To test this hypothesis, we cloned the BCR3 region in sense and antisense orientation in the pGL3 reporter vector and evaluated transcriptional activity of the constructs by transient transfections in HeLa cells. Our results shown that the sense orientation BCR3 construct up-regulate luciferase gene expression (p<0.005). Interestingly, we no observed significative changes in transcriptional activity with the antisense orientation BCR3 construct. Therefore, our data demonstrate that the BCR3, located in the intron 5 of the *RUNX1* gene, exhibit promoter activity.

FONDECYT 1130697

111. Rheb interacts with LKB1 kinase; implications on LKB1 functions

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The small GTPase Rheb activates mTORC1, which promotes cell growth and is implicated in metabolic deregulation in cancer cells. The LKB1-AMPK pathway senses intracellular energy levels, and negatively regulates Rheb-mTORC1 signaling in response to metabolic stress. However, our group recently demonstrated that Rheb activates AMPK through an mTORC1-independent mechanism. Additionally, we also showed that Rheb interacts with LKB1 kinase, and requires its expression to activate AMPK. These observations suggest that a negative feedback loop between Rheb and AMPK exists, but how Rheb activates AMPK remains elusive. We now studied how Rheb interaction with LKB1 affects LKB1 function and consequently AMPK activity. By GST-pull down assay, we demonstrated that the inactive and active forms of Rheb indistinctly interact with LKB1, indicating that LKB1 is not an effector of Rheb. In addition, Rheb was unable to directly affect LKB1 kinase activity as demonstrated by a non-radioactive in vitro kinase assay. However, we found that Rheb stabilizes LKB1 protein. Since LKB1 is found in complexes with proteins that control its catalytic activity and stability, our results suggest that Rheb participates of these complexes to modulate LKB1 function, and consequently the activation of AMPK. Further studies will aim at defining whether Rheb interacts with proteins of the LKB1 complexes and how these interactions could affect the activation of AMPK by Rheb. Funding FONDECYT 1120923

112. Polyglucosan molecules induce apoptosis via the *intrinsic pathway* in male germ cells

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Glycogen is considered the main source of glucose for different tissues; however, the accumulation of aberrant polyglucosan molecules, weakly branched and hyperphosphorylated could promote degeneration and cellular death. Previously, we have reported that accumulation of glycogen in testis from transgenic animals overexpressing a superactive form of glycogen synthase (GS) promotes apoptosis of premeiotic cells and fibrosis signals in the seminiferous tubules. The hyperactivation of endogenous GS in the cell line GC1 promotes accumulation of glycogen and caspase3 activation. The aim of this work was to identify the structural characteristics of the glycogen storage in these cells and the possible mechanism involved in the pro-apoptotic activity. By spectrophotometric analysis, we have identified that glycogen synthesized by superactive form of GS or by hyperactivation of endogenous GS overexpressing PTG (*Protein Targeting to Glycogen*) is a poorly branched polyglucosan, showing a 80nm shift respect to iodine spectra of purified glycogen. In addition, the immunodetection of cleaved caspase3/9 suggests that cellular death induced by polyglucosan molecules is selective affecting mainly GC1 and not 42GPA9 Sertoli cells. These results are correlated with changes in intracellular ATP and cytochrome C content in GC1 cells after the polyglucosan accumulation, suggesting mitochondrial impairment and the activation of an intrinsic apoptotic pathway.

113. Association of VDR with the nuclear matrix: Identification of specific domains

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The vitamin D receptor (VDR) regulates transcription through its interaction with coactivator proteins and forming multiprotein complexes. The components of the regulatory complexes as well as the region of VDR necessary for the interactions remain to be identified. On the other hand VDR binds to the nuclear matrix, a scaffold structure that retains proteins in specific sites of the nucleus allowing processes including transcription. In osteoblastic cells, we performed transient expression of mutant versions of the VDR followed by vitamin D stimulation. Then, nuclear matrix intermediary filament preparation *in situ* and immunocytochemistry analysis using specific antibodies were carried out to determine the nuclear matrix localization. We find that the VDR requires binding of the hormone to localize at the nuclear matrix fraction and to form multiprotein complexes that regulate transcription. We also analyzed the regions of the VDR that contribute to localization at the nuclear matrix.

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114. Inhibition of Andes hantavirus entry into the cell

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Andes virus (ANDV) is a highly pathogenic virus that causes Hantavirus Pulmonary Syndrome in humans. To enter cells, ANDV must fuse its envelope membrane with host cell membranes of the endocytic pathway. Previously we showed that ANDV Gc corresponds to the viral fusion protein that shares characteristics with class II fusion proteins. The ectodomain of this class of fusion proteins is composed of three domains rich in beta sheets. Class II fusion proteins are inhibited by exogenous domain III (DIII) when present at low pH. Here, we tested if this inhibitory strategy allows the inhibition of ANDV. To this end, we synthesized ANDV DIII based on our previous model structure. The protein domain was soluble, presented disulfide bridges and beta sheet secondary structure content, confirming the previous *in silico* predictions. ANDV Gc domain III inhibited ANDV glycoprotein mediated cell-cell fusion and ANDV cell entry. Different to the described inhibition of class II fusion proteins by DIII, ANDV Gc DIII bound to ANDV particles at pH 7, pointing towards novel strategies for its therapeutic use. A model for ANDV inhibition by Gc DIII is discussed.

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115. Decrease in NAD⁺/NADH ratio produces mitochondrial dysfunction and prevents development of cardiomyocyte hypertrophy

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NAD⁺ is a key co-factor for energy production due its role in the citric acid cycle and the electron transport chain. However it also serves as substrate for various enzymes, including PARP-1 and class III HDACs. Drops in NAD⁺ levels along with a rise in ROS are detected in hypertrophied cardiomyocytes. Exogenous administration of NAD⁺ prevents cardiomyocyte hypertrophy. These evidences suggest that diminishing NAD⁺ levels would result in a redox imbalance, and possibly cardiomyocyte hypertrophy. In this study, NAD⁺ decrease effects were evaluated in cultured rat cardiomyocytes using the nicotinamide phosphoribosyltransferase inhibitor FK866. Cardiomyocytes were cultured with FK866 1-10 μ M and/or norepinephrine (NE) 10 μ M for 48 h. Cell viability and ROS levels were determined using propidium iodide and dihydrorodhamine 123, respectively. NAD⁺ and ATP levels were assessed by commercial kits. Sarcomerization and cell area were evaluated by fluorescence microscopy using phalloidin staining. The results obtained show that FK866 diminishes NAD⁺ and ATP levels without affecting cell viability. FK866 decreases ROS basal levels and prevents both the sarcomerization as well as cell size increase in cardiomyocytes treated with NE. These results suggest that NAD⁺ plays an essential role for the hypertrophic response in cardiomyocytes.

Anillo ACT1111, FONDECYT 1120212 (SL), 3110114 (RT) and 3130749 (CP).

116. Vitamin C recycling and the protective function of the astrocyte against neuronal death induced by oxidative stress and dehydroascorbic acid

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Vitamin C is an antioxidant molecule found in high concentration in the nervous system; in physiologic conditions his reduced form predominates, Ascorbic acid (AA). When vitamin C fulfills his antioxidant function, it is produced dehydroascorbic acid (DHA). DHA is incorporated by the astrocytes through the GLUT1 transporter, to be reduced intracellularly to AA, and to be finally liberated to the extracellular medium. This phenomenon is known as the vitamin C recycling. This study evaluates the importance of vitamin C recycling, by the use of co-culture experiments (Neurons-astrocytes), quantifying the protective capacity of the astrocyte, against neuronal death. The neurons (N2a cell line) were treated with H₂O₂ and DHA. DHA increases death in stressed neuronal cells. Additionally, the presence of astrocytes and DHA recycling reverses the cell death of stressed neurons. Taken together, DHA increases the death of stressed neurons and astrocytes are essential in neuronal antioxidative defense.

117. Biochemical and cellular characterization of the Golgi apparatus protein GOLPH3 in human breast cancer cells

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Breast cancer represents the most frequent and deadly cancer for women in Chile, and worldwide. Despite enormous advances on the understanding of this type of cancer, little is known about the different aspects of the secretory pathway in breast cancer tumorigenesis, and in particular on the contribution of the Golgi apparatus. GOLPH3 is a highly conserved phosphoprotein of the Golgi originally found in proteomic analyses. Importantly, GOLPH3 is overexpressed in several tumor types including breast, lung, melanoma, ovarian, prostate, and glioblastoma, but its function in normal or malignant cells is unknown. GOLPH3 is a peripheral membrane protein that is enriched at the trans-Golgi network, and that rapidly exchanges between cytosolic and Golgi-associated pools. We compared GOLPH3 from two human breast cancer cells with the ultimate goal to understand the putative role that GOLPH3 might play in tumorigenesis. ZR-75 cells of ductal epithelial carcinoma, and MDA-MB231 cells of adenocarcinoma were used for biochemical and cellular analyses of GOLPH3 by immunoblot, fluorescence microscopy, and live cell imaging. In this work we report the serendipitous finding that an accidentally obtained GOLPH3 mutant is predominantly associated to transport vesicles, and how this feature could be related to a gain of function in tumor cells. Supported by Fondecyt 1130710.

118. ATP induces altered gene expression in adult fibers from C57 mice with cancer

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Introduction: A murine model of cancer was used to study muscle atrophy which determines progressive weight loss. We hypothesize that ATP-induced gene transcription is altered for both interleukin-6 (IL-6) and apoptosis genes in fibers isolated from skeletal muscle of tumor bearing animals.

Materials and Methods: Adult fibers were isolated from mouse flexor digitorum brevis (FDB) from C57 mice with melanoma cancer. ATP release measurements were performed using a Luciferin/Luciferase kit. Isolated fibers were stimulated with 10uM ATP, and mRNA of genes was assessed by real time PCR.

Results: Mice with cancer showed an elevated ATP release in resting conditions (n=4) 933.5 ± 103.7 , controls (n=4) 337.9 ± 34.9 pmoles/ugRNA, $p < 0.0001$. The IL-6 mRNA is elevated at baseline in the muscle fibers from mice with cancer (n=16) 4.16 ± 1.16 compared to controls (n=12) 1.59 ± 0.55 , $p < 0.05$. Fibers stimulated with ATP show elevated mRNA content of PUMA (n=11) 5.76 ± 1.88 and BIM (n=11) 1.82 ± 0.3 , compare to baseline (n=11) 0.94 ± 0.37 and (n=12) 0.7 ± 0.14 , respectively.

Discussion: In muscle fibers both increased basal ATP release and ATP stimuli induce gene expression which could be related with muscle wasting in this model of cancer cachexia.

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119. Galectin-1, a protein involved in the polymerization of the Reissner Fiber?

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Introduction Reissner Fiber (RF) is a filamentous structure that extends through the aqueduct of Sylvius (SA), fourth ventricles and the central canal of the spinal cord. RF continuously grows by the addition of glycoproteins released by the subcommissural organ (SCO) located at the entrance of SA. Absence of RF in the SA leads to congenital hydrocephalus. The aim of the present investigation was to analyse the protein composition of RF to help to understand the mechanism of polymerization of the proteins forming RF, a process that is poorly understood.

Methods. Bovine RF was obtained. RF proteins were solubilized with different media (urea, guanidine-HCl), deglycosylated and digested. Resulting peptides were analysed by mass spectrometry (MS). The MS information was confirmed by western blotting and immunofluorescence, using the antibodies: AFRU (against RF glycoproteins), anti-P15 (specific marker of SCO-spondin, the main constituent protein of RF) and anti-galectin1.

Results. Malditoff analyses showed the presence in RF extracts of SCO-spondin, galectin-1, S100 protein and endothelin-2. The immunochemical studies confirmed the presence in RF of SCO-spondin, a glycoprotein of 450 kDa and Galectin-1 and a lectin of 14 kDa.

Conclusions. Since Galectin-1 is a protein involved in adhesion via glycan bridging, it may be postulated that it bridges SCO-spondin molecules allowing their polymerization and, consequently, formation of RF.

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120. *Trypanosoma cruzi* induces cellular proliferation and differentiation in the human trophoblast

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Chagas' disease is caused by the protozoan *Trypanosoma cruzi* (*T. cruzi*). However the transmission rate is low suggesting the presence of local antiparasitic mechanism. The epithelial turnover of the trophoblast could be one of them.

In order to determine whether the parasite is able to induce proliferation and differentiation in the trophoblast, we incubated HPCVE or BeWo cells in presence and absence of *T. cruzi* trypomastigotes (low concentration) and respective positive controls during 2, 24 and 48 hours. DNA synthesis was assayed by BrdU incorporation by spectrometry and immunocytochemistry. Mitotic index was determined in DAPI stained samples and cell growth by detection of NORs. Differentiation of the trophoblast was determined by the detection of hCG by spectrometry, immunohisto(cito)chemistry and Western blotting. Additionally, we determined the fusion index in the BeWo cells by the Two Colour Fusion Assay. Parasites were identified inside cells by their nuclear and kinetoplast morphology. Statistical significance was analyzed by ANOVA followed by Dunnett's post-test.

T. cruzi induces a significant increase in cellular proliferation, hCG expression and secretion in HPCVE and BeWo cells, the latter also increases their fusion index.

We conclude, that the parasite may increase the trophoblast turnover by induction of differentiation of the trophoblast.

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121. Arabidopsis Sirtuin 1 is a Histone Deacetylase and Participates in the Regulation of Flowering Time.

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The sirtuin family of proteins appears highly conserved among organisms from all kingdoms. Their enzymatic activity can produce the deacetylation or ADP-rybosilation of target proteins, using NAD⁺ as a cosubstrate. This activity responds to changes in the NAD⁺/NADH ratio, so they have been proposed as sensors of the metabolic state of the cell and the evidence has shown that they modulate processes such as transcriptional activity and key metabolic pathways. However, little is known about the two sirtuin genes present in most plants and their function. Previously we have determined that SRT1 is a nuclear protein and characterized an *Arabidopsis thaliana* insertional mutant of sirtuin 1 of which no homozygous mutant plants can be obtained, and it also presents alterations in gametophyte development. In this work, we generated overexpression lines of *SRT1* that, when compared with wild type plants, show a delay in flowering. On the other hand, the heterozygous mutant line flowers earlier than wild type, suggesting that Sirtuin 1 activity regulates the transition to the reproductive state. Furthermore, we analyzed the levels of acetylation of histone H3 in these lines, observing a correlation between diminished acetylation of the lysines 9 and/or 14 with the delay in the flowering time. Financed by Fondecyt 1100601, Núcleo Milenio en Genómica Funcional de Plantas P10-062-F.

122. MEF26, a PPR protein necessary for efficient RNA Editing at two sites in two mitochondrial transcripts in *Arabidopsis thaliana*.

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RNA editing is a specific post-transcriptional modification that occurs in vascular plant's plastid and mitochondrial transcripts by C-to-U conversions. In *Arabidopsis*, 34 editing sites are known for chloroplasts, whereas more than 500 cytidines are modified in mitochondrial transcripts. In the last years, several *trans*-factors essential for organelar RNA editing have been identified. A protein family of great importance for this process is the Pentatricopeptide Repeat Proteins (PPR). In the present work, we have characterized a PPR protein called MEF26 (Mitochondrial Editing Factor 26). First, we found an exclusively mitochondrial destination by fusing the N-terminal of MEF26 to GFP. Over 350 mitochondrial editing sites and all chloroplast editing sites were analyzed by SNaPshot in homozygous mutant plants and their wild type equivalents, revealing a decreased editing at specific site in mitochondrial transcripts. The *mef26* mutant presented deficient editing at two editing sites in two transcripts: *nad4-166* and *cox3-311*. Complementation of *mef26* mutant plant with MEF26 restore editing at those sites, confirming the role of this PPR protein as an editing factor. Therefore, the identification of new PPR proteins relevant to RNA editing in *Arabidopsis thaliana* is an interesting approach for understanding this post-transcriptional modification. Acknowledgements: FONDECYT-1100601, Núcleo-Milenio-P10-062-F, ApoyoTesis-CONICYT-N°24100161.

123. A Key Enzyme Behind the Bug?: Characterization of *Candidatus phytoplasma* AYWB Malic Enzyme

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Phytoplasmas are insect-vectored plant pathogens that infect high-value agricultural crops. They are obligate parasites that survive and replicate intracellularly within both insect and plant hosts. As a consequence of genome reduction, their metabolic capabilities are hardly impaired, resulting in a strict nutrient dependence on their hosts. It is not clear which is the preferred carbon source, as sugars do not seem to be the best option according to the metabolic pathways deduced from genome analysis. However, a pathway for malate conversion to acetate, which includes malic enzyme as the first enzyme, appears an appropriate alternative. In this work, we characterized the recombinant malic enzyme encoded in *Ca. P. asteris* strain AYWB. Our studies show that the enzyme is active with both NAD and NADP as cofactors, with maximum activity at pH 8.2, and following a Bi-Ter steady-state random mechanism. Besides, the enzyme is activated by glutamine and glutamate, which are highly abundant in plant phloem. Interestingly, the enzyme is activated by ADP but inhibited by ATP, giving a strong indication of its participation in the bacterial energetic metabolism. Finally, phylogenetic analyses show that this enzyme follows the species evolution, and that it has not been acquired by horizontal gene transfer. Overall, our results provide evidence on the functionality of this malic enzyme, which could play a key role in phytoplasma pathogenesis

124. Root architecture and gene expression in response to salt stress in chilean quinoa genotypes

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Optimum growth in quinoa (*Chenopodium quinoa* Willd.) occurs in soil containing 100-200 mM NaCl, while 300mM NaCl allows plants to complete their life cycle, however, leads to yield reduction. Our aim is to unravel how quinoa responds to salt stress by modification of plant architecture and functional traits, agronomic (yield) and molecular (gene expression modulation) parameters. This research is based on "in vitro" and field experiments to describe the different response strategies to salt stress in quinoa Chilean genotypes. Germination and root/shoot growth were evaluated in six genotypes: R49, PRJ, PRP, VI-1, Villarrica and BO78 treated with 0, 100 and 300 mM NaCl. During the establishment and growth of seedlings germinated in 300 mM NaCl, the biomass of seedlings and the ratio root/hypocotyl length were differentially affected in all genotypes. Moreover, root architecture of quinoa is modified in response to NaCl, especially in the apical meristem and later in the abundance of secondary roots compared to controls. We selected three genotypes (R49, VI-1 and Villarrica) for gene expression analysis. Genes related to responses to salt stress such as *NHX*, *SOS*, *NCED* and transcription factors regulating these genes, such as *DREB*'s and *MADS*, were differentially expressed. The results indicate that the high salt tolerance observed in quinoa is strongly dependent on applied treatment as well as biogeographical provenance of the selected genotypes.

125. Cloning and characterization of a xyloglucan endo-transglycosylase/hydrolase gene of radiata pine seedlings

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Cell wall is a dynamic structure that can be modified due to different conditions during the plant growth. Stem inclination in conifers induces biochemical changes and chemical modifications in cell wall that allow plant stem reorientation. Different enzymes take part in cell wall remodelling, including xyloglucan transglycosylase/hydrolase(XTH). This enzyme modifies xyloglucans, the main hemicellulose compound in primary cell wall of pine by two different activities the hydrolase, transglycosylase or both. A partial *XTH* gene was previously identified as differentially expressed in a subtractive suppressed hybridization library from radiata pine. Full-length cDNA of *Pr-XTH1* was isolated and cloned in order to characterize and determine the specific activity. A full length *Pr-XTH1* comprised an open reading frame of 894bp encoding a protein of 288 amino acids. Phylogenetic and gene structure analysis showed that *Pr-XTH1* belong to group I close to poplar XTH crystal 1UMZ. Genes belong to this group have the XET activity. To confirm the transglycosylation activity of Pr-XTH1, the coding sequence was cloned in *P. pastoris* and the recombinant protein obtained was used to perform enzymatic assays. A strong transglycosylation activity from extracted recombinant Pr-XTH1 was quantified. **Acknowledgments** Was supported by Fondecyt N°s 1120635 and 11121170. C.V. thanks to U. Talca for his doctoral fellowship. P.R. was supported by postdoctoral PBCT-PSD61 project.

126. Characterization of *Solanum lycopersicum* defense response to pathogen attack under the influence of nitrogen availability

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Nitrogen (N) is the main limiting nutrient for plant growth and yield in agriculture, with profound effects in different developmental programs. It is well known that nitrate and other N metabolites affect leaf growth and flowering time, among other plant developmental processes. In addition, the plant N nutritional status influences its ability to respond effectively when challenged to plant pathogens. Although significant progress has been made in the identification and characterization of defense-response genes, the molecular mechanism, signaling and the regulatory networks involved in the interaction between N and pathogen responses are poorly understood. To address this question, we evaluated *Solanum lycopersicum* defense response to the bacterium *Pseudomonas syringae* and the fungus *Botrytis cinerea* under contrasting nitrogen regimes. N availability alters Tomato susceptibility to both pathogens. To identify potential candidate regulatory gene networks mediating the plant defense response associated with nitrogen nutrition, we used GENIUS a new machine learning algorithm to infer functional gene networks. Experiments are underway to validate key components of both N regulation and the defense response, which we identified with our bioinformatics approach. These results are contributing to a better understanding of the convergence points between biotic stress and N status and metabolic regulation in plants. Milenio-P10-062-F, Fondecyt-11110095 and 3110127.

127. *ERF115* gene codes for a transcription factor involved in tolerance to salinity stress in *Arabidopsis thaliana*

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Salinity of soils is an important abiotic stress that affects plants impairing agricultural productivity. Although many players involved in salt tolerance of plants have been described, there are still components that remain unknown. We identified *ERF115*, a gene coding for a putative transcription factor that belongs to the AP2 family protein. *ERF115* expression is induced in roots by high salt treatments and functional analysis of an homozygous mutant line showed to be less tolerant than WT plants to salinity stress. *erf115* line was complemented with two different constructs to overexpress ERF115 protein fused to Citrine (*pUBQ10::ERF115-Citrine*) and to cMyc (*p35S::ERF115-cMyc*). Several homozygous lines showing different *ERF115* transgene constitutive expression levels were selected. . Analysis of *pUBQ10::ERF-Citrine* lines by confocal microscopy shows that *ERF115* produces a protein that localized in the nuclei. ERF115 is able to activate transcription, as detected by using a yeast one-hybrid assay. To assess whether the complemented mutant lines were able to recover the WT phenotype, we evaluated the tolerance to salt stress and the expression of genes which we previously determined are affected in the mutant line. Our results suggest an important role for ERF115 in the tolerance to salinity stress, by regulating genes involved in the stress response. Financed by FONDECYT 1100656; Millennium Nucleus for Plant Functional Genomics P10-062-F.

128. Functional promoter analysis of *GRXC9*, a gene activated by a non-canonical salicylic acid-dependent pathway in *Arabidopsis*

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Salicylic acid (SA) is one of the key hormones involved in defense responses against environmental conditions. *GRXC9* gene belongs to a group of genes early and transiently activated by SA. *GRXC9* expression is also activated by different stress conditions by a SA-dependent manner and is partially dependent of the transcriptional coactivator NPR1. We are interested to elucidate the mechanism controlling the activation of this gene by SA. *In silico* analysis of *GRXC9* promoter, allow us identified two putative SA-responsive *as-1-like* elements in its proximal region. These elements have been described as targets of transcription factors of the bZIP TGA family. By using different constructs of *GRXC9* promoter fused to *GUS*, we show that both *as-1-like* elements are needed for *GRXC9* SA-mediated induction. The expression of *GRXC9* is abolished in the triple *tga2/5/6*. Interestingly we found that in wild type plants TGA2 and TGA3 are constitutively bound to *GRXC9* promoter; however only after SA stimuli the RNA polymerase II is recruited to the promoter. This effect isn't observed in the *tga2/5/6* triple mutant plants, indicating that RNA pol II recruitment is dependent of SA and TGA class II factors. Our results indicate that under basal conditions TGAs are bound to *GRXC9* promoter and the SA stimuli produces a change in TGA complex allowing the recruitment of RNA pol II and the transcriptional activation of *GRXC9*. Supported by FONDECYT 1100656 & Millennium Nucleus P10-062-F.

129. Identification of pollen specific promoters from *Arabidopsis thaliana* and their use to generate male gametophyte ablation.

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The mature pollen grain displays a highly specialized function in angiosperms, accordingly, the male gametophyte development involves many cell activities and mechanisms, making it a complex and unique process in plants. In order to accomplish this, during the pollen development, a whole new transcription program starts indicating the switch from a sporophytic to a gametophytic tissue, involving the expression of many pollen specific genes. With the search in microarrays databases we selected, according to their expression profile, five candidates for pollen specific genes (*PSG*), and confirmed this through RT-PCR. A transcriptional fusion where performed between the putative promoters of the *PSG* genes and the *GUS* reporter gene, in order to determinate the spatial and temporal expression pattern, this showed *GUS* activity exclusively confined to the pollen grain in *Arabidopsis* transgenic lines. The expression of a cytotoxin under the transcriptional control of the *PSG* promoters generated pollen specific ablation. Through the selection of genes that shown a pollen specific expression profile in microarrays experiments, we were able to identify promoter regions that confers pollen specific expression, providing a powerful tool for the expression of genes exclusively in pollen during microgametogenesis, like the generation of male sterility. Funded by Fondecyt 1120766 and UNAB DI-74-12/R.

130. Modular hyperthermostable bacterial endo- β -1,4-mannanase: molecular shape, flexibility and new structural insights

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Endo- β -1,4-mannanase from *T. petrophila* (TpMan) is a hyperthermostable enzyme that catalyzes the hydrolysis of β -1,4-mannoside linkages in various mannan-containing polysaccharides. A recent paper reported that TpMan is composed of a GH5 catalytic domain joined by a long linker to a carbohydrate-binding domain, the same paper presents the crystal structure of GH5 catalytic domain, at this moment, there is no three-dimensional structure determined for entire TpMan. Little is known about the conformation of the TpMan enzyme as well as the role of the length and flexibility of the linker on the spatial arrangement of the constitutive domains. We report the first structural characterization of the entire TpMan by SAXS in order to shed light on the low-resolution model, overall dimensions, and flexibility of this enzyme. The results are consistent with a linker with a compact structure and that occupies a small volume with respect to its large number of amino acids. The results indicate that the linker is a compact domain structurally very similar to immunoglobulin-like β -sandwich domain. The results are consistent with a model where TpMan is a flexible molecule composed of three distinct domains. Our findings reveal that TpMan has a maximum dimension similar to the maximum dimension of others two-domain carbohydrases, this means that the central domain in the case of TpMan certainly optimizes the geometry between the others two domains with respect to the substrate.

131. Dissection of the Mechanism of Protein Unfolding and Translocation by the AAA+ Protease Machine ClpXP

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Proteases of the AAA+ family play crucial roles maintaining protein homeostasis in the cell by degrading misfolded/damaged proteins. ClpXP, an ATP-dependent protease from *E. coli*, is considered an archetypal for the study of AAA+ proteases. I use high-resolution optical tweezers to study the mechanisms of protein unfolding and polypeptide translocation in ClpXP. I probed the effect on ClpXP operation of: i) sequence composition of the protein substrate, and ii) mutations in the GYCG loops, regions in the ClpX pore that make direct contacts with the substrate and drive translocation. My current data shows that both small and bulky amino acids in the substrate being translocated reduce ClpX translocation rate and induce a pause. This suggests that the main feature in the substrate that affects ClpX translocation is actually the size of the amino acid side chains. We propose a model where ClpX tightly grips its substrate by additional contacts of the GYVG loops with amino acid side chains of the substrate. If the substrate is composed of small amino acids, then poor contacts with the substrate might be established. On the contrary, large amino acids in the substrate may provide significant contacts with the GYVG loops, but at the same time may hinder the movements of the GYVG loops that drive translocation due to filling of the narrow ClpX pore, resulting in a state where the motor is stalled and therefore translocation temporally ceases.

132. Biochemical characterization of metal-nucleotide binding in an ADP-dependent phosphofructokinase from *Pyrococcus horikoshii*.

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Some archaea use a modified Embden-Meyerhof pathway that employs an ADP-dependent glucokinase (GK) and phosphofructokinase (PFK) instead of classic ATP-dependent kinases. These enzymes are classified as members of the ribokinase superfamily, whose structural analysis reveal highly conserved motifs related with their common catalytic mechanism: the GXGD motif, involved in activation of the phosphoryl acceptor substrate for nucleophilic attack; the NXXE motif (DXXE in some kinases) involved in binding of the metal-nucleotide complex essential for enzyme activity. In this work, we evaluated the role of the highly conserved residues of the DXXE motif and two residues, L340 and L431, proximal to the ribose and adenine rings of Mg-ADP, in the enzyme kinetics of the ADP-dependent PFK from *Pyrococcus horikoshii* (phPFK) by alanine scanning mutagenesis. The D287A mutant did not show differences in its kinetic parameters compared to the wild type enzyme, while the E290A mutant showed a 500-fold decrease in *k_{cat}*. For L431A, we observed a 10-fold increase on *K_m* for Mg-ADP, while the L340A mutant causes a 5-fold increase on *K_m* for fructose-6-P. These results suggest that E290, and not D287, plays a critical role in coordinating the metal and stabilizing the nucleotide in the binding pocket, while L340 and L431 are important in the establishment of an adequate space that limits the movement of the nucleotide in its binding pocket to allow phosphate transfer. Fondecyt 1110137

133. Structure of the dimerization domain of Bcy1, the regulatory subunit of the cAMP dependent protein kinase from *Saccharomyces cerevisiae*, and interaction with a peptide derived from IRA2, a GTPase activating protein involved in the cAMP signalling pathway.

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A prototypic member of the Ser/Thr kinase family is the cAMP dependent protein kinase (PKA). In most organisms it exists as an inactive holoenzyme, consistent of two catalytic (C) subunits and a regulatory (R) subunit dimer. When extracellular signals trigger cAMP synthesis, two molecules bind each R subunit, releasing fully active C subunits.

In mammals, PKA holoenzyme is confined to specific subcellular compartments by high affinity interaction between the dimerization and docking (D/D) domain of R₂ and an amphipathic α – helix of scaffold proteins belonging to the A Kinase Anchoring Proteins (AKAPs) family.

In yeast, PKA is involved in the regulation of processes related to nutrient source and availability, response to stress, etc. No yeast AKAPs have been described to date. We have already reported the interaction of Bcy1 with a 20 aminoacid peptide of IRA2 and have obtained a low resolution structure of the D/D domain of Bcy1. Here we present a spectroscopic and crystallographic study addressed to gain insight into this interaction. The IRA2 peptide folds into an amphipathic α – helix, and interacts with Bcy1 with nM affinity. Crystallization trials on Bcy1 D/D domain were successfully performed. Crystals diffracted at 2.5 Å resolution, and belong to the P2₁ space group, with four dimers in the asymmetric unit. The first approaches to solve the structure by molecular replacement methods showed structural differences with the D/D domains of mammals.

134. Identification of a pro-amyloidogenic region in microcin E492, a pore-forming bacteriocin.

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Microcin E492 (MccE92) is a low molecular weight antimicrobial peptide (<10KDa) produced by *K. pneumoniae* RYC492 that is post-translationally modified with a salmochelin-like molecule at the C-terminal. Its mechanism of action involves the recognition of outer membrane siderophore receptors through the salmochelin-like molecule, translocation to the periplasm and pore formation into the inner membrane of the target cell. MccE492 can form amyloid in vitro as well as in vivo. MccE492 modified form presents a delayed kinetics of amyloid formation compared to the unmodified form. To study the importance of sequence determinants called "pro-amyloidogenic regions" that play a key role over the aggregation propensity of amyloidogenic proteins, we performed bioinformatics analysis of MccE492. A possible pro-amyloidogenic region was identified, and a deletion mutant between residues 54 to 63 was generated. The in vitro aggregation kinetics of the mutant as well as the wild type MccE492 was followed using thioflavin T and label free fluorescence measurements. We observed a significant decrease of aggregation ability of the mutant Δ 54-63, but interestingly this mutation did not abolish the pore-forming ability of MccE492 tested on liposomes. Supported By FONDECYT Grant 1100141.

135. Characterization of the microcin E492 pore-forming activity using liposomes

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Microcin E492 (MccE492) is a pore-forming bacteriocin produced by *K. pneumoniae* RYC492 which genetic determinants were cloned and expressed in *E. coli*. MccE492 is also amyloidogenic being capable of generating amyloid fibrils in vivo and in vitro.

MccE492 pore-forming kinetics was determined using liposomes from *E. coli* lipids that were loaded with quenched fluorophores. Pore formation was followed by an increase of the fluorescence over time due to the release and dequenching of the fluorophores.

MccE492 pore-forming kinetics was pH dependent with 3 plateaus: a maximal activity at pH 3-4; a lower activity at pH 5-7; and no activity at pH $\geq$ 7.5. Crosslinking studies revealed the presence of dimers or trimers in solution at pH $\leq$ 7. Formation of tetramers and pentamers using fluorescence anisotropy and crosslinking were observed at pH $\geq$ 7.5. MccE492 amyloid aggregation occurs at pH $<$ 7, which suggests that the active form is the amyloidogenic prone. On the other hand, MccE492 pore-forming kinetics at pH 7 showed two phases, one initial fast followed by a slow phase. Through the Hill equation we determined that both phases were cooperative, with a Hill coefficient of 4.6 for the fast phase and 2.6 for the slow one. The fast phase is congruent with MccE492 annular pentamer observed by electron microscopy. The use of HFIP, a solvent used to dissolve pre-aggregates completely eliminated the slow phase, and augmented the fast phase.

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136. On the tertiary-quaternary coupling of the allosteric activation of glucosamine-6-P deaminase from *Escherichia coli*.

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E. coli glucosamine 6- P deaminase (EC 3.5.99.6) catalyses the conversion of GlcN6P into Fru6P and NH₃. It is an allosteric enzyme activated by *N*-acetylglucosamine 6-P. The structures of its two extreme allosteric conformers (T and R) are well known. Its allosteric kinetics in solution has been carefully described and the T-R transition dependence on the activator concentration is consistent with a conformational selection mechanism. Multiple evidences indicate the existence of tertiary sub-states of each quaternary conformer, but their connection with quaternary transition was not yet explored. To study these sub-states we isolated the local structural changes, abolishing the T-R transition by nano-encapsulating the T conformer in silica sol-gels. The enzyme used was a mutant form containing a fluorescent quenching-generating pair of Cys and Trp designed and constructed to operate as a conformational reporter. With this spectral tool we studied the relaxation kinetics of tertiary transitions. The results show that T state is strongly biased by tertiary low affinity conformation (t conformation), and support an induced fit tertiary activation mechanism.

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137. Insights into cross-reactivity of allergens: structure, dynamics and stability of allergen Gad m 1 free and in complex with single chain variable fragment by solution NMR Spectroscopy

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Allergenic proteins are able to stimulate an inappropriate IgE production in atopic individuals. Gad m 1 is the major allergen from *Atlantic cod* and belongs to β -parvalbumin protein family, which are the most important fish allergens and their high cross-reactivity is the cause of the observed polysensitization in allergic patients. Despite extensive efforts, the complete elucidation of β -parvalbumin-IgE complexes has not been achieved yet. In this work, we solved the solution structure of Gad m 1 using NMR spectroscopy. Concomitantly, scFv interaction studies were performed, using chemical shift perturbation assays. We compared our results with other sites mapped in homologous parvalbumins: Cyp c 1, Sco j 1 and Gad c 1. Residues around positions 30 to 40 matched epitopes mapped for the three allergens. Residues around position 50 to 60 were only observed for Gad m 1 and Gad c 1, while residue around 80 was mapped on Cyp c 1. Residues 31-33 and 75-78 exhibit increased R_2/R_1 ratios. These residues are located in two regions mapped by CSP. The big ratios observed may be due to chemical exchange between bound and free forms. This work will present for the first time the solution structure of a fish β -parvalbumin as well as the characterization of Gad m 1-scFv interaction as a first and crucial step in the development of hypoallergenic vaccine and to a better understanding of the molecular interactions between allergens and IgE's.

138. Effects of chaotropic agents on the activity and aggregation of the bacteriocin gicin A

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In recent years, some bacteriocins emerged as an alternative in food industry due to their potent and selective activity against pathogenic bacteria. Because of the above the need to study the stability of bacteriocins activity arises. Microcins produced by the Gram negative bacteria show a high stability to denaturing agents, this is the case of gicin A, a bacteriocin synthesized by *Pseudomonas aeruginosa* O400 that has activity against Gram positive bacteria, which has shown to be resistant to high temperatures. In order to study the stability of this polypeptide, we observed the effects of some denaturing agents on its activity and aggregation. For this, gicin A was purified from its producing strain by hydrophobic exchange chromatography and was then incubated for 2 to 4 hours in solutions containing different concentrations of urea and guanidinium chloride and they were tested on a lawn of indicator bacteria. Controls of gicin A were performed in absence of denaturing agents, without prior incubation and controls of solutions without gicin A. Finally the effect of chaotropic agents on the aggregation of the gicin A was determined by measuring the sample absorption at 600 nm. The results show that incubation of gicin A in presence of chaotropic agents, tends to increase its activity. According to the results obtained, it could be postulated that gicin A is a peptide without defined structure and does not require it to carry out its action.

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139. Structural changes in *E. coli* hosphofructokinase-2 upon subunit dissociation induced by chemical, temperature and mutational perturbations.

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Phosphofructokinase-2 (Pfk-2) is a dimeric enzyme that follows a three-state folding mechanism involving an expanded monomeric intermediate with lesser secondary structure than its native state. This intermediate is accumulated during cold denaturation and guanidine-induced denaturation at 0.85M guanidine-HCl. Although biophysical characterizations by global spectroscopy suggest these species resemble each other, they differ in the fluorescence obtained for bound ANS. Recently, we described a hydrogen-deuterium exchange mass spectrometry (HXMS) strategy to follow local structural changes during cold denaturation of Pfk-2, proving that its intermediate state is solvent-penetrated. Here we use HXMS to describe structural changes in Pfk-2 upon subunit dissociation induced by solvent, temperature and mutational perturbations of the protein-protein interface. A single-point mutant (L93A), monomeric at protein concentrations below 40 μ M, has a Stokes radius 35% smaller than the thermally or chemically induced monomeric intermediate, as seen by sedimentation velocity. HXMS proves the chemically induced intermediate is more solvent accessible than the cold denatured one, while the extent of isotope exchange for L93A is similar to the native dimer, except for residues 44-69, 118-126 and 154-164 directly connected to the interfacial domain. These results suggest the monomer structure is locally unstable and its stabilization depends on dimer formation. FONDECYT 1110137

140. Structural Basis of CCR2 Sulfotyrosine Recognition by the human beta-defensin 6

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Human β -defensins (hBDs) are believed to function as alarm molecules that stimulate the adaptive immune system when a threat is present. In addition to its antimicrobial activity, defensins present other activities such as chemoattraction of a range of different cell types to the sites of inflammation. We have solved the structure of the hBD6 by NMR spectroscopy that contains a conserved β -defensin domain followed by an extended C-terminus. We use NMR to monitor the interaction of hBD6 with microvesicles shed by breast cancer cell lines and with peptides derived from the extracellular domain of CC chemokine receptor 2 (Nt-CCR2) possessing or not possessing sulfation on Tyr26 and Tyr28. The NMR-derived model of the hBD6/CCR2 complex reveals a contiguous binding surface on hBD6, which comprises amino acid residues of the α -helix and β 2- β 3 loop. The microvesicle binding surface partially overlaps with the chemokine receptor interface. NMR spin relaxation suggests that free hBD6 and the hBD6/CCR2 complex exhibit microsecond-to-millisecond conformational dynamics encompassing the CCR2 binding site, which might facilitate selection of the molecular configuration optimal for binding. These data offer new insights into the structure-function relation of the hBD6-CCR2 interaction, which is a promising target for the design of novel anticancer agents.

141. Structural and kinetic studies of an uncommon allosteric Glucosamine 6-P deaminase

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Glucosamine 6-phosphate (GlcN6P) deaminase (EC 3.5.99.6) catalyses the conversion of GlcN6P into Fru6P and NH₃; it plays a key role in the regulation of amino sugar metabolism due to its allosteric properties and the control of its gene expression. Most of the best-known GlcN6P deaminases have a Rossman-like fold, but the enzyme from *Shewanella oneidensis* (NagBII) has an entirely different fold, the SIS (sugar isomerase) fold. In spite of their structural difference, deaminases from *E. coli* and *S. oneidensis* show both homotropic cooperativity and allosteric activation by *N*-acetyl-GlcN6P (GlcNAc6P). However, *S. oneidensis* enzyme presents kinetic cooperativity. We obtained its structural model on basis on the crystallographic structure of its homolog from *S. denitrificans*; the enzyme is a homodimer, with one active site per subunit, but a single allosteric site located in the intersubunit space. These sites were both localised by molecular docking and subsequently verified by site-directed mutagenesis and their stoichiometry confirmed by direct binding experiments. We analysed the conformational changes produced by either GlcN6P or GlcNAc6P binding, by spectrometric and computational analysis. We demonstrated that homotropic cooperativity and allosteric activation by GlcNAc6P depend on different molecular mechanisms and that they can be experimentally dissociated by enzyme nanoencapsulation in silica gel. (Supported by Papiit, UNAM and Conacyt, México).

142. The energetic cost of knotting a polypeptide chain in its unfolded state determined by optical tweezers.

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The unfolded state of a protein is considered as a highly fluctuating state of the polypeptide chain, but its tendency to form entangled structures like knots has not been quantified. In this work, we determined the energetic cost of threading a designed knotted protein. The bacteriophage P22 ARC repressor is a homodimer, which can be converted into a single-chain monomer (mARC) adding a 15-residue glycine-rich linker to connect both subunits. Structural models show that flexible glycine-rich linker have the potential to create a knotted or untied topology by movements around the protein structure. Using optical tweezers, we explored the folding mechanism of mARC pulling their C and N-terminal extremes. Analysis of 537 unfolding events obtained from eleven molecules of mARC show two populations of proteins characterized by contours lengths of 37 (7 fibers) and 43 nm (4 fibers). These values agree with the expected size of the polypeptide being stretching from a knotted (37 nm) or unknotted (43 nm) conformation. Furthermore, thermodynamic analysis obtained from the knotted or unknotted conformation of mARC indicates that the unfolded knotted conformation is destabilized by 6 kcal/mol with respect to the unknotted fully unfolded conformation. This situation contrasts with respect to naturally-occurring knotted proteins where knots prevail in the unfolded state. Fondecyt 11110534.

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143. Insights into the role of Pro475 in the ion permeation process of Shaker K-channel using non-equilibrium molecular dynamics.

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Shaker is a low-conductance K-channel in which single-point mutations of Pro475, a residue near the internal pore entrance, promote 6- to 8-fold higher unitary current than in the WT channel. In order to understand the role of Pro475 in the K⁺ conductance process we performed molecular dynamics simulations of WT, P475D and P475Q mutants, which altogether account for more than 0.5 us of simulation. External electric fields were applied at +1.2 V to simulate the patch clamp experimental conditions. Applying an external electric field, a higher number of outward K⁺ transport events are observed. The Pro475 properties involved in the K⁺-conductance process were defined analyzing K⁺ current in terms of channel properties including electrostatic potential, permeation events number, K⁺ dehydration processes and K⁺ sites inside the pore. A single charged residue, at the 475 position, (P475D mutant) produces an effect on ion density, attracting K⁺ ions into the cavity site and allowing a faster permeation. We have identified that current differences are not defined by changes on secondary structure; instead, mutations change ion concentration and diffusion rate by modulating the electrostatic potential in the pore. This study provides new perspectives to understand the ion conductance in K-channels. This work is supported by FONDECYT grant 1131003, CONICYT doctoral fellowship (To R.S.) and Millennium Institute-CINV

144. Corner spider's Phospholipase D antibodies in subjects without loxoscelism and its relation with bacterial phospholipases D phosphodiesterases.

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Phospholipases D family of *Loxosceles* spider posses a highly conserved glycerophosphoryl diester phosphodiesterase (GDPD) domain, and similarities between *Loxosceles* PLDs and bacterial PLDs have been documented. We evaluated the epitope profile between phospholipases D phosphodiesterases of *Escherichia coli*, *Salmonella enterica* and *Clostridium perfringens*, and compared it with the epitope profile of the phospholipase D isoform 1 of *L. laeta* (LIPLD1) with the aim to respond the previously observation of the presence of *L. laeta* phospholipase D specific antibodies in sera from subjects without loxoscelism. We found that the pooled sera and individuals sera from this group detected specifically the venom of *L. laeta* and the recombinant LIPLD1 by dot-blot, immuno-blot and ELISA. Also, IgGs purified from sera and immuno-selected against the venom of *L. laeta* recognized the three bacterial extract by immuno-blotting. Additionally, the three bacterial extract has phospholipase D activity against sphingomyelin. The continuous epitope profile of *E. coli* and *S. enterica* present the high similarities between both, but minor epitope similarities with the continuous epitope profile of LIPLD1. However, the discontinuous epitope profile of LIPLD1 showed that conserved amino acid present in all the bacterial phospholipase D sequences studied are involved. Our data, allow us to guide on the origin of these antibodies with cross-reactive in individuals without loxoscelism.

145. Insights into the mechanism for DNA capture and transport by a type II topoisomerase is provided by a high-resolution structure of an 'open' topoisomerase II-DNA complex.

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Type II topoisomerases perform essential roles in DNA replication, chromosome segregation, and recombination[1-3] and are important antibacterial and anticancer targets[4-7]. Type II topoisomerases regulate DNA supercoiling and chromosome segregation via an ATP-driven DNA strand passage mechanism. However, the paucity of structures for native full-length proteins has been a significant obstacle in defining the reaction pathway. Here we present a high resolution X-ray crystal structure of an 'open clamp' complex of a type II topoisomerase [8], the key complex engaged in DNA capture and transport. [1] Gellert, M., Mizuuchi, K., O'Dea, M. H. & Nash, H. A. (1976) *Proc. Nat. Acad. Sci. USA* **73**, 3872-3876. [2] Schoeffler, A.J. & Berger, J.M. (2008) *Q. Rev. Biophys.* **41**, 41-101. [3] Vos, S.M., Tretter, E.M., Schmidt, B.H. & Berger, J.M. (2011) *Nat. Rev. Mol. Cell. Biol.* **12**, 827-41. [4] Collin, F., Karkare, S. & Maxwell, A. (2011) *Appl. Microbiol. Biotechnol.* **92**, 479-97. [5] Drlica, K., Malik, M., Kerns, R.J. & Zhao, X. (2008) *Antimicrob. Agents Chemother.* **52**, 385-92. [6] Nitiss, J.L. (2009) *Nat. Rev. Cancer* **9**, 338-50. [7] Pommier, Y., Leo, E., Zhang, H. & Marchand, C. (2010) *Chem. Biol.* **17**, 421-33. [8] Laponogov, I., Veselkov, D.A., Crevel, I.M.T., Pan, X-S., Fisher, L.M & Sanderson, M.R. *Nucleic Acids Res.* in press.

146. Biochemical Mechanisms of Protein Oxidative Modifications in Membranes: Connecting Lipid Peroxidation with Amino Acid Oxidation

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Protein oxidative posttranslational modifications are usually associated to pathologies and the aging process. Much of the oxidative damage is performed by reactive free radicals that lead to the formation of amino acid-derived radicals. In the case of membrane proteins, we have recently proposed that lipid peroxidation-derived radicals constitute preferential one-electron oxidants. Herein, we studied the biochemical mechanisms of tyrosine oxidation in a model system of tyrosine-containing transmembrane peptides incorporated to phosphatidyl choline liposomes of different fatty acid composition exposed to peroxynitrite, oxo-metal complexes and peroxy radicals. Tyrosyl radicals were detected by EPR-spin trapping and oxidative modifications including 3-nitrotyrosine, 3,3'-dityrosine, 3-hydroxytyrosine analyzed after HPLC separation and UV-vis, fluorimetric and MS detection. The bioanalytical data plus kinetic analysis indicate that lipid peroxy- and lipid alkoxyl radicals react with tyrosine to yield tyrosyl radicals. Experiments at variable oxygen levels (10-200 µM) indicate that oxygen fuels the tyrosine oxidation process by its participation in the propagation phase of lipid peroxidation. Moreover, the concomitancy of lipid- and tyrosine-derived radicals leads to the formation of Diels-Alder-type addition products. Our data support a general biochemical mechanism by which lipid peroxidation and amino acid oxidation are connected processes in membranes.

147. Enzymes stepping on land mines

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It has been one hundred years since Michaelis and Menten characterized the substrate concentration dependence of an enzyme-catalyzed reaction. In the intervening years, detailed catalytic mechanisms have been established for many different enzyme families, and it has been well established that enzymes increase reaction rates by lowering the activation energy of chemical processes. Although some enzymes are known to form transient covalent structures with their substrates, it is generally accepted that they are unaffected by the reaction process. Recently, it has been shown that the diffusivity of enzymes increases in a substrate-dependent manner during catalysis. Although this observation has been reported and characterized for several different systems, the precise origin of this phenomenon is unknown. In this presentation we quantitatively demonstrate the mechanistic link between enhanced diffusion and the heat released in the reaction using single molecule fluorescence correlation spectroscopy data analyzed within the framework of a stochastic theory. We show that the magnitude of the diffusion change is proportional to the enthalpy of the reaction. This study offers a novel perspective on the effect of chemical reactions on their catalysts and suggests future experiments to explore how the local heat released at each turnover affect an enzyme's internal degrees of freedom.

148. Unraveling the function of thioredoxin-interacting protein (TXNIP) through structural studies

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The thioredoxin-interacting protein (TXNIP) belongs to the α -arrestin protein family. TXNIP functions as a cell growth and transcription repressor, metabolic regulator, modulator of inflammatory response, cardiac function and cell signaling and apoptosis. It is also the only member of the α -arrestin family capable of binding to thioredoxin (Trx). TXNIP also plays a crucial role in several pathological conditions such as cancer and cardiovascular diseases, increasing the interest in studying its structure, dynamics and interaction with cellular targets. Our goal is to solve the solution structure of TXNIP using different constructions for each structural domain, called here D1 (17 kDa) and D2 (15 kDa), to understand how the interaction with human thioredoxin (hTrx) regulates glucose uptake. The native and cysteine-mutated ¹⁵N and ¹⁵N/¹³C D1 and D2 have been expressed and purified. We collected NMR spectra for assignment and structure determination of D1 and almost complete resonance assignments were achieved. We also probed the interaction of D1 with both oxidized and reduced hTrx by chemical shift mapping. Both domains are capable to bind to hTrx separately. For D1-hTrx interaction, we construct a structure model of the complex based on NMR chemical shift perturbation. The structure revealed a different binding site than the previous one identified by another group using point mutations. Acknowledgments: FAPERJ, CNPq, INBEB, CNPq-Science w/o Borders

149. Changes in kinase and phosphatase activities in PC12 cells cultured with HTLV-1 infected cell conditioning medium.

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HTLV-1 causes Adult T-cell leukemia (ATL) and HTLV-I associated myelopathy/Tropical Spastic Paraparesis (HAM/TSP). Mechanisms underlying the axonal neurodegeneration of cortico-spinal tracts in HAM/TSP patients are still unknown. Some reports point to viral protein Tax as responsible of the neurodegenerative process. Our Laboratory has developed an *in vitro* model to produce decrease in the neurite length of PC12 cells during their differentiation to neuronal type in presence of HTLV-I infected cells culture media (MT-2 cell line). MT-2 culture medium contains Tax and proteolytically cleaved sSEMA-4D, that coimmunoprecipates with Tax. This semaphorin produces growth cone collapse. Tax-sSEMA4D complex could be involved in the effect seen in neurite growth, mediated by the activation of its receptor, Plexin-B1. Experiments using anti-Tax and anti-SEMA4D demonstrated the involvement of both proteins in the decrease of the neurite length in our model. Cell lysates of PC12 cells cultured in the presence of MT-2 conditioned medium showed no changes in GSK3 β activity, a decrease in CDK5 activity and an increase the inactive form of protein phosphatase 2A (PP2A). This imbalance between phosphatases and kinases could account for decreased levels of phosphorylated Tau at Thr181 (substrate of PP2A and CDK5) also found in PC12 cells cultured in these conditions.

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150. Electrical stimulation induces interleukin 6 expression in skeletal muscle through extracellular ATP, by activating Ca²⁺ signals and an interleukin 6 autocrine loop

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Interleukin 6 (IL6) is a relevant myokine, highly expressed in skeletal muscle cells upon exercise. We assessed IL6 expression evoked by electrical stimulation (ES) or extracellular ATP, as a known mediator of the excitation-transcription mechanism in skeletal muscle, and studied signaling pathways involved in this process.

Either ES of rat myotubes or 100 μ M ATP increased both IL6 expression and pSTAT3 levels in a process inhibited by 100 μ M suramin and 2U/ml apyrase. ATP also evoked IL6 expression in both isolated skeletal fibers and whole FDB muscles. ATP increased IL6 release up to 10 fold. STAT3 activation evoked by ATP was abolished by the JAK2 inhibitor HBC. Blockade of secreted IL6 with a neutralizing antibody, or pre-incubation with the STAT3 inhibitor VIII, reduced in 70% STAT3 activation evoked by ATP. Inhibitor VIII also reduced by 70% IL6 expression evoked by ATP, suggesting a positive IL6 loop. In addition, ATP increased up to 60% the protein levels of SOCS3, a negative regulator of IL6 signaling pathway. On the other hand, intracellular calcium chelation or blockade of IP₃-dependent calcium signals abolished STAT3 phosphorylation evoked by either extracellular ATP or ES.

These results suggest that expression of IL6 in stimulated skeletal muscle cells is mediated by extracellular ATP and nucleotide receptors, involving IP₃-dependent calcium signals as an early step that triggers a positive IL6 autocrine loop.

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151. Human RIC-8B overexpression increases the intracellular levels of cAMP in HEK293T cells in response to isoproterenol.

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The Gα subunit from heterotrimeric G proteins participate in many signaling pathways regulating different cellular functions. In particular, Gsα activates the adenylyl cyclase, which in turn catalyzes the synthesis of the universal second messenger AMPc. In this pathway, RIC-8B has been identified as a guanine nucleotide exchange factor (GEF) that potentiates the Gsα signaling, also stabilizing and preventing its degradation. In order to evaluate the effect of human RIC-8B (hRIC-8B) overexpression in the cAMP pathway mediated by Gsα under isoproterenol induction, experiments using an inducible system called "Tet3G-System" in HEK293T culture cells were carried out. HEK293T cells were transfected with a doxycycline-dependent hRIC-8B overexpression vector, and then the temporal effect of hRIC-8B overexpression over the cAMP intracellular levels using a luciferase assay system was studied. Our results did not show any significant increase in the cAMP levels under RIC-8B overexpression. However, when we used a specific cAMP biosensor, we observed that RIC-8B overexpression increases the intracellular levels of cAMP when the pathway is activated by isoproterenol. On the other hand, when we used both cAMP quantification methods, we noticed that the overexpression of Gsα increased the cAMP levels by itself. Together our results indicate that hRIC-8B increases the intracellular levels of cAMP potentiating the signaling pathway in response to isoproterenol.

152. Glucagon-Like Peptide-1 changes mitochondrial dynamics and metabolism in vascular smooth muscle cells (VSMC) by a PKA/Drp-1 signaling pathway.

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Mitochondrial dynamics involve fusion and fission processes that control mitochondrial metabolism. Mitochondrial dynamics is tightly regulated by fusion (Mfn-1/2 and Opa-1) and fission (Drp-1, Fis-1) proteins. Mitochondrial fission can be regulated by PKA-dependent phosphorylation in Drp-1 Ser 637, blocking Drp-1 migration to mitochondria. Whether GLP-1, an incretin used in diabetes mellitus treatment, regulates mitochondrial dynamics by controlling Drp-1 activity in VSMC remains unexplored. Our results show that GLP-1 100 nM activated PKA and increased Drp-1 phosphorylation in Ser 637 in an embryonic smooth muscle cell line from rat aorta (A7r5). Moreover, GLP-1 induced mitochondrial fusion which was prevented by H-89 (a PKA specific inhibitor). GLP-1 augmented mitochondrial membrane potential by 27% (flow cytometry with JC-1) and oxygen consumption by 43% (Clark electrode). Both parameter relied on a GLP-1-dependent PKA activation because they were abolished by H-89 pretreatment. In summary, our data suggest that GLP-1 induced a PKA-dependent phosphorylation and inactivation of Drp-1, triggering mitochondrial fusion and enhancing mitochondrial metabolism.

FONDECYT 1110180 (MC), Anillo ACT1111 (MC, SL), CONICYT PhD fellowship (GT).

153. Insulin promotes cardiomyocyte mitochondrial fusion by increasing Opa-1 expression through the Akt-mTOR-NFκB pathway

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Insulin controls cardiac metabolism by regulating glucose uptake and mitochondrial function. We have recently shown that insulin acutely promotes mitochondrial fusion in cultured cardiomyocytes, improving mitochondrial function. The aim of this work was to investigate the mechanism by which insulin triggers mitochondrial fusion. Our results showed that treatment of cardiomyocytes with 10 nM insulin for 3 h increased Opa-1 fusion protein mass and mRNA levels. Mitochondrial elongation and the increase in Opa-1 levels were abrogated by preincubation with either actinomycin D or cycloheximide, suggesting that insulin acts by increasing Opa-1 synthesis rather than inhibiting its degradation. Inhibition of Akt or mTOR abolished insulin-induced mitochondrial fusion and Opa-1 levels up-regulation. *In silico* analysis revealed the presence of an evolutionarily conserved NFκB binding site in the Opa-1 promoter. Since the Akt-mTOR pathway regulates the transcription factor NFκB, we evaluated whether this factor is implicated in the effects of insulin on mitochondrial dynamics. Inhibition of NFκB by pharmacological and molecular tools abrogated Opa-1 increase and mitochondrial elongation. In conclusion, these data suggest that insulin-induced mitochondrial fusion in cardiomyocytes depends on Opa-1 expression by the Akt-mTOR-NFκB pathway. Supported by Anillo ACT-1111 and FONDECYT 1120212 (SL). VP and CLC hold a CONICYT fellowship

154. Angiotensin-(1-9) regulates cardiomyocyte mitochondrial calcium by AT2 receptor

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Angiotensin-(1-9) is a novel peptide of the non-canonical renin-angiotensin system with anti-cardiac hypertrophic action. In the present work, we investigate the role of Ca²⁺ signaling in the hypertrophy inhibited by Ang-(1-9). Ca²⁺ plays a major role in contraction, cell energy metabolism and gene expression in the heart. Our data showed that Ang-(1-9) itself does not have a direct effect on cytoplasmic or mitochondrial Ca²⁺ signals but decreases cytoplasmic Ca²⁺ response triggered by norepinephrine (NE). Ang-(1-9) increased mitochondrial Ca²⁺ transients evoked by NE or histamine by activating the AT2 receptor. We also investigate by immunocytochemistry whether the increases in mitochondrial Ca²⁺ were due to a closer interaction between this organelle and the endoplasmic reticulum (ER). Our results show that Ang-(1-9) prevents the loss of interaction between mitochondria and ER induced by NE. We concluded that Ang-(1-9) modulates NE-dependent cytoplasmic Ca²⁺ levels by improving the efficiency of Ca²⁺ entry into the mitochondria. Supported by Anillo ACT1111, FONDECYT 1120212, 1100874 and FONDEF D1111122.

155. Reducing membrane cholesterol induces glucose transport through GLUT4 and generates calcium transients in skeletal muscle: a possible treatment for insulin resistance?

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Cholesterol is enriched in the skeletal muscle transverse tubule (TT), a network of plasma membrane invaginations where translocation of GLUT4 glucose transporters takes place in response to insulin. We tested whether feeding mice a high fat diet (HFD), together with producing insulin resistance, increased TT cholesterol content and modified insulin-induced glucose uptake. Male C57BL/6J mice were fed a normal or a HFD (60% fat) for 8 weeks. HFD-fed mice displayed higher body weight and insulin resistance. Insulin-stimulated glucose uptake in fibers from HFD-fed mice was lower than in controls whereas cholesterol levels in triads from HFD-fed mice were 30% higher. Pre-incubation with Methyl- β -cyclodextrin (M β CD) to decrease membrane cholesterol reduced Akt phosphorylation and increased both basal and insulin-induced glucose uptake in muscle fibers from controls or HFD-fed mice. M β CD treatment restored normal glycemia and insulin sensitivity in HFD mice, even after Akt or CaMKII inhibition. Indinavir, a GLUT4 antagonist, inhibited the effects of M β CD. M β CD increased membrane GLUT4 content and elicited intracellular calcium signals inhibited by Dantrolene, which inhibits the interaction Cav1.1/RyR1. Dantrolene reduced M β CD-mediated glucose uptake. Our findings imply a novel role of both TT cholesterol content and intracellular calcium on GLUT4 translocation and glucose uptake, suggesting a possible therapy for insulin resistance. FONDECYT 3110105; ACT1111.

156. Deciphering Arabidopsis Calmodulin-binding Protein IQ67-Domain (IQD) roles

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Ca²⁺ is a fundamental second messenger in cells. Calmodulins and Calmodulins-like proteins are among the more widespread Ca²⁺ sensors. A novel class of putative plant-specific Calmodulin/Calmodulin-like-targets is the IQ67-Domain (IQD) family protein. IQD members share a unique domain of multiple in tandem Calmodulin retention motifs, are structurally diverse in molecular mass but have some properties reminiscent of RNA binding proteins. Surprisingly little is known about their biological roles. In a very drought tolerant species *Portulaca oleracea* we previously identified, by a transcriptomic approach, the induction of a transcript ortologous to *Arabidopsis thaliana* IQD32 after withholding water. To decipher the role of this protein, we started to study it and two other members of the family (IQD28 and -30) in *A. thaliana*. The transcripts encoding them displayed the greatest expression in roots, followed by seeds, flower, stems and leaves. The response to different abiotic stress conditions was tested; their expression was greatly induced (12-fold) in roots after 3 hs of mannitol or NaCl treatments and decreased after 72 hs. Similarly in leaves, their expression increased after 3 hs of abscisic acid and manitol and reduced thereafter. We have cloned and expressed IQD28, -30 and-32 recombinant proteins and are currently testing their ability to bind calmodulin as well as looking for their targets.

157. Study of the Ca²⁺ independent spontaneous postsynaptic currents at *Drosophila*'s neuromuscular junction

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Spontaneous neurotransmitter release (SNTr), first described in frog neuromuscular junction (NMJ; Fatt & Katz, 1952), includes an external Ca²⁺ independent component (Katz & Miledi, 1963) of large postsynaptic spontaneous events (Thesleff & Molgó, 1983) whose frequency is increased by botulinic toxin, dbcAMP, caffeine or cytoskeletal disorganization (Tabti, et al. 1986). cAMP induced SNTr in absence of Ca²⁺ has been described in *Drosophila* third instars NMJ using FM1-43 dye (Kuromi & Kidokoro, 2001), but electrophysiological properties of these SNTr remain obscure. Previous studies indicate synaptic vesicles at this NMJ organize in functionally different pools: the ready releasable pool (RRP) and the reserve pool (RP) (Kuromi & Kidokoro, 1989), where RPs present pharmacological properties similar to spontaneous Ca²⁺ independent NTr in rat NMJ. RP compromise the largest pool of synaptic vesicles in nerve terminals (Delgado et al, 2001), and are proposed to be released under strong synaptic demand. In order to clarify if RP synaptic vesicles originate Ca²⁺ independent SNTr we characterized the Ca²⁺ independent spontaneous postsynaptic currents (SPSCS) recorded from *Drosophila* NMJ under voltage clamp and show that cAMP or octopamine, absent of external Ca²⁺, induce higher frequency of SPSC and the occurrence of large charge content release episodes, revealing the secretion of different neurotransmitter laden vesicles whose associated proteins we expect to characterize.

158. Novel candidate regulators of lipid droplet metabolism revealed by high throughput and chemogenomic analyses

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Lipid droplets are dynamic intracellular organelles that store the neutral lipids. Manipulation of their content is important to attain high levels of triacylglycerol for biotechnological purposes. Acetyl-CoA carboxylase (ACCase) is a key enzyme in fatty acid synthesis and its activity is correlated with the amount of TAG synthesized. The signaling pathways that govern lipid droplets dynamics are not well understood. To discover novel ACCase regulators we screened a ~4860 knockout yeast library for differential content of lipid droplets and for tolerance to an ACCase specific inhibitor. By a chemiogenomic approach we were able to build an hybrid network by combining genetic/physical gene-gene interactions and drug-gene interactions which allowed us to map new genes that interact with ACCase. By overlapping these results to those obtained in the lipid droplet screen we identified 12 novel genes whose deletion led to a positive correlation between ACCase activity and intracellular lipid content. Among the novel regulators we found that the Ser-Thr phosphatase Sit4 modulates an important regulator of ACCase, the Snf1/AMPK kinase. We also describe the ubiquitin proteasome-dependent degradation pathway as novel regulator of acetyl CoA carboxylase activity. Our results provide a network that can now be manipulated to regulate TAG content in yeast.

159. Crosstalk between Toll-like receptor 4 and autophagy in L6 myotubes

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Toll-like receptor 4 (TLR4) is responsible for sensing lipopolysaccharide (LPS) from gram-negative bacteria and contributes to the innate immune response. Increased LPS levels have been detected in chronic inflammatory diseases such as obesity and diabetes. Stressors ranging from nutrient deprivation to immune signaling can induce degradation of cytoplasmic material by autophagy. In muscle cells, LPS induces autophagy by activating of TLR4. We hypothesized that there is a crosstalk between autophagy and TLR4 in skeletal muscle cells. We studied the effect of autophagy on TLR4 levels in L6 myotubes exposed to LPS or/and starvation. Autophagy was assessed by processing of LC3-I to LC3-II and p62 protein levels by Western blot (WB). TLR4 protein levels also were determined by WB. Our results show that LPS (5 ng/mL, a physiological concentrations) did not trigger autophagy during TLR4 activation in L6 myotubes. By contrast, these cells exposed to starvation plus LPS increased the LC3 processing and decreased p62 levels. Moreover, L6 myotubes exposed to starvation plus LPS for 6 h decreased TLR4 protein levels. These results indicate that autophagy induced by starvation plus LPS degraded the TLR4 suggesting that there is a crosstalk between TLR4 signaling and autophagy in L6 myotubes.

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160. Expression of structural proteins of a Mayaro Virus in insect cells: application in serological diagnosis and molecular studies.

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Mayaro Virus (MAYV), an arbovirus member of the family Togaviridae, genus Alphavirus, is widespread in tropical South America, being endemic in rural areas surrounded by forests. MAYV is associated with a dengue-like illness with rash, fever, and severe arthralgia, being difficult to distinguish MAYV infection among emergent arboviruses by symptoms. Recently, we obtained a complete nucleotide sequence of a MAYV from wild dispersed (D) genotype (MAYV-Ac) isolated from an illness patient from Acre, Brazil. The aminoacid primary sequence of the MAYV capsid (C) protein is highly homologous to the Alphavirus core protein, a chymotrypsin-like peptidase essential to virus budding. Comparative modeling studies on the C protein from MAYV of D and limited (L) genotypes showed slightly structural differences which can be related to specific virus genotypes host interactions. Thus, in order to obtain MAYV antigens for specific serological diagnosis, and to study the molecular characteristics of MAYV from D and L genotypes, we expressed the C and the Envelope 1 (E1) structural proteins from MAYV-Ac in Sf9 insect cells using recombinant baculoviruses. Until now, this is the first molecular study on MAYV by employing eukaryotic heterologous expression system. Acknowledgment: FAPESP (Grant 2009/11.347-6)

161. Metabolic remodeling in breast cancer: implications in the design of potential anti-cancer agents

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Cancer cells present a different participation of glycolysis and OXPHOS to determinate the intracellular ATP pool compared with no-malignant cells and can remodel the participation of both metabolic pathways according to cellular requirements. In cancer progression, several changes in mitochondrial bioenergetics are exhibited, such as accumulation of Krebs cycle intermediates (KCi) and hypofunction of electron transport chain (ETC). In this context, small molecules that act on bioenergetics can exhibit anti-cancer effects. We identified in a screening based in structure-activity relationship studies, a novel compound, alkyl hydroquinone (P1) that uncoupling OXPHOS. In mitochondria isolated from mammary adenocarcinoma TA3 cells, P1 increases oxygen consumption rate (OCR) in respiratory state-4o without inhibitory effect on ADP-sensible respiration. In human breast cancer MCF7 and metastatic MDA-MB-231 cells, P1 produces increase of OCR, a decrease of intracellular NADH and a decrease of ATP only at earliest exposition time (< 30 minutes) since after long exposition time (4 hours) ATP levels are normalized. Moreover, glucose availability is responsible to determinate the effect of P1 compound on cell proliferation. This phenomenon is also observed for classic inhibitors of Complex-I (rotenone) and ATP synthase (oligomycin). These results suggest that re-oxidation of KCi by uncoupling of OXPHOS is a potential target to obtain anti-cancer effects.

162. The differential expression of Non-Coding Mitochondrial RNAs can be used as a diagnostic tool for Cervical Cancer

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The WHO estimates that by 2020 cervical cancer will increase by 25%, being the main cause of this cancer a persistent infection by Human Papillomavirus. The classic diagnosis is a Papanicolaou test, however, the efficiency of this test is hampered by a high number of false negatives. Our research group has characterized a family of Noncoding Mitochondrial RNAs (ncmtRNAs) comprised of sense (SncmtRNA) and antisense molecules (ASncmtRNAs), which show a differential expression depending on the cell proliferative status. Normal proliferating cells express both transcripts, whereas in tumor cells only the sense transcript is detected. Our current research is based in determining the expression pattern of these ncmtRNAs correlating it to biomarkers, such as p16 and PCNA, in formalin-fixed paraffin-embedded tissues diagnosed as Normal, LSIL, HSIL and Invasive Cervical Carcinoma. Preliminary results show a difference in the expression of the ncmtRNAs between the different grades of the disease, where ASncmtRNA was found down-regulated in HSIL and Invasive Cervical Carcinoma with up to a 3-fold decrease in the integral optical density. Interestingly, in some patients the SncmtRNA transcript was detected in the cell nucleus of the tumour whereas in the squamous epithelium this transcript was found in the cytoplasm, suggesting an implication in the progression of the disease and in the survival of the patient. (FONDEF D10I1090; CCTE-PFB-16; CONICYT Scholarship-Masters Studies)

163. Cx43 hemichannel activity induced by cytokines and high glucose inhibit the glucose transport sensitive to insulin in human endothelial cells

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Metabolic actions of insulin to promote glucose disposal are mediated by its vascular actions in endothelium. Indeed, nitric oxide released from endothelium in response to insulin-stimulation increases blood-flow improving glucose uptake. Inflammation plays fundamental roles on insulin resistance, while it enhances hemichannel activity and consequently increases cellular susceptibility to insults. Therefore, we investigated whether TNF- α /IL-1 β or INF- γ in normal or high glucose affect hemichannel activity and/or glucose transport in insulin stimulated endothelial cells. EAhy-926 human endothelial-cells were treated for 72h with TNF- α and IL-1 β (10 ng/ml) or IFN- γ (2.5 ng/ml) in the presence of normal (5 mM) or high glucose (25 mM), followed by 45 min of incubation with 1 μ M insulin. Then, hemichannel activity and glucose transport were evaluated by ethidium and 3-O-methyl-[3H]-glucose uptake, respectively. Only treatment with cytokine in high glucose increased Etd uptake (~250%) in a connexin43 hemichannel-dependent manner. Moreover, these treatments reduced 3-O-methyl-[3H]-glucose uptake (~80%) and extracellular nitrite levels (~75%) induced by insulin, and currents. Therefore, connexin43 hemichannels may be novel therapeutic targets to reduce insulin resistance associated to endothelial dysfunction.

164. Establishment of primary cultures of advanced cervical cancer as a model for selective antisense therapy

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It is estimated that about 530,000 women are diagnosed with cervical cancer and about 50% die of this disease. The development of new and effective therapies with low side effects has been a challenge in the past years. Our laboratory described a family of noncoding mitochondrial RNAs (ncmtRNA) consisting of sense (SncmtRNA) and antisense (ASncmtRNA) transcripts, which have a differential expression depending on the proliferative state of the cell. Normal proliferating cells express sense and antisense molecules, whereas in tumor cells only sense is detected. We have found that oligonucleotide treatment targeting the antisense molecule in cervical cancer cell lines triggers 60-80% of cell death. On the other hand, the same treatment does not induce death or transforming events in normal cells, which indicates a safe treatment. Cell lines are widely used in cancer research, however these do not resemble the *in vivo* tumor physiology, therefore it is necessary to carry out studies in cell models derived from patients. In this work, we set up three models of advanced cervical cancer primary cultures, which express p16^{INK4a}, CK17 and are HPV16+. Treatment efficiency was evaluated demonstrating a cytostatic and an apoptotic effect (~30%) by MTT and Annexin V assays respectively. These results demonstrate the efficiency of the treatment on cervical carcinoma primary cultures, which are more representatives to an *in vivo* tumor. (FONDEF D10I1090 – CCTE-PFB16 CONICYT)

165. Exome-capture sequencing strategy for the identification of genetic risk factors of cholesterol gallstone disease in Chilean families with early onset disease.

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Cholesterol gallstones disease (GSD) is one of the most prevalent gastrointestinal disorders in the Chilean population, with a high genetic susceptibility. Here, we present an exome-wide analysis of Chilean families with early GSD onset in order to uncover new genetic variants of functional impact which could explain the etiology of GSD. Twenty-four DNA samples from 6 Chilean individuals: 6 young GSD index cases plus their parents and one second relatives (16 with and 8 without GSD) were analyzed by using TruSeq Exome Enrichment kit followed by Illumina HiSeq2000 sequencing platform. Alignment to the reference genome (GRCh37) and variant calling (SNP and InDels) were performed using the SAMtools software. Variant filtering and annotation yielded 34 exonic-nonsynonymous SNPs shared in at least 6 GSD individuals. These variants fell in 28 genes including 3 genes related with gallbladder pathophysiology: the Mucin 4 gene (MUC4) and Mucin 5 B gene (MUC5B), both components of the mucin gel of the gallbladder; and within the Cholecystokinin-2 receptor gene (CCKR2), involved in gallbladder motility. Our results suggest the existence of coding SNPs with potential functional impact on disease-related genes, providing new insights into the genetic susceptibility of this prevalent digestive disorder. Funding: FONDAF-CRG 15090007 and FONDECYT grant 1130303 (JFM) and 1100942 (GVD).

166. Identification of Copy Number Variants (CNV) from exome-capture sequencing in Chilean families with early-onset cholesterol gallstone disease

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Gallstones Disease (GSD) constitutes the third most common cause of medical discharges in Chile. Although high rates of heritability are observed and a defect in lipid metabolism is recognized, the genetic factors that contribute to its development are not completely understood. Here we have studied Copy Number Variants (CNV) -defined as polymorphic genomic portions (>1kb) that can behave as deletions or multiple gains- among Chilean families with early-onset GSD. A set of 24 individuals (16 cases, 8 controls) grouped in 6 families with the presence of 1 index case of early-onset GSD (before age of 20), were subjected to whole exome sequencing with the Illumina TrueSeq Exome enrichment kit. Raw reads per sample were aligned to the reference human genome GRCh37 and the output was introduced to XHMM software to detect CNV based on the read depth observed for each exonic region. We detected 910 CNV among the 24 samples analyzed, 124 of which were considered novel (13.6%). These CNVs affect 824 genes including several genes involved in lipid metabolism such as the ATP-binding cassette, sub-family B, member 11 and UDP glucuronosyltransferase 2 family, polypeptide B7. The present work will contribute to understand the genetic hallmarks of this prevalent gastroenterological disorder. Funding: FONDAF-CRG 15090007 and FONDECYT grants 1130303 (JFM) and 1100942 (GVD).

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167. Wnt/ β -catenin signaling regulates Nlgn3 expression in hippocampal neurons.

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The adhesion molecule Neuroligin-3 (Nlgn3) has an important role in the function and maturation of synapses and mutations in this gene are associated with autism. The Wnt/ β -catenin pathway is important for central nervous system development and is involved in different prevalent neurological disorders like Alzheimer's disease, schizophrenia and autism. We studied whether Wnt/ β -catenin pathway regulates Nlgn3 transcription on mouse and rat hippocampal neuron, in the absence/presence of activators Wnt3a or LiCl. Nlgn3 protein and mRNA levels were determined by Western Blot and qPCR, respectively. A ~2,0 Kb regions of Nlgn3 promoter from mouse and rat with putative TCF/LEF binding elements (TBE) were cloned and their activity in response to Wnt/ β -catenin signaling were measured through serial deletions (reporter assays) and ChIP assays. Wnt/ β -catenin stimulation of hippocampal neurons induced a dose and time-dependent increase in the expression of Nlgn3 transcripts and proteins. Through reporter assays mimicking active Wnt/ β -catenin signaling, we observed an enhancement in the promoter activity of Nlgn3 constructs. Finally, we confirmed the interaction of β -catenin/TCF complexes on TBE sites in the Nlgn3 promoter by ChIP. Our results link the transcription of the Nlgn3 gene to Wnt/ β -catenin signaling and serve to underscore the importance of this gene in autism. Funding: UNAB DI-194-12/I to MM; FONDECYT 1100942 and FONDAP-CRG 15090007 to GVD.

168. Interplay between cholinergic and glutamatergic transmission are essential to induce long-lasting synaptic enhancement of gabaergic synapses

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In the last years, growing experimental evidence shows that induction of synaptic plasticity in interneurons play an important role in the network oscillation patterns and brain plasticity, in which the cholinergic modulation of GABAergic synaptic efficacy may be crucial. According to role of acetylcholine (ACh) in the synaptic physiology and brain function, our previous works have showed that temporal and spatial interaction of endocannabinoids (eCBs) and cholinergic system in synaptic terminal of cholecystokinin positive (CCK+) hippocampal interneurons are necessary to induce activity-dependent GABAergic long-term depression in CA1 pyramidal neurons. Here, we show, using electrophysiological recordings hippocampal slices that muscarinic acetylcholine (mAChR) and metabotropic glutamate (mGluR) receptors synergistically induce long-term potentiation (LTP) in insensitive-eCBs GABA synapses. We observed that: i) the ACh generated a transient presynaptic inhibition of GABAergic transmission, ii) the ACh followed by high frequency stimulation of Schaeffer collaterals induce a strong LTP in GABA synapses iii) the LTP is blocked by mAChR or mGluR antagonist. These results suggest that cooperative signaling via mACh and mGluR could be providing a mechanism by which the long-term plasticity of inhibitory synapses can modulate the learning machinery of the brain.

169. Differentiation of humans adipose-derived mesenchymal stem cell (hASC) into insulin producing cells (IPC).

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Introduction: Adipose-derived mesenchymal stem cells (ASC) are a pluripotent lineage, which can be differentiate to several cell types of endo, ecto and mesoderm origin. Recently, ASC has been proposed as a source for the generation of functional insulin producing cells (IPC), to be used eventually in therapy in type I diabetes (T1DM). Our aim was to generate functional IPC from ASC, expressing pancreatic β cell specific markers and function. **Materials and Methods:** ASC were obtained from lipoaspirated fat tissue from human donors. ASC were differentiated to IPC in vitro using a two-stage protocol based on a combination of low and high-glucose DMEM, supplemented with activinA, Nicotinamide y GLP1. The process was monitored by studying the expression levels of protein such as pancreatic and duodenal homeobox 1 (PDX1), Neurogenin 3 (NGN3), Insulin (INS), along with insulin secretion in response to external glucose. **Results:** IPC exhibited nuclear localization of β cell proteins such as PDX1 and NGN3, that they localized in the cytoplasm of undifferentiated ASC. Moreover, IPC actively express INS, compared to ASC that don't express. Also, IPC responded with secretion of INS in response to high external glucose. **Discussion:** IPC generated by ASC exhibit multiple characteristics of pancreatic β cells. Future research with these cells aims to test their function in in vivo models of T1DM. Financial Support: Proyecto Contingencia 2010, VID, U. de Chile.

170. Multipotent mesenchymal stromal cells are renoprotective in both type 1 and type 2 diabetes mellitus mice

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The most detrimental complication of diabetes mellitus (DM) is diabetic nephropathy (DN), a clinical syndrome comprised of kidney damage and increased risk for cardiovascular disease. The pathological sequence in the progression of DN secondary to type 1 DM (T1DM) or type 2 DM (T2DM) follows the same general pattern. Nevertheless, patients with T2DM have additional factors that exacerbate renal damage, making DN secondary to T2DM even more complex than DN secondary to T1DM.

Multipotent mesenchymal stromal cells (MSCs) are one of the most promising tool due to their renoprotective potential.

The aim of our work was to evaluate whether the renoprotective effect of MSCs depends on diabetes etiology (T1DM vs T2DM).

We used C57BL/6 mice rendered diabetic by the administration of streptozotocin (DN derived from T1DM) or chronically exposed to high fat diet (DN derived from T2DM). Animals received intravenously the vehicle or a single dose of MSCs (0.5×10^6) at the time where microalbuminuria was present, but renal structures still unmodified, 3 months later, animals that received the vehicle presented macroalbuminuria and renal morphological alterations including mesangial expansion, podocyte loss and increased glomerulosclerosis index. In contrast, diabetic animals that received MSCs presented basal albuminuria and normal renal histology.

Our data shown that the irrespective of DM etiology, MSC administration have a renoprotective potential. FONDECYT 1120133

171. Inhibition of IP3R causes hyperpolarization and decreases mitochondrial fusion

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Mitochondrial uptake of 1,4,5- inositol trisphosphate receptor (InsP(3)R)-released Ca²⁺ is fundamentally required to provide optimal bioenergetics by providing sufficient reducing equivalents that support oxidative phosphorylation. Mitochondrial Ca²⁺ uptake occurs at regions of close apposition of mitochondria with endoplasmic reticulum (ER) Ca²⁺ release sites, driven by the inner membrane voltage generated by proton pumping of the respiratory chain. Ca²⁺ uptake is mediated by the so-called uniporter complex. Absence of this Ca²⁺ transfer disrupts oxidative phosphorylation, lowers cellular ATP, and activates AMP kinase-dependent pro-survival autophagy. Mitochondria are highly motile organelles that constantly undergo fission and fusion. Impairment of mitochondrial dynamics is associated with mitochondrial dysfunction. Dysfunctional mitochondria produce more reactive oxygen species (ROS), particularly the superoxide anion (O₂⁻) and hydrogen peroxide (H₂O₂). How inhibition of InsP(3)R Ca(2+)-release with xestospongine B affects mitochondrial dynamics and ROS production is unknown. Here we determine that inhibition of InsP3R with Xestospongine B (XeB) hyperpolarizes mitochondria, and reduces fusion leading to mitochondrial fragmentation.

172. Decreasing Ski levels is a key event in the oncogenic mechanism of Aurora A kinase.

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Aurora A (AURKA) is a kinase required for centrosome maturation and separation and entry into mitosis. This kinase is overexpressed in a variety of tumors and this overexpression leads to centrosome amplification, abnormal mitotic spindle formation and consequent aneuploidy. Previous studies from our laboratory described the transcriptional co-repressor Ski as a target for AURKA. Ski localizes in the nucleus and has been found associated to centrosomes. Interestingly, our studies show that reduction of this protein results in centrosome amplification, multipolar mitotic spindles and aneuploidy, resembling the effect of AURKA overexpression. These observations led us to test the hypothesis that phosphorylation of Ski by AURKA promotes the degradation of Ski and this, in turn, is part of the oncogenic mechanism of this kinase. Thus, we performed *in vitro* kinase assays followed by mass spectrometry to recognize the sites phosphorylated by AURKA in Ski. We found that Ser326 and Ser383 were *in vitro* target sites for AURKA and phosphorylation of Ski in these sites reduced protein half-life *in vivo*. Moreover, overexpression of Ski significantly decreased AURKA oncogenic effect as assessed by centrosomes amplification, mitotic index and cellular transformation. Our results indicate that the Ski protein is an important target for the oncogenic effects of AURKA.

173. Protective effects of trimetazidine from palmitate-induced cardiomyocyte lipotoxicity

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Trimetazidine (TMZ) is a β -oxidation (β ox) inhibitor used for heart failure and stable angina treatment, but its molecular mechanism remains controversial. Our previous data show that low concentrations of TMZ induce mitochondrial fusion and increase mitochondrial metabolism in cardiomyocytes, while palmitate has opposite effects. We hypothesized that TMZ protects mitochondrial function from palmitate lipotoxicity *in vitro*. Cardiomyocytes were treated with BSA-complexed palmitate (25 nM free, 3-6 h) with or without TMZ (1 μ M, 24h). Mitochondrial morphology (confocal microscopy), oxygen consumption (polarography), ATP (luminescence), lipid accumulation and ceramide production (fluorescence microscopy/mass spectrometry), were assessed. Palmitate decreased mitochondrial volume (49%, $p < 0.05$, $n=3$), oxygen consumption (19%, $p < 0.05$, $n=3$) and ATP levels (16%, $p < 0.05$, $n=3$), while the pretreatment with TMZ protected from these effects. Palmitate had no effects on lipid accumulation, but TMZ increased the number (3.4 fold, $p < 0.05$, $n=3$) and area (4.8 fold, $p < 0.05$, $n=3$) of lipid droplets. Also, palmitate induced sphinganine (3.1 fold, $p < 0.01$, $n=4$) and C16-ceramide (2.6 fold, $p < 0.05$, $n=4$) production, which was blunted by TMZ. We conclude TMZ protective effects against palmitate toxicity depend on cellular changes on lipid handling in lipotoxic conditions. ANILLO ACT 1111, FONDECYT 1120212 and 1090727. JK, VP CONICYT fellowships.

174. ApoA-I natural variant G26R triggers a pro-inflammatory response in murine macrophages.

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Human apolipoprotein A-I (apoA-I) is the major protein in High Density Lipoprotein particles (HDL) and plays a principal role in the Reverse Cholesterol Transport and endothelial function. Some apoA-I natural variants, like G26R, are involved in systemic amyloidosis, affecting different organs and with variable severity. We previously reported that apoA-I G26R is less stable than the Wild type protein (Wt) under physiological conditions, and that the inflammatory scenery can induce pro-amiloidogenic processing in both Wt and G26R. Here we set out to answer whether this variant can trigger an inflammatory response as part of the pathogenic pathways. We show that apoA-I G26R induces the release of Reactive Oxygen Species (ROS) and the pro-inflammatory cytokines TNF- α and IL-1 β from murine macrophages *in vitro*. Moreover, we obtain similar results in the Nitric oxide production, cyclooxygenase (COX)-2 and inducible nitric oxide synthase (iNOS). Interestingly, macrophage activation was not evident under the same experiment conditions when incubated with other apoA-I amyloidogenic variant (Lys 107-0). Altogether these results show that several mechanisms, involving protein stability and cellular activation are involved with different depth in apoA-I induced amyloidosis.

175. Increased activity and expression of ecto-5'-nucleotidase (CD73) in glioblastoma stem-like cells.

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Glioblastoma multiforme (GBM) is a highly malignant brain tumour. Studies have shown glioblastoma stem-like cells (GSCs) to be the only tumorigenic population and responsible for the extreme chemoresistance in this tumour. An essential obstacle for chemotherapy of GBM is the presence of multiple drug resistance (MDR) transporters, essentially Mrp1. The activity of ecto-5'-nucleotidase (CD73) is essential for the production of extracellular adenosine and there is a correlation between the overexpression of CD73 and MDR transporters. Our main objective was to determine the role of CD73 and adenosine receptors on the regulation of Mrp1 in GSCs. We identify a high expression of CD73 and adenosine A3 receptors in GSCs. Concurrently, CD73 mediated AMPase activity was found notably increased in GSCs. Moreover, using a specific inhibitor of CD73 activity (AOPCP) or an adenosine A3 receptor antagonist (MRS1220), we observed a significant decrease in the expression and activity of Mrp1. The viability of GSCs was decreased upon inhibition of the activity of CD73 together with the exposure to antitumor drugs substrates of Mrp1. It is urgent today, to evaluate new therapeutic alternatives for recurrence of GBM. In this context, a feasible way of chemo-sensitizing GSCs could be the inhibition of CD73 activity or the use of adenosine A3 antagonists to reverse the extreme refractoriness to anticancer drugs mediated by Mrp1, in this tumor. Supported by Fondecyt N°1121121.

176. Neuroprotective effects of the local delivery of XBP1s into the *Substantia Nigra* of adult mice.

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Introduction: Recent studies in Parkinson's Disease (PD) mouse models and patient-derived samples suggest an involvement of endoplasmic reticulum (ER) stress in the disease process. X-Box binding protein 1 (XBP1) is a major transcription factor controlling adaptation to ER stress. Here we tested the possible beneficial effects of artificially enforcing XBP1s expression in a toxin-induced model of PD. **Materials and Methods:** We performed unilateral stereotaxic injections into the *Substantia Nigra* of adult wild type mice with adeno associated viral vectors overexpressing XBP1s and evaluated dopaminergic neuron survival after the exposure to the neurotoxin 6-hydroxydopamine through immunohistological analysis. **Results:** Overexpression of an active form of XBP1 in the *Substantia Nigra* of mice led to a high expression of *xbp1s* mRNA in this brain region and triggered a UPR transcriptional response *in vivo*. XBP1s overexpression resulted in a reduced level of striatal denervation and increased survival of dopaminergic neurons after exposure to 6-hydroxydopamine. **Discussion:** Our results indicate that XBP1s expression has an important neuroprotective activity in dopaminergic neurons. **Acknowledgments:** FONDECYT #3120146 (G.M.), #1100176, FONDEF D1111007, FONDAP #15010006, ACT1109, Millennium Institute #P09-015- and Michael J. Fox Foundation for Parkinson Research.

177. Long-Term ω -3 Fatty Acids Supplementation Restore both GABAergic Synaptic Transmission and Memory impaired of Chronically Stressed Rats.

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Chronic stress induces CA3 hippocampal atrophy, impairs the spatial memory while the ω -3 supplementation improves the spatial memory. The aim of this study was to evaluate effect of ω -3 fatty acids supplementation on memory and synaptic plasticity in rats. Male *Sprague-Dawley* rats were randomly assigned to three experimental groups: 1) Control, 2) Vehicle, and 3) ω -3, rats supplemented with ω -3. Each experimental group was divided into two subgroups: one of which was not subjected to stress while the other was subjected to a restraint stress protocol. Afterward, memory, morphology of CA3 neurons, and synaptic transmission in the hippocampus were analyzed by Y-Maze, Golgi stain and path clamp recordings respectively. Both memory impairment and dendritic atrophy of the stressed rats was restored by ω -3 supplementation. In addition, we observed that restraint stress decreased the probability GABA releases in hippocampal pyramidal neurons, whereas ω -3 supplementation prevented these stress-dependent changes in the synaptic efficacy. Our results demonstrate that ω -3 supplementation prevented the memory impairment, the dendritic atrophy of CA3 neurons, and the imbalance of hippocampal excitation and inhibition produced by restraint stress.

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178. POLYCYSTIN-1, a new mechanosensor to regulate cardiac hypertrophy via L-type calcium channel

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Increases in L-type calcium channel (LTCC) activity are critical to load-induced cardiac hypertrophy. Yet, underlying mechanisms are unknown. Polycystin-1 (PC1) functions as a mechanosensor in different cell types and is present in cardiomyocytes. We hypothesized that PC1 is a mechanosensor in heart and regulates cardiac hypertrophy. Mechanical stress was induced both *in vivo*, using transverse aortic constriction (TAC), and *in vitro* using hypo-osmotic solution (HS). Whereas control mice manifested robust TAC-induced increases in cardiac mass (HW/BW increased 80%), PC1 KO mice manifested no significant growth (HW/BW increased 27%) respect the WT. At baseline, PC1 KO mice manifested decreased cardiac function (%FS 65±4 WT vs. 40±3 KO). HS stimulation induced hypertrophy in cultured neonatal rat ventricular myocytes (NRVM) in a manner dependent on LTCC activity. Knockdown of PC1 blocked this hypertrophy. Conversely, over-expression of a C-terminal fragment of PC1 was sufficient to trigger NRVM hypertrophy. HS stimulation of NRVMs increases in α 1C protein levels, a response which was prevented by PC1 knockdown. MG132, a proteasomal inhibitor, rescued HS-induced increases in α 1C protein levels despite PC1 knockdown. Polycystin-1 is a novel cardiomyocyte mechanosensor which functions to stabilize α 1C protein.

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179. Characterization of glioblastoma stem-like cells in relation to the multiple drug resistance phenotype.

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Glioblastoma multiforme (GBM) carries the worst prognosis of tumors of the central nervous system. One of the main reasons for the failure of chemotherapy treatment is the phenomenon of multiple drug resistance (MDR), which is conferred by an overexpression of transporters of the ABC family, that eject anticancer drugs back into the extracellular space. We have demonstrated that Mrp1 is the transporter mostly expressed in biopsies of human GBM. In this type of aggressive tumors, a subpopulation of cells, termed glioblastoma *stem cells* (GSCs), has also been described be responsible for the growth and chemoresistance of the tumors. The main aim of this study was to characterize GSCs and to determine their relation with the MDR phenotype in GBM. We determined that GSCs express *stem cell* markers, CD133 and CD44, elevated levels of Mrp1 and Mrp3 transporters and cell proliferation markers. In addition, we also saw a marked decrease in cell viability and proliferation with inhibitors of the Mrp1 transporter. Likewise, our results obtained by flow cytometry show that chemosensitization would be being mediated mainly by the GSCs/CD44+ subpopulation, as these cells had a greater content of the Mrp1 transcript (75%). An efficient means of inhibiting chemoresistance in this tumor is by inhibiting the expression or activity of MDR transporters, specifically in the GSCs/CD44+ subpopulation, where the Mrp1 transporter is found to be overexpressed. Financed by Fondecyt 1121121.

180. The Inhibition of NF-kappaB activation by Sodium tungstate modulates the inflammation in diabetic kidney rats.

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Diabetes mellitus induces significant complications in human patients, among these diabetic nephropathy (DN). Sodium tungstate (NaW) has been described as a potent normoglicemiant by exerting an insulin mimetic action. In the present study, we investigate the effect of NaW and its mechanism action on renal inflammation in streptozotocin-induced diabetic rats model of type I diabetes mellitus. After 4 months of diabetes, kidney damage was characterized by biochemical and anatomic pathology in treated control and diabetic groups. Protein analyses were performed by Western blot and immunostaining. The subunit p65 of NFkB was significantly higher in diabetic animals compared with control animals. The increase of p65 subunit in nuclear extract and p-IkB in cytosolic extract were greatly attenuated by NaW in the diabetic kidneys. This data correlates with the decrease of inflammation and inhibition of the kidney damage detected by decrease of the accumulation of pro-inflammatory proteins and macrophages. These results indicate that this drug could be effective treatment to inhibit renal inflammation in diabetic nephropathy. In conclusion NaW is able to inhibit the activation of NFkB and expression of pro-inflammatory proteins characteristic of this disease suggesting that this drug could be effective treatment for human patient with advanced stages of diabetic nephropathy. (CONICYT 24110230; FONDECYT 1131033).

181. Optimal IFN- γ secretion in human NK cell is dependent on PGC1 α mitochondrial biogenesis induced by IL-2

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Natural killer cells are a subpopulation of lymphocytes which play an important role in the immune response. They are fundamental in the clearance of viral infections and malignant cells through their two main activities: cytotoxic and secretory activity. IL-2 stimulates both NK cell functions the secretory and cytotoxic activity. Previously, we have observed an increase in mitochondrial mass upon NK cell activation by IL-2 suggesting that this organelle might be important for NK cell function. PGC1- α is an important cofactor for mitochondrial biogenesis and its overexpression is associated with an increase in mitochondrial mass. We postulate that the increase in NK cell mitochondrial mass by IL-2 stimulation is mediated by PGC1 α and this should be necessary for INF γ secretion. To address this hypothesis, purified human peripheral NK cells were treated with PGC1 α siRNA or control siRNA in the presence of either 50UI/mL (control) or 2000 UI/mL rhIL-2. Our results demonstrated that incubation with 2000 UI/mL rhIL-2, increased NK cytotoxicity, INF γ secretion, mitochondrial mass and PGC1- α expression. However, when we used PGC1- α siRNA, NK cells did not increase mitochondrial mass in response to IL-2 (2000 UI/ml). Moreover, under this condition NK cells showed a decrease in IFN- γ secretion. Accordingly, we can conclude that IL-2-induced NK cell mitochondrial biogenesis is mediated by PGC1 α and this is relevant to sustain an optimal IFN- γ secretion.

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182. Docking and MM-GB/SA calculations in predicting the binding modes and binding free energies of biphenyl amide derivatives as P38 kinase inhibitors

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In this work the binding modes and binding free energies, for a series of congeneric compounds, have been determined by means of docking and MM-GB/SA calculations, respectively. The target protein was the kinase p38 that is implicated in disease processes associated with inflammation and progressive cartilage and bone destruction in rheumatoid arthritis. Two MM-GB/SA protocols were compared and their performance is discussed in terms of their capability to rank and predict the experimental affinity reported for compound series studied.

Docking validation study was able to reproduce the binding mode of crystallographic structures in the reference complexes. In general, in their complexes with p38 α all the compounds established a hydrogen bond (HB) with the hinge region within the active site, between the amide carbonyl of the inhibitors and the backbone amide NH of residue Met109. Additionally, the oxadiazole ring docked in the ATP-site, forming close contacts with Leu75 and Phe169. It formed two HB with the backbone NH atoms of Asp168 and/or Phe169. Our MM-GB/SA protocols, for rescoring docking poses, proved to be effective in ranking the congeneric set studied and they potentially would be used to predict biological activity of novel p38 inhibitors. ACKNOWLEDGEMENTS: We are indebted to FONDECYT and Government of Chile, which supported this research through projects No 11100177 and No 1100542.

183. Molecular simulations suggest an allosteric contribution to the DNA-binding cooperativity of Oct4 and Sox2

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Oct4 is a key regulator of stem cell pluripotency. It has two independent DNA-binding domains known as POU specific (POU_S) and POU homeodomain (POU_{HD}) which are connected by a short linker. In pluripotent cells, Oct4 binds cooperatively with Sox2 to a composite DNA motif formed by the juxtaposition of their individual binding sites. On this DNA motif, Sox2 only interacts with the POU_S domain of Oct4. The enhancer of the *utf1* gene bears a variant of the composite motif which is recognized specifically by Oct4 but not by its homologs such as the ubiquitously expressed transcription factor Oct1. Remarkably, the ability of Oct4 to bind this regulatory sequence strongly correlates with its capacity to maintain pluripotency. Here, we performed molecular simulations of Oct4 or Oct1 bound to the enhancers of the *utf1* and *hoxb1* (consensus composite motif) genes in the presence and absence of Sox2. We found that Sox2 strongly modifies the dynamics and DNA-specificity of both domains of Oct4. This effect is almost absent in the case of Oct1. In addition, the free energy profiles of unbinding of the individual domains of Oct4 from the *utf1* enhancer show that the affinity of both domains of Oct4 is increased in the presence of Sox2 even though Sox2 only interacts with the POU_S domain. Therefore, we propose that Sox2 affects the DNA-binding properties of the POU_{HD} domain through an allosteric mechanism.

184. A new model of TPK4 potassium channel.

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Tandem-pore K⁺ channel family encompasses five of the fifteen genes that are able to code for K⁺ channels in *A. thaliana*. Four members of this family (TPK1, 2, 3, and 5) reside in the vacuolar membrane, while TPK4 is localized in the plasma membrane of Arabidopsis pollen.

Typical potassium channels consist of four subunits each contributing two transmembrane domains and one pore region. However, TPK channels form dimers consisting of two identical subunits; each one contains a duplicated Kir-like structure with four transmembrane domains and two pore loops. The duplicated structure is also presented in mammalian K_{2P} channels. In a recent work, Marcel et al. constructed a model of TPK4 based on the structure of KcsA channel. They identified essential residues for TPK4 channel rectification using this model. Very recently, in 2012, the crystallographic structures of K_{2P} channels TWIK-1 and TRAAK were determined. These structures should help to investigate TPK channel structures and to identify the residues involved in functional characteristics. Sequence alignment indicates that TWIK-1 is a better template than KcsA to create a homology model of TPK4. However, our results suggest that TWIK-1 is not enough to create a reliable model, and a better option is a multiple alignment. Considering the new TPK4 model, we explain some characteristics of TPK4 that influence its interaction with lipids and propose some ideas about the pH sensing.

185. Two chromophorylated linkers from the phycobilisome of *Gracilaria chilensis*

Lobos, F¹, Becerra, P¹, Bruna, C¹, Bunster, M¹, ¹Laboratorio de Biofísica Molecular, Facultad de Ciencias Biológicas, Universidad De Concepción. Phycobilisomes (PBS) are accessory light-harvesting complexes found in cyanobacteria and red algae that capture and transfer light energy to the photosystem II. This process occurs efficiently due to the features that both chromophores and proteins from the complex present. PBS consist mainly of phycobiliproteins (PBPs), but besides them, PBS have several linker proteins distributed throughout the complex. These proteins are mostly in charge of the PBP assembly and stabilization. However, some linkers are chromophorylated, just like PBPs. Two examples are the 33-kDa γ subunit of R-phycoerythrin (γ^{33}) and the core-membrane linker (L_{CM}). The former is associated to hexamers of the PBP R-phycoerythrin, while the latter is a terminal energy acceptor acting as an anchor between the PBS and the thylakoid membrane. Unlike PBPs, structural and functional information about these kind of linkers is scarce. Due to this, it is impossible to determine their roles in the energy transfer process. Therefore, the present work seeks to elucidate some sequence and structural aspects of γ^{33} and L_{CM} . Both genes from *Gracilaria chilensis* were sequenced, phylogenetic analyses, domain assignment and chromophorylation sites prediction were performed. Furthermore, these sequences were used to propose preliminar structural models of γ^{33} and the globular domain of L_{CM} . Funding: FONDECYT 113.0256 and DIUC 211.037.012-1.

186. Homology Modeling of Calcium Channel $Ca_v1.2$ in Open Conformation: Structural characterization of the selectivity filter from atomic-level perspective.

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Voltage-gated calcium channels (Ca_v) play key roles in cell physiology. Calcium entry through Ca_v initiates and controls a large variety of cellular functions. Ca_v can be divided into two subgroups based on their activation threshold: low voltage activated (LVA) and high voltage activated (HVA); the latter denominated as L-type calcium channel. In the absence of crystallographic structures, a structural model of voltage-gated calcium channels $Ca_v1.2$ in open conformation has been built, using homology modeling techniques. The model of the pore area of the $Ca_v1.2$ (UniProtKB P15381) was based in a related crystal structure of the voltage-gated sodium channel of the marine bacterium *Magnetococcus* sp. (PDBID 4F4L). To build the channel transmembrane segments, the crystal structure of voltage-gated sodium channel of the *Arcobacter butzleri* (PDBID 3RVY) was used. Using the new model, we can elucidate structural features of the selectivity filter of the channel. Also, by Molecular Dynamics Simulations and Free Energy Methods, we can do predictions that will allow to determine calcium binding sites in the pore of the channel. Results confirm at atomic-level the relevance of the ring of glutamates in the formation of a calcium binding site in the filter of L-type Ca^{2+} channel. Acknowledgement: M-M.V. thanks to CONICYT doctoral fellowship. M-B.V. and G-N.F. thanks to FONDECYT 1131003. All authors thanks to CINV, Millenium Initiative 09-022-F.

187. Use of BLAST as a tool for mapping *Xenopus laevis* scaffolds onto *Xenopus tropicalis* chromosomes: Is it the right tool? How well it performs?

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Xenopus laevis and *Xenopus tropicalis* are two related clawed frog species. Both are important models for cell development biology and genetics, respectively. Their common ancestor is estimated to have lived around 65.5 Million years ago. Although the assembled scaffold sets for each frog species cover almost 90% of their genomes, the number of gaps is almost intractable. Given the high number of scaffolds in *X. laevis* genome, over 400 000, BLAST would be a convenient tool for binning/mapping them to *X. tropicalis* chromosomes due to its high speed and low computational costs. These features make worth assessing its performance. We have mapped the largest *X. laevis* scaffolds on *X. tropicalis* chromosomes using two different techniques: 1. BLAST, which is local alignment tool and 2. Cgaln, which is Coarse grained (global) alignment tool and our reference. In this work, we have evaluated BLAST in terms of classifier performance respect to chromosome binning, scaffold mapping and respect of computing costs. The low identity between the species might render BLAST unsuitable, and although faster than Cgaln, BLAST derived scaffold mapping is less accurate than the one derived from Cgaln. However, in terms of binning, BLAST does a very good job with a generally low prediction error rate. Therefore, depending on the biological question, BLAST might be the best choice of tool even with a very low identity between sequences.

188. Addition of non-native electrostatic interactions in coarse-grained and all-atom structure-based models for protein folding and binding.

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The energy landscape theory of protein folding states that the native ensemble of structures of a protein corresponds to the global minima of a funnel shaped landscape where trapping by non-native interactions is infrequent. Thus, in current structure-based potentials all interactions stabilize the native state, while non-native interactions are neglected. However, experimental evidence shows non-native electrostatic interactions play a key role in protein stability and unspecific interaction with charged ligands, such as DNA. To model the effect of electrostatic interactions in protein folding and binding, we included charged interactions for the residues ASP, GLU, ARG, and LYS in structure-based models of different levels of coarse-graining. The electrostatic potential was represented either by Coulomb or Debye-Hückel models, while maintaining the native bias of the structure-based energy function unperturbed. Using NTL9 as a model, we observed good correlation between experimental and simulated changes in protein stability for 12 charge-neutralizing mutations using all-atom models, showing that E38Q is the most destabilizing mutant and K19M the most stabilizing one. For protein folding and unspecific binding, coarse-grained simulations showed an acceleration of the folding rate of Arc repressor when DNA is present, consistent with experimental evidence. FONDECYT 1130510.

189. Characterization of NAD(P) binding sites using distance dependent knowledge-based potentials

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The nicotinamide adenine dinucleotides, NAD and NADP, are cofactors that mediate the production and consumption of reducing power in a huge variety of reactions of the metabolic network in all organisms. Despite the structural similarity of these cofactors, the NAD(P) binding proteins display an impressive capacity of molecular recognition, since they are capable to discriminate for the presence of the 2'-phosphate group. In this work, we aimed for a better understanding of the recognition determinants in NAD(P) binding protein through the derivation of distance dependent knowledge-based potentials generated from the whole repertory of known NAD(P) complexes. A protein database was build by selecting the better resolution structures of each protein family that binds NAD(P), showing a complete ligand in extended conformations. We present cases in which these potentials can be used to predict the preference of dual enzymes and mutants with reversed cofactor specificity, represent the difference in Gibbs free energy between wild type and mutant enzymes and identify enzymes in which these cofactors are tightly bound. FONDECYT 1121170.

190. Comparisons between *Xenopus tropicalis* and *Xenopus laevis* genome and protein sequences: large repetitions, inversions and evolutionary implications.

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Xenopus tropicalis and *Xenopus laevis* are two related African clawed frogs, used as models for cell development studies. Their common ancestor is estimated to have lived around 65.5 My ago. In spite of their phenotypic similarity, their evolutionary divergence is similar to the divergence between human and mouse, around 75 My. *X. tropicalis* genome is 1.7 billion base pairs long and has 20 diploid chromosomes. On the other hand, *X. laevis* has 3.1 billion base pairs long in its allotetraploid genome of 36 diploid chromosomes. Both species code for more than 30 000 proteins. In order to better understand the evolutionary relationships between the two species, a comparison at the nucleotide and protein sequence levels was carried out. In this work we have mapped the scaffolds of *X. laevis* on the chromosomes of *X. tropicalis*, estimating the overall identity between the species genomes through sampling couples of corresponding blocks of 5,000 nucleotides. We have also assessed the overall identity of protein sequences. Finally, we have estimated the amount of large (over 5Kbp) repetitions and inversions in those genomes. We contrast the high similarity between these two clawed frog species in terms of protein sequences compared to the overall much lower similarity between their DNA sequences.

191. Adsorption of multi-protein complexes characterized by atomic force microscopy imaging and nanomechanical simulations

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Adsorption is a physicochemical process where biomolecule conformational changes occur after interacting with surfaces. Adsorption is key for many biological and technological processes including multi-protein membrane interaction and biosensors development. However mechanisms of adsorption studied at the single molecule level are still in its infancy. Purified P2X receptors in the presence or absence of anti-subunit IgG antibodies were incubated in mica and imaged via atomic force microscopy (AFM). The adsorption degree for the IgG antibody was significant after 30 sec of incubation and reached a maximum at 10 min incubation. IgG antibodies bound to P2X receptors induced a decrease of the receptor adsorption, which was dependent on the number of IgG molecules. Using nanomechanical simulations of the individual IgG antibodies, P2X receptors and receptor-antibody complexes, conformational changes associated to the adsorption process at real time have been recorded. Also it has demonstrated that biomolecules with larger accessible areas facing the surface correspond to the most likely orientation for adsorption. Taken together these results represent the first experimental single molecule approach to characterize the adsorption of multi-protein complexes. Funded by Millennium Science Initiative P10-035F, Fondecyt 1120169 and Anillo ACT 1108 grants.

192. Structure-based prediction of transcription factor binding sites using a numerical optimization technique

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Protein-DNA binding is of utmost importance since it is involved in cellular processes such as gene expression and cell division. Since the first DNA-protein structure was solved at atomic resolution, our knowledge about how the recognition is carried out has increased notably. Several studies have been reported, where sequence and structural information has been used to predict protein-DNA binding specificities. Despite sequence-based methods are widely used, they are still not very accurate, exhibiting poor sensitivity/specificity trade offs.

We have developed a structure-based method for predicting binding sites in the DNA. This method relies on software for the 3D modelling of protein-DNA complexes and on statistical potentials, which estimate the protein-DNA stability from the 3D structure. We have included this approach in a protocol for predicting binding sites of transcription factors at the DNA level. The core of the protocol is a Metropolis-Montecarlo simulation, a random sampling method for modelling systems with many coupled degrees of freedom, that allows us to reduce the sampling space as well as to obtain DNA sequences that bind with a medium-high or high affinity.

Here we report this protocol and show that we can obtain ensembles of DNA sequences with high fitness for the protein-DNA complex under study and predict transcription factors binding sites at DNA level with this information.

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193. Rational Design of Dendrimer-Based Nanoparticles as Transfection Reagents: from atomic-scale perspective to transfection efficiency.

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The development of safe and effective nucleic acid - delivery systems is one of the most important challenges in the implementation of therapies. Many vectors have been designed for delivering genes and protect them from nucleases. Non-viral based transfection reagents, including lipids, polymers, and nanoparticle-based compounds, have been developed to overcome the limitations of viral vectors and improve the transfection efficiency. Dendrimers have emerged as potential gene carriers due to their cationic charges and customizable structure. In this work, we introduce an efficient workflow for rational design of dendrimers as transfection reagents, which involves interaction between atomic-scale studies, chemical synthesis, and biological characterization. Thus, we have designed dendrimer-based nanoparticles functionalized with biocompatible groups and molecular dynamics studies were performed to characterize dendrimer-oligonucleotide complexes. Then, in order to achieve an experimental validation of our new functionalized dendrimers as effective transfection reagents, cytotoxicity and transfection efficiency were evaluated through flow cytometry and fluorescence microscopy. Studies of cellular uptake of dendrimers conjugated with Quantum Dots using TIRF Microscopy were also described. Results support that our new designed dendrimers are efficient gene-vectors. V.M.M thanks CONICYT doctoral fellowship, Fraunhofer Chile Research and Millenium Initiative (P09-022-F).

194. Identification and *in silico* analysis of membrane proteins of different isolates of *Piscirickettsia salmonis*: Insights in to survival strategies and pathogenicity.

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Piscirickettsia salmonis is the etiologic agent of Salmon Rickettsial Septicaemia (SRS) or Piscirickettsiosis, disease affecting salmon production in different latitudes with particular impact in southern Chile, where is a persistent pathogen responsible for major economic losses. Despite the importance of this pathogen is scarce the information about their physiology and other relevant aspects of their life cycle and pathogenicity. Using various public databases and bioinformatics tools we have identified, classified and analyzed the predicted membrane proteins in the genomic sequences of different isolates of *P. salmonis*. In all isolates under study we have identified a large number of transporter proteins, as amino acid transporters, heavy metal transporters, ion transporters and others. We have also analyzed and compared the sequences of these proteins, identifying the differences between isolates under study. This study provides a comprehensive bioinformatic analysis of membrane proteins present in the genomes of *P. salmonis*, which could play a key role in the processes of survival and pathogenicity of this bacterium.

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195. Characterization of binding affinity, transport and control release of Angiotensin 1-7 using dendrimer technology.

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Angiotensin 1-7 (Ang1-7) is a bioactive heptapeptide of interest for the treatment of circulatory system, skeletal muscle and nervous system diseases. Ang1-7 is involved in processes that regulate blood pressure and neurotransmitter reuse. In order to use Ang1-7 as a therapeutic agent is required avoid its degradation and increasing its bioavailability at the zone of action. In this work we propose the use of dendrimers as carrier of Ang1-7. Dendrimers are branched macromolecules at nanoscale with demonstrated capability of protection and transport of diverse molecules such as drugs, DNA, RNA and peptides. Here, we studied the interaction between Ang1-7 and the PAMAM (poly amido amine) dendrimer. The characterization of the binding affinity, protection and transport are obtained using affinity assays, degradation kinetics and cellular assays, all this experimental information are analyzed at molecular level using molecular simulations. This preliminary study will allow us to understand the structural properties that modulate the control release of Ang1-7 through the use of dendrimers functionalized. Acknowledgement: F, G-N thanks to FONDECYT 1131003, Anillo Científico ACT1107, FCR Innova- Chile CORFO (FCR-CSB 09CEII-6991). C, C-V thanks to Association-Francaise Contre Les Myopathies AFM #16670; FONDECYT #1120380 and 3130593.

196. The genetic canonical code enhances the stability of the primary structure of proteins subject to punctual random mutations.

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The canonical genetic code reduces the effect due to either punctual mutation or translation error. This feature allows a little changes of the primary structure of protein through the evolution. Using computational simulation, we investigated the impact of different genetic codes on the primary structure under one neutral evolution process.

We observed that the canonical genetic code enhances the stability of the primary structure of proteins respect to random genetic codes. And, besides, that this effect does not depend of both the length and the amino acid profile of the proteins. We believe that this effect depends on the distribution of amino acids in the groups of synonymous codons in the genetic code used. This methodology will allow exploring the characteristics of the genetic codes underlying this property, and test other genetic codes that could be more robust than the canonical.

**197. Expression and characterization of a GH 62
alpha-L-arabinofuranosidase from *Aspergillus
fumigatus* WMO**

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Jaime Eyzaguirre)

Aspergillus fumigatus, like many other fungal saprophytes, has a xylanolytic enzyme system, which has not been well studied. In an attempt to better understand this system we have identified genes of potential glycosyl hydrolases in *A. fumigatus* WMO. One such gene shows 99% identity with the gene *afu2g12770* from *A. fumigatus* Af293, annotated as coding for an arabinofuranosidase belonging to family GH62, which has not been studied biochemically. The gene has 999 bp, without introns and codes for a protein of 332 residues including a 16 residue signal peptide. The gene was cloned and expressed in *Pichia pastoris* and the recombinant enzyme was purified by cation exchange chromatography. The enzyme was assayed against a number of p-nitrophenyl glycosides; activity was found only with p-nitrophenyl alpha-L-arabinofuranoside and p-nitrophenyl beta-D galactofuranoside (4% compared to the other). The molecular weight of the enzyme is estimated at 35 kDa by SDS-PAGE, while the optimum pH range is between 4.0 to 5.0 and the optimal temperature is 37 °C at pH 4. Grant support: FONDECYT 1100084 and 1130180 and UNAB DI-61-12/R.

**198. Properties of the electron transport chain of
*Burkholderia tropica***

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In the actuality, more than 60 *Burkholderia* species have been recognized, and have recovered from soil, water, plant rhizosphere, roots, shoots, human, animal and other sources. *Burkholderia tropica* is a facultative aerobic Gram negative rod originally isolated from the rhizosphere, rhizoplane, stems and roots of corn plants and sugarcane. This bacterium have ability to fix nitrogen, solubilize phosphate, produce siderophores, exoheteropolisacáridos and have great potential for agriculture.

Here we report our preliminary results concerning to some bioenergetics properties of *B. tropica* Ppe8^T cultured under O₂-limited conditions. The aim of the work is to get insight into the composition of the respiratory system *B. tropica*.

We found that purified cell membranes were able to oxidize NADH, succinate, glucose and ascorbate plus TMPD; however, oxidation rate by succinate and glucose was more rapid than that evoke by NADH. Differences in the kinetics of the respiration with succinate, glucose, and ascorbate-TMPD in presence KCN, suggested the presence of a KCN-sensitive terminal oxidase. Cytochromes b and c were spectroscopically detected after reduction with physiological or artificial electron donors.

This work was supported by grant from Universidad Libre Barranquilla, Colombia and Universidad Nacional Autónoma de México (UNAM)

199. Beyond glycogen synthesis: functional characterization of glycogen synthase in Sertoli cells

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Several metabolic enzymes have been classified as RNA binding proteins and as mRNA expression regulators. Sertoli cells maintain nutritional needs of germ cells by providing lactate from glucose. After glucose incorporation, only 1-3% is converted to glycogen suggesting low levels of glycogen synthase. Additionally, expression levels of muscular glycogen synthase (MGS), isoform present in these cells, do not correlate with the low levels of glycogen. The aim of this work was to functionally characterize and explore a new feature of MGS in primary culture of Sertoli cells and the cell line 42GPA9. MGS was highly phosphorylated (Ser640) and the activity rates were very low, indicating that the enzyme is practically inactive. These results were corroborated in infected cells that overexpress Protein Targeting to Glycogen (PTG) to stimulate glycogen synthesis, showing that MGS is present but inactive. By analysing the subcellular localization and possible protein interactions, we found that the enzyme co-localized and co-immunoprecipitated with AGO2. In addition, we studied MGS interaction with mRNAs by RNA immunoprecipitation. By a microarray of the immunoprecipitated extracts, we identified several mRNAs encoding intracellular signalling cascades, transcriptional and translational activators. These results suggest that MGS could play an unknown role in the regulation of mRNA expression in Sertoli cells.

200. Dynamic Localization of Glucokinase and its Regulatory Protein in Hypothalamic Tanycytes

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Glucokinase (GK), the hexokinase involved in glucose sensing in pancreatic b cells, is also expressed in hypothalamic tanycytes, the glia that cover the ventricular walls of the basal hypothalamus and are in contact with neuroendocrine arcuate nucleus neurons. GK is regulated at the post-translational level by the GK regulatory protein, GKRP, in liver. Here, we cloned both GK and GKRP expressed in primary culture of tanycytes. GK, expressed in *E. coli*, was functional, displaying a cooperative kinetic with a $S_{0.5}$ of 10 mM. GKRP, expressed in *S. cerevisiae*, inhibited GK activity *in vitro* with a K_i 0.2 μ M. We also analyzed GK localization *in vitro* and *in vivo* in response to glucose using confocal microscopy and Western blot analysis. *In vivo* GK localization in hypothalamic and liver tissues of the same rats under several glycemic conditions was also compared. Whereas increased GK nuclear localization was observed in the hyperglycemic condition, it was primarily localized in the cytoplasm in hepatic tissue. Interestingly, both GK and GKRP translocate from the cytoplasm to the nucleus in response to high glucose concentration in tanycytes. These data were confirmed using Western blot analysis of nuclear and cytosolic extracts. Results indicate that GK undergoes short-term regulation, determined by intranuclear compartmentalization. Thus, in tanycytes, GK can act as a molecular switch to arrest cellular responses to increased glucose. GRANT FONDECYT 1100705

201. FoxO transcription factors in a diabetic cardiomyopathy model

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Type 2 diabetes mellitus (T2DM) is a worldwide healthcare problem. Cardiovascular disease is the primary cause of death in patients with T2DM and obesity. In this context, diabetic cardiomyopathy is an important pathological condition responsible of cardiac injury. FoxO family of transcription factors regulates metabolism, oxidative stress response and cell death. FoxO1 and FoxO3a are downstream targets of insulin, which negatively regulates FoxO proteins through PI3K/Akt signaling pathway. Insulin resistance and T2DM lead to unregulated activation of FoxO, which result in severe metabolic disturbances in the heart (Battiprolu et al. J Clin Invest 2012). To study novel downstream target of FoxO1 in cardiac tissue under metabolic stress, we set up the experimental model of T2DM induced by a high fat diet (HFD). HFD-fed mice manifested increases in body weight, fasting serum glucose and serum insulin. In addition, cardiac hypertrophic growth with augmented cross-sectional area of left ventricle cardiomyocytes and increased abundance in hypertrophic markers including contractile protein β -myosin heavy chain, recapitulating the main features of diabetic cardiomyopathy. In this model, we observed activation of FoxO1/3a and increased mRNA expression of downstream target genes. With these results we can focus now to the search of downstream targets regulated by FoxO. CONICYT PhD fellowship (CV, MN), Anillo ACT 1111 (SL), FONDECYT 1120212 (SL), 11090301 (AE).

202. Activity of AMP-activated kinase (AMPK) and glycogenolytic enzymes in the final pH of *Longissimus thoracis* muscle in steer carcasses.

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The presence and activity of AMPK before slaughter is necessary to achieve an efficient postmortem glycolysis in mouse muscle and to decrease the final pH value. The meat industry requires obtaining a low final pH value (<5,8), as it improves its shelf-life, sensory quality, price and trade options. On the contrary, the dark cutting condition is developed when the final pH (24h postmortem) of steer muscle remains high (pH>5,9). Muscle samples (*Longissimus thoracis*) were taken from 150 steer carcasses at 0,5 and 24 h after slaughter. Nineteen samples with high pH (>5,9) values and 19 samples with normal pH (<5,8) values were selected from steers originated at the same farm to determine glycogen, lactic acid and glucose+glucose-6-P levels and AMPK, glycogen phosphorylase (GP) and glycogen debranching enzyme (GDE) enzymatic activity. The substrate concentrations were higher in normal carcasses. GDE activity did not show significant differences between groups. GP showed a significant increase in its activity in carcasses with normal pH at 24 h with respect to 0,5 h (p<0,05). Finally, AMPK activity in normal samples showed an increase of more than 3-fold (p<0,01) in comparison to samples obtained from high pH carcasses at 0,5 and 24 h. A direct relation could exist between the final pH of *L. thoracis* muscle and the glycogen content at slaughter, while its postmortem metabolism would be controlled by an increase in GP activity after 24 h and a constant activity of AMPK.

203. Different effects of progesterone and estradiol on native and its chimeric isoform aldosterone synthase that cause Familial Hyperaldosteronism type 1 (FH-1): Studies *in vitro*

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The FH-1 is caused by unequal recombination of genes encoding enzymes 11 β -hydroxylase and aldosterone synthase (ASN), resulting in a hybrid gene CYP11B1/B2 that expresses a chimeric enzyme (ASC) which generates high amounts of aldosterone causing hypertension. Aldosterone levels rise during pregnancy, but in pregnant women with FH-1, their hypertensive condition either remains unchanged or may even improve. **Aim** To investigate whether female sex steroids modulate the activity of ASN or ASC enzymes *in vitro*. **Methods** HEK-293 cell line transiently transfected with full cDNA of ASN or ASC were used as enzyme source. Progesterone and Estradiol (0.6-10 μ M) effect on both enzymes activities were assessed using deoxycorticosterone (0.6-30 μ M/24h) as substrate. **Results** In our model, both enzymes displayed similar apparent kinetic parameters (ASN: Km=1.056 μ M, Vmax=30.12 μ M/24h; ASC: Km=1.060 μ M, Vmax=22.69 μ M/24h, n= 5 p=ns, Mann-Whitney test). Progesterone inhibited aldosterone production by ASN and ASC (ASN: IC₅₀= 2.240 μ M and ASC: IC₅₀= 3.907 μ M), while estradiol had no effect. Kinetic data and molecular modeling studies of steroids binding of these enzymes suggest that Progesterone acted as a competitive inhibitor on ASN and ASC. **Conclusions** Our results show a new role of progesterone in order to inhibit the biosynthesis of aldosterone, protecting women from developing hypertension during pregnancy. **Funding** FONDECYT 1130427, FONDEF CA12110150 & IMII P09/016-F grants.

204. Biocompatible microcapsules as carriers of L-asparaginase, an approved enzyme drug in therapy of leukemia

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Asparaginases (ASNase) catalyze the deamidation of the amino acid asparagine to aspartate and ammonia. Bacterial ASNases as therapeutic enzymes are used in the treatment of various blood cancers to deplete from serum free L-asparagine which is essential for leukemic cells. A major limitation of ASNase is related to the so-called silent inactivation of the enzyme through the patient's immune system. This inactivation of ASNase manifests itself in a blood serum half-life of only about one day. Loading on biocompatible nanoparticles and encapsulation potentially protects the enzyme from the immune system and from degradation by proteases.

We report on a novel approach for encapsulation of the eukaryotic yeast cytoplasmic asparaginase. We capitalize on the use of the Layer-by-Layer (LbL) method of microcapsule formation. This technique allows biomolecules to be efficiently adsorbed onto colloidal core particles which serve as templates for the consecutive adsorption of several layers of oppositely charged polymers. We have used calcium carbonate particles as core templates for protein adsorption, with subsequent coating with cationic poly-L-arginine and anionic dextran sulfate layers. The protein-containing cores were treated with EDTA under mild conditions resulting in the removal of CaCO₃. Our work suggests that efficient encapsulation in combination with successful enzyme engineering could set the basis for novel ways of alternative blood cancer treatment.

205. Reconstruction of last common ancestor of arginases and agmatinases

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Arginase catalyzes the hydrolysis of L-arginine in ornithine and urea, and agmatinase catalyzes the hydrolysis of agmatine (decarboxylated arginine) in putrescine and urea. Both enzymes belong to ureohydrolases superfamily and they are similar in their substrates, urea production, Mn²⁺ requirement and conservation in aminoacid residues for the catalysis and metal ions stabilization and have a high conservation in their tertiary structure. However, both enzymes are highly specific for their respective substrates. In this work, we used phylogenetic methods to search the last common protein ancestor between arginases and agmatinases that, hypothetically, would catalyze the hydrolysis of both substrates. In this work 88 protein sequences were analyzed with software Mr.Bayes v1.3.2 (Bayesian inference) and Mega v5.1 (maximum likelihood), considering all members of ureohydrolases superfamily. The results indicate the presence of an ancestral node highly supported (p=0,99%). In addition, we performed an analysis of discrete characters to evaluate the Bayes factor, considering the last common ancestor node and the specifically node for arginases and agmatinase, using the matrix of phylogenetic trees with Bayestrans software and we generate structural model for three nodes. The last common ancestor of arginase and agmatinase, would be an enzyme with high probability to be specialist in arginine hydrolysis (p=0,93%) and agmatinase activity would be an evolutionary gain.

206. All paths lead to Rome: Evolutionary convergence and divergence of K⁺ channel blocking toxins

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Potassium channels are key players in a number of physiological processes, no wonder then that venoms from cone snails, scorpions, sea anemones, snakes and spiders are all known to be rich sources of K⁺ channels modulators. Most of these toxins exert their function via a common molecular mechanism that involves the blockade of ion conduction by direct binding onto the external mouth of K⁺ channels, leading to an abnormal cellular depolarization. Most K⁺ channel blocking toxins characterised to date belong to one of four protein families: i) CSab motif containing proteins, ii) the ICK fold, iii) the Kunitz-type protease inhibitors and iv) the CRISPs. While each of these toxin types are represented by large families that arose through extensive gene duplications and resulted in a diversity of structural forms (divergence), the "pharmacophores", i.e. the molecular determinants of K⁺ channel blockade, in these structurally unrelated types are common to all (convergence). In order to better understand these contrasting evolutionary mechanisms, we reconstructed the evolutionary histories of all four K⁺ channel blocking toxin types and evaluated the nature of selection sculpting their molecular evolution. We illustrate the functional divergence and evolutionary convergence in K⁺ channel blocking toxins through a series of examples.

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207. Local properties of the genetic code could determine global stability conditions on transcription or translation errors.

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Stability in some physical and chemical properties of amino acids, with respect to encoding codon changes, could be a driving force for the evolution of the genetic code. It has been shown that the stability found in the standard genetic code is overcome only in no more than one in a million random codes (given fixed sets of synonymous codons). We have studied whether these stability properties of the standard genetic code could be explained by local properties, i.e.: some property of one amino acid encoded by a codon xyz could be determined by the corresponding properties of the amino acids whose encoding codons are neighbor of xyz, such as we have verified for the hydropathy index property.

208. The inhibition of protein degradation affects the dynamics of cytosolic histones H3 and H4

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The DNA in eukaryotic cells is compacted with the help of nucleosomes, which are comprised of two copies of each of the histones: H2A, H2B, H3 and H4, around which 147 base pairs are wrapped. The synthesis of the histones H3 and H4 occurs in cytoplasmic ribosomes followed by the translocation to the nucleus. Our group recently described a complex cascade of events, where cytosolic histones establish their post-translational modifications and the correct folding with the assistance of histone chaperones. However, little is known about cytosolic histone degradation. In yeast, histones H3 and H4 are degraded by the proteasome, and recently one group suggested that in mammals the chaperone-mediated autophagy is also involved. In order to investigate this, we used inhibitors of proteasome and autophagy to treat HeLa cells. Cytosolic extracts derived from treated cells were biochemically fractionated, and the distribution of cytosolic H3/H4 complexes analyzed by Western blot. We found that upon inhibition of protein degradation, the dynamics of histones H3/H4 are affected as well as some of the chaperones associated with the histones. Funded by FONDECYT 1120170, Basal Project PFB16.

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209. The full-length mRNA of human T-cell leukemia virus type 1 (HTLV-1) harbors and IRES element that is dependent on the ribosomal protein S25

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In this study we demonstrate that the 5'untranslated region (5'UTR) of the capped HTLV-1 full-length mRNA harbors an IRES. Cap-independent translational activity was demonstrated using dual luciferase bicistronic mRNAs in rabbit reticulocyte lysate system, in mammalian cell culture, and in *Xenopus laevis* oocytes. The possibility that expression of the downstream cistron in the bicistronic constructs resulted from an alternatively spliced transcript or from cryptic promoter activity associated with the 5'UTR of the HTLV-1 mRNA was evaluated. The ribosomal protein S25 (RPS25) was shown previously to be required for IRES-mediated translation, while knockdown of RPS25 had no effect on cap-dependent translation. Therefore it is significant that the HTLV-1 IRES was found to be dependent on RPS25 for full activity. The HTLV-1 IRES demonstrated sensitivity to edeine, indicating that recruitment of the initiation complex can occur internally yet recognition of the initiation codon requires scanning ternary complexes. FONDECYT 1130270 y P09/016-F Iniciativa Científica Milenio del Ministerio de Economía, Fomento y Turismo. Eduardo Olivares was supported by UCH0604 MECESUP-USACH doctoral fellowships.

210. Role of RCO-1 in circadian transcriptional regulation in *Neurospora crassa*.

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N. crassa is a model organism for the study of circadian clocks, which control endogenous rhythms of different processes, including gene expression. Though the mechanisms by which the oscillator works have been studied in the past decades, little is known about how the central clock controls the cyclical expression of key processes such as conidiation. Importantly, different mutant strains that present alterations in conidiation have been characterized, leading to the identification of *rco-1*, among others genes. RCO-1 is homologous to *S. cerevisiae* Tup1, a transcriptional regulator that works as a key co-repressor. Since conidiation is strongly affected in this KO strain and since the expression of luciferase-based circadian reporters is impaired in this mutant, we evaluated the role of RCO-1 in the control of circadian gene expression. Using transcriptional and translational reporter fusions we have observed that the expression of this protein fluctuates daily. Based on literature information, putative targets genes were chosen, and their expression levels evaluated by RT-qPCR. In the $\Delta rco-1$ mutant, we observe an alteration in the expression pattern of several genes, for which we have generated luciferase transcriptional fusions to evaluate if their transcription is rhythmic and dependent on RCO-1. In addition, through mass-spec we have started to identify proteins that physically interact with RCO-1. CONICYT, GO-21100309, Fondecyt 1131030.

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211. Unveiling Novel Transcriptional Networks behind the Circadian Clock of *Neurospora crassa*.

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The circadian rhythms are endogenous phenomena present in a wide range of organisms and sustained by a cellular autonomous molecular circuit. All the organisms in which circadian studies have been performed share a common molecular architecture within this circuit, that is based on a positive element able to activate the expression of a negative one that repress the action of the former, inhibiting its own expression. Studies in mammals, *Drosophila* and *Arabidopsis* have shown transcriptional networks (TNs) capable of supporting the central molecular circuit. In *Neurospora crassa* the clock central circuit has been well characterized but the participation of other TNs in this system is unknown. In our lab, we are trying to find novel TNs capable to sustain the circadian clock in *N. crassa*. For this purpose, we performed a global search of these networks, analyzing on a genome-wide basis a luciferase circadian reporter in all the KO strains (~300) for transcriptional regulators (TRs) in *N. crassa*. After this examination, we obtained a set of TRs capable to affect the oscillations in *N. crassa*, confirming their effect with phenotypic analysis. With this information in conjunction with the DNA binding preference for the most part of the TRs available in *N. crassa*, we are now designing novel networks behind the central oscillator. We expect that this strategy will provide us a new vision of the circadian system in this model organism. Fondecyt 1131030 Conicyt 21110734.

212. Circadian clock and carbon catabolite repression in *Neurospora crassa*: insights into the role of the CRE-1 transcription factor in the crosstalk of two regulatory systems.

Díaz, R¹., Larrondo, L¹., ¹Departamento de Genética molecular y microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica De Chile. (Sponsored by Luis F. Larrondo)

Neurospora crassa is a model organism for circadian studies. Genome-wide studies suggest that about 20% of the *Neurospora* transcripts are under circadian control and interestingly; many of them are related to metabolism. Indeed, important cellular processes under circadian control like conidiation are subjected to metabolic control. However, the transcriptional mechanisms involved in transferring both the temporal information and the nutritional status of the cell to the conidiation pathway components, has not been clearly determined. Despite this, the idea of a crosstalk between metabolism and the circadian clock has emerged as a transversal concept in the last years. We are interested in elucidating the transcriptional mechanisms interconnecting circadian and metabolic processes, particularly regarding organismal fitness. Thus, we are analyzing carbon catabolite repression (CCR) and cellulolytic capabilities in a circadian context, using *N. crassa* as a model. Evaluating the role of CRE-1, a crucial transcription factor involved in several important cellular processes as cellulose degradation and carbon catabolic repression, we found evidence that suggests CRE-1 as a link between both pathways, as it appears to be important for both CCR and circadian control of target genes. These data provide insights on how the circadian clock is influencing *Neurospora* physiology. **Acknowledgments** Grant FONDECYT 1131030 and CONICYT fellowship Grant AT-24121510.

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213. Phytoene synthase genes of *Malus domestica* codify for functional enzymes that are expressed differentially during fruit ripening.

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Carotenoids are isoprenoid molecules that act as accessory pigments in photosynthesis and protect plants against photooxidative damage. They are vitamin-A precursors and provide antioxidants properties. A key step in carotenoid synthesis is catalyzed by phytoene synthase (PSY).

Malus domestica is one of the most worldwide consumed cultivated fruits, mainly because of its high nutritional value, however the carotenoid composition in the flesh of a mature fruit is very low (0.10 mg/g dw). In order to understand this, by using apple EST and GDR databases we identified four candidate genes for PSY, that through *in silico* analysis, we observed they share conserved domains at the aminoacidic level. qRT-PCR expression analysis indicate that there is a tissue and developmental differential expression of these genes in leaves and during fruit ripening. *MdPSY2* and *MdPSY3* are preferentially expressed in fruit and leaf respectively while *MdPSY1* and *MdPSY4* are scarcely expressed. *MdPSY3* is induced and *MdPSY2* is repressed during fruit ripening. Through bacterial complementation we determined the functionality of *MdPSY2* and *MdPSY3* and of a *MdPSY2* variant that loses a 18bp region in its coding sequence. Moreover, PSY::GFP fusion protein permits us to determine its expected plastidial localization. We conclude that *MdPSY3* and *MdPSY2* are functional and may not be the key point that prevents the carotenoid accumulation in fruits of *M. domestica*. Founded by FONDEF D1011022

214. Characterization of the BcFRQ protein and its role in the *Botrytis cinerea* circadian clock.

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Our research focuses on the phytopathogen *Botrytis cinerea*, a fungus that affects over 200 plant species in a necrotrophic way, generating many agronomical and monetary losses. The main model in chronobiology is the non-pathogenic fungus *Neurospora crassa*, whose clock is built by three main components that form the transcriptional/translational feedback loop characteristic of a circadian oscillator. These encoding genes, *wc-1*, *wc-2*, and *frequency (frq)* are present in the *B. cinerea* genome (*bcwc-1*, *bcwc-2* and *bcfrq*), as previous studies in our lab showed. Our results also demonstrate that the transcriptional levels of *bcfrq* oscillate in constant conditions, in agreement with a gene that is controlled by a circadian clock. To further asses the role of BcFRQ we generated knock out (KO) strains for this gene. Phenotypical analyses of the KO strains reveal abnormal colonial morphologies, and altered conidial banding under light:dark cycles. Through the generation of a V5 tagged strain we aim to evaluate oscillations in BcFRQ levels, in order to confirm its dynamics as a clock controlled state variable. Finally, we expect to perform a co-immunoprecipitation assay with this strain, to elucidate the BcFRQ interactome and build a proper clock model for this economically relevant fungus. Fondecyt 1131030.

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215. Potential use of the β actin promoter of chinese hamster cells in the expression system of recombinant proteins

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β -actin gene (ACTB) promoter contains a CpG island, which regulates silencing of constitutive genes. We aimed to investigate whether, in a recombinant system, this promoter reverses the typical silencing of recombinant CMV promoter upon their chromosomal integration. Based on the firefly/renilla luciferase (FIR/REN) expression system, we generated the pACTB and pCMV constructions, which correspond to the Chinese Hamster ACTB and human CMV promoters, by cloning into pGL4.17 vector. Transient expression was performed by transfection of CHO-K1 cells and FIR/REN activity measure after 48 h. Stable expression was performed by transfections, isolation of antibiotic resistant clones, and FIR activity measure after several cell culture passages. In transient expression assays, pACTB vector activity was four times lower than that of pCMV. However, in stable expression assays after 15 cell culture passages, the pACTB clones reached an average 2.5 times increased FIR activity compared to pCMV clones. Moreover, a high dispersion was detected for the ACTB promoter activity, allowing the selection of extremely active clones. Therefore, although the basal ACTB promoter activity is lower than that of the CMV promoter, once it is integrated into the genome, it can achieve higher activity levels. We believe that the use of ACTB promoter in recombinant expression systems, allows isolating clones with higher levels of expression than the CMV promoter. *Financed by FONDEF D09I1190.*

216. The *Piscirickettsia salmonis* infection *in vitro* on salmon cell line induced changes in the gene expression of type 4b Secretion System.

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Piscirickettsia salmonis is the etiological agent of the disease called Salmon Rickettsial Syndrome (SRS). Several vaccines are used to prevent the onset of the disease, which have not been effective in controlling the disease. Seeking better antigens for vaccine development, proteins belonging to Secretion System Type 4b (SST4b) were found in the genome of the bacterium. Thus, to evaluate the expression during kinetics infection of genes *dotG*, *dotH* and *sdhA*, specific primers were designed to detect by conventional PCR and real-time on SHK-1 cell cultures. Standardized primers detect at conventional PCR the presence of those three specific transcribed genes characteristic of SST4b. Different levels of signal intensity were observed on agarose gels, which give a sign of a differential expression during infection. In Real-Time PCR, short or long times post-infection we observe the variation levels of transcript normalized against his own bacteria gene, 23S. These changes are not continuous throughout the whole infection may be due to the characteristic of the secretion system, working as injectisome, translocating effector proteins into the cytoplasm of the infected cell, establishing an optimal environment for the growth and intracellular survival of bacteria. Proyecto Tesis Doctoral financiado por Fondap- INCAR 15110027, Gastos Operacionales beca Conicyt Folio 21110631/2013.

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217. Sumoylation modulates vitamin D-mediated transcription in osteoblasts.

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The vitamin D receptor (VDR) modulates gene expression in response to 1 α ,25-dihydroxy vitamin D₃ (VD₃). Binding of this ligand to the C-terminal of VDR promotes a conformational change that allows interaction with transcriptional coactivators, including members of the p160/SRC family and DRIP205 subunit of the DRIP/TRAP/Mediator complex. It has been described that interaction of nuclear receptors with transcriptional coactivators can be modulated by sumoylation of both the receptor and coactivator moieties and which affects transcriptional activity. We have analyzed the aminoacidic sequence of VDR finding putative sumo interacting motifs (SIM) at the C-terminus domain. We explored the contribution of sumoylation during transcriptional regulation of the VD₃-responsive osteocalcin (OC) gene in osteoblastic cells by combining transient over-expression, RT-PCR, GST and His pull down and co-IP studies. We found that endogenous OC gene transcription is decreased by forced expression of the sumoylation machinery components SUMO1, E1-Ubc9 and E3-Piasy, in the presence of VD₃; sumoylated proteins bind to VDR during VD₃ stimulation through the SIMs at the ligand binding domain and finally, that SRC-1 binds to sumoylation machinery components and that is sumoylated during VD₃ response. Our results indicate that, following exposure of osteoblastic cells to VD₃, sumoylation plays an important regulatory role at VD₃-responsive genes.

218. Biochemical and Functional Analysis of CREB and C/EBP β binding sites in the human RIC-8B gene promoter

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The RIC-8B protein is a positive modulator of G α s subunit, and regulates the cAMP signaling pathway. This pathway is present in different tissues and its pleiotropic effect observed after activation can be explained in part by protein modulators with differential tissue-specific and developmental stage-dependent expression pattern, such as RIC-8B. In order to get insight into the mechanisms involved on transcriptional regulation of the human RIC-8B gene, we studied its promoter region and identified binding sites for CREB (CRE sites) and C/EBP (C/EBP sites) transcription factors. These sites are conserved in the mouse promoter, where only binding of C/EBP β has been reported. Here, we demonstrate the binding of CREB1 to CRE elements present in this promoter by EMSA assays using nuclear extracts from two different human cell lines and recombinant human CREB1. The binding of C/EBP β to the C/EBP elements was confirmed through EMSA using recombinant human C/EBP β . We also performed DNaseI-Footprinting assays and confirmed the binding sites previously identified. Finally, promoter activity analysis by luciferase assays shown that mutation of these sites significantly decrease the overall activity of the promoter, supporting the previous findings of the regulatory action of C/EBP β and suggesting a role of CREB in the regulation of RIC-8B gene expression. Funding Acknowledgements: FONDECYT 1090150 and 1110803, CONICYT 24121186.

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219. Characterization of a circadian clock in *Botrytis cinerea* and its role in pathogenesis using *Arabidopsis thaliana* as a plant model.

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Botrytis cinerea is a necrotrophic fungus that infects over 200 plant species, ranking as the second most important phytopathogen according to its scientific-economic importance. Although, it has been suggested that the outcome of a plant-pathogen interaction could have daily oscillations, the importance of a circadian clock has only been addressed in the plant, but not in the pathogen. Therefore, we have started to characterize - in connection with pathogenesis - the *B. cinerea* clock, which is composed of the FRQ protein and a transcriptional complex formed by WC-1 and WC-2. Our results indicate that *bcfrq* mRNA presents daily oscillations in a light-dark cycle and in constant darkness (DD), that are lost in a *bcwc-1 KO* strain. We have observed oscillatory levels of the BcFRQ protein under temperature cycles and DD. Both the *bcfrq* mRNA and BcFRQ protein anticipate cyclical-environmental changes, a key characteristic of circadian behavior. Interestingly, we have also observed an impaired infection process using a *bcfrq* and a *bcwc-1 KO* strains. Moreover, we demonstrate that the outcome of the plant pathogen interaction using a necrotrophic fungus varies with the time of day. These results provides the first evidence indicating the existence of a circadian clock in this pathogenic fungus, raising the possibility that it may utilize the clock to synchronize key elements of pathogenesis. CONICYT, AT-24121100, Fondecyt postdoc 3110127, Fondecyt 1131030.

220. Characterization of transcriptional regulation of *MYBA1*, mediated by glucose, during fruit ripening of *Vitis vinifera*.

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The grape berry development is divided into three stages: the formation stage; the lag phase, in which the fruit changes color (*veraison*), and the ripening stage. During *veraison*, begins the glucose and fructose accumulation and the color change is due to the beginning of anthocyanins biosynthesis.

Sugars have been studied not only as an energy source, but as a signaling molecule able to control gene expression. It has been demonstrated that glucose and fructose induced the anthocyanins biosynthesis in cell cultures of grape. This induction could be mediated by transcription factors, between which, MYBA1 is very important, since it regulates the expression of UFGT, an enzyme that catalyses the last step in the anthocyanins biosynthesis. During berry ripening, the transcript levels of *MYBA1* begin in *veraison*, the moment in which the sugar levels increased.

In order to know whether *MYBA1* is transcriptionally regulated by glucose or fructose, *in silico* analysis were done in its promoter region, and sugar response elements were found, which indicates that it could be regulated by sugar.

Also, sugar treatments to fruit disks and cells cultures of grape were performed, and it was found that *MYBA1* is induced by glucose.

Finally grape leaves was transformed with the promoter of *MYBA1* fused to the *uidA* gen (GUS) and was possible demonstrate that this gene is transcriptionally regulated by glucose.

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221. SRS transcriptome: a useful resource for understanding host-pathogen interaction.

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Piscirickettsia salmonis is one bacterial agent affecting the Chilean Atlantic salmon, however little is known about the interaction between the host and the pathogen from a molecular point of view. We use high-throughput sequencing of individuals bearing different genetic resistance backgrounds in order to characterize the expression of genes involved in this process. The brujin graph assembly gave an average length of 921 bp and N50 length of 1,656 bp. The BLAST revealed that a quarter of the contigs were from *Salmo salar*. A total of 21,982 annotations were selected. Gene ontology analysis was performed and all transcripts were classified into functional categories, assigning a total of 108,513 GO terms. The principal biological process of ontology was cellular and metabolic processes, response to stimulus and biological regulation. In terms of cellular components, the main category was intracellular followed by organelle and macromolecular components. These results show that the interaction of the host and the pathogen is complex in terms of the processes involved, such that not only immunological components explained the transcriptome after the infection. This is the first large scale analysis of the transcriptome response of Atlantic salmon, providing a valuable resource for better understanding of host-pathogen interaction. Furthermore, this give scope for selecting genes that are not obvious candidate genes, that are expressed after the infection.

222. Post-transcriptional control of gene expression.

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The post-transcriptional lives of RNA transcripts are remarkably diverse, play a central role in biological regulation and still rich with opportunities for discovery. I will focus on two recent findings from my research group. First, I will present evidence for abundant, developmentally regulated circular RNA isoforms in animals, plants, fungi and protozoa, and describe some of their features. Second, I will present some surprising new insights into the process of translation elongation from a new analysis of data from deep sequencing of ribosome-protected mRNA fragments, which allows us to separately measure the kinetics of decoding and translocation at each codon.

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223. Histone modifications at the MLH1 promoter respond differentially to 5-aza-dC treatment

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MLH1 gene has important functions in DNA repair and it has been shown to be silenced in certain types of cancer. Lack of MLH1 expression results in the accumulation of errors in the cell DNA, facilitating cancer progression. DNA methylation and histone modifications are epigenetic mechanisms by which gene expression is regulated. The DNA demethylating agent 5-aza-deoxycytidine (5-aza-dC) is been used in cancer therapy resulting in re-expression of some genes. However, the effect of this compound on histone modifications is not well known. In this study we analyzed the effect of 5-aza-dC on histone modifications at the MLH1 promoter in HeLa and HL-60 cells. To this end, we used ChIP to evaluate enrichment of histone marks associated with transcriptional activation (H3K4Me y H3K4Me3) and repression (H3K9Me, H3K9Me3 and H3K27Me) at the MLH1 promoter. Our results show that MLH1 promoter exhibit different histone modification pattern in HL-60 and HeLa cells in basal condition. 5-aza-dC increases association of H3K4Me3, H3K9Me1 and H3K9Me3 in HL-60 and decreases association of H3K4Me3 and H3K9Me1 in HeLa cells. Moreover, expression of MLH1 after treatment is observed in HL-60 but not in HeLa cells. Taken together, our results demonstrate that histone modifications associated with MLH1 promoter region differentially respond to 5-aza-dC treatment in the two cell lines analyzed and that this difference correlates with gene re-expression.

FONDECYT 1130697

224. Development of a sandwich Enzyme-Linked Fluorescence Assay (ELFA) for detecting cathelicidin from Rainbow trout fry

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Opportunistic pathogens like IPNV, *Flavobacterium* and *Saprolegnia* cause diseases and dead in larval stage of salmonids farming, generating high economic losses. To prevent and control diseases, the industry has invested in the use of immunoprophylaxis methods such as vaccination and immunostimulation. However, evaluate the effectiveness of these strategies is necessary to establishing the ability of immune response in larvae. Cathelicidins are a well-known family of antimicrobial peptides (AMPs), which have been identified in rainbow trout. An increase of their mRNA expression after bacterial infection suggests that they play a key role in fish innate immunity. Therefore these AMP is considered as a potential immunological marker to assess immune response capability in fish. Therefore, the aim of this work was to develop a sandwich ELFA for detecting cathelicidin, thus two monospecific polyclonal antibodies were developed in mouse and rabbit; the specificity of both antibodies was confirmed by Western blot where only one 14,3 kDa immunoreactive band was observed and the sensitivity of the ELFA assay was established in the order of 70 ng/mL. With this assay we were able to detect Cathelicidin from 34 to 56 days post hatching indicating that larvae have innate immune response capability. Therefore this assay may be a useful indicator of disease susceptibility, providing a new, sensitive tool for rapid screening of larvae population health. PIIC 2012, UTFSM. Becario CONICYT.

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225. Selection of scFv constructs that bind to human MICA using multiple antigenic presentation systems and Phage Display library

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NK cells play an important role in the control of tumor cells. They express the activating receptor NKG2D, which recognizes MHC class I polypeptide-related sequence A (MICA) ligand, expressed on the surface of several tumor cells. NKG2D-MICA interaction triggers NK cell cytotoxic activation, resulting in lysis of target tumor cells. However, the generation of high levels of soluble MICA (sMICA), a tumor evasion strategy, has been reported in patients with several types of cancer. Serum accumulation of sMICA could interfere with the recognition of surface MICA in tumor cells and thus compromise NK cell-mediated immune surveillance. The aim in this work was to develop a specific scFv construct against human MICA, selected with specific peptides. For the selection of antibodies against the binding site of MICA, we performed Phage Display using a library of human scFv and multiple antigen presentation systems, which consists in a lysine core with 8 branches of peptides. We analyzed the affinity of single clones for MICA peptides by ELISA using the scFv fused to M13 phage. The specificity of the scFv for recombinant MICA was analyzed by ELISA and Western Blot. We obtained scFv constructs with different affinity for MICA peptides. Furthermore, these scFv recognized native MICA using tumor cell lines. Therefore, it is possible to select scFv with synthetic peptides by Phage Display to obtain human scFv against specific regions of target proteins. IDeA FONDEF CA12I10023

226. Chromatographic analysis and functional characterization of synovial fluid from rheumatoid arthritis patients

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Rheumatoid arthritis (RA) is an autoimmune disease characterized for joint inflammation and cartilage damage. Even though progress has been achieved regarding RA immunopathogenesis, antigens that trigger the disease are not fully described. Since synovial fluid (SF) from RA patients contains a diversity of proteins, it could be an autoantigen reservoir as potential source for dendritic cell (DC) loading in a cell-based therapy approach. The aim of this study is to characterize the protein composition of RA patients' SF and elucidate its antigenic potential using co-cultures of SF-pulsed DC and autologous effector CD4⁺ T cells from RA patients. For this purpose, RA patients' SF samples were analyzed through anion exchange chromatography. We demonstrated that protein profiles, do not present a significant variation between different samples. On the other hand, in a functional assay where peripheral blood mononuclear cells (PBMC) from RA patients and healthy subjects were stimulated with SF samples from RA or osteoarthritis patients, we detected significant difference in T cell proliferation when RA SF was used as antigen source. Finally, we observed that CD4⁺ T cells co-cultured with SF protein fractions-pulsed DC from patients with RA acquired different cytokine and proliferation profiles. These results encourage the use of chromatography SF protein fractions as antigen source for T-cell proliferation assays. Supported by FONDECYT 1100102 and MIII-P09/016-F.

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227. IL-10 regulates mica expression on the surface of gastric adenocarcinoma cells

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NKG2D is a co-activating receptor expressed on NK cells that binds to a variety of ligands (NKG2DL), including MICA, MICB and ULBPs. These cell surface glycoproteins are overexpressed under cellular transformation, thus playing an important role in cell-mediated immune response to tumors. Tumor cells can interfere with NKG2D-mediated activity through several mechanisms, such as NKG2DL cell surface shedding. Interleukin 10 (IL-10), which signals through the transcription factor STAT3, is an immunosuppressive cytokine expressed by many tumors and it has been described that this transcription factor is constitutively active in cancer. In this work, we aimed to describe the role of IL-10 and its signalling pathway on the expression and shedding of MICA/B. Cell lines derived from gastric adenocarcinoma, AGS, MKN-45, HTB-135 and prostate adenocarcinoma LNCap, were stimulated with exogenous IL-10 for 48 h and STA21 (STAT3 inhibitor) for 24h. Cell surface expression of MICA and MICB was assessed by flow cytometry and soluble MICA and MICB were measured by ELISA. We observed that IL-10 downregulates MICA cell surface expression on MKN45 and AGS cells in a dose-dependent manner. Also, STA21 increases MICA and MICB cell surface expression and the secretion of their soluble form, giving a potential role for STAT3 in the regulation of these glycoproteins.

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228. Rab14 regulates antigen presentation by major histocompatibility complex type I (MHC-I) in dendritic cells infected with *Chlamydia trachomatis*

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Chlamydia is an obligate intracellular bacterium that replicates inside a vesicle called *inclusion*. This pathogen manipulates Rab GTPases, master controllers of vesicular transport, to ensure its survival and replication. Dendritic cells are the most powerful antigen presenting cells of the immune system. We have shown that *Chlamydia trachomatis* intercepts Rab14-vesicles to acquire host lipids necessary for its growth and multiplication. Recently, it has been determined that an efficient cross-presentation requires that the exogenous antigen containing-phagosome interacts with Rab14-endosomes. Our hypothesis is that *C. trachomatis* recruits Rab14 not only as a strategy for nourishment, but also to interfere with the process of antigen presentation. By confocal microscopy, we observed that the transferrin receptor (RTf) is recruited to the inclusion immediately after bacterium invasion and remains throughout its development. Furthermore, we observed that Rab14 is associated with the plasma membrane at the entry site of the bacterium. Later, Rab14 is recruited to the chlamydial inclusions and remains throughout the entire cycle. The knock-down of Rab14 impairs the bacterial invasion. In addition, Rab14 silencing causes a redistribution of MHC-I molecules and interferes with their transport towards the plasma membrane, assessed by flow cytometry. These data suggest that Rab14 plays a role not only for bacterial growth but also for its hiding from immune system.

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229. The CD1d-natural killer T cell axis in the immune response against human gastric adenocarcinoma

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CD1d is a class I-like HLA molecule that presents glycolipid antigens to natural killer T (NKT) cells, a modulatory subset of T lymphocytes. In normal conditions and several disease settings, CD1d is expressed on antigen-presenting cells, among other cells. Nevertheless, the role of CD1d and NKT cells in gastric cancer (GC), the second most common cause of cancer death worldwide, still remains unclear. We studied, by flow cytometry, the expression of CD1d on antigen-presenting cells and the frequency and cytokine production by circulating and tumor-infiltrating NKT cells from GC patients. Gastric mucosa of GC patients and peripheral blood from healthy donors were used as controls. Although no significant difference in the percentage of peripheral, tumor and mucosal-infiltrating NKT cells was observed, high levels of IL-10 and low or absent production of IFN- γ and TNF- α by NKT cells were detected in all tissues of GC patients. CD1d expression was detected on B lymphocytes and monocytes infiltrating the tumor and adjacent mucosa, as well as in the blood of GC patients. We also found that, in blood samples, CD1d expression was significantly higher in GC patients than in healthy donors. Our results provide evidence that the regulatory function of NKT cells in GC, possibly triggered by interaction with CD1d-expressing cells, may dampen the anti-tumor immune response, thus contributing to tumor evasion.

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230. Differential expression of cell-surface MICA/B and CD44 in the tumor tissue and gastric mucosa of patients with gastric adenocarcinoma

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Gastric cancer (GC) is the second most common cause of cancer death worldwide. Natural killer cells are cytotoxic effectors that express NKG2D, an activating receptor that recognizes MICA and MICB, cell-surface glycoproteins that are overexpressed in tumor cells. CD44, a receptor for extracellular matrix, is involved in cell adhesion and migration, and predicts a poor prognosis for GC. We determined, by immunohistochemistry, the expression of MICA/B and CD44 in the tumor and gastric mucosa of patients with advanced GC who had undergone gastrectomy. Sections of paraffin-embedded tissues were incubated with anti-human MICA/B or CD44 antibodies, and positive staining was measured in tumor and mucosal glands and lamina propria cells. Gastric mucosa from a bariatric patient was used as a non-tumor control. Tumor and mucosal tissues of GC patients presented increased expression of MICA/B and CD44 as compared to the bariatric control. Furthermore, in gastric tumor and mucosa, MICA/B was predominantly expressed in the mucosal glands, while CD44 staining was largely evidenced in the lamina propria, indicating that these markers may not be expressed on the same cell types. Our results confirm previous evidence for a differential distribution of MICA/B and CD44 expression in GC patients, which may help understand the interaction of gastric adenocarcinoma cells with the immune system at advanced stages of the disease.

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231. *In vitro* evaluation of the cellular immune response of *Mytilus chilensis* hemocytes in primary culture exposed to Saxitoxin.

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Saxitoxin (STX) is a neurotoxin produced by dinoflagellates like *Alexandrium*, and cause VPM in humans after ingestion of contaminated shellfish. Since these filter-feeding bivalves are able to concentrate these toxins without an apparent effect, we have focused our attention on the effect of STX on immune response of these bivalves. In marine invertebrates, hemocytes are the main effector cells of the immune cellular response, and which have been shown to play a fundamental role in the recognition of foreign particles and among other processes such as defense against pathogens. In this study we evaluated the response *in vitro* on cell cultures of *Mytilus chilensis* hemocytes and the effect of exposure to different concentrations of STX. Among the parameters measured on the cell culture, it was determined no effect on cell viability of this toxin at any concentration (1-100 nM) at 24 hours exposure (MTT). However, the superoxide anion production assessed by nitroblue tetrazolium (NBT) and phagocytic activity evaluated using flow cytometry showed variations compared to control. Superoxide anion production increases when hemocytes are exposed during the first 4 hours to STX (1-100 nM), which decreases at 16 hours. Similarly, there was a significant decrease in phagocytic activity in the presence of saxitoxin (1-100 nM) for each exposure time (4 and 16 h). Together, these results confirm the existence of an immune response of hemocytes in culture after STX exposure

232. Increased Reactive Oxygen Species is associated with an altered NFkB-dependent IL-6 gene expression.

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Duchenne Muscular Dystrophy (DMD) is a genetic disease, caused by mutations in dystrophin gene. DMD pathology is associated by an increased production of reactive oxygen species (ROS). The aim of this work was to study the alterations in ROS production elicited by electrical stimuli and its relation with NF-kB activity, in both *wt* and *mdx* skeletal muscle cells. Tetanic electrical stimulation (TES) induced higher ROS production in *mdx* than *wt* myotubes, measured by CM-DCF fluorescence. ROS production was blocked by apocynin and gp91ds-tat in both *wt* and *mdx* muscle cells, suggesting an up regulated NOX2 activity. Moreover, the mRNA expression of p47^{phox} and gp91^{phox} were increased in *mdx* myotubes. TES induced NF-kB activation in *wt* myotubes, whereas in *mdx* there was a significant reduction of NF-kB activity. H₂O₂ induced a dose-dependent effect in NF-kB activation in *wt* and *mdx* myotubes. In *wt* myotubes, NF-kB activity was increased at 50 mM without any effect at 100-200 mM. However, in *mdx* there was a dose-dependent reduction in NF-kB activity, with a significant effect at 100 μM and 200 μM. Finally, TES had a differential effect in IL-6 gene expression in both *wt* and *mdx* myotubes. TES increased IL-6 mRNA in *wt* myotubes, whereas in *mdx*, there was a significant reduction in IL-6 mRNA levels. This data suggests that *mdx* cells show an altered NF-kB-dependent gene expression in response to depolarization, due to a dysregulation in ROS production by NOX2.

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233. Glucose effect on expression of carotenogenic genes and pigment synthesis in a *Xanthophyllomyces dendrorhous* *mig1*⁻ mutant.

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The red basidiomycete yeast *X. dendrorhous*, has the property to synthesize astaxanthin and to ferment glucose. As glucose is the preferred carbon source in various microorganisms, it exerts a repressive effect on the expression of genes involved in other sugar and secondary metabolism. This phenomenon is known as Catabolite Repression and it has been suggested that it would be operating in carotenoid biosynthesis in *X. dendrorhous*.

Mig1 is a glucose dependent transcriptional repressor described in various yeasts that recognize specific DNA sequences in the promoter of regulated genes. A *MIG1* homologous gene was found in the *X. dendrorhous* genome by bioinformatics analyses and a *mig1*⁻ mutant strain was constructed. The effect of this mutation was evaluated in the yeast carotenoid production and the pigment composition was determined by RP-HPLC. In addition, several homologous genes known to be repressed by glucose in other yeasts such as *INV* and *grg2*, were identified in its genome, which had putative Mig1 binding sites in their promoter region. Mig1 binding sites were also detected in at least 3 carotenogenic genes (*crtYB*, *crtI* and *crtS*). The glucose effect on the expression of these genes in wild-type and *mig1*⁻ strains, is being analyzed by RT-qPCR at different times after glucose addition to cultures.

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234. The cytoplasmic dynein complex is important for Murine Leukemia Virus Infection

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Retroviruses are obligate intracellular parasites; they deliver their genome into the cell nucleus and integrate it into the host genome where it is transcribed by cell machinery to generate mRNAs and new infectious viruses. Each step of this process requires the assistance of multiple host proteins. How retroviruses deliver their DNA into the nucleus of the host cells is poorly understood.

Many different viruses utilize the dynein complex for its transport along the microtubules, how the motor protein is recruited to the incoming viruses is poorly understood. Moreover, nothing is known about the use of the dynein complex by MLV.

Using a combination of immunoprecipitation and mass spectrometry, we have found that microtubule motor cytoplasmic dynein intermediate chain interacts with viral cores of Murine Leukemia virus (MLV) at early times of infection, suggesting a direct interaction between the incoming viral particles and the dynein complex. We propose that this interaction is relevant for MLV infection, because silencing of the cytoplasmic dynein intermediate chain reduced it. We are trying to understand how other members of the cytoplasmic dynein complex are participation in MLV infection.

An understanding of the strategies used by viruses to bind cytoplasmic dynein will provide important clues to design or develop new anti-retroviral drugs that specifically disrupt intracellular viral trafficking.

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235. Genetic and Structural analysis of *Porphyromonas gingivalis* Lipopolysaccharide and its role in antimicrobial compounds resistance.

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Porphyromonas gingivalis has been proposed as a key etiological agent in periodontitis, a multifactorial infectious disease that causes inflammation in tooth supporting tissues. Over 90% of the Chilean adult population has characteristic clinical signs of this pathology. Among its main virulence factors there is **the lipopolysaccharide (LPS)**, a membrane molecule important in Gram-negative bacteria able of being recognized by membrane receptors of the immune system cells (Toll-like receptors 2 and 4) causing Pro-inflammatory cytokine secretion like interleukins 6 and 8, as a defense mechanism to invasion. In our laboratory we characterize the LPS of different clinical isolates of *P. gingivalis* from healthy and chronic periodontitis patients, regarding their LPS production and some important genes in LPS synthesis. For these purposes, genes were amplified by PCR and LPS was purified and visualized on polyacrylamide gels stained with silver. Furthermore, we relate the presence or absence of the LPS with the resistance to antimicrobial compounds by inhibition test or antibiogram. Our results indicate that the LPS from **healthy patients have no polymeric O-antigen** unlike patients with periodontitis. Also, we note that the presence of O-antigen confers differential resistance to different antimicrobial compounds. Furthermore, it was found that clinical isolates from healthy patients **doesn't have the gen for the ligase**, an important protein in the last steps of LPS synthesis.

236. Development of an enzyme-linked immunosorbent assay for evaluating the efficacy of vaccines against SRS

Sandoval, R¹, Espinoza, C², Valenzuela, K¹, Cárcamo, J¹, Yañez, A¹, ¹Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral De Chile, ²Ciencias Veterinarias Universidad De Concepción. (Sponsored by Dr. Alejandro Yañez)

The Salmonid Rickettsial Septicaemia (SRS) is one of the most damaging syndrome that affects salmon farming in Chile. The causal agent of this pathology is *Piscirickettsia salmonis* and vaccination remains the best way to reduce the economic impact caused by this disease. Several vaccines are used based in bacterins and recombinant proteins. However, the modulation of the humoral immune response has not been determined. Serological methods are the best option to evaluate the immune response achieved by administration of vaccines. Thus, an Enzyme-Linked ImmunoSorbent Assay (ELISA) was developed to detect specific IgM against *P. salmonis* antigens from fish serum. Different extracts of *P. salmonis* were used as the coating antigen. Standardization was carried out with sera of healthy and infected fishes. The signals of these positive and negative serums were significantly different. Finally, we evaluate 3 prototypes SRS vaccines. We injected 3 groups of fish with these formulations. Later, the fish of each group were challenged with a lethal dose of *P. salmonis*. Sera obtained were tested through ELISA to detect specific IgM response. Only one prototype was efficient to stimulate the specific acquire immune systems and also was highly efficient to protect against SRS. These results validate a new platform to evaluate the effectiveness of SRS vaccines through determination of IgM specific anti-*P. salmonis*. INNOVA 11IDL2-10523, Fondap-INCAR 15110027

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237. Characterization of gene clusters involved in the biosynthesis of new compounds in freshwater cyanobacteria.

Soto-Liebe, K¹, Fuentes-Valdés, J^{1,2}, Vásquez, M¹, Arce-Johnson, P¹, ¹Genética Molecular y Microbiología, Ciencias Biológicas, Pontificia Universidad Católica De Chile. ²Ingeniería Química y Bioprocesos, Facultad de Ingeniería, Pontificia Universidad Católica De Chile. (Sponsored by Patricio Arce-Johnson)

Cyanobacteria are photosynthetic microorganisms and some of them are capable of synthesizing biologically active secondary metabolites that are highly toxic for animals. The biosynthetic pathways of the five major cyanotoxins: microcystin, nodularin, saxitoxin, cylindrospermopsin and anatoxin have been genetically and biochemically elucidated, all belonging to NRPS-PKS, PKS or PKS-like biosynthetic pathways. *C. raciborskii* CS-505 is a cylindrospermopsin producing cyanobacterium, that contain a NRPS cluster of 37.6 kb (cluster *cyp*), probably involved in the synthesis of a new peptide named cylindropeptolide (CYP). With the aim of characterizing a new NRPS biosynthetic gene cluster and predicting the corresponding molecular structure of the peptide product, we developed a primer set based on the *cyp* cluster and assays in 15 cyanobacterial strains. We presented the sequences obtained in at least 10 cyanobacteria. The presence of a similar cluster in *Anabaena* sp. 90 (99% amino acid sequence similarity), which does not contain *cypE*, indicates the existence of a non-characterized molecule in this strain. Nonetheless, there is a difference in the order in which the genes are oriented, which may generate variations in amino acid condensation, forming analogues with similar molecular weight, but with different amino acid configurations. Acknowledgements: Postdoctoral FONDECYT-CONICYT N° 3130681, Consortium Algae fuels 09CTE16861-02, PhD CONICYT fellowship.

238. Phenoloxidase activity and its relationship with the specificity and selectivity in bipartite cyanolichens of the genus *Peltigera*

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In the bipartite lichens from the genus *Peltigera*, the lichenizable fungus (mycobiont) is associated with a cyanobacterial photobiont (cyanobiont). Various enzymatic activities probably associated with protection against pathogens, antibiosis and pedogenesis have been quantified from lichens. In members of the order Peltigerales, enzymes with phenoloxidase activity, like laccases and tyrosinases, have been described. On the other hand, phylogenetic analyses of the symbiotic pairs allow the mycobiont specificity and selectivity calculation. 50 lichen thalli were collected from two lenga beech forests in Coyhaique. DNA was extracted from each sample and the molecular markers 28S and 16S rDNA amplified to fungi and cyanobacteria, respectively. Specificity and selectivity were calculated based on the number of cyanobiont OTUs (OCs) related to each mycobiont OTU (OM), weighted by the genetic distance and the frequency of each pair. Besides, for a representative from each OM, phenoloxidase activity was determined by spectrophotometry. Marked differences were observed in the phenoloxidase activity of the 6 OMs tested, which is apparently not directly related to the mycobiont specificity/selectivity. However, it was found that OMs which exhibit low mycobiont phenoloxidase activity are associated with the most abundant OC. Therefore, the role of these enzyme activities would be more related to the mycobiont functions than with the partner selection. FONDECYT 11100381.

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239. Cell Division Proteins Interact with PBP2b and Contribute to Morphogenesis in *Streptococcus pneumoniae*

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The penicillin-binding proteins (PBPs) are enzymes involved in cell wall synthesis and cell division in *Streptococcus pneumoniae*. We have previously described that Cp1015 strain containing *pbp2b* mutations (from clinical strains that confer β -lactam resistance) showed a pneumococcal subpopulation with bacillary shape, atypical septum formation and positioning, frequent asymmetrical divisions, and a PBP2B and FtsZ delocalization. These results suggested that PBP2b is involved in essential processes related to cell shape determination and cell division. By two-hybrid assays, we revealed that PBP2b interacted with different proteins, such as ErzA, MreC, RodA and FtsA, which were involved in cell division, but in other bacteria with different cell division mechanisms to *S. pneumoniae*. To determine the contribution of these proteins in pneumococcal morphogenesis, we constructed the *mreC*, *rodA*, *ftsA* and *erzA* mutants by insertion-duplication mutagenesis using the pEVP3 vector. These mutants displayed small colonies, fitness and morphological alterations. We also detected cell-wall biosynthesis alterations by vancomycin-fluorescein staining in all mutants. These results suggested that ErzA, MreC, RodA and FtsA could contribute, together with PBP2b, to determine the pneumococcal cell shape of *S. pneumoniae*.

240. Preliminary characterization of a bacteriocin-like compound produced by an Antarctic strain of *Pseudomonas*.

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Bacteria inhabiting extreme ecosystems have been recognized as valuable sources of novel bioproducts. Production of inhibitory compounds against pathogenic bacteria by *Pseudomonas*, a dominant taxa in Antarctic, has been widely demonstrated. The abundance of bacteriocin genes in *Pseudomonas* suggests that these proteinaceous compounds are secreted in various niches as anti-competitor compound, however, to our knowledge, only a few have been described from Antarctic strains. Thus, the aim of this work was to identify bacteriocin-like compounds (BLC) produced by Antarctic *Pseudomonas* spp. All strains tested produced antimicrobial activity against *S. aureus*. The inhibition halos were clearer on King B medium (KB) than LB, however, only 12B14 strain was able to secrete the antimicrobial growing in liquid and solid medium. Given the suppression of pathogens by *Pseudomonas* through siderophore-mediated competition for iron, we evaluated the pyoverdine secretion and antimicrobial activity produced by 12B14 strain growing in KB with Fe (III). Addition of iron inhibited the pyoverdine production but did not eliminate the inhibitory effect against *S. aureus*. Finally, to gain an insight into the chemical structure of the antimicrobial compound, we tested its thermo-stability and sensitivity to proteases, resulting in high tolerance to heat and loss of activity after proteinase K treatment, similar to some BLC previously described. Acknowledgments. CORFO and CONICYT Fellowship Program.

241. Antibacterial activity determination of cobalt(III) and copper(II) complexes with carbohydrate ligand.

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For several years, our research group has focused its interest in the coordination chemistry of transition metals with ligands having oxygen and/or nitrogen donors and their association with the activity and/or modulation of biological processes. In this work we present the study of the antibacterial properties of two metal complexes with sugars: [Co(1,10-phenanthroline)₂ Sucrose](I₃)₂ and [Cu(acetate)(CH₃-α-DMAP).

The formulas of these complexes are proposed based on circular dichroism, UV-Vis absorption spectroscopy and ¹H-NMR in solution studies. The antibacterial activity of these complexes was evaluated by discs assay, using a panel of Gram-negative bacteria (*E. coli*, *S. enterica* sv Enteritidis, *K. pneumoniae* and *P. aeruginosa*) and a panel of Gram-positive bacteria (*E. faecalis*, *B. cereus*, *Micrococcus* sp. and *S. aureus*).

The results indicated that only Co(III) complex exhibits antibacterial activity against species tested. In contrast, Cu(II) complex has no activity for any of strains used. On the other hand, we determined the minimum inhibitory concentration of Co (III) complex and evaluated its effect on *E. coli* HB101 reporter strain, using a colorimetric method on plate. The results of this assay indicated that Co(III) complex act through a bacteriostatic effect on the reporter strain. We also assessed the *in vitro* cytotoxicity this complex against HEK293 cells and found to possess low cytotoxicity on eukaryotic cells.

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242. Analysis of different DNA mismatches on RecA-catalyzed recombination *in vitro*

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Homologous recombination plays key roles in the generation of genetic diversity and DNA repair in both prokaryotes and eukaryotes. In bacteria, the initial stages of recombination are catalyzed by RecA, which promotes the alignment of two DNA sequences and initiates strand exchange between them. Previous studies have demonstrated that RecA is able to sense mismatches generated during the alignment of partially divergent DNA sequences preventing its recombination. The aim of this work is to study the effect of different DNA mismatches, as well as its number and distribution on the efficiency of recombination catalyzed by RecA of *Pseudomonas aeruginosa*. The kinetics of the RecA-catalyzed strand exchange reaction was determined *in vitro* by fluorescence resonance energy transfer using synthetic oligonucleotides. Here, we show that the position of a single G-T mismatch influences the RecA-catalyzed strand exchange. In addition, the presence of three G-T mismatches severely reduced recombination. These effects were strongly dependent on the type of mismatch (G-T, T-T or C-T). We obtained similar results with RecA from *Escherichia coli* and the eukaryotic homologue, Dmc1. Thus, our results reveal that RecA is sensible to the mismatch type as well as its location and number during the search for homology, a property conserved in other recombinases from bacteria and eukaryotes.

243. Complete nucleotide sequence of a widely dispersed lineage of a Mayaro Virus isolated from a febrile illness patient resident in a farming settlement in the Brazilian State of Acre.

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As part of a collaborative study on emergent arboviruses in Ramal do Granada, the largest farming settlement in the Brazilian State of Acre, we isolated a Mayaro Virus (MAYV) from the blood of a febrile illness patient. MAYV, an arbovirus member of the family Togaviridae, genus Alphavirus, is widespread in tropical South America, being endemic in rural areas surrounded by forests. Its transmission cycle is sylvatic including vertebrate and invertebrate hosts where the main mosquito vectors are *Haemagogus spp* and the vertebrate hosts are terrestrial mammalian and birds. MAYV is associated with a dengue-like illness. Difficulties in distinguish MAYV infection among emergent arboviruses by symptoms, the possibility of its transmission by *Aedes aegypti* associated to the recent demographic changes and new lifestyles throughout South America, make out this arbovirus as one of the major emerging disease in the neotropics. Molecular phylogenetic studies have recognized two MAYV lineages: genotypes widely dispersed (D) and limited (L). The complete nucleotide sequence of the Acre isolated MAYV genome showed high homology to the MAYV D genotype which is more than 15% divergent from L genotype at the nucleic acid level. Molecular genetic analysis of MAYV isolates can provide clues as to its maintenance patterns and geographic distribution in South America. Acknowledgment: FAPESP (Grant 2009/11.347-6)

244. Vascular effect of the proanthocyanidins (PAs) from seed and skin *País* grape extracts in human chorionic vein

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Proanthocyanidins (PAs) from grape seeds and skins have been associated with *in vitro* inhibition of angiotensin I-converting enzyme (ACE) activity. We evaluated the effect of the mean degree of polymerization (mDP) of PAs from seeds and skin extracts from *País* grapes in the vasorelaxation of human chorionic vein. Extracts purification were performed in a size exclusion medium [Toyopearl TSK HW-40 (F)] to obtain rich PAs fractions from *País* seeds and skin grapes (Quillón, Chile). For the chemical characterization, fractions were submitted to degradation by acid catalysis in the presence of phloroglucinol and subsequent analysis by RP-HPLC. Vascular reactivity was assessed in a wire miograph system using human chorionic vein rings in the presence of thromboxane A2 analog (U46619) and hydrogen peroxide (H₂O₂). A total of nine purified fractions (F1S to F6S from seeds; F4P to F6P from skins) were obtained and characterized. PAs fractions have mDP ranging from 2 to 17 in seeds and 7 to 49 in skins. The pretreatment of human chorionic veins with F5P and F6P (100 µM) significantly (p<0.05) decreased chorionic vein vasoconstriction in the presence of U46619 (33% and 78%, respectively) or H₂O₂ (86% and 81%, respectively) compared to control. Our results showed that vascular protective effects of grape seeds and skins extracts were positively related to their mDP. Skin polymeric fractions (F5P and F6P) might be of interest like a potential antihypertensive phytodrug.

245. Role and Regulation of Ryanodine Receptor Expression in Synaptic Plasticity (LTP)

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The ryanodine receptor (RyR) channels present in intracellular Ca²⁺ storage organelles are ubiquitously expressed in many types of cells. By mediating Ca²⁺ release from these stores, RyR channels participate in a variety of important Ca²⁺ signaling processes. In particular, the endoplasmic reticulum of hippocampal neurons possesses the three mammalian RyR isoforms (RyR1, RyR2 and RyR3). Herein we tested the effects of RyR inhibition on LTP induction by theta burst stimulation (TBS) in the CA3-CA1 circuit of rat hippocampal slices, and determined the associated changes in RyR mRNA and protein levels by Q-PCR and Western blot analysis, respectively. Our results show that RyR inhibition prevented LTP induction by TBS, but did not affect paired-pulse responses suggesting a postsynaptic mechanism. LTP induction by TBS produced after 1 h a significant increase in RyR1 and RyR2 mRNA levels and in RyR2 protein content, but did not modify RyR3 mRNA and protein levels. Inhibition of RyR activity by pre-incubation of slices with inhibitory ryanodine prevented both the induction of LTP and the increased RyR1/RyR2 expression. We interpret these results as an indication that RyR-mediated Ca²⁺ release plays a central role in hippocampal synaptic plasticity. **Support:** BNI-09-015F; FONDECYT 1100052; Beca MECESUP; Millennium Scientific Initiative (IC-09-022-P and BNI P-09-015F).

246. Identification and characterization of interleucin-like growth factors in the human pathogen *Trichomonas vaginalis*.

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INTRODUCTION: *Trichomonas vaginalis* is the causative agent of human trichomoniasis, a sexually transmitted human infection, with ~170 million cases occurring annually worldwide. Cellular proliferation is a critical step in parasite colonization of the urogenital epithelium. However, the molecular mechanisms involved on *T.vaginalis* proliferation are not well understood. Observation of our laboratory have shown an exponential cell growth, when the initial inoculum is ranged between 250,000 to 500,000 parasites mL⁻¹ whereas at lower cell number, proliferation was not observed. **MATERIAL AND METHODS:** *T. vaginalis* growth factors in the genome of the parasite, will be cloned and expressed. Tests of proliferation, antisense oligonucleotides, confocal immunofluorescence, electronic microscopy and pulse-chase assays, will be realised. **RESULTS:** *T. vaginalis* cultures were incubated with parasite culture supernatants obtained after 4h incubation, the parasite number was increased in 50-60% when compared with the controls incubated with Diamonds medium alone. A dose-dependent increase of the parasite number (37-60%) was observed in trichomonas cultures incubated with the recombinant growth factors respect to controls incubated without it. **DISCUSSION:** These observations allow us to suggest that *in vitro T. vaginalis* proliferation is inoculum dependent and could be induced by protozoan secreted growth factors.

247. Different roles of the Andes hantavirus Gc cytoplasmic tail and transmembrane region in virus-cell fusion

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Entry of enveloped viruses into a host cell is generally driven by fusion of virus-cell membranes, a process that involves multiple consecutive steps, among them a hemifusion intermediate in which the outer monolayers are merged while the inner monolayers are still intact, followed by fusion pore formation and extension. This process is driven by viral fusion proteins that contain different functional domains such as fusion peptide, stem region, transmembrane region (TM) and cytoplasmic tail (CT). Different studies have shown that TM and CT of class I and class III fusion proteins play an important role in driving complete mixing of membranes and fusion pore expansion whilst still less information is available for TM and CT of class II fusion proteins. The fusion activity of hantaviruses has been associated with the Gc envelope glycoprotein that shares characteristics with class II fusion proteins. In this work, the role of Gc-TM and Gc-CT in virus-cell fusion was assessed. To this end, different deletions and residue substitutions of Gc-CT and Gc-TM were produced. The results show that all mutants except Gc-Δ7TM were expressed and accumulated at the cell surface. Further, Gc-ΔCT and Gc-Δ1TM mediated fusion, whilst the residual mutants lost fusion activity in cell-cell fusion assays. This data indicates that Gc-CT is dispensable whilst the length and specific residues of Gc-TM are key for the fusion activity of Gc. Funding: FONDECYT 1100756 and CONICYT PFB-16.

248. Cell bioassay as a toxicological tool for detection of paralytic shellfish poison.

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The paralytic shellfish poison (PSP) is a group of neurotoxins present in shellfish contaminated with toxic microalgae *Alexandrium catenella*, which main analogue is Saxitoxin (STX). The development and implementation of *in vitro* toxicological methods more sensitive than mouse bioassay, are of international concern. The aim of this study was to evaluate the cell bioassay developed by Manger et al. (1993), which uses mouse neuroblastoma cells (Neuro2a), ouabain and veratridine as complementary toxins to detect the presence of standards PSP. Two different VPM extraction protocols were evaluated in real samples: AOAC 2005.06 and an alternative extraction protocol, and were compared by HPLC. STX dose-response curve was established to characterize the cellular response for PSP for detection of PSP in cell culture in real samples. Using the AOAC 2005.06 extraction protocol we obtained 170 µg eq. STX / 100g shellfish, and using the alternative extraction protocol we obtained 101 µg eq. STX / 100g shellfish, 30% lower compared to the AOAC 2005.06. Manger cell bioassay responds in a dose-dependent manner to the presence of standards of PSP with an EC50 of 3.5 ng / mL for STX. When the real sample was extracted with AOAC protocol and evaluated with the cell bioassay, the PSP concentration detected was 168.5 µg eq. STX / 100g shellfish. This indicates that the cell bioassay is useful for detection of PSP in real samples. This research was supported by FONDEF Di1140

249. Conjugation of CdTe-GSH Quantum Dots to IgG and IgY for immunoassays

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Immunoanalytical methods are widely used by research and diagnostics laboratories due to their potential to detect almost any target molecule. One of the great advances in this field in the past decades has been the development of fluorescently labeled IgGs as secondary detection reagent. Fluorescent labelling allows multiplexing, fluorescent emissions in different wavelengths can be selectively detected using optical filters and therefore more than one signal can co-exist in an assay without interfering. Currently, a limiting factor in the penetration of this technology to research laboratories in emerging economies is the cost of these reagents. We have developed inexpensive fluorescently labeled secondary antibodies using quantum dots (QDs) to label chicken immunoglobulins (IgYs) and commercial donkey anti-goat IgGs. Chicken total IgYs were purified from egg yolk through precipitation with Polyethylene Glycol (PEG) and donkey anti-goat IgGs were obtained commercially from Rockland Immunochemicals. CdTe-GSH QDs were functionalized with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide-hydrochloride (EDC) and N-hydroxysuccinimide (NHS) in borate buffer. Functionalized, amine reactive QDs were then crosslinked to Chicken total IgYs and donkey anti-goat IgGs. Both IgG and IgY conjugation to QDs were confirmed by SDS-PAGE. These labeled immunoglobulins and the functionality of QD-labeled IgGs was verified by Western Blot using Goat IgG as the target molecule.

250. Improved method for the biomimetic synthesis of biocompatible fluorescent nanoparticles for multiplex analytical assays

Valderrama, I¹, Barra, P¹, Acuña-Rougier, C¹, Pérez-Donoso, J², Gutiérrez, M¹, ¹Biolmaging Lab NEON - Creative Labware, ²Nanobiotechnology Lab, School of Biological Sciences, UNAB. (Sponsored by María Antonieta Valenzuela)

Quantum Dots (QDs) are semiconductor nanoparticles (NPs) that emit fluorescent signal at different wavelengths (λ) depending on their size when they are excited by UV light. Most QDs are very expensive because of complicated synthesis methods, and they are not compatible with living matter because of their high toxicity. Our group developed an inexpensive biomimetic synthesis method that produces less toxic biocompatible QDs. This work aims to use these QDs for multiplex determination of analytes, yet this method produces QDs with a broad bandwidth that does not allow for selective documentation of individual signals. Through variations in the synthesis protocol we aim to reduce the bandwidth of these QDs and increase their range of emission wavelengths without affecting their biocompatibility. We modified the reactant concentrations, incorporation times, synthesis temperature, buffers composition, and synthesis pH of the synthesis protocol and achieved a 30% reduction on bandwidth and an average increase in intensity of 95%. DLS studies show that the reduction of bandwidth is associated with a decrease in particle size range. Finally, through Minimum Inhibitory Concentration (MIC) studies using *E. coli* and *S. aureus* we determined that these NPs have a 60% decrease in toxicity and thus may be more biocompatible than the ones synthesized with the previous method.

251. Spectrophotometric Mobile Technologies for Science and Education

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In the current technological context it seems incredible that in the educational field some of the most widely distributed innovations of our time are not being used. For this reason, Lab4U is developing a mobile App (SpectroCell) that is capable of using the camera as a sensor, allowing a smartphone or tablet to be used as a spectrophotometer. This seeks to bring a tool into the classrooms and homes of thousands of students and scientists who will be able to learn spectrophotometric techniques and analyse samples in inaccessible places, where the presence of a common spectrophotometer it is not viable. The App needs a single picture for its calibration curve and samples to be analysed. Samples are dropped on a surface as individual drops; subsequently the App identifies the droplets on the surface, the known concentrations was entered as additional input. RGB and HSV values of each pixel were determined to calculate the RGB and HSV value for each drop, next a nonlinear regression of the calibration droplets was performed by the App to obtain a calibration curve; then the sample's RGB or HSV average values was interpolated to deliver the sample's final concentration. Our results indicate a calibration plot with a R=0,995, suggesting that SpectroCell can be used as a basic spectrophotometer. Thus, Lab4U pretends to give a new approach to Science Education where thousands of scientists will be able to have a hands-on Lab equipment in their pocket.

252. Development of an inexpensive imaging platform and consumables for multiplex western blotting

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Immunoassays, such as western blot, have migrated from monochromatic film-dependent techniques to multicolor applications that allow multiple target detection by using discriminable optical characteristics of labeling fluorophores. The adoption of this advance in the field by researchers in developing countries has been slow given the prohibitive cost of instruments and consumables. We have developed an inexpensive documentation system for the selective digital imaging of secondary antibodies labeled with custom made quantum dot fluorophores that will allow researchers to detect up to three different analytes in a protein blot. Our system can be commercialized for a fifth of the price of current instruments, thus allowing widespread access of this technology to researchers in Latin-America. By using a cooled CCD camera, bandpass optical filters, consumable UV light source and a dark box setup we are able to selectively document the fluorescent signal from three differentially labeled secondary antibodies, thus detecting three different proteins in a western blot setup. The signal intensity obtained from our system allows quantitative measurement of an analyte, determined by a dilution standard curve of a protein sample. This system provides comparable analytical capacity to that of a film-dependent chemiluminescent western blot, yet allowing multiplex detection.

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253. E-cadherin-dependent regulation of Caveolin-1-enhanced Rac1 activation and migration in metastatic cancer cells.

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At later stages of cancer, elevated Caveolin-1 (CAV1) levels are associated with enhanced cell migration and metastasis. Both events are modulated by the expression of E-cadherin, which potentiates CAV1 tumor suppressing activity, and suppresses CAV1-enhanced metastasis *in vivo*. However, the mechanisms underlying these E-cadherin-mediated functions and specifically how E-cadherin reduces CAV1-enhanced Rac1 activation and cell migration is not known. B16-F10 mouse melanoma and HT29(US) human colon cancer cells (lacking both CAV1 and E-cadherin) were stably transfected with CAV1 alone or co-transfected with E-cadherin and CAV1. Protein expression was monitored by western blotting. Cell migration was evaluated in transwell assays. Rac1 activation was determined using pull down assays. In both B16-F10 and HT29(US) cells, expression of CAV1 increased cell migration and Rac1 activation. Co-expression of E-cadherin with CAV1 blocked cell migration and Rac1 activation induced by CAV1. Presence of E-cadherin reduced CAV1-enhanced Rac1 activation and migration of metastatic cancer cells.

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254. Mechanism of Trypanosoma gambiense resistance to human serum.

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Trypanosoma brucei gambiense accounts for 97% of human sleeping sickness cases. Human immunity to African trypanosomes is due to two serum complexes designated trypanolytic factors (TLF-1 and -2), which both contain haptoglobin-related protein (Hpr) and apolipoprotein L-I (apoL1). While Hpr association with haemoglobin (Hb) allows TLF-1 binding and uptake via the receptor TbHbHbR, TLF-2 enters independently of TbHbHbR. ApoL1 kills trypanosomes by insertion into endosomal/lysosomal membranes. Here we report that T. b. gambiense resists TLFs via a hydrophobic β -sheet of the T. b. gambiense-specific glycoprotein (TgsGP), which prevented apoL1 toxicity and induced stiffening of membranes. Two additional features secured resistance to TLFs: reduction of sensitivity to apoL1 requiring cysteine protease activity and TbHbHbR inactivation due to a L210S substitution. According to such defense mechanism, transgenic expression of T. b. brucei TbHbHbR in T. b. gambiense did not cause parasite lysis in normal human serum. However, these transgenic parasites were killed in hypohaptoglobinemic serum, following high TLF-1 uptake in the absence of haptoglobin (Hp) that competes for Hb and receptor binding. TbHbHbR inactivation preventing high apoL1 loading in hypohaptoglobinemic serum may have evolved because of the overlapping endemic area of T. b. gambiense infection and malaria, the main cause of hemolysis-induced hypohaptoglobinemia in Western and Central Africa.

255. Caveolin-1 promotes migration and invasion of metastatic cancer cells via a novel Rab5-Rac1 signalling axis

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Caveolin-1(CAV1) is a membrane protein that promotes cell migration and metastasis, augments Rac1 activation and binds to Rab5, a master regulator of early endosome dynamics implicated in migration. Whether the CAV1-Rab5 connection is relevant to cell migration remains to be determined. In the human colon cancer cells HT-29(US) and mouse melanoma cells B16F10, CAV1 was introduced by stable transfection with the plasmid pLacIOP(Cav-1). In MDA-MB-231 breast cancer cells, endogenous CAV1 expression was reduced by transduction with CAV1-specific shRNA constructs. The effect of CAV1 on Rab5 activation in these metastatic cell was assessed using R5BD pull down assays. The participation of Rab5 in CAV1-dependent Rac1 activation was determined by lentivirus-mediated transduction with shRNAs specific for Rab5 followed by Rac1 activity measurements using GST-PBD pull-down assays. Cell migration was evaluated in transwell assays. Invasiveness was determined in matrigel assays. Our results suggest that CAV1 promoted Rab5 and Rac1 activation, cell migration and invasion. Also, shRNA-mediated Rab5 knock-down reduced CAV1-enhanced Rac1 activation, cell migration and invasion. Finally, we propose a novel signaling axis, whereby CAV1 activates Rab5 and subsequently Rac1 to promote cell migration and invasion. **Acknowledgements:** FONDAP 15010006, FONDECYT 1130250 (AFGQ). FONDECYT 1110149; BNI P09-015-F (LL), FONDECYT 11100287, CONICYT 79090021(VT); CONICYT PhD fellowship (JD).

256. Mitochondria-ER communication is disrupted in cardiomyocyte hypertrophy

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Cardiomyocyte hypertrophy is a major predictor of progressive heart disease and an adverse prognosis. Pathological cardiomyocyte hypertrophy is characterized by increases in cell size and protein synthesis, changes in the organization of sarcomeric structure, energy homeostasis and disruptions in Ca²⁺ levels. This ion is a critical regulator of cardiac myocyte function. We investigate here the regulation of mitochondria-ER communication *in vitro*. The results showed a reduced coupling of both organelles during norepinephrine-dependent hypertrophy in cultured rat cardiomyocytes. Significant decreases in InsP₃R-dependent mitochondrial Ca²⁺ uptake and levels of the mitochondrial calcium uniporter (MCU), while increased NOGO-B protein levels were also observed in hypertrophied cardiomyocytes. The decrease of the protein Mfn2 involved in mitochondria-ER interaction was sufficient to stimulate cardiomyocyte hypertrophic response in the absence of an hypertrophic stimulus. Collectively these results suggest that mitochondria-ER communication plays a key role in the development of cardiac hypertrophy. Supported by Anillo ACT1111 and FONDECYT 1120212 (SL), FONDECYT 15010006 (EJ), FONDECYT 3110114 (RT) and FONDECYT 3130749 (CP).

257. Estrogen and progesterone receptors in normal canine mammary tissue during the reproductive cycle

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The estrogen and progesterone receptors (ER, PR) concentrations were investigated in mammary gland of normal bitches in proestrus (n = 3), estrus (n = 2), diestrus (n = 4) and anestrus (n = 3). Cycle stages were classified by vaginal cytology and circulating levels of progesterone (P). Progesterone concentrations were determined by radioimmunoassay. The ER and PR determinations were performed by ligand binding assay. The ligands used were 3H-E2 for ER or 3H-ORG-2058 for PR, while nonlabelled ligands were diethylstilbestrol and ORG-2058 respectively. The ER, PR and P concentration (mean±pooled s.e) were analyzed by ANOVA and was considered significant p≤0.05. The P concentration was higher in diestrus than in proestrus, estrus and anestrus (52±11, 0.93±13, 2.81±15, 0.25±13 ng/mL; respectively). There was a significant effect of stage of the reproductive cycle on the ER and PR concentration. The ER and PR concentrations (fmol/mg proteins) were lower in diestrus than proestrus and estrus (20±3.2, 63±3.6, 61±4.5; for ER and 20±2.6, 28±2.9, 44±3.7; for PR respectively). The ER concentrations also were lower in diestrus than anestrus (52±3.7) while for PR were similar (14±3.0). The lower ER and PR concentrations found in diestrus may be explained by the inhibitory effect of the higher P circulating levels. This is consistent with the known up- and down-regulation exerted by the estrogen and progesterone on the receptors expression.

258. Activation of the kinin B1 receptor induces cellular migration of estrogen-sensitive breast cancer cells

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We have previously shown that stimulation of the kinin B1 receptor (B1R) increases proliferation and release of metalloproteinases 2 and 9 in breast cancer cells lines, effects that depend on epidermal growth factor receptor (EGFR) activity and subsequent ERK1/2 MAPK phosphorylation. Now, our goal was to determine whether activation of the B1R with its specific agonist, Lys-des[Arg⁹]bradykinin (LDBK), is able to induce a migratory and invasive phenotype in MCF-7 estrogen-sensitive breast cancer cells. This phenotype is characterized by a decrease in the levels of E-cadherin and an increase of α -smooth muscle actin (α -SMA).

Cells were stimulated for 72 h with increasing concentrations of LDBK prior to migration assays and evaluation of E-cadherin and α -SMA levels, two proteins that regulate cell migration. LDBK (100 nM) produced an increase between 2 to 4 folds in cell migration, which correlates with a decrease in the levels of E-cadherin, and increased levels of α -SMA. Preliminary results show that this event could depend on EGFR transactivation, given that migration rate was significantly reduced (75%) when the inhibitor of EGFR-tyrosine kinase activity, AG1478 was used.

These results suggest that the kinin B1R may be an important mediator of migration in estrogen-sensitive breast cancer cells, a functional activity that is crucial for metastasis.

Supported by FONDECYT

259. Generation of stable breast cancer cell lines with silenced or augmented vitamin C transporter expression

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The role of vitamin C in cancer remains controversial, with different groups supporting apparently irreconcilable positions against or in favor of using vitamin C as an anticancer agent. An important finding made during the study of the relationship between vitamin C and cancer is the altered expression of the ascorbic acid transporter SVCT2 in breast cancer cell lines and tumor breast tissue. In this work, we aimed to produce stable breast cancer cell lines with silenced or augmented SVCT2 protein expression to address the issue of the pathophysiology of vitamin C in cancer cells. We constructed two viral packaging vectors (pLCW/shRNA-SVCT2 and pLCW/shRNA-scrambled control), using a shRNA anti SVCT2 sequence that decreases by more than 80% SVCT2 protein expression when transfected in HEK-293 cells. Each vector was co-transfected with three helper plasmids in HEK-293FT cells, and the lentiviral particles were used to infect breast cancer cell lines, which resulted in efficient silencing of SVCT2 protein expression. In parallel, breast cancer cell lines were transfected with a plasmid encoding SVCT2, and clones were selected for high SVCT2 expression. These tools are being used to study the role of SVCT2 in the resistance of breast cancer cells to oxidative stress and anticancer treatment.

260. Effect of LDL-Ox on genes related to inflammatory process in macrophages cells

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Isolated LDL fraction from human plasma was peroxidized *in vitro* with Cu⁺⁺. THP1 human cells (as monocytes and macrophages) were treated with LDL-Ox for 4, 8, 12 and 24 h. Monocytes were transformed into macrophages by adding PMA in the culture medium. We evaluated the expression of genes involved in the inflammatory (TNF α , FAT/CD36) and anti inflammatory process (11 β HSD1, MMR). The mRNA level of different genes was measured by quantitative real-time PCR. In THP1-monocytes, FAT/CD36 and ACAT1 gene expression related to LDL-Ox incorporation, fatty acid esterification and formation of foam cells increased at 8 h; however, THP1-macrophages needed longer time (24 h LDL-Ox treatment) to show the increment. Results showed, again, an increase of pro-inflammatory marker TNF α at short time incubation in THP1-monocytes and longer time in THP-1 macrophages. The 11 β HSD1 expression indicates the reduction of cortisone to cortisol (glucocorticoid active form). In THP1-monocytes, the effect of LDL-Ox promotes a significant increase of 11 β HSD1 expression at all the times tested; however, in THP1-macrophages the increment was only at short times. The increase of the gene F4/80 (macrophages marker) and the decrease of MMR (anti-inflammatory macrophages marker) suggest that longer LDL exposition times promoted the proliferation of pro-inflammatory macrophages population. In conclusion, monocytes showed a more rapid pro-inflammatory response than macrophages type.

261. The Antisense Non Coding Mitochondrial RNAs modulates expression of Cell Cycle and Survival Genes

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The family of non-coding mitochondrial RNAs (ncmtRNAs) which displays differential expression between cancer and normal cells has been studied in our laboratory as a tool to generate a selective cancer therapy. Knockdown of ASncmtRNAs in mouse and human cancer cell lines induces massive apoptotic death, without affecting viability of normal cells. Apoptosis is preceded by cell proliferation inhibition, displaying downregulation of Cyclins B1 and D1. This treatment also induces down-regulation of Survivin, a member of the inhibitor of apoptosis (IAP) family, which is upregulated in virtually all cancers. Additionally, Bcl-2, another apoptosis-related protein, is relocated to the nucleus, which has been reported to induce apoptosis. Interestingly, the localization of Bcl-2 to mitochondria depends on the protein FKBP38, which is downregulated in tumor cells by the same treatment. These results show that selective tumor cell death by this approach is brought about by a simultaneous "attack" on different fronts, showing promise for the application of this molecular target for an efficient and safe cancer therapeutic strategy. (Grants: PhD Scholarship, Conicyt, Fondecyt 1110835, Conicyt, Chile and INOVA-Corfo 12IEAT-16317).

262. DNA double strand breaks on *RUNX1* gene are induced by etoposide treatment of differentiated blood cells

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Treatment of cancer patients with topoisomerase II inhibitors such as etoposide has as known side effect development of secondary leukemia. About 10% of the patients with secondary leukemia present the chromosomal translocation (8;21), which involves *RUNX1* and *ETO* genes. All the breakpoints mapped in the *RUNX1* gene for t(8;21) are clustered in three regions (named BCR1-3) located in intron 5 of the *RUNX1* gene. Interestingly, one of this BCR (BCR3) co-localize with both a DNase I hypersensitive site and a topoisomerase II cleavage site. A molecular mechanism to explain the DNA double strand breaks (DSBs) formation during etoposide treatment propose that DNA damage is the result of radicals produced by myeloperoxidase activity. HL-60 cells and neutrophils are known to express myeloperoxidase. Therefore, we hypothesize that in progenitor as well as differentiated blood cells etoposide treatment induces specific DNA DSBs at *RUNX1* gene locus. To test this hypothesis we treated HL-60 and blood cells with increasing concentrations of etoposide and assessed DNA integrity at BCR3 using quantitative real time PCR. Our results show that leucocyte treatment with etoposide induces DSBs in intron5 of the *RUNX1* gene. Therefore, our results demonstrate that etoposide induced chromosomal damage traditionally associated to bone marrow cells also occur in differentiated blood cells.

FONDECYT 1130697

263. Doxorubicin inhibits autophagy in cultured rat cardiomyocytes

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Doxorubicin (DOX) is one of the most widely used and successful anticancer drugs with a dose-dependent cardiotoxicity. The mechanism underlying DOX-induced cardiotoxicity is not completely understood. The main toxic mechanism involves oxidative stress but recent studies show that antioxidants do not reduce cardiotoxicity, suggesting that other mechanisms might contribute to this toxic effect. Autophagy is a vital process in the heart, participating in the removal of dysfunctional components and serving as a catabolic energy source during times of starvation. Autophagy serves to protect cells and may also contribute to cell damage. In this study, we evaluated the long term effects of DOX on cardiomyocyte autophagy and its relationship to cell viability. Cultured rat cardiomyocytes were stimulated with DOX 1 μ M for 24 h and autophagy markers LC3-II and p62 were analyzed in condition of autophagy flux. The results showed that DOX treatment for 24 h inhibits autophagy. The LC3-II levels were also diminished even in presence of Bafilomycin A (Baf A). Furthermore, p62 levels remained reduced in presence of Baf A, which could suggest that DOX activates other degradation pathways. Stimulation of DOX for 24 h also increased the percentage of cell death compared to untreated cardiomyocytes. Therefore, we conclude that the inhibition of autophagy by DOX is associated to cardiomyocyte death. Supported by grant Anillo ACT1111 (to SL) and CONICYT PhD fellowship (to MP)

264. Physiological effects of adenosine in podocytes and in diabetic glomerulopathy

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Introduction. It has been described that diabetic glomerulopathy progresses with alterations of the podocyte cells function. The most remarkable feature is the deterioration of the filtration barrier. Interestingly, the nucleoside adenosine increases locally during the progression of diabetic glomerulopathy, probably having a pathogenic role. Our aim will be to determine the role of adenosine in mediating the dysfunction of podocyte cells.

Results. Primary cultured podocytes expressing specific markers were obtained from glomeruli of rats. Exposure of podocytes to adenosine leads to the activation of RhoA and actin redistribution. The expression of adhesion molecule integrin α 3 was decreased upon treatment with adenosine. Both effects were blocked using an antagonist of the adenosine A_{2B} receptor subtype. Furthermore, adenosine induced changes in protein composition in the slit diaphragm. Using an antagonist of adenosine A_{2B} receptor *in vivo*, the glomerular expression of VEGF and nephrin were restored and the increased levels of proteinuria normalized in diabetic rats. *Conclusions.* Adenosine mediates the dysfunction of podocyte cells by inducing the rearrangement of the cytoskeleton and slit diaphragms. Both effects are relevant in affecting the integrity of the glomerular filtration barrier because *in vivo* intervention using an antagonist of adenosine A_{2B} receptor ameliorates the proteinuria in diabetic animals.

265. 5-aza-2'-deoxycytidine treatment induced changes in histone acetylation pattern at the p16 and MLH1 promoters in HL-60 and HeLa cells.

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DNA methylation and histone modifications are two epigenetic mechanisms involved in gene regulation. Importantly, the levels and patterns of these epigenetic marks are altered in cancer. During the past few years, the DNA demethylating agent 5-aza-2'-deoxycytidine (5-aza-dC) has been used as an alternative treatment for certain types of cancer. Several studies have shown that 5-aza-dC treatment induces re-expression of some tumor suppressor genes in cancer cell lines. Among the genes that are usually silenced during the transformation processes are the p16 and MLH1 which are involved in cell cycle regulation and DNA repair respectively. We hypothesize that global DNA demethylation induced by 5-aza-dC treatment result in changes in histone H3 and H4 acetylation at the promoter region of p16 and MLH1 genes. To test this hypothesis we evaluated changes in histone acetylation at the p16 and MLH1 gene promoters in HeLa and HL-60 cells treated with 5-aza-dC by chromatin immunoprecipitation (ChIP) assays. Our results show that 5-aza-dC treatment induced changes in gene expression that are accompanied by an altered pattern of acetylated histone associated with the promoter region of the corresponding gene. Notably the changes both at the expression level and at histone acetylation status are gene and cell specific. FONDECYT 1130697

266. Antioxidant properties of the *Pinus radiata* bark extract in salmonid cell lines *in vitro*.

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Fish *enteritis* is one of the inflammatory diseases associated with the presence of soybean meal in aquatic feed. The inflammation in the *enteritis* increases the levels of reactive oxygen species producing fish digestive mucosa damage. We evaluated the bioactive properties of the *Pinus radiata* bark extract *in vitro* by the determination of the extract's cytotoxicity and antioxidant activity. Cytotoxicity of the extract was measured in a salmonid cell line (CHSE-214), using the MTT cell viability assay. The antioxidant extract capacity was evaluated using DCFH-DA method in salmonid cell lines (SHK-1 and ASK), to determine intracellular levels of reactive oxygen species. In addition, the capacity of the *P. radiata* extract to protect DNA from oxidative damage was assessed using the comet assay. The *P. radiata* bark extract demonstrated to be innocuous up to 50µg/mL. Also, presented an antioxidant activity close to 90% at 50µg/mL, higher than vitamin C: 60% and the commercial *P. pinaster* extract Pycnogenol: 10%, at the same concentrations. Also, 100µg/mL of the extract showed a protective effect against DNA damage in SHK-1 cells exposed to hydrogen peroxide (50µM-100µM) preventing DNA fragmentation. The preincubation with the extract decreased the comet assay parameters as tail DNA, tail moment, and olive tail moment. The antioxidant properties of the *P. radiata* bark extract suggest that it could be used to counter the effects caused by the reactive oxygen.

267. P27Kip1 is involved in autophagy-dependent survival of Tsc2-null cells during rapamycin treatment.

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Tuberous sclerosis complex (TSC) is a disorder that results from inactivating mutations in the *tsc1* or *tsc2* genes, and is characterized by development of benign tumors in a variety of organs. Rapamycin and its analogs are the most common drug therapies for TSC. Tumor shrinkage has been reported for specific manifestations of TSC, but on withdrawal from rapamycin, these tumors rapidly regain size. Autophagy is one of the cellular processes known to be activated upon rapamycin treatment. Current evidence identified that autophagy activation is necessary for the survival of TSC tumor cells. However, it is still unknown the molecular mechanism by which autophagy is activated during rapamycin treatment of these cells. To investigate it, we used Tsc2-null MEF cells, and analyzed autophagy activation by the expression and cellular distribution of LC3, a well-known autophagy marker. Survival was determined by quantification of ATP levels, and by fluorescence-based cell membrane integrity assay. We found that the cell cycle inhibitor p27kip1 (p27) is involved in autophagy activation during rapamycin treatment of Tsc2-null cells. Moreover, p27-dependent activation of autophagy is necessary for the survival of these cells. *These results highlight a potential survival mechanism that could explain in part the inefficacy of the rapamycin treatment of TSC tumors.* Funded by FONDECYT 1120923

268. Emotional stress increases cFOS and Arc early gene products: correlation with mRNAs and protein levels of Matrix Metalloproteinase (MMP) 9 in Rat hippocampus.

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Stress modifies the neurocircuitry and behavior probable involving transcriptional activation of immediate early genes (IEG) to control genes related to synaptic plasticity. **Objective:** We examine whether acute restraint stress increases glutamate neurotransmission contributing to regulation of mRNAs, in particular metalloproteinase (MMP)9 considering its role in excitatory synapse remodeling in rat hippocampus. **Methods:** Male Sprague-Dawley rats were stressed by restraint during 0.5 h or 2.5 h, sacrificed immediately after stress session or 6 h and 24 h after stress. Hippocampi were processed for mRNA and protein extraction. **Results:** The expression of the IEG mRNA activity-regulated cytoskeleton-associated protein (Arc) increases 3 fold in parallel with a rise in Arc protein (1.5 fold) observed only during the first 30 min of stress; suggesting an increased excitatory neurotransmission during stress procedure. Also we observed that the expression of IEG mRNA cFos increases at least 7 fold in comparison to the rise observed in its putative target gene MMP-9 (twice) only during 30 min of stress. A rise in MMP-9 protein levels and activity was observed immediately after stress procedure in contrast to the sustained increase in the processing of β -dystroglycan, a specific MMP-9 substrate. It will be necessary to demonstrate whether or not the MMP-9 mRNA translation is an activity-dependent process in the stress model. FONDECYT 1120528.

269. Analysis of SNAP/NSF complex in the mouse oocyte physiology

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Following fertilization, cortical granules (CG) undergo exocytosis (CGE) to release their content into the perivitelline space, avoiding polyspermy and ensuring normal embryonic development. Several studies suggest that CGE is a SNARE-mediated process; however, the molecular mechanism is still incomplete. We hypothesized that SNAP/NSF complex regulates CGE. RT-PCR results showed that α - and γ -SNAP (but not β), and NSF are expressed in mouse oocyte. WB analyses indicates that these proteins are present during oocyte maturation and egg activation. Immunolocalization reveals that all identified proteins are predominantly observed in the CG-enriched cortical region. Furthermore, we showed that microinjection of specific antibodies against either α -SNAP or NSF inhibits SrCl₂-stimulated CGE in a dose-dependent manner. To further confirm these results *in vivo*, we started the characterization of a spontaneous α -SNAP mutant mouse known as hyh. So far, we have determined that hyh female have a strongly reduced *in vivo* fertility and, under hormonal ovary stimulation, hyh female mice yield four times less oocytes than wild type mice. In addition, mature oocytes from hyh mice exhibit a reduced density and an atypical distribution of CG. Altogether, our results strongly suggest that α -SNAP/NSF complex regulates CGE, and that a failure of SNAPs and/or NSF might have consequences still not uncovered.

270. Sall2 - NUA1 relationship in cell survival under metabolic stress.

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Sall2 is a poorly characterized transcription factor, member of the Spalt gene family involved in neurogenesis and differentiation. Interestingly, Sall2 is deregulated in various cancers suggesting a role in the disease. However, the role of Sall2 in tumorigenesis is still controversial. Previous work from our laboratory suggests that Sall2 plays a role in cell survival under genotoxic stress. Here, we investigated the role of Sall2 in cell survival under metabolic stress as metabolic deregulation promotes cell transformation. We found that Sall2 expression is necessary for the survival of MEF cells under metabolic stress, as cell survival was significantly decreased in Sall2- deficient MEFs. Bioinformatics analysis of putative Sall2 targets identified NUA1, a kinase involved in tumor progression and cell survival. qPCR and Western blot analysis of NUA1 indicated that its expression is decreased in Sall2-deficient cells supporting a relationship between Sall2 and NUA1. Furthermore, the survival of MEFs under metabolic stress was decreased by shRNA-induced depletion of NUA1. The survival rate of NUA1 depleted MEFs was similar to that of Sall2-deficient MEFs. These data suggest that Sall2 targets NUA1 to promote cell survival under metabolic stress. FONDECYT 1110821.

271. Chronic stress - inadaptability and its relationship with imbalance in mirnas levels in rat hippocampus

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OBJECTIVES: This study compare changes in the levels of miRNAs in rat hippocampus during transition from an adaptive response induced by acute restraint stress with a maladaptive promoted by chronic stress.

METHODS: Male Sprague-Dawley rats were stressed by restraint during 0.5h or 2.5h, sacrificed immediately after the stressor event. Other groups of animals were subjected to stress (2.5h/ day) for 1-7(subchronic) and 14 days (chronic) and sacrificed 24 hours after the last stress event. The hippocampi were dissected and RNAs were obtained with commercial kit.To analyze the levels of microRNAs we used miRNAs Arrays chip and RT-qPCR.

RESULT: We found that restraint stress induces an increase in the levels of neuronal immediate-early gene (IEG) *c-fos* and *Arc* mRNAs at 0.5h and after 7 days (2.5h/day). Furthermore, analysis of miRNAs showed an increase in 132-miRNA, 134-miRNA and 138-miRNA during 0.5 and 2,5h of stress. However in subchronically and chronically stressed animals the levels of 132-miRNA, 134-miRNA returns to control value; in contrast 138-miRNA decreased in comparison to control.

CONCLUSION: We conclude that the restraint stress produces differential changes in the levels of microRNAs 132, 134 and 138 in hippocampus. These studies may be crucial to understand the pathophysiology effect of stress especially mood disorders.

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272. Electrical stimuli release atp to increase ros production via p2y1-nox2 in skeletal muscle fibers

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Introduction During exercise, skeletal muscle produces ROS via NOX2 which induces some adaptations associated with contractile activity; the signals involved in this mechanism are debated. ATP is released from skeletal muscle during electrical stimulation and can signal autocrinely through purinergic receptors, we hypothesized that it may influence ROS production. The aim of this work was to measure ROS production induced by electrical stimulation and extracellular ATP in adult skeletal muscle fibers. **Material and Methods** 8-10 weeks old male C57BL/6J micewere used. ROS Production was evaluated by CM-DCF probe and for a plasmid that encodes for fluorescent HyPer protein. All experiments were performed in at least three different mice and results are expressed as the mean $\pm$ S.E.M. *p **Results** Electrical stimulation triggered a ROS transient increase in muscle fibers. The ROS production was mimicked by extracellular ATP and was prevented by CBX and suramin, antagonists of pannexin channel and purinergic receptor respectively. MRS2365, a P2Y1 receptor agonist induced a large signal while UTPyS (P2Y2 agonist) elicited a much smaller signal that was mimicked using ATP plus MRS2179, an antagonist of P2Y1. **Discussion** Electrical stimulation releases ATP into the extracellular medium, which is necessary to increase ROS production via P2Y1-NOX2. Financed by Anillo ACT 1111, Fondecyt 1110467

273. Sortin2 effect analysis in the development of lateral roots in different mutants of *A. thaliana*

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Plant root system modifies its structure by inducing root growth and developing lateral roots (LR) in a postembryonic process. It has been shown that the hormone auxin tunes LR development depending on the SCF^{TIR} auxin receptor. However, we have described a novel pathway with independence of the SCF^{TIR} by inducing endocytic trafficking using the compound Sortin2 in *Arabidopsis*. Our goal is to determine the molecular mechanisms activated by Sortin2 to induce such developmental program and its dependence on endocytic trafficking. A genetic approach looking for *Arabidopsis* mutants with different sensitivities to Sortin2 would contribute to tackle this goal. We have selected known mutants with impairments in protein trafficking to determine their Sortin2 sensitivity. In order to evaluate hormonal regulation of Sortin2-induced LR, mutants with defect in hormone signaling were tested. The analyzed mutants have different sensitivities to Sortin2. Interestingly, mutants in genes involved on phospholipases transport and intracellular trafficking are resistant to Sortin2 LR-induction. Also, five mutants result in hypersensitivity to Sortin2 whose LR index is 3 times higher than wild type plants. Alternatively, we are performing a genome-wide Sortin2 suppressor screening that will bring molecular players of the Sortin2 mode of action inducing LR. We expect by this means to unravel the role of protein trafficking in postembryonic LR development and its regulation in plants. FONDECYT 1120289

274. Differential effect of restraint stress on dorsal and ventral rat hippocampus: changes in mRNA levels of plasticity markers and glutamate receptors subunits

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Stress produces structural and functional changes in the brain, and is associated with some psychiatric disorders such as depression. Hippocampus is a structure related to neuroplasticity, and is particularly sensitive to stress. The evidence indicates that the ventral and dorsal hippocampus differ in their function. While the dorsal hippocampus (posterior in primates) has been mainly related to cognitive functions, the ventral hippocampus (anterior in primates) is related to stress response, emotion and affect. Both hippocampal fractions differentially contribute in various processes of memory and learning. Restraint stress causes changes in mRNA levels of certain markers of neural plasticity, as well as on subunits of ionotropic glutamate receptors (NMDA and AMPA). There is evidence that the expression of these receptors is modified in response to stress in rat prefrontal cortex and this could be related to plastic changes produced in acute and chronic stress. In this work we compare the changes in mRNA levels of plasticity markers and subunits of glutamate receptors such as AMPA and NMDA in the ventral and dorsal hippocampus of rats subjected to acute and chronic stress by restriction of movement. This information contributes to a better understanding of the molecular changes involved in the opposite effects elicited by acute and chronic stress in rat hippocampus. FONDECYT 1120528.

275. Understanding the role of Sall2 in the regulation of cell cycle.

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Sall2 is a member of the *Spalt*-like gene family of transcription factors. Members of this family have been associated with inherited developmental disorders, but the cellular role and targets of Sall2 have been poorly characterized. By bioinformatic analysis, we found putative binding sites for Sall2 in various cyclins promoters, suggesting a role for Sall2 in the regulation of cell cycle. Accordingly, we showed that Sall2 mRNA expression in mouse embryonic fibroblasts (MEFs) oscillates during the cell cycle, reaching its peak in the S phase. In parallel, we analyzed mRNA expression of cyclins B, D1/D2 and E1/E2 throughout the cell cycle. The expression profile of these cyclins in wild type MEFs was compared to that of Sall2-null MEFs. We found that Sall2-null MEFs show altered expression profile of these cyclins, but most significantly show increased mRNA and protein expression of cyclin E1. In addition, by luciferase transcriptional assays, we demonstrated that overexpression of Sall2 inhibits cyclin E1 promoter activity. These results suggest that Sall2 negatively regulates cyclin E1 gene expression, supporting a role for Sall2 in cell cycle progression. Funded by FONDECYT Regular 1110821

276. Metabolomic profiling during ripening of peach fruits: common and distinct metabolic processes among peach varieties

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Rosaceous fruits display a great variety of flavours, textures and metabolites with nutrition and health-related properties. During ripening of peach (*Prunus persica* L. Batsch), the complex network of metabolites is dramatically altered, affecting the nutritional quality, flavor and aroma. The sugars/organic acids ratio at the ripe state, significantly contributes to the overall flavor of the fruit. Here, metabolic profiling during ripening of 15-peach varieties was assessed by Gas Chromatography–Mass Spectrometry. Data obtained at harvest and ripened stages reveal clear metabolic shifts among varieties. During ripening, some sugars were increased and others were not modified: xylose and fucose increased in all varieties, while maltose, sucrose and trehalose did not change their relative levels. The increase of fucose could be related to cell wall degradation during softening. With respect to sugar alcohols, myo-inositol and maltitol did not vary during ripening, while galactinol, an important compound involved in plant-tolerance to various stresses, was dependent on the variety. The pattern of variation of organic acids and amino acids was diverse. Putrescine was drastically reduced during ripening, in agreement with its potential role as a senescence inhibitor. Overall, data obtained highlight singular metabolic programs for peach ripening, which may allow the identification of key factors related to agronomic traits of this important crop species

277. Phenylpropanoids biosynthesis pathway are activated in response to stem inclination in seedlings of radiata pine

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Tree inclination response is a widely studied biological phenomenon, however the molecular mechanism is still unknown. Addressed to characterize the response to inclination stress in radiata pine, seedlings were tilted at 45°. Based on a Suppressive Subtractive Hybridization (SSH) transcriptomic approach, eight libraries were generated. Bioinformatics analyses revealed that many genes showed differential expression. One of the most interesting groups of genes was the related to the phenylpropanoids compounds. Phenylpropanoids biosynthetic genes analyzed by qPCR showed an induction after inclination, in a temporal and spatial differential manner along the stem. In addition to the analysis of expression profile, an auxin response gene, which is repressed in presence of the hormone, showed a differential expression pattern along and across the stem. Additionally, flavonols quercetin and kaempferol showed an accumulation according to the expression profile observed for their biosynthetic genes. The results suggest that auxin distribution is involved in the inclination response according to the Cholodny-Went hypothesis, indicating a concerted activation of genes that generates a misbalance in local auxin distribution that finally induces stem reorientation. The results obtained leading to a greater understanding of the molecular mechanism that governs the biological process. This project was supported by FONDECYT (11121170, 1120635) and PBCT anillo ACT-41.

278. Phenylpropanoid metabolites are required in the Antarctic ecotype of *Colobanthus quitensis* (Kunth) Bartl. to tolerate UV-B radiation

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Colobanthus quitensis is the only one dicot plant in maritime Antarctica, where is exposed to different environmental conditions, such as low temperatures, high salinity and high UV-B environment. In laboratory conditions, *C. quitensis* was exposed to low, medium and high UV-B intensity, in presence and absence to aminooxiacetic acid (AOA), an inhibitor of phenylalanine ammonialase (PAL) -the key enzyme of phenylpropanoid pathway-. The results show that the plants with inhibited PAL at medium and high UV-B intensity have oxidative damage and photosystem II (PS II) efficiency reduced respect to the low UV-B intensity. Plants under low UV-B intensity increase PAL activity and phenolic metabolite concentrations. These results suggest that the phenylpropanoid pathway may be the key in the tolerance of *C. quitensis* to the high UV-B in Antarctica.

279. Ectopic application of EXLX1 increases grain weight in *Triticum aestivum*

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Expansins are proteins that induce cell wall expansion. These proteins were discovered in the 1990's and were classified into alpha and beta expansins. The exact action mechanism of these proteins is poorly understood and attempts of overexpressing them in heterologous systems remain unsuccessful. As an alternative way to replace their activity in field experiments, the protein named EXLX1 from *Bacillus subtilis* is interesting because it was found to possess a structural similarity with the plant expansins. This protein is encoded by the gene *yoaJ* and exhibits similar characteristics with the beta expansins. This protein is used by *Bacillus subtilis* to colonize the plant root and enhance plant-bacteria interaction. In the present work we cloned this protein as described in Georgelis et al. 2011, with a hexa histidine tag, we expressed it in *Escherichia coli* BI21 (DE3) and successfully purified it. We performed an assay in *Triticum aestivum* (cv. Bacanora) and we found that the ectopic application of EXLX1 promotes an increased weight in the grains. Funding: Fondef "Formulados de expansinas para aumentar el rendimiento del trigo" (D091125)

280. Development of an *in vitro* culture protocol for micropropagation of Chilean strawberry (*Fragaria chiloensis*)

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There is an interest to develop Chilean strawberry (*Fragaria chiloensis* (L.) Mill) into a commercial crop due to its organoleptic qualities such as aroma, flavor and white-pink coloration. Local farmers use plants propagated through runners which reduces production and spread pathogens. We proceed to develop a protocol for its *in vitro* culture with the objective of generating high productivity disease-free plants. Lateral buds of the stolon were used as starting material and several asepsis, multiplication and acclimation treatments were analyzed to determine the best conditions for its culture. NaOCl at 0,4% for 8min generated the lowest oxidation to the buds which allowed their fast activation, while 2μM zeatin in Murashige & Skoog (MS) medium generated a great number of plantlets ready to be acclimated in a short time. Rooting and *ex vitro* acclimation supplemented with an MS solution generated an optimal root growth and survival in greenhouse. Plantlets showed a temporal rejuvenation during their *in vitro* culture, which could be caused due to hormonal adjustments. Our protocol allows the production of over 100 plantlets per bud in a period of 5 months which develop without malformations, generating stolons and floral buds the next year. This work was supported by the FONDECYT 11110171 project.

281. The effect of tetracycline in the natural antioxidant properties of chilean unifloral honeys.

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Chilean honeys are produced from several native vegetables species with interesting biological properties.

In many cases, it is possible to find certain kind of undesirable chemical compounds, such as antibiotics used for veterinary purposes owing to the treatments for improving the production of honeys and other bee products. This fact may affect the original properties of a given honey.

Honey of Tevo (*Retanilla trinervia*) with free content of antibiotic was used for this study. Three samples were fortified with increasing amounts of tetracycline (5 µg/mL; 10 µg/mL and 15 µg/mL, respectively). A fourth sample remained without fortification (control sample).

The Chromatographic analysis showed an important modification in the retention time of the phenolic compounds. This change was related with the increased concentration of the added tetracycline. The antioxidant activity assessed by FRAP assay was slightly stronger in samples with higher antibiotic's fortification. The antiradical activity determined by DPPH assay showed no significant differences among the obtained results.

In this work, it is done the assessment of the changes in the chromatographic profiles caused by an antibiotic.

Finally, it is discussed the correlation with the modification of the natural antioxidant properties and the fortification of samples with tetracycline.

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282. Analysis of VvNAC-1: a NAC transcriptional activator found induced in grapevine's compatible viral interactions

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The NAC transcription factor family is found only in flowering plants. They are associated with a wide range of process such as leaf and root development, and tolerance to biotic and abiotic stresses. Some NAC Transcription factors share a common domain of 20 amino acids called NACRD (NAC repressor domain), which has been recently reported as an inhibitor of transcriptional activation.

In this work, we cloned and characterized the VvNAC-1 gene from *Vitis vinifera* which is induced in viral infected leaves and berries. We demonstrated the nuclear localization of the protein fused with GFP. Modeling of VvNAC-1 DNA binding domain showed that it has similar architecture with NAC crystallized proteins. The sequence analysis revealed an imperfect NACRD within the N terminal of VvNAC-1. To address whether the VvNAC-1 it's a transcriptional activator, and if it's putative NACRD is functional, we constructed several VvNAC-1 mutants and perform transactivation and repression assays on yeast, using b-gal as a quantitative reporter.

Using GrapeHunt we found genes that have high enrichment of NAC binding elements in their promoters. Some of those genes were induced in the infected tissues and are associated with processes such as RNA binding, RNA metabolism and defense.

To identify direct target genes of NAC-1 we cloned promoters of putative target genes and perform an activation assay in *planta*, using luciferase as a reporter.

Acknowledgments: FONDECYT 1100709

283. Functional Characterization of Pollen-Specific Kinases Trough the Generation of Knock-down Transgenic Plants Expressing Artificial microRNAs (amiRNAs).

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The pollen grain is a three-celled organism that is derived by stereotypical cell divisions. Inside the anther, the sporogenous initial cells undergo meiosis to form a tetrad of cells that is enclosed in a thick callose wall. Every microspore enlarges and goes through an asymmetric mitosis that results in two cells, the larger cell also called vegetative cell that will form the pollen tube and a smaller cell, called generative cell, that is engulfed inside the cytoplasm of the vegetative cell, then undergoes a second mitosis to form two sperm cells. During fertilization, the pollen tube transports directionally the sperm cells to the ovule to produce the double fertilization event. Little is known about signal transduction pathways and molecular components involved in these processes. Using microarray data, we identified 4 genes that encode for protein kinases and were named PSK1 to 4 (for Pollen Specific Kinase). To functionally characterize these genes, we've generated transgenic plants expressing artificial microRNAs using a pollen specific promoter. amiRNAs has been successfully used in plants to silence endogenous genes and they are generated by exchanging the miRNA sequence of endogenous precursors with artificial sequences to create constructs targeting the gene of interest. Analysis of pollen development, germination and tube elongation will be performed to establish the physiological role of these genes. Funded by Fondecyt 1120766 and UNAB DI-74-12/R.

284. Transcriptional and post-transcriptional regulation of four pollen-specific genes coding for protein kinases in *Arabidopsis thaliana*

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Mature Pollen grains of *Arabidopsisthaliana*, poses two haploid sperm cells within a large haploid vegetative cell. During fertilization the vegetative cell produces a pollen tube, which grows rapidly a tip structure that drives along the stigma the sperm cells into the embryo sac where one sperm cell fertilizes the egg to form the zygote, and the other fertilizes the central cell to produce the endosperm. Despite the biological relevance of this event, little is known about the molecular mechanisms that regulates and directs pollen development and function. Using microarray data we have previously identified 4 genes encoding kinase proteins (PSK1 to 4, for *POLLEN SPECIFIC KINASE*) that are expressed exclusively during the last stages of pollen development, germination and pollen tube elongation. Sequence analysis of their promoter regions showed the presence of several pollen-specific *cis* elements and the putative vegetative repressor *Germline Restrictive Silencing Factor* (GRSF). In order to characterize the specific regulation of these genes we have designed a series of 5' deletions, based on the sequence analysis, fused to β -glucuronidase gene (GUS) to isolate the role of each *cis* element on the spatio-temporal expression of PSK genes.

285. Effects of the plant growth-promoting bacteria *Burkholderia phytofirmans* PsJN in the auxin-signalling pathway gene expression in *Arabidopsis thaliana* plants

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Plant growth-promoting rhizobacteria (PGPR) are soil microorganisms that induce positive effects in plants, such as increased growth or reduced stress susceptibility. The molecular mechanisms underlying these interactions have not been studied in depth. We have previously shown that the PGPR *Burkholderia phytofirmans* PsJN increases growth, modifies the ontogeny and induces transcriptional changes in hormone related-genes pathways in *Arabidopsis thaliana* plants. In this work we studied the effect of short time-PsJN treatments in the expression of genes involved in the synthesis, perception, transport or signaling pathway of the auxin hormone in *A. thaliana*. The expression patterns of these genes were analyzed in a temporal and spatial context. Then, we realized cluster analyses to identify group of genes with early or late regulation, finding that some of these genes presented up or down-regulation as soon as 2 hours after inoculation, while others changed their expression patterns later at 24 hours after treatment. These changes correlate with an increase in different growth parameters in the plants. These results suggest that early changes on auxin-related genes expressions could be involved in the plants phenotypes that are observed later in plant ontogeny. Fondecyt 11121306, NM-PFG (P10-062-F).

286. Functional analysis of the grapevine *VvMYB24* gene reveals its possible role in anther development.

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MYB proteins constitute a superfamily of transcription factors involved in processes such as cell differentiation, flavonoid synthesis and stress responses. In *Arabidopsis*, *AtMYB24*, a member of the R2R3-MYB family, is expressed mainly in flowers and, together with *AtMYB21*, it has been associated with anther development. Transgenic plants over-expressing (OE) *AtMYB24* present dwarf flowers, non-dehiscent anthers, abnormal pollen grains and male sterility, while *AtMYB24* mutants do not show any phenotype due to its redundancy with *AtMYB21*. We characterized the R2R3-MYB family in grapevine and identified a gene with significant homology to *AtMYB24*, which was named *VvMYB24*. Here, we characterized the *VvMYB24* gene. *VvMYB24* coding region was cloned from *Vitis vinifera* cv. C. Sauvignon flowers. Its sequence showed the conserved signatures from the R2R3 MYB family, specially the C-terminal domains belonging to the Subgroup 19. *VvMYB24* OE *Arabidopsis* plants were generated and lines with different expression levels were selected for further studies. Vegetative growth was similar in both wild-type and OE lines. However, reproductive growth was compromised in these lines, including premature anther maturation, reduced filament elongation, and presence of senescent ovules. Together, these results suggest that *VvMYB24* could be the *AtMYB24* ortholog gene in grapevine. Acknowledgement: Fondecyt 1100709 and Millennium Nucleus in Plant Functional Genomics P06-009-F.

287. Identification of salicylic acid-inducible genes coding for Glutathione S-transferases with antioxidant properties in the defense response to stress in *Arabidopsis thaliana*.

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Plants are constantly exposed to several types of biotic and abiotic stress conditions that lead to increases in reactive oxygen species (ROS) levels altering the cellular redox state. Although ROS are required as key signals for defense response, their levels must be controlled to avoid oxidative damage. Salicylic acid (SA) is a key hormone in the establishment of the defense response to stress in plants, and it has been shown that it is essential for both, the production and the contention of ROS. Here we assess the antioxidant role of SA in the defense response. SA induces the expression of a group of genes coding for proteins with antioxidant and detoxifying function, among them we are interested in evaluate the role of Glutathione S-transferases (GSTs). After a bioinformatics analysis we selected 8 GST genes which expression is induced under different stress conditions where SA is involved. We have confirmed the expression pattern by real-time PCR under basal and stress conditions in wild-type plants and its dependency of SA in plants that are deficient in SA biosynthesis. Our results indicate that SA induces the expression of a set of GST genes in a spatio/temporal specific manner, suggesting a particular role for them in controlling ROS levels in the defense response. Supported by FONDECYT (1100656) and Millennium Nucleus for Plant Functional Genomics (P10-062-F).

288. Biochemical and structural characterization of a α -D-galactopyranosidase from the pectinolytic fungus *Penicillium purpurogenum*

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The filamentous fungus *P. purpurogenum* degrades plant cell walls by means of cellulolytic, xylanolytic and pectinolytic enzymes. Among the enzymes degrading pectin is α -D-galactopyranosidase which acts on α -1,6 bonds present in galactooligosaccharides. GALP1, a α -D-galactopyranosidase of *P. purpurogenum*, was heterologously expressed in *Pichia pastoris* and purified. The enzyme is active against p-nitrophenyl- α -D-galactopyranoside, melibiose, raffinose and stachyose, with respective Km values of 0.12, 0.04, 3.96 and 4.74 mM. The activity is inhibited by high concentrations of galactose, a competitive inhibitor. The structure of GALP1 was built by comparative modeling, validated and refined with molecular dynamics simulation (MDS). The model structure is composed of 16 β strands and 17 helices (11 α helices and 6 3_{10} helices), the catalytic residues are D133 and D225 at a distance of 10.1 Å. A 2 ns MDS showed that D133 and D225 residues are exposed to a small pocket, allowing their interaction with substrates. Conformational interaction between GALP1 and several ligands was explored by molecular docking. The most favoured predicted substrate orientation was observed for melibiose (-5.0 kcal/mol of affinity energy), and the lowest for stachyose (-3.4 kcal/mol). There is a perfect coincidence between the kinetic studies and molecular docking analysis. Support: FONDECYT 110084 and 1130180; UNAB DI-61-12/R; CONICYT Fellowship to L.M. (24120932), Anillo ACT-1110.

289. Effects of oxidation of lysozyme by hypohalous acids and haloamines on enzymatic activity and aggregation

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Hen egg white lysozyme (HEL) an antibacterial enzyme is a prototype protein for studying the physical and chemical events that underlie the formation of amyloid fibril aggregates. Here, we studied alterations in enzymatic activity and aggregation provoked by oxidation of HEL by hypochlorous acid (HOCl), hypobromous acid (HOBr) taurine chloramine (Tau-NHCl), taurine monobromamine (Tau-NHBr), and taurine dibromamine (Tau-NBr₂). In Add of only 4-fold molar excess of Tau-NHBr or Tau-NBr₂ to HEL caused complete depletion of its intrinsic fluorescence, whereas HOCl and HOBr caused 40%–50% bleaching. The selective effect of bromamines on tryptophan residues had a direct effect on enzymatic activity; bromamines were about two-fold more effective as inhibitors of HEL than the acid precursors. The oxidation of HEL by HOCl and HOBr was more effective regarding the aggregation of the protein, which was evidenced by increased turbidity, Rayleigh scattering, and anisotropy. The aggregation of HEL suggested the formation of amyloid fibrils as measured by the thioflavin assay. In conclusion, the capacity of Tau-NHBr and Tau-NBr₂ as inhibitors of the bactericidal activity of HEL could depict a role in the exacerbation of pulmonary infection, since leukocytes are rich sources of both taurine and HOBr. Moreover, the oxidation of HEL by just a small excess of hypohalous acids, a condition that could be found in inflammatory sites may represent a new pathway for initiation of aggregation

290. Study of lipid transport system in South American tarantula *Grammostola rosea*.

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Lipids due to its hydrophobic nature are transported in the hemolymph mainly by lipoproteic fractions. In the present work we studied the components of lipid transport of the spider *Grammostola rosea*. Through ultracentrifugation two lipoproteic fractions are isolated, one of high density named Gr-HDL ($\delta = 1.13\text{--}1.15\text{ g/ml}$) and the other of very high density called Gr-VHDL ($\delta = 1.23\text{--}1.25\text{ g/ml}$). Gr-VHDL in hemolymph is majority in relation to Gr-HDL. In this sense Gr-VHDL fraction has 98.6% of hemolymphatic proteins, transporting 89.3% of lipids circulating in hemolymph. Both lipoproteic fractions possess phospholipids such as majority lipids and 18:1, 16:0, 18:2 and 18:0 as the major fatty acids. Through *in vitro* assays with different ¹⁴C-lipid, it was observed that Gr-VHDL takes up mainly free fatty acids and triacylglycerols unlike that observed for Gr-HDL in relation to phosphatidylcholine. Through electrophoresis it was observed that Gr-VHDL has three apolipoproteins: a predominant band of 68 kDa and two others of 99 and 121 kDa. Gr-HDL displayed a predominant band of 93kDa and other minority of 249 kDa.

In conclusion, this study reports lipid and proteic characterization of the components of the lipid transport system in tarantula, using *G.rosea* as a model. The role of each protein in relation to lipid uptake is sustained by *in vitro* assays.

This work was supported by grants from: CONICET-PIP no. 075 and PICT no. 2010-1270, Argentina.

291. Cysteine residues are important determinants of functional and folding properties of the ascorbic acid transporter-2, SVCT2.

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SVCT2 belongs to the nucleobase/ascorbic acid transporter family NSCT2, is composed of 650 amino acid residues and the transport mechanism involves the transport of one ascorbate and two sodium ions per transport cycle. SVCT2 contains 14 transmembrane segments (TMs) grouped in two domains; a central domain formed by TMs 1-4 and 8-11 that contains an hydrophilic transport pore, and a second domain acting as a substrate-selectivity filter formed by TMs 5-7 and 12-14. We have initiated a systematic alkylation and site-directed mutagenesis study of cysteine residues to elucidate the structure-function of SVCT2. The alkylation studies showed that PCMB and NEM affect SVCT2 activity by reducing the transport V_{max} , without changes in K_m , probably by interruption of conformational changes that govern transport activity through long-range effects. Site-directed mutagenesis studies indicate that cysteine residues 113, 129, 187, 401 and 426 are important molecular determinants that govern the transport kinetic constants, probably by affecting the structural integrity of the pore-containing central domain. Functional and co-localization studies of a mutant SVCT2 without cysteine residues revealed that cys-less SVCT2 is a non-functional transporter that is trapped intracellularly. Overall, these results suggest a crucial role for cysteine residues in the folding and traffic of SVCT2 to the plasma membrane.

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292. Point mutations in GTP hydrolysis sites of both bacterial tubulin monomers (BtubA/B) causes loss of their function due to a low filament formation.

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Microtubules (Mts) are subcellular structures that play a crucial role in multiple functions. They are composed by the α/β tubulin heterodimer that was considered a cytoskeleton component exclusive of eukaryotes. However, it has been detected in *Prostheobacter* genus a well-defined bacterial cytoskeleton, which has among its components the bacterial microtubules. Its subunits are BtubA and BtubB which have high similarity with the beta and alpha monomers of tubulin. In our laboratory it was demonstrated that they are dynamic structures that show dynamic instability and treadmilling. To understand the dependence of this behavior on the GTP hydrolysis, in this work the T7 "loop" mutant proteins of BtubA/B monomers were overexpressed in bacteria and purified by ion exchange columns. The T7 "loop" is responsible for the GTP hydrolysis, that is involved in the depolymerization of BtubA/B and its structural homologs. The *in vitro* assays show a dynamic loss due to a low polymerization capacity, which suggests that the mutation of this site would disrupt the longitudinal interaction preventing the dimer formation, or the interaction between dimers. Supported by FONDECYT GRANT. 1130711.

293. Sequence evolution of the intrinsically disordered and globular domains of the adenovirus E1A oncoprotein

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Virus manipulate the host cell cycle through interaction with key host proteins. These interactions are mainly mediated by linear sequence motifs of 3 to 8 function-determining residues, often found within intrinsically disordered domains. The small DNA tumor virus adenovirus induces cell transformation in several model animals by the action of the Early 1A and 1B oncoproteins. The HAdV5 E1A gene encodes 2 major proteins that only differ in an internal sequence of 46 residues. The larger E1A consist of 5 regions: The less conserved N-terminal region, and four conserved regions designated CR1 to CR4. CR3 is absent in the smaller E1A. We used over 100 E1A sequences from known adenovirus types to construct sequence alignments. 4 E1A regions are found to be intrinsically disordered: N-terminal, CR1, CR2 and CR4, while CR3 is predicted to be globular. We study conservation and coevolution of the disordered regions and CR3. The intrinsically disordered regions of E1A show a high degree of conservation along its whole length without any long stretches of low conservation. Sequence analyses of this regions revealed 7 functional linear motifs, which partly explains the conservation pattern. Sequence conservation in the CR3 region can be explained by its main functions of zinc and target binding and structure stabilization. Additional cysteine residues proximal to the zinc-binding site may allow redox regulation of E1A function.

294. FtsEX interaction Network in the Bacterial Divisome.

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Bacterial Division is an important and complex process. The machinery (divisome) involved in this process includes more than 12 essential proteins, which are transcriptional, spatial and temporal regulated. The divisome formation can be separated in 2 steps. The first step involves the formation of the proto-ring with the proteins ZipA, FtsA and FtsZ, and in the second step the rest of the proteins are recruited sequentially to the septal ring. The first complex recruited in the second step is FtsEX, which poses structural and sequence similarity to an ABC transporter, where FtsE correspond to the ATP binding subunit and FtsX to the transmembrane subunit. Later proteins depend on the recruitment of FtsEX to be recruited. ATP binding to FtsE is critical for the division, but not in the formation of the divisome. We have expressed and purified FtsE and FtsX. The structural characterization has been performed by spectroscopic techniques like CD and fluorescence and we have determined the dissociation constants of the interaction of these proteins with the divisome network using MST.

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295. A CYSTEINE-LESS FORM OF THE GLUT2 GLUCOSE TRANSPORTER

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Glucose, one of the most important and ubiquitous biological in nature, is a polar molecule that requires transporter proteins to pass through the plasma membrane. The facilitative GLUT carriers are the most abundant glucose transporters since they are expressed in every cell including epithelial ones. The family of human GLUT carriers comprised 14 members of whom GLUT1 is the most characterized both structurally and functionally. GLUT2 is the main transporter in the liver and also participates in intestinal glucose absorption; therefore it has an important role in glucose homeostasis. However, there is no detailed information about its structure or kinetic properties. We aimed to prepare a GLUT2 derivative devoid of cysteine residues (GLUT2 C-less) to dispose of a model protein to deal with structural and functional studies. This construct was designed *in silico*, expressed in both *Xenopus laevis* oocytes and in CHO-K1 cells, and we analyzed some of its kinetic and regulatory properties. GLUT2 C-less is active, showing similar kinetic characteristics to those of native GLUT2 and is affected by known inhibitors, as wild GLUT2 does. Our data suggest that this derivative will be useful to address sophisticated studies of the structure and functional characteristics of the human GLUT2 transporter (Financed by FONDEF D0711171, FONDEF D1111131 and FONDECYT 1130386).

296. γ -tubulin-CCT interaction. A theoretical and experimental structural approach.

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The eukaryotic CCT chaperonin has a peculiar mechanism of specificity. It has been proposed that CCT has coevolved with its substrates, gaining, loosing or transforming the structures involved in the inter-molecular interaction. Within the CCT's substrates there are the tubulin family members. The specificity of this interaction has reached the point of that the tubulins are unable to fold to its native state in absence of CCT. We have performed an evolutionary approach to find sequence regions of γ -tubulin responsible of the interaction with CCT. Correlated mutation analysis together with sequence harmonic analysis were used to find these regions in both counterparts. Using the sequences of the predicted regions we expressed and purified three point mutants of γ -tubulin. One mutation was located in the main predicted interaction region (the T7 loop between H7 and H8), The other mutation was done immediately outside the predicted region (the M loop between S7 and H9) and a double mutant with both mutations. The mutant proteins were used for *in vitro* γ -tubulin-CCT interaction experiments supported by electron microscopy image analysis, and *in vivo*, by microinjecting the mutant proteins into zebrafish early embryos. We have demonstrated that mutations in the predicted regions of γ -tubulin significantly decrease the quantity of binding with CCT and modify its behavior inside the cell, supporting our theoretical analysis. Supported by FONDECYT and Fundación Carolina

297. Structural and Functional Characterization of Cargo-Binding Sites on the μ 4-Subunit of Adaptor Protein Complex 4

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Adaptor protein (AP) complexes facilitate protein trafficking by playing key roles in the selection of cargo molecules to be sorted in post-Golgi compartments. Four (AP-1 to AP-4) AP complexes contain a medium-sized subunit (μ 1- μ 4) that recognizes YXX Φ sequences, which are sorting signals in transmembrane proteins. A conserved, canonical region in μ subunits mediates recognition of YXX Φ -signals by means of a critical aspartic acid. Recently we found that a non-canonical YXX Φ -signal on the cytosolic tail of the Alzheimer's disease amyloid precursor protein (APP) binds to a distinct region of the μ 4 subunit of the AP-4 complex. In this study we aimed to determine the functionality of both binding sites of μ 4 on the recognition of the non-canonical YXX Φ -signal of APP. We found that substitutions in either of both binding sites abrogated the interaction with the APP-tail in yeast-two hybrid experiments. Characterization by isothermal titration calorimetry showed instead loss of binding to the APP signal with only the R283D substitution at the non-canonical site, in contrast to a decrease in binding affinity with the substitution D190A at the canonical site. We solved the crystal structure of the C-terminal domain of the D190A mutant bound to this non-canonical YXX Φ -signal. Together, our analyses support that the functional recognition of the non-canonical YXX Φ -signal of APP is limited to the non-canonical site of μ 4. Supported by FONDECYT 1100896.

298. Structural and thermodynamics characterization of variants from cardiac troponin C present in cardiomyopathic patients

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Troponin C (TnC) is the subunit of the troponin complex and regulates Ca^{2+} sensitivity. It is composed by two domains connected by an alpha helix. Mutations in TnC have implicated phenotypic characteristics as hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), and restrictive cardiomyopathy (RCM). Ventricular hypertrophy and fibrillation is common in HCM subjects and is associated with the presence of TnC variants including A8V, D145E, C84Y and A31S. In the other side, DCM is manifested by a ventricular dilation and wall thinning and variants were also reported as Y5H, M103I and I148V. The main goal of this work is to investigate the stability of these mutants in the presence or absence of Ca^{2+} using an ensemble of thermodynamic and structural approaches. Circular dichroism data against urea titration reveal a greater stability and prominent cooperative effect in N-domain variants as compared to those from the C-domain and wild-type. An automatic molecular modelling procedure revealed side-chain orientations that may explain this different behavior between the N- and C-domain variants. In addition small angle X-ray scattering data reveal that, in the absence of Ca^{2+} , mutants have a lower stability as compared to the wild-type protein, an effect not observed in the presence of Ca^{2+} . These observations open up new avenues for the comprehension of the complex behavior of HCM and DCM that has heretofore been not evaluated at structural level.

299. Structural features of potassium channel blocking by AcaIII1425, a novel toxin from sea anemone

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The venom of sea anemones is a known source of bioactive compounds, including peptides that act on voltage-gated ion channels. A novel toxin, AcaIII1425, was isolated from the venom of *Anthopleura cascaia*. It was demonstrated that this toxin selectively blocks rKV1.1, rKV1.6 and rKV4.3 potassium channel subtypes, being more potent in rKV1.6. To gain more insights into its specificity, we solved the three dimensional structure of AcaIII1425 using solution Nuclear Magnetic Resonance (NMR). It presents the typical cysteine-knot structural motif with three disulfide bonds, a three-stranded β -sheet and a small α -helix. Toxin dynamics was also probed by the acquisition of NMR relaxation parameters. Moreover, we studied AcaIII1425 interaction with rKv1.6 channel using peptides corresponding to specific sequence signatures of the channel: the selective filter, the post-filter region and the turret. The last one is believed to be the region responsible for the specificity of toxins, as it is not conserved between known potassium channels. The toxin was able to bind the turret peptide at pH 5.8 and the interacting residues were mapped using chemical shift variations. A docking model was obtained based on the NMR experimental parameters and it allowed us to depict the structural basis for the toxin interaction and specificity.

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300. NMR mapping of Human β -defensin 6 interaction with glycosaminoglycan: a model for extracellular matrix complex

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Human β -defensins (hBD) plays important roles in the innate and adaptive immune system when a threat is present. Defensins present other activities such as chemoattraction of a range of different cell through specific chemokine receptors and this chemoattraction could be regulated by glycosaminoglycan (GAG). In the present study we use NMR spectroscopy of ¹⁵N hBD6 to map the binding site for a synthetic pentasaccharide fondaparinux, a highly sulfated heparin mimetic. Chemical shift mapping of the interaction reveals a contiguous binding surface on hBD6, which comprises amino acid residues of the α -helix and the β 2- β 3 loop. The dissociation constant of the hBD6/GAG complex was determined by NMR spectroscopy to be $4 \pm 3 \mu\text{M}$. The formation of the hBD6/GAG complex was also confirmed by the overall decrease in ¹⁵N R₁ concomitant with an increase in ¹⁵N R₂ indicating an increase in the overall tumbling time of the protein. The pentasaccharide fondaparinux binds to hBD6 inducing dimerization and partially overlaps with the binding site for the chemokine receptor CCR2. The structural and dynamic data obtained in this study provide insights into the recognition of GAG by human defensin.

301. Prion Protein Aggregation Triggered By Neutrophil Extracellular Traps (NETs)

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Prion diseases are fatal neurodegenerative protein-misfolding diseases related to the prion protein (PrP). The constitutive cellular isoform of PrP (PrP^C) can be converted into its abnormal, β -sheet-rich isoform (PrP^{Sc}) undergoing aggregation. PrP^{Sc} can be transmissible and infectious. The evidences suggest that adjuvant factors may play a role in the conversion. Our group previously reported that DNA can convert PrP^C into the β -sheet conformation leading to aggregation. Neutrophil extracellular traps (NETs) are large DNA webs decorated with histones and granule proteins released by activated neutrophils. Recently, amyloid fibrils-associated NETs were found in tissues of patients with other protein-misfolding diseases. Moreover, NETs were reported of being released in lymphoid tissues which are described as an important site for prion replication leading to the spread of the disease to the brain. We asked if DNA present in NETs could induce PrP^C aggregation *in vitro*. Murine recombinant PrP was added into the supernatants from activated human neutrophils containing NETs. Aggregation was measured by light scattering and electron microscopy. Instantaneous aggregation was detected. It gradually declined remaining detectable after 1 hour. The aggregates showed amorphous morphology and absence of fibrils. Thus, NETs can trigger a stable aggregation of PrP^C and are an intriguing factor that must be appraised in the studies concerning prion propagation mechanisms.

302. Substrate specificity and fold evolution in the 6-phosphogluconate dehydrogenase family

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The members of the 6-phosphogluconate dehydrogenase (6PGDH) family catalyze the last step of the oxidative branch of the pentose phosphate pathway, oxidatively decarboxylating the 6-phosphogluconate (6PG) using NAD(P) as cofactors. The fold of this family is composed by a N-terminal dinucleotide binding domain and a C-terminal helicoidal domain that binds 6PG and establishes the interfacial surface for oligomeric interactions. Furthermore, although the helicoidal domain could be composed by single or duplicated hemi-domains in different members, the oligomeric packing follows similar trends suggesting that the structural determinants for the 6PG binding pocket are also conserved. Our phylogenetic results indicate that the duplication-fusion event of C-terminal hemidomain precedes the segregation of dinucleotide specificity traits in the family. We used 6PGDH from *Gluconobacter suboxydans* (Gs6PGDH) and *Escherichia coli* (Ec6PGDH) as representatives of single and duplicated-hemidomain members. Initial velocity studies were performed using wild type and mutant forms. Our results suggest that although the presence of the NRX₃K motif in the dinucleotide-binding pocket of Ec6PGDH correlates with a strong preference for NADP, the replacement for residues of a putative NAD motif, did not increase the affinity for NAD. Finally, homology modeling of Gs6PGDH and molecular docking were used to compare with Ec6PGDH the interactions at the 6PG binding pocket. FONDECYT1121170

303. Identification of residues conforming an allosteric regulatory site on the GLUT1 transporter

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Glucose transporter GLUT1 facilitates basal glucose transport in most animal cells. This is the most studied of the 14 members of the glucose transporter GLUT family. Multiple evidences indicate that several molecules structurally unrelated to sugars, such as cytochalasin B, flavones, isoflavones and methylxanthines, inhibit the functional activity of GLUT1. Kinetic studies have shown that there are binding sites accessible on the transporter outer (exofacial) surface that are essential for inhibition. Using an *in silico* high quality structural model of GLUT1 we identify potential functional sites and their constituent amino acid residues by performing molecular docking of various GLUT1 ligands. On this study, we tested the sensitivity of single mutants of some of these residues against quercetin, pentoxifylline and cytochalasin B, using trans-zero glucose entry assays in a transient expression system in HEK293 cells. We identified mutants that are refractory to inhibition by methylxanthines, without disturbance of other regulatory properties. This confirms that there is an allosteric site on the human GLUT1 exofacial surface explaining the inhibition of its activity by methylxanthines; this also putatively identifies amino acid residues conforming this regulatory site.

304. Physicochemical study of the transcriptional repressor NagC from *Escherichia coli*

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NagC is a transcriptional repressor of the ROK family. It regulates the transcription of the divergent *nagE-BACD* operon involved in the amino sugar metabolism and is essential for cell growth under amino sugars as the sole carbon source and also for the cell wall recycling. It has a Helix-Turn-Helix DNA binding domain at its N-terminal end. *N*-acetylglucosamine 6-phosphate (GlcNAc6P) binding site is near its the C-term end of the protein. The ligand GlcNAc6P acts as an inducer and dissociates NagC from DNA, allowing the expression of the divergent operon. Their DNA binding sites sequence are well known [Plumbridge, 2012] but its crystal structure and GlcNAc6P binding constants remain to be studied. Different conditions of ionic strength and acidity have been tested looking for better NagC stability. In this work we have purified the NagC protein and established the working conditions *in vitro* to study GlcNAc6P binding. A single tryptophan-quenching signal shows a slow change upon addition of GlcNAc6P; 45 minutes after the inducer addition, the biphasic relaxation kinetics is complete. This kinetics are complex and difficult interpretation. In the first phase a K_d , calculated from the fluorescence amplitude at different ligand concentrations, gave a K_d of $60 \pm 3 \mu\text{M}$. The same K_d was also obtained by direct binding experiments using ³H-GlcNAc6P. When the fluorescence stabilizes on the second phase it shows a K_d of $6.0 \pm 0.3 \mu\text{M}$.

305. A novel method to characterize interactions among biomolecules in living cells

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Studying interactions among biomolecules is very relevant in biology. Two parameters that characterize these interactions are the complex stoichiometry (CS) and its formation constant (Kf). Here, we present a novel method to evaluate these parameters in living cells which is based on the Number and Brightness (N&B) assay⁽¹⁾. This fluorescence microscopy technique provides the average number of moving, fluorescent molecules and their brightness at every pixel of images; this last parameter is related to the self-association state of the molecules. Previously, we have studied the mechanisms involved in the ligand-dependent homo-dimerization of the glucocorticoid receptor (GR) by using 1-color N&B. The activated GR interacts also with another transcription factors and co-regulators. To study hetero-interactions, we developed a 2-color N&B-based method that consists of analyzing the brightness in both channels as a function of the molecules concentrations. To test the method, we performed computer simulations, where we recovered the CS and the Kf of the simulated systems. The brightness maxima give the CS, while the Kf is given by the slopes. Also, we analyzed GR-mCherry and GR-GFP interactions in living cells. Our results are compatible with dimers formation with a very high Kf. We conclude that it could be a useful method to apply in our system of interest and generally for studying protein-protein interactions *in vivo*.

⁽¹⁾Digman *et al* (2008) Biophys J 94:2320-32

306. Combined inhibition of protein kinase CK2 and cyclooxygenase-2 is highly detrimental for viability of colon cancer cells. Potential pharmacological targets for diagnosis and treatment of colon cancer.

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Protein kinase CK2 and cyclooxygenase-2 (COX-2) have been related with progression and metastasis of colon cancer by regulating the Wnt/ β -catenin signaling pathway. Therefore, CK2 and COX-2 combined inhibition was performed in colon cancer cells. Expression of survivin, an Inhibitor of Apoptosis Protein family member, was evaluated by RT-PCR and western blot in colon cancer cells (HT29-ATCC, DLD-1 and HT29-US) that replicates increased malignity. CK2 and COX-2 catalytic activities were inhibited with TBB and celecoxib, respectively. Viability and apoptosis were determined by the MTS® and Caspase-Glo® assays, respectively. Elevated survivin expression was detected in colon cancer cells. Inhibition of CK2 and COX-2 significantly down-regulated cell viability, as observed by diminished proliferation and increased apoptosis. CK2 and COX-2 inhibition also correlated with decreased mRNA and protein levels of survivin. Altogether, these results identify to CK2 and COX-2 as relevant factors in cancer cell viability and malignancy. Moreover, CK2 and COX-2 are expected to be potentially interesting pharmacological targets in colon cancer diagnosis and treatment.

307. Thy-1-induced migration requires $\beta 3$ integrin expression and activation of the PI3K/Rac1 pathway in primary astrocytes

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Neuronal Thy-1 induces spreading, adhesion and migration of DI TNC1, but not primary astrocytes. This cyclic process engages $\alpha V\beta 3$ Integrin and Syndecan-4 receptors. Interestingly, upon damage to the central nervous system both receptors are up regulated in the brain. However, whether astrocytes respond to Thy-1 in an inflammatory environment is unknown. Thus, we evaluated whether TNF α -treated primary astrocytes respond to Thy-1, as reported for DITNC1 cells. In addition, we studied signaling mechanisms triggered downstream of $\alpha V\beta 3$ Integrin. We used primary astrocytes treated or not with TNF α and performed immunofluorescence analysis to evaluate cell polarization and cell adhesion, whereas migration was tested in wound-healing assays. $\beta 3$ Integrin levels were evaluated by immunoblotting and signaling events tested using genetic and pharmacological inhibitors. Results indicate that in primary astrocytes, $\beta 3$ Integrin expression increased after TNF α treatment. TNF α also render astrocytes responsive to Thy-1 as evidenced by increased adhesion, polarization and migration. Our studies indicate that DI TNC1 astrocyte migration involves FAK/PI3K, Tiam1 and Rac1 activation. Also, for TNF α -treated primary astrocytes treatment with PI3K or Rac1 inhibitors diminished cell migration. These observations are likely to be relevant to wound healing and regeneration. FONDECYT 1070699 and 1110149 (LL), 1090071(AFGQ); FONDAP 15010006 and ACT1111 (AFGQ); ICMP09-015-F (LL).

308. Light-dependent activation of Transient Receptor Potential (TRP) channels C5, A1 and V1 in *Ulva compressa* (Chlorophyta) exposed to copper excess

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Ulva compressa is a copper-tolerant macroalga that dominates copper-enriched coastal environments in northern Chile. *U. compressa* cultivated *in vitro* with a sub-lethal concentration of copper (10 μ M) showed increases in intracellular calcium at 2, 3 and 12 h of exposure. These increases require the entry of extracellular calcium and the activation of Voltage-Dependent Calcium Channels (VDCC). In order to study the potential activation TRP channels allowing calcium and other ions entry leading to membrane depolarization and the subsequent activation of VDCC, the alga was exposed to 250 μ M copper and to inhibitors of TRP A1, C5 and V1 (HC-30031, SKF-96365 and capzasepin) and 250 μ M copper for 20 min. Increases of intracellular calcium were observed at 4, 9 and 12 min which were inhibited by SKF, HC and capzasepin, respectively, indicating the activation of TRPC5, A1 and V1 by copper excess. Unexpectedly, the efficient activation of the latter channels required the exposure of the alga to the sun light. In order to analyze this effect, photosynthesis was inhibited with DCMU, an inhibitor of photosystem II, leading to the inhibition of copper-induced activation TRPC5, A1 and V1. Thus, copper induces a light-dependent activation of TRP C5, A1 and V1 channels in the marine alga *U. compressa*. This work was financed by Fondecyt 1130118 to A.M.

309. Characterization of a multiprotein complex containing PANNEXIN-1 in a muscle cell line

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Pannexin-1 (Panx1) releases ATP to the extracellular space in skeletal muscle cells in response to electrical stimulation. ATP is a relevant mediator between membrane depolarization and gene expression. We propose that this signaling requires coordination between several signaling molecules, among them DHPR, Panx1 and nucleotide P2Y receptors assembled as a multiprotein complex. Our aim is to characterize these protein-protein interactions in an L6 cell line stably expressing Panx1-STRIP-tag recombinant protein, as a model to unveil this signaling complex. Overexpression in L6 myotubes was induced with 5mM sodium butyrate and Panx1 recombinant protein expression was assessed by immunoblot and immunofluorescence. Protein-protein interactions were evaluated by co-immunoprecipitation and confocal microscopy with colocalization analysis. Panx1, DHPR and P2Y2 all co-immunoprecipitated in these cells together with Dystrophin and PI3K. Colocalization assays confirmed these results with high Mander's coefficients. L6 cells stably expressing a recombinant Panx1 correctly assemble a multiprotein complex with DHPR, P2Y₂, Dystrophin and PI3K, as previously described for the endogenous proteins in myotubes and adult fibers. Therefore, this new cell line constitutes an ideal model to purify and characterize the molecular components of this protein complex involved in Excitation-Transcription coupling. Fondecyt-1110467-11100454; Conicyt-22120686; ACT-1111

310. Key roles of sPLA₂ in the inflammatory response during retinal injury

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The aim of the present study was to analyze the implications of Group V secretory phospholipase A₂ (sPLA₂) in cyclooxygenase (COX)-2 and nuclear factor kappa B (NF-κB) regulation working with an *in vitro* model of age-related macular degeneration. Retinas incubated with increasing iron concentrations (50, 200 and 800 μM Fe²⁺) showed augmented levels of lipid peroxides and a decrease in cell viability. Iron content measured by ICP increased in a metal concentration-dependent manner, such increase being 400% (200 mM Fe²⁺) and 1100% (800 mM Fe²⁺) with respect to controls. sPLA₂ localization in the cytosolic fraction and its association with COX-2 increased as a function of iron concentration. However, COX-2 activity in sPLA₂ immunoprecipitates was inhibited in retinas exposed to iron. In iron-overloaded retinas, p65 (RelA) and p50 NF-κB nuclear localization was higher than that observed in controls. The inhibition of sPLA₂ (YM 26734) restored the nuclear localization of NF-κB subunits to control levels. Results from the present study suggest that sPLA₂ has multiple intracellular targets that could be related to inflammatory responses by its participation in NF-κB and COX-2 regulation.

311. Interleukin-6 induces ErbBs receptor phosphorylation, ERK1/2 pathway activation and utrophin expression in dystrophic myotubes.

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Duchenne muscular dystrophy (DMD) is a neuromuscular disease originated by mutations in the dystrophin gene. A promising pharmacological treatment deals with functional substitution of dystrophin by utrophin, a structural homolog that might be able to compensate dystrophin absence in DMD muscle fibers. It has been described that both interleukin-6 (IL-6) and neuregulin-1 (NRG-1) induce utrophin expression in skeletal muscle. We investigated a possible functional link among IL-6, NRG-1 and utrophin, in normal (C57) and dystrophic (*mdx*) skeletal muscle cells. Western Blot analysis allowed us to demonstrate that IL-6 (100 ng/ml) induces NRG-1 receptor phosphorylation (ErbB2/ErbB3) in both cellular types, and a transient increase on ERK1/2 phosphorylation only in dystrophic myotubes. Semiquantitative PCR showed that IL-6 treatment increases utrophin mRNA levels just in *mdx* myotubes. We observed that utrophin mRNA induction was abolished by BAPTA-AM (an intracellular Ca²⁺ chelator), GM6001 (a general metalloproteinase inhibitor), genistein (a general protein tyrosine kinase inhibitor) and PD-158780 (an ErbB receptor tyrosine kinase inhibitor) treatment. Our results suggest that IL-6 induces utrophin expression in *mdx* myotubes, through NRG-1/ErbBs signalling cascade activation. Soluble NRG-1 elicited by proteolytic processing of transmembrane NRG-1 might induce ErbBs phosphorylation and ERK1/2 pathway activation, leading to utrophin upregulation. FONDECYT 11100267

312. Incretin GLP-1 promotes endoplasmic reticulum – mitochondria communication in vascular smooth muscle cells by activating a PKA/Mitofusin-2 pathway

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Coupling of endoplasmic reticulum (ER) with mitochondria is a mechanism by which the function of these organelles can be controlled, mainly through calcium transfer. This process relays on the tethering protein Mitofusin-2 (Mfn2). This communication has been recently associated to the regulation of smooth muscle cells (SMC) phenotype and function, leading to beneficial effects on the cardiovascular system. Given that the incretin hormone GLP-1 also has positive functions on vascular system, we tested the effects of this hormone on ER-mitochondria coupling on the A7r5 rat aortic cell line. Here we show that GLP-1 100 nM diminishes ER-mitochondria distance, evaluated as an increase in Manders' colocalization coefficient. In parallel, GLP-1 facilitates Ca²⁺ entry to mitochondria after histamine stimulation, a hormone that releases Ca²⁺ from the ER. Moreover, GLP-1 increases Mfn2 levels through a PKA-dependent mechanism. Accordingly, enhanced Ca²⁺ intake to mitochondria in GLP-1-treated cells was completely blunted by PKA inhibition or Mfn2 siRNA treatment. These data show that GLP-1 promotes functional coupling between ER and mitochondria by activating a PKA/Mfn2 pathway, suggesting a new mechanism by which incretin could control VSMC function. Supported by FONDECYT 1110180 (MC), Anillo ACT1111 (SL, MC). GT holds a CONICYT fellowship.

313. Insulin/NF- κ B/VCAM-1 signaling pathway in the protection to cardiac ischemia.

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In the heart, insulin controls key functions such as metabolism, muscle contraction and cell death. The transcription factor NF- κ B seems to be protective against ischemia (ISC), however, both its role in the insulin pathway and its target genes are still poor understood. Our previous results showed that VCAM-1 is surprisingly present in cardiomyocytes. Because VCAM is also regulated by NF- κ B we investigate whether VCAM-1 has a protective role in cardiomyocytes under ISC and if insulin/NF- κ B regulates VCAM-1 levels in these cells. To test these points, cultured rat cardiomyocytes were treated with or without siRNA VCAM-1 and/or insulin (10 nM) and then submitted to simulated ISC. Our results show that insulin stimulated phosphorylation (S536) and nuclear translocation of NF- κ B in normal conditions, and that ISC decreased VCAM-1 levels, but insulin abolished its. Insulin prevented cardiomyocyte death induced by ISC and this protective effect was abolished in cells treated with VCAM-1 siRNA, moreover levels of pAkt induced by insulin in ISC were decreased with VCAM-1 siRNA. In summary, insulin activates NF- κ B in normal condition, the insulin/VCAM-1 signaling pathway has a protective role against ISC. Future work should evaluate if this increase of VCAM-1 levels depends of insulin/NF- κ B. This study represents the first evidence of a protective role of VCAM-1 in cardiomyocytes.

314. Herp regulates insulin response in skeletal muscle cells

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Homocysteine-inducible ER stress protein (Herp), an ER membrane resident protein inducible under stress, participates in the ER associated protein degradation, regulates the Ca²⁺ homeostasis and protects the cell under stress conditions. Recently, a work shows that Herp knockout mice is intolerant to glucose load. Although skeletal muscle is the primary target tissue for insulin-dependent glucose uptake and plays a key role in type 2 diabetes mellitus, the role of Herp in this tissue remains unexplored. Here we evaluate the role of Herp in rat L6 myotubes exposed to insulin. Our results show that Herp knockdown in L6 myotubes decreased 30% 2-deoxyglucose uptake induced by insulin. These effects were associated to a decrease in Ser473-Akt phosphorylation (50%), without changes in the levels of phosphorylated insulin receptor. We conclude that Herp is required for an adequate insulin response in skeletal muscle cells.

315. Effect of transcriptional factor FoxO1 on gene expression of metabolic enzymes mediated by testosterone in cardiomyocytes

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Elevated testosterone concentrations induce cardiac hypertrophy. Hypertrophy requires metabolic adaptations to fulfill energy demands associated with cell growth; however the mechanisms for these adaptations are not fully described. The transcription factor Forkhead box protein O1 (FoxO1) has important roles on energy homeostasis. FoxO1 localization is regulated by Akt, which induces FoxO1 translocation from nucleus to cytoplasm, producing activation or inactivation of genes. Here, we studied whether testosterone induces modifications in phosphorylation and cellular localization of FoxO1 leading to changes in the expression of metabolic genes. A potential crosstalk between Akt/FoxO1 and androgen receptor (AR) was evaluated using pharmacological inhibitors and siRNA. Testosterone increased FoxO1 phosphorylation (Ser256) and its nuclear export, which were abolished by Akt inhibition but not by AR inhibition. Testosterone increased the mRNA levels of Hexokinase II and long-chain acyl-CoA dehydrogenase, which were increased by siRNA-FoxO1. Noteworthy, siRNA-FoxO1 *per se* led to changes in transcription levels of various metabolic enzymes as FPK, PDK4 and GLUT4. These results suggest that testosterone induce FoxO1 migration to cytoplasm via Akt, inducing changes in the expression of metabolic genes. Thus, testosterone via FoxO1 could be involved in a metabolic adaptive mechanism required for cardiomyocyte hypertrophy. FONDECYT 1120259.

316. CaMKII increases MEF2 activity to induce cardiomyocyte hypertrophy by testosterone

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In cardiomyocytes, testosterone induces hypertrophy through androgen receptor-dependent and -independent pathways. The Ca²⁺/calmodulin-dependent protein kinase II (CaMKII) is involved in cardiac hypertrophy and controls the activity of MEF2. However, the role of CaMKII/MEF2C pathway on cardiomyocyte hypertrophy is unknown. Here we studied whether testosterone effects on CaMKII participate in MEF2 activity and the subsequent cardiomyocyte hypertrophy. Testosterone (100 nM) increased phosphorylation of CaMKII (Thr286) an event that correlated with increases in CaMKII activity. Long-term stimulation with testosterone increased MEF2 activity, which was blocked by CaMKII inhibitors (AIP and KN-93). Testosterone increased a number of markers for cardiomyocyte hypertrophy such as cell size among others. The increase in these hypertrophy markers was blocked by inhibition of CaMKII (AIP and KN-93). In summary, these results suggest that in the development of cardiomyocyte hypertrophy induced by involve an increased CaMKII activity, in order to activate downstream targets, like MEF2. These parallel mechanisms used by testosterone showing that a complex network of signaling pathways is activated by this anabolic hormone in cardiac cells. FONDECYT 1120259.

317. Amyloid- β peptide oligomers impair spatial learning and decrease c-fos and RyR2 hippocampal protein levels

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Introduction: Amyloid- β peptide oligomers (A β Os) reduce synaptic protein expression in the hippocampus and disrupt learning and memory processes. Here, we investigated whether bilateral injection of A β Os into rat hippocampus impaired spatial learning, tested with the "Oasis maze" task, and evaluated changes in c-fos, RyR2 and RyR3 protein content. **Methods:** Animals were trained in the Oasis Maze task following bilateral injection of A β Os or saline. Rats were submitted during 6 consecutive days to the task, which involved searching for reward in 1 out of 21 equidistant wells on a circular arena provided with visual cues. All sessions were video recorded and analyzed using a Matlab routine. After this task, rats were perfused and their brains were removed and sliced in a frozen microtome for immunohistochemistry analysis of c-fos, RyR2 and RyR3 protein contents. Fluorescence images were captured by fluorescence microscopy and analyzed with ImageJ software. **Results:** Rats injected with A β Os exhibited increased number of errors, decreased hit rate and increased walked/expected distance ratio. Impairment in performance was paralleled by a decrease in RyR2 and c-fos protein contents without alterations in RyR3 protein levels. **Discussion:** These results indicate that A β Os reduce hippocampal content of the early gene c-fos and RyR2 synaptic proteins and affect spatial memory. **Support:** BNI-09-015F; FONDECYT 11110322 & 1100052; FONDAP 15010006, CENEM-ICM-P10-001-F.

318. Unveiling the death mechanism induce by InsP3R inhibition in a cancer model.

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Mechanisms that regulate cell metabolism are a fundamental requirement for cell viability. Normally differentiated cells rely primarily on mitochondrial oxidative phosphorylation to generate the energy needed for cellular processes. Recently we identified an unexpected and essential role for constitutive InsP3R-mediated Ca²⁺ release in maintaining cellular bioenergetics. The absence of mitochondrial uptake of InsP3R-released Ca²⁺ results in the inactivation of the mitochondrial enzyme pyruvate dehydrogenase leading to a decrease of both reducing equivalents and oxidative phosphorylation, which in turn activates AMPK and macroautophagy as a compensatory pro-survival mechanism. Unexpectedly this mechanism seems to be essential for cancer cells, since the inhibition of the InsP3R with Xestospongine B (XeB) causes cell death. Here we explored the mechanism responsible of cell death observed after InsP3R inhibition in two cancer derived breast cell lines (MCF7 and T47D). To determine the participation of autophagy, apoptosis or necroptosis, we pharmacologically blocked each of these pathways in the presence of XeB and determined the viability of the cells using different assays. Our results show that autophagy works as a survival mechanism, apoptosis is minimal and necroptosis does not play a role in cell death observed after InsP3R inhibition. The release of the transcription factor HMGB1 from the nucleus indicates that cell death is caused by necrosis.

319. Role of mitochondria in neuronal Ca^{2+} and ROS signals induced by amyloid- β peptide oligomers

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Amyloid- β peptide oligomers (A β Os) induce reactive oxygen species (ROS) generation, elicit persistent Ca^{2+} signals due to Ca^{2+} entry and subsequent amplification by ryanodine receptor (RyR)-mediated Ca^{2+} release, trigger mitochondrial fragmentation and reduce RyR2 expression. RyR inhibition blocks these effects, highlighting the essential role of RyR-mediated Ca^{2+} release on the responses induced by A β Os. Here, we studied in primary hippocampal neurons if the general antioxidant N-acetyl-cysteine (NAC) and the mitochondrial antioxidant EUK134 affected the mitochondrial fragmentation, and the RyR2 decrease induced by A β Os. Neurons were transfected with mito-Pericam to detect mitochondrial Ca^{2+} levels, were loaded with MitoTracker to detect mitochondrial fragmentation, or were incubated with MitoSox or transfected with HyPerMito to measure mitochondrial superoxide and H_2O_2 levels, respectively. Fluorescence images were acquired by confocal or spinning disk microscopy; RyR2 expression was studied by q-PCR. **Results:** A β Os promoted mitochondrial Ca^{2+} uptake and increased superoxide and H_2O_2 levels. NAC or RyR inhibition by ryanodine prevented A β Os-induced mitochondrial Ca^{2+} -uptake; both NAC and EUK-134 prevented the mitochondrial fragmentation and NAC, the RyR2 decrease induced by A β Os. We propose that A β Os-induced RyR activation requires ROS and that the antioxidants NAC and EUK-134 prevent noxious effects of A β Os. **Support:** FONDECYT11110322, 1100052; BNI P-09-015F.

320. Maternal obesity-induced endoplasmic reticulum stress reduces migration in human umbilical vein endothelial cells

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Maternal obesity (MO) is associated with fetal programming of adverse cardio-metabolic outcome in the offspring. Obesity produces endoplasmic reticulum (ER) stress and chemically induced ER stress is associated to impairment of cellular migration. We studied ER stress markers and their involvement in cellular migration of human umbilical vein endothelial cells (HUVEC) from pregnancies with MO. We analyzed activation of ER stress pathway (PERK, ATF6 and PKR) and *in vitro* migration scratch test in primary cultures of HUVEC from pregnancies with MO (HUVEC-OB) or normal nutritional state (HUVEC-N). Increased P-PERK and its downstream kinase P-eIF2 α (~3 and ~2 fold, respectively) were detected by western blot in HUVEC-OB, compared to HUVEC-N. PKR, another ER stress-activated protein, was also increased (~0,5 fold) in HUVEC-OB. In addition, an elevated nuclear location of the transcription factor ATF6 was seen in HUVEC-OB. Finally, HUVEC-OB showed a reduced cellular migration capacity, a phenomenon that was reversed by a chemical chaperone treatment that inhibits ER stress. Thus, MO was associated to ER stress-related impairment of cell migration in HUVEC. FONDECYT (1121145, 1110977, 1090594), CONICYT (ACT-73 PIA).

321. Short-term high-fat diet induces mitochondrial Ca^{2+} mishandling and insulin resistance in skeletal muscle prior to mitochondrial damage

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The mitochondrial function quickly responds to energy-dense food supply in order to deal with the demand but the role of mitochondrial Ca^{2+} has not been understood. Male C57BL/6J mice were fed a normal chow diet (NCD) or a high fat diet (HFD) for 1 week. The insulin resistance was evaluated by IPGTT, fasting glucose and HOMA-IR. GLUT4myc-eGFP was electroporated in the *Flexor digitorum brevis* muscle (FDB). Insulin induces an increase in cytoplasmic and mitochondrial Ca^{2+} signal in adult fibers. The uncoupler FCCP, releases Ca^{2+} from mitochondria to cytoplasm. Insulin-dependent mitochondrial Ca^{2+} uptake is decreased in fibers from short-term HFD fed mice while insulin-dependent cytoplasmic Ca^{2+} increase appear to be faster and stronger. In fibers from NCD fed mice, insulin-dependent mitochondrial Ca^{2+} uptake was inhibited by xestospongin B, a specific IP_3R inhibitor. Using TMRE⁺ in the non-quenching mode (5 nM), we found that the $\Delta\Psi_m$ was larger in fibers from short-term HFD fed mice v/s NCD derived fibers. Using TMRE⁺ in quenching mode (100 nM), we found that the TMRE⁺ released after uncoupler stimuli was higher in fibers from short-term HFD fed mice v/s NCD fed mice. The glucose analogue uptake (2-NBDG) and the redistribution of GLUT4myc-eGFP induced by insulin were decreased in presence of xestospongin or ruthenium red (MCU inhibitor). This effect suggest a retrograde regulation of mitochondrial Ca^{2+} uptake over insulin signaling. FONDECYT3110170;1110467;ACT1111

322. Effect of statins on microRNA expression signature in HepG2 cells

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Statins are widely used drugs for treatment of hypercholesterolemia and cardiovascular risk reduction. Its action mechanism is through competitive and selective inhibition of the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase, blocking intracellular lipid synthesis. In different cellular models, statins modify the expression of genes involved in cholesterol homeostasis. However, today there is no information about the post-transcriptional control exerted by the non-coding RNAs named microRNAs (miRNAs) in the regulation of cholesterol, especially in response to statins. Thus, the present study aimed to investigate the effect of statins therapy on the expression pattern of microRNAs using an *in-vitro* model of liver hepatocellular cells (HepG2) submitted to therapy with 2 different statins. The microRNA expression profiling of HepG2 cells treated with 2 different statins (simvastatin and atorvastatin) was carried out. Expression was examined using a microarray chip containing 2019 probes. When using simvastatin therapy, HepG2 cells showed a total of 9 miRNA differentially expressed, whereas for atorvastatin therapy, the expression pattern of 35 microRNAs was deregulated. These deregulated microRNAs can constitute promising biomarkers to evaluate the response to statins or future therapeutic targets. **Financial support:** FAPESP - Brazil (N° 2011/21967-1), CNPq - Brazil (N° 473485/2012-5), CONICYT-Fellowship, Chile (21090417) and FONDECYT - Chile (N° 1130675).

323. Grafting of subcommissural organ (SCO) explants to diminish or repair abnormalities in brain development associated to congenital hydrocephalus.

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Introduction: The SCO secretes glycoproteins into the cerebrospinal fluid. This secretion promotes neural growth and differentiation. The aim of this investigation was to investigate whether the grafting of SCO explants would have a beneficial effect on congenital hydrocephalus and abnormal neurogenesis.

Materials and methods: Explants of the bovine SCO were cultured for 12 weeks. The conditioned media (CM) were studied by western blotting. Explants either were processed for immunohistochemistry or grafted into the lateral ventricle (LV) of HTx rats. The following primary antibodies were used: AFRU (SCO-glycoproteins) and b3-tubulin (neuroblast).

Results: After 3 weeks in culture, explants formed spheres containing a mix of AFRU positive and immunonegative ependymocytes. The CM contained AFRU-reactive and immunonegative proteins. The study of the brain of grafted animals showed an increased proliferation of neural precursors in the LV containing grafting explants.

Conclusions: (1) SCO explants retain their secretory capacity *in vitro* and *in vivo* for at least 12 weeks. So they are a good source of neurotrophic factors. (2) Grafting of SCO explants have a beneficial effect on postnatal neurogenesis. A question is yet unsolved: Which are the compounds secreted by AFRU-immunonegative ependymocytes?

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324. Identification of Novel 11 β -HSD1 Inhibitors with Combined Ligand- and Structure-Based Virtual Screening

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The enzyme 11 beta-hydroxysteroid dehydrogenase type 1 (11 β -HSD1) converts cortisone to cortisol in a NADPH dependent manner. Overexpression of 11 β -HSD1 in key metabolic tissues is related to the development of type 2 diabetes, obesity, hypertension and metabolic syndrome. Using crystal structures of human 11 β -HSD1 in complex with inhibitors as source of structural information, a combined ligand and structure-based virtual screening approach was implemented to identify novel 11 β -HSD1 inhibitors. A selected group of compounds was identified *in silico* and further evaluated in cell-based assays for cytotoxicity and 11 β -HSD1 mediated cortisol production inhibitory capacity. The expression of 11 β -HSD1 and 11 β -HSD2 in human LS14 adipocytes was assessed during differentiation. Biological evaluation of 39 compounds in adipocytes and cortisol quantification by HPLC-MS/MS identify compounds that exhibit 11 β -HSD1 mediated cortisol production inhibitory activity with potencies in the low micromolar range. These compounds represent starting points for the development of more active derivatives with higher efficacies targeting intracellular cortisol levels in type 2 diabetes and metabolic syndrome. **Acknowledgments:** FONDEF CA12i10150, FONDECYT 1130427& IMII P09/016-F grants, OpenEye Software for academic license and the DTP/NCI for kindly providing the compounds screened in this study.

325. PGC1 α and mitochondria biogenesis in activated human NK cells from elderly donors

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Immunosenescence is defined as the state of dysregulated immune function that contributes to the increased susceptibility to infection, cancer and autoimmune disease. In this context, it has been described that the relative risk for the development of infection increase with the decrease in NK cell activity. Moreover, a low NK cell activity has been associated with short survival due to infection in older people. Previous studies from our laboratory have shown that during IL-2 stimulated NK cell function takes place an increase in mitochondria mass, as well as mitochondrial membrane potential both associated with an increase in the expression of PGC1 α , transcriptional cofactor involved in mitochondrial biogenesis. To assess this response in older people we negative purified NK cells from healthy elderly donors (> 70 years old). Our results showed that NK cells from elderly donors did not increase mitochondrial mass in response to IL-2 activation. Moreover, IL-2 induced a decrease in mitochondrial membrane potential compared to control. Interestingly, PGC1 α also decrease when NK cells were incubated with IL-2, suggesting that a failure in signaling may be responsible for mitochondrial dysfunction in NK cells from elderly donors. Accordingly, we can conclude that in NK cell from elderly donors, IL-2 failed to induce mitochondrial biogenesis which was related with a decreased PGC1 α . Financial support: Fondecyt 11110401, Proyecto Bicentenario PDA-20.

326. Use of biomimetic CdTe-GSH Quantum Dots in photodynamic therapy (PDT): understanding the molecular mechanism of phototoxicity

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Photo Dynamic Therapy (PDT) is a novel medical treatment against cancer and bacterial/fungal infections based on the use of a photosensitizing agent (PS), light and O₂. Currently, there is great interest in developing new PS agents to improve efficiency of treatments. Fluorescent nanoparticles or Quantum Dots (QDs) have been tested for PDT applications due to their unique optical properties. However, high levels of toxicity associated to metal release, and high production costs limit their use. Recently, our lab developed a simple and economic method for chemical synthesis of CdTe-GSH QDs mimicking biological conditions. The essential feature of this method lies in the use of the biological thiol glutathione (GSH) as reducing and stabilizing agent.

The goal of this work was to evaluate the phototoxicity mechanism of two CdTe-GSH QDs (emission λ =500 and 600 nm) for potential applications on PDT. Interestingly, obtained results indicate that most biocompatible QDs (λ =500 nm) display a greater PD effect. Experiments to determine cadmium release, production of Reactive Oxygen Species (ROS) and QDs phototoxicity on *E. coli* mutant strains in genes involved in ROS and Cd⁺² defense, revealed that PD effect observed in green QDs is consequence of ROS generation.

Altogether, obtained results indicate that CdTe-GSH QDs synthesized by green chemistry represent a promising and cost effective alternative for biomedical applications against cancer and microbial infections.

327. Changes in proteins related with calcium homeostasis in skeletal muscle from HFD fed mice

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Introduction. Metabolic syndrome is characterized by dyslipidemia, hypertension, central obesity and insulin resistance; all of them are consequences of obesity due to progressive incorporation of unhealthy diets. Skeletal muscle is one of the main target tissues affected, because it is the main tissue responsible for the insulin-stimulated glucose disposal. Mice fed with high-fat diet (HFD) during 12 weeks are a widely used model for both systemic and muscle-specific insulin resistance. Calcium is necessary for glucose uptake in skeletal muscle and the main proteins involved in intracellular calcium homeostasis are: ryanodine receptor (RyR), IP₃ receptor and sarcoplasmic reticulum calcium ATPase (SERCA).

Aim: To measure both levels and distribution of SERCA, IP₃R and RyR in skeletal muscle from HFD fed mice and to study the correlation between intracellular fat and metabolic fiber type. **Methodology:** We measured protein levels of IP₃ receptor, SERCA and ryanodine receptor in skeletal muscle through western blot analysis, in control and HFD-fed mice. All serial cuts were stained with hematoxylin and eosin, oil red and SDH.

Results, we observed an increase in the number of SDH-stained fibers in skeletal muscle from HFD fed mice, associated to oil red positive stain in these fibers. Apparently this phenotype change is also associated with a decrease in SERCA expression. No changes in IP₃R and RyR were observed.

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328. Photophysical study of the interaction between CdTe-GSH fluorescent nanoparticles synthesized by green chemistry and chlorine e6 for potential applications on Photo Dynamic Therapy (PDT).

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Fluorescent semiconductor nanoparticles or Quantum dots (QDs) display unique optical and electronic properties. The main problems of their use in therapeutic treatments are related with its high toxicity and production costs. Recently, our laboratory developed a chemical CdTe-GSH QDs synthesis protocol emulating biological conditions (biomimetic). QDs synthesized by this method exhibit high biocompatibility and low production costs, which favors their potential biological applications. In a previous work we determined that QDs synthesized by the biomimetic method generate reactive oxygen species when are excited (PD effect). Based on this, a possible way for increase the PD effect is through Förster Resonance Energy Transfer (FRET) mediated by photosensitizers (PS). In this work we studied the interaction between our QDs and chlorine e6 (Ce6), a PS with high PD effect and photostability. Green (emission at 500 nm) and red QDs (emission at 600 nm) were optically and physically characterized. FRET efficiency and Förster radius between QDs and Ce6 were calculated by the *Steady State* method and by spectrum overlap analysis. Furthermore, experiments of photobleaching showed that production of singlet oxygen was increased indicating that the PDT effect is enhanced by FRET. Obtained results indicate that QDs synthesized by green chemistry, transfer energy efficiently to Ce6 (EE:63%) improving the PD effect.

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329. Sodium tungstate mediated ERK phosphorylation in HK-2 cells and rat kidney.

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Sodium tungstate (NaW) is a potent anti-diabetic agent. When NaW is administered orally, is able to normalize glycaemia in many animal models of type 1 and 2 diabetes. It also increases the total amount and translocation of GLUT4 transporter in muscle and restores the glucose hepatic metabolism in streptozotocin-induced diabetic rats. Unfortunately, the molecular mechanisms of tungstate action are not well defined. Thus, we studied the effect of NaW in the insulin signaling transduction cascade in a human cell line (HK2) and diabetic rat kidney. We observe that ERK activation in HK-2 cells was mediated by sodium tungstate, showing a different profile to ERK phosphorylation mediated by insulin. Similarly, the NaW treatment in controls and diabetic rat shows a strong activation of this MAPK in renal tissue, with different distribution compared to insulin therapy. These findings raise an approach to establish the possible mechanism of action of this drug by enhancing the proliferation properties in damaged diabetic kidney (FONDECYT 1131033).

330. Angiotensin-(1-9) decreases blood pressure and cardiac damage induced by deoxycorticosterone by Mas and AT2 receptor independent mechanism.

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Previously, we showed that angiotensin (Ang)-[1-9] reduces blood pressure (BP) and cardiovascular remodeling in a rat model of Ang II-induced hypertension. These effects were mediated by AT2 receptor. In this study, we explored whether the chronic administration of Ang-(1-9) decreases both BP and cardiac damage in a rat model of low renin hypertension through an AT2 receptor-dependent mechanism. Our results showed that in uninephrectomized deoxycorticosterone acetate (DOCA)-salt hypertensive rats significantly increase the systolic BP (SBP, 66%) and diastolic BP (DBP, 60%), heart weight (HW, 30%), cardiomyocyte area (CA, 34%) and perimeter (CP, 16%), collagen volumetric fraction (CVF, 89%), cardiac inflammation (CI, 70%) and decrease the plasma renin activity (96%), as compared with control rats. Ang-(1-9) significantly decreased SBP (22%), DBP (25%), HW (15%), CA (16%), CP (10%), CVF (33%) and CI (64%). Ang-(1-9) effects were not modified either by A779 (Mas receptor antagonist) nor PD123319 (AT2 receptor antagonist). In conclusion, Ang-(1-9) reduced BP and cardiac damage in a model of low renin hypertension. These effects seem not be mediated by Mas and AT2 receptors. Supported by Fondecyt 1100874, Fondef D1111122 and Conicyt 24110203.

331. A role for the Ca²⁺-dependent repressor DREAM in the onset and progression of Huntington disease

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Deregulated intracellular Ca²⁺ homeostasis underlies synaptic dysfunction and is a common feature in neurodegenerative processes. DREAM/calsenilin/ KChIP-3 is a multifunctional Ca²⁺ binding protein with specific functions in different subcellular compartments. In the nucleus, the Ca²⁺-free form of DREAM binds tightly to DRE sequences in the DNA and controls the expression of several genes related to Ca²⁺ homeostasis, neuronal excitability and neuronal survival. DREAM mutants unable to respond to Ca²⁺ and/or cAMP will disturb gene regulation leading to changes in the physiology of the synapses that might be determinant for or predispose to neuronal damage and death. We have used transgenic mice over expressing dominant active DREAM mutants, i.e. insensitive to Ca²⁺, and DREAM deficient mice to assess the role of DREAM in the onset of unbalanced motor coordination and neurodegenerative processes found in chemically- or genetically-induced mouse models of Huntington disease (HD). In addition, we have tested drugs able to bind to DREAM for an effect on the onset and progression of motor dysfunction in the R6/2 mouse model of HD. Funded by grants from Fundacion LaCaixa, CEE (LSHM-CT-2004-512039), Era-Net-SAF2008-04753-E y CIBERNED.

332. Chimeric Protein GLUT4myc-eGFP *in vivo* electroporated in *Flexor digitorum brevis* muscle allows to quantify real time translocation from vesicles to sarcolemma

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Overweight and obesity are risk factors in the development of insulin resistance and type 2 Diabetes. Skeletal muscle is a major target organ of insulin in glycemic control. Insulin stimulates glucose uptake by translocation of GLUT4 glucose transporters to the membrane and transverse tubule. This process is impaired in insulin resistance or diabetic models.

Male C57BL/6J mice were fed a normal or a high fat diet (HFD) for 8 weeks. The insulin resistance was evaluated by OGTT, fasting glycaemia, plasma insulin, and HOMA-IR. GLUT4myc-eGFP was electroporated in the *Flexor digitorum brevis* muscle.

In control mice, the subcellular localization of GLUT4myc-eGFP in basal state showed a vesicular organization. After insulin, GLUT4myc-eGFP migrated to the sarcolemma and transverse tubules. These processes were dependent on inositol 1,4,5-trisphosphate (IP3) receptor activation, since insulin-dependent 2-NBDG uptake was decreased in presence of inositol trisphosphate receptor inhibitor, xestospongin B. HFD mice in basal conditions showed the presence of large, compact vesicles that were not affected by insulin stimulation. GLUT4myc-eGFP allows to efficiently study GLUT4 translocation and insulin resistance in muscle fibers and opens new horizons in the study of the mechanisms in skeletal muscle involved in the onset of diabetes. Founding: FONDECYT 3110170; 110467; ACT 1111

333. Sodium tungstate decreases the progression of renal damage through inhibition of fibrosis and apoptosis in diabetic rat kidney.

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Chronic diabetes induces several associated complications in patient including diabetic nephropathy (DN). Tungstate treatment to streptozotocin (STZ) – induced diabetics rats causes a decrease in blood glucose concentration and ameliorates experimental diabetes. In this study, we evaluate the effect of sodium tungstate (Na₂WO₄) in the progression of renal damage in long term STZ-induced diabetic rats. The anatomopathological analysis showed glomerular damage since 4 months of diabetes and increases constantly to 8 months. At the final stage we found damage in glomeruli by observing increased glomerular sclerosis (16%). Moreover, tubular damage is the first to appear beginning in the second month of induction of diabetes. Surprisingly, sodium tungstate reduced the progression of nephropathy on early and long-treated diabetic rats. In addition, we observe that the increase in the protein levels of fibrinogen and α -SMA produced by renal damage were reverted by treatment with sodium tungstate. Similarly, this treatment was able to decrease the number of apoptotic nuclei present in diabetic rat kidney. These results show that sodium tungstate is an agent that can improve the processes involved in diabetes-mediated renal damage (FONDECYT 1131033).

334. Diabetes modifies extracellular metabolism and physiological effects of adenosine in renal proximal epithelial tubule cells.

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Introduction. The progression of diabetic kidney disease is associated with extensive renal fibrosis. Interestingly, kidneys isolated from the equilibrative nucleoside transporter 1 knockout mice (*ent1*^{-/-}) showed higher levels of interstitial collagen and α -SMA positive cells. Furthermore, increasing levels of adenosine have been quantified in plasma of patients affected by diabetic nephropathy and in urine from experimental models of diabetes. Our aim was to study diabetes-mediated alterations on adenosine handling in the proximal tubule epithelial cells. *Results.* Renal proximal tubules were isolated from kidneys of healthy and diabetic rats. The [³H]-adenosine uptake activity mediated by ENT1 was decreased in tubules from diabetic rats. Beside, the extracellular AMP hydrolyzing activity was increased by diabetes. Adenosine, the general antagonist of adenosine receptors, NECA, and TGF- β increased the expression of phenotypic transition markers α -SMA and fibronectin in HK2 cells *in vitro*. The epithelial to mesenchymal transition (EMT) induced by TGF- β can be blocked by using an antagonist of the adenosine A₃ receptor subtype. *Conclusions.* An increased AMPase activity and a lower uptake of adenosine leading to extracellular accumulation seem to be the main contributors in raising the levels of the nucleoside under diabetes. Furthermore, adenosine is an inductor of EMT in proximal epithelial tubule cells.

335. Molecular dynamics simulation and site-directed mutagenesis suggest a ternary complex mechanism for the catalysis of alcohol acyltransferase of mountain papaya fruit

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Aroma in *Vasconcellea pubescens* fruit is determined by esters, which are produced through an esterification reaction catalyzed by alcohol acyltransferase (VpAAT1). VpAAT1 protein structure displayed the conserved HxxxD motif facing the solvent channel in the center of the structure. To gain insight of the role of these catalytic residues, kinetic and site-directed mutagenesis studies were carried out. Based on dead-end inhibition studies, the kinetic mechanism could be described in terms of a ternary complex mechanism. Kinetic results showed that the lowest K_M value was obtained for hexanoyl-CoA. Additionally, the most favoured predicted substrate orientation was observed for hexanoyl-CoA, showing a coincidence between kinetic studies and molecular docking analysis. Substitutions of H166A, D170A, D170N and D170E were *in silico* evaluated. The shape and size of the solvent channel of mutant proteins showed major differences. Molecular docking and molecular dynamics simulations were able to describe unfavoured energies for the interaction of the mutant proteins with different alcohols and acyl-CoAs. Finally, *in vitro* site-directed mutagenesis of H166 and D170 induced the loss of activity in VpAAT1 mutant enzymes, confirming the functional role of both residues for activity, being H166 directly involved in catalysis.

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336. "Towards the analysis of key specificity determinants in DNA recognition by the transcription factors MarA, SoxS and Rob in Enterobacteria".

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Transcription factors (TF) play an important role in biology; they are proteins that bind specific sequences of DNA, thus controlling the biological regulation of gene expression within the cell. MarA, SoxS and Rob are TF that belong to the AraC-XylS family, have a similar 3D structure and are capable to cross talk between them. These TF, in their DNA binding regions, contain a bipartite helix-turn-helix DNA-binding domain, which recognizes asymmetric DNA sequences (not palindromic) of around 20 base pairs long denominated marbox.

In this work we used bioinformatics tools in order to search, map, annotate and analyze the binding sites of MarA, Rob and SoxS in several bacterial genomes. Compiling known marbox sequences from different publications, we performed a search for marboxes in the genome group of enterobacteria, allowing from 0 to 3 mismatches and considering the sequence context where the matches occurred. Through this analysis we inferred new potential marbox sites in other enterobacteria. In addition with this new information, we also performed a sequence analysis and clustering of the marbox sites. Finally, the aminoacidic sequences from MarA, SoxS and Rob in several enterobacteria were used to perform correlated mutation analyses, and propensity tables were calculated for these and other TF with DNA. With all these analysis performed, we move a step forward in the inference of the key specificity determinants of DNA recognition by these important TF.

337. Determination of putative binding sites on human $\alpha 7$ nicotinic acetylcholine receptor to positive allosteric modulators type I and II through computational studies

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Nicotinic Acetylcholine Receptors (nAChR) is a family of ligand gated ionic channels present in neurons located at the nervous systems. In the last decades, nAChRs located in the neuronal cells have become the focus of investigations because it has been reported that their abnormal function is associated with various psychiatric disorders and neurological diseases. Positive allosteric modulators (PAMs) for the Nicotinic Acetylcholine receptors act enhancing the activity of endogenous ligand, helping on treatment of these disorders, but not all binding sites of PAM described are known. For this reason we searched possible ligand binding sites on models of human $\alpha 7$ nAChR, then a molecular dynamic simulation was done with the purpose of select binding sites stable through time and at least a high-throughput virtual screening was performed in order to determine if those PAMs present affinity with the predicted binding sites. This information is intended to prove useful for the rational design of new drugs that shown better performance even in the framework of poly-pharmacology

338. *Acidithiobacillus* phylogenomics: understanding ecophysiology in extreme acidic environments by whole genome evolutionary reconstruction

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Acidithiobacilli are acidophilic, chemolithoautotrophic bacteria that are characterized by their ability to derive energy from inorganic sources such as sulfur, iron and to resist high concentrations of heavy metals and extremely low pHs. They are found in acid mine drainage and as part of consortia of microorganisms used for the commercial extraction of metals. The availability of several genome sequences for representatives of this genus is generating new opportunities for the assessment of evolutionary processes associated with the gain/loss of functional properties. These novel opportunities also are proving useful for assessment of ecophysiological interactions and for providing new clues about their controversial taxonomic classification. To address these issues, we present a comparative genomic analyses of seven isolates for which we have defined the pan and core-genome across the genus evolutionary gradient. This information has allowed us to identify essential, dispensable and unique genes present within the genus and to delineate the trends of gene family expansion, duplication and reduction in *Acidithiobacilli*. We also performed an ancestral genome reconstruction to infer species genome differentiation in order to explain how major functional features present in each member have evolved across this evolutionary gradient. Ack: CONICYT doctoral fellowship and Basal CTE PFB16, FONDECYT 1130683 D.H., 11110434 J.V. and InnovaChile-CORFO (FCR-CSB 09CEII-6991)

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339. Study of sequence/structure/function relationships of catalytic domain of DNA polymerases using multiple sources of structural comparison.

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DNA polymerases are a very ancient gene family involved in the preservation of the genetic information and, with a high degree of structural and sequence divergence. For that reason they are a very challenging case in bioinformatics. They are currently classified in 6 families according to their sequence similarity, but very few efforts have been made to explore structural relationships among them. Structural alignments of proteins allow the exploration of deep relationships in families with high divergence like the observed in DNA polymerases. In this work we have performed a comprehensive and detailed comparison of the catalytic domain of DNA polymerases using a methodology that uses multiple source of structural information. Our results show that they are a very diverse family of proteins, suggesting that each family A and B should be divided in two classes. Also, our methods allowed the detection of relationships between catalytic domains from different families that cannot be detected by sequence comparison methods. Finally a map of relationships was produced synthesizing the relationships between catalytic domains of DNA polymerases from different families. The map suggests that the catalytic domains should have arisen in at least three independent events in the history of evolution. Acknowledgement: FONDECYT 1110400, ICM N° P09-016-F

340. Assessing the importance of cooperativity of hydrogen bonding through Density Functional Theory calculations

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DFT-PBE calculations have been carried out on both finite and infinite systems composed by linear small molecules that follow the pattern $H-X\equiv X'$ ($X = C, N, Si$ or P) to measure the hydrogen bond interaction energy. Computation results demonstrated that these kinds of systems do exhibited a cooperative behavior, which was reflected by the overall exceeding of the interaction energies from what would be expected a pairwise additivity. Noncovalent interactions can exhibit cooperative effects that are important to several molecular phenomena, and therefore have been subject of study by several research groups during the last years. Hydrogen bond is a widely examined electrostatically-driven interaction that plays a key role in many biological processes, and its cooperativity has been addressed as well. We have extended the same hydrogen bond cooperativity concepts, revised in those small molecules, to biological relevant systems as the protein kinases and their interaction with small organic molecules. The initial recognition interaction is supposed to be driven through hydrogen bond interaction between ligands and protein kinase hinge region. It is expected that H-bond cooperativity does play an important role in the estabilization of protein-ligand interaction. ACKNOWLEDGMENT: We are indebted to FONDECYT and Government of Chile, which supported this research through project N° 11100177.

341. Transcriptomic analysis of TRPM4 gene signatures in prostate cancer patients and their potential role in the progression of the disease.

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Previous studies have described an overexpression of the transient receptor potential melastatin 4 (TRPM4) channel in a variety of human cancer. Our experiments in prostate cancer lines have shown that TRPM4 regulates the β -catenin pathway and epithelial-mesenchymal transition (EMT). To advance our understanding of the role of TRPM4 in prostate cancer progression, a bioinformatic analysis of ten public transcriptomic datasets were used in order to characterize the coexpression groups of genes and the TRPM4 mRNA expression across these datasets, comparing control and tumoral patient samples. Additionally, COSMIC and Oncomine databases were used to identify possible chromosomal abnormalities related to TRPM4 in prostate cancer. Our result shows, in 8 datasets, significant differences in TRPM4 expression profile between the tumoral and controls samples, which it's correlated with the progression of the disease. Moreover, a coexpresión analysis (*K-means clustering*; *K:20*) using PANTHER and DAVID databases, shows a significant enrichment of the oncogenic WNT signaling pathway and cancer ontologies, which additionally correlated with the expression levels of prognosis markers of the disease. These results suggest that during cancer progression in human patients, TRPM4 may modulate biological processes related to the WNT signaling pathway, which could explain the role of TRPM4 in carcinogenesis and progression of prostate cancer.

342. *In silico* studies to understand structure/function of exopolyphosphatase from *Escherichia coli*

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Escherichia coli exopolyphosphatase (PPX) hydrolyzes polyphosphate (polyP) in a processive way to yield PolyP_(n-1) and orthophosphateP_i. Putative catalytic residues and the Mg²⁺ binding pocket were suggested, by the interaction mode PolyP is not very clear. Based on sulfate ions present in the crystal and electrostatic potential a putative region to bind long chain of PolyP was described^{1,4}.

Here, we made an evolutionary trace³ study in order to analyze possible binding sites for polyP. The results shown here let us to propose several sites where polyP may interact and reach the active site. Both active site and an electropositive channel contain well conserved residues consistent with those predicted in^{1,4}. Possible new sites located in the N-termini where found, one of them is consistent with the one described in¹, and the other with described in⁴. One of the sites described in¹ is poorly conserved.

To characterize the binding of polyP to these sites we pretend to perform molecular dynamics. Considering the lack of parameters available for this ligand, in first place we optimized a chain of 4 residues of polyP using quantum mechanics (QM) and optimize the parameters of the molecule, so the molecular mechanics (MM) energy profile resembles those calculated by QM.

Unbinding studies will be carried out in order to understand in which way polyP interact with PPX, and we will correlate the theoretical studies experimentally, in quasiprocesive studies².

343. Increased local flexibility of the forkhead domain of FOXP2 transcription factor is required for domain swapping.

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The P subfamily of forkhead box (FOX) transcription factors is composed by four members (FoxP1-4). The crystallographic structure of the forkhead DNA-binding domain of FOXP2 (fhFOXP2) shows both monomer and dimer species, where the latter is formed by domain swapping (DS). In this association mechanism, an oligomer reaches its native state by interchanging segments between one or more subunits, while reestablishing the native contacts intermolecularly and thus recapitulating the native state seen for the monomer. As a general DS mechanism, it has been proposed that the domain swapped dimer is connected with its monomeric conformation by the denatured state. In order to study DS mechanism of fhFOXP2 proteins we performed Gō-model molecular simulations using coarse-grained structure-based models. These simulations showed an energy minimum that includes two dimer conformations: one observed in the crystallographic structure and an alternative conformation that only differs in the dihedral configuration of the hinge region. Also, simulations using AMBER empiric force field showed that there are two flexible regions in the protein: the hinge region and the DNA recognition region. Since the hinge region has been characterized by NMR-experiments as important for DS mechanism in FOXP1, these results suggest this increased flexibility is a conserved feature required for DS in the FOXP subfamily. FONDECYT 1130510.

344. Distribution modeling for Chilean Amphibian species: a MAXENT approach

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Amphibians are probably a symbol of the crisis of biodiversity loss today. In Chile there are 60 species of amphibians, 34 of which are endemic. Here we modeled the geographic distribution of amphibian species in Chile, and projected the expected changes under global change scenarios available for Chile. Distribution modeling was performed using the maximum entropy approach implemented in the MAXENT software. To focus on climatic factors that may constrain amphibian distribution, we used climate layers from the Wordclim database. Specifically, we used temperature (annual maximum, mean and minimum values) and precipitation (average total annual value and average monthly values for january, april, july and october). We also included topographic and hydrographic variables. Adjusted distribution models were characterized by good fits, as indicated by the receiver operator characteristic curve and its area under the curve index (AUC). This indicated that the adjusted models were able to capture the relationship between environmental variables and geographical distribution. Modeled distributions were contrasted with the degree of conservation protection available today, and the expected degree of coverage by these protected areas under different climate change scenarios. We discuss the validity of existing conservation and protection measures for amphibians in Chile in the light of the results obtained.

345. Molecular Dynamics Simulations of Current-Voltage in BK and Shaker K⁺ Channels.

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K⁺ channels are membrane proteins that allow rapid and selective flow of K⁺ ions across the membrane governing the electrical properties of the cell. These voltage-gated K⁺ channels are involved in generation and propagation of electrical impulses in nervous systems to regulate gene expression, and release of hormones and neurotransmitters, among other functions. The K⁺ channels have a selectivity filter (SF) composed by a highly conserved sequence of five amino acids that form the narrowest part of the channel. Despite the fact that the structure of the SF is conserved among K⁺ channels, different K⁺ channels show different conductance rates. Thus, the features that control the ion flux are still unknown. In order to study the conductance mechanism, molecular dynamics simulations (MD) for two K⁺ channels with very disparate conductance rates were examined: BK channel, which has a large conductance of 250 pS, and Shaker channel, which presents a low conductance rate of 18 pS. To characterize the ion permeation process from inside to outside of the cell, an external electric field (V=1200mV) was applied to both K⁺ channel systems. The current, ion density and the order of waters were calculated determining that the experimental results were clearly rationalized at atomic level in the MD simulations. Acknowledgement: VM and RS thanks to CONICYT doctoral fellowship. This work was supported by FONDECYT 1131003 (FGN) and CINV (Millenium Initiative, 09-022-F)

346. Towards an accurate identification of DNA mobile elements in eukaryote genomes.

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DNA mobile elements can move DNA segments inside a chromosome or between chromosomes. They are characterized by a sequence that encodes a transposase protein of about 300 aminoacids and flanking it, short DNA segments that are inverted repeated terminals. Transposition may affect, in the short term, gene regulation, and in the long term, organisms' evolution. There are 12 superfamilies of eukaryotic DNA mobile elements. Each superfamily is characterized by the degree of protein conservation amongst its members. Nonetheless, the similarity between members of a superfamily is low. Because of the high inter and intra-species diversity, identifying DNA mobile elements is a hard task. Currently, there are no methods that can accurately predict DNA mobile elements. In this work we develop sensitive and selective models called HMM (Hidden Markov Models) Profiles generated from the rigorous curation of the protein sequences of each of the superfamilies of eukaryotic DNA mobile elements. The HMM profiles developed in this work show high true positive rates, and low false positive rates. Those values suggest that the models are good at identifying protein sequences corresponding to transposases, and also that they are good discriminating them between families, and amongst unrelated sequences. These models are the first step towards identifying DNA mobile elements, and they could be used in future works to analyze the influence they may have in eukaryotic genomes.

347. A beta-complex statistical four body contact potential combined with a hydrogen bond statistical potential recognizes the correct native structure from protein decoy sets

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We present a new four-body knowledge-based potential for recognizing the native state of proteins from their misfolded states. This potential was extracted from a large set of protein structures determined by X-ray crystallography using Beta-Mol, a software based on the recent theory of the beta-complex (β -complex) and quasi-triangulation of the Voronoi diagram of spheres. This geometric construct reflects the size difference among atoms in their full Euclidean metric; property not accounted for in a typical 3D Delaunay triangulation. The ability of this potential to identify the native conformation over a large set of decoys was evaluated. Experiments show that this potential outperforms a potential constructed with a classical Delaunay triangulation in decoy discrimination tests. The addition of a statistical hydrogen bond potential to our four-body potential allows a significant improvement in the decoy discrimination, in such a way that we are able to predict successfully the native structure in 90% of cases.

348. Carbon use in cyanobacteria from genus *prochlorococcus* in the anoxic marine zone from the eastern-south pacific: a metagenomic analysis

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The cyanobacteria of the genus *Prochlorococcus* are the most abundant photosynthetic organisms in the ocean and the planet. Currently there are different strains of these small microorganisms (diameter less than 1 μ m) isolated in culture, many of them with their genomes sequenced, becoming a model for ecophysiology and marine genomics studies. Populations of *Prochlorococcus* phylogenetically different to their relatives in culture with genetic and physiological characteristics still unknown, have also been found in anoxic marine zone (AMZ) of the Southeast Pacific Ocean, where the dissolved oxygen is undetectable and light conditions are limited. Through a metagenomic study based on environmental DNA from the abovementioned AMZ was possible to assembly some genomic fragments of *Prochlorococcus* with the goal of identifying some genes that may indicate potential metabolic adaptations to low light or oxygen absence. The *aspC* gene (aspartate aminotransferase) was detected in *Prochlorococcus* metagenome fragments, this gene would allow to *Prochlorococcus* to get metabolic intermediates, electrons and reducing power through a TCA cycle shunt. Also, the presence of a glucose transporter (*melB*) and genes associated with glucose alcoholic fermentation suggest that this organism would be able to use organic carbon as alternative source of carbon and energy when the absence of light demanded. Funding acknowledgements: Gordon & Betty Moore Foundation and FONDECYT 1130784

349. Comparative three-dimensional structure modeling of protein-DNA complexes with MODELLER: application to the prediction of transcription factor DNA binding specificity

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Any tool capable of providing structural information can be potentially used for a better understanding of a biological process. Recently, we have implemented a computational tool for the comparative three-dimensional modeling of duplex DNA structures, based on the MODELLER software suite. The implemented protocol generates full atom DNA structures, keeping a final conformation as close as possible to the chosen template, while optimizing base replacements, and generating models with proper stereochemistry. In this work, the modeling pipeline is further extended towards the comparative modeling of protein-DNA complexes. 18 DNA geometrical restraints have been extracted from a non-redundant set of protein-DNA complex structures, and were used for guiding the optimization of several protein-DNA complexes, using the MarA-DNA complex crystal structure as reference template. For binding specificity assessment, 3D complex models with different DNA sequences were generated and evaluated with statistical potentials, as a computational scoring and selection criterion. High binding affinity sequences known in MarA-regulated genes were used to assess the accuracy of this protocol on transcription factor DNA binding prediction. Results obtained in this work sustain the promissory use of this new comparative protein-DNA complex structure modeling and evaluation protocol as a suitable and general tool for the *in silico* prediction of protein-DNA binding specificity.

350. Prediction and structural characterization of copper (Cu 2 +) binding sites in the Kunitz trypsin inhibitor 3 (KTI3) protein expressed in poplars.

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Excessive accumulation of copper (Cu²⁺) in soil inhibits the plant growth, affecting its cellular processes. Recent studies with poplars subjected to Cu²⁺ stress allowed identifying a set of differentially expressed genes. Among them, *PdKTI3*, encoding the Kunitz trypsin inhibitor 3 protein, was highly up-regulated. From that result, it is postulated that PdKTI3 would have a protective role, as a Cu chelating protein. To understand the mechanisms involve in that process, the PdKTI3 structure was modelled. This involved a search for structural patterns that for identifying potential binding sites. To determine the specific interaction of the metal at the candidate sites, quantum mechanics (QM) was applied to characterizing the energy and structure of the atoms involved in the protein-metal complex. From the detailed description of the Cu binding ability of the PdKTI3 protein, it is expected to enhance the future development of plants for phytoremediation of this metal.

This work was supported by the FONDECYT grant number 11110214.

351. Synthetic Toxins Based on Dendrimers as Ion Channel Blocker

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Ion channels play an important role in sensory physiology; this makes them attractive molecular targets for treatment of various diseases. Inactivation of these channels by natural toxins currently has a wide range of biomedical applications, since cosmetics products until inhibition of chronic pain. Crystallographic information toxin-ion channel complex, shows that a lysine plays an important role in blocking the S0 and S1 site selectivity filter of a K⁺ channel (PDB ID:2A9H). Based on the physicochemical principles that govern the interaction between toxins and extracellular section of ion channels, polyamidoamine dendrimer was proposed as an ion channel blocker -or synthetic toxin- and we studied the activity of this synthetic particle in order to ascertain their ability to emulate toxin properties. The ability of the dendrimer as a potential blocker of Shaker K⁺ channel and TRPV1 channel was studied using electrophysiological assays and Molecular Dynamics Simulations, obtaining an atomic-level perspective of the specific interactions that guide the binding between dendrimer and ion channels. Also, structural characterization of this complexation will allow *in-silico* design of first generation of synthetic toxins based in dendrimers. Acknowledgement: VM thanks to CONICYT doctoral fellowship. This work was supported by FONDECYT 1131003, Anillo Científico ACT1107, FCR Innova-Chile CORFO (FCR-CSB 09CEII-6991) and CINV (Millenium Initiative, 09-022-F)

352. Differential properties of four α-L-arabinofuranosidases secreted by the fungus *Penicillium purpurogenum*.

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The fungus *P. purpurogenum* secretes a variety of xylanolytic enzymes, including four arabinofuranosidases. This work focuses on a biochemical analysis of these enzymes: Three enzymes (ABF1, 2 and 3) have been purified to homogeneity from *P. purpurogenum* culture and the gene of ABF4 has been expressed in *Pichia pastoris* and the enzyme was purified and characterized. The molecular weights, as determined by SDS-PAGE, are 58 000, 70 000, 50 700 and 68 100 for ABFs1, 2, 3 and 4, respectively. Their kinetic properties have been analyzed using the synthetic substrate p-nitrophenyl-α-L-arabinofuranoside; all follow Michaelis-Menten kinetics with KM values of 0.16 mM for ABF1, 98 μM for ABF2, 0.65 mM for ABF3 and 1.57 mM for ABF4. Optimal temperatures are 60 °C for ABF2 and 50 °C for ABFs1, 3 and 4; pH optima are 4.0 for ABF1, 4.6 for ABF4 and 5.0 for ABFs2 and 3. ABF1 and 4 are debranching enzymes liberating arabinose from sugar beet arabinan and arabinoxylan, and show synergism when incubated with endoxylanase A from *P. purpurogenum*. ABF2 does not act on these substrates and only acts on short-chain arabinooligosaccharides while ABF3 shows activity on arabinoxylan. None of the enzymes acts significantly on debranched arabinan. These results suggest that the four ABFs have a complementary function in hemicellulose biodegradation. Support: FONDECYT: 1100084, 1130180 and Post-Doctoral Fellowship MC. Ravanal 3120032; UNAB: DI 03-10/R and 61-12/R.

353. Characterization of extracellular amylase, cellulase, xylanase and chitinase activities, from cultures of different Antarctic yeast species.

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Yeasts are found in numerous environments including the Antarctica, considered as one of the most extreme environments for life. We have a collection of yeasts isolated from soil and water samples of sub-Antarctic region, which are being studied in relation to their ecological importance and potential biotechnological applications. In this work, yeast species with high amylase, cellulase, xylanase and chitinase activities, determined in "*in vivo*" assays, were selected for more characterizations of their enzyme activities. For that, the cell-free supernatants from liquid cultures of yeasts were concentrated by ammonium sulfate precipitation. These protein extracts were evaluated for the enzyme activities aforementioned under different pH and temperature conditions. By this approach the optimal conditions for enzyme activities for each yeast species were determined, important input for ecological analysis and for the evaluation of potential uses in industrial applications.

Fondecyt 1130333

354. Autophagy contributes to germ cell survival during a glutathione depletion condition.

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Autophagy is the major intracellular degradation system by which cytoplasmic proteins and organelles are delivered and degraded in the lysosome. In fact, autophagy acts as a pro-survival mechanism during oxidative stress or nutrient deficiency. The development and survival of male germ cells depend on their close contact with Sertoli cells and the antioxidant capacity of the seminiferous tubule. Glutathione (GSH) plays a significant role in the antioxidant defences of the spermatogenic epithelium. In this work, we evaluated whether autophagy is involved in germ cell survival during severe GSH deficiency. We depleted GSH content with buthionine sulfoximine (BSO) in mouse germ cell line GC-1, without inducing cell death. Autophagy was assessed by processing and subcellular distribution of the endogenous protein LC3. Immunoblot analysis showed a consistent increase of LC3-II and by immunofluorescence we observed an accumulation of autophagic vesicles in GSH depleted conditions. This process did not affect the activity of AMP-activated protein kinase (AMPK) or ATP content. However, inhibition of autophagy decreased ATP content in GSH depleted germ cells. These results suggest that GSH deficiency triggers an AMPK-independent autophagy in germ cells as an adaptive stress response.

355. Mitochondrial complex I inhibition as inducer of cell death in human acute lymphoblastic leukemia

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Acute lymphoblastic leukaemia accounts for around 75% of all childhood leukaemia and chemotherapy is still the most used treatment. In cancer cells, the metabolism is shifted to aerobic glycolysis, but its still highly dependent on the TCA cycle. Intra mitochondrial Ca^{2+} regulates the activity of three dehydrogenases from the TCA cycle and, consequently, the production of reduced equivalents and biomolecules bricks. Ca^{2+} enters to mitochondrial matrix by MCU, driven by the negative transmembrane potential. This potential is generated by the activity of three complexes of the electron transport chain, and the modulation of the activity of this process will affect the bioenergetics fate of the cell. Recently we described the anticancer activity of 3-hydroxybakuchiol (3-OHbk), a rare molecule capable of inhibits the electron flow by interaction with the electron transport chain complex I in a murine model. This interaction caused cell death by depletion of intracellular ATP. Here, we show that this new molecule inhibits specifically complex I in a human acute lymphoblastic leukaemia model causing several a cellular bioenergetics crisis, apparently mediated by a disruption of Ca^{2+} homeostasis. Notably, this drug cause cell death only in cancer derivate cells presumable by an inefficient autophagy.

356. Interaction of agmatinase-like protein with Mn^{2+} and Zn^{2+} ions

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Agmatinase-like protein (ALP) hydrolyzes agmatine in putrescine and urea. Agmatine has been associated to neurotransmitter, anticonvulsant, antineurotoxic and antidepressant actions in the brain. ALP sequence not contain typical aminoacid residues of urea hydrolase family that stabilize the metal activator Mn^{2+} and exhibits in the C-terminal a LIM-domain that coordinate two Zn^{2+} ions. Previously we found that LIM-domain truncated ALP increases 30 times its efficiency and isolated LIM-domain had inhibitory effect to truncated-ALP but not over wild-type and the inhibition was competitive. Now we found that C453 residue is critical in stabilization of Zn^{2+} ions in LIM-domain an in inhibitory effect on ALP, replacement of this residue by alanine produce an enzyme without Zn^{2+} ions and with kinetic characteristic like truncated-ALP. About to Mn^{2+} requirement to ALP activity, we determinate one Mn^{2+} by subunit of ALP and the H205 would be critical for interaction of this ion. ALP-wild type, truncated-ALP, and mutants H205A and H435A increased significantly its activity by pre-incubation to 65°C with MnCl_2 , we propose that in this conditions ALP incorporate a second Mn^{2+} ion in its active site. ALP-mutant H127A not increases its activity in this condition, therefore this residue must be critical in interaction with second Mn^{2+} . Us results reinforce an auto inhibitory role for the LIM-domain and demonstrate Mn^{2+} requirement to ALP activity. Grant Fondecyt 1120663

357. The Limch1 protein has agmatinase activity.

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Agmatine is a polyamine which has important physiological and pharmacological roles, such as modulates the insulin release from pancreatic cells, renal sodium excretion, enhances the analgesic effect of morphine and attenuates its dependency, in the last years, agmatine has been associated to neurotransmitter actions and is enzymatically degraded by agmatinase. The knowledge about mammal agmatinases is scarce, and cloned human agmatinase not present *in vitro* activity. In our laboratory we have cloned and characterized a rat brain protein with agmatinase activity, this protein doesn't belong to ureohydrolase family, and has been denominated agmatinase like protein (ALP). It was immunohistochemically detected in the hypothalamic region and hippocampal astrocytes and neurons. ALP contains 523 aminoacid residues and database analyses indicate that its part of carboxyl extreme of a protein denominated LIMCH1, this protein presents two isoforms of 1085 (I) and 902 (II) aminoacid residues. Both proteins contain a LIM-domain in his carboxyl end and isoform I present a calpain homology domain in the amino end. In this work we cloned both isoforms and at now, expression of isoform II we detected agmatinase activity and similar to *E. coli* agmatinase, its activity required Mn²⁺ and *K_m* to agmatine is 4 mM, according to obtained to ALP. These findings reinforce the importance of these proteins in regulating the agmatine concentration in brain mammals. Fondecyt 1120663.

358. A novel fungal phytase active at low temperatures

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The presence of high amounts of phytic acid (PA) in plant feedstuff for monogastric animal, especially fish, has brought economic and environmental problems. As it is not bio-assimilated, PA causes malnutrition in the fishes and when excreted promotes eutrophication of the environment by favoring a high proliferation of unwanted algae and microorganism. Attempts to supplement plant feedstuff with phytase, an enzyme that hydrolyzes PA to inorganic phosphorus plus inositol-phosphates to make phytic phosphorus assimilable by fishes, had failed since commercially available enzymes work best at temperatures above than those found in live fish and in breeding areas. To identify a phytase active at low temperature (14°C) we screened a library of microorganisms to select a fungus that grows using PA as the sole source of phosphorus. In solid and liquid media this microorganism releases an extracellular phytase with high activity at 14°C. The enzyme has a molecular mass >30 kDa and exhibit a *K_M* value for the PA of about 30 µM. This phytase with high activity at low temperatures has great potential for feed applications, especially in aquaculture (Financed by FONDEF CA1210022, DID-UACH S-2012-46).

359. Bioinformatics and Functional Analysis of 2-aminophenol Metabolic Pathway in *Burkholderia xenovorans* LB400

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2-aminophenol (2-AP) is a toxic pollutant produced from industrial activities. 2-AP degradation by bacteria is useful for the clean-up of polluted environments. *Burkholderia xenovorans* strain LB400 encodes the 2-aminophenol catabolic pathway. The present report describes a bioinformatic analysis. Strain LB400 and other 2-AP and nitrobenzene-degrading bacteria has a conserved *amn* genetic cluster, with 4 genes (*amnBACD*) to convert 2-AP into 4-oxalocrotonate releasing ammonium as nitrogen source, a second conserved gene cluster (*amnFEHG*) which is similar to catechol pathway to convert 4-oxalocrotonate in acetyl Co-A to uses it as carbon and energy source, a transcriptional regulatory gene (*amnR*) and an unknown function gene (*amnJ*). Functional analysis showed 2-AP degradation concomitant with picolinic acid (PA) production. PA was identified by HPLC analysis with a commercial standard. This secondary molecule produced spontaneously from 2AP metabolism had inhibitory effect on LB400 growth (MIC of 2 mM). LB400 cultures with 2-AP as sole nitrogen source reduced their PA production (0.2 mM) allowing its growth. However, when 2-AP was used as sole carbon source PA increases (0.5 mM), inhibiting LB400 growth. Overall these results indicate a novel functional aromatic central catabolic pathway in LB400 strain. We gratefully acknowledges financial support from FONDECYT (1020221 and 1070507), Center for Nanotechnology and Systems Biology and USM (131109, 130948) grants.

360. Preliminary purification and kinetic characterization of different isoforms of *Trametes versicolor* laccases

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Laccases are multicopper oxidases widely distributed in most kingdoms. Interest in these enzymes has grown because of their utility in various industrial applications.

This work describes the separation of different isoforms of *Trametes versicolor* laccase by liquid chromatography using different resins. The kinetic constants of the different pools were preliminarily compared using reduced ABTS as the substrate.

T. versicolor extract (Sigma- Aldrich) was used given its ability to produce several laccase isoforms. The purification was performed using a hydrophobic interaction column (PhenilSepharose[™], GE Healthcare) and laccase activity in the different fractions was determined spectrophotometrically by measuring the production of oxidized ABTS at 436 nm. The chromatogram indicates that c.a. 70% of total protein is lost the front without laccase activity, and three peaks are found showing laccase activity, accounting for 5, 10 and 15% of total protein. The second and third peaks were pooled and loaded on an ion exchange column (HiTrap-Q-Hp[™], GE Healthcare) in order to separate them completely. In this case, 90% of total laccase activity appears in the front, and the rest is found on 3 peaks with similar specific activities. The fractions comprising each single peak of the hydrophobic interaction and ion exchange columns were pooled and analyzed by SDS-PAGE and kinetic parameters were obtained by initial velocity measurements.

361. Possible role of WNT3A ligand in the regulation of muscle glycogen synthase (MGS) in germ line GC-1 (spermatogonia type b) and GC-2 (spermatocytes)

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A coordinate proliferation and differentiation of germ cells takes place in the seminiferous epithelium. Currently, Wnt/b-catenin signaling pathway has been linked to the development and maturation of testicular tissue. The activation of this pathway induces sequestration of GSK-3 β , preventing the phosphorylation of β -catenin. It is unknown whether this inhibition generates an effect on other targets of GSK-3 β such as glycogen synthase. The objective of this study was to determine whether Wnt3a ligand inhibits GSK-3 β using two germ cell lines GC-1 and GC-2, and its probable effect on muscle isoform of glycogen synthase (MGS). GC-1 and GC-2 were exposed to Wnt3a ligand (100ng/ml) for 6 h and total and nuclear protein extracts were analyzed by Western blot, immunodetecting MGS, β -catenin and their phosphorylated states. In total cell extracts of Wnt3a treated cells, a decrease in the phosphorylation of both, β -catenin and MGS was observed. However, in nuclear extracts, an accumulation of total β -catenin and a decrease in the total MGS was evidenced. Additionally, glycogen content increased in this condition. With these results we are able to suggest that the Wnt3a ligand is capable of modulating the action of GSK-3 β and its substrates, with potential implications in glycogen synthesis and physiology of these cells. FONDECYT-1110508, FONDECYT 3130449, DID 1330-32-09.

362. Complex interactions of evolutionary models explain the actual diversity of relaxin and hemoglobin gene families

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From a genetic standpoint, gene duplication is recognized as a key biological event in generating the raw material for evolution to act on. After duplication, the functional and regulatory divergence between the resulting gene copies is thought to contribute to the generation of evolutionary novelties. Successive rounds of gene duplication give rise to group of genes with similar biological function and DNA sequence, today known as gene families. Although is well known that gene families represent an important fraction of our genome, their evolution has been subject of controversy for many years. In the literature four main models have been proposed to describe their evolution as an evolutionary unit, among them, the divergent and concerted evolutionary models are thought to be the most important ones. In this work I'll present evolutionary studies using two different gene families as a model in order to highlighting the complex interactions of different evolutionary models in shaping the genomic diversity observed in extant species.

363. Functional novelty in enzyme evolution: Paleomolecular study of the ADP-dependent sugar kinases family.

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Gene duplication is proposed as a motor mechanism by which promiscuous function in enzymes can specialize and generate specific enzymes. The ADP-dependent sugar kinases family has two specificities; phosphofructokinase (PFK) and glucokinase (GK), being most of the experimentally characterized enzymes highly specific for glucose (GKs) or fructose-6P (PFKs). These enzymes have been found in three archaeal order of *Euryarchaeotas*; *Thermococcales* (GKs and PFKs), *Methanosarcinales* (GKs and PFKs), *Methanococals* (PFKs) and in eukaryotes (GKs). Interestingly, the enzyme from *Methanocaldococcus jannaschii* has been described as a promiscuous enzyme capable to phosphorylate both sugars. In this work we inferred the sequence and resurrected experimentally several ancestral enzymes of this family. We found that specific enzymes derived from promiscuous ancestors. Characterization of current proteins showed that the bifunctional trait is conserved in PFKs enzymes of the *Methanococcales* and *Methanosarcinales* order. To explain the phylogeny topology of the family tree and the presence of one or both homologues in different archaea groups, we propose a mechanism based only in horizontal gene transfer, in the absence of gene duplication events, in contrast to the proposed mechanism for this family. These results highlight the importance of horizontal gene transfer as an important mechanism to generate functional novelty in enzyme evolution. FONDECYT 1110137 Beca CONICYT 24121448.

364. Effect of histone deacetylase- and demethylase-inhibitors over replication and transcriptional activity of the Hepatitis B Virus.

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Infection with the Hepatitis B virus (HBV) is a major cause of liver disease and hepatocellular carcinoma. The HBV genome replicates its DNA in the nucleus of the infected hepatocytes via an intermediary known as covalently closed circular DNA (cccDNA). The cccDNA is the replicative intermediate responsible for the persistent infection of hepatocytes and it serves as template for the transcription of all viral RNAs. Within the hepatocyte nucleus, the cccDNA is organized as a minichromosome, associated with cellular proteins such as histones. Our hypothesis is that the viral chromatin also plays a regulatory function and modulates transcription and replication of HBV. In this work, we have focused on the role of histone deacetylases and demethylases over the viral replication and its transcriptional activity. We used an *in vitro* culture system reflecting the HBV viral replication cycle, in the presence or absence of sodium butyrate and pargyline, inhibitors of histone deacetylases and demethylases, respectively. We observed that the treatment with pargyline decreased the viral replication, whereas sodium butyrate increased it. This correlated with changes on histone modifications on several viral promoters. Together, our results show a role for histone modifications as contributors of the HBV life cycle progression, opening an alternative for developing new therapies against the virus. Funded by ANILLO ACT119, FONDECYT 1120170, Basal PFB16, USS 2011-005-R.

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365. Genome-wide screen implicates lipid metabolism in transcriptional regulation of Protein Kinase A subunits

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Transcriptional regulation is a key mechanism controlling the response of cells to diverse stimuli. The identification of regulators mediating these signals is a crucial step to elucidate how cell fate is regulated. In *S. cerevisiae*, three genes encode the PKA catalytic subunit, *TPK1*, *TPK2*, *TPK3*; while the regulatory subunit is encoded by only one gene, *BCY1*. We have used a dual reporter gene system to survey the yeast deletion collection in order to identify regulators of PKA subunits promoters. Positive and negative transcription regulators were classified into functionally related groups based on GO. This analysis revealed an enrichment in genes with roles in lipid and phosphate metabolisms, cytoskeleton organization and transcription regulation. Results of the genetic screen were validated by using β -galactosidase reporter assay and qRT-PCR. We report the results obtained from different strains lacking genes involved in PA-mediated regulation of phospholipid synthesis. *TPK1* and *TPK2* promoters activities were increased in *ino2 Δ* , *ino4 Δ* and *dgk1 Δ* strains, while decreased in *opi1 Δ* cells. *BCY1* promoter showed the opposite behavior. The activities of *TPK1*, *TPK2* and *BCY1* promoters responded to the presence of inositol in the culture medium. *TPK3* promoter showed no change, neither in inositol supplemented culture nor in the different deletion strains. These results implicate glycerolipid metabolism in the differential regulation of PKA subunits transcription

366. Epigenetic regulation of the ribosomal cistron seasonally modulates enrichment of H2A.Z- and H2A.Zub- in response to different environmental inputs in carp (*Cyprinus carpio*)

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The histone variant H2A.Z is essential in higher eukaryotes, and its incorporation within chromatin is a relevant process for gene expression. However, the dual positive and negative roles of H2A.Z in gene regulation still remain unclear. We have reported that acclimatization in carp fish (*Cyprinus carpio*) involves cyclical seasonal gene reprogramming as an adaptation response to its natural environment, when rRNA synthesis and processing are profoundly affected. The aim of this study was to describe the presence of several H2A.Z subtypes in carp, and assess the role of H2A.Z on the ribosomal cistron. This work reports the transcriptional expression of four different H2A.Z subtypes. A novel H2A.Z.7 was found, which corresponds to a tissue-specific histone subtype that contains seven amino acid residues longer than the canonical H2A.Z. Particularly, H2A.Z enrichment through the ribosomal cistron was significantly higher during summer, when rRNA transcription and processing are highly active, than it was in winter. In addition, ubiquitinated-H2A.Z (H2A.Zub) was strongly enriched on regulatory regions of the ribosomal cistron in summer-acclimatized carp. Our study revealed seasonally-dependent H2A.Z enrichment for active ribosomal cistron during the carp adaptation. Moreover, seasonal H2A.Zub enrichment appears as a specific mechanism contributing to the regulation of chromatin architecture under natural conditions.

367. The hepatitis C virus (HCV) IRES Domain III participates in the recruitment and positioning of the eukaryotic Ribosome.

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Translation initiation of the hepatitis C virus (HCV) messenger RNA (mRNA) is dependent on an internal ribosome entry site (IRES). The HCV IRES is a RNA structure composed of three major domains, II, III, and IV all essential for the assembly of competent 80S ribosome. Domain III is required for 40S ribosomal subunit and eIF3 binding. Previously our laboratory showed that mutations (G266A, G268U, G266A/G268U) within the III_d subdomain hinders IRES activity without altering overall RNA structure. This new study was designed to determine the molecular basis of the observed inhibition of IRES activity. Through "SHAPE" analysis we confirm that, although the structure of loop III_d is altered by the mutations, the overall structure of the IRES remains unchanged. A biochemical study showed that all mutants recruit the 40S ribosomal subunit with only a slightly reduced efficiency and equally promote 80S complexes formation. Toeprinting and SHAPE footprint analysis revealed sharp differences on the positioning of the 40S ribosomal subunit and the 80S ribosome assembly on the IRES. Considering that HCV IRES requires the assembly of the 80S ribosome directly on the initiation codon for proper initiation of protein synthesis, data suggest that the initiation complexes recruited on the mutant IRESs are misplaced in a less productive conformation thus, hindering translation.

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368. Analysis of Expression Profiles of Cell Cycle and DNA Repair Genes upon Knockdown of the Antisense Noncoding Mitochondrial RNA

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Non-coding RNAs (ncRNAs) have been increasingly found to participate in virtually all cellular processes, regulating key functions such as cell cycle, survival, metabolism and death. Our group described a family of ncRNAs of mitochondrial origin, derived from the 16S gene. These mitochondrial non-coding RNAs (ncmtRNAs) contain inverted repeats covalently joined to the 5' end of the sense (SncmtRNA) or the antisense (ASncmtRNA) 16S mitochondrial RNAs. Consequentially, these transcripts adopt stem-loop structures with very long double stranded RNA regions. By *in situ* hybridization, these transcripts show a differential pattern depending on proliferation status; while normal proliferating cells express both S and ASncmtRNAs, in tumor cells the AS transcript is practically undetectable and in resting cells neither is detectable. Hence, these RNAs seem to be involved in cell cycle progression. Blocking the ASncmtRNA with chemically modified complementary oligonucleotides (ASO) causes massive apoptotic death of tumor cells, preceded by cell cycle arrest, whereas normal cells are not affected. This strategy is therefore being used to develop a safe and effective anti-cancer therapeutic approach, with promising results. In this work we investigated the effects of this ASO (1537S) on the expression profiles of cell cycle and DNA repair genes in cancer cell lines (H292 and SK-MEL-2) by quantitative PCR Arrays (qPCR Arrays, SA Biosciences). Funded by Fondecyt Grant 1110835.

369. Role of ASH2L and NCOA6 in glucocorticoid-mediated apoptosis in leukemic cells

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Glucocorticoids are frequently used in the treatment of hematopoietic diseases due to its proapoptotic properties, representing an alternative and promising therapy in leukemia. We have already observed that leukemic U937 cells undergo apoptosis after dexametazone (Dex) treatment and this effect correlates with the downregulation of the antiapoptotic isoform Bcl-X_L. Moreover, ASH2L (H3K4 methylase activity) and NCOA6 (steroid receptor coactivator) showed to be necessary for programmed cell death in response to Dex. The main goal of this project is to study the role of ASH2L and NCOA6 proteins as putative epigenetic modulators in glucocorticoid-mediated apoptosis in human leukemic cells. Here we show that no downregulation of bcl-X_L mRNA is observed when ASH2L or NCOA6 are silenced. We observed that the glucocorticoid receptor (GR) and ASH2L coimmunoprecipitate in the presence of Dex in wt cells, but this endogenous interaction is lost when NCOA6 expression is silenced. We also evaluated the recruitment of GR to hormone-response elements located in the intron of *bcl-X* gene (+18, +42, +58 kb from +1). Interestingly, GR is already recruited to chromatin in the absence of hormone (0,6% input) and when Dex is added the receptor is displaced (0,2% input). In conclusion, our results suggest a concerted epigenetic mechanism for transcriptional regulation during glucocorticoid-mediated apoptosis in leukemia. Funding acknowledgements UBACYT IJ 2011-13 and PIP Joven 2012-14

370. The eukaryotic RNA binding protein Mex3A stimulates translation of the Andes virus (ANDV) SmRNA

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Eukaryotic messenger RNAs (mRNAs) have a 5'cap structure and most a 3'poly(A) tail. Both elements participate in translation initiation, mediating mRNA circularization through interactions between translation initiation factor 4G (eIF4G), cap binding protein eIF4E and poly(A) binding protein PABP. The capped mRNA of the Small segment (SmRNA) of the Andes Hantavirus (ANDV) lacks a poly(A) tail, therefore, translation initiation of the ANDV SmRNA is PABP independent. In absence of viral proteins, translation initiation of ANDV SmRNA is cap, eIF4G, and 3'UTR dependent. These observations lead us to hypothesize that circularization of the ANDV SmRNA is mediated by yet unidentified cellular proteins. Through an RNA affinity chromatography approach, ANDV 3'UTR-protein complexes were isolated, and the proteins identified by mass spectrometry. The RNA binding protein Mex3A was identified, and its possible role in the translation of ANDV SmRNA was evaluated both in absence and presence of the viral nucleocapsid (N) protein. Results show that during translation initiation, Mex3A binds the 3'UTR and, together with the ANDV N protein, stimulates translation of the viral mRNA. ECV has a CONICYT Doctoral fellowship (Doctoral y Apoyo). Funded by P09/016-F Iniciativa Científica Milenio, Ministerio de Economía, Fomento y Turismo. NIH/International Collaborations in Infectious Disease Research (ICIDR) 2U01AI045452-11.

371. Characterization of Iron Metabolism Genes of *Piscirickettsia salmonis*.

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Salmonid Rickettsial Septicaemia is a systemic disease caused by *Piscirickettsia salmonis*. Experimental data have shown that one of the most important requirements for PS is high iron availability. A highly virulent strain of *Piscirickettsia salmonis* (AUSTRAL-005) was sequenced and 6 of the identified genes are involved in siderophore synthesis, 12 in siderophore transport, 2 in ferrous iron transport, 23 in porphyrin metabolism, and mRNA level of these genes was detected by qPCR, supporting their functionality in PS. Partial genome information from 12 different Chilean isolates of PS were compared and the ferric uptake regulator (*fur*), a global transcriptional regulator responsible of controlling the intracellular concentration of iron, was identified as a common element. *Fur* binds to DNA sites, called *Fur* boxes, are palindromic AT rich sequences with the consensus (NATWAT)₃. Among Chilean *Piscirickettsia salmonis*, 8 isolates (including the AUSTRAL-005) have 5 *Fur* boxes and 4 isolates (including the type strain LF-89) have only one *Fur* box, that is located downstream of short chain dehydrogenase and ferroxidase genes. A phylogenetic tree based on the *fur* gene sequence was done. Since *Fur* is a known virulence factor and potential target for new antibiotics is necessary to characterize and study its regulation to propose new therapeutic targets for the design of drugs that reduce SRS mortality.

372. Florfenicol-resistant *P. salmonis*: characterization of ABC and RND family efflux pump involved in resistance.

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Piscirickettsia salmonis is the etiologic agent of the salmonid rickettsial septicemia (SRS) which constitutes one of the main problems in farmed salmonid and marine fishes. Florfenicol, a bacteriostatic belonging to the group of phenicols, is one of the antibiotics most commonly used to control SRS, however the abuse of this antibiotic could bring collateral damage to the salmon industry, as the generation of new resistant strains of *P. salmonis*. This phenomenon may be triggered by the expression of certain genes involved in resistance. These genes are Bacterial efflux transporters classified into five major superfamilies, based on the amino acid sequence and the energy source used to export their substrates. Through bioinformatics analysis, we identified several genes of two families: the ATP-binding cassette superfamily (ABC) and the resistance-nodulation-cell division superfamily (RND). For evaluate changes in the expression of these resistance-markers in the *P. salmonis* genome, we realized induction kinetics with several antibiotic concentrations at different times in culture of *P. salmonis*. Interestingly the results obtained by qPCR assays show that there is an increase in the relative expression of these transporters over time. This finding may explain the low susceptibility of the bacteria to the drug and eventually would give a potential new therapeutic target for the treatment of SRS. FONDAP-INCAR 15110027.

373. Expression of small RNAs in response to oxidative stress in *Acidithiobacillus ferrooxidans*.

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Translational control of gene expression by small non-coding RNAs (sRNA) has been described in several microorganisms. In *Escherichia coli*, sRNA RyhB and OxyS participate in the iron metabolism and oxidative stress response respectively. Neither RyhB nor OxyS have been predicted in the genome from the bioleaching bacterium *Acidithiobacillus ferrooxidans*. What small RNAs respond to oxidative stress in this microorganisms?. To answer this question, two approaches, based on deep sequencing of RNAs (Solexa/Illumina) combined with a bioinformatic analysis, were designed to identify sRNAs in intergenic regions (IGR) that changed their expression in response to hydrogen peroxide (100 µM). 1. Possible promoters and transcription terminators were predicted using bioinformatic tools. Four out of fourteen IGR that contain possible promoters and terminators were selected for further analysis. We observed that predictions were consistent with expression data. Levels of sRNAs were validated by RT-PCR. 2. A group of IGR that increased their expression more than twice in response to hydrogen peroxide were selected and the same bioinformatic analysis was performed. Five out of twenty five IGR were analyzed. Additionally, a tRNA-like RNA that is induced by hydrogen peroxide that is encoded in a mobile genetic element was identified. Possible targets for translational regulation by the identified sRNAs are in the process of identification. FONDECYT Grant 1110203 and 1120746.

374. The transcriptional co-repressor Ski regulates the expression of a cohort of pericentromeric genes at the early G₁ phase of the cell cycle

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Ski is a nuclear protein that is part of transcriptional co-repressor complexes containing histone deacetylase activity. Ski is expressed throughout the cell cycle, reaching its highest levels in mitosis. At this stage, Ski is located in the pericentromeric region of some mitotic chromosomes. In order to assess whether the presence of Ski in those regions affected the expression of underlying genes immediately after mitosis is completed, we compared overall gene expression in Mouse Embryonic Fibroblasts (MEF) *Ski*^{-/-} and *Ski*^{-/-} MEFs with Ski reconstituted (*Ski*^{-/-}hSKI) synchronized at early G₁. Total RNA was extracted and Cy3/Cy5 labeled cDNA was hybridized MEEBO slide. Analysis of differentially expressed genes at G₁, revealed that about 60% of genes downregulated by Ski were at chromosomes 9, 13 and 17. Among these, three pericentromeric genes (*mmp-3*, *mmp-10*, *mmp-13*) located in a cluster at chromosome 9. Microarray results were validated by real-time RT-PCR. Overall, the presence of Ski correlated with a decrease in H3Lys9 acetylation and an increase in H3Lys9-3me levels in mitotic and G₁ cells. At chromosome level, acetylation of histone H3 at Lys9 (H3Lys9) was decreased in regions that co-localized with Ski. These results suggest that Ski bookmarks pericentromeric genes during mitosis by epigenetic modifications, repressing the expression of those genes at the beginning of the next cell cycle (early G₁). Supported by FONDECYT 1120222

375. p53 and SP1 transcriptionally regulate the SALL2 transcription factor.

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SALL2 is a transcription factor found deregulated in various cancers, which suggest it plays a role in the disease. Knowledge of SALL2 regulation should help to understand its deregulation in cancer. Previous reports indicate that SALL2 gene is regulated by WT1 and AP4 transcription factors. Recently, we identified that the p53 tumor suppressor represses Sall2 expression in a context-specific manner. However, the mechanism for this repression remains unclear. Here, we performed Electrophoretic mobility shift assay to investigate whether p53 directly binds to the SALL2 promoter. In addition, by using Sodium butyrate (NaBt) and Trichostatin A histone deacetylase inhibitors (HDACi), we investigated HDAC function on SALL2 promoter regulation. We show that p53 specifically binds to the SALL2 promoter. HDACi significantly increased the promoter activity suggesting that acetylation is important for SALL2 regulation. Finally, since one of the main NaBt targets is SP1, and the SALL2 promoter contains putative SP1 sites, we tested the effect of SP1 overexpression on SALL2 promoter activity. Data show that SP1 overexpression increased SALL2 promoter activity. Our finding indicates that direct binding of p53 is needed for Sall2 repression, and suggests SP1 acetylation as a positive mechanism for SALL2 regulation, adding knowledge to SALL2 gene regulation and potential mechanisms for its deregulation in cancer. Funded by FONDECYT 1110821

376. Experimental validation of mirrorRNAs in human transcriptome

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The existence of a special type of natural antisense transcripts (NATs), which are perfectly complementary to mature mRNAs over several exons, including splice sites, has been reported. We refer to these NATs as "mirrorRNAs". However, there are reports that suggest that this RNAs could be an experimental artifact. Previously, we performed a bioinformatic analysis of human cDNAs, ESTs and strand specific RNA-Seq data to search and characterize mirrorRNAs. We now report the experimental validation, using different approaches, of putative mirrorRNAs selected in the bioinformatic analysis. We used 3' Rapid Amplification of cDNA Ends (3' RACE) and 5' RNA Ligase Mediated Rapid Amplification of cDNA Ends (5' RLM-RACE). We also performed RNase protection assay with RT-PCR detection (RPA-RT-PCR) to detect double stranded RNA molecules. Our results experimentally validate the presence of mirrorRNAs in human cell lines and human tissues. Our data suggest that mirrorRNAs are the result of RdRP activity. Currently, we are conducting knockdown experiments in the HEK293T cell line to detect proteins that could participate in mirrorRNA biogenesis, and specially to find the protein responsible for the putative RdRP activity in human cells.

377. Regulatory role of C/EBP β isoforms and chromatin remodeling complexes in transcriptional regulation of the human albumin gene.

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C/EBP β is a transcription factor involved in different cellular processes. In humans, this protein exists as three isoforms, derived from alternative translation of the same mRNA. It has been described that this factor can act as transcriptional activator or repressor, depending on the participant isoform and other nuclear proteins. We have recently described that the hC/EBP β isoforms physically interact with the ACF complex, which belongs to the ISWI subfamily of chromatin remodeling complexes. In order to explore functional roles of this interaction on transcriptional regulation, we studied the effect of C/EBP β isoforms and the ACF complex on the activity of the albumin gene, for which independent studies suggest a regulatory involvement of both C/EBP β and ACF. qRT-PCR analyses of RNA samples obtained from HepG2 cells transfected and co-transfected with vectors encoding hC/EBP β isoforms showed that these isoforms generate a differential effect on the expression of the albumin gene and a competition between the largest and the shortest isoform. Furthermore, overexpression and knockdown of SNF2H (catalytic subunit of the ACF complex) showed a repressive effect of this protein on the expression of this gene. The results obtained suggest that C/EBP β isoforms exert a differential effect on the expression of the albumin gene and a negative regulatory role of the ACF complex. Fondecyt 1130818.

378. Generation of expression vectors containing core promoters with TATA box and 5'top motifs, initiator, motif ten element and downstream promoter element.

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Eukaryotic transcription of protein coding genes is achieved by the RNA Polimerase II. This process is regulated by a wide variety of factors, including general transcription factors, co-regulator factors, specific binding proteins to promoters and/or enhancers, and chromatin modifier and remodeler complexes. Ultimately, the signals of these factors converge to the promoter during transcription initiation. Thereby, the core promoter, which is typically about 100 nucleotides long and encompasses the transcription initiation site, is a crucial element in gene expression. For a long time, it was thought that all core promoters operated via a common mechanism, but it was found that this region is structurally and functionally very different due to the presence of sub-regions or motifs which confer it specific properties. Here, we describe the design of expression vectors that contain core promoters with motifs such as TATA box, 5'TOP (5'-Terminal oligopyrimidine tract), Inr (initiator), MTE (motif ten element) y DPE (downstream promoter element), besides incorporating an intronic region, and we analyse the transcription process, and the enhancement of the luciferase gene expression in CHO-K1 cells. The resulting synergy between subregions allows high transcriptional expression which is useful in the production of recombinant antibodies. Financed by FONDEF D09I1190.

379. Epigenetic regulation complexes including WDR5, EZH2, PRMT5, NO66 and UTX control transcription of Runx2.

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Transcription factor Runx2-Il/p57 is essential for osteoblast lineage commitment. Expression of Runx2-Il/p57 is controlled by the P1 promoter and is accompanied by changes in chromatin structure as well as in histone H3 and H4 covalent modification. To identify the enzymes responsible for this specific epigenetic pattern; C2C12 cells were stimulated with BMP2 or horse serum (HS) for 72 hours to induce osteoblast or myoblast differentiation, respectively. To knockdown WDR5, EZH2, PRMT5, NO66 and UTX expression in C2C12 cells, we used specific lentiviral-driven shRNAs. Binding of these enzymes to the Runx2 gene P1 promoter sequence was determined by Chromatin immunoprecipitation (ChIP). We find that silencing of WDR5 and UTX in BMP2 stimulated cells drastically decrease Runx2-Il/p57 expression and prevents osteoblast differentiation. ChIP studies show that WDR5 and UTX are recruited to the Runx2 gene P1 promoter in BMP2-treated C2C12 cells. In contrast, when these cells are stimulated with HS, we find that NO66, EZH2 and PRMT5, are enriched at the P1 promoter. Our results indicate that the epigenetic regulators, WDR5, EZH2, PRMT5, NO66 and UTX have a critical role during control Runx2 gene transcription. The enzymatic activities associated with these regulatory components may be establishing an epigenetic environment at the P1 promoter that facilitates binding of the transcription machinery or the recruitment of complexes that mediate gene silencing.

380. Characterization of histone H3 translocation complexes.

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Histones H3 and H4 are basic proteins of low molecular weight whose function is to package the DNA into chromatin. Our previous work showed that once H3 and H4 mRNAs are translated, the histone polypeptides go through a successive cascade of maturation events that comprises four protein complexes. One of these complexes is the so-called Complex IV, in which the histones H3 and H4 are associated to Importin4, the protein that is the responsible of mediating the translocation of the histones H3 and H4 into the nucleus. The aim of this work is to characterize this protein complex. Toward this, we have purified Complex IV through multistep biochemical fractionation of S100 extract derived from HeLa cells. We followed each of the purification steps by western blot against histone H3. We will present evidences of the existence of two types of Complex IV, both associated to Importin 4, but with different histone chaperones. In addition, we will show that histone H3 have different patterns of post-translational modifications. Funded by FONDECYT 1120170, Basal Project PFB16.

381. TIM-1: A candidate marker of human regulatory B cells

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Introduction: Typically, B cells have been known as precursors of antibody-producing cells, however these cells are also capable of secreting cytokines and influencing other cell populations. Recently, a population of B cells secreting IL-10, known as regulatory B cells (Bregs), have acquired particular significance. These cells are enriched within the transitional B cell population in humans. The receptor T cell immunoglobulin and mucin domain-1 (TIM-1), which induces activation and proliferation of T cells, has recently been identified as a specific marker of murine Bregs. Currently, it is not known if TIM-1 is expressed on human B cells and whether this molecule can be induced along with their ability to secrete IL-10. **Materials and methods:** B cells were isolated from healthy donors' peripheral blood and cultured for 48 hours in the presence of agonists for Toll-like receptor 9 and B cell receptor, to subsequently determine the expression of TIM-1 and IL-10 on naive, memory and transitional B cell populations by flow cytometry. **Results:** TIM-1 is constitutively expressed within the CD19⁺CD24^{hi}CD38^{hi} transitional B cell population, and its expression is increased, along with IL-10, after stimulation, mainly on transitional B cells, but also in a lesser degree on memory and naive B cells. **Conclusion:** TIM-1 can be eligible as a suitable marker for human Bregs, in order to isolate these cell for immunotherapeutic interventions. **Grants:** Fondecyt 1121100, 11121497

382. FcγRIIB modulates IL-10 secretion by regulatory B cells: Implications in systemic sclerosis

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Systemic sclerosis (SSc) is an autoimmune disease characterized by an extensive fibrosis of skin and organs. Activated B cells have a major role on its pathogenesis through the secretion of cytokines and disease-associated antibodies. Recently, an IL-10-secreting subset of B cells that participates in tolerance, called regulatory B cells (Bregs), has been described. In humans, Bregs are characterized with a CD19⁺CD24^{hi}CD38^{hi} phenotype. FcγRIIB is a receptor expressed on B cells that delivers an inhibitory signal by recognizing the Fc fraction of IgG from immune complexes bound to B cell antigen receptor (BCR). This inhibition occurs due to the dephosphorylation of intermediaries of the signaling cascade of the BCR complex, such as Akt kinase. Our aims were to study the effect of FcγRIIB on the IL-10 secretion by Bregs after BCR activation, and to analyze the frequency of Bregs population and the expression of FcγRIIB in these cells in SSc patients. We show that stimulation of B cells with the F(ab)'2 fraction of an anti-BCR antibody induces a higher IL-10 production compared to the complete immunoglobulin. We also observed an increased population of Bregs in SSc patient, with a higher FcγRIIB expression, related to controls. These results suggest that FcγRIIB expressed on Bregs inhibits IL-10 secretion, and that SSc patients may have a dysregulation in IL-10 production due to an increased FcγRIIB expression on Bregs.

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383. Galectins are involved in infection caused by the intracellular pathogen *Chlamydia trachomatis*

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Chlamydia trachomatis is an obligate intracellular Gram-negative bacterium that modulates relevant eukaryotic processes as an adaptive strategy. Pathogenicity of *Chlamydia* can be partially explained by an altered immune response. Galectins are a family of highly conserved proteins in evolution, with a carbohydrate recognition domain with affinity for beta-galactosides (Gal b-NAcGlc 1-4), that have an active role in immunity, inflammation and cancer. We propose to investigate whether glycans and galectins are involved in *Chlamydia*-host cell interaction. In infected cells, expression of Galectin-1 increases. By confocal microscopy, we observe an intracellular redistribution of Galectin-1 and its recruitment to the chlamydial inclusion at some stages of development. These proteins might play a role in establishing infection and intracellular persistence of *Chlamydia*.

384. Tolerogenic dendritic cells generated with dexamethasone and monophosphoryl lipid A modulate antigen-specific memory CD4+ T cell responses in a pro-tolerogenic direction.

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Dendritic cells (DCs) are antigen-presenting cells that initiate and modulate immune responses. Depending on their maturation state DCs are able to induce immunity or tolerance. In their tolerogenic stage (tDCs), DCs are considered a promising therapeutic tool for T cell-mediated pathology control by T cell deletion, anergy induction, cytokine deviation, and/or regulatory T cells induction. Recently, we described a 5-day protocol for generation of monocyte-derived tDCs with dexamethasone and monophosphoryl lipid A (MPLA-tDCs), characterized by low expression of costimulatory molecules, an IL-10^{high}/IL-12^{low} profile, CCR7/CXCR4 upregulation and reduced ability to stimulate CD4+ T cells, inhibiting their pro-inflammatory cytokine secretion. Furthermore, we studied the antigen-specific modulation of memory CD4+ T cells by MPLA-tDCs in terms of proliferation using CFSE dilution method, and cytokine production by intracellular staining and ELISA. We demonstrated that after CD4+ memory T cells were co-cultured with MPLA-tDCs, they exhibited a reduced proliferation rate, a diminished pro-inflammatory cytokines production and an increased IL-10 secretion. In addition, CD4+ memory T cells displayed a hyporesponsiveness to re-stimulation with autologous mature DCs evidenced by low proliferation and cytokine expression. Our data support that MPLA-tDCs are able to modulate memory T cells responses in a pro-tolerogenic direction. Funded by FONDECYT 1100102 and MIII-P09/016-F.

385. Phenotypic characterization of murine tolerogenic dendritic cells generated with dexamethasone and monophosphoryl lipid A and preliminary evaluation of their therapeutical properties in a mice model of arthritis

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Dendritic cells (DCs) are professional antigen-presenting cells considered pivotal orchestrators of the immune responses. In their tolerogenic state, which can be enhanced *in vitro*, they have proved to modulate several experimental models of autoimmunity. Although two clinical trials using tolerogenic DCs to treat rheumatoid arthritis (RA) patients are being currently conducted, there are no reports about their safety and effectiveness to date. In this context, the use of the collagen-induced arthritis (CIA) murine model, the most extensively applied experimental model of RA, for tolerogenic DC effectiveness testing seems very useful. We describe an 8-day protocol for bone marrow-derived DC generation, modulated *ex vivo* with dexamethasone, an immunosuppressive drug, and the non-toxic LPS-derivative monophosphoryl lipid A (MPLA), producing MPLA-tDCs. MPLA activates DCs and endows them with a stable tolerogenic profile, which should resist the pro-inflammatory milieu detected by MPLA-tDCs after administered. Murine MPLA-tDCs displayed reduced expression of CD86 costimulatory and CD40 functional activator molecules and low secretory levels of IL-6, a pro-inflammatory cytokine. When MPLA-tDCs were injected in mice with established CIA in a preliminary experiment, they were able to modulate the autoimmune process. Our results suggest that MPLA-tDCs may be a promise for future RA cell-based therapies. Funded by CONICYT 791100001, FONDECYT 1100102 and MIII-P09/016-F.

386. Role of Complement resistance Protein in *Trypanosoma cruzi* virulence

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Complement Resistant Proteins (CRP), are known as virulence factor involved on *Trypanosoma cruzi* resistance to complement lysis (CL). We used the *T. cruzi* H510 clone to demonstrate the role of CRP on virulence. One variant of H510 clone was cultured on mice by 25 years, selecting a virulent forms of the clone named H510^{vir}. The other, was cultured by 25 years on axenic medium selecting an avirulent form (H510^{avir}). Here, trypomastigotes and epimastigotes from H510^{vir} and H510^{avir} were evaluated respect their susceptibility to CL. Then, parasites were incubated with two fold dilutions of fresh human sera used as source of complement. In addition, expresion of CRP was evaluated by transcriptomic and proteomic approaches. When CL assays were performed with epimastigotes a hundred percent of H510^{avir} parasites were lysed while H510^{vir} displayed a 60% of susceptibility. On the other hand, trypomastigotes displayed less susceptibility to CL where H510^{vir} parasites were less lysed than H510^{avir} trypomastigotes (20% v/s 60% of lysis). Transcriptomic and proteomic approaches shown that CRP was more expressed on H510^{vir} trypomastigotes than in H510^{avir} ones.

These results reinforce the idea that CRP are involved on parasite virulence which is based on the parasite capacity of resist CL.

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387. *Helicobacter pylori* increases NK cell response by induction of NKG2DL surface expression in gastric adenocarcinoma cells

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NKG2D ligands (NKG2DL) are inducible molecules expressed in stressed cells. Upon recognition, these molecules allows damaged cells to be eliminated by Natural Killer (NK) cells. Modification in their expression levels in tumor cells has been associated to immune evasion and tumor establishment. *Helicobacter pylori* (*H. pylori*) infection has been related with gastric cancer (GC), but the mechanism associated to its influence on tumor development are still under study. Our aim was to describe the effect of HP on NKG2DL expression on gastric adenocarcinoma cell lines and its influence on NK cell response. For this purpose, MKN-45 cells were incubated with Heat killed *H. pylori* (HKHP) and later co-incubated with purified peripheral NK cells from healthy donors for cytotoxic assays. NKG2DL expression and NK cell activation were analyzed by flow cytometry. HKHP induced MICA, ULBP2-5-6 and ULBP4 cell surface expression in adenocarcinoma cells, which favored NK cell activation, as determined by increased CD107a expression on these effector cells. These results suggest that *H. pylori* can induce NK cell cytolytic activity, which may explain, in part, why its presence in tumor microenvironment could trigger a chronic NK cell response associated with NK cell anergy and immune evasion by tumor.

388. Contribution of a conserved metal motif to the antimicrobial activity of trout hepcidin

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Hepcidin is an antimicrobial peptide and a hormone produced in the liver. It is a cysteine-rich peptide with a highly conserved beta-sheet structure. Recently, we describe the hepcidin expression in liver of rainbow trout and its inducibility by iron overloading and LPS. Thus, in this work, we analyzed the importance of its oxidized and reduced state in the conformation and antimicrobial activity and the relevance of the presence of a conserved binding metal motif. The molecular mass of the synthetic peptide was confirmed by MALDI-TOF. Antimicrobial activity assays showed that the oxidized peptide is better than the reduced peptide against the important salmon fish pathogen *Piscirickettsia salmonis*. Moreover, we proved that this peptide is able to induce DNA hydrolysis in the presence of ascorbate and CuCl₂. When the same experiments were carried out using a variant with truncated N-terminus no DNA hydrolysis was observed. Finally, confocal analysis of *P. salmonis* culture exposed to trout hepcidin coupled with rhodamine revealed the intracellular location of this peptide. Our results suggest that correct folding of hepcidin is required for its antimicrobial activity and most likely the metal-binding site (ATCUN motif) present in its N-terminus is involved in the oxidative damage to macromolecules.

Acknowledgements: CONICYT.

389. Generation of tolerogenic monocyte-derived dendritic cells from rheumatoid arthritis (RA) patients aimed at providing a treatment for RA.

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RA, an incurable systemic inflammatory disease resulting from an autoimmune response to unknown self-antigens, is characterized by chronic joint synovial inflammation and progressive cartilage and bone destruction. RA process involves both an altered antigen presentation by antigen presenting cells, priming auto-reactive CD4⁺ T cells, and also an imbalance between effector and regulatory T cells. In the collagen-induced arthritis (CIA) murine model, the most extensively applied experimental model of RA, we proved that a single inoculation of antigen-loaded tolerogenic DCs (t-DCs) were able to inhibit the established CIA. Encouraged by these results, in a 5-day protocol, using dexamethasone as immunomodulatory agent, and monophosphoryl lipid A (MPLA) as tDCs activator, we generated monocyte-derived tDCs from RA patients (MPLA-tDCs). We demonstrated that MPLA-tDCs exhibited a semi-mature surface phenotype and an anti-inflammatory cytokine secretion profile. In addition, when CD4⁺ T cells were co-cultured with MPLA-tDCs, they displayed reduced allogeneic CD4⁺ T cell stimulatory abilities, and the percentage of actively proliferating IFN γ -producing CD4⁺ T cells was significantly reduced compared with that exhibited by CD4⁺ T cells co-cultured with mature DCs. These data support that MPLA-tDCs could be considered as potential tools for a future RA therapy. Funded by FONDECYT 1100102 and MIII-P09/016-F.

390. Alternative electron donor in P450s systems of the carotenogenic yeast *Xanthophyllomyces dendrorhous*

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Cytochrome P450s (P450s) are enzymes involved in the oxidation of many metabolites that depend on a P450 reductase to receive electrons from NADPH. However, alternative electron donors from NADH via cytochrome b5 reductase and cytochrome b5 (Cytb5R and Cytb5, respectively), have been described. In *X. dendrorhous*, *crtR* encodes the P450 reductase and 13 P450 genes have been identified, including *crtS* (astaxanthin synthase) and *CYP61* (22-sterol desaturase), which are involved in astaxanthin and ergosterol biosynthesis, respectively. The *crtR* mutant does not synthesize astaxanthin, but although less than wild type, it is able to produce ergosterol suggesting that alternative electron donors complement this mutation. The aim of this work was to identify and characterize the genes encoding Cytb5 and Cytb5R in the genome of *X. dendrorhous*, determine their expression by RT-qPCR and to analyze cytochrome c reductase activity in wild-type and *crtR* mutant strains after 24, 38, 72 and 120 h of culture. The *CYTB5R* and *CYTB5* genes were identified and their transcript levels were 2-fold higher in *crtR* than in the wild-type strain. The cytochrome c reductase activity was similar in microsomes from both strains, but dependent on different cofactors: NADPH in wild type and NADH in the *crtR* strain. These results support that the Cytb5R-Cytb5 via is operative in *X. dendrorhous*, supporting ergosterol but not astaxanthin biosynthesis.

391. Role of a general transcription repressor involved in catabolite repression in the regulation of carotenogenesis in *Xanthophyllomyces dendrorhous*.

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Xanthophyllomyces dendrorhous is a carotenogenic yeast that produces astaxanthin, a pigment of commercial interest due to its use in aquaculture and pharmaceutical industries. Actually, the carotenoid biosynthetic pathway and the expression profile of carotenogenic genes in *X. dendrorhous* are known. However, there is a lack of knowledge regarding regulatory aspects of this process. It has been demonstrated that glucose has a repressive effect on carotenogenesis, which may be explained in part by transcriptional repression of carotenogenic genes. It is known that catabolic regulation is one of the most important regulatory mechanisms in yeasts, which is widely conserved. Possible regulatory genes in this yeast, including those involved in catabolite repression like *MIG1*, *GTR1* and *GTR2* were identified in our laboratory by bioinformatic methods. Due to the above-mentioned, it is interesting to study their possible role on the carotenogenesis regulation process in *X. dendrorhous*. The aim of the present study was to characterize the role of the *GTR1* gene that encodes a general transcription repressor in the inhibition of carotenogenesis mediated by glucose. The effect of the *gtr1* mutation in the yeast resulted in a higher carotenoid production and changes on the expression of carotenogenic genes when strains are cultured in presence or absence of glucose. Funding: Fondecyt 1100324. Cordova, P. is CONICYT doctoral fellowship.

392. The *ICY1* gene of *Saccharomyces cerevisiae* is involved in ammonium uptake during alcoholic fermentation

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Saccharomyces cerevisiae is the main microorganism responsible for alcoholic fermentation. The consumption of nitrogen in this process is of great importance since it is found in limiting quantities and its deficiency produces stoppage and lagging of the fermentation. We compared the transcriptional profiles of genetically related strains which differ in their nitrogen consumption and identified genes with differential expression which could explain the different consumption levels of this compound, between them in the gene *ICY1*. With the aim of confirming the effect of this gene on the consumption of nitrogen, we evaluated its expression in wine yeast during the first stages of fermentation in low (60 mg/L) and normal (300 mg/L) conditions of assimilable nitrogen. The results indicate that the transcript levels of this gene decrease in low assimilable nitrogen condition. Furthermore, we constructed a wine strain derived from the industrial strain EC1118 as a null mutant in this gene as well as one that overexpressed it. Our results suggest that the expression of gene *ICY1* is regulated by the amount of nitrogen available in the must and it is involved in the consumption of ammonium, given the increase in the consumption of this nitrogen source observed in the null mutant strain, and a decrease in the consumption of this compound in strain overexpressing the gene in both conditions.

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393. Single- and dual-species biofilms of *Klebsiella pneumoniae* and *Escherichia coli* clinical isolates from patients with catheter-associated urinary tract infections

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Urinary tract infections associated with long-term catheterization are polymicrobial, and biofilms constitute a bacterial strategy for persistence on catheter surfaces. In a retrospective study performed at a public hospital from Ciudad Autónoma de Buenos Aires, we found *K. pneumoniae* and *E. coli* frequently co-isolated from patients with polymicrobial bacteriuria. We studied the ability of these *K. pneumoniae* and *E. coli* clinical strains to form single- and dual- species biofilms. Biofilm quantification was performed through total biomass quantification by crystal violet staining. From the analysis of single-species biofilms, it was evident that *K. pneumoniae* biofilms developed faster and had a higher total biomass than those of *E. coli*. Moreover, the number of cultivable cells being recovered from *K. pneumoniae* biofilms was 3-times higher than for *E. coli* biofilms at all the time-points assayed (from 2 to 72 h). In dual-species biofilms, development of total biomass was similar to that observed for *K. pneumoniae* in single-specie biofilms. Cultivability assays using selective media evidenced that *K. pneumoniae* constituted more than 90 % of the biofilm population after 24 h. Moreover, a decrease in *E. coli* cultivability in dual-species biofilms, compared to single-specie biofilms, was observed. Our findings suggest that the interaction between *K. pneumoniae* and *E. coli* in dual-species biofilms resulted in a detrimental effect over *E. coli*.

394. Interaction between biofilm matrix components in *Rhizobium leguminosarum*

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Rhizobia are soil bacteria that infect root tissue and induce the development of nodules on leguminous plants under conditions of nitrogen starvation. The ability of rhizobia to grow in varied environmental conditions is probably due to its ability to produce different types of biofilm. The main component of the extracellular matrix of the biofilm developed on a glass surface is an acidic exopolysaccharide or EPS that provides support to the biofilm structure. Proteins secreted by a type I secretion system (PrsDE) have been shown to be important for biofilm development. Interestingly, some of them possess one or more characteristic Ra/CHDL (cadherin like-) domains that confer carbohydrate binding ability. Among them, we have characterized RapA2, a unipolar calcium binding lectin that specifically recognizes the EPS and the glycanases Ply that regulate the length of EPS molecules in the extracellular medium. A characteristic distribution of EPS molecules length is important for the development of an ordered polysaccharide network. Thus, *Ply* mutants are unable to form a typical biofilm. On the other hand, anomalous levels of RapA2 affect the production of surface acidic polysaccharides. A model depicting the interaction of RapA2, *Ply* glycanases and acidic polysaccharides during the progress of biofilm matrix development is presented. *Supported by CONICET and Agencia de Promoción Científica y Tecnológica-FONCYT*

395. Structural-functional analysis of *Helicobacter pylori* the HU protein at acidic pH

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The HU protein is the only histone-like bacterial protein in *H. pylori*. HU binds and bends DNA, a function associated with DNA integrity and regulation of gene expression. HU structure shows an α -coil crowned by extended β -strands that form an arm causing two sharp DNA kinks. HU participates in acid stress response mechanisms. We have shown that HU is overexpressed by *H. pylori* under acid stress, and that it is down-regulated when tested in an acid-sensitive mutant strain. We designed, cloned and purified two substitution mutant proteins, HU_{m2}: K3A/S27D and HU_{m3}: K62R/V63N/P64A, to conduct in vitro analyses. Wild-type and the mutant proteins formed dimers and tetramers in solution. Both mutant proteins bind DNA with less affinity and HU_{m3} was unable to induce DNA compaction. This suggests that amino acids 62-64, which interact with the DNA minor groove, are crucial for DNA bending and compaction. DNA protection seems to be related with the DNA-binding ability because the three proteins protected DNA from endonucleolytic cleavage and from oxidative stress damage. The *H. pylori* *hup* negative mutant has both a lower rate of growth at pH 7.0 than the wild type strain, and an acid sensitive phenotype at pH 5.5. Gene expression qPCR studies in the *hup* negative mutant showed that its acid sensitive phenotype could be associated with lack of induction of *speA* and *ureA* genes whose products have been involved in acid resistance mechanisms. FONDECYT 1120126

396. Assessment of the protein interactions of Acy, a new membrane protein involved in Z ring orientation during cell division of the filamentous cyanobacterium *Anabaena* sp. PCC 7120.

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Anabaena sp. PCC 7120 (*Anabaena*) is a filamentous cyanobacterium capable of fixing nitrogen through differentiated heterocysts. Filaments grow through vegetative cell division in one plane, forming long trichomes. *all2320* encodes a protein, hereafter Acy (30kDa), present exclusively in filamentous cyanobacteria. In previous work, we analyzed the phenotype of the non-fully segregated *Anabaena* *all2320*-mutant, where the Z ring was located in a different axis. Domain search with bioinformatic program SMART showed that Acy contains a transmembrane domain and a coiled-coil domain, where other proteins can be associated. Our laboratory is particularly interested in Acy interactions and its role in cell division of filamentous cyanobacteria. Total protein extract from exponential phase *Anabaena* cultures were used to analyze the protein interactions. First protein complexes were separated by BN-PAGE and their respective subunits were visualized using a second dimension SDS-PAGE. We produced a polyclonal antibody (anti-Acy) to perform the immunodetection through Western Blot analysis, detecting that Acy is associated with 3 different proteins, forming a complex of around 66 kDa. In order to identify the protein complex, the respective bands were cut and sequenced by MALDI-TOF. Further *in vivo* analysis will be performed in *Anabaena* using BIFC protocols with the purpose of confirming these results. (Grant: FONDECYT 1131037).

397. Involvement of some *Cupriavidus pinatubonensis* JMP134 aromatic catabolism pathways in degradation of root exudates and rhizosphere colonization of *Arabidopsis thaliana*.

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Cupriavidus pinatubonensis JMP134, a highly versatile bacterium in terms of catabolic ability, is known to possess 11 out of 12 described aromatic compounds degradation bacterial pathways. Recent reports have demonstrated that *C. pinatubonensis* is able to establish colonies in the rhizosphere of *Arabidopsis thaliana*, presumably using its exudates as source of carbon and energy. Studies using catabolic pathways mutants have shown that simultaneous mutation of *pcaIJ*, *mmIFG* and *scoAB* genes (keys in the β -ketoadipate pathway), decreases rhizospheric colonization of *A. thaliana* and exudates utilization. We sought to expand this knowledge preparing and using JMP134 strain mutants in genes *hmgA* and *abmG*, encoding key steps in homogentisate and anthranilate pathways, respectively; *bphQ* (involved in organic acids-induced catabolic repression of aromatic compounds degradation) and *pcaH* (protocatechuate degradation pathway). Our results showed that strains $\Delta hmgA$ and $\Delta abmG$ do not significantly alter rhizospheric colonization of *C. pinatubonensis*, or growth on exudates, with the exception of strain $\Delta bphQ$, which showed a decreased growing rate in exudates. Surprisingly, $\Delta pcaH$ mutant showed an increase in fresh weight of *A. thaliana*. We suggest that certain routes of aromatic compounds catabolism contribute to interaction between *A. thaliana*-*C. pinatubonensis* JMP134.

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398. Immune response and invasiveness ability of intracellular *Escherichia coli* isolated from Inflammatory bowel diseases patients: invasiveness in eukaryotic cells.

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Background: Adherent-invasive *Escherichia coli* (AIEC) strains have been associated to Crohn's disease (CD) pathogenesis. The aim of this study is to evaluate the genetic difference and invasiveness *in vitro* of intracellular *E. coli* (IEC) strains isolated from Chilean patients and to determine the induction of immune response in macrophages. Methods: IEC strains from intestinal biopsies were isolated from 8 CD patients, 2 UC and 1 control using gentamicin protection assay. The strains were characterized for genetic heterogeneity by PCR and pulsed-field gel electrophoresis (PFGE), respectively. Invasive and replicative ability was assessed by infections of epithelial cells and macrophages with strains *in vitro*, respectively. The secretion of proinflammatory cytokines in macrophages exposed to bacterial isolates was determined by ELISA. Results: Strains from different patients are heterogeneous, but those from the same patient are closely related (over 95%). The ability to invade epithelial cells and survive is variable in different strains, but in most cases is higher than commensal *E. coli*. IEC strains are able to induce the secretion of cytokines such as TNF- α , IL-6 and IL-1 β in macrophages. Conclusions: Intracellular *E. coli* strains studied have different virulence genes that give the ability to invade and survive in eukaryotic cells and induce an innate immune response.

399. Root colonization and long-range signaling by *Burkholderia phytofirmans* PsJN contribute differentially to saline stress tolerance in *Arabidopsis thaliana*.

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Environmental stress limits the growth of plant crops worldwide. However, interaction with certain plant growth promoting rhizobacteria (PGPR) improves the rate and extent of plant growth in the presence of environmental challenge. *Burkholderia phytofirmans* PsJN is a well-studied PGPR, reported to produce hormone phytostimulation of different plant hosts, which could result from bacterial activity in contact with plant tissues, and/or from long range signaling among partners, without the need of actual root colonization. We reported that strain PsJN increases salt tolerance in *Arabidopsis thaliana*, but the bacterial functions involved in this effect are yet unclear. The aim of this work was to assess the role of direct plant-bacteria contact on plant tolerance to saline stress, comparing growth and response to salinity in *A. thaliana* inoculated *in vitro* with strain PsJN, and in plants co-cultured with the bacterium held in a separate compartment, relative to salt-treated plants grown without *B. phytofirmans*. Additionally, the contribution of specific bacterial colonization functions was assessed using two PsJN mutant derivative strains. Our results indicate that PsJN-induced salinity tolerance in *Arabidopsis* is only partially dependent on tissue colonization, suggesting that phytostimulation by *B. phytofirmans* is mediated by a combination of alternative, and probably complementary, growth promotion mechanisms. Fondecyt 11121515, 11121306, NM-PFG (P10-062-F).

400. *Burkholderia phytofirmans* PsJN improves growth of *Arabidopsis thaliana* under drought stress conditions.

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Drought is one of the most limiting environmental factors affecting establishment and survival of terrestrial plants, producing huge losses in crop productivity worldwide. A few bacterial strains, belonging to the plant growth promoting rhizobacteria (PGPR) group, have been described to associate with several plants, increasing their tolerance to specific biotic and abiotic stresses. *Burkholderia phytofirmans* PsJN is able to promote growth of a number of plant hosts, increasing their growth rate and, in certain cases, their tolerance to chilling and salinity. The aim of this work was to explore the effect of *B. phytofirmans* inoculation on plant tolerance to drought stress, evaluating the response of colonized *Arabidopsis thaliana* when subjected to varying stress intensities. Plants inoculated with strain PsJN were cultured under different irrigation conditions (25, 50, and 100%), and leaf growth rate and total biomass were compared to those of non-inoculated plants. To evaluate the effect on osmotic stress, plants were grown *in vitro*, in the presence of osmotic solutes as Mannitol and PEG. Our results suggest that strain PsJN inoculation in *A. thaliana* increases plant growth under drought conditions, improving biomass yield in 50 – 70% relative to control plants. In addition, biomass yield was equivalent for inoculated plants on all irrigation treatments, suggesting a marked reduction in overall water requirements. FONDECYT 11121515, NM-PFG (P10-062-F).

401. MutS controls the action of DNA Polymerase IV

Margara, L¹., Argaraña, C¹., Monti, M¹., CIQUIBIC-CONICET, Dpto Química Biológica, Fac. Ciencias Químicas, Universidad Nacional de Córdoba- República Argentina. (Sponsored by SECYT-UNC, Mincyt-Cba, CONICET, ANPCyT)

MutS contributes to the DNA replication fidelity by recognizing mispairs and recruiting factors involved in the Mismatch Repair (MMR). Interaction of MutS with the replication processivity factor β clamp has been proposed to target MutS to areas of active DNA replication to initiate MMR. We demonstrated that the MutS- β clamp interaction does not play a role in the MMR of *Pseudomonas aeruginosa* by characterizing a strain (*mutS^b*) which encodes a MutS mutant that does not interact with β clamp. Although the *mutS^b* strain exhibits mutation rates similar to that of the parental strain, it shows a change in the mutation spectrum which is compatible with the activity of the low fidelity DNA polymerase IV (PolIV). We propose that MutS could be limiting the action of PolIV by its interaction with β clamp. To test this hypothesis, we first analyzed the mutation spectrum in PolIV deficient strains. This assay confirmed that PolIV is responsible for the change of the mutation spectrum in the *mutS^b* strain. Then, we examined the effect of increasing PolIV expression levels on mutagenesis. Mutation rates were increased and PolIV-associated mutations were dominant in the mutation spectrum of the *mutS^b* strain. This effect depended of the catalytic activity and interaction with β clamp as overexpression of PolIV-D8A and PolIV- Δ 5 mutants did not increase mutation rates, respectively. Conversely, mutagenesis was slightly promoted by PolIV in the parental strain.

402. Assembly and arrangement of the DNA translocase SpoIIIE for the chromosome segregation during the sporulation of *Bacillus subtilis*

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Chromosome segregation is one of the most essential aspects of bacterial cell division. In sporulating *Bacillus subtilis*, SpoIIIE, which is a DNA translocase and membrane-anchored protein, localizes as a focus in the division septum and exports a circular chromosome from the mother cell into the developing forespore. The functional architecture and organization of SpoIIIE at the septum to translocate DNA is still controversial. Here, using a combination of genetics, biochemistry, bioinformatics and single molecule microscopy (optical tweezers, atomic force microscopy, AFM, and photoactivatable localization microscopy, PALM), we demonstrate that the motor domain of SpoIIIE is a fast DNA translocase (~4000 bp/s) that assembles as a hexamer and in the presence of the membrane domain assembles into a larger protein complex with a molecular weight of dodecamer. Modeling of the SpoIIIE motor domain shows that the hydrophobic interaction might play an important role to maintain the ring structure. Moreover, *in vivo* SpoIIIE organizes as a two complexes at the division septum, one located in the mother cell and the other in the forespore when visualized by PALM. These results support the DNA translocation model in which two hexamers of SpoIIIE interact to form a DNA crossing channel across the sporulation septum.

403. In vitro characterization of cytotoxicity of the antimicrobial polypeptide, gicina A on normal and tumor cells.

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Bacteriocins show potential applications in the food industry and in the pharmaceutical industry. Gicina A is a bacteriocin, produced by *P. aeruginosa* O400 which has antimicrobial activity against Gram positive bacteria. It is relevant to study the cytotoxic effect of bacteriocins on human eukaryotic cells. To characterize the cytotoxic effect of gicina A, its hemolytic potential was measured on human erythrocytes, on peripheral blood mononuclear cells (PBMC) and on HEK 293 cell line. Nisin was used as a low toxicity control, due it is used as food preservative. The results show a similar hemolytic activity between gicina A and nisin, measured by hemoglobin release with a concentration 100-fold minimum inhibitory concentration (MIC) both bacteriocins. The cytotoxicity of gicina A on PBMC is low as the percentage of live cells does not decrease after 24 h of exposure, a decrease was observed only after 48h and 72h of exposure to gicina A with values of 100-fold the MIC for this. The effect of nisin on these same cells shows an increase in the percentage of live cells with a concentration 100-fold the MIC. Finally its cytotoxicity was characterized on HEK293. It was observed that gicina A shows a greater cytotoxicity than nisin, as the percentage of live cells decreased and with gicina A and nisin for 72h exposure to the peptides and with a concentration of 100-fold the MIC.

404. Effect of Enriched Environment on the Auditory Attention of Stressed Rats

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Chronic stress induces dendritic atrophy on the rat auditory cortex and impairs auditory attention. The aim of this study was to analyze whether an enriched environment (EE) improves auditory attention of stressed rats. Male *Sprague-Dawley* rats were subjected to EE or standard environment (SE) during the experiment. Animals were trained in a two-alternative choice task (2-ACT), a behavioral paradigm to study auditory attention in rats. Trained animals that reached a performance over 80% of correct trials in the 2-ACT were randomly assigned to the following experimental groups: control, stress, control + EE, stress + EE (n=9 for each group). To analyze the effects of restraint stress and EE on the auditory attention, trained rats of all groups were subjected to 50 2-ACT trials one day before and one day after of the stress period. A difference score was determined by subtracting the number of correct trials after from those before the stress protocol (DS-CT). Chronic stress significantly impaired the auditory attention and EE improved this effect. As well, control rats subjected to EE had lower levels of DS-CT than those rats that lived in a SE. Our results show that EE exposure improves the stress effects on auditory attention.

405. Characterization of proteases produced for *Pseudoalteromonas issachenkonii* and *Flavobacterium* sp., obtained from rey jorge island, antártica

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The proteases are hydrolytic enzymes that can be used for biotechnological purposes. Nevertheless, the enzymes that are being used at present have an optimal temperature of catalysis around 60°C, being inefficient in many applications. The search and characterization of proteases secreted by psychrophilic and psychrotolerant microorganisms may result on enzymes with improved properties, due to metabolic adaptations to low temperatures. Microorganisms with these features are found in Antarctica. In the present work, 12 Antarctic strains were evaluated, eight were able to produce proteases on solid medium (using skin-milk agar), and two were selected for their special properties: *Pseudoalteromonas issachenkonii* (*Pi*) and *Flavobacterium* sp. (*Fsp.*). The protease-production capacity in liquid medium (measured through azocasein assays) in both strains was better at 10°C, at incubation time over 72 hours. The optimal conditions for the proteolytic activity of the extracts was 35°C and pH 9 for *Pi* and 25°C and pH 8 for *Fsp.* Using specific inhibitors, it was determined that *Pi* produced serine proteases, and *Fsp.* produced metalloproteases. The catalytic properties were also determined. Our results suggest the presence of proteases with better adaptations to low temperatures, and biotechnological potential. Projects: INNOVA BIO-BIO N° 12.250; INACH T-17-08 and Facultad de Ciencias Biológicas, Universidad de Concepción.

406. Kinetic studies of the hydrolysis of pectin by commercial pectinases

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There has been a recent interest in developing citrus waste biorefineries. A key process in such a biorefinery is the hydrolysis of pectin to D-galacturonic acid, which is a precursor of various chemical intermediates with industrial applications. Due to the resistance of the pectin backbone to chemical hydrolysis, the best way to obtain D-galacturonic acid is via enzymatic hydrolysis of the pectin by pectinases. Although pectinases have been used in the food industry for many years, relatively little attention has been given to the kinetics of the saccharification of pectin. Our group has initiated studies into the kinetics of hydrolysis of pectin by commercial pectinase preparations. There is a significant deceleration in the rate of the release of reducing sugars over the first ten minutes of the reaction, even though the degree of hydrolysis is still relatively low. Although several authors have attributed this deceleration to product inhibition, we have demonstrated that this is not the case. Two possible alternative explanations are the exhaustion of easily hydrolysable bonds or the stalling of enzymes on the pectin molecule. Since the addition of an additional aliquot of enzyme during the reaction only leads to a temporary increase in reaction rate, stalling appears to be the most likely explanation. We are currently undertaking more detailed studies to confirm whether this is indeed the cause of the deceleration.

407. Evaluation of the antimicrobial and antiproliferative effect of venom of Chilean tarantulas and Chilean Scorpions.

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Chile has a rich fauna of arachnids (Mygalomorphae) and scorpions distributed throughout its geography, whose venoms can be exploited because of its biotechnological potential. We evaluated the antimicrobial and antiproliferative effect of the venom of different species of Chileans tarantulas and scorpions. The tarantula genders studied were *Euathlus*, *Grammostola*, *Paraphysa*, *Aphonopelma* and *Olygoxistre*, were purchased commercially and were maintained in appropriate arcnarios. The species of Chileans scorpions were *Bothriurus keiserlingi* and *Caraboctonus keiserlingi*. SDS-PAGE electrophoretic separation of venoms showed a significant presence of peptides smaller than 10 kDa. In addition it was observed that the protein profile was very similar between the different species of tarantulas. Antiproliferative effect was shown with the venom of *Grammostola*, *Paraphysa* and *Euathlus*, which were able to decrease the proliferation of Vero cells in culture. In contrast, the venom of the both species of scorpions showed no significant antiproliferative activity on Vero cells. Moreover, all tested tarantula venom, showed different levels of antimicrobial activity against *Staphylococcus aureus*, with a higher antimicrobial effect shown by the venoms of *Grammostola porteri* and *Grammostola rosea*. These results provide us to guidance for the assessment of purified fractions of the venoms and subsequent evaluation of peptides, which have a high biotechnological potential.

408. ECM-specific caveolin-1 phosphorylation on tyrosine 14 promotes melanoma migration

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Increased expression of Caveolin-1 (CAV1) and phosphorylation on tyrosine-14 is associated with enhanced migration and metastatic potential of tumor cells. Extracellular matrix (ECM) interactions of cells via integrins play an important role in migration and metastasis. Here we evaluated how CAV1 phosphorylation on tyrosine-14 relates to migration and spreading of B16F10 melanoma cells on different ECM surfaces. CAV1-enhanced persistency and directionality of migration on both fibronectin and laminin correlated with increased tyrosine-14 phosphorylation. Moreover, CAV1-enhanced migration was ablated by expression of CAV1(Y14F), but not CAV1(Y14E). Also, CAV1 increased cell spreading on fibronectin and focal adhesion disassembly in a tyrosine-14-dependent manner and surface expression of beta1 and alpha5 integrins. In summary, CAV1-enhanced migration on specific ECM surfaces requires tyrosine-14 and is linked to increased cell spreading, focal adhesion disassembly and integrins surface expression.

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409. Functional study of *Toxoplasma gondii* small heat shock protein 20

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Toxoplasma gondii is a protozoan parasite which belongs to the phylum Apicomplexa. It is known that synthesis of small heat shock proteins (sHSP) can be triggered under conditions of stress. Our laboratory has studied *T. gondii* HSP20 which localizes to the inner membrane complex (IMC). This localization is achieved by N-terminal palmitoylation. Experiments with the palmitoylation inhibitor 2-bromopalmitate show changes in TgHSP20 localization from the parasite's pellicle to the cytosol. Similar results can be obtained with a non-palmitoylatable TgHSP20 version. In addition, we generated a Tghsp20 gene deletion (TgHSP20-KO) strain to address TgHSP20 function. TgHSP20 showed to be dispensable for parasite survival and these parasites showed no growth defect. However, the TgHSP20-KO strain showed a mild invasion phenotype at low temperature. Additionally, the TgHSP20-KO strain displayed a lower invasion rate than the parental strain after treatment with agents that affect membrane fluidity. This suggests that TgHSP20 might be involved in maintenance of membrane fluidity and that this condition is necessary for invasion. Further characterization of the phenotype of this mutant under a variety of environmental stress conditions is ongoing.

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410. Estrogen receptors concentrations in canine mammary tumors

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The aim of this study was to investigate the concentration of estrogen receptors (ER) in canine mammary tumors. The tumors were classified as benign (n=3), malignant (n=8) and hyperplasia (n=2). The ER determinations were performed by ligand binding assay. The ligand used was 3H-E2 while nonlabelled ligand was diethylstilbestrol. The ER concentrations and the apparent dissociation constant (Kd) values were analyzed by ANOVA. The Kd values for ER were similar in tumors benign, malignant and hyperplasia (0.75±0.20, 0.52±0.13 and 0.89±0.28 nM, respectively). There was a significant effect of tumor on the ER concentration (P≤0.002). In the malignant tumors the ER concentrations were lower than in the benign (P≤0.0006) and in the hyperplasia (P≤0.04) (10±1.9, 29±3.2 and 16±3.9 fmol/mg proteins, respectively). The ER concentrations in the benign tumors and in the hyperplasia were similar. The Kd values for ER found were similar, suggesting that variations in responsiveness of tumors to estrogen may not depend on changes of affinity, but rather on the binding capacity (ER concentrations). Lower ER concentrations in tumors malignant than in the benign were found in agreement with the results reported for mammary canine tumor. The higher ER concentrations found in benign mammary tumors suggests that it could establish a complementary treatment to surgery with anti estrogenic drug.

411. Distribution of matrix metalloproteinase-2 (MMP-2) in sheep cervix during the oestrous cycle

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The aim of this work was determine the histological distribution of matrix metalloproteinase-2 (MMP-2) isozyme in the ovine cervix during the oestrous cycle. Cranial (Cr) and Caudal (Ca) cervical zones were obtained from Corriedale adult ewes at Days 1 (n=7), 6 (n=5) or 13 (n=7) after oestrus detection (Day 0). The distribution of MMP-2 was assayed by immunohistochemistry using a polyclonal antibody (hMMP-2, cs-8835, Sta. Cruz Biotech., Inc.CA-USA). Positive and negative MMP-2 nuclei were counted (400X) in 1000 cells in each stromal compartment: superficial and deep fold stroma and superficial and deep wall stroma. The percentage of MMP-2 positive cells (%MMP-2) was calculated and analyzed by ANOVA (Mixed Proc, SAS). The MMP-2 immunostaining was associated mainly to active fibroblasts, some to inactive fibroblasts and occasionally to leucocytes. The %MMP-2 (mean±sem) was lower on Cr (0.97±0.27) than on Ca (2.83±0.43), (P<0.0004). The %MMP-2 trend to be higher on Day 1 than on Day 13 in Cr (1.56±0.40 and 0.38±0.50, respectively, P=0.080), whereas was lower on Days 1 and 6 than on Day 13 in Ca (2.76±0.61, 1.43±1.05 and 4.30±0.43), (P<0.006). The results suggest that the active fibroblasts may be involved in cervical collagenolysis, with a greater potential in the Ca zone. The percentage of MMP-2 positive fibroblasts could be differentially hormone-modulated during the oestrous cycle depending on the cervical zone.

412. Stimulation of the kinin B1 receptor activates specific signaling pathways and transactivates the epidermal growth factor receptor (EGFR) in normal and neoplastic human keratinocytes

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Wound healing and cancer are two conditions in which keratinocytes proliferate and migrate after they become activated by growth factors like EGF. Our aim was to investigate whether kinin B1 receptor (B1R) stimulation results in transactivation of the EGFR and at the same time regulates cell proliferation. Stimulation of normal and neoplastic keratinocytes with a B1R agonist produced transactivation of EGFR and phosphorylation of ERK1/2 MAPK, effects that were completely blocked by AG1478, an inhibitor of EGFR-tyrosine kinase or by a negative mutant of EGFR. Different concentrations of the B1R agonist did not change the incorporation of bromodeoxyuridine in normal and neoplastic keratinocytes; moreover, cells stimulated with 100 nM of the agonist displayed a lower proliferation rate than untreated cells. Analysis of cell cycle confirmed that normal keratinocytes did not progress to S or G₂ phases after stimulation with the B1R agonist. Stimulation of the kinin B1R results in activation of specific signaling pathways including EGFR transactivation. In addition, B1R activation in normal and neoplastic keratinocytes may be an important event to modulate EGF-dependent cell proliferation during wound healing and cancer. Supported by FONDECYT 3130385, DID-UACH, Chile

413. Stimulation of kinin B1 receptor induces expression/release of vascular endothelial growth factor (VEGF) and metalloproteases (MMPs) in tumor microenvironment cells

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Progression of breast tumors toward a malignant phenotype is highly dependent on interactions within tumor microenvironment which is composed by tumor cells, resident and infiltrating non-tumor cells, growth factors and components of the extracellular matrix. Our aim was to determine whether stimulation of the kinin B1 receptor (B1R), expressed by cells of this microenvironment, increase the expression/release of VEGF and gelatinases known as MMP-2 and MMP-9. Human neutrophils and macrophages stimulated with 10 nM of a kinin B1R agonist for 1-3 h increased both expression and release of VEGF as determined by western blotting and ELISA. Fibroblasts and endothelial cells also increased the expression levels of VEGF but, after 6 h of stimulation. Activity of MMPs increased in supernatants of fibroblasts and endothelial cells stimulated for 1 h with the B1R agonist as determined by zymography. Our results demonstrate that B1R activation increases both expression and release of VEGF and MMPs in neutrophils, macrophages, fibroblasts and endothelial cells, elements which are part of tumor microenvironment and may contribute to angiogenesis and dissemination of cancer cells. FONDECYT 11090292, DID-UACH, Chile.

414. HERP: a novel regulator of cardiomyocyte hypertrophy

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HERP is an endoplasmic reticulum (ER) membrane protein linked to ER-associated degradation. In a neuronal model, HERP has also shown to regulate Ca²⁺ homeostasis, preventing the degradation of the inositol triphosphate receptor (IP3R) during ER stress. Although HERP has been reported in the heart, its function remains not understood in cardiomyocytes. Pathological cardiac hypertrophy occurs in response to a chronic activation of the sympathetic system which leads to the expression of a hypertrophic phenotype by the activation of the α 1-adrenergic receptor/IP3R/Ca²⁺ signaling pathway. In order to determine whether HERP regulates cardiac hypertrophy, cultured rat cardiomyocytes were treated with a siRNA against HERP in the absence or presence of the hypertrophic agonist norepinephrine (NE). Our data showed that the down regulation of HERP levels produces hypertrophy, increasing sarcomerization, perimeter, area and beta-myosin heavy chain levels in a similar manner to that observed with NE. Such hypertrophic phenotype might be related to the increase in protein levels of the IP3R seen under HERP knock-down. This study proposes a new role for HERP in the heart and establishes a new potential therapeutic target for the treatment of cardiac diseases.

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415. Rab39-vesicular transport is usurped by *Chlamydia trachomatis* to acquire host cell lipids

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Chlamydia trachomatis is the causal agent of the most frequent bacterial sexually-transmitted diseases worldwide. This obligate intracellular pathogen exploits different host trafficking pathways to acquire essential molecules for its survival. Rab proteins comprise a family of GTPases that controls eukaryotic cell traffic. This study analyzes the participation of Rab39 in the course of chlamydial infection. We defined Rab39a localization in multivesicular bodies, late endosomes and a subset of acidic vesicles. By confocal microscopy, we observed that Rab39a associates with the chlamydial inclusion throughout its development. Rab39a colocalizes with two bacterial proteins, IncA and IncG, at the chlamydial inclusion membrane. Overexpression of GFP-Rab39a WT and its positive mutant (GFP-Rab39a Q72L) increases the chlamydial inclusion size. Our results demonstrate that Rab39a decorates vesicles carrying lysobisphosphatidic acid and sphingolipids, and plays a role in the delivery of these molecules from host multivesicular bodies to the chlamydial inclusion. Furthermore, silencing of Rab39a by siRNA reduces bacterial multiplication and infectivity, assessed by the counting of inclusion forming units. Likewise, Rab39a silencing significantly decreases lipid acquisition from infected host cells by these bacteria. These data suggest that *Chlamydia trachomatis* selectively usurps Rab39a pathway to acquire essential lipids for its development, growth and replication.

416. Purinergic and glutamatergic interplay in the upregulation of astrocyte-neurons signaling in the epileptic hippocampus

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The role that astrocytes play in synaptic plasticity and pathophysiological processes has been widely accepted. Reactive astrocytes have been considered as hallmark in several neurological diseases, including epilepsy. Recently, we have shown that in kindling-model of epilepsy, reactive astrocytes exhibit a Ca²⁺-based hyperexcitability that increases the spontaneous glutamatergic gliotransmission and the efficacy of CA3-CA1 synapses. However, the signaling pathways implicated in these upregulation are unknown. Here, by using electrophysiological and Ca²⁺-imaging in combination with pharmacological tools, we studied two possible altered pathways in reactive astrocytes of kindled hippocampal slices and their functional interaction: 1) Glutamate gliotransmission: the blockade of mGluR5 with MPEP increases the PPF index whereas the astrocytic Ca²⁺ signal remains unaffected; 2) Purinergic pathways implicated in astrocyte Ca²⁺ signal: in presence of the non-selective P2Rs antagonist and specific antagonist of P2Y1R MRS2179, astroglial Ca²⁺ waves were strongly reduced in parallel to an increase in the PPF index; 3) The effect of MPEP over synaptic efficacy was occluded in presence of MRS2179. We suggest that in reactive astrocytes from epileptic hippocampus, the P2Y1R activation regulates Ca²⁺-dependent release of glutamate from astrocytes and subsequently upregulate the synaptic efficacy via mGluR5.

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417. *In vivo* distribution of candidate receptors for Andes Hantavirus entry into susceptible cells

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Hantaviruses are rodent-borne single-strand negative sense RNA viruses that belong to *Bunyaviridae* family. Once transmitted to humans via aerosols, they can produce severe disease such as Hantavirus Pulmonary Syndrome or Hemorrhagic Fever with Renal Syndrome. Hantaviruses are composed of a lipid envelope in which two glycoproteins are anchored: Gn and Gc. These proteins are responsible for mediating viral cell entry. Based on *in vitro* studies, several receptors have been proposed to be involved in hantavirus cell attachment or entry, among them β_3 integrin, DAF/CD55 and gC1qR/p32. The present work aims to assess candidate receptors that are involved *in vivo* in Andes virus (ANDV) entry into susceptible cells. To this end, tissues from lungs and kidneys of ANDV-infected rodents were analyzed using immunohistochemistry techniques. In kidneys, all three candidate receptors were present in the medulla renal; however in the renal cortex, only the presence of β_3 integrin was detected. In lungs, DAF/CD55 and gC1qR/p32 were detected in bronchioles and alveoli, but not β_3 integrin. Studies are under way to correlate these results with the distribution of ANDV infected cells among these tissues.

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418. Obesity in pregnancy alters human equilibrative nucleoside transporters 1 expression and intracellular distribution in human umbilical vein endothelial cells

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Human umbilical vein endothelial cells (HUVEC) take up adenosine mainly (~80%) via human equilibrative nucleoside transporters 1 (hENT1). hENT1 activity is altered in pathologies in pregnancy (e.g., gestational diabetes) causing fetal endothelial dysfunction. Since obesity is associated with endothelial dysfunction, a supraphysiological gain of weight in pregnancy (OP) could become a condition altering fetal endothelial function. We evaluated whether hENT1 expression, transport activity and intracellular distribution are altered in HUVEC from OP. Adenosine transport (31-250 μ M, 3 μ Ci/ml, 25°C, 20 seconds) in the absence or presence of 1 μ M and 10 μ M nitrobenzylthioinosine (NBTI), hENT1 protein abundance, mRNA expression levels and hENT1 distribution by immunofluorescence were measured in HUVEC primary cultures from pregnant women whose pregnancy courses with a physiological increase in weight (N, normal) or with OP. hENT1-mediated adenosine transport is unaltered in OP compared with N group. However, hENT1 protein abundance and mRNA expression are increased (~3-fold) in OP compared with N group. Moreover, hENT1 pattern of distribution is mainly perinuclear in OP compared with N group. Thus, OP is a pathological condition that could associates with altered adenosine handling in human fetal endothelium.

419. Silencing effect of Cathepsin L variant on HCT116 cell proliferation

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We have studied a variant of cathepsin L protease homologous to SpH protease, a kind of protease initially described in sea urchin. This variant of cathepsin L is entitled SpH-Cathepsin-L and it has been described also in cancer cells. This particular protease has a molecular weight of 60 kDa and it has a nuclear localization during S phase of the cell cycle; it colocalizes with alpha-tubulin during mitosis in colorectal cancer and cervical cancer cell lines. SpH-Cathepsin L was also identified at the colorectal cancer HCT116 cell line. With the purpose of analyzing the effect of cathepsin L variant (SpH-cathepsin L) on cell proliferation of HCT116 cell line we decided to silence protease gene using siRNA technique, to measure cell proliferation by means of bromodeoxyuridine (BrdU) addition and to finally analyze cell viability as well by means of MTT assay. Obtained results show that SpH-cathepsin L expression silencing when treated with specific siRNA designed in our laboratory affects cell proliferation. It was concluded that this particular cathepsin L variant expression decrease is related to a low rate of HCT116 cell proliferation. Besides, it was shown that used siRNA did not generate a cytotoxic effect in employed cell line. Based on these results it was concluded that SpH cathepsin L participates in proliferative processes at the HCT116 cell line, which is consistent with results obtained with other variants of cathepsin L.

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420. Prostaglandin E2 disrupts association of caveolin-1 to β -catenin/E-cadherin complex, allowing β -catenin release and promoting malignant progression of colon cancer cells

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Prostaglandin (PGE2) synthesis by cyclooxygenase-2 (COX-2) and β -catenin/Tcf/Lef signaling are both up-regulated in colorectal cancer. In cells, β -catenin associates at the cell surface with E-cadherin in a complex that also includes the tumor suppressor protein caveolin-1 (CAV1) (CEB: Caveolin-1/E-cadherin/ β -catenin complex). We previously showed that PGE2 increases nuclear β -catenin localization/activity. However, the mechanism by which PGE2 releases β -catenin from E-cadherin in the CEB complex in colon cancer cells, as well as the role of CAV1 in this process is not known. IP assays were used to detect CAV1 in the CEB complex in DLD-1 colon cancer cells. PGE2 supplementation was used to disrupt the CEB complex. Up-regulation of survivin, β -catenin/Tcf-Lef transcriptional activity, cell proliferation and apoptosis were evaluated by Western blotting, reporter assays, the MTS assay and using the Caspase Glo kit, respectively.

Our data demonstrate that the CEB complex in DLD-1 colon cancer cells is disrupted by medium supplementation with PGE2. Interestingly, PGE2 precludes caveolin-1 (CAV1) association with E-cadherin/ β -catenin at the cell surface and promoted dissociation of CAV1 and β -catenin from the complex, which increased β -catenin release and hence nuclear transcription of target genes and proliferation, but reduced apoptosis. These observations shed light on how PGE2 may promote progression of colon cancer cells.

421. Obesity in pregnancy decreases no synthesis due to inactivation of enos in human umbilical vein endothelium

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Obesity (developed) during pregnancy (OP) is a condition that mothers with normal weight (body mass index (BMI): 18.5-24.9) at the beginning of gestation suffer a supraphysiological gestational weight gain during pregnancy ending with obesity (BMI $\leq$ 30). Even when obesity is correlated with high cardiovascular risk, studies on endothelial dysfunction in OP are not available. We evaluated whether OP is associated with nitric oxide (NO) synthesis alteration. L-[³H]Citrulline formation (100 μ M, 2.7 μ Ci/ml, 37°C, 30 min), endothelial NO synthase (eNOS) protein abundance and mRNA expression, and eNOS phosphorylation were measured in human umbilical vein endothelial cells (HUVEC) from normal (N (n=6)) or OP (n=6)). The NOS-dependent L-citrulline formation was reduced in OP (~96%) compared to N. OP was also associated with lower eNOS expression (~83% mRNA, ~50% protein abundance), higher inhibitory (p-Thr⁴⁹⁵), but unaltered stimulatory (p-Ser¹¹⁷⁷) phosphorylation of eNOS compared to cells from N. The p-Ser¹¹⁷⁷/p-Thr⁴⁹⁵ was lower in OP due to decreased eNOS activation (~70%). In conclusion, OP is a pathological condition that results in lower NO synthesis due to eNOS inactivation in the fetoplacental vein endothelium. Support: CONICYT (ACT-73 PIA, AT-24120944), FONDECYT (3130583, 1110977, 11110059). CONICYT-PhD fellowship (EG-G, TS). PUC-Faculty of Medicine PhD fellowship (RS).

422. Rheb regulation of p27KIP promotes metabolic stress-induced autophagy in colon cancer cells.

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Rheb is a small GTPase that couples growth factor signaling to activation of the mammalian Target of Rapamycin Complex 1 (mTORC1), thereby regulating key processes of cellular metabolism. The activity of Rheb/mTORC1 is controlled by the TSC2 tumor suppressor, whose deficiency causes pathology characterized by tumors in many organs. We reported that activation of Rheb induces mTORC1-independent cytoplasmic localization of the cell-cycle inhibitor p27KIP (p27) in TSC2-null and colon cancer cells. We now analyzed the relevance of this function of Rheb in colon cancer cells. We found that Rheb depletion decreased cell survival under serum deprivation, which was correlated with decreased autophagy. Since Rheb regulates p27 in colon cancer cells, we analyzed whether this regulation was associated with the biological effects of Rheb. We demonstrated that p27 depletion decreased survival and autophagy of colon cancer cells. In addition, we found that Rheb regulation of autophagy depends on the expression of p27. Altogether, our results suggest that Rheb regulation of p27 is important for autophagy and survival of cancer cells under stress conditions. Since mTORC1 inhibitors are used for the treatment of cancer, our work highlights biological consequences of mTORC1-independent responses driven by Rheb in cancer cells, which could help to identify mechanisms of tumor cells resistance to mTORC1 inhibitors. DIUC 210.037.011-1.0 and FONDECYT 1120923

423. Effects of the blockade of astrocyte to astrocyte signaling on synaptic transmission in hippocampal slices

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Astrocytes exhibit both spontaneous and evoked excitability based in intracellular Ca²⁺ elevations, triggering gliotransmitters release that modulates both neuronal excitability and synaptic transmission. Astroglial Ca²⁺ elevations can propagate through several intracellular and extracellular pathways, mainly IP₃-mediated and ATP-mediated signaling, respectively. However, the role of this kind of paracrine signaling in the modulation of synaptic efficacy is not clear. Using astroglial Ca²⁺ imaging and field potential recordings combined to pharmacological tools, we studied the effects of the blockade of astrocyte-astrocyte signaling over spontaneous astroglial Ca²⁺ oscillations and their consequences on glutamatergic neurotransmission in hippocampal slices. The blockade of connexin 43 hemichannels or P2Y receptors –both preferably expressed in astrocytes– reduced the number of spontaneous astroglial Ca²⁺ oscillations. Moreover, these antagonists increased the paired pulse facilitation index, indicating that the probability of neurotransmitter release is also decreased. This evidence suggests that astrocyte to astrocyte signaling is required for the regulation of synaptic efficacy at glutamatergic synapses of CA3-CA1.

424. Herp protects Hela cells from oxidative stress-induced cell death

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Herp, an endoplasmic reticulum (ER) protein expressed in response to ER stress, osmotic stress or deregulation of Ca²⁺ homeostasis, exerts cytoprotective effects. It is not understood whether Herp is also regulated by oxidative stress and which specific signaling pathways mediates this effect. Our results showed that protein levels of Herp increased in response to oxidative stress, within 30 min in HeLa cells or MEF cells treated with H₂O₂. Cell death, assessed by PI and flow cytometry, was significantly higher in Hela cells knockdown for Herp (Sh-Herp) treated with H₂O₂ in comparison with control cells (Sh-Luc). This effect was abolished by the intracellular Ca²⁺ chelator BAPTA-AM or the IP₃ receptor antagonist xestospongine B (XeB), suggesting that this effect was dependent of intracellular Ca²⁺ stores and IP₃ receptor. Intracellular Ca²⁺ kinetics shows that Sh-Herp HeLa cells exhibited a greater and more sustained Ca²⁺ increase over time than Sh-Luc HeLa cells. Similar results were observed in mitochondrial Ca²⁺ kinetics. Sh-Herp cells showed higher basal oxygen consumption and increased lactate production, leading to a higher ATP production. Sh-herp cells showed increased mitochondrial metabolism dependence. Cell viability recovered when Sh-herp cells were treated with methyl-pyruvate before exposure to H₂O₂. Collectively, these results suggest that Herp regulates energy metabolism of Hela cells by controlling Ca²⁺ transfer from the ER to the mitochondria.

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425. Insulin and Testosterone regulate glucose uptake by GRP78 in human endometrial stromal cells

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Hyperandrogenemia and hyperinsulinemia are associated with polycystic ovarian syndrome (PCOS). It remains unknown whether androgen and insulin compromise endometrial endoplasmic reticulum (ER) function in PCOS patients. We evaluate whether long exposure of testosterone or insulin affects GRP78 protein levels (an ER-stress marker) and 2-deoxy-glucose uptake in immortalized human endometrial stromal cells. Cells were cultured with testosterone or insulin, 100nM each by 24/48-h and challenged to 20-min insulin 100nM prior to 5-min [³H]2-deoxy-D-glucose-pulse or were homogenized for GRP78 and phospho-Akt-Ser473/total-Akt protein ratio western-blot analysis. Our results showed a 50% decrease of glucose-uptake in cells exposed to testosterone for 48-h vs basal and insulin-stimulated cells (p<0.05). The ratio of phospho-Akt-Ser473 was similar between testosterone and insulin exposed cultures at 24 or 48-h. Testosterone-treated cells, but not with insulin, exhibited 30% lesser GRP78 content at 24 and 48-h vs basal (p<0.05). Cultures treated with a siRNA-GRP78 with or without insulin 20-min stimuli, showed a 30% reduction of glucose-uptake but higher phospho-Akt levels vs non-siRNA treated cells (p<0.05). The results suggest that an excess of testosterone, as it is present in PCOS patients, compromise glucose availability by reducing GRP78 protein levels in our cell model.

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426. Sall2 transcription factor deficiency is associated with decreased p53- dependent apoptosis induced by genotoxic insults.

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Sall2 is a poorly characterized transcription factor, member of the SPALT family, involved in development. Several studies suggest a relationship between Sall2 and cancer; however, the role of Sall2 in the disease remains unknown. Recently, we demonstrated that activated p53 tumor suppressor decreases Sall2 levels through a transcriptional mechanism, supporting a relationship between them. To understand the functional relationship between Sall2 and p53 we investigated whether lack of Sall2 affects p53-dependent apoptosis induced by the chemotherapeutic drug doxorubicin. We used mouse embryonic fibroblasts (MEFs) derived from Sall2- deficient or Sall2- deficient/p53 knock-in embryos. Our results show that in Sall2- deficient MEFs the apoptotic response induced by the treatment is barely detectable. Western blot and qPCR analysis showed that the decreased apoptotic response in Sall2- deficient MEFs was correlated with lower levels of expression of the pro-apoptotic genes BAX and NOXA. In addition, the levels of total and activated p53 were decreased in both Sall2 deficient MEFs models. Importantly, Sall2- deficient mice exposed to gamma radiation also showed decreased apoptosis in radiosensitive tissues, confirming our studies on primary cells. Altogether our results suggest that Sall2 is necessary for the p53- dependent apoptosis induced by genotoxic insults; and give insight into Sall2 relationship with p53 that could be relevant to cancer. FONDECYT 1110821.

427. Nifedipine treatment reduces atp release, cell death and damage in dystrophic MDX muscles

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Duchenne Muscular Dystrophy (DMD) is a recessive X-linked genetic disorder, caused by mutations in the dystrophin gene in both humans and mdx mice. We have demonstrated that ATP signaling is altered in mdx fibers, including increased release of ATP to the extracellular medium and activation of muscle fiber death. The aim of this work was to study the potential therapeutic effect of Dihydropyridine Receptor (DHPR) blockage using nifedipine in DMD. Mdx mice were treated with daily intraperitoneal injections of nifedipine (1mg/Kg) for 1 week. This resulted in decreased [Ca²⁺]_i measured *in vivo*, in blood CK levels and also reduced the expression of the pro-apoptotic gene Bax in diaphragm muscle. In order to determine the participation of extracellular ATP levels in the [Ca²⁺]_i, we treated mdx isolated fibers with apyrase (2U/mL), an enzyme that metabolizes extracellular ATP. This treatment significantly reduced [Ca²⁺]_i at similar levels obtain with nifedipine treatment. This result is correlated with a marked decrease in ATP release in mdx fibers isolated after nifedipine treatment. We hypothesize that nifedipine, acting on the DHPR, reduces basal ATP release, decreasing purinergic receptor activation that results in a decrease in [Ca²⁺]_i in mdx skeletal muscle cells. These data suggest that nifedipine can be used as a therapeutical tool to reduce muscle damage observed in mdx muscles. Founding: Fondecyt 1110467, ACT1111, AFM14562, AT-24110211

428. Differential regulation of somatolactin genes in response to endocrine disruptors in pituitary of *Cyprinus carpio*.

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Changes in aquatic environment affect fish gene expression and with the aim to develop hypophyseal factors as early alert biomarkers for changes in the aquatic environment we characterized somatolactin (SL) in *Cyprinus carpio*. SL, a fish specific pituitary hormone belonging to prolactin (PRL) superfamily, is involved in background adaptation, osmoregulation, reproduction and fatty acid metabolism. Two SL genes (a and b) exist in carp and transcripts of both were detected and cloned. Expression of SLa and SLb was measured in response to treatment with 17- β -estrogen or ZnCl₂ by RT-qPCR. Interestingly both genes increased significantly expression in response to Zinc but only SLb responded to estrogen. In addition, the proximal promoter region of SLa and b was cloned. Binding of nuclear factors using extracts from carp pituitary was detected by electro mobility shift assays at potential sites in the SLa promoter for Pit-1, estrogen receptor and MTF-1/Sp1. These data suggest that SL seems to be involved in the response to estrogen and heavy metals and could serve as early indicator of endocrine disrupting effects to assess biological relevant changes in the aquatic environment.

429. Peach Leaf Curl Disease: study of *Taphrina deformans*-*Prunus persica* interaction

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Taphrina deformans is a harmful fungus that causes Peach Leaf Curl on peach trees (*Prunus persica*) in most of the cultivation areas of this fruit species. The disease affects leaves, buds, fruits and flowers. Leaf Curl affects yield and plant longevity. It first appears in spring as reddish areas on developing leaves; these areas become thickened and puckered, causing leaves to curl and severely distort. Affected leaves later turn yellow or brown and usually abscise. Only a few cultivars are considered tolerant. In this work by using a resistant and a susceptible genotype a first approach was conducted to characterize the interaction. On one hand, early stages of infection were studied by using asymptomatic leaves and PCR analysis was used to identify infected and uninfected samples of both genotypes. On the other hand, leaves of the susceptible genotype exhibiting different degrees of the disease were separated in the asymptomatic and symptomatic areas and analyzed. Symptomatic areas, irrespectively of the degree of the disease, displayed lower chlorophyll a and b, carotenoids, anthocyanins and phenolic compounds contents than the asymptomatic areas. The susceptible genotype showed higher levels of carotenoids and phenolics than the resistant. We are currently conducting histological, biochemical and molecular studies, which include 2D-DIGE and metabolic profiling by GC-MS that will help us to reveal the complex interplay of *T. deformans* with its host plant.

430. *Burkholderia phytofirmans* PsJN primes *Arabidopsis thaliana* plants enhancing saline stress tolerance

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High salinity leads to a series of molecular, biochemical, physiological and morphological changes that affect plant growth and productivity. In this context, plants respond with a variety of mechanisms that contributes to salt tolerance. PGPR are soil microorganisms that colonize the rhizosphere of many plant species exerting beneficial effects including growth promotion and reduced susceptibility to stresses such as high salinity. We have observed that the PGPR *Burkholderia phytofirmans* PsJN increases salt tolerance in *Arabidopsis thaliana* plants. Here, we evaluated the effect of this bacterium on physiological, metabolic and transcriptional parameters related to salt tolerance in *A. thaliana*. Also we assessed the effect of three PsJN mutant derivatives in plants, finding that these mutations were relevant in the parameters of rosette diameter and fresh weight of plants under salt stress. We also determined that the inoculation should occur before the plants were exposed to the stress condition and that there is a direct relationship between the duration of the plant-bacteria interaction and the enhancement of salt tolerance in inoculated plants. Fondecyt 11121306, 11121515, NM-PFG (P10-062-F).

431. Characterization of the defense response given by the *loci* Run1 and/or Ren1 in *Vitis vinifera* against *Erysiphe necator*

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Vitis vinifera is an important crop worldwide. This specie is susceptible to the infection of the biotrophic pathogen *Erysiphe necator*, causal agent of powdery mildew. To date, several resistant *loci* have been mapped in grapevine; among them, Run1 and Ren1 were the first two dominant *loci* described that are associated with the resistance to *E. necator*, depriving fungal development during infection. Bioinformatic approaches previously published have shown that these *loci* co-segregate with a cluster of putative R genes that might mediate the defense response. In previous work, we have introgressed these *loci* into a *V. vinifera* population, in which resistant phenotypes have been observed in greenhouse conditions. Using microsatellite analysis, we have selected four different lines (Run1⁺, Ren1⁺, Run1⁺Ren1⁺ and Run1⁻Ren1⁻) from this population. In order to evaluate whether Run1 and Ren1 induces an R-gene mediated response, we have detected common cellular events associated with this defense against biotrophic pathogens in these introgression lines, such as cell wall fortification, ROS induction and a localized cell death at the infection site. Currently, we are further investigating this response in more detail to fully understand if the resistance conferred by Run1 and Ren1 is mediated by an R-gene mechanism. Acknowledgements: Programa de Mejoramiento Genético de Vides Consorcio de la Fruta-CORFO and Millennium Nucleus in Plant Functional Genomics P10-062-F.

432. The transcription factor ZINC FINGER PROTEIN 7 is a direct target of the histone acetyltransferase GCN5 in *Arabidopsis thaliana*.

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GENERAL CONTROL NON-REPRESSIBLE 5 (GCN5) appears to be an important histone acetyltransferase required for gene expression involved in many development pathways in plants and animals. Mutations in *Arabidopsis thaliana* GCN5 (AtGCN5) show various pleiotropic defects as a consequence of affecting the activity of plant stem cell populations (meristems). The phenotypes of these mutant plants include dwarfism, loss of apical dominance, aberrant meristem function, disrupted root and leaf development and defects in the floral organ identity. Although AtGCN5 plays an essential role in chromatin modification and transcriptional regulation, its mode of action is still not understood. Proteins involved in chromatin remodelling control the development of plants and animals through directly regulating the expression of specific developmental transcription factors. Using a CHIP-seq approach, we identified a ZINC FINGER PROTEIN 7 (ZFP7) as a transcription factor which expression is regulated directly by AtGCN5. ZFP7 is highly expressed in leaves, roots and seedling but not in inflorescence and flowers. The ZFP7 protein has two EAR motifs and interacts with the corepressor TOPLESS in yeast, suggesting that ZFP7 is a transcriptional repressor. Functional analysis will reveal the role of ZFP7 in plant development and their genetic interaction with AtGCN5 in *arabidopsis thaliana*. Acknowledgment: Postdoctoral fellowship STIPAS and Programa Mecesup2

433. Family affairs: interactions among protein phosphatases with Kelch-like domains in *Arabidopsis thaliana*.

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Protein phosphatases with Kelch-like domains belong to a subfamily of the PPP Ser/Thr phosphatases, found only in green organisms and in alveolates. They are formed by three different domains: an N-terminal Kelch-like β -propeller domain, probably involved in protein-protein interactions, a C-terminal catalytic domain closely related in sequence to PP1, and an intermediate domain of unknown affiliations or functions. *Arabidopsis thaliana* encodes four members of the family, dubbed BSL1 to 3 and BSU1; the first three are highly conserved, whereas the latter is particularly divergent. We have previously shown that BSL2 is able to form homotypic interactions, an unusual feature in the PPP family, and described that both the catalytic and the intermediate domains are necessary. We here extend these studies to the other paralogs. We found that the behavior of some of the other members of the family characteristically differs, correlating with their phylogenetic relationships: in BSL1, the intermediate domain is dispensable, and BSU1 appears to be unable of self-interactions. Although the formation of homo- and heterotypic interactions seems to be a common feature of this family of proteins, the determinants of interaction vary.

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434. Hormonal control of polar auxin transport during initial grapevine berry development

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Auxin is a plant hormone that controls fruit formation since it promotes flower to fruit transition. In tomato fruits optimal levels of auxin are required, since when polar auxin transport (PAT) is blocked, fruit formation is inhibited. Auxin induces PAT in several tissues of *Arabidopsis*, while gibberellin, another fruit promoting hormone, induces PAT in poplar. Given that the content of both phytohormones changes along grapevine berry formation, they could be controlling PAT. So, we wondered whether PAT is important for grapevine berry formation and how is it regulated. First, we inhibited auxin transporters activity, which resulted in berry abscission. Next, we quantified changes in PAT and observed a decrease during initial development. This was consistent with the decrease in the transcript abundance of putative grapevine polar auxin transporters, *VvPINs*. We were also able to detect *VvPIN* localization through immunofluorescence. The observed changes in PAT could be explained by hormonal regulation, so first we measured gibberellin and auxin content using LC-MS/MS and then we performed auxin and gibberellin treatments. We found that gibberellin represses PAT while auxin activates it. Taking together, our results show that PAT is important during fruit formation and that changes in PAT could be explained by hormonal regulation.

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435. Loss of function of NADP-malic enzyme 1 results in lower sensitivity to aluminum

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(Sponsored by CEFOBI And ANPCyT)

In acidic soils, aluminum (Al) toxic species are solubilized causing root growth inhibition. The release of organic acids that chelate these ions plays a central role in plant Al tolerance. In *A. thaliana*, malate is the organic acid excreted to a greater extent. It has been demonstrated the importance of malate channels in this mechanism, but little is known about the metabolic changes underlying excretion. To determine if malic enzyme (ME), which catalyzes the oxidative decarboxylation of malate might be involved in this process, *Arabidopsis* mutant lines of each gene of the ME family were analyzed in response to Al. Plants lacking NADP-ME1 presented lower inhibition of root elongation and an increase in root malate levels after Al treatment. This increased tolerance was not seen in mutants of the other isoforms and could be related to the particular and confined localization of this protein in roots. Moreover, quantification of transcript levels showed a specific decrease in the expression of nadp-me1 in wild type seedlings exposed to Al. Thus, NADP-ME1 could be involved in regulating the levels of malate in the cytosol of the root apex and its loss may result in an increase in the content of this organic acid being available for excretion. Additionally, this isoform may be affecting the levels of signaling molecules of Al stress such as ROS or aminoacids

436. Methyl jasmonate treatment induces changes in fruit ripening by modifying the expression of several ripening genes in *Fragaria chiloensis* fruit

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The role of methyl jasmonate (MeJA) during ripening of non climacteric fruits is not clearly understood. We proceed to analyse the effect of 10 and 100µM MeJA on several physiological parameters and gene expression changes during ripening in a synthetic media of *Fragaria chiloensis* fruit at 2, 5 and 9 days. MeJA transitorily increased loss of firmness, SSC/TA ratio, color acquisition and anthocyanin biosynthesis at day 5 with 100 µM MeJA, and lignin accumulation at days 2 and 9 with 10 µM. We found a high correlation between the higher expression of several genes related to anthocyanin (PAL and UFGT) and lignin biosynthesis (CCR, CAD and POD27) with their higher accumulation, and between the higher expression of hemicellulose modifying genes (EG and XTH) with the lower firmness at day 5 and higher hemicellulose modification at day 9. MeJA increased the expression of several ethylene (AOS and ACS) and jasmonate biosynthesis genes (LOZ, AOS and OPR3), which correlated with the higher expression of the lignin biosynthesis genes and with the higher expression of the anthocyanin biosynthesis and hemicellulose modifying genes, respectively. In conclusion, MeJA accelerates several processes during ripening of *F. chiloensis* due to a higher expression of several genes involved in them, mediated in part through higher expression of ethylene and jasmonate biosynthesis genes. This work was supported by the FONDECYT 11110171 and 3100031 projects.

437. Bioactive phenolic compounds of chilean unifloral honeys inherited from floral specific source

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Chile produces several kind of honey owing to the presence of a great endemic native flora. Those bee products have important biological properties inherited from specific floral sources. The detection of certain chemicals compounds has allowed to certify the origin of honey and other beehive products. One group of those chemicals is the family of phenolics compounds. Those molecules are involved in the natural capabilities and they are useful as bio markers, because they are originated as products of the secondary metabolism of melliferous plants responsible of the botanical origin of honey.

Ethanollic extracts of unifloral honey and flowers of Tevo (*Retanilla trinervia*) were analyzed by UPLC-MS/MS, in order to identify any phenolic compound with potential use as chemical marker and biological active compounds.

The analyses showed the presence of Gallic Acid, Caffeic Acid, trans-Ferulic Acid, Rutine and Naringenine among the most abundant compounds. Other Phenolics were detected but they have not identified (Less than 10 % of total phenolics). Those results suggest that bee is capable to modify the initial content of nectar, but several compounds remain unchanging through of the elaboration process of honey. In this work, we discuss the phenolic profiles obtained for establishing a "fingerprint" of those molecules in unifloral honeys.

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438. Role of the E3 sumoligase SIZ1 in the regulation of salicylic acid biosynthesis in *Arabidopsis*.

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SUMOylation is a type of post translational modification that involves covalent attachment of a SUMO (Small Ubiquitin-like Modifier) peptide to target proteins affecting their functions. SUMOylation process requires three enzymes: E1, E2 and E3. SIZ1 is an E3 sumoligase enzyme from *Arabidopsis* involved in various stress responses. *siz1* mutant plants show accumulation of salicylic acid (SA) under basal conditions. SA is involved in stress responses. Accumulation of SA in *siz1* plant is correlated with an increased expression of genes involved in SA biosynthesis, such as *ICS1*, *EDS1*, *PAD4*, *EDS5*, *CBP60g* and *SARD1*. This suggests involvement of SIZ1 in a negative control mechanism of SA biosynthesis. On the other hand, EIN3 is involved in the ethylene signaling and in the repression of SA biosynthesis. EIN3 was described in a massive screening of SUMOylation targets, but the function of this modification in its activity remains unclear. In this context, we propose that the expression of one or more genes involved in SA biosynthesis is repressed under basal conditions, in a mechanism depending of EIN3 SUMOylation. To evaluate this hypothesis, first we evaluated the expression of genes involved in SA biosynthesis in *siz1* and *ein3 eil1* mutant plants. Then we evaluated the participation of *siz1* in the SUMOylation of EIN3 through an *in vivo* SUMOylation assay. This analysis will allow us identifying new targets of SIZ1 that can explain the SA accumulation in *siz1* mutant.

439. Saline stress induces activation of the early jasmonate responses through a COI1-dependent signaling pathway in *Arabidopsis thaliana* roots

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The build-up of salts on the surface of soil is a widespread agricultural problem affecting world's irrigated cropland. Understanding plant tolerance to salinity is therefore relevant. Jasmonate (JA) is a critical hormone in plant responses to biotic and abiotic stresses. We asked the question whether JA-dependent gene activation could be triggered by salt stress in *Arabidopsis thaliana* roots. To test our hypothesis, we analyzed the effect of salinity (150 mM NaCl for 3 h) on the JA responses by quantifying the relative transcript levels of several early JA responsive genes in roots by RT-qPCR assays. We observed up-regulation of transcripts for seven JAZ genes encoding for JA signaling regulators and five JA biosynthetic pathway genes. Noteworthy, the expression of JAZ8 and LOX3 was highly up-regulated by salt (36- and 116-fold, respectively) and at higher levels than observed in roots treated with JA, suggesting that the JA signaling pathway is fully activated by salt stress in roots. Moreover, *coi1* mutant plants defective in JA receptor function showed a 54-95% reduction in transcript levels of JA-responsive genes compared with WT plants after salt treatment. Together, these results uncover a previously unknown COI1-dependent crosstalk between salt-triggered stress response and the JA signaling pathway in *Arabidopsis* roots. This work was funded by FONDECYT grant No. 1120086.

440. Gene expression of the Homeobox and GRF transcription factor families on early stages of fruit development in *Vitis vinifera*.

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The initiation of grape fruit growth known as fruit set is observed at 5 days after anthesis (DAA), while ovarian development begins with a period of cell division that follows until the 12 DAA. Complete fruit development depends on cell expansion, which occurs after a successful pollination and fertilization. Currently, the possible regulatory mechanisms of early stages of fruit development are not clearly understood. For this, a transcriptional analysis by RNAseq of berry early developmental stages of *Vitis vinifera* was performed. Different stages were analyzed (0DAA, 2DAA, 6DAA and 12DAA) and differences in expression levels of members of homeobox and GRF transcription factors were found. Differential gene expression for VIT_04s0023g01330, VIT_08s0007g04200, VIT_16s0098g01170, VIT_15s0048g02870 and VIT_16s0098g01080, VIT_09s0002g01350 VIT_02s0025g04910 of Homeobox and GFR TF, respectively, were validated using quantitative real-time PCR. These results suggest the involvement of Homeobox and GRF families TF during early events of grape development, supporting the idea that the transcription factors are a major target for understanding regulatory processes on plants, in response to development.

441. Effect of oxidative stress on the vernalization response using several *Arabidopsis* ecotypes that express the *FRI/FLC* module

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Flowering in plants is under a tight genetic and environmental control, and the most important pathways regulating this process are the photoperiod and vernalization. Both converge in the master regulatory gene *FLOWERING LOCUS T (FT)*, which encodes a small protein produced in the leaf vasculature that travels to the shoot apical meristem, activating the expression of the floral meristem identity genes. During vernalization, *FLOWERING LOCUS C (FLC)* promoter is remodeled and the gene becomes transcriptionally silenced, allowing *FT* expression. Most laboratory strains of *Arabidopsis*, as Col-0, 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 sub-optimal cold treatments. *proFLC:GUS* transgenic plants show a reduction in GUS histochemical activity when grown on salicylic acid (SA), which indicate that this response is controlled at the transcriptional level. Indeed using qRT-PCR, we found that levels of *FLC* transcripts decreased after the treatment with SA and methyl viologen (MeV). Taken together our results suggest that mild oxidative stress could promote flowering in northern accessions of *Arabidopsis* through inactivation of *FLC*. Funded by Fondecyt 1120766 and UNAB DI-23/10-R.

442. Molecular and functional characterization of 4 Pollen Specific Kinase coding genes (*PSK1-4*) from *Arabidopsis thaliana*.

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

443. High throughput screening of small bioactive molecules that alter polarized root hair growth

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Arabidopsis root hairs are tip growing tubular formations that elongate from specialized epidermal cells. Tip growth relies on highly active and polarized exocytic and endocytic membrane trafficking. Root hairs are easily accessible for imaging which facilitates the analysis of tip growth phenotypes. These characteristics make root hairs an excellent system for screenings to identify novel compounds that alter polarized growth and ultimately endomembrane polarized trafficking. Here, we present a high-throughput screen using collections of small molecules to identify root hair elongation-altered phenotypes by automated microscopy. The selected bioactive compounds were subsequently scored in a secondary screen for their effects on different trafficking pathways using plants expressing GFP fusion marker proteins. The perturbed localization of the plasma membrane-associated tip focused markers GFP-ROP2 and GFP-ROP6 was analyzed. Additionally, a secreted version of GFP was scored for alterations in protein secretion. Its retention on intracellular compartments was also evaluated. As a result, we selected a set of bioactive molecules capable of altering polarized root hair tip growth and a subset of compounds that directly affects specific endomembrane trafficking markers. These collections of compounds will provide an excellent approach to connect root hair polarized growth phenotypes with their correlated endomembrane trafficking processes. Support Fondef IDeA CA12I10206

444. The transcription factors WRKY7, 11 and 17 have a role in the transcriptional repression of UPR responding genes during the establishment of immune responses in Arabidopsis thaliana

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The endoplasmic reticulum is the organelle where one third of the cell proteins are synthesized. An increment in the synthesis of proteins leads to a decrease in the folding capacity and results in the accumulation of unfolded proteins triggering the Unfolded Protein Response (UPR). The pathogen attack triggers the UPR an event that depends on salicylic acid. This process leads to the establishment of systemic acquired resistance (SAR). The fine tuning of UPR-responding genes is important because continuous activation of the UPR lead to programmed cell death. Nevertheless, the components involved in the deactivation of this response are barely known. Recently, WRKY7, -11 and -17 have been shown to be involved in the negative regulation of plant immunity. Since these proteins bind calmodulin *in vitro*, an interesting scenario involving calcium as activator of these transcription factors during the establishment of SAR is suggested.

In order to assess the role of those transcription factors, we analyzed the expression of UPR-responding genes by Q-PCR after treatments with SA in wild type and knockout mutant plants, determining the expression kinetics. Additionally, we analyzed the interaction between WRKY7, -11 and -17 with calmodulin and calcium using a calmodulin overlay assay. These findings will be relevant given that very few examples of genes being activated and repressed during a UPR have been reported.

Fondecyt 11121387

445. Endocytosis dynamics synthetically induced leads lateral root initiation most likely changing cell fate in *Arabidopsis thaliana*

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Eukaryotic cells have membranous organelles that built the Endomembrane System (ES). ES is responsible for protein trafficking and fate both highly regulated. Protein trafficking is essential for cell function impacting high and dynamically tissues and the whole organism. Plant ES is directly involved in plant organogenesis. Lateral roots (LR) develop post-embryonically and constitute a fundamental organ for plant physiology. Recently our laboratory has described a link between protein trafficking and LR development. By means of using the synthetic compound Sortin2 we published that endocytosis dynamics induction promotes LR formation independently of the auxin receptor SCF^{TIR}. Continuing our understanding how this process takes place we have found that Sortin2-LR induction depends of the auxin-transducer SLR indicating that Sortin2-activated pathway shared component with auxin pathway. However Sortin2 induces more cell division than auxin maxima events strongly suggesting that it induces an early stage of LR initiation. Indeed Sortin2-LR initiation depends on endocytic traffic towards to the vacuole. Sortin2-LR induction does not depend on its effect at the main root meristem, since Sortin2 is able to induce LR initiation when applied locally in the primary root. Overall our results strongly suggest the existence of a LR initiation-signaling pathway that relies in protein relocation within the ES in *Arabidopsis thaliana*. FONDECYT 1120289

446. Quorum Sensing of *Burkholderia phytofirmans* (PsJN) plays a role in colonization and plant growth promotion of *Arabidopsis thaliana*.

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Bacterial signaling known as quorum sensing (QS) system allows the expression of specific genes at high cell densities. Some plant growth promotion rhizobacteria, such as *Burkholderia phytofirmans* PsJN, possess two QS systems mediated by *N*-acyl homoserine lactones (AHL). Strain PsJN is able to colonize and promote the growth of *Arabidopsis thaliana* plants. In this work, the role of strain PsJN QS systems in colonization and growth promoting of *A. thaliana* plants was investigated. Two mutants in AHL synthase enzyme, *bpl.1* and *bpl.2*, corresponding to QS systems encoded in the chromosome and chromid of strain PsJN, respectively, were analyzed. Compared with the wild type strain, the *bpl.1* and *bpl.2* mutants produce significantly lower levels of 3-hydroxy-C8-AHL and the *bpl.2* mutant does not produce C14-3-oxo-AHL. We evaluated the ability of these mutants to colonize and promote growth of *A. thaliana* plants *in vitro*. Results showed significantly less rhizospheric colonization by the *bpl.1* mutant compared with wild type strain and the *bpl.2* mutant. Plants inoculated with the *bpl.1* mutant did not present improvement in plant growth parameters, as detected with the *bpl.2* mutant and the wild type strain. Analyses of selected gene expression in *bpl.1* and *bpl.2* mutants showed differences in motility genes compared with the wild type strain. These results indicate a differential regulation of colonization and plant growth promotion by QS systems in plant growth promotion of *A. thaliana* by *B. phytofirmans* PsJN.

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447. Synthesis and degradation of the phytohormone indole-3-acetic acid in *Burkholderia phytofirmans* PsJN, a plantgrowth-promoting β -proteobacterium

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Indole-3-acetic acid (IAA) belongs to a class of plant hormones known as auxins that play a key role in growth and development of plants. However, not only plants can synthesize IAA since many bacteria associated with them are also able to produce it, related with phenomena such as phytostimulation. Several pathways have been proposed for IAA biosynthesis in bacteria, with tryptophan as the main precursor. Additionally, it has been reported that bacteria degrade IAA, describing a set of genes associated with this ability, although the function and regulation of these genes is presently unknown. It is known that plants can use multiple mechanisms to achieve auxin homeostasis, including biosynthesis and catabolism. In this context it has been proposed that bacteria which simultaneously possess genes related to the synthesis and degradation of IAA would control plant-bacteria associations. This is the case of the β -proteobacterium *Burkholderia phytofirmans* PsJN, able to establish beneficial interactions with plants, which encodes several putative IAA biosynthetic routes and one degradation pathway, as predicted through bioinformatic analysis. The present study assessed the functionality of both IAA pathways. It was observed that the strain has the ability to synthesize IAA in the presence of tryptophan, and it is capable of use IAA as a sole source of carbon and energy. These results also suggest that there are complex regulatory mechanisms that allow the two processes operate simultaneously, depending on the requirements of the bacterium. FONDECYT 1110850, NM-PFG P10-062-F.

448. Nitration of Peroxiredoxin 2. Structural changes and potential consequences on redox signaling.

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Peroxiredoxins (Prx) are a large family of thiol-dependent peroxidases that reduce hydroperoxides at expense of thioredoxin or other disulfide reductases. Prxs are not only antioxidant enzymes but their extraordinary reactivity and specificity for hydroperoxides makes them ideal sensors of endogenous H₂O₂ and the first step in H₂O₂-induced signaling pathways. Prx2, a typical 2-Cys Prx, is present as a homodimer with a unique intermolecular redox-active disulfide center or a decamer (arrange of five dimers). Oxidation of the reactive Cys to sulfenic acid inside the decameric reduced protein results in conformational changes at the active site needed for the attack of the resolving Cys to form the intermolecular disulfide. This conformational transition needed for catalysis imposes a kinetic pause that can result in overoxidation of the active-site Cys, a modification that has been linked to redox signaling pathways. Post-translational modifications of Prx that could affect their activity, redox state, oligomeric structure or interaction with other proteins, will undoubtedly affect redox signaling by H₂O₂. We herein report on the effects of Prx2 modification by peroxynitrite treatment, mainly nitration of tyrosines, that alter the dynamic equilibrium between oligomers in favor of the dimer, and notably increase its peroxidase activity along with resistance to overoxidation by H₂O₂, most likely by favoring disulfide formation.

449. Influence of pH on the conformational changes of hyperthermostable bacterial endo- β -1,4-mannanase and relevance for enzymatic activity

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Endo- β -1,4-mannanase from *T. petrophila* (TpMan) is an important enzyme involved in the degradation of mannan. Here, biophysical properties of TpMan and its catalytic domain (TpManGH5) were characterized as a function of pH and correlated with the enzymatic activity. As the pH decreases from 7.5 to 4, the TpManGH5 and TpMan structures remain well defined. However, TpMan showed a spatial reorganization subtle pH-dependent between the catalytic domain and carbohydrate-binding domain. The electrophoretic mobility (μ_e) data were converted to zeta potential values, which allowed one to determine the isoelectric point (pI). Finally, using the hydrodynamic diameter and the μ_e data allowed the determination of the number of uncompensated charges on enzymes surfaces. Our results indicate that the net electric charge (N_e) of the carbohydrate-binding domains plus the central domain should be approximately close to the N_e of TpManGH5. Thus, the spatial reorganization subtle pH-dependent observed for TpMan is determined by the electrostatic repulsion between the constitutive domains. At pH values above the pI the data demonstrate that the global structure of the TpManGH5 and TpMan remain well defined and have N_e negative suitable for maintaining a good activity. At pH 4 TpManGH5 and TpMan structures also remain well defined, however, with N_e positive that decrease the activity.

450. Crystal structure of GumB, an outer membrane lipoprotein essential for secretion of xanthan

Jacobs, M¹., Bianco, M¹., Salinas, S¹., Ielpi, L¹., ¹Laboratorio de Genética Bacteriana Fundación Instituto Leloir. Xanthan, an extracellular polysaccharide secreted by *Xanthomonas campestris*, is involved in several cellular processes and has a broad range of applications in industry. Here we report the 2.54 Å resolution structure of the GumB tetramer, an outer membrane lipoprotein encoded in the *gum* operon, which is essential for the secretion of xanthan. Overexpression, purification and crystallization of GumB were achieved replacing its signal peptide by a His₆-tag. GumB structure shows features also presented in other membrane proteins involved in polysaccharide secretion. Each protomer of GumB has two domains, both of them showing structural homology with the periplasmic domains D1 and D2 of Wza, an outer membrane lipoprotein involved in the secretion of group I capsular polysaccharide in *E. coli*. The RMSD value between D1 domains of both proteins (residues 44-119 and 89-169 of GumB and Wza, respectively) was of 1.7452 Å and between D2 domains (residues 120-204 and 171-253 of GumB and Wza, respectively) was 1.2151 Å. Moreover, GumB tetramer has an electropositive region due to the presence of lysines and arginines which could interact with the negatively charged xanthan. This characteristic has also been observed in AlgE, a β -barrel outer membrane protein which possesses an electropositive pore to allow secretion of alginate. Acknowledgments: Work was supported by CONICET and ANPCyT (Argentina).

451. Structural bases of hexacoordination in globins

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Human Myoglobin (Mb) and hemoglobin are relevant examples of pentacoordinated (5c) heme proteins and many of their biological functions involve the coordination of exogenous ligands in the sixth position of the heme group. On the other hand, in several globins direct coordination of HisE7 to the iron atom leads to the formation of a hexacoordinated (6c) globin, as noted in neuroglobin (Ngb) and cytoglobin, among others. Ngb, which is expressed in vertebrates nervous system, has been the subject of many investigations in the last years, but its function is still unclear. In our previous work, we have studied by computer simulation the 5c $\leftrightarrow$ 6c transition in wild type Ngb, and analyzed the main differences between this protein and Mb, observing that the flexibility of the CD region is a key factor in the hexacoordination process. In this work, by mean of a theoretical-experimental approach, we have studied comparatively Ngb and Mb structure and dynamics as well as those of chimeric proteins exchanging their CD region. Herein we present results of classical molecular dynamics, free energy profiles for the 5c $\leftrightarrow$ 6c process, UV-VIS spectroscopy and Stopped Flow and Flash Photolysis analysis. Results suggest that each chimeric protein shifted its coordination state according to its CD region, as shown for the partially hexacoordinated myoglobin or the subtly pentacoordinated Ngb, confirming our hypothesis about the key role of the CD loop.

452. Protein structure of C24 gene from *Bizionia argentinensis* resembles the phage T4 long tail fiber receptor-binding tip: a new role for a recent insertion or a novel marine bacteriophage?

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JUB59 represents a new bacterial strain isolated from the Antarctic sea surface. It was grouped into the Flavobacteriaceae family and considered as a novel species of the genus *Bizionia*, for which the name *Bizionia argentinensis* (BA) was proposed. The genome of BA was recently decoded and constitutes a relevant source for the discovery of new proteins showing biological activity under extreme conditions of low temperatures. In order to identify suitable targets for structure determination among the proteins codified in the BA genome, a protocol based on bioinformatics analysis and NMR screening was set up. By this method we were able to select proteins that given its solubility and folding became good candidates for structural determinations. From an initial pool of 50 target proteins we selected several candidates. Here, we show the crystal structure of one of the selected targets, the protein C24, revealing a previously unknown sequence/structure pair. We describe the overall structure of C24 and its individual domains. Comparing the 3D structure of C24 with existing databases, we found that C24 presents a similar overall shape to the bacteriophage T4 long tail fiber receptor-binding tip. In addition, we studied the C24 mRNA expression levels in BA and the presence of the C24 gene in several strains of different genera within Flavobacteriaceae and Proteobacteria. Taken together, these data allow us to discuss the possible biological role of C24.

453. Intramolecular Dynamics within the N-Cap-SH3-SH2 Regulatory Unit of the c-Abl Tyrosine Kinase Reveal Targeting to the Cellular Membrane

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c-Abl is a key regulator of cell signaling and is under strict control via intramolecular interactions. The c-Abl regulatory mechanisms involve post-translational modifications that act as switches, e.g., intermolecular phosphorylation / dephosphorylation of Y412 and Y245 and G2-myristate association/dissociation with/from the C-terminal cleft of the kinase domain via the N-Cap segment. In this study, we address changes in the intramolecular dynamics coupling within the c-Abl regulatory unit by presenting its N-terminal segment (N-Cap) with an alternative function in the cell as c-Abl becomes activated. Using small angle X-ray scattering, nuclear magnetic resonance and confocal microscopy, we demonstrate that the N-Cap and the Src homology (SH) 3 domains acquire μ s-ms motions upon N-Cap association with the SH2-L domain, revealing a stabilizing synergy between these segments. The N-Cap-myristoyl tether likely triggers the protein to anchor to the membrane because of this flip-flop dynamic, which occurs in the μ s-ms time range. This segment not only presents the myristate during c-Abl inhibition but may also trigger protein localization inside the cell in a functional and stability-dependent mechanism that is lost in Bcr-Abl+ cells, which underlie chronic myeloid leukemia. This loss of intramolecular dynamics and binding to the cellular membrane is a potential therapeutic target.

454. Tubulin-Colchicine binding inhibition by Phenstatin derivatives: 1,4-Dihydroxy-2-aroynaphthalenefuran-2-carbaldehyde and 1,4-Dihydroxy-2-aroynaphthalene 1H-pyrrole-2-carbaldehyde

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Phenstatin derivatives, 1,4-Dihydroxy-2-aroynaphthalenefuran-2-carbaldehyde (NFB) and 1,4-Dihydroxy-2-aroynaphthalene 1H-pyrrole-2-carbaldehyde (NPY), were assayed for tubulin polymerization inhibition and presented a dose-dependant effect with an IC₅₀ of 5.2 and 2.1 mM respectively. The sites location for NFB and NPY was performed through molecular docking analysis and molecular dynamics simulation, they were located in the pocket of colchicine binding site. The amino acids involved in the colchicine interaction were not the same that those for the compounds, suggesting that their binding sites were not structurally coincident, although they showed a small overlapping. The binding constants (K_d) for NFB and NPY to tubulin were 4.1 and 1 mM respectively. In order to obtain experimentally the localization of the binding site of NFB and NPY, kinetic experiments, following the fluorescence of tubulin-colchicine complex formation were performed in the presence of these compounds. The initial velocities at fixed concentrations of colchicine, were measured. Modified Dixon and Cornish-Bowden plots showed a competitive type of inhibition, K_i values of 14.6 and 4.6 mM for NFB and NPY respectively. The competitive inhibition behavior showed that NFB and NPY share the pocket of colchicine binding site and validated our bioinformatics and thermodynamic analysis. Financed by FONDECYT # 1130711 and EC SFP # 223431.

455. Identification of amino acid residues conforming an exofacial allosteric site on the human hexose transporter GLUT1

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Glucose transporter GLUT1 facilitates basal glucose transport in most animal cells. This is the most studied of the 14 members of the human glucose transporter GLUT family. Multiple evidences indicate that various molecules structurally unrelated to sugars, such as cytochalasin B, flavones, isoflavones and methylxanthines, inhibit the functional activity of GLUT1. Kinetic studies have shown that there are binding sites accessible on the transporter outer (exofacial) surface that are essential for inhibition. Using an *in silico* high quality structural model of GLUT1 we identify potential functional sites and their constituent amino acid residues by performing molecular docking of various GLUT1 ligands. On this study we tested the sensitivity of single mutants of some of these residues against quercetin, pentoxifylline and cytochalasin B, using trans-zero glucose entry assays in a transient expression system in HEK293 cells. We identified mutants that are refractory to inhibition by methylxanthines, without disturbance of other regulatory properties. This confirms that there is an allosteric site on the human GLUT1 exofacial surface related to the inhibition of its activity by methylxanthines and putatively identifies amino acid residues conforming this regulatory site (Financed by FONDEF D07i1171, D11i1131 and FONDECYT 1130386).

456. All1765, All1769 and All1770 proteins; a putative protein complex in membranes of *Anabaena* sp. PCC7120.

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Important aspects of the development of the multicellular filamentous cyanobacteria are still unclear. *all1765*, an exclusive gene of filamentous cyanobacteria, encodes a hypothetical protein in *Anabaena* sp. PCC7120. This protein has high similarity to proteins that belong to the type II secretion pathway (TII SP). Five genes with unknown function are arranged in the proximity of *all1765* forming a cluster, which shows synteny in a wide number of filamentous cyanobacteria. In summary, we suggest that All1765, All1769 and All1770 form a protein complex in *Anabaena* sp. PCC7120 membranes. Our aim is to identify the subcellular localization and the function of these proteins. Membrane localization was determined by immunogold staining using a polyclonal antibody against All1765 generated by us. A real time RT-PCR shows a significant overexpression of *all1765* and *all1769* in diazotrophy. We have also generated a mutant strain for *all1765* in *Anabaena* sp. PCC 7120. This strain compared to wild type strain showed a significant resistance to the filament fragmentation in diazotrophy. We also purified different protein constructs for antibody generation and crystallization. We already have All1769 crystals in analysis. These results suggest a function of this protein in mechanisms involved in filament fragmentation and a probable regulation by nitrogen. We are currently investigating the *all1769* and *all1770* genes with the same approaches described above for *all1765*.

457. Functional multimeric complexes of the svct2 ascorbic acid transporter

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Oligomerization is important for the function of membrane transporters. SVCT2 is a sodium-coupled transporter with an apparent ascorbic acid K_m of 20 μ M. We analyzed SVCT2 quaternary structure and its effect on ascorbic acid transport. Cross-linking with paraformaldehyde followed by western blot showed formation of protein complexes with molecular masses consistent with dimer, tetramer and superior order structures. To gain information about the role of quaternary structure on function, we transiently co-expressed in HEK-293 cells SVCT2 and SVCT2mutants (altered kinetics, apparent K_m for ascorbic acid > 100 μ M) at different molecular ratios. As the ratio SVCT2:SVCT2mutant changed from 1.0:0.0 to 0.8:0.2, 0.6:0.4, 0.4:0.6, 0.2:0.8, and 0.0:1.0, a single functional component resembling SVCT2 was observed at ratios > 0,5 (molar excess of SVCT2), and a single functional component resembling SVCT2mutant was observed at ratios < 0,5 (molar excess of SVCT2mutant), without evidence of two functional components, while the two expected functional components were observed in HEK-293 cells co-expressing SVCT1 and SVCT2. These results indicate that SVCT2 oligomerize into higher order structures, that the subunit composition of the oligomer affects the kinetics of transport, and that the specificity of the process impedes the formation of mixed SCVT1-SVCT2 oligomers. Conicyt Doctoral Fellowships, Doctoral Thesis Fellowship 24121614, and Fondecyt projects 1090501 and 1130842.

458. Mutations in residue F380 in the S6 transmembrane helix uncouples voltage sensor activation to BK channel gating

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Large conductance Ca^{2+} and voltage-activated K^+ channels (BK) opening is mediated by a cross-talk of modular voltage and Ca^{2+} sensors. BK open probability is enhanced by increasing cytosolic Ca^{2+} concentration and/or depolarization. These stimuli activate sensors that are coupled by allosteric interactions to channel gating. Here, we report that point mutations of a phenylalanine (F380A and F380Y) located in the S6 transmembrane helix produces large changes of the open probability-voltage curve with minor changes in gating current. Interpretation of our results using an allosteric model, suggests that the F380 mutations modifies the channel closed-open equilibrium and uncouples voltage and calcium sensors from channel opening. Based on these functional studies, we propose a structural model that sheds light on the molecular nature of the coupling between the voltage sensor activation and pore opening. Moreover, due to the large rightward shift of open probability-voltage curve, the mutation F380A could be used for detailed voltage sensor studies in the presence of permeant cations.

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459. Cold-adapted enzyme from the ADP-dependent sugar kinase family: characterization and comparison with a hyperthermophilic homologue.

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Most of the currently described Archaea are extremophiles, where the largest proportion of their biomass found on the biosphere are cold adapted organisms. These organisms, also called psychrophiles, have evolved to synthesize enzymes capable of performing basic biological activities at low temperatures. This ability is attributed to its high structural flexibility and low thermal stability. In this work we characterized the ADP-dependent phosphofructokinase from *Methanococcoides burtonii*, which constitutes the first study of a cold-adapted enzyme in this family. Interestingly, besides fructose 6-phosphate the enzyme was able to phosphorylate glucose. This cold-adapted enzyme shows higher specific activity at low temperatures than its hyperthermophilic homologous from *Thermococcus litoralis*. In addition, the Km values for glucose in the cold-adapted enzyme increased with temperature, while the opposite trend is observed in the hyperthermophilic enzyme. The activation energy of the phosphorylation reaction catalyzed by the psychrophilic enzyme is approximately 3 kcal/mol lower than the one determined for the reaction catalyzed by its hyperthermophilic homologue. These results show that the cold-adapted enzyme compensates the low kinetic energy of its environment with lower activation energies for the phosphorylation reaction performed at low temperatures. Fondecyt 1110137.

460. Determination of the stoichiometry of interacting 5-HT_{3A} and P2X₂ receptors by atomic force microscopy

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5-HT_{3A} and P2X₂ ionotropic receptors mediate communication between neurons, through rapid synaptic transmission. Although it has been demonstrated a crosstalk between both receptors, its stoichiometry has remained still unknown. HEK 293 cells were transfected with plasmid DNA of 5-HT₃ and P2X₂ receptors tagged with Myc/His-6 and GFP, respectively. Membrane fractions were solubilized and incubated in Ni²⁺-agarose beads. Proteins were eluted with increasing concentrations of imidazole for purification. Purified 5-HT_{3A}-P2X₂ receptor complexes were analyzed by immunoblotting and atomic force microscopy (AFM) imaging using tapping mode. The frequency distribution of molecular volumes of 5-HT_{3A}-P2X₂ receptor complexes has shown multiple populations of stoichiometric ratios. These results suggest that polydisperse complexes could regulate synaptic transmission efficiency. Funded by Millennium Science Initiative P10-035F, Fondecyt 1120169 and Anillo ACT 1108 grants.

461. Structural studies of the connexin 26/30 hemichannels via atomic force microscopy imaging

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Connexins (Cx) are hexameric hemichannels that mediate intercellular communication and extracellular transport. Permeability data suggest that connexins can form heteromeric hemichannels; however their stoichiometry and subunit arrangement are unknown. HEK 293 cells were co-transfected with Cx26-His6 and Cx30 cDNAs, and later Ni²⁺ agarose beads were used to purify Cx 26/30 hemichannels from cell membrane fractions. Presence of the heteromeric Cx 26/30 was confirmed by mass spectrometry analysis. Purified samples were imaged by air tapping mode atomic force microscopy (AFM). Molecular volumes of the Cx 26/30 hemichannel complexes have shown a particle population centered at 350 nm³, which is within the volume range expected to heterohexameric Cx based on their molecular weights. The next step will visualize Cx 26/30 hemichannels decorated with anti-subunit antibodies by AFM and in consequence determine both the number of each subunit in the complex and its subunit arrangement. Funded by Millennium Science Initiative P10-035F, Fondecyt 1120169 and Anillo ACT 1108 grants.

462. Molecular determinants of the functional and structural properties of the ascorbic acid transporter-2, SVCT2

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The sodium-coupled ascorbic acid transporter-2 (SVCT2) belongs to the nucleobase/cation symporter 2 (NCS-2) family. We described in our laboratory a folding 3D model for SVCT2 based on the crystal coordinates of the bacterial UraA transporter. In this 3D model, SVCT2 presents a topology characterized by having 14 transmembrane segments (TMS) grouped in two domains, a central domain formed by TMS 1,2,3,4 and 8,9,10,11 containing the hydrophilic transport pore, and a second domain acting as a substrate-selectivity filter formed by TMS 5-7 and 12-14. We used the 3D model of SVCT2 refined by molecular dynamics (5ns), to perform molecular docking studies with the substrate L-ascorbic acid in order to identify amino acid residues possibly involved in both substrate binding and its translocation along the central transport channel. These studies led to the identification of six amino acid residues, present in three TMS (TMS2, TMS8 and TMS10) of the central domain that contains the transport channel, as possible structural components that participate in SVCT2 interaction with its substrate. Each residue was replaced with alanine using site-directed mutagenesis, and the resulting mutant proteins were expressed in HEK-293 cells and analyzed for protein expression, cellular localization and functional properties. Our results indicate that these residues are important structural elements that affect the function of SVCT2. Funding: Fondecyt projects 1130842.

463. *In vivo* counting of single flagellar motor protein, FlIM, using photoactivated localization microscopy

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Photoactivated localization microscopy is a fluorescence technique based on the photoactivatable fluorescent proteins (PA-FPs) and has the ability to quantify proteins. However, the intrinsic blinking of PA-FPs mEos2 and Dendra2 leads to an over-counting error, which constitutes a major obstacle for their use as molecular counting tags. Here, we introduce a kinetic model to describe blinking and show that Dendra2 photobleaches three times faster and blinks seven times less than mEos2, making Dendra2 a better PALM tag than mEos2 for molecular counting. We present a method that quantifies the total counting error and minimizes it by balancing over- and under-counting. This new method establishes that Dendra2 is better for counting purposes than mEos2, allowing us to count *in vitro* up to 200 molecules in a diffraction-limited spot with a bias smaller than 2% and an uncertainty less than 6% within 10 minutes. Finally, we demonstrate that this counting method can be applied to protein quantification *in vivo* by counting the bacterial flagellar motor protein FlIM fused to Dendra2.

464. Thy-1-induced migration requires $\beta 3$ Integrin expression, ATP release and P2X7R activation in TNF α - treated primary astrocytes.

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Thy-1 is an abundant neuronal glycoprotein that binds to the astrocyte receptor $\alpha \beta 3$ Integrin triggering ATP release and activation of P2X7 receptors (P2X7R). These events promote cell adhesion and migration of DL-TNC1 astrocytes. However, in primary astrocytes, Thy-1 has no such effects, possibly due to the absence of $\beta 3$ Integrin expression, which reportedly increases in inflammation. We hypothesized that primary astrocytes become responsive to Thy-1 by increasing $\beta 3$ Integrin protein levels. To test this, GFAP, a marker for reactive astrocytes, and $\beta 3$ Integrin levels were evaluated by western blotting. Also, $\beta 3$ Integrin was either knocked-down or overexpressed in primary astrocytes and the effects of these changes were tested in wound-healing assays. The role of ATP or P2X7R in migration was evaluated using either Apyrase or the antagonist BBG, respectively. The pro-inflammatory cytokine TNF α induced expression of $\beta 3$ Integrin and enhanced migration of primary astrocytes in response to Thy-1. When $\beta 3$ Integrin expression was suppressed prior to TNF α treatment, astrocytes did not respond to Thy-1. In addition, overexpression of $\beta 3$ Integrin sufficed to permit Thy-1-induced migration without TNF α treatment. Finally, Thy-1-enhanced migration required P2X7R activation and extracellular ATP. Acknowledgements: CONICYT 21090323 and 24001198 (AA), FONDECYT 1110149 (LL) and 1130250 (AFGQ); BNI P09-015-F (LL) and Anillo ACT1111 (AFGQ); BNI postdoctoral fellowship (AC).

465. Liver X receptor: effect of two cholestenic acid analogues on mouse mammary epithelial cells

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Liver X receptors (α and β) are oxysterol-activated transcription factors that belong to the nuclear hormone receptor superfamily. LXRs are involved in many physiological functions such as lipid de novo synthesis and excretion, cell proliferation and apoptosis. In mammary gland, LXRs not only control lipid levels during lactation but also has antiproliferative and proapoptotic effects. Our aim was to evaluate the role of LXR activation on a mouse mammary epithelial cell line (HC11) and to assay in vitro activity of two novel cholestenic acid analogues: 27-nor and 27-nor Δ 24 3β -hydroxy-5-cholestenic acids. HC11 cells are broadly used as a mammary cell differentiation and apoptosis model. EGF protects these cells from apoptosis when cultured in serum free medium mainly throughout AKT survival pathway activation. Both LXR α/β are expressed in all HC11 differentiation stages. The cholestenic acid derivatives showed antagonistic activity when co-incubated with the LXR agonist GW3965 in cells transfected with a luciferase reporter vector responding to LXR. Moreover, these compounds also inhibited GW3965 mediated expression induction of several genes involved in lipogenesis and cholesterol efflux. Surprisingly, preliminary results also suggest that these analogues decrease EGF mediated AKT activation on HC11 cells, becoming important tools to understand LXRs participation on mammary gland milk lipid production, cell proliferation and death.

466. Role of phospholipase D pathway in the inflammatory response of the retinal pigment epithelium cells.

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The retinal pigment epithelium (RPE) plays an important immunological role in the retina and it is involved in many ocular inflammatory diseases that may end in loss of vision and blindness. In this work the role of the phospholipase D (PLD) pathway in the inflammatory response of the human RPE cells (ARPE-19) exposed to bacterial lipopolysaccharide (LPS) was studied. ARPE-19 were exposed to LPS (10 μ g/ml) for 24 and 48 h. LPS treatment reduced cell viability by 15% after 48 h and increased NO production by 1 and 1.8 fold after 24 and 48 h, respectively. Furthermore, 24 h LPS treatment strongly induced cyclooxygenase-2 (COX-2) expression and the activation of protein kinase C (PKC α/β) and extracellular signal-regulated kinase (ERK1/2). Transfected cells with EGFP-PLD plasmids showed the typical subcellular localization of PLD1 (perinuclear) and PLD2 (plasma membrane). Untransfected cells expressed both PLD isoforms. LPS treatment increased PLD activity (measured as phosphatidylethanol formation) by 80% with respect to the control. The presence of the PLD1 inhibitor (EVJ 0.15 μ M) or the PLD2 inhibitor (APV 0.5 μ M) reduced LPS-induced PKC α/β activation and COX-2 induction but only PLD2 inhibition reduced ERK1/2 activation. Moreover, the inhibition of PLD2 and ERK1/2 restored cell viability to control levels. Our results demonstrate for the first time the participation of the PLD pathway in the inflammatory response of RPE cells exposed to LPS

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467. A novel auto-phosphorylation mechanism of PKA activity regulation in *Saccharomyces cerevisiae*

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In the AGC family of serine/threonine kinases, the activities of catalytic subunits are regulated by multiple phosphorylation. In *S. cerevisiae*, the catalytic subunit of PKA is encoded by three genes: TPK1, TPK2 and TPK3; while the regulatory subunit is encoded by the gene BCY1. Pkh1 (homolog of mammalian PDK1) phosphorylates the activation loop of Tpk1(Thr²⁴¹) in newly synthesized Tpk1 independently of the carbon source. Pkh inactivation reduces the interaction Tpk1-Bcy1 without affecting the specific kinase activity of Tpk1. We have described that Tpk1 increases its phosphorylation status during glucose stimulus by an autophosphorylation mechanism. Here we demonstrate that Ser¹⁷⁹ is a residue target of glucose induced phosphorylation only on Tpk1 active molecules. Studies evaluating the relationship between Thr²⁴¹ and Ser¹⁷⁹ phosphorylation indicated that the negative charge on Ser¹⁷⁹ did not affect Thr²⁴¹ phosphorylation status, while low phosphorylation on Thr²⁴¹ increased the phosphorylation status of Ser¹⁷⁹ *in vivo*. The kinetic effects of adding a negative charge on Ser¹⁷⁹ were measured using purified Tpk1^{S179D} and Tpk1^{S179A}. Phosphomimetic Tpk1^{S179D} mutant showed a 20-fold increase in V_{max} compared with Tpk1^{S179A}, however its K_m for a peptide substrate and the $A_{0.5}$ for cAMP activation of the holoenzyme were not significantly affected. Therefore, we demonstrate that an autophosphorylation mechanism controls PKA activity in response to glucose availability

468. Bag3 regulates LC3 expression to increase basal autophagy in HeLa cells

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Autophagy is a degradation process where damaged proteins and/or organelles are trapped by double membrane vacuoles that subsequently are fused with lysosomes. Chaperones are required to select damaged proteins, but remains unknown how chaperones regulates autophagy. Here we studied whether the co-chaperone Bag3 (Bcl2-associated athanogene) controls autophagy signaling pathways. To test this, HeLa cells were treated with siRNA for Bag3 or Bag3-expression plasmid, LC3-I and/or LC3-II levels or expression were determined by Western blot and immunofluorescence, respectively. Bag3 knockdown in HeLa cells decreased both protein levels of LC3I and LC3II, which do not reverse by bafilomycin A treatment. Proteasome inhibition with MG132 neither reverses LC3 reduction in Bag3 knockdown cells. No changes in mRNA Bag3 levels (assessed by qPCR) were also observed. These results suggest that Bag3 does not control LC3 degradation neither mRNA level of LC3. Bag3 overexpression confirmed these results. Our data also show that Bag3 knockdown reduces AKT-mTOR-p70S6K activity, a signaling pathway controlling protein translation. Collectively, our novel results suggest that Bag3 co-chaperone regulates basal autophagy controlling LC3 translation in HeLa cells. Supported by Anillo ACT1111 (SL) and CONICYT 24110051 (AR). AR holds a CONICYT PhD fellowship.

469. Ryanodine receptor-mediated Ca^{2+} signals and ROS are key components of synaptic plasticity promoted by the neurotrophin BDNF

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Dendritic spine remodeling is an integral part of the cellular changes underlying learning and memory. We have reported that primary hippocampal neurons display fast spine growth and new spine formation after exposure to brain-derived neurotrophic factor (BDNF). Inhibition of ryanodine receptor (RyR) activity prevents these effects, highlighting the requirement for RyR-mediated Ca^{2+} release, which is redox-sensitive, in BDNF signaling. Here we investigated the roles of the RyR2 isoform and of reactive oxygen species (ROS) on BDNF-induced spine remodeling, and studied if RyR inhibition modified endoplasmic reticulum (ER) Ca^{2+} -content. To determine dendritic spine morphology or ROS generation, primary hippocampal neurons were transfected with RyR2 shRNA and loaded with calcein, or were transfected with the Hyper-cyto H_2O_2 sensor protein, respectively. To measure ER Ca^{2+} content, cells loaded with Fluo4-AM were tested with thapsigargin. Our results indicate that BDNF induces ROS generation, that selective inhibition of RyR2 expression or treatment with the antioxidant N-acetylcysteine suppresses BDNF-induced spine remodeling, and that ER Ca^{2+} -content does not decrease following 1 h incubation with inhibitory ryanodine to prevent RyR activity. These combined results suggest that RyR2-mediated Ca^{2+} signals and ROS are key components of the complex neuronal plasticity processes induced by BDNF. FONDECYT (3120093, 11110322, 1100052) and BNI P-09-015F.

470. Role of intercellular adhesion molecule-1 (ICAM-1) in cardioprotective actions of insulin.

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Intercellular adhesion molecule-1 (ICAM-1) is a cell adhesion molecule of the immunoglobulin family involved in inflammatory processes. This protein is essential for other non-immune functions like heart development and homeostasis. Null mice for this protein are not viable. Our previous data showed that ICAM-1 is expressed in cultured rat cardiomyocytes and insulin increased its levels. On the other hand, insulin exerts cardioprotection by triggering anti-apoptotic effects. Here we studied whether insulin and ICAM-1 exert protective effect in cardiomyocyte under ischemia. These cells were stimulated with insulin before or during simulated ischemia. At the end of 8 h ischemia, cell viability and ICAM-1 levels were determined by Trypan blue exclusion or TUNEL and Western blot, respectively. Incubation with 10 nM insulin during ischemia produced a significant reduction of apoptotic cell death and increased ICAM-1 expression. siRNA treatment for ICAM-1 during ischemia in the presence or absence of insulin increased cell death by apoptosis. We concluded that insulin has cytoprotective effect in cardiomyocytes and this action could be associated with ICAM-1. Fondecyt 1110346 (LG)

471. The TRPM4 channel regulates the Epithelial-Mesenchymal transition in prostate cancer cells.

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The Transient Receptor Potential Melastatin 4 (TRPM4) is a Ca²⁺ activated and voltage-dependent monovalent cation channel, which depolarizes the plasma membrane, thereby modulating Ca²⁺ influx through Ca²⁺-permeable pathways. TRPM4 overexpression has been described in a number of cancers, including prostate cancer, suggesting that TRPM4 promotes cancer progression in these cells. Nevertheless, the molecular mechanisms exerted by TRPM4 in the progression of prostate cancer remain unknown. In this work we analyze the consequences of TRPM4 knockdown in the expression of epithelial-mesenchymal transition (EMT) markers (including CDH1, CDH2, VIM, CTNNB1, FN1, ZEB1, SNAI1 and SLUG) and biological processes such as cellular migration and proliferation. Experiments performed in PC3 and LnCAP prostate cancer cells show that after TRPM4 knockdown a significant change in the expression pattern of EMT marker genes is observed. These changes point toward a reversion of the EMT process in these cells. To define the underlying mechanisms, we study the activity of the β -catenin and GSK3- β signaling pathway in these cells. TRPM4 knockdown cells have decreased total β -catenin protein levels, and an increase in S33, S37 and T41 β -catenin phosphorylation as well as an increase in S9 GSK3- β inhibitory phosphorylation, suggesting that a downregulation of components of the canonical WNT signaling pathway could explain the relationship between TRPM4 and EMT.

472. Angiotensin-(1-9)/AT2 receptor signaling pathway a novel regulator of cardiomyocyte mitochondrial dynamics

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Pathological cardiac hypertrophy is triggered by chronic stress induced by neurohumoral stimuli such as epinephrine (NE). This biological response is characterized by increases in sarcomer number and cardiomyocyte size among changes in gene expression and energy metabolism. Mitochondria form a dynamic network whose morphology and function depends on fusion and fission events. Our laboratory previously showed that NE induces cardiac hypertrophy and mitochondrial fission. Angiotensin-(1-9) is a novel anti-hypertrophic peptide of the non-canonical renin-angiotensin system. In this study we investigate whether angiotensin-(1-9) modulates mitochondrial dynamics by preventing NE-induced mitochondrial fission. The results showed that cardiomyocytes treated with angiotensin-(1-9) (100 mM for 1 to 24 h) induced mitochondrial fusion associated with a decrease in the fission protein Drp1 migration to mitochondria, without changes in levels of the fusion proteins Opa1 and Mfn2. Mitochondrial fission stimulated by NE 10 μ M 24 h was prevented by angiotensin-(1-9). The AT2 receptor antagonist PD 123319, but not the Mas receptor antagonist A779, prevented mitochondrial fusion induced by angiotensin-(1-9). In summary, angiotensin-(1-9)/AT2 receptor signaling pathway modulates cardiomyocyte mitochondrial dynamics and prevents mitochondrial fission induced by NE. Supported by Anillo ACT 1111, FONDECYT 1120212, 1100874 and FONDEF D111122.

473. Testosterone increases PGC-1 α activity in rat cardiomyocytes inducing a PPAR- α mediated response

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PGC-1 α is a key factor for cardiomyocyte hypertrophy. Physiological and pathological stimuli can induce hypertrophy in cardiac tissue, with a differential response involving PGC-1 α . Testosterone induces hypertrophy in cardiomyocytes, but the mechanisms involved are still under investigation. Here we studied the role of PGC-1 α in testosterone-induced cardiomyocyte hypertrophy. Cardiomyocytes in primary culture were transfected with PGC-1 α -Luciferase and treated with testosterone alone or with different inhibitors for 24 h. Testosterone increased PGC-1 α -luciferase at 24 h. AICAR (an AMPK activator) also increased PGC-1 α -luciferase, but testosterone did not produce a further increase. Compound C (AMPK inhibitor), Xestospongin C (antagonist of IP₃ receptor) and GW6471 (a PPAR- α inhibitor) abolished the effects of testosterone on PGC-1 α -Luciferase. PGC-1 α and PPAR- α regulate the energy substrates used by the cardiomyocytes. Here, testosterone stimulation by 12 h increased both PPAR- α and PPAR- β protein levels. Inhibition of PPAR- α by GW6471 abolished PPAR- β increase. Testosterone regulates PGC-1 α and PPAR- α , which are implicated in the energy homeostasis. Because these proteins can modify the intracellular energy environment, we hypothesized that these metabolic pathways have important roles during the change from normal to cardiomyocyte hypertrophy. Thus, a new energetic balance is produced to increase glycolysis or β -oxidation. FONDECYT 1120259

474. Angiotensin-(1-9) modulates Akt via the angiotensin II receptor type 2 (AT2R).

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Angiotensin-(1-9) (Ang-(1-9)) is a peptide member of the renin-angiotensin system, present in human and rat plasma at picomolar concentration. Ang-(1-9) plasma levels increase early after myocardial infarction and in animals treated with angiotensin-converting enzyme inhibitors. Recent studies from our group have shown that Ang-(1-9) prevents cardiovascular remodeling and has anti-hypertensive effects *in vivo*. However, the receptor implicated in these effects is unknown. We hypothesized that Ang-(1-9) activates the vascular angiotensin II type-2 receptor (AT2R). **OBJECTIVE:** To determine whether Ang-(1-9) activates the AT2R of rat aorta and modulates the Akt activity. **METHODS AND RESULTS:** Endothelium-intact aortic rings from male Sprague Dawley rats (180-200 g body weight) were incubated in the presence of Ang-(1-9) (0.1 pM to 1 nM) for 5 min. We observed a concentration-dependent inhibition of p-AKT(Ser473)/AKT. Ang-(1-9) EC₅₀ for p-AKT(Ser473)/AKT inhibition was 20.3 pM, (R², 0.93), with a maximal effect observed at 1 nM (P<0.05). In a second set of experiments we observed that the effect of 1 nM Ang-(1-9) was blocked by the AT2R antagonist PD123319 (1 μ M), but it was not affected by the AT1R receptor antagonist losartan (1 μ M). The incubation in the presence of a specific AT2R agonist (CGP42112, 100 nM) decreased p-AKT(Ser473)/AKT, similar to the effect of Ang-(1-9). **CONCLUSION:** Angiotensin-(1-9) is an angiotensin II type-2 receptor agonist in rat aortic tissue.

475. DNA glycosilase and AP endonucleases. presence, activity and involvement in *T. cruzi* DNA repair and infection persistence

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Trypanosoma cruzi, the etiological agent of Chagas' disease, survives the oxidative damage to its DNA exerted by ROS/ RNS generated by its hosts. In recent eukaryotes oxidative DNA damage is mainly repaired by the base excision repair (BER) pathway.

By enzymatic assays using appropriate substrates as well as by western blot and immunofluorescence analysis the presence, activity and localization of two AP endonucleases (TcAP1 and TcAP2) was assessed in *T. cruzi* extracts. In addition, the subcellular localization of a *T. cruzi* DNA glycosilase (TcNTH1) was determined by immunofluorescence. Viability of parasites transfected with expression vectors of those three enzymes was analyzed using the MTT assay.

The three enzymes which are proper of the DNA BER pathway are present in the *T. cruzi* nuclei. TcAP1 and TcAP2 AP endonuclease activities were detected. Viability of parasites over expressing TcAP1 is increased when chased with O₂H₂ or NOO⁻, mainly under sustained exposition to these oxidative agents.

We propose that the DNA repair BER pathway is activated when *T. cruzi* is exposed to ROS/RNS, allowing its survival and leading to parasite persistence in its hosts.

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476. FLAP endonuclease. Presence and activity in cell proliferation and DNA repair in *Trypanosoma cruzi*

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T. cruzi survives damage produced by reactive species present in the insect vectors and the host mammals, probably by repair of its DNA through the base excision repair pathway (BER). In recent eukaryotes FLAP endonucleases are involved both in DNA repair and replication by processing DNA intermediaries presenting a nucleotide flap.

The activity of a *T. cruzi* FLAP endonuclease (TcFEN1) was determined using a DNA substrate generated by annealing three different oligonucleotides to form a double-stranded DNA with a 5' flap in the middle. The 5' ends of the flap-containing oligonucleotide or a complementary oligonucleotide were labeled with P³². This substrate was used to detect FLAP activity in *T. cruzi* extracts. An expression vector (pTREX-TcFEN1-GFP) was generated, transfected to the parasite and the recombinant protein evidenced by fluorescence microscopy and western blot.

TcFEN1 activity was detected in the three cellular forms of the parasite. The peak of activity was at pH 8.0 and at 37°C. The substrate cut by the enzyme could be ligated generating a repaired product. TcFEC1-GFP was located in the nucleus. Transfected parasites showed higher proliferative activity than control ones.

The presence of a flap endonuclease activity in the three cellular forms of *T. cruzi* as well as its nuclear location are indicative of the existence of the DNA base excision repair long pathway in this parasite.

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477. Gene polymorphism of Mb1951c ORF, in *Mycobacterium bovis* field isolates

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M. bovis is the ethiological agent of bovine tuberculosis (BTB), a zoonosis that can spread to humans upon the inhalation of aerosols from infected cattle or through the ingestion of unpasteurized milk. In Chile, BTB is a chronic, endemic disease thus representing a major animal health problem due to the huge economic losses derived from livestock deaths, trade restriction and a threat to humans. Recent evidences have suggested a relation among the differences in the capacity of the pathogen to disseminate and the types of *M. bovis*. With the aim to establish the different genotypes of *M. bovis* prevalent in Chile, we have recently done a survey of genetic polymorphism in a collection of Chilean field *M. bovis*

isolates, taking into account several regions of the *M. bovis* genome encoding hypothetical virulence factors. Here we report the finding of a highly polymorphic locus, Mb1951c gene, encoding a putative membrane protein belonging to the PPE family. It has been speculated that members of this family are important for early host-pathogen interactions. Characterization of the cloned Mb1951c PCR fragment derived from different field isolates, showed that the Mb1951c ORF undergoes deletions. Sequence analysis demonstrated that those deletions were originated from recombination events among repeat sequences present within the gene.

Our results suggest that the polymorphism in the ORF Mb1951c accounts for a selective pressure triggered by interactions between *M. bovis* and its host. We believe that shorter versions or null expression of the protein in those isolates might represent an evolutionary advantage caused by in vivo selection.

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