CHILEAN SOCIETY FOR CELL BIOLOGY

XXV ANNUAL MEETING
November, 1st – 5th, 2011
Puerto Varas

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CHILEAN SOCIETY FOR CELL BIOLOGY
XXV ANNUAL MEETING

NOVEMBER, 1st-5th, 2011
PUERTO VARAS

P R O G R A M

TUESDAY, NOVEMBER 1st, 2011

09:00 – 13:00 **Registration**
Poster Mounting Session I: N° 1 to N° 85
Convention Center Foyer

11:30 – 12:30 **Technical Lectures - Calbuco Room**
WITec GmbH and Genexpress
Combining High Resolution Optical and Scanning Probe Microscopy
Andrea Jaub. Senior Application Scientist, WITec GmbH, Germany

Technical Lectures - Tronador Room
Eppendorf and Arquimed
Resource Optimization of PCR Equipment, Influence of Pipetting Techniques and Residues to Obtain Reproducible Results
Josely Chiarela. Eppendorf, Brasil

12:30 – 13:30 **Technical Lectures - Calbuco Room**
LifeTechnologies and GrupoBios SA
Attune® Acoustic Focusing Cytometer and New Molecular Probes® Reagents for Cell Biology with a Focus on Violet Laser Capabilities
Michael Olszowy, Director, Flow Cytometry Systems, LifeTechnologies

13:00 – 14:30 **Lunch**

14:30 – 15:30 **Inauguration**

PLENARY LECTURE LUIS IZQUIERDO FERNANDEZ
Volcanes Room - Chair: Maria Rosa Bono

FROM RIBOSOMES TO PSYCHOSIS. Alfonso González. Departamento de Inmunología Clínica y Reumatología, Fac. Medicina. Centro de Envejecimiento y Regeneración (CARE). Fac. Ciencias Biológicas. Pontificia Universidad Católica de Chile.

15:30 – 17:30 **Oral Presentations I**
Volcanes Room - Chair: Maria Rosa Bono and Co-Chair: Mauricio Gonzalez

***In vivo* ANALYSIS OF CELLULAR TOXICITY MECHANISMS OF ALPHA-SYNUCLEIN AGGREGATES AND POTENTIAL THERAPEUTIC DRUGS IN *Caenorhabditis elegans*.** A. Minniti[4], I. Alfaro[2], S. Bernales[2,3] and **R. Aldunate[1]**. [1]Escuela de Biotecnología, Universidad Santo Tomás, [2]Fundación Ciencia para la Vida, [3]Medivation Inc., [4]Fac. Ciencias Biológicas, P. U. Católica de Chile.

UNDERSTANDING THE NETWORK BETWEEN Th17 CELLS, REGULATORY T CELLS AND MYELOID DERIVED SUPPRESSOR CELLS IN THE TUMOR MICROENVIRONMENT. Sarah Núñez[1], Juan José Sáez[1], Mario Rosemblatt[1,2], María Rosa Bono[1] and Daniela Sauma[1,2]. [1]Departamento de Biología, Facultad de Ciencias, Universidad de Chile; [2]Fundación Ciencia para la Vida. sarah.nunez.c@gmail.com

MEGALIN PROTEOLYTIC PROCESSING AND PHOSPHORYLATION IS AFFECTED BY OCRL1 DOWN REGULATION. Lisette Sandoval[1], Antonella De Matteis[2] and María Paz Marzolo[1]. [1]Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile. [2]Telethon Institute of Genetics and Medicine, 80131 Naples, Italy. mmarzolo@bio.puc.cl

REPROGRAMMING OF A HUMAN CARCINOMA CELL LINE BY ZEBRAFISH EMBRYONIC MICROENVIRONMENTS. Leonel Muñoz, Germán Reig, and Miguel Concha. Laboratory of Experimental Ontogeny - LEO, ICBM, Faculty of Medicine, University of Chile; and Biomedical Neuroscience Institute, Santiago, Chile. lamunoz@med.uchile.cl; mconcha@med.uchile.cl

LACONIC, A GENETICALLY ENCODED FRET NANOSENSOR FOR LACTATE. [1,2]**Alejandro San Martín**, [1,2]Sebastián Ceballo, [3]Wolf B. Frommer and [1]L. Felipe Barros. [1]Centro de Estudios Científicos (CECs), Valdivia, [2]Universidad Austral de Chile, Valdivia, & [3]Carnegie Institution of Washington. aalejo@cecs.cl

RUNX2 PROMOTES MIGRATION AND INVASION IN HUMAN OSTEOSARCOMA CELL LINES. **Óscar Vega[1,2],** Claudia Lucero[1,2], Julio Tapia[2], Marcelo Antonelli[2], Mercedes Lopez[1,3], Flavio Salazar-Onfray[1,3], Gary S Stein[4], Andre van Wijnen[4], Mario Galindo[1,2]. [1]Millennium Institute on Immunology and Immunotherapy, [2]Programa de Biología Celular y Molecular, [3]Prode Inmunología, ICBM, Facultad de Medicina, Universidad de Chile. [4]Department of Cell Biology and Cancer Center, University of Massachusetts Medical School, USA. mgalindo@med.uchile.cl

ROLE OF SYNDECAN-4 IN WNT/PLANAR CELL POLARITY PATHWAY DURING MOUSE DEVELOPMENT. **Noelia Escobedo[1],** Marjorie Farías[1], Osvaldo Contreras[1], Hector Carrasco[1], Uyen Tran[2], Oliver Wessely[2], Andrew Copp[3] and Juan Larraín[1]. [1]Center for Aging and Regeneration, Millennium Nucleus for Regenerative Biology, Faculty of Biological Sciences, P. Universidad Católica de Chile; [2]Department of Cell Biology, Louisiana State University, Health Sciences Center, EEUU; [3]Institute of Child Health, University College London,UK. jlarrain@bio.puc.cl

TESTOSTERONE INCREASES GLUCOSE UPTAKE THROUGH GLUT4 AND CaMKII/AMPK PATHWAY IN RAT CARDIOMYOCYTES. **Carlos Wilson,** Ariel Contreras, Katherine Montoya, Rodrigo Maass and Manuel Estrada. Facultad de Medicina, ICBM, Universidad de Chile. carloswr@gmail.com

17:30 – 18:30 **Coffee Break – Exhibitors – Poster Viewing: Session I**
Convention Center Foyer

18:30 – 19:30 **PLENARY LECTURE CENTER FOR AGING AND REGENERATION (CARE)**
P. UNIVERSIDAD CATOLICA DE CHILE
Volcanes Room - Chair: Nibaldo C. Inestrosa

A NANOSCALE VIEW INTO THE DYNAMIC OF AMPA RECEPTOR ORGANIZATION IN SYNAPSES. **Daniel Choquet.** Institut Interdisciplinaire de Neuroscience, UMR 5297 CNRS-Université de Bordeaux. dchoquet@u-bordeaux2.fr

19:30 – 20:30 **BEST THESES AWARDS FUNDACION CHILENA PARA BIOLOGIA CELULAR**
Volcanes Room - Chair: Federico Leighton and Co-Chair: Maria Rosa Bono

UNDERGRADUATE
FELIPE BELTRAN GONZALEZ, Biochemistry, Universidad Austral de Chile.
GLUT3 is a key component in glucose transport modulation by ascorbic acid.
Thesis Advisor: Maite Castro, PhD

GRADUATE
CARLA BITTNER HOFMANN, PhD in Sciences, Mention in Cell & Molecular Biology.
Universidad Austral de Chile
Acute modulation of astrocytic glucose metabolism by neuronal signals.
Thesis Advisor: L. Felipe Barros, PhD

20:30 **Dinner**

22:00 – 23:30 **Poster Presentations - Session I: N° 1 to N° 85**
Convention Center Foyer
Coordinators: Alejandra Alvarez, Maite Castro, Pedro Zamorano

(1) HEMICHANNELS FORMED BY PANNEXIN1 ARE ACTIVATED BY EXTRACELLULAR ATP IN ADIPOCYTES. **Fernández PE,** Benvenuto MA, Sáez JC. Departamento de Fisiología, Pontificia Universidad Católica de Chile. paolafernandez.bq@gmail.com

(2) ROLE OF mTOR PATHWAY IN POST-MITOTIC TRANSLATION OF INHERITED RUNX2 MRNA IN MOUSE PRE-OSTEOBLASTS CELLS. **Alejandra Aránguiz**[1], Nelson Varela[1], Marcelo Antonelli[1], Zhang Ying[3], Gary Stein[3], Andre van Wijnen[3] y Mario Galindo[1]. [1]Programa de Biología Celular y Molecular, ICBM, Facultad de Medicina, Universidad de Chile. [3]Department of Cell Biology and Cancer Center, University of Massachutsetts Medical School, USA. mgalindo@med.uchile.cl

(3) ACTIVATION OF THE UNFOLDED PROTEIN RESPONSE (UPR) ENHANCES MOTOR RECOVERY AFTER SPINAL CORD INJURY. **Vicente Valenzuela[1,2,3],** Eileen Collyer[3], Donna Armentano[4], Geoffrey Parsons[4], Felipe A. Court[3,5] and Claudio Hetz[1,2,5]. [1]Biomedical Neuroscience Institute and [2]Center for Molecular Studies of the Cell, Institute of Biomedical Sciences, University of Chile. [3]Millennium Nucleus in Regenerative Biology (MINREB), Catholic University of Chile. [4]Department of Molecular Biology, Genzyme Corporation, USA. [5]Neurounion Biomedical Foundation. chetz@med.uchile.cl, fcourt@bio.puc.cl

(4) PHOSPHOENOLPYRUVATE CARBOXYKINASE EXPRESSION IN A CELLS FROM HUMAN AND MOUSE PANCREAS. **Romina Bertinat[1]**, Fabián Pardo[1], Cristian Carrasco[2], Karen Jaramillo[1], Juan Carlos Slebe[1] and Alejandro J. Yáñez[1]. Instituto de Bioquímica y Microbiología, Universidad Austral de Chile[1]; Unidad Anatomía Patológica, Hospital Base Valdivia[2], Valdivia, Chile. romibert@gmail.com

(5) ANALYSIS OF THE EPIGENETIC RESPONSE UNDER CONDITIONS OF CELLULAR ENERGY RESTRICTION DURING THE ACCLIMATIZATION OF *C. carpio*: THE eNosC COMPLEX. **Fernández de la Reguera C.,** Nardocci G., Morales J., Molina A., Vera MI., Alvarez M. L. de Biología Celular y Molecular, F. de Ciencias Biológicas, U. Andrés Bello, Viña del Mar, Chile. malvarez@unab.cl; c.fernandezde.c@uandresbello.edu

(6) TISSUE FACTOR PATHWAY INHIBITOR (TFPI) AND TISSUE FACTOR (TF) CO-LOCALIZE IN LIPID RAFTS (LR) OF PLATELET MEMBRANES: MECHANISM TO INHIBIT PLATELET PROCOAGULANT (PCA) ACTIVITY. **González César**, Matus Valeria, Pereira Jaime, Mezzano Diego, Panes Olga. Department of Hematology-Oncology, School of Medicine, P. Catholic University of Chile. cesar.gonzalezg@usach.cl (Sponsor: V. Velarde)

(7) INVOLVEMENT OF CONNEXIN26 HEMICHANNELS IN PURINERGIC SIGNALING AND THEIR POSSIBLE CONTRIBUTION IN OTOTOXICITY. **Figueroa VA.[(1,3)],** Jara O.[(1)], Martínez AD.[(1)], Fiori M.[(2)], Altenberg GA.[(2)], and Sáez JC.[(1,3)]. [(1)]Centro Interdisciplinario de Neurociencias de Valparaíso (CINV), Universidad de Valparaíso, [(2)] Department of Cell Physiology and Molecular Biophysics and Center for Membrane Protein Research, Texas Tech University Health Sciences Center, [(3)]Departamento de Fisiología, P. Universidad Católica de Chile. vaniafigueroa@gmail.com

(8) INCREASE COPPER LEVELS IN AN HEPATOMA CELL LINE MIMICKING NPC1 PHENOTYPE. **Mary Carmen Vázquez[1]**, Mauricio González[2] and Silvana Zanlungo[1]. [1]Departamento de Gastroenterología, Facultad de Medicina, P. Universidad Católica de Chile. [2]Laboratorio de Bioinformática y Expresión Génica, INTA, Universidad de Chile. mc.vazquez.rodriguez@gmail.com

(9) EVALUATION OF THE CYTOTOXIC EFFECT OF A HYDROALCOHOLIC EXTRACT OF *Ruta graveolens* IN THE HEK 293 AND HUVEC CELL LINES. **Ignacio Jofré[1,3]**, Fernando Romero[1], Jenny Rudlinger[1], Karina Mansilla[1], Jorge Parodi[2], Raúl Salvatici[1], Patricia Navarrete[1]. [1]Center of Neurosciences and Peptides Biology- BIOREN, University of La Frontera, Temuco, Chile. [2]Laboratory of Molecular Neurobiology, FONDAP-CRCP, Pontificia Universidad Católica de Chile. [3]Biotechnology career, University of La Frontera, Temuco, Chile. Partially supported by Dirección de Investigación, University of La Frontera. ignaziojf@gmail.com

(10) HIGH LEVELS OF LEPTIN DECREASES CILIARY ACTIVITY, THROUGH THE ACTIVATION OF NOS IN CILIATED CELLS OF THE RAT OVIDUCT. **Carolina Oses[1]**, Maria Paz Hernández[1], Daniela Careño[1], Carmen Llados[1], Manuel Villalón[1]. [1]Department of Physiology, Faculty of Biological Sciences. Pontificia Universidad Católica de Chile, Santiago, Chile. cdoses@uc.cl

(11) NEURONAL ENDOPLASMIC RETICULUM ORGANIZATION AND MODULATION OF CALCIUM SIGNALS. **Figueroa C[1]**, San Martín C[3], Couve A[2], Härtel S[1], Ramírez O[1]. [1]SCIAN-Lab,

[2]Laboratory of Cellular and Molecular Neurobiology, [3]Molecular Studies Center of the Cell, ICBM, Faculty of Medicine, U-Chile. carolina.figueroa.a@gmail.com

(12) NEOGENIN 1 (Neo1): A TARGET OF SONIC HEDGEHOG SIGNALLING PATHWAY IN HUMAN NEUROBLASTOMA CELLS. **Natalie Espinoza G.[1]**, Luis A. Milla[1] and Verónica Palma[1]. [1]Laboratory of Stem Cells and Development. Faculty of Sciences, University of Chile, Santiago, Chile. nat.espinozagiacomozzi@gmail.com

(13) A FAILURE IN ASCORBIC ACID HOMEOSTASIS IS RESPONSIBLE FOR THE METABOLIC IMPAIRMENT IN HUNTINGTON'S DISEASE. [1]**Felipe Beltran,** [1]Macarena Solis, [2]Rene Vidal, [13]Carlos Cepeda, [1]Ilona I. Concha, [2]Claudio Hetz, [3]Michael Levine, [1]Maite A. Castro. [3]Semel, UCLA; I. Ciencias Biomedicas, U. Chile; I. Bioquímica y Microbiología, UACh.

(14) DIMEBON PROTECTS FROM CELL DEATH-INDUCED BY OXIDATIVE STRESS AND ALPHA-SYNUCLEIN OVER-EXPRESSION, AND DECREASES ALPHA-SYNUCLEIN PROTEIN LEVELS IN A PARKINSON´S DISEASE CELL MODEL. **Iván E. Alfaro[1]**, Luz Delgado[1], Dania Valdovinos[1], Andrew Protter[1,2], Sebastián Bernales[1,2]. [1]Fundación Ciencias Para la Vida, Santiago, Chile. [2]Medivation Inc., CA, USA. alfobioq@gmail.com

(15) REDUCTION OF Aβ-OLIGOMERS IN PLASMA OF ALZHEIMER TRANSGENIC MICE TREATED WITH A c-Abl INHIBITOR. **Estrada LE[1]**, Chamorro D[1], Inestrosa NC[2], and Alvarez AR[1]. [1]Laboratorio de Señalización Celular, [2]CARE, Depto. de Biología Celular y Molecular, FCB, P. Universidad Católica de Chile. lestrada.biotech@gmail.com

(16) THE cJUN-AMINO TERMINAL KINASE (JNK) REGULATES INTERNALIZATION AND RETROGRADE APOPTOTIC KILLING OF THE p75 NEUROTROPHIN RECEPTOR (p75) IN SYMPATHETIC NEURONS (SCGS). Escudero CA[1], **Cabeza C[1]**, Galleguillos C, Uzma S[2], Maloney M[3], Carter BD[2], Mobley W[3,4], Bronfman FC[1]. 1Millennium Nucleus in Regenerative Biology (MINREB), Facultad de Ciencias Biológicas. Pontificia Universidad Catolica de Chile, Chile. 2Department of Biochemistry, Vanderbilt University, USA. 3Department of Neurology, Stanford University, USA. 4Department of Neurosciences, UCSD, USA. caescude@gmail.com

(17) CHARACTERIZATION CDNF EXPRESSION BY LENTIVIRAL VECTORS AS A VEHICLE FOR THE POTENTIAL TREATMENT OF PARKINSON'S DISEASE. **Sheyla Guzmán**, Jorge Escobar, Pedro Zamorano. Laboratorio de Neurobiología, Facultad de Ciencias de la Salud, Universidad de Antofagasta. zamorano@uantof.cl

(18) cAMP-EPAC SIGNALING IS INVOLVED IN THE DEVELOPMENT OF THE AXON. **Pablo Muñoz-Llancao[1]**, Daniel R. Henriquez[1], Martina Schmidt[2] and Christian Gonzalez-Billault[1]. (1)Laboratory of Cell and Neuronal Dynamics, Department of Biology, Faculty of Sciences and Institute for Cell Dynamics and Biotechnology (ICDB), Universidad de Chile, Santiago, Chile. (2)Department of Molecular Pharmacology, University of Groningen, The Netherlands.

(19) CHARACTERIZATION OF AN *in vitro* MODEL OF MOTOR NEURON DISEASE. Cristina Pinto[1], Nelson Osses[2], **Juan Pablo Henríquez[1]**. [1]Department of Cell Biology, University of Concepcion, and [2]Institute of Chemistry, Catholic University of Valparaiso, Chile. jhenriquez@udec.cl

(20) WNT-5A LIGAND INDUCES FISSION-FUSION OF MITOCHONDRIA AND PROTECTS HIPPOCAMPAL NEURONS FROM Ab OLIGOMER NEUROTOXICITY. Juan A. Godoy**, Macarena S. Arrázola** and Nibaldo C. Inestrosa. Centro de Envejecimiento y Regeneración (CARE). Departamento de Biología Celular y Molecular. Facultad de Ciencias Biológicas. Pontificia Universidad Católica de Chile, Santiago, Chile. msarrazo@puc.cl

(21) TREHALOSE TREATMENTS ALLEVIATE AND ATTENUATE ALS PROGRESSION POSSIBLY THROUGH AUTOPHAGY ACTIVATION. **Castillo K.[1,2,3]**, Lopez E [1,2,3] , Nassif M [1,2,3], Matus S [1,2,3] and Hetz C. [1,2,3]. [1]Biomedical Neuroscience Institute, Faculty of Medicine, [2]Center for Molecular Studies of the Cell, Institute of Biomedical Sciences, University of Chile, Santiago, Chile. [3]Neurounion Biomedical Foundation, Santiago, Chile. karencitakc@gmail.com

(22) ROLE OF ApoER2 IN REGENERATION OF THE PERIPHERAL NERVOUS SYSTEM (PNS) AFTER NERVE DAMAGE. **Joaquín Cerda**, María Luisa Benítez, Felipe A. Court[1] and María Paz Marzolo[2]. Millennium Nucleus in Regenerative Biology (MINREB), P. Universidad Católica de Chile, Santiago, Chile. [1]fcourt@bio.puc.cl, [2]mmarzolo@bio.puc.cl

(23) MOTOR IMPAIRMENT OF A BRAIN SPECIFIC ERp57 KNOCKOUT MOUSE MODEL. **Andreu C**.[1,2],Valenzuela V.[1,2],Woehlbier U.[1,2],Irrazábal T.[1,2] and Hetz C[1,2,3]. [1]Biomedical Neuroscience Institute, [2]Center for Molecular Studies of the Cell, Institute of Biomedical Sciences, University of Chile, [3]Neurounion Biomedical Foundation, Santiago, Chile. chetz@med.uhile.cl

(24) BIPHASIC EFFECTS OF COPPER ON NEUROTRANSMISSION IN RAT HIPPOCAMPAL NEURONS. **Christian Peters**, Braulio Muñoz, Fernando Sepúlveda, Juan Urrutia, Mauricio Quiroz, Sandra Luza, Giancarlo V. De Ferrari, Luis G. Aguayo, Carlos Opazo. Laboratorio de Neurobiometales, Departamento de Fisiología, Facultad de Ciencias Biológicas, Universidad de Concepción, Chile. cpeters@udec.cl

(25) αVβ3 INTEGRIN EXPRESSION IN RAT BRAIN UPON INJURY. **Soto, C.**[1,2], Rojas-Mancilla, E.[1,2], Alvarez, A.[1,2], Hermosilla, T.[1], Díaz, E. [2], Herrera-Marschitz, M.[2] and Leyton, L.[1,2]. [1]Centro de Estudios Moleculares de la Célula (CEMC), [2]Biomedical Neuroscience Institute, ICBM-Facultad de Medicina, U de Chile.

(26) GLIAL CELL TYPE DEFINES MORPHOLOGICAL AND TEMPORAL CHARACTERISTICS OF AXONAL DEGENERATION. **Alejandra Catenaccio**, Jaime Alvarez and Felipe A. Court. Millennium Nucleus in Regenerative Biology (MINREB), Catholic University of Chile and Neurounion Biomedical Foundation. fcourt@bio.puc.cl, alecatenaccio@gmail.com

(27) GLIAL SUBVENTRICULAR TUMORS INDUCED IN THE NEUROGENIC NICHE INCREASE GENERATION OF NEUROBLASTS. **Nery Jara**, Federico Rodríguez and Francisco Nualart. Neurobiology and Stem Cells Laboratory, Department of Cell Biology, University of Concepción. neryalejara@udec.cl

(28) NH_4^+ AS A POSSIBLE SIGNAL LINKING NEUROTRANSMITTER RECYCLING WITH FAST GLYCOLYTIC STIMULATION IN ASTROCYTES. **[1,2]Rodrigo Lerchundi** and [1]L. Felipe Barros. [1]Centro de Estudios Científicos (CECs), Valdivia, Chile & [2]Universidad Austral de Chile, Valdivia, Chile. rlerchundi@cecs.cl

(29) SUBCELLULAR LOCALIZATION ANALYSIS OF XTRIC-8A AND ITS PARTICIPATION IN CELL POLARITY IN *X. tropicalis* EMBRYOS. **Cecilia Arriagada[1],** Maria V. Hinrich[1] and Marcela Torrejón [1]. [1]Laboratory of Genetic and Molecular Biology, Department of Biochemistry and Molecular Biology, University of Concepción, Chile**.** ceciarriagada@udec.cl

(30) HETEROCHRONY AND HETEROTOPY IN THE EVOLUTION OF BRAIN ASYMMETRY DEVELOPMENT BETWEEN ZEBRAFISH AND MEDAKA. Iskra Signore[1,2], **Geraldine Vásquez**[1,2], Javiera Ríos[1,2], Alexander Jares[3], Alicia Colombo[1], and Miguel Concha[1,2]. [1]Laboratory of Experimental Ontogeny - LEO, ICBM, Faculty of Medicine, University of Chile; [2]Biomedical Neuroscience Institute - BNI, Santiago, Chile; [3] Yale University, USA. iskra.signore@gmail.com; mconcha@med.uchile.cl

(31) ETHANOL EXPOSURE DISRUPTS CELL MIGRATION AND PRIMARY CILIA STRUCTURE IN DEVELOPING EMBRYOS. **Katica Boric[1],** [2]Eduardo Couve[2], Patricio Orio[1] & Kathleen Whitlock[1,2]. [1]CINV, Universidad de Valparaíso, Chile. [2]Universidad de Valparaíso. kboric@gmail.com

(32) GENERATION A MODEL OF OXIDATIVE STRESS INDUCTION USING ZEBRAFISH AND EVALUATION OF ANTI-OXIDANT, PROTECTIVE AND REGENERATIVE ACTIVITY OF RESVERATROL. **Marjorie Alvarez[1]**, Tomas Egaña[2], Miguel L. Allende[1]. 1.FONDAP Center for Genome Regulation, Facultad de Ciencias, Universidad de Chile. 2.Department of Plastic Surgery and

Hand Surgery, Faculty of Medicine, Technical University of Munich. Munich, Germany. marjorie.alvarez@ug.uchile.cl

(33) SYNDECAN-4 AND FIBRONECTIN, FOCAL ADHESION COMPONENTS, REGULATE WNT/b-CATENIN SIGNALING. Pablo Astudillo, Héctor Carrasco and Juan Larraín. Center for Aging and Regeneration (CARE), P. Universidad Católica de Chile. prastudi@uc.cl

(34) AVERSIVE MEMORY IN *Drosophila melanogaster* LARVA CAN BE MODIFIED BY LIGHT ACTIVATION OF CHANNELRHODOPSIN-2. Paula Burgos, Karina Palma & Jorge M Campusano. Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad de Chile. paulaburgos.s@gmail.com, jcampusano@bio.puc.cl (Sponsor: E. Aliaga).

(35) INFLAMMATION RESOLUTION BY RETROGRADE MIGRATION OF NEUTROPHILS AFTER LOCALIZED TISSUE DAMAGE IN ZEBRAFISH LARVAE. Oscar Peña, **Nicole Reynaert,** Miguel Allende. FONDAP Center for Genome Regulation, Facultad de Ciencias, Universidad de Chile. nicole.reynaert@googlemail.com; allende@uchile.cl

(36) PHENOTYPIC CHANGES INDUCED BY LOW-OF- FUNCTION OF PATCHED-RELATED DURING EMBRYOGENESIS OF *Drosophila melanogaster*. Carmen Bolatto y Verónica Cambiazo. Laboratorio de Bioinformática y Expresión Génica INTA-Universidad de Chile. Depto. de Histología y Embriología, Facultad de Medicina- UdelaR, Uruguay. cbolatto@fmed.edu.uy, vcambiaz@inta.cl

(37) HYPOXIA-INDUCIBLE FACTOR-1 ALPHA IS NECESSARY FOR THE POSTERIOR LATERAL LINE CELL-PROLIFERATION ON ZEBRAFISH. Barros M, Reyes A.E. Facultad de Ciencias Biológicas. Universidad Andrés Bello. Avda. República 217, piso 4. Santiago, Chile. arielreyes@unab.cl

(38) CHARACTERIZATION OF PELADO DURING ZEBRAFISH DEVELOPMENT. Solís C[1]., Feijóo CG[1] and Glavic A[2]. (1) Facultad de Ciencias Biológicas, Universidad Andrés Bello, Chile. (2) Facultad de Ciencias, Universidad de Chile, Chile.

(39) CALCIUM AND cAMP SIGNALING IN THE PROTHORACIC GLAND AND ITS ROLE IN THE CIRCADIAN TIMING OF *Drosophila* ECLOSION. Angelina Palacios-Muñoz and John Ewer. Laboratory of Neurogenetics and Development, Interdisciplinary Center of Neuroscience of Valparaíso, University of Valparaíso, Chile. angelina.palacios@cinv.cl

(40) TESTOSTERONE INDUCES HYPERTROPHY IN SKELETAL MUSCLE CELLS BY ACTIVATING BOTH mTOR/p70S6K PATHWAY AND THE CLASSIC ANDROGEN RECEPTOR. Basualto-Alarcón C., Jorquera G., Estrada M. and Jaimovich E. Centro de Estudios Moleculares de la Célula, ICBM, Facultad de Medicina, Universidad de Chile, Santiago, Chile. carlabasualto@gmail.com

(41) CTGF INDUCES INFLAMMATION IN THE SKELETAL MUSCLE. Cabrera D., Morales MG., Cabello-Verrugio C. and Brandan E. Laboratory of Cell Differentiation and Pathology, CARE. Department of Cell and Molecular Biology, Catholic University of Chile. dacabrer@puc.cl

(42) ISOLATION OF RAT SKELETAL MYOFIBERS AND DENERVATION, BUT NOT IMMOBILIZATION OF A FAST SKELETAL MUSCLE PROMOTES AN INCREASE IN CONNEXINS LEVELS. L.A. Cea, M.A. Riquelme, A. Vargas, J.C. Sáez. Departamento de Fisiología, Pontificia Universidad Católica de Chile, Luisceapisani@gmail.com

(43) MOLECULAR TOOLS TO CHARACTERIZE MULTIPROTEIN COMPLEXES CONTAINING $P2Y_2$ RECEPTORS AND PANNEXIN-1 CHANNELS. Buvinic, S[1,2] and Jaimovich, E[2]. [1]Facultad de Odontología; [2]Centro de Estudios Moleculares de la Célula, ICBM, Facultad de Medicina. Universidad de Chile, Santiago, Chile. sbuvinic@u.uchile.cl

(44) EARLY UPTAKE OF VITAMIN C UP-REGULATES ITS SVCT2 TRANSPORTER AND STIMULATES MYOGENESIS. Jorge Ojeda, Daniel Sandoval, Marcela Low, Juan Pablo Henriquez**.**

Department of Cell Biology, Faculty of Biological Sciences, University of Concepcion, Concepcion, Chile. jhenriquez@udec.cl

(45) ATP REDUCES THE UP-REGULATION OF CONNEXINS 39, 43, AND 45 IN CULTURED ADULT SKELETAL MYOFIBERS. **Cisterna B.A.,** Cea L.A., Riquelme M.A., and Sáez J.C. Departamento de Fisiología, P. Universidad Católica de Chile. bcisterna@uc.cl

(46) UNVEILING A MULTIPROTEIN COMPLEX INVOLVED IN EXCITATION-TRANSCRIPTION COUPLING IN NORMAL AND DYSTROPHIC SKELETAL MUSCLE. **Almarza, G.**[1], Valladares D.[1], Jaimovich E.[1] and Buvinic, S[1,2]. [1]Centro de Estudios Moleculares de la Célula, ICBM, Facultad de Medicina, [2]Facultad de Odontología. Universidad de Chile, Santiago, Chile. gonzalo.almarza@gmail.com

(47) AMP-ACTIVATED PROTEIN KINASE (AMPK) PATHWAY IS INVOLVED IN TESTOSTERONE-INDUCED CARDIOMYOCYTE HYPERTROPHY. **Katherine Montoya,** Carlos Wilson, Rodrigo Maass and Manuel Estrada. ICBM, Facultad de Medicina, Universidad de Chile. iestrada@med.uchile.cl

(48) VASOPRESSIN-INDUCED PROLIFERATION OF VASCULAR SMOOTH MUSCLE CELLS INVOLVED THE EGFR TRANSACTIVATION AND ACTIVATION OF ERK SIGNALING PATHWAY. **Marianne Brenet R.** Carolina Villanueva M., Pamela Carmona R., Carlos B. González. Instituto de Fisiología. Facultad de Medicina. Universidad Austral de Chile. Marianne.brenet@alumnos.uach.cl

(49) ATP-DEPENDENT GLUCOSE UPTAKE IN SKELETAL MUSCLE CELLS. [*†]**Osorio-Fuentealba C**., [†]Contreras-Ferrat AE., [†]Altamirano. F., [†]Espinosa A. and [†]Jaimovich E. [*]Facultad de Medicina, Universidad Finis Terrae, [†]Centro de Estudios Moleculares de la Célula, ICBM, Facultad de Medicina, Universidad de Chile, Santiago.

(50) CROSS TALK BETWEEN ApoER2 AND THE NEUROTROPHINS RECEPTORS: EFFECT OF NEUROTROPHINS AND REELIN ON PROTEOLYTIC PROCESSING OF ApoER2. **Jorge Larios Y.**, Francisca Bronfman C., María Paz Marzolo C. Millennium Nucleus in Regenerative Biology (MINREB), Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile. jalarios@uc.cl, fbronfman@bio.puc.cl, mmarzolo@bio.puc.cl

(51) COX-2 EXPRESSION IS REGULATED BY CK2 THROUGH THE WNT/□-CATENIN PATHWAY IN HUMAN EMBRYONIC CELLS. **Pablo Cabello**[1], Roger Yefi[1], Ignacio Niechi[1], Eduardo Silva[1], Diego A. Rodriguez[2], Daniela P. Ponce[1], Katherine Marcelain[2], Ricardo Armisen[2], Andrew F.G. Quest[2] & Julio C. Tapia[1,2]. [1]Cell Transformation Laboratory, [2]Institute of Biomedical Sciences (ICBM), Faculty of Medicine, University of Chile. jtapia@med.uchile.cl

(52) CO-INCUBATION WITH TOLL LIKE RECEPTORS ACTIVATORS, LPS AND DNA-CPG, INDUCED A SYNERGISTIC INCREASE OF CILIARY BEAT FREQUENCY IN RESPIRATORY CILIATED CELLS. **Daniela Carreño**[1], Claudia González[2], Carolina Oses[1], María Paz Hernández[1], Carmen Llados[1] and Manuel Villalón[1]. [1]Faculty of Biological Sciences; [2]Hospital Clínico. Pontificia Universidad Católica de Chile. dvcarren@uc.cl

(53) FUNCTIONAL CHARACTERIZATION OF CARGO-BINDING SITES ON MU-SUBUNITS OF ADAPTOR PROTEIN COMPLEXES. **Esteban Corales**[1]**,** Yimo Lin[1], Juan Bonifacino[2], James Hurley[3], Patricia Burgos[1], and Gonzalo Mardones[1]. [1]Instituto de Fisiología, Facultad de Medicina, Universidad Austral de Chile, Valdivia, Chile, and [2]Cell Biology and Metabolism Program, NICHD, and [3]Laboratory of Molecular Biology, NIDDK, NIH, Bethesda, MD, USA. gamardon@gmail.com

(54) INHIBITION OF PHOSPHATIDIC ACID HYDROLISIS CHANGES THE ENDOCYTIC TRAFFICKING AND SIGNALING OF ONCOGENIC EGFRvIII. **Apud M.,** Shaughnessy R.[1,2], Otero C. [1,2], Metz C, Soza A.[1,2], González A.Departamento de Inmunología Clínica y Reumatología, Fac.Medicina, Centro de Envejecimiento y Regeneración (CARE), Fac.Ciencias Biológicas, Pontificia Universidad Católica de Chile. apud.mari@gmail.com

(55) KININ B2 RECEPTOR STIMULATION AMELIORATES EPITHELIAL MESENCHYMAL TRANSITION INDUCED BY ALBUMIN IN RENAL EPITELIAL CELLS. **Cárdenas A,** Campos J, Ehrenfeld P, Ardiles L, Figueroa CD. Laboratorio de Nefrología e Instituto de Anatomía, Histología y Patología, Universidad Austral de Chile. arelicardenas0@gmail.com

(56) NSPA AND NOT THE RIBOSOMAL P0 PROTEIN IS THE CELL SURFACE TARGET OF ANTI-P AUTOANTIBODIES ASSOCIATED WITH LUPUS PSYCHOSIS. **Espinoza S.**[1,2], Segovia F.[1,2], Bravo-Zehnder[1,2] M., Massardo L.[1], González A.[1,2]. Departamento de Inmunología Clínica y Reumatología, Facultad Medicina[1], Centro de Envejecimiento y Regeneración (CARE), Facultad Ciencias Biológicas[2], Pontificia Universidad Católica de Chile. csespino@gmail.com

(57) LEPTIN INCREASES CILIARY ACTIVITY AND AFFECT THE RESPONSE TO CHEMICAL SIGNALS IN THE RESPIRATORY EPITHELIUM. **Maria Paz Hernández**, Carolina Oses, Daniela Carreño, Carmen Llados, Manuel Villalón. Department of Physiology, Faculty of Biological Sciences. Pontificia Universidad Católica de Chile, Santiago, Chile. mpherna2@uc.cl

(58) CK2 UP-REGULATES COX-2 EXPRESSION AND THEREBY INCREASES VIABILITY AND INVASIVENESS OF COLON CANCER CELLS. **Roger Yefi[1]**, Ignacio Niechi[1], Eduardo Silva[1], Pablo Cabello[1], Diego Rodriguez[2], Daniela P. Ponce[1], Katherine Marcelain[2], Ricardo Armisen[2], Andrew F.G. Quest[2] & Julio C. Tapia[1,2]. [1]Cell Transformation Laboratory, [2]Institute of Biomedical Sciences (ICBM), Faculty of Medicine, University of Chile. jtapia@med.uchile.cl

(59) THE ACTIVATION OF KEY SIGNALING PATHWAYS IN BREAST CANCER CELLS INVOLVES TRANSACTIVATION OF THE EGFR BY THE KININ B1 RECEPTOR. **Andrade Y,** Ehrenfeld P, Plasencia M, Cardenas A, Matus CE, Pavicic F, Figueroa CD**.** Institute of Anatomy, Histology and Pathology. Universidad Austral de Chile. ingridehrenfeld@uach.cl

(60) IL-10 DECREASES MICA CELL SURFACE EXPRESSION IN GASTRIC ADENOCARCINOMA CELL LINES. **Garrido-Tapia M**, Hernández CJ, Ribeiro CH, Kramm K, Molina MC. Laboratorio de Evasión Inmune. Programa Disciplinario de Inmunología (ICBM). Facultad de Medicina, Universidad de Chile. macagarrido@gmail.com

(61) *REPRIMO*, A CANDIDATE BIOMARKER FOR EARLY DIAGNOSIS AND RESPONSE TO TREATMENT OF HUMAN GASTRIC CANCER. **Maturana MJ.**[1], Torres V.[1], Olivares W.[1], Cerda E.[1], Padilla O.[1], Garrido M.[1], Aguayo F.[3], Calvo A.[2], Ferreccio C.[1], Corvalán AH.[1]. [1]Hematology-Oncology Pontificia Universidad Católica de Chile, [2]CRS-San Rafael, SSMSO, [3]Virology Department, Universidad Chile. mariaj.maturana@gmail.cl (Sponsor: G. Owen)

(62) LACTATE EFFECT ON MITOCHONDRIA AND GLUCOSE UPTAKE IN HUMAN CANCER CELLS. **Viviana Ahumada[1,2]**, María Cabrera[1], Claudio Acuña-Castillo[1], Dante Miranda[2], Margarita Montoya[1]. 1.Department of Biology. Faculty of Chemistry and Biology. University of Santiago of Chile. 2.Faculty of Chemical and Pharmaceutical Sciences. University of Chile. margarita.montoya@usach.cl

(63) DEVELOPING AN RNAi-BASED GENETIC ADJUVANT SILENCING STAT3 THAT COUNTERACTS TUMORASSOCIATED TOLERANCE. **Nicole Rojas-Colonelli**, Jonathan Roco, Andrés Herrada, Octavio Aravena, Manuel Varas, Alvaro Lladser. Laboratory of Gene Immunotherapy, Fundación Ciencia Para la Vida. alvaro.lladser@bionova.cl

(64) AN ASSAY TO PERSONALIZE CHEMOTHERAPY IN OVARIAN CANCER PATIENTS. **M.L. Bravo[1,5]**, P. González[1,5], S. Kato[2,5], M. Garrido[2], S. González[3], J. Pizarro[3], M.I. Barriga[3], H. Leon[4], E. Bustamante[4], M.A Cuello[2,5], G. I. Owen[1,5]. [1]Facultad de Ciencias Biológicas, [2]Facultad de Medicina, Pontificia Universidad Católica de Chile, [3]Hospital Sotero del Rio, [3]Fundacion Arturo López Pérez [5]Biomedical Research Consortium of Chile (BRMC).

(65) SALMONID SELENOTRANSCRIPTOME: *IN SILICO* AND *IN VIVO* CHARACTERIZATION. **Francisco Altimiras,** Rodrigo Pulgar y Verónica Cambiazo. Laboratorio de Bioinformática y Expresión Génica, INTA, Universidad de Chile and Center for Genome Regulation (CRG). fjaltimiras@gmail.com

(66) PROTEOMIC ANALYSIS OF PROTOSCOLEX PROTEINS FROM *Echinococcus granulosus*. **María Pía García**[a], Christian Hidalgo[a], Henrique Bunselmeyer Ferreira[b], Ulf Hellman[c], Norbel Galanti[d] and Rodolfo Paredes[a,*]. [a]Escuela de Medicina Veterinaria, Facultad de Ecología y Recursos Naturales, Universidad Andrés Bello. [b]Centro de Biotecnologia, UFRGS, Brazil. [c]Ludwig Institute for Cancer Research Ltd., Sweden. [d]Programa de Biología Celular y Molecular, ICBM, Facultad de Medicina, Universidad de Chile. rparedes@unab.cl

(67) MITOCHONDRIAL DYNAMICS IN ERYTHROPOIETIC CELLS IS MODULATED BY COPPER. **Rodrigo Bustos,** Yancing Rossel, Alvaro Elorza. Universidad Andrés Bello**.** aelorza@unab.cl

(68) HANTAVIRUS LIKE-PARTICLES: A TOOL FOR DISEASE PREVENTION AND VIRUS-CELL ENTRY STUDIES. **[1,2]Acuña, R.,** [1]Cifuentes, N., [1]Bulling, M. and [1]Tischler, N.D. [1]Fundación Ciencia para la Vida and [2]Universidad Andrés Bello. rodrigo.acuna.bq@gmail.com

(69) EFFECT OF EGG YOLK IgY ANTIBODIES AGAINST *Piscirickettsia salmonis* INFECTION IN ATLANTIC SALMON SHK-1 CELLS. **Oliver C[1].,** Valenzuela K[1]., Silva H[1]., Oyarzún F[1]., Pontigo JP[1]., Álvarez C[1]., Olavarría VH[1]., Amthauer R[1]., Romero A[2] and Yáñez AJ[1]. [1]Instituto de Bioquímica y Microbiología, [2]Instituto de Patología Animal. Universidad Austral de Chile, Valdivia, Chile. cristianoliver7@gmail.com

(70) MODEL SYSTEMS FOR ACCURACY TESTING OF SKELETON METHODS FOR BIOLOGICAL STRUCTURES. **Alcayaga L**[13], Santibáñez F[1], Ramírez O[1], Palma K[12], Jara J[13], Concha M[1], Hitschfeld N[3] and Härtel S[1]. [1]SCIAN-Lab, [2]LEO, BNI, ICBM, Faculty of Medicine, [3]DCC, FCFM, U-Chile. lalcayag@dcc.uchile.cl

(71) GENERATION OF *Escherichia coli* K12 Xth NULL MUTANT. **José Delgadillo**, Sofia Sepúlveda, Ulrike Kemmerling, Norbel Galanti, Gonzalo Cabrera. Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile. gcabrera@med.uchile.cl

(72) SHORT TOLEROGENIC DENDRITIC CELLS PROTOCOL REPRESSES T CELLS PROLIFERATION AND EFFECTOR T HELPER PROFILES INDUCTION. **Falcón-Beas C[1,2]**, Tempio F[1,2], Pesce B[1,2], Aguillón J[1,2], Pereda C[1,2], Saffie C[1,2], Salazar-Onfray F[1,2], López M. N[1-3]. [1]Millennium Nucleus on Immunology and Immunotherapy; [2]Disciplinary Program of Immunology, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile; [3]Research Support Office, University of Chile Clinical Hospital. cfalcon@med.uchile.cl

(73) ANTIBODIES GENERATED AGAINST *Trypanosoma cruzi* CALRETICULIN S-DOMAIN DETECTS PUTATIVE MURINE CALRETICULIN. **[1]González, A.,** [1]Valck, C., [1]Maldonado, I., [1]Ramirez, G., [2]Galanti, N., [1]Ferreira, A. [1]Immunology Disciplinary Program, [2]Program of Cellular and Molecular Biology, Faculty of Medicine, University of Chile, Chile. aferreir@med.uchile.cl

(74) TUMOR NECROSIS FACTOR DIFFERENTIALLY MODULATES HUMAN Th1 AND Th17 CELLS. **B. Pesce,** J.C. Aguillón, M. Cuchacovich, D. Catalán. Immune Regulation and Tolerance Research Group, Programa Disciplinario de Inmunología, ICBM, Facultad de Medicina, Universidad de Chile. www.irtgroup.cl**,** bpesce@gmail.com

(75) STUDY OF THE FUNCTIONAL PLASTICITY IN TUMOR-SPECIFIC MEMORY T CELLS STIMULATED WITH DIFFERENT TYPES OF DENDRITIC CELLS. **Mora Gabriela**, Tittarelli A., Ramirez M., Pereda C., Ortiz C., Tempio F., Falcon C., López, M.Salazar-Onfray F. Tumor Immunology Laboratory, University of Chile. gabribau@gmail.com

(76) MODULATION OF SIGNALING PATHWAYS BY DOPAMINE RECEPTORS D3 AND D5 IN CD4+ T-CELLS. **Dafne Franz[1]**, Hugo González[1], Carolina Prado[1] and Rodrigo Pacheco[1,2]. [1]Fundación Ciencia para la Vida and [2]Universidad San Sebastián. Santiago, Chile. dafne.franz@gmail.com

(77) INVOLVEMENT OF P2X7R IN ANTIGEN CROSS-PRIMING, *in vivo*. **Ximena López**, Bélgica Villegas, Yohana Labra, Javier Mena, Víctor Lazo, Ronny Hernández, Alejandro Torres,

Alejandro Escobar, Margarita Montoya, Mónica Imarai, Claudio Acuña-Castillo. Universidad de Santiago de Chile. claudio.acuna@usach.cl

(78) DEVELOPMENT OF AN ANTIBODY AGAINST CD28 TO IDENTIFY T LYMPHOCYTES OF RAINBOW TROUT *(Oncorhynchus mykiss)*. **Soto-Aguilera S**, Valenzuela B, Maisey K, and Imarai M. Laboratorio de Inmunología, Centro de Biotecnología Acuícola (CBA), Facultad de Química y Biología, Universidad de Santiago de Chile. sarita.soto@usach.cl

(79) *Trypanosoma cruzi* CALRETICULIN INHIBITS THE CLASSICAL PATHWAY OF COMPLEMENT ACTIVATION IN *Gallus gallus*. **Paula Abello,** Carolina Valck, Ismael Maldonado, Hector Hidalgo, Arturo Ferreira. Disciplinary Immunology Program, ICBM. University of Chile. aferreir@med.uchile.cl

(80) ASSESSMENT OF THE VIABILITY OF OLIGODENDROCYTES FROM THE PROGENY GESTATED IN MOTHERS WITH THYROID HORMONE DEFICIENCY IN RESPONSE TO INFLAMMATORY MOLECULES. **Eduardo Albornoz**[1,3], Claudia Cortes[1,3], Pablo Cisternas[1,3], Pablo Gonzalez[1,3], Carlos Pizarro[1,3], Leandro Carreño[2,3], Susan Bueno[2,3], Eliseo Eugenin[4], Joan Berman[4], Alexis Kalergis[2,3] and Claudia Riedel[1,3]. [1]Facultad de Ciencias Biológicas, Universidad Andrés Bello. [2]Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, PUC. [3]Instituto Milenio de Inmunología e Inmunoterapia. [4]Albert Einstein College of Medicine of New York. edua.albornoz@uandresbello.edu

(81) SOLUBLE TOLL LIKE RECEPTOR 2 (sTLR2) IS PRODUCED BY METALLOPROTEASE ECTODOMAIN TLR2 SHEDDING. **Langjahr, P.E**.; Rubio, E.; Díaz-Jiménez, D.; Hermoso, M.A. Laboratorio de Inmunidad Innata, Programa D. de Inmunología, ICBM, Facultad de Medicina, Universidad de Chile. plangjhar@med.uchile.cl

(82) CHARACTERIZING THE DNA-SENSING SIGNALING PATHWAYS OF THE INNATE IMMUNITY THAT MEDIATE THE INDUCTION OF ANTITUMOR T CELL IMMUNITY ELICITED BY DNA VACCINES: THE ROLE OF NF-κB. **Jonathan Roco**[1], Maarten Ligtenberg[2], Andrés Herrada[1], Rolf Kiessling[2], Alvaro Lladser[1]. [1]Laboratory of Gene Immunotherapy, Fundación Ciencia para la Vida, Santiago, Chile. [2]Immune and Gene Therapy Unit, CancerCentrum Karolinska, Karolinska Institutet, Stockholm, Sweden. alvaro.lladser@bionova.cl (Sponsor: P. Valenzuela)

(83) ALLOGENEIC PLGA PHAGOSOMES DECREASE THE ALLOIMMUNE RESPONSE *IN VIVO*. **Yessia Hidalgo**[1], Paulina Ruiz[1], Paula Maldonado[1], Cinthia Silva[1], Mario Rosemblatt[1,2], María Rosa Bono[1]. Laboratorio de Inmunología, Facultad de Ciencias, Universidad de Chile[1]. Fundación Ciencia para la Vida[2]. yhidalgo@ug.uchile.cl

(84) LOW PLASMA LEVELS OF 2-METHOXYESTRADIOL (2-ME) IN EARLY PREGNANCY OF PATIENTS THAT WILL DEVELOP PREECLAMPSIA (PE). **Pérez-Sepúlveda A**, Torres MJ, Valenzuela FJ, Larraín R, Galaz J, Valenzuela I, Soto MJ, Figueroa-Diesel H, Illanes S. Departamento de Obstetricia & Ginecología y Laboratorio de Biología de la Reproducción. Facultad de Medicina, Universidad de los Andes, Santiago. sillanes@uandes.cl (Sponsor: U. Wyneken).

(85) REVISITING GLUCOSE TRANSPORTER 1 AND 3 THROUGH LIVE CELL FLUORESCENT MICROSCOPY. **Aníbal I. Acuña**, Ilona I. Concha, Maite A. Castro. Instituto de Bioquímica y Microbiología, UACh. macastro@uach.cl

WEDNESDAY, NOVEMBER 2nd, 2011

08:00 **Poster Mounting Session II: N° 86 to N° 170**
Convention Center Foyer

09:00 – 10:30 **SYMPOSIUM INSTITUTE FOR CELL DYNAMICS AND BIOTECHNOLOGY (ICDB) UNIVERSIDAD DE CHILE - COOPERACION INTERNACIONAL FONDECYT 1095089**
Calbuco Room - Chair: Christian Gonzalez-Billaut

"CELLULAR AND MOLECULAR FUNCTIONS OF CYTOSKELETON IN THE NERVOUS SYSTEM"

ACUTE EFFECTS OF ACTIN ON THE POSTSYNAPTIC DENSITY. **Thomas A. Blanpied**, School of Medicine, University of Maryland.

Thy-1/INTEGRIN-MEDIATED BIDIRECTIONAL SIGNALING BETWEEN NEURONS AND ASTROCYTES. **Lisette Leyton.** Laboratorio de Comunicaciones Celulares, Centro de Estudios Moleculares de la Célula (CEMC), Biomedical Neuroscience Institute (BNI), ICBM, Facultad de Medicina, Universidad de Chile.

MICROTUBULE ASSOCIATED PROTEIN (MAP1B) IN INVOLVED IN DENDRITIC SPINE DEVELOPMENT AND NEUROTRANSMISSION. **Christian González-Billault,** Laboratory of Neuronal and Cell Dynamics, Department of Biology, Universidad de Chile and Institute for Cell Dynamics and Biotechnology (ICDB) Santiago, Chile.

SYMPOSIUM
"MEMBRANE TRAFFICKING AT SYNAPSES: FROM NEURONAL TRANSMISSION TO IMMUNITY (I)"
Tronador Room - Chair: Ana Maria Lennon

THE VESICULAR SNARE SYNAPTOBREVIN IS REQUIRED FOR SEMAPHORIN 3A AXONAL REPULSION. Kathleen ZYLBERSZTEJN[1,2], Maja PETKOVIC[1,2], Andrea BURGO[1,2], Marie DECK[3], Sonia GAREL[3], Séverine MARCOS[4], Evelyne BLOCH-GALLEGO[4], Fatiha NOTHIAS[5], Guido SERINI[6], Dominique BAGNARD[7], Thomas BINZ[8] and **Thierry GALLI[1,2].** [1]University Paris Diderot, Sorbonne Paris Cité, Jacques Monod Institute, CNRS UMR7592, Program in Development & Neurobiology, Paris, 75013 France. [2]INSERM ERL U950, 'Membrane Traffic in Neuronal & Epithelial Morphogenesis', Paris, 75013 France. [3]Ecole Normale Supérieure, IBENS, INSERM U1024, CNRS UMR8197, Paris, 75005 France. [4]Cochin Institute, University Paris Descartes, CNRS UMR8104, Department in Genetic and Development, INSERM U567, Paris, 75005 France. [5]'Axon Regeneration and Growth', PMSNC, INSERM U952, CNRS UMR 7224, University Pierre and Marie Curie, Paris, 75005 France. [6]University of Torino School of Medicine, Candiolo, Torino, 10100 Italy. [7]INSERM U682, Strasbourg, 67000 France. [8]Institute of Biochemistry, Medizinische Hochschule Hannover, Hannover, 30001 Germany.

Cdc42 CONTROLS THE BALANCE BETWEEN KISS-AND-RUN AND FULL FUSION OF SECRETORY VESICLES BY REGULATING MEMBRANE TENSION. Marine Bretou[1,2], Ouardane Jouannot[1], Claire Desnos[1], Paolo Pierobon[2], Isabelle Fanget[1], Nathanaël Larochette[1], Pierre Gestraud[3,4,5], Marc Guillon[6], Valentina Emiliani[6], Stéphane Gasman[7], Ana-Maria Lennon-Duménil[2] and **François Darchen[1].** [1]Centre National de la Recherche Scientifique, Université Paris Descartes, Sorbonne Paris Cité, UMR8192, 45 rue des Saints-Pères, 75270 Paris cedex 06, France. [2]Inserm U932, Institut Curie, 12 rue Lhomond, 75005, Paris, France. [3]Institut Curie, Paris F-75248, France. [4]Inserm, U900, Paris F-75248, France. [5]Ecole des Mines ParisTech, Fontainebleau, F-77300 France. [6]INSERM U603, CNRS UMR 8154, Université Paris Descartes, Sorbonne Paris Cité, 45 rue des Saints-Pères, 75270 Paris cedex 06, France. [7]CNRS/UPR3212, INCI, Université Strasbourg.

POLARIZED SECRETION OF LYSOSOMES AT THE B CELL SYNAPSE COUPLES ANTIGEN EXTRACTION TO PROCESSING AND PRESENTATION. Maria-Isabel Yuseff[1,2], Anne Reversat[1,2], Danielle Lankar[1,2], Jheimmy Diaz[1,2], Isabelle Fanget[3], Paolo Pierobon[1,2], Stéphane

Gasman[4], François Darchen[3], Claire Desnos[3] and **Ana-Maria Lennon-Duménil[1,2].** [1]Institut Curie, Centre de Recherche, Paris F-75248, France. [2]INSERM Unité 932, Immunité et Cancer, Paris, France. [3]CNRS/Université Paris Descartes UMR8192, 45 rue des Saints-Pères, 75006, Paris, France. [4]CNRS/UPR3212, INCI, Université Strasbourg, France.

10:30 – 11:30 Coffee Break – Exhibitors – Poster Viewing: Session II
Convention Center Foyer

11:30 – 13:30 Oral Presentations II
Volcanes Room - Chair: Andrew Quest and Co-Chair: Nelson Osses

DEVELOPMENT OF NEW BIOINFORMATICS TOOLS FOR THE ACCURATE PREDICTION OF TRANSCRIPTION FACTOR BINDING SITES. Tomás Norambuena, Alex W. Slater and **Francisco Melo**. [1]Molecular Bioinformatics Laboratory, Millennium Institute on Immunology and Immunotherapy, Alameda 340, Santiago, Chile. [2]Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Alameda 340, Santiago, Chile. fmelo@bio.puc.cl

ACUTE TREATMENT WITH ANTI-NEOPLASTIC DRUGS INDUCED CAVEOLIN-1 UP-REGULATION AND INCREASED MIGRATION VIA A MEK/ERK-DEPENDENT PATHWAY IN COLON CANCER CELLS. Díaz-Valdivia, N., Leyton, L., Quest, AFG. CEMC, Facultad de Medicina, Universidad de Chile. aquest@med.uchile.cl

TMBIM3/GRINA IS A CONSERVED UNFOLDED PROTEIN RESPONSE (UPR) TARGET GENE THAT CONTROLS APOPTOSIS THROUGH THE MODULATION OF ER CALCIUM HOMEOSTASIS. Diego Rojas-Rivera,[1,2,3] Ricardo Armisén,[2] Alicia Colombo,[3] Gabriela Martínez,[1,2,3] Diego Rodríguez,[1,2,3] Rosario Rizzuto,[5] Geert Bultynck,[4] Miguel L. Concha,[3] Jimena Sierralta,[3] Andrés Stutzin,[1,2] Claudio Hetz [1,2,3]. [1]Neurounion Biomedical Foundation, Santiago, Chile, [2]Center for Molecular Studies of the Cell, Institute of Biomedical Sciences, University of Chile, [3]Biomedical Neuroscience Institute, Faculty of Medicine, University of Chile, [4]Katholieke Universiteit Leuven**,** Belgium and [5]University of Padova, Italy. diegorojasrivera@gmail.com

BONE MORPHOGENETIC PROTEIN 2 INHIBITS NEURITE OUTGROWTH OF MOTOR NEURON-LIKE NSC-34 CELLS AND UP-REGULATES ITS TYPE II RECEPTOR. Francisca Benavente[1], Margarita Parada[1], Cristina Pinto[2], Juan Pablo Henríquez[2], and **Nelson Osses[1].** [1]Instituto de Química, Pontificia Universidad Católica de Valparaíso. [2]Departamento de Biología Celular, Universidad de Concepción. nelson.osses@ucv.cl

RESVERATROL INHIBITS Cdk5 ACTIVITY THROUGH REGULATION OF p35 EXPRESSION. Elias Utreras, Anita Terse, Jason Keller, Michael Iadarola and Ashok Kulkarni. Functional Genomics Section, NIDCR, NIH, USA. elias.utreras@uchile.cl

DETERMINATION OF THE ROLE OF p53-RELATED PROTEIN KINASE (PRPK) IN AXON ELONGATION. Villarroel D.[1], Henriquez D.[1], Glavic A.[2] and González-Billault C.[1]. [1]Cellular and Neuronal Dynamics Laboratory and [2]Center for Genome Regulation, Department of Biology, Faculty of Sciences, Universidad de Chile, Santiago, Chile. davidbiovilla@gmail.com

C/EBPβ-MEDIATED RECRUITMENT OF SWI/SNF IS A MECHANISM FOR RIC-8B GENE REPRESSION DURING OSTEOBLAST DIFFERENTIATION. **Rodrigo Aguilar**, Aníbal Arce, Berta Henríquez, Hugo Sepúlveda, Martín Montecino. Center for Biomedical Research and FONDAP Center for Genome Regulation, Universidad Andres Bello, Santiago, Chile. raguilarmaureira@hotmail.com

WNT/b-CATENIN SIGNALING ENHANCES RUNX1 TRANSCRIPTIONAL ACTIVITY IN HEMATOPOIETIC CELLS. Medina M, Pérez E, Gajardo I, Ugarte GD, De Ferrari GV. Center for Biomedical Research, Faculty of Biological Sciences and Faculty of Medicine, Andres Bello University, Chile. gdeferrari@unab.cl

13:30 – 15:30 Lunch

15:30 – 16:30 PLENARY LECTURE FUNDACION CIENCIA PARA LA VIDA AND MIFAB
Volcanes Room - Chair: Patricia Burgos

NAVIGATING THE CELLULAR LANDSCAPE WITH NEW OPTICAL PROBES, IMAGING STRATEGIES AND TECHNICAL INNOVATIONS. **Jennifer Lippincott-Schwartz**. Eugene Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892.

16:30 – 17:30 Coffee Break – Exhibitors – Poster Viewing: Session II
Convention Center Foyer

17:30 – 19:30 Oral Presentations III
Volcanes Room - Chair: Monica Imarai and Co-Chair: Alvaro Elorza

TOXIC FACTORS RELEASED BY ALS-LINKED MUTATED SOD1 ASTROCYTES INDUCE MOTONEURON PATHOLOGY AND DEATH BY TARGETING SODIUM CHANNELS. **Elsa Fritz**[1,4], Fabiola Rojas[1,4], Constanza Riquelme[4], Camila Segura[4], Rodolfo Madrid[2], Felipe Court[3], and Brigitte van Zundert[4]. [1]Universidad de Concepción; [2]Universidad de Santiago de Chile; [3]Pontificia Universidad Católica de Chile. [4]Universidad Andrés Bello. bvanzundert@unab.cl

MMP-14 PRODUCED BY BONE MARROW-DERIVED CELLS SHEDS EPITHELIAL ENDOGLIN MODULATING THE MIGRATORY PROPERTIES OF HUMAN BREAST CANCER CELLS. **Tobar N[1].,** Toyos M[1]., Quintanilla M[2]., Bernabeu C.[3] and Martínez J.[1]. Laboratorio de Biología Celular y Molecular, INTA, Universidad de Chile. [2]Instituto de Investigaciones Biomédicas and [3]Centro de Investigaciones Biológicas, CSIC, Madrid, Spain. nicolastobar@gmail.com

CROSS-TALK BETWEEN Ca^{+2}, NO AND H_2O_2 IN THE MARINE ALGA *Ulva compressa* (*Chlorophyta*) IN RESPONSE TO COPPER EXCESS. **Alberto González**, M. Josefa Henríquez, Rodrigo A. Contreras and Alejandra Moenne. alejandra.moenne@usach.cl (Sponsor: M. Imarai)

INFECTIOUS PANCREATIC NECROSIS VIRUS IN SALMONIDS: PERSISTENCE AND IMMUNOLOGICAL RESPONSE. **Reyes-Cerpa S[1]**, Toro-Ascuy D[1], Montero R[1], Cottet L[2], Reyes-López FE[1], Sandino AM [2], Imarai M[1]. [1]Laboratorio de Inmunología, [2]Laboratorio de Virología. Centro de Biotecnología Acuícola. Facultad de Química y Biología. Universidad de Santiago de Chile. sebastian.reyesc@usach.cl

COPPER OVERLOAD IN THE ERYTHROPOIETIC CELL LINE K562 GOVERNS CELL FATE ALONG WITH CHANGES IN MITOCHONDRIAL PHYSIOLOGY. **Lina Ruiz,** Yancing Rossel, Rodrigo Bustos, Alvaro Elorza. Universidad Andrés Bello. aelorza@unab.cl

PANNEXIN HEMICHANNELS CONTRIBUTE TO Ca^{2+} DYNAMICS DURING ATP-INDUCED MIGRATION IN CONFINEMENT. **Sáez PJ[1],** Vargas P[2], Lennon-Duménil AM[2] and Sáez JC[1]. [1]Departamento de Fisiología, Santiago, Chile and [2]INSERM U932, Institut Curie, Paris, France. pjsaez@uc.cl

ANTI-P AUTOANTIBODIES ENHANCE CYTOSOLIC CALCIUM AND NEUROTRANSMISSION IN HIPPOCAMPAL NEURONS BY CROSS-REACTING WITH CELL SURFACE NSPA. **Fabian Segovia-Miranda**[1,2], Jorge Parodi[1], Felipe Serrano[1], Noelia Escobedo[1], Marcela Bravo-Zehnder[1,2], Pedro Zamorano[3], Juan Larraín[1], David Valenzuela[4], Loreto Massardo[2], Nibaldo C. Inestrosa[1], Alfonso González[1,2]. Centro de Envejecimiento y Regeneración (CARE), Fac. Ciencias Biológicas[1]. Departamento de Inmunología Clínica y Reumatología, Facultad Medicina[2]. Pontificia Universidad Católica de Chile. Departamento Biomédico, Universidad de Antofagasta de Chile[3]. Regeneron Pharmaceuticals Inc[4].

INCREASED RESTING CALCIUM LEVELS ACTIVATE NF-kB IN DYSTROPHIC (*MDX*) MYOTUBES. **Altamirano F.[1],** López JR.[2], Allen PD[2] and Jaimovich E[1]. [1]Centro de Estudios

Moleculares de la Célula, ICBM, Facultad de Medicina, Universidad de Chile, Santiago, Chile and [2]Department of Anesthesia, Brigham and Women's Hospital, Harvard Medical School, Boston, USA. fcoaltamirano@gmail.com

19:30 – 20:30 PLENARY LECTURE CENTRO FONDAP DE REGULACION DEL GENOMA UNIVERSIDAD DE CHILE, P. UNIVERSIDAD CATOLICA DE CHILE Y UNIVERSIDAD ANDRES BELLO
Volcanes Room - Chair: Miguel Allende

IMMUNE MODULATION OF MAMMALIAN REGENERATION. Nadia Rosenthal. Mouse Biology Unit, EMBL-Monterotondo Outstation, Rome**.** Heart Science Centre, Imperial College London**.** Australian Regenerative Medicine Institute, Monash University, Melbourne.

20:30 Dinner

22:00 – 23:30 Poster Presentations Session II: N° 86 to N° 170
Convention Center Foyer
Coordinators: Maria de los Angeles Garcia, Christian Gonzalez, Ariel Reyes

(86) DIFFERENTIAL REGULATION OF TACE/Adam17 PROMOTER BY TRANSCRIPTION FACTOR RUNX2. Héctor Araya[1,2], Oscar Vega[1,2], Nelson Varela[1,2], Marcelo Antonelli[2], Ricardo Moreno[3], Juan Pablo Rodríguez[4], Gary S Stein[5], Andre van Wijnen[5] , Julio Tapia[2], Mario Galindo[1,2]. [1]Millennium Institute on Inmunology and Inmunotherapy. [2]Programa de Biología Celular y Molecular, ICBM, Facultad de Medicina, Universidad de Chile. [3]Unidad de Reproducción y Desarrollo, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile. [4]INTA, Universidad de Chile. [5]Departament of Cell Biology and Cancer Center, University of Massachusetts Medical School, Worcester, Massachusetts. mgalindo@med.uchile.cl

(87) REGULATION OF N-TYPE Ca^{2+} CHANNELS BY THE INTERACTION WITH THE LIGHT CHAIN 1 (LC1) OF MICROTUBULE ASSOCIATED PROTEIN B (MAP1B). Henríquez, D.R.[1], Gandini, M.A.[2], Sandoval, A.[2], Felix, R.[2] and González-Billault, C.[1]. [1]Laboratory of Cell and Neuronal Dynamics, Department of Biology, Universidad de Chile, [2]Departmento de Biología Celular, Cinvestav-IPN, México. darohenga@gmail.com

(88) HEMICHANNELS PARTICIPATE IN MAST CELLS DEGRANULATION INDUCED BY ANTIGEN RECOGNITION. Harcha PA, Sáez JC. Departamento de Fisiología, P. Universidad Católica de Chile. paharcha@puc.cl

(89) ROLE OF α6β1 INTEGRIN IN SECRETION, ORGANIZATION, ADHESION AND SURVIVAL OF DIFFERENTIATED 3D ACINI OF SALIVARY GLANDS CELLS: IMPLICATIONS IN SJÖGREN'S SYNDROME. Urra H, Cortés J, Bahamondes V, Castro I, Barrera MJ, Aguilera S, Molina C, Leyton C, Alliende C, González S, and González MJ. ICBM Facultad de Medicina Universidad de Chile. hery.urra@gmail.com

(90) THE HEDGEHOG (Hh) PATHWAY MODULATES MATURATION OF CHONDROCYTES *in vitro* THROUGH NEOGENIN 1. João Francisco Botelho, Cynthia Villarroel, Pablo Lois and Verónica Palma. Laboratory of Stem Cells and Development, Facultad de Ciencias, Universidad de Chile. chicobotelho@gmail.com

(91) EXPRESSION OF AKT/PKB IN STREPTOZOTOCIN-INDUCED DIABETIC RAT KIDNEY. Marcos Soto, Romina Bertinat, Pamela Silva, Pamela Kairath and Alejandro Yañez. Instituto de Bioquímica y Microbiología, Universidad Austral de Chile (UACH). marcossoto85@hotmail.com

(92) REGULATION OF RUNX2 DURING CELL CYCLE AND ITS EFFECT IN THE PROLIFERATION OF OSTEOSARCOMA CELL LINES. Claudia Lucero[1,2], Oscar Vega[1,2], Mariana Osorio[1,2], Julio Tapia[2], Marcelo Antonelli[2], Gary S Stein[3], Andre van Wijnen[3] and Mario Galindo[1,2]. [1]Millennium Institute on Immunology and Immunotherapy, [2]Programa de Biología Celular y Molecular, ICBM, Facultad de Medicina, Universidad de Chile, [3]Department of Cell Biology and Cancer Center, University of Massachutsetts Medical School, USA. mgalindo@med.uchile.cl

(93) PANNEXIN MEDIATED COUPLING OF OLIGODENDROCYTES AND OF A CELL LINE DERIVED FROM HUMAN OLIGODENDROGLIOMA. **Paola A. Soto[1]**, Paola Fernández[1], Maximiliano Rovegno[1], Agustín D. Martínez[2], Bruno Cisternas[1], Felipe Court[1], Alex Vielma[2], Oliver Schmachtenberg[2], Michael V.L. Bennett[3] and Juan C. Sáez[1,2]. [1]Departamento de Fisiología, Pontificia Universidad Católica de Chile, Santiago, Chile and [2]Instituto milenio, CINV, Valparaíso, Chile. [3]Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY, USA.

(94) ANALYSIS OF A FRACTION OF HUMAN RECOMBINANT FSH THAT INDUCES CELL DEATH. **Orellana RF**, Ríos M, Ortiz ME, Owen GI and Velásquez EV. Faculty of Biological Sciences, Pontificia Universidad Católica de Chile and Chilean Institute of Reproductive Medicine (ICMER), Santiago, Chile.

(95) QUANTIFICATION OF ACTIVE ORGANIZATION AND DIFFUSION WITHIN THE ENDOPLASMIC RETICULUM (ER). **Briones L**[1], Ramírez O[1], Scheer J[1], Moraga H[1], Jara J[13], Osorio-Reich M[1], Asahi T[4], Ortega J[4], Couve A[2], and Härtel S[1]. [1]SCIAN-Lab, [2]Couve-Lab, BNI, Faculty of Medicine, [3]DCC, [4]CMM, FCFM, U. of Chile. lbriones@med.uchile.cl

(96) GLUCOSE INCREASES INTRACELLULAR FREE Ca^{2+} IN TANYCYTES VIA ATP RELEASED THROUGH CONNEXIN 43 HEMICHANNELS. **Juan A. Orellana[1]**, Pablo J. Sáez[1], Christian Cortés-Campos[2], Roberto J. Elizondo[2], Kenji F. Shoji[1], Susana Contreras-Duarte[1] Vania Figueroa[1], Victoria Velarde[1], Jean X. Jiang[3], Francisco Nualart[2], Juan C. Sáez[1] and María A. García[2]. [1]Departamento de Fisiología, Pontificia Universidad Católica de Chile, Santiago, Chile; [2]Departamento de Biología Celular, Universidad de Concepción, Concepción, Chile; [3]Department of Biochemistry, University of Texas Health Science Center, San Antonio, TX, USA.

(97) AT EARLY STAGE OF DIABETIC NEPHROPATHY (DN), RAF KINASE INHIBITOR PROTEIN (RKIP) DOWNREGULATION PRODUCE AN UNUSUAL THICKNESS OF GLOMERULAR BASEMENT MEMBRANE (GBM). **Fabian Pardo**, Romina Bertinat, Juan Carlos Slebe and Alejandro Yáñez. Instituto de Bioquímica y Microbiología, Universidad Austral de Chile. fabian.pardo@gmail.com

(98) BMP-2 ON MESENCHYMAL STEM CELLS (MSCS) ADIPOGENIC DIFFERENTIATION. **Oscar Donoso**, Nelson Osses*, Ana María Pino, Mireya Fernández, Juan Pablo Rodríguez. Laboratorio de Biología Celular y Molecular, INTA, Universidad de Chile. *Facultad de Ciencias. P. Universidad Católica de Valparaíso. oscardonosomora@gmail.com

(99) COTRANSIN INHIBITS p58 AND ERdj3 TRANSLOCATION AND DOWNREGULATES THE UNFOLDED PROTEIN RESPONSE. **Ureta, G.[1,2],** Amoroso, A.[3], McCullahg, E.[1], Taunton,J.[4], Snapp,E.[5], Bernales,S.[1]. 1.Fundación Ciencia para la Vida. 2.Universidad Andrés Bello. 3.Universidad San Sebastián. 4.University of California. 5.Albert Einstein College of Medicine. g.ureta.diaz@gmail.com

(100) ROLE OF CHOLESTEROL AND MLN64 IN MITOCHONDRIAL DYSFUNCTION AND OXIDATIVE STRESS IN NIEMANN-PICK TYPE C DISEASE MODELS. **Elisa Balboa**, Nuria Matías, Carlos Fernandez-Checa, Silvana Zanlungo. Departamento de Gastroenterología, Facultad de Medicina, Pontificia Universidad Católica de Chile. ebalboa@gmail.com

(101) MOLECULAR CHARACTERIZATION AND DEVELOPMENTAL EXPRESSION OF CG6234 PROTEIN FROM *Drosophila melanogaster*. **Carlos Chacón,** Christian Hodar and Verónica Cambiazo. Laboratorio Bioinformatica y Expresión Génica, INTA-Universidad de Chile and Center for Genome Regulation (CRG). chacon.biotec@gmail.com

(102) ROLE OF THE NEUROTROPHIN BRAIN DERIVED NEUROTROPHIC FACTOR (BDNF) IN LATERAL LINE NERVE REGENERATION IN ZEBRAFISH LARVAE. **Moya J.**, Villegas R., Sánchez M., Peña O. and Allende M. FONDAP Center for Genome Regulation, Facultad de Ciencias, Universidad de Chile. allende@uchile.cl

(103) SPINAL CORD INJURY AND REGENERATION IN *Xenopus laevis*. **Dasfne Lee-Liu[1,3],** Mauricio Moreno[1], Leonardo Almonacid[2], Ricardo Tampe[1], Marcia Gaete[1], Francisco Melo[2], Juan

Larrain[1]. [1]Center for Aging and Regeneration and Millennium Nucleus in Regenerative Biology, Pontificia Universidad Catolica, Santiago, Chile; [2]Molecular Bioinformatics Laboratory, Millennium Institute on Immunology and Immunotherapy, Santiago, Chile; [3]Faculty of Chemical and Pharmaceutical Sciences, Universidad de Chile, Santiago, Chile. dasfne@gmail.com

(104) TRANSIENT INACTIVATION OF MYOSTATIN IN DIFFERENT DEVELOPMENTAL STAGES REGULATES DIFFERENTIALLY MYOGENESYS IN ZEBRAFISH. **Navarro C.,** Valdés J.A. and Molina A. Universidad Andrés Bello. Chile. amolina@unab.cl

(105) GENETIC ANALYSIS OF ECLOSION HORMONE FUNCTION IN *Drosophila* ECDYSIS BEHAVIOR. **Eileen Kruger**, Wilson Mena & John Ewer. Centro Interdisciplinario de Neurociencia de Valparaíso. Universidad de Valparaíso. eileen.kruger@cinv.cl

(106) TARGET GENES OF DPP/BMP SIGNALING PATHWAY REVEALED BY TRANSCRIPTOME PROFILING IN THE EARLY *D. melanogaster* EMBRYO. **Calixto Domínguez,** Alejandro Zúñiga, Carlos Chacón, Michael Pino y Verónica Cambiazo. Laboratorio Bioinformatica y Expresión Génica, INTA-Universidad de Chile and Center for Genome Regulation (CRG). cdportilla@yahoo.com

(107) MAGUK PROTEINS PARTICIPATES IN *Drosophila* OLFACTORY LEARNING. **Claudia Molina** and Jimena Sierralta. ICBM, Faculty of Medicine, Universidad de Chile and BNI. cmolina@med.uchile.cl

(108) Daam1 CONTROLS MORPHOGENESIS OF THE HABENULO-INTERPEDUNCULAR CIRCUIT IN ZEBRAFISH. Alicia Colombo, **Álvaro Díaz-Briceño**, Lorena Armijo, and Miguel Concha. Anatomy and Developmental Biology Program, Institute of Biomedical Sciences, University of Chile, Santiago, Chile. acolombo@med.uchile.cl; mconcha@med.uchile.cl

(109) ODOR-MEDIATED REGULATION OF OLFACTORY RECEPTOR EXPRESSION IN ZEBRAFISH. **Cristian Calfún[1,2],** Maegan Rivard[3], Juanita Astudillo[1,2], & Kathleen Whitlock[1,2]. 1.CINV, Universidad de Valparaiso, Chile. 2.Centro de Genómica de la Célula, Núcleo Milenio. Universidad de Valparaíso. 3. MBG, Cornell University USA. cristian.calfun@gmail.com

(110) HIF-1α IS REQUIRED FOR THE NORMAL FORMATION AND ARBORIZATION OF THE TRIGEMINAL GANGLION. **Santander L**. and Reyes A.E. Laboratorio Biología del Desarrollo, Facultad de Ciencias Biológicas, Universidad Andrés Bello, Santiago, Chile. arielreyes@unab.cl

(111) CTIP1/Bcl11a PARTICIPATES IN THE DETERMINATION OF NEURONAL IDENTITY IN THE DEVELOPING NEOCORTEX. Cánovas J., Berndt F.A., Oliva C., Sierralta J., **Kukuljan M**. Programa de Fisiología y Biofísica, ICBM, e Instituto Milenio de Neurociencias Biomédicas, Facultad de Medicina, Universidad de Chile. kukuljan@med.uchile.cl

(112) THE ROLE OF THE UNFOLDED PROTEIN RESPONSE IN AXONAL REGENERATION AFTER SCIATIC NERVE INJURY. **Oñate M**.[1,2,3], Court F.A.[3,4] and Hetz C.[1,2,4]. [1]Biomedical Neuroscience Institute and [2]Center for Molecular Studies of the Cell, Institute of Biomedical Sciences, University of Chile. [3]Millennium Nucleus in Regenerative Biology (MINREB), Catholic University of Chile. [4]Neurounion Biomedical Foundation. chetz@med.uchile.cl, fcourt@bio.puc.cl

(113) BMP SIGNALLING MODULATES PROLIFERATIVE POTENTIAL OF ADULT SPINAL CORD DERIVED NEURAL STEM CELLS. **Emilio Méndez**, Alejandro Erices. Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile. eemendez@uc.cl

(114) META-ANALYSIS OF GENOME-WIDE ASSOCIATION STUDIES (GWAS) IN ALZHEIMER'S DISEASE. **Bustos B**, Pérez E and De Ferrari GV. Center for Biomedical Research. Faculty of Biological Sciences and Faculty of Medicine, Universidad Andres Bello, Chile. gdeferrari@unab.cl.

(115) *ANDROGRAPHOLIDE* PREVENT THE DECREASE OF SYNAPTIC PROTEINS AND INDUCES LTP IN BRAINS OF A DOUBLE TRANSGENIC MODEL OF ALZHEIMER'S

DISEASE, POSSIBLY BY A MECHANISM INVOLVING CANONICAL *WNT* PATHWAY. **Cheril Tapia-Rojas**, Felipe G. Serrano and Nibaldo C. Inestrosa. Centro de Envejecimiento y Regeneración (CARE), Departamento de Biología Celular y Molecular, Pontificia Universidad Católica de Chile. cctapia1@uc.cl

(116) INTERNALIZATION AND TRAFFICKING OF THE p75 NEUROTROPHIN RECEPTOR (p75). EVADING THE LATE ENDOSOMAL ROUTE TOWARDS MULTIVESICULAR BODIES SPECIALIZED FOR EXOSOMAL RELEASE. **Galleguillos C[1]**, Escudero CA[1], Parraguez JI[1], Uzma S[2], Lopez-Verrilli MA[1], Carter BD[2], Court FA[1], Bronfman FC[1]. 1Millennium Nucleus in Regenerative Biology (MINREB), Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile.

(117) EPIGENETIC MECHANSIMS THAT CONTROL PSD95 GENE EXPRESSION IN DEVELOPING HIPPOCAMPAL NEURONS. Fernando Bustos[1,2], Berta Henríquez[2], Rodrigo Aguilar[2,3], David González[2], **Martín Montecino[2,3], Brigitte van Zundert[2]**. [1]University of Concepción; [2]Center for Biomedical Research, Andres Bello University, Santiago; [3]FONDAP Center for Genome Regulation. fbustosf@gmail.com

(118) METABOLIC MODULATION BY ASCORBIC ACID IN NEURONS UNDER GLUTAMATERGIC ACTIVITY DOES NOT RELY ON THE ANTIOXIDANT PROPERTIES OF THIS MOLECULE. **[1]María Paz Miró,** [1]Felipe Beltran, [1]Aníbal I. Acuña, [1]Ilona I. Concha, [2]Michael Levine, [1]Maite A. Castro. [2]Semel Institute, UCLA; [1]Instituto de Bioquímica y Microbiología, UACh. macastro@uach.cl

(119) ATF-4 DEFICIENCY PROTECTS AGAINST AMYOTROPHIC LATERAL SCLEROSIS ASSOCIATED TO AN ALTERATION OF THE PROTEIN DISULPHIDE ISOMERASE (PDI) FAMILY EXPRESSION PATTERN AND A CHANGE IN CELLULAR REDOX STATE. **Matus S**.[1,2,3], Lopez E.[1,2,3], Hetz C.[1,2,3]. [1]Biomedical Neuroscience Institute, Faculty of Medicine. [2]Center for Molecular Studies of the Cell, Institute of Biomedical Sciences, University of Chile, Santiago, Chile. [3]Neurounion Biomedical Foundation, Santiago, Chile. mmatus@med.uchile.cl

(120) ASSESSMENT OF THE AGGREGATION PROPERTIES OF PICCOLO AND BASSOON POLY-GLUTAMINE (PQ) DOMAINS: TOWARDS A PRION-LIKE MECHANISM FOR PROTEIN RECRUITMENT TO THE SYNAPSE. **Jaime Villalobos**, Yocelin Cruz, Viviana Torres, Pedro Zamorano. Laboratorio de Neurobiología, Facultad de Ciencias de la Salud, Universidad de Antofagasta. zamorano@uantof.cl

(121) COPPER REDUCES Aβ OLIGOMERIC SPECIES AND AMELIORATES NEUROMUSCULAR SYNAPTIC DEFECTS IN A *C. elegans* MODEL OF IBM. **Daniela L. Rebolledo[1]**, Rebeca Aldunate[1,2], Alicia N. Minniti[1], Nibaldo C. Inestrosa[1]. [1]CARE, P. Universidad Católica de Chile. [2]Escuela de Biotecnología. Universidad Santo Tomás. dvreboll@uc.cl

(122) CLONING AND EXPRESION OF A NOVEL NEURON SURFACE PROTEIN, NSPA, INVOLVED IN PSYCHIATRIC LUPUS. **Bravo-Zehnder M**[1,2], Segovia F.[1,2], Jurado A[2], Zamorano P[3], Massardo L.[1,2], González A.[1,2]. Departamento de Inmunología Clínica y Reumatología, Facultad Medicina[1]. Centro de Envejecimiento y Regeneración. Fac. Ciencias Biológicas[2]. Pontificia Universidad Católica de Chile. Departamento Biomédico Universidad de Antofagasta de Chile[3]. mbravo@med.puc.cl

(123) A ROLE OF Beclin1 IN THE REGULATION OF AUTOPHAGY IN AMYOTROPHIC LATERAL SCLEROSIS. **Nassif M**. and Hetz C. Biomedical Neuroscience Institute, Center for Molecular Studies of the Cell, Institute of Biomedical Sciences, University of Chile, Neurounion Biomedical Foundation, Santiago, Chile. chetz@med.uhile.cl

(124) MYELIN ASSOCIATED PROTEINS BLOCK MIGRATION OF OLFACTORY ENSHEATHING CELLS: AN IN VITRO STUDY USING SINGLE CELL MIGRATION AND TRACTION FORCE MICROSCOPY ANALYSIS. Sara Nocentini*, **Diego Reginensi***, Simón Garcia, Patricia Carulla, María Teresa Moreno-Flores, Francisco Wandosell, Xavier Trepat, Ana Bribian, José A. del Río. Molecular and Cellular Neurobiotechnology. Institute for Bioengineering of Catalonia (IBEC), Spain. *These authors contribute equally to this study. diego.reginensi@gmail.com

(125) BDNF INDUCES DENDRITIC BRANCHING OF HIPPOCAMPAL NEURONS THROUGH A RAB11-DEPENDENT MECHANISM: DOES THE SAME MECHANISM ACCOUNT FOR AXONAL OUTGROWTH? **Andrés González**, Oscar Lazo, Carlos Flores and Francisca C Bronfman. Millennium Nucleus in Regenerative Biology (MINREB). Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile. augonzal@uc.cl

(126) A RETINOIC ACID-DEPENDENT CHECKPOINT IN THE DEVELOPMENT OF CD4$^+$ T CELL-MEDIATED IMMUNITY. **Karina Pino-Lagos[1]**, Yanxia Guo[1], Chrysothemis Brown[3], Matthew P. Alexander[1], Raul Elgueta[3], Kathryn A Bennett[1], Victor De Vries[1], Elizabeth Nowak[1], Rune Blomhoff[4], Shanthini Sockanathan[5], Roshantha A Chandraratna[6], Ethan Dmitrovsky[2] and Randolph J Noelle[1, 3*]. 1.Department of Microbiology and Immunology, Dartmouth Medical School and Norris Cotton Cancer Center, Lebanon, NH 03756, USA. 2. Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, NH 03755, USA. 3.King's College London, King's Health Partners, Medical research Council (MRC) Centre of Transplantation, Guy's Hospital, London SE1 9RT, UK. 4.Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo. Oslo, Norway. 5.The Solomon H. Snyder Department of Neuroscience, Johns Hopkins University, School of Medicine, Baltimore, MD 21205, USA. 6.IO Therapeutics, Santa Ana, CA 92705, USA.

(127) THE AXONAL ENDOPLASMIC RETICULUM AND GABA$_B$1a TRAFFICKING. **Viviana Valdés**[1,2], Christoph Schmidt[3] and Andrés Couve[1,2]. [1]Physiology and Biophysics, ICBM and [2]Biomedical Neuroscience Institute (BNI), Facultad de Medicina, Universidad de Chile, Santiago, Chile. [3]Georg-August-Universität Fakultät für Physik, Göttingen, Germany. vivianavaldes@gmail.com

(128) EXERCISE MODEL CHARACTERIZATION TO ASSESS MUSCLE ADAPTATIONS TO ATP-MEDIATED INTERLEUKIN-6 EXPRESSION IN MICE. **Fernández, R.[1]**, Galgani, J.[2], Jaimovich, E.[1] and Buvinic, S.[1,3]. [1]Centro de Estudios Moleculares de la Célula, ICBM, Facultad de Medicina, [2]Departamento de Nutrición, Facultad de Medicina and [3]Facultad de Odontología. Universidad de Chile, Santiago, Chile. rodrigolicaf@gmail.com

(129) MUSCLE STEM-CELL THERAPY IS IMPROVED BY REDUCING THE FIBROSIS ASSOCIATED TO MUSCULAR DYSTROPHIES. **Jaime Gutiérrez**, Cabrera D., Morales MG., Brandan E. Laboratory of Cell Differentiation and Pathology, CARE. Department of Cell and Molecular Biology, Catholic University of Chile. jagutiep@puc.cl

(130) THE TRANSCRIPTION FACTORS NFAT, CREB AND SMAD2/3 ARE DIFFERENTIALLY REGULATED BY MYOSTATIN/IGF-1 DURING MYOBLAST DIFFERENTIATION. Sylvia Flores, Rodrigo Zuloaga, Andrea Retamales, Alfredo Molina, **Juan Antonio Valdés**. Universidad Andrés Bello, Facultad de Ciencias Biológicas, Laboratorio de Biotecnología Molecular. Santiago, Chile. jvaldes@unab.cl

(131) ALTERATION OF IL-6 SIGNALING IN UREMIC SKELETAL MUSCLE. **Dünner N.**, Venegas F., Peña JP., Coronado F., Michea L. and Jaimovich E. Center for Molecular Studies of the Cell. ICBM, Facultad de Medicina, Universidad de Chile. dailea_nd@yahoo.com

(132) THE VITAMIN C TRANSPORTER SVCT2 IS DOWN-REGULATED DURING EARLY POST-NATAL DEVELOPMENT OF SLOW SKELETAL MUSCLE FIBERS. **Daniel Sandoval,** Marcela Low, Jorge Ojeda, Jaime Teneb, Francisco Nualart and Juan Pablo Henríquez. Department of Cell Biology, Faculty of Biological Sciences, Universidad de Concepcion, Concepcion, Chile. jhenriquez@udec.cl

(133) TRANSFORMING GROWTH FACTOR-β STIMULATES MAMMARY MYOFIBROBLASTS DIFFERENTIATION THROUGH NOX4 INDUCTION AND JNK ACTIVATION. **Toyos M.,** Tobar N., and Martínez J. Laboratorio de Biología Celular y Molecular, INTA, Universidad de Chile. marcetoyos@gmail.com

(134) DIFERENCIAL GENE EXPRESSION BETWEEN NORMAL AND MDX MOUSE FIBERS INDUCED BY ATP SIGNALING. **Valladares D.,** Almarza G., Jaimovich E. Centro de Estudios Moleculares de la Célula, ICBM, Facultad de Medicina, Universidad de Chile.

(135) UBIQUITIN-PROTEASOME SYSTEM MEDIATED STABILITY REGULATION OF PAX7 AND ITS ROLE IN ADULT MUSCLE SATELLITE STEM CELL FUNCTION. **Francisco J. Bustos**[1], John Yates III[2] and Hugo C. Olguín[1]. [1]Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile. [2]Dept. of Chemical Physiology, The Scripps Research Institute, La Jolla, USA. fubustos@uc.cl (Sponsor: E.O. Campos).

(136) PROCESSING, RELEASE AND NUCLEAR RELOCALIZATION OF THE INTRACELLULAR TAIL DOMAIN OF BONE MORPHOGENETIC PROTEIN RECEPTOR II (BMPRII). **Margarita Parada**[1], Juan Pablo Henríquez[2] and Nelson Osses[1]. [1]Instituto de Química, Pontificia Universidad Católica de Valparaíso. [2]Departamento de Biología Celular, Universidad de Concepción. nelson.osses@ucv.cl

(137) ALTERED PRO-INFLAMMATORY GENE EXPRESSION IN DYSTROPHIC *MDX* SKELETAL MUSCLE CELLS. **Henriquez C.**, Altamirano F. and Jaimovich E. Centro de Estudios Moleculares de la Célula, ICBM, Facultad de Medicina. Universidad de Chile, Santiago, Chile. carlos.henriquez@ug.uchile.cl

(138) ACTIVATION OF THE KININ B1 RECEPTOR INCREASES THE EXPRESSION AND RELEASE OF MATRIX METALLOPROTEASE-9 FROM HUMAN HaCaT KERATINOCYTES. **Matus CE,** Mejia AJ, Ehrenfeld P, Pavicic MF, Figueroa CD. Instituto de Anatomia, Histologia y Patologia, Universidad Austral de Chile. matus.carola@gmail.com

(139) MINIMAL DOMAIN OF WNT3A ABLE TO ACTIVATE WNT/b-CATENIN SIGNALING. **Burgos CF**[1,2], Peralta A[1], Martinez J[2] and De Ferrari GV[1,2]. [1]Center for Biomedical Research, Faculty of Biological Sciences and Faculty of Medicine, Universidad Andrés Bello, Santiago, and [2]Department of Biochemistry and Molecular Biology, Faculty of Biological Sciences, Universidad de Concepción, Chile. gdeferrari@unab.cl

(140) ROLE OF INTRACELLULAR CALCIUM CHANNELS RYR, IP3R AND NAD(P)H OXIDASE IN INSULIN-INDUCED GLUT4 TRANSLOCATION AND GLUCOSE UPTAKE IN SKELETAL MUSCLE CELLS. **Contreras-Ferrat A.**[1,2], Vasquez C.[1], Espinosa A.[1], Lavandero S.[1], Klip A.[2], Jaimovich E.[1]. [1]Centro de Estudios Moleculares de la Célula, Universidad de Chile, Santiago, Chile [2]Hospital for Sick Children, Research Institute, Toronto, ON, Canada. acontreras@med.uchile.cl

(141) TURNOVER OF AMYLOID PRECURSOR PROTEIN CARBOXY TERMINAL FRAGMENT BETA (C99) IN LYSOSOMAL COMPARTMENTS. **Andrés Rivera-Dictter**[1], Hianara Bustamante[1], Vanessa Muñoz[1], Viviana Cavieres[1], Juan S. Bonifacino[2], Gonzalo Mardones[1], and Patricia Burgos[1]. [1]Laboratorio de Biología Celular y Molecular, Instituto de Fisiología, Facultad de Medicina, Universidad Austral de Chile, Valdivia, and [2]Cell Biology and Metabolism Program, NICHD, National Institutes of Health, Bethesda, MD USA. burgospa@gmail.com

(142) FLUCTUATIONS IN BRAIN EXTRACELLULAR ASCORBIC ACID CONCENTRATION COULD DRIVE CHANGES IN THE AVAILABILITY OF SVCT2 AT PLASMA MEMBRANE. [1]**Magdalena Esparza**, [1]Aníbal I. Acuña, [1]Carlos Kramm, [2]Carlos Toro, [2]Sebastián Brauchi y [1]Maite A. Castro. [1]Instituto de Bioquímica y Microbiología, [2]Instituto de Fisiología, Universidad Austral de Chile. macastro@uach.cl

(143) GALECTIN-8 INDUCES EGFR ACTIVATION, ENDOCYTOSIS AND CELL PROLIFERATION IN HELA CELLS. **Remziye Döger,** Ronan Shaughnessy, Andrea Soza, Alfonso Gonzalez. Departamento de Inmunología Clínica y Reumatología, Fac. Medicina. Centro de Envejecimiento y Regeneración (CARE). Fac. Ciencias Biológicas. Pontificia Universidad Católica de Chile. remziye.doger@gmail.com

(144) TH1 AND TH17 PROFILES INDUCTION ARE ASSOCIATED WITH IMMUNOLOGICAL RESPONSES AND LONG-TERM PATIENT SURVIVAL ON MELANOMA PATIENTS TREATED WITH DENTRITIC CELLS BASED IMMUNOTHERAPY. Falcón-Beas C[1,2], Tempio F[1,2], Pesce B[1,2], Aguillón J[1,2], Pereda C[1,2], Saffie C[1,2], Salazar-Onfray F[1,2], **López M.N[1-3].** [1]Millennium

Nucleus on Immunology and Immunotherapy; [2]ICBM, Faculty of Medicine, University of Chile; [3]Research Support Office, University of Chile Clinical Hospital. melopez@med.uchile.cl

(145) DOPAMINE RECEPTOR D5 EXPRESSED ON IMMUNE CELLS PROMOTES CD4$^+$ T-CELL-MEDIATED AUTOIMMUNITY. **Carolina Prado[1,2]**, Francisco Contreras[1,2], Rodrigo Pacheco[1]. [1]Fundación Ciencia para la Vida and [2]Universidad Nacional Andrés Bello. Santiago, Chile. carolinapradot@gmail.com

(146) INHIBITION OF HANTAVIRUS ENTRY INTO THE CELL. **[1,2]Barriga., G.P.** and [1]Tischler, N.D. [1]Fundación Ciencia para la Vida and [2]Universidad Andrés Bello, Santiago, Chile. gonzalo.barriga@gmail.com

(147) AtPRP3 BUT NOT AtPRP1 IS ACTIVELY ENDOCYTOSED FROM THE CELL WALL AT THE ROOT HAIR GROWING TIP. **Rodriguez-Furlan, Cecilia I** (A), Orellana, Ariel (A), Tierney, Mary (B). (A):Plant Biotechnology Center, Universidad Andres Bello. (B):Plant Department, Vermont University. cec.rodriguez.f@uandresbello.edu

(148) ANTI-CD115 ANTIBODIES: TOOL FOR CHARACTERIZATION OF MACROPHAGES-LIKE CELLS OF RAINBOW TROUT. **Maisey K[1]**, Torres-Undurraga C[1], Wang T[2], Secombes CJ[2] and Imarai M[1]. [1]Laboratorio de Inmunología, Centro de Biotecnología Acuícola (CBA), Universidad de Santiago de Chile. [2]Scottish Fish Immunology Research Centre University of Aberdeen, UK. kmaisey@cba-usach.cl

(149) CALRETICULIN DETECTION IN SALIVA OF DOMESTIC DOGS (*Canis lupus familiaris*). **Coddou, F.,** Weinberger, K., Duaso, M.L., Valck, C., Ramírez, G., Maldonado, I., Ferreira, A. Disciplinary Immunology Program, Biomedical Sciences Institute, Faculty of Medicine, University of Chile. aferreir@med.uchile.cl

(150) INDUCIBLE REGULATORY T CELLS PRODUCE FACTORS THAT GENERATE REGULATORY T CELLS *in vitro*. **C. Fuentes[1]** C. Moore[2], M.R. Bono[1], M. Rosemblatt[1,2]. [1]Facultad de Ciencias, Universidad de Chile, [2]UNAB y Fundación Ciencias para la Vida. camifuentes@gmail.com

(151) PRODUCTION OF ANTI-CD3e ANTIBODIES: A TOOL FOR CHARACTERIZATION OF SALMONID LYMPHOCYTES. **Montero R**, Valenzuela B, Maisey K, Imarai B. Laboratorio de Inmunología, Centro de Biotecnología Acuícola. Facultad de Química y Biología, Universidad de Santiago de Chile. ruth.montero@usach.cl

(152) DEXAMETHASONE AND MONOPHOSPHORYL LIPID A STIMULATION GENERATES TOLEROGENIC DENDRITIC CELLS WITH AN *in vitro* STABLE IMMUNOREGULATORY PHENOTYPE IN HEALTHY VOLUNTEERS. **Lorena Hoyos,** Paulina García, Rodrigo Morales, Bárbara Pesce, Karina Pino-Lagos, Diego Catalán, Juan Carlos Aguillón. Programa Disciplinario de Inmunología, ICBM, Facultad de Medicina, Universidad de Chile. www.irtgroup.cl; lorehoyosp@gmail.com

(153) *Neisseria gonorrhoeae* EFFECTS ON THE MATURATION OF DENDRITIC CELLS. **Bélgica Villegas-Valdés**, Sebastián Reyes-Cerpa, Kevin Maisey, Alejandro Escobar, Mónica Imarai, Claudio Acuña-Castillo. Universidad de Santiago de Chile. claudio.acuna@usach.cl

(154) REPRIMO REDUCES TUMORIGENIC CHARACTERISTICS OF HUMAN GASTRIC CANCER CELL LINES. **Olivares W.[1]**, Torres V.[1], Leguina A.[1], Maturana MJ.[1], Montecinos V.[1], Aguayo F.[2], Corvalán AH.[1]. [1]Hematology-Oncology Department, Pontificia Universidad Católica de Chile. [2]Virology Department, Universidad Chile. wildapucv@gmail.com (Sponsor: A. Quest)

(155) CHEMOTHERAPEUTIC NUCLEOSIDE ANALOG TRANSPORTER HUMAN ENT1 (EQUILIBRATIVE NUCLEOSIDE TRANSPORTER 1) EXPRESSION AND ACTIVITY IS MODULATED BY PEROXISOME PROLIFERATOR ACTIVATED-RECEPTORS (PPARs) IN OVARIAN CANCER CELLS. **Trinidad Montero[1]**, Sumie Kato[2], Dusan Racordon[4], M[a] Loreto Bravo[4], Gareth Owen[4], Mauricio Cuello[2], Miguel Bronfman[3] and Andrea V Leisewitz[1]. [1]Hematology-

Oncology Department, [2] Division of Obstetrics and Gynecology, School of Medicine,[3] Cellular and Molecular Biology Department, [4]Physiology Department, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile, Santiago, Chile.

(156) DIFFERENTIAL EXPRESSION PATTERN OF STROMA CELL MARKERS AND VEGF-A IN PRIMARY CULTURED STROMA FROM BENIGN AND PROSTATE CANCER EXPLANTS. **Javier Cerda[1]**, Ignacio San Francisco[2], Alejandro Godoy[3], Viviana Montecinos[1]. [1]Hematology-Oncology and [2]Urology Departments, Pontificia U. Católica de Chile. [3]Department of Urology, Roswell Park Cancer Institute, Buffalo NY. jcerda.infante@gmail.com (Sponsor: MA García).

(157) ANALYSIS OF NKG2D LIGANDS EXPRESSION ON TUMOR CELLS FROM GASTRIC CANCER PATIENTS. **Kramm K.,** Ribeiro C. H., Bustamante M, Garrido Tapia M., Hernández C. J., Collazo N., Sotelo P., Zúñiga R. and Molina M.C. Laboratory of Immunosurveillance and Immune Evasion. Disciplinary Program of Immunology, Faculty of Medicine, Universidad de Chile. karinakv@ug.uchile.cl

(158) NOVEL ANDROGEN RECEPTOR PURE ANTAGONIST MDV3100 PREVENTS NUCLEAR RECEPTOR TRANSLOCATION AND INDUCE TUMOR REGRESSION IN MICE MODEL OF CASTRATE-RESISTANT PROSTATE CANCER. J. Guerrero[1], **F. Gomez[1]**, I.E. Alfaro[1], A.A. Protter[2], S. Bernales[1,2]. [1]Fundación Ciencia para la Vida, Santiago, Chile. [2]Medivation Inc., San Francisco, CA, USA. fcogomezm@gmail.com

(159) STATINS COUNTERACT THE LEPTIN INDUCED MIGRATION AND INVASIVENESS IN HUMAN EPITHELIAL OVARIAN CANCER CELLS BY INHIBITION OF RHOA SIGNALLING PATHWAY. **Díaz D[1]**., Kato S[1]., Cuello M[1]. [1]Division of Obstetrics and Gynecology, Pontificia Universidad Católica de Chile. diazsilva.dm@gmail.com

(160) THE ANTI-TUMORIGENIC ACTION OF 2-METHOXYESTRADIOL IS INHIBITED BY SULFONATION IN THE BREAST CANCER CELLS. **Vargas MF[1,3]**, Spink DC[2] y Owen GI[1,3]. [1]Facultad de Ciencias Biológicas, Universidad Católica de Chile, Chile; [2]Wadsworth Center, SUNY, USA. [3]Biomedical Research Consortium of Chile (BMRC).

(161) MOLECULAR ANALYSIS OF THE rDNA TRANSCRIPTIONAL REGULATION DURING THE SEASONAL ADAPTATION OF *Cyprinus carpio* FISH. **Fumeron R.,** Dupré G., Molina A., Vera MI., Alvarez M. Laboratorio de Biología Celular y Molecular, Facultad Ciencias Biológicas, Universidad Andrés Bello, Viña del Mar, Chile. malvarez@unab.cl

(162) TWO DIMENSIONAL ELECTROPHORESIS PATTERNS FROM BOTH FERTILE AND INFERTILE *Echinococcus granulosus* CYSTS. **Christian Hidalgo**, Rodolfo Paredes. Laboratorio Salud de Ecosistemas, Escuela de Medicina Veterinaria, Facultad de Ecología y Recursos Naturales, Universidad Andrés Bello. rparedes@unab.cl

(163) EXPRESSION AND ACTIVITY OF TcAP1, A REPAIR DNA ENDONUCLEASE, IN *Trypanosoma cruzi*. **Iván Ponce**, Sofia Sepúlveda, Lucía Valenzuela, José Delgadillo, Santiago Ramírez, Soledad Sierra, Paula Bahamondes, Natalia Muñoz, Ulrike Kemmerling, Gonzalo Cabrera, Norbel Galanti. Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile. ivponce@ciq.uchile.cl

(164) MULTIPONTECIALITY OF HUMAN YOLK SAC ENDODERMAL CELLS. **Sulz L,** Godoy C, Pereda J. Escuela de Medicina, Universidad de Santiago de Chile, Santiago, Chile. lorena.sulz@usach.cl, carlos.godoy@usach.cl, jaime.pereda@usach.cl (Sponsor: P. Orihuela)

(165) INSULIN STIMULATION OF HUMAN CATIONIC AMINO ACID TRANSPORTER 1-MEDIATED L-ARGININE TRANSPORT INVOLVES A_{2A} ADENOSINE RECEPTORS IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS. **Guzmán-Gutiérrez E**, Westermeier F, Salomón C, Leiva A, Casanello P, Sobrevia L. Cellular and Molecular Physiology Laboratory (CMPL) & Perinatology Research Laboratory (PRL), Division of Obstetrics and Gynecology, School of Medicine, Faculty of Medicine, Pontificia Universidad Católica de Chile, P.O. Box 114-D, Santiago, Chile. elguzman@uc.cl

(166) TONIC INHIBITION OF THE RhoA/ROCK PATHWAY IS NOT PART OF THE MECHANISMS UNDERLYING MYOMETRIAL QUIESCENCE DURING PREGNANCY. **Garmendia LR,** Delpiano AM, Poblete JA, Cuello MA, Carvajal JA. Laboratorio de Medicina Materno-Fetal. Departamento de Obstetricia y Ginecología, Facultad de Medicina, Pontificia. U. Católica de Chile. liliana.garmendia.c@gmail.com

(167) ASSESSMENT OF THE A357D ACID SPHINGOMYELINASE GENE MUTATION FREQUENCY IN THE CHILEAN POPULATION. **Pérez MJ[1]**, Robledo F[1,] Castro J[1], Martínez P[1], Acuña M[1], Schuchman E[2], Miquel JM[1], Mabe P[3], Zanlungo S[1]. [1]Gastroenterology Department, Medicine Faculty, Pontificia Universidad Católica de Chile, [2]Department of Genetics & Genomic Sciences, Mount Sinai School of Medicine, New York, [3]Neurology Unit, Dr. Exequiel González Cortés Children's Hospital, Santiago de Chile. mjperez@uc.cl

(168) PHYSICAL AND FUNCTIONAL ASSOCIATION OF GLYCOPROTEIN VI (GPVI) AND TISSUE FACTOR (TF) IN HUMAN PLATELETS. **Valenzuela JG**, Panes O, Román P, Pereira J, Mezzano D, Matus V. Department of Hematology-Oncology, School of Medicine, P. Catholic University of Chile. (Sponsor: V. Velarde)

(169) INTEGRIN EXPRESSION DURING THE MENSTRUAL CYCLE IN THE FALLOPIAN TUBE. **Soto V.**[1], Solar P. [1,2], Cárdenas H.[1.2], Velásquez LA.[1,2]. [1]Laboratorio de Inmunología de la Reproducción, USACH. [2]Centro para el Desarrollo de la Nanociencia y la Nanotecnología (CEDENNA). (Sponsor: R. Moreno).

(170) SURFACE ADAM17 IS RELATED TO XENOESTROGENS–INDUCE GERM CELLS APOPTOSIS. **Paulina Urriola-Muñoz**, Raúl Lagos-Cabré, Magdalena Díaz and Ricardo D Moreno. Pontificia Universidad Católica de Chile, Facultad de Ciencias Biológicas, Departamento de Fisiología. paurriol@uc.cl

THURSDAY, NOVEMBER 3rd, 2011

08:00 **Poster Mounting Session III: N° 171 to N° 255**
Convention Center Foyer

09:00 – 10:30 **SYMPOSIUM CENTER FOR AGING AND REGENERATION**
P. UNIVERSIDAD CATOLICA DE CHILE
Calbuco Room - Chair: Nibaldo C. Inestrosa

"**TRAFFIC OF SYNAPTIC RECEPTORS"**

REGULATION OF NEURONAL DENDRITOGENESIS BY NMDA RECEPTORS AND THEIR UNDERLYING SCAFFOLDING AND SIGNALLING PROTEINS. **van Zundert, B**. Centro de Investigaciones Biomédicas (CIB), Fac. Ciencias Biológicas y Fac. Medicina, Universidad Andrés Bello, Santiago. bvanzundert@unab.cl

THE ROLE OF TROPHIC FACTORS AND TRAFFICKING MOLECULES IN ANTIDEPRESSANT ACTION. **Ursula Wyneken.** Laboratorio de Neurociencias, Universidad de los Andes, uwyneken@uandes.cl

CaMKII TRIGGERS THE DIFFUSIONAL TRAPPING OF SURFACE AMPARs THROUGH PHOSPHORYLATION OF STARGAZIN. **Patricio Opazo** and Daniel Choquet. Institut Interdisciplinaire de NeuroScience IINS, Bordeaux, France.

REGULATION OF KAR PLASTICITY AND TRAFFICKING BY CaMKII AND INTERACTION WITH PSD95. Mario Carta, Patrizio Opazo, Julien Veran, Daniel Choquet, Christophe Mulle and **Françoise Coussen.** Institut Interdisciplinaire de Neuroscience, UMR 5297 CNRS-Université de Bordeaux.

SYMPOSIUM CENTRO DE ESTUDIOS MOLECULARES DE LA CELULA (CEMC)
UNIVERSIDAD DE CHILE
Tronador Room - Chair: Andrew Quest

"**FOCUS ON GASTRIC CANCER"**

TRANSCENDING CLASSIC PARADIGMS IN CANCER – E-CADHERIN IS A MOLECULAR SWITCH THAT DEFINES CAVEOLIN-1 FUNCTION *IN VIVO*. **Andrew F. G. Quest.** Laboratorio de Comunicaciones Celulares, Centro de Estudios Moleculares de la Célula (CEMC), Facultad de Medicina, Universidad de Chile. aquest@med.uchile.cl

Stat3 PROVIDES A DRUGABLE SIGNALING NODE LINKING INFLAMMATION TO GASTROINTESTINAL CANCER. **Matthias ERNST.** Ludwig Institute for Cancer Research, Melbourne, VIC 3055, Australia**.**

REPRIMO, A NOVEL BIOMARKER AND POTENTIAL TUMOR SUPPRESSOR GENE IN GASTRIC CANCER. **Alejandro Corvalán**, Dept. Hematology-Oncology, P. Universidad Católica de Chile.

10:30 – 11:30 **Coffee Break – Exhibitors – Poster Viewing Session III**
Convention Center Foyer

11:30 – 13:30 **Oral Presentations IV**
Volcanes Room - Chair: Miguel Allende and Co-Chair: Ricardo Moreno

NEURONAL ENDOPLASMIC RETICULUM (ER) IMAGING WITH SUPER-RESOLUTION OPTICAL FLUCTUATION IMAGING (SOFI). **Omar Ramírez[1],** Felipe Santibáñez[1], Dirk Haehnel[3], Andrés Couve[2], Jörg Enderlein[3] & Steffen Härtel[1]. [1]SCIAN-Lab, [2]Laboratory of Cellular and Molecular

Neurobiology, BNI, ICBM, Facultad de Medicina, U-Chile. [3]III. Physikalisches Institut, Universität Göttingen, Germany. oramirez@med.uchile.cl

SYNERGISTIC EFFECT OF PLASMID pcDNA-SURV IN CONJUNCTION WITH THE PARASITIC CALRETICULIN ON B16F10 TUMOR MODEL *IN VIVO*. **Aguilar L.**[1,2], Lobos L.[2], Quest A.F.G.[2], Ferreira A.[1]. [1]Laboratorio de Inmunología de la Agresión Microbiana, [2]Laboratorio de Comunicaciones Celulares, ICBM, Universidad de Chile. aferreir@med.uchile.cl

ADAM17, AS A NEW CANDIDATE IN THE MECHANISM OF XENOESTROGENS-INDUCED APOPTOSIS IN TESTICULAR GERM CELLS. **Raúl Lagos-Cabré**, Paulina Urriola-Muñoz, Pablo Sáez, Juan C. Sáez and Ricardo D. Moreno. Pontificia Universidad Católica de Chile, Facultad de Ciencias Biológicas, Departamento de Fisiología. rclagos@uc.cl

THE ROLE OF MATRIX METALLOPROTEINASE 9 (MMP9) AND PI3K/AKT SIGNALING IN INFAMMATORY RESPONSE IN ZEBRAFISH LARVAE. **Oscar Peña,** Mario Sánchez, Nicole Reynaert, José Moya, Miguel Allende. FONDAP Center for Genome Regulation, Facultad de Ciencias, Universidad de Chile. oscarpena@ug.uchile.cl; allende@uchile.cl

THE DENDRITIC ENDOPLASMIC RETICULUM AND CONVENTIONAL KINESIN DEFINE A NON-CANONICAL TRAFFICKING MODALITY FOR $GABA_B$ RECEPTORS. **José Ignacio Valenzuela**[1,3], Matías Jaureguiberry[1,3,4], Omar Ramírez[2,3], Thomas Blanpied[1], and Andrés Couve[1,3]. [1]Physiology and Biophysics, [2]Anatomy and Development, ICBM and [3]Biomedical Neuroscience Institute (BNI), Facultad de Medicina, Universidad de Chile, Santiago, Chile. [4]School of Biochemistry, Faculty of Biological Science, Universidad Andrés Bello, Santiago, Chile. [5]Department of Physiology, University of Maryland School of Medicine, Baltimore, MD. joseignacio.v@gmail.com

UBIQUITIN-DEPENDENT PROTEASOMAL DEGRADATION AND CLEAVAGE BY γ-SECRETASE COMPETE FOR THE AMYLOID PRECURSOR PROTEIN CARBOXY TERMINAL FRAGMENT BETA (C99). **Hianara Bustamante**, Andrés Rivera-Dictter, Viviana Cavieres, Vanessa Muñoz, Gonzalo Mardones, and Patricia Burgos. Laboratorio de Biología Celular y Molecular, Instituto de Fisiología, Facultad de Medicina, Universidad Austral de Chile, Valdivia. burgospa@gmail.com

***In vivo* AND *in vitro* FUNCTIONAL CHARACTERIZATION OF RhoGEF3, A NEW GUANINE NUCLEOTIDE EXCHANGE FACTOR (GEF) OF *Drosophila melanogaster*.** **Alejandro Zúñiga**, Leandro Farías and Verónica Cambiazo. Laboratorio de Bioinformática y Expresión Génica, INTA-Universidad de Chile & Center for Genome Regulation (CRG). jano@inta.uchile.cl

AUXIN-INDEPENDENT LATERAL DEVELOPMENT INDUCED BY A CELLULAR TRAFFICKING DISRUPTING-DRUG. **Perez P[1,2],** Norambuena L[1,2]. [1]Plant Molecular Biology Laboratory, Faculty of Science, University of Chile. [2]Plant Cell Biotechnology Millennium Nucleus. lnorambuena@uchile.cl

13:30 – 15:30 Lunch

15:30 – 17:30 Oral Presentations V
Volcanes Room - Chair: Enrique Brandan and Co-Chair: Francisco Nualart

ANGIOTENSIN-(1-7) REDUCES FIBROSIS AND IMPROVES FUNCTION IN DYSTROPHIC SKELETAL MUSCLE. **Acuña MJ**[1], Vío CP[2], Cabello-Verrugio C[1,3] and Brandan E[1]. [1]Laboratorio de Diferenciación Celular y Patología; [2]Laboratorio de Fisiología, CARE, P. Universidad Católica de Chile. [3]Centrode Genética Humana, Clínica Alemana-Universidad del Desarrollo. ebrandan@bio.puc.cl

DEHYDROASCORBIC ACID MODULATES GLYCOLITIC, PENTOSE-PHOSPHATE PATHWAY AND GLUTATHION ACTIVITY IN ASTROCYTES. **Pedro Cisternas,** Carmen Silva-Alvarez, Karina Oyarce, Paula Llanos and Francisco Nualart. Department of Cell Biology, University of Concepcion. pecister@udec.cl

REGULATION OF DENDRITIC BRANCHING BY BDNF-INDUCED INCREASE OF RAB11 ACTIVITY IN HIPPOCAMPAL NEURONS. **Oscar M. Lazo**[1], Maria Ascano[2], Rejji Kuruvilla[2], Andrés Couve[3] and Francisca C. Bronfman[1]. [1]Millennium Nucleus in Regenerative Biology (MINREB), Facultad de Ciencias Biológicas. Pontificia Universidad Católica de Chile. [2]Department of Biology. Johns Hopkins University. USA. [3]ICBM, Universidad de Chile. omlazo@uc.cl

MCT1 KNOCKDOWN IN HYPOTHALAMIC GLIAL CELLS AND THEIR EFFECT IN THE EXPRESSION OF NEUROPEPTIDES THAT CONTROL FOOD INTAKE. **Cortés-Campos C**, Elizondo R, Nualart F, García MA. Departamento de Biología Celular, Facultad de Ciencias Biológicas, Universidad de Concepción. mgarcia@udec.cl

INHERENT GROWTH HORMONE RESISTANCE IN FISH SKELETAL MUSCLE IS MODULATED BY THE NUTRITIONAL STATUS AND IS CHARACTERIZED BY HIGH CONTENTS OF TRUNCATED GHR, IMPAIRMENT IN THE JAK2/STAT5 SIGNALING PATHWAY AND LOW IGF-I EXPRESSION. **Eduardo N. Fuentes**[a], Ingibjörg Eir Einarsdottir[b], Juan Antonio Valdes[a], Marco Alvarez[c], Alfredo Molina[a], Björn Thrandur Björnsson[b]. [a]Laboratorio de Biotecnología Molecular, Universidad Andrés Bello, Chile, [b]Fish Endocrinology Laboratory, University of Gothenburg, Sweden. edua.fuentes@gmail.com

ROLE OF PK17E AND RECS1 IN THE REDISTRIBUTION OF CADHERIN DURING *Drosophila* WING EPITHELIAL REMODELING. **Alvaro Glavic**. Center for Genome Regulation, Facultad de Ciencias, Universidad de Chile.

DENDRITIC AND STROMAL CELLS FROM THE SPLEEN OF LUPIC MICE PRESENT PHENOTYPIC AND FUNCTIONAL ABNORMALITIES. **A. Gleisner**[1], P.A. Reyes[2,3], M. Rosemblatt[1,2,3], M.R. Bono[1]. [1]Facultad de Ciencias, Universidad de Chile, [2]Universidad Andrés Bello, [3]Fundación Ciencia para la Vida. alejandra.gleisner@gmail.com

SCHWANN CELL TO AXON TRANSFER OF EXOSOMES PROMOTES AXONAL GROWTH AND REGENERATION. **M. Alejandra Lopez-Verrilli**, Felipe A. Court. Millennium Nucleus in Regenerative Biology (MINREB), Catholic University of Chile and Neurounion Biomedical Foundation. alejanddra@gmail.com, fcourt@bio.puc.cl

17:30 – 18:30 **Coffee Break – Exhibitors – Poster Viewing Session III**
Convention Center Foyer

18:30 – 19:30 **PLENARY LECTURE COOPERACION INTERNACIONAL DE FONDECYT (1100557)**
Calbuco Room - Chair: Maria Rosa Bono

REGULATION OF PLASMA CELL DIFFERENTIATION AND SURVIVAL IN AUTOIMMUNITY. **Loren D. Erickson**, Ph.D. Department of Microbiology, University of Virginia, Charlottesville, Virginia, USA.

PLENARY LECTURE NUCLEO MILENIO EN BIOLOGIA REGENERATIVA (MINREB)
Tronador Room - Chair: Alejandro Erices

HUNTINGTIN FROM EVOLUTION TO STEM CELL BIOLOGY AND TRANSPLANTATION. **Elena Cattaneo**, Coordinator NeuroStemcell (www.neurostemcell.org). Department of Pharmacological Sciences and Centre for Stem Cell Research (UniStem), University of Milano, Italy. Laboratory web site: www.cattaneolab.it - UniStem web site: www.unistem.it

19:30 – 21:00 **Society Members Meeting**
Lobby Room

20:30 **Dinner**

22:00 – 23:30 Poster Presentations Session III: N° 171 to N° 255
Convention Center Foyer
Coordinators: Marcela Hermoso, Mónica Imarai, Rodolfo Paredes

(171) RUNX2 REGULATES MMP EXPRESSION AND MODULATES CELL MIGRATION AND INVASION IN OSTEOSARCOMA CELLS. Karina Villegas[1,2], Oscar Vega[1,2], Marcela Hernández[3], Jorge Gamonal[3], Gary S Stein[4], Andre van Wijnen[4] and Mario Galindo[1,2]. [1]Millennium Institute on Immunology and Immunotherapy, [2]Programa de Biología Celular y Molecular, ICBM, Facultad de Medicina, Universidad de Chile. [3]Laboratorio de Biología Periodontal, Facultad de Odontología, Universidad de Chile. [4]Departament of Cell Biology and Cancer Center, University of Massachusetts Medical School, Worcester, Massachusetts. mgalindo@med.uchile.cl

(172) DIFFERENTIAL EXPRESSION OF GENES RELATED TO AUTOPHAGY AND METASTASES IN ADVANCED OVARIAN CANCERS. [1,5]**Racordon D**, [1,5]Erices R, [1,5]Bravo M, [1,5]Gonzalez P, [3]Bustamante E, [4]Pizarro J, [2,5]Kato S, [2,5]Cuello M.A, [1,5]Owen G. I. [1]Faculty of Biological Sciences, [2]Faculty of Medicine, Pontificia Universidad Católica de Chile. [3]FALP, [4]Hospital Sotero del Rio, [5]Biomedical Research Consortium of Chile.

(173) EFFECT OF IGF2 OVEREXPRESSION ON TUMORIGENICITY OF HUMAN OVARIAN CARCINOMA CELLS. **Jurriaan Brouwer-Visser**, Suzan K. Chao, Ricardo D. Moreno and Gloria S. Huang. Albert Einstein College of Medicine, Bronx, New York and Pontificia Universidad Católica de Chile. jurriaan.brouwer@einstein.yu.edu

(174) ONCOGENIC KINASE AURORA-A DECREASES STABILITY OF THE TRANSCRIPTIONAL CO-REPRESSOR SKI. **Solange Rivas[1],** Jocelyn Mosquera[1], Leandro Torres[1,4], Ricardo Armisén[2,4], Julio C. Tapia[3,4], Michael J Hayman[5], Katherine Marcelain[1,4]. [1]Programa de Genética Humana, [2]Fisiopatología, [3]Biología Celular y Molecular, ICBM, Facultad de Medicina, Universidad de Chile. [4]Centro de Estudios Moleculares de la Célula, Facultad de Medicina, Universidad de Chile. [5]Microbiology and Molecular Genetics Department, Stony Brook University, USA. kmarcelain@med.uchile.cl (Sponsor: J.C. Tapia)

(175) PHYSICOCHEMICAL AND CYTOTOXICOLOGY CHARACTERIZATION OF POLYFUNCTIONAL POLYMERIC NANOPARTICLES DESIGNED FOR CANCER TREATMENT AND DIAGNOSIS. **Solar P**.[1,2], Vilos C.[1,2], Juica N.[1], Moreno M.[2], González G.[2], Cárdenas H.[1,2], Velásquez LA.[1,2]. [1]Laboratorio de Inmunología de la Reproducción, USACH. [2]Centro para el Desarrollo de la Nanociencia y Nanotecnología (CEDENNA)**.** paula.solar@usach.cl (Sponsor: R. Moreno).

(176) A LENTIVIRUS ENCODING RNAI TARGETING A NON-CODING MITOCHONDRIAL RNA INHIBITS MELANOMA TUMOR GROWTH AND METASTASIS IN A MOUSE MODEL *in vivo*. **Manuel Varas**[1,2,3], Alvaro Lladser[1], Nicole Farfan[1], Pablo D.T. Valenzuela[1,2,3], Luis O. Burzio[1,2,3]. [1]Fundación Ciencia para la Vida; [2]Andes Biotechnologies S.A; [3]Facultad de Ciencias Biológicas, Universidad Andrés Bello. manu.varas@uandresbello.edu

(177) RAB5 ACTIVATION IS REQUIRED FOR CAVEOLIN-1 ENHANCED TUMOR CELL MIGRATION. **Jorge Díaz[1, 2],** Lisette Leyton[1], Andrew F.G. Quest[1], Vicente Torres[1,2]**.** [1]Centro de Estudios Moleculares de la Célula, Facultad de Medicina, Universidad de Chile. [2]Laboratorio de Biología Celular y Molecular, Departamento de Ciencias Básicas y Comunitarias, Facultad de Odontología, Universidad de Chile. aquest@med.uchile.cl, vatorres@med.uchile.cl

(178) NEURAL TENEURINS ARE EXPRESSED IN HUMAN TUMORS AND TUMOR-DERIVED CELL LINES. **[1]Ziegler A.,** [1]di Capua G., [1]Oyarzún J.E., [2]Roa I.E., [3]Brañes J.A., [3]Casanello P., [1]Repetto G. [1]Center for Human Genetics, Faculty of Medicine, Clínica Alemana-Universidad del Desarrollo; [2]Division of Pathology, Clínica Alemana de Santiago; Perinatology [3]Research Laboratory (PRL) & Cellular and Molecular Physiology Laboratory (CMPL), Division of Obstetrics and Gynaecology, School of Medicine, Pontificia Universidad Católica de Chile. aziegler@udd.cl (Sponsor: C. Cabello)

(179) INHIBITION OF CAVEOLIN-1 PHOSPHORYLATION ON TYROSINE-14 IS ASSOCIATED WITH REDUCED MIGRATION, INVASION AND METASTASIS OF B16F10 MELANOMA CELLS. **Ortiz R.J*,** Urra H*, Lobos, L., Leyton L*, Quest AFG*. *Laboratorio de Comunicaciones Celulares, Centro de Estudios Moleculares de la Célula (CEMC), Facultad de Medicina, Universidad de Chile. aquest@med.uchile.cl

(180) RUNX2 EXPRESSION MODULATES ADHESION OF OSTEOSARCOMA CELLS TO HUMAN PULMONARY ENDOTHELIAL CELLS AND POSITIVELY CORRELATES WITH METASTATIC POTENTIAL. **Francisco Villanueva[1,2],** Óscar Vega[1,2], Mercedes Lopez[1,3], Flavio Salazar-Onfray[1,3], Gary Stein[4], Andre van Wijnen[4], Mario Galindo[1,3]. [1]Millennium Institute on Immunology and Immunotherapy, [2]Programa de Biología Celular y Molecular, [3]Programa Disciplinario de Inmunología, ICBM, Facultad de Medicina, Universidad de Chile. [4]Department of Cell Biology and Cancer Center, University of Massachusetts Medical School, USA. mgalindo@med.uchile.cl

(181) REGULATION OF ENDOTHELIN CONVERTING ENZYME-1 (ECE-1) EXPRESSION BY THE CYCLOOXYGENASE-2/PROSTAGLANDIN E2/β-CATENIN AXIS IN COLON CANCER CELLS. **Eduardo Silva[1]**, Pablo Cabello[1], Luis R. Cataldo[1], Roger Yefi[1], Ignacio Niechi[1], Daniela P. Ponce[1], Ricardo Armisen[2], Cristina Fernandez[3] & Julio C. Tapia[1,2]. [1]Cell Transformation Laboratory, [2]Institute of Biomedical Sciences (ICBM), Faculty of Medicine, and [3]Department of Anatomopathology, HCUCH, University of Chile. jtapia@med.uchile.cl

(182) REGULATORY T CELLS FROM PATIENTS WITH GASTRIC CANCER PRODUCE IL-10, A CYTOKINE THAT MODULATES NK CELL FUNCTION. **Ribeiro CH**, Kramm K, Bustamante M, Garrido-Tapia M, Hernández C, and Molina MC. Laboratorio de Inmunovigilancia y Evasión Inmune, Programa Disciplinario de Inmunología, Facultad de Medicina, Universidad de Chile. chager@med.uchile.cl

(183) STUDY OF THE MIGRATORY CAPABILITY OF TUMOR ANTIGEN PRESENTING CELLS (TAPCELLS) UNDER DIFFERENTIAL EXPRESSION OF CCR7. **Ortiz Carolina**, González F, Mora Gabriela, Salazar-Onfray F. Programa Disciplinario de Inmunología, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile.

(184) THE ANTI-INFLAMMATORY SOLUBLE ST2 PROTEIN IS INDUCED BY GLUCOCORTICOIDS IN ULCERATIVE COLITIS. Lucía Núñez-Aguilera, **David Díaz-Jiménez**, Marjorie de la Fuente, Karen Dubois, Francisco López-Kostner, Rodrigo Quera, Marcela Hermoso. Innate Inmunity Lab, Immunology Program, ICBM, Universidad de Chile. lenunez@med.uchile.cl

(185) *Helicobacter pylori* INCREASES MICA AND ULBP-2 EXPRESSION ON GASTRIC ADENOCARCINOMA CELLS. **Hernández CJ**, Garrido-Tapia M, Kramm K, Ribeiro CH, Molina MC. Laboratorio de Evasión Inmune. Programa Disciplinario de Inmunología (ICBM), Facultad de Medicina, Universidad de Chile. carojimena@gmail.com

(186) NPC2 PROTEIN INCREASES CHOLESTEROL CRYSTALLIZATION IN MODEL BILE. **González L**, Castro J, Amigo L, Miquel JF, Zanlungo S. Departamento de Gastroenterología, Facultad de Medicina, Pontificia Universidad Católica de Chile. ldgonzal@uc.cl

(187) ACTIVATING THE b–CATENIN PATHWAY MODULATES THE PHENOTYPE OF DENDRITIC CELLS. **César Oyarce[1]**, Andrés Herrada[1], Andrew F.G. Quest[2] and Alvaro Lladser[1]. [1]Laboratory of Gene Immunotherapy, Fundación Ciencia para la Vida, Santiago, Chile. [2]Laboratory of Cellular Communication, Center for Molecular Studies of the Cell (CEMC), Facultad de Medicina, Universidad de Chile, Santiago, Chile. alvaro.lladser@bionova.cl

(188) *Pseudomonas aeruginosa* INDUCES DIAPAUSE FORMATION IN *Caenorhabditis elegans*: A LINK BETWEEN ANTIBACTERIAL IMMUNITY AND RNAi. Chavez F[1], Pollak B[2], Ortiz J[1] and **Calixto A**[2]. [1]Department of Biology, Faculty of Sciences, University of Chile. [2]Center of Aging and Regeneration (CARE), P. Catholic University of Chile. acalixto@bio.puc.cl

(189) GENE EXPRESSION PROFILE IN MELANOMA PATIENTS TREATED WITH IMMUNOTHERAPY BASED IN DENDRITIC CELLS. **Garcia T**, Tittarelli A, Villablanca A, Pereda C, Matthäus F, López M, Hoheisel J, Gebicke-Haerter, Salazar F. Tumor Immunology Laboratory, Faculty of Medicine, University of Chile. tgarcia@med.uchile.cl

(190) ISA VIRUS INFECTION ALTERS THE CELLULAR REDOX BALANCE IN PRIMARY SALMON KIDNEY CELLS. **Víctor Olavarría** and Alejandro Yáñez**.** Laboratorio de Metabolismo y Biotecnología, Instituto de Bioquímica y microbiología, Universidad Austral de Chile. Campus Isla Teja, Valdivia, Chile. volavarria@gmail.com

(191) IDENTIFICATION OF CD8+ CELLS IN LYMPHOID ORGANS OF SALMONIDS. **Valenzuela B**., Rodríguez F.E., Maisey K., Imarai M. Laboratorio de Inmunología, Centro de Biotecnología Acuícola. Facultad de Química y Biología, Universidad de Santiago de Chile. beatriz.valenzuelam@usach.cl

(192) PRODUCTION OF VLPs OF INFECTIOUS PANCREATIC NECROSIS VIRUS (IPNV) BY USING BACULOVIRUS SYSTEM. René A. Manríquez, Melina V. Villalba, Freddy A. Calderón, **Juan G. Cárcamo**. Laboratorio de Bioquímica Farmacológica, Instituto de Bioquímica y Microbiología, Universidad Austral de Chile. gcarcamo@uach.cl

(193) REGULATION OF CYTOKINE EXPRESSION IN SALMONIDS SUBJECTED TO OXIDATIVE STRESS BY OVERCROWDING. **Rodríguez FE**., Cappelli, C., Valenzuela B., Imarai M. Laboratorio de Inmunología, Centro de Biotecnología Acuícola. Facultad de Química y Biología, Universidad de Santiago de Chile. felipe.rodriguezti@usach.cl

(194) GENOME-WIDE SURVEY OF GENE EXPRESSION RESPONSE TO *Piscirickettsia salmonis* IN RESISTANT AND SUSCEPTIBLE FAMILIES OF *Salmo salar*. **Rodrigo Pulgar,** Christian Hodar, Verónica Cambiazo. Laboratorio de Bioinformática y Expresión Génica, INTA, Universidad de Chile and Center for Genome Regulation (CRG). rpulgar@gmail.com

(195) IN SEARCH OF MARKERS FOR THE IDENTIFICATION OF LYMPHOID CELLS IN ZEBRAFISH. **Rubio S.,** Wittamer V., Bertrand J., Menares E., Allende M., Traver D., Rosemblatt M. Fundación Ciencia para la Vida, Universidad Andrés Bello; Facultad de Ciencias, Universidad de Chile; Santiago Chile; University of California, San Diego, USA. solangerubio@gmail.com

(196) GALECTIN-8 ENHANCES B CELL ANTIGEN PRESENTATION. Yuseff MI.[3], Soza A.[1,2] **Pardo E**.[1,2], Lennon-Duménil A.M.[3] and González A.[1,2]. Centro de Envejecimiento y Regeneración (CARE), Fac. Ciencias Biológicas[1]. Departamento de Inmunología Clínica y Reumatología, Fac. Medicina[2]. Pontificia Universidad Católica de Chile. Institut Curie, Paris, France[3].

(197) A KININ B1 RECEPTOR AGONIST INDUCES THE EXPRESSION AND RELEASE OF IL-4 FROM HUMAN HACAT KERATINOCYTES. **Mejia AJ,** Matus CE, Pavicic F, Ehrenfeld P, Figueroa CD. Instituto de Anatomía, Histología y Patología. Facultad de Medicina. Universidad Austral de Chile. ajmejiam@gmail.com

(198) RhoGEF3, A NEW GUANINE EXCHANGE FACTOR OF *Drosophila melanogaster*, IS INVOLVED IN TRACHEAL SYSTEM DEVELOPMENT. **Leandro Farías,** Alejandro Zúñiga and Verónica Cambiazo. Laboratorio de Bioinformática y Expresión Génica, INTA-Universidad de Chile and Center for Genome Regulation (CRG). leafariasag@gmail.com

(199) PARTICIPATION OF dp115 IN CELL PROLIFERATION IN *Drosophila melanogaster*. **Consuelo Ibar[1]** and Álvaro Glavic[1]. [1]Laboratorio de Biología del Desarrollo, Faculty of Science, University of Chile. con.ibar@gmail.com

(200) TISSUE DYNAMICS AND TENSILE PROPERTIES UNDERLYING THE FORMATION OF CELLULAR ROSETTES IN AN *in vivo* MODEL OF EPITHELIAL ORGANOGENESIS IN ZEBRAFISH. Eduardo Pulgar[1,2,3], Felipe Santibañez[2,3], **Ricardo Figueroa**[1,3], Justin Steinfeld[1], Luis Briones[2,3], Steffen Hartel[l2,3], Miguel Concha[1,3]. [1]Laboratory of Experimental Ontogeny – LEO and

[2]SCIAN-Lab, ICBM, Faculty of Medicine, University of Chile; Biomedical Neuroscience Institute, Santiago, Chile. eduardopulgar.bioq@gmail.com; mconcha@med.uchile.cl

(201) PERSISTENT EXPOSURE OF ZEBRAFISH LARVA TO OXYTETRCYCLINE INDUCES CHRONIC INFLAMMATION AND DECREASES THE CELL REGENERATION CAPACITY. **Barros-Becker F[1].,** Pulgar A[1]., Romero J[2]., Feijóo CG[1]. 1Departamento de Ciencias Biológicas, Universidad Andrés Bello. 2INTA, Universidad de Chile. fbarros86@gmail.com

(202) ROLE OF THE CHEMOKINE SDF1A IN COLLECTIVE CELL MIGRATION AND AXON PATHFINDING IN ZEBRAFISH. **Mardones, Camila[1]**; Valdivia, Leonardo[1]; Young, Rodrigo[2]; Wilson, Stephen[2]; Allende, Miguel[1]. [1]FONDAP Center for Genome Regulation, Universidad de Chile. [2]University College London, UK. camilamardonesk@gmail.com

(203) *Prokineticin2* DISRUPTION IN ZEBRAFISH RESULT IN KALLMANN SYNDROME RELATED ANOMALIES. **Joaquín Letelier**[1,2] & Kathleen Whitlock[1,2]. [1]Centro Interdisciplinario de Neurociencias Valparaíso, [2]Center for Genomics of the Cell, Facultad de Ciencias, Universidad de Valparaíso. joaquin.letelier@cinv.cl

(204) MACROH2A2 AFFECTS CELL PROLIFERATION IN RETINAL PROGENITORS. **Guajardo L**. and Reyes A.E. Laboratorio de Biología del Desarrollo, Facultad de Ciencias Biológicas, Universidad Andrés Bello, Av. República 217, piso3. Santiago, Chile. arielreyes@unab.cl

(205) *Enterctopus megalocyathus* EYE LENS STRUCTURE: AN EVIDENCE OF GROWTH. **María José Villegas**, Erick Baqueiro and Rodolfo Paredes. Escuela de Medicina Veterinaria, Facultad de Ecología y Recursos Naturales, Universidad Andrés Bello. rparedes@unab.cl

(206) Akt Inh-IV INDUCES UPR INDEPENDENT OF Akt CLASSICAL PHOPHORYLATIONS. **Jennifer Alfaro[1]**; Daniela Perez[1], Carolina Urrutia[1], Matías Blaustein[2], Alejandro Colman-Lerner[2] and Sebastian Bernales[1]. [1]Fundación Ciencia para la Vida. [2]IFIByNE, Universidad de Buenos Aires-CONICET. jennifer.alfaro.formas@gmail.com

(207) FUNCTIONAL ANALYSIS FROM A PROTEOMIC AND YEAST TWO-HYBRID SCREENING FOR NEW IRE1ą INTERACTINĢ PROTEINS. **Diego Rodriguez,** Hery Urra, Daniel Henriquez, Tomas Vaisar, Christian Gonzalez-Billault, Laurie Glimcher and Claudio Hetz. [1]Biomedical Neuroscience Institute, Faculty of Medicine, [2]Center for Molecular Studies of the Cell, Institute of Biomedical Sciences, University of Chile, Santiago, Chile. chetz@med.uchile.cl

(208) SODIUM-DEPENDENT VITAMIN C TRANSPORTER 2 (SVCT2) AVAILABILITY AT PLASMA MEMBRANE IS IMPAIRED IN HUNTINGTON'S DISEASE MODELS. **[1]Carlos Kramm**, [1]Aníbal I. Acuña, [1]Magdalena Esparza, [2]Carlos Toro, [1]Ilona I. Concha, [2]Sebastián Brauchi y [1]Maite A. Castro. [1]I. Bioquímica y Microbiología, [2]I. Fisiología, UACh. macastro@uach.cl

(209) A ROLE OF MUTANT PROTEIN DISULFIDE ISOMERASES IN AMYOTHROPIC LATERAL SCLEROSIS PATHOGENESIS. **Woehlbier U**.[1,2,3], Gonzalez-Perez P.[4], Irrazábal T.[1,2,3], Colombo A.[1], Diaz A.[1] Sagredo A.[2], Armisen R.[2], Concha M.[2], Brown R.H.[4], Hetz C.[1,2,3]. [1]Biomedical Neuroscience Institute, Faculty of Medicine, University of Chile, Santiago, Chile, [2]Center for Molecular Studies of the Cell, Institute of Biomedical Sciences, University of Chile, Santiago, Chile, [3]Neurounion Biomedical Foundation, Santiago, Chile, [4]University of Massachusetts, Worchester, USA.

(210) UP-REGULATION OF NEUROTROPHIC FACTORS AND RELATED MOLECULES IN THE CONTRALATERAL MOTOR CORTEX AFTER UNILATERAL FOCAL STROKE. **Claudia Pissani[1,2]***, Katherine Stack[1,2]*, Macarena Vargas[1], Gareth I. Owen[1], Francisca C Bronfman[1,2]. [1]Physiology Department, Pontificia Universidad Católica de Chile. [2]Millennium Nucleus in Regenerative Biology (MINREB). *Both contributed equally. chpissan@uc.cl , stacklkatherine@gmail.com

(211) DOPAMINE RECEPTOR D3 FACILITATES INFILTRATION OF CD4+ T-CELLS IN THE CENTRAL NERVOUS SYSTEM AND NEURODEGENERATION OF DOPAMINERGIC NEURONS IN A MOUSE MODEL OF PARKINSON'S DISEASE. **Hugo Gonzalez[1,2]**, Carolina

Prado[1,2], Francisco Contreras[1,2],Rodrigo Pacheco[1]. [1]Fundación Ciencia para la Vida and [2]Universidad Nacional Andrés Bello. Santiago, Chile. h.gonzalez.v10@gmail.com

(212) IN THE SEARCH OF NOVEL FUNCTIONS FOR CDK5. Erick Contreras-Vallejos[1], **Cristina Olmos[1]**, Alex di Genova[2], Alejandro Maass[2], Elías Utreras[1,3], Ashok Kulkarni[3] and Christian Gonzalez-Billault[1].[1]Laboratory of Cell and Neuronal Dynamics, Department of Biology and ICDB, Faculty of Sciences, and [2]Mathematical Modeling Center (CMM), Faculty of Physical and Mathematical Sciences, Universidad de Chile. [3]NIDCR, NIH, Bethesda, USA. erickcontreras@gmail.com

(213) FUNCTIONAL ROLE OF THE UNFOLDED PROTEIN RESPONSE IN HUNTINGTON'S DISEASE. **Vidal, R.**[1,2,3], Figueroa A.[1,2,3] and Hetz, C.[1,2,3]. [1]Biomedical Neuroscience Institute, Faculty of Medicine, [2]Center for Molecular Studies of the Cell, Institute of Biomedical Sciences, University of Chile, Santiago, Chile, [3]Neurounion Biomedical Foundation, Santiago, Chile. renevidalg@gmail.com

(214) α-CHEMOKINES MODULATE DIFFERENTIATION OF NEURAL STEM CELLS FROM SPINAL CORD. **Cristi, F.**[1,2], Erices A.[1]. [1]Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, and [2]Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile. fcristi@bio.puc.cl

(215) PPAR BETA/DELTA AGONIST INDUCES CELL PROLIFERATION AND REGULATES SOX2 LEVELS IN MOUSE ADULT NEURAL PRECURSOR CELLS. **Bernal C.,** Araya C., Bronfman M. Departamento de Biología Celular y Molecular. Pontificia Universidad Católica de Chile. CARE-FONDAP. mbronfman@bio.puc.cl

(216) PROLIFERATION OF MURINE MIDBRAIN NEURAL STEM CELLS DEPENDS UPON AN ENDOGENOUS SONIC HEDGEHOG (Shh) SOURCE. **Víctor Hugo Cornejo**, Constanza Martínez, Pablo Lois and Verónica Palma**.** Laboratory of Stem Cells and Development, Facultad de Ciencias, Universidad de Chile. vcornejocorona@ug.uchile.cl

(217) SPINAL CORD REGENERATION IN *Xenopus* TADPOLES PROCEEDS THROUGH ACTIVATION OF SOX2 POSITIVE CELLS. Marcia Gaete, Rosana Muñoz, **Mauricio Moreno,** Natalia Sánchez, Ricardo Tampe, Esteban Contreras, Juan Larraín. Center for Aging and Regeneration, Millennium Nucleus in Regenerative Biology, Faculty of Biological Sciences, P. Universidad Católica de Chile. mmoreno777@gmail.com

(218) STUDY OF XtRic-8A DURING THE NEURAL DEVELOPMENT OF *X. tropicalis*. **Gabriela Toro**, Lester Riquelme, Juan Olate, and Marcela Torrejón. Laboratory of Molecular Genetics, Biochemistry and Molecular Biology Department, Universidad de Concepción, Chile. gabrielatoro@udec.cl

(219) THE NEOGENIN 1 (Neo1) RECEPTOR IS CONTROLLED IN SONIC HEDGEHOG (Shh) DRIVEN MEDULLOBLASTOMAS. **Luis A. Milla[1]**, Brandon Wainwright[2] and Verónica Palma[1]. [1]Laboratory of Stem Cells and Development. Faculty of Sciences, University of Chile, Santiago, Chile; [2]Institute for Molecular Bioscience, University of Queensland, Australia. lmillab@gmail.com

(220) LOCAL TRAFFICKING OF VOLTAGE-GATED SODIUM CHANNELS IN PERIPHERAL AXONS. **Carolina González[1,2]**, Felipe Court[3,4] and Andrés Couve[1,2]. [1]Physiology and Biophysics, ICBM and [2]Biomedical Neuroscience Institute (BNI), Facultad de Medicina, Universidad de Chile, Santiago, Chile. [3]Faculty of Biological Sciences, and [4]Millenium Nucleus for Regenerative Biology, P. Universidad Católica de Chile. carolags@gmail.com

(221) TRANSCRIPTIONAL REGULATION OF P2X3 RECEPTORS IN NOCICEPTIVE NEURONS. **Emilio Diaz**[1,3], Rodolfo Madrid[2], Martín Montecino[3], Brigitte van Zundert[3]. [1]Universidad de Concepción; [2]Universidad de Santiago de Chile; [3]Centro de Investigaciones Biomédicas, Universidad Andrés Bello.emdiaz@udec.cl

(222) ETHANOL EFFECT IN GLYCINE RECEPTOR IS INHIBITED BY Gbg BLOCKING PEPTIDES. **San Martín, L.**, Cerda, F., Aguayo, L., Guzmán, L. Department of Physiology, University of Concepcion, Concepcion, Chile. loresanmartín@udec.cl

(223) REGULATION OF ERC AGGREGATION BY SRPK2/3 IS MEDIATED THROUGH THE COILED-COIL (CC) DOMAINS. **Yocelin Cruz**, Dolly Araya, Cristina Araya, Natalia Oro, Fernanda Olivares, Jonathan Bijman, Pedro Zamorano, Viviana Torres. Laboratorio de Neurobiología, Facultad de Ciencias de la Salud, Universidad de Antofagasta. viviana.torres@gmail.com

(224) TGF-β1 MODULATES MAPK AND NF-κB SIGNALING BY INCREASING MKP-1 LEVELS IN GLIAL CELL CULTURES. **Betsi Flores** and Rommy von Bernhardi. Department of Neurology, Faculty of Medicine, Pontificia Universidad Católica de Chile. rvonb@med.puc.cl

(225) PLASTICITY OF ASTROCYTES AND ALDOC SECRETION FOLLOWING ANTIDEPRESSANT TREATMENT. **Alejandro Luarte,** Rodrigo Herrera-Molina, Mauricio Sandoval, Ursula Wyneken. Laboratorio de Neurociencias, Universidad de los Andes. uwyneken@uandes.cl

(226) MATERNAL THYROID HORMONE DEFICIENCY CAUSES ALTERATIONS IN NEURONS AND ASTROCYTES IN THEIR OFFSPRING. [1,2]**Pablo Cisternas*,** [1,2]Pablo Gonzalez*, [1,2]Gabriela Zuñiga*, [1,2]Claudia Cortés, [1,3]Leandro Carreño [1,3]Susan Bueno, [1,3,4]Alexis Kalergis, [1,2]Claudia Riedel. [1]Millennium Institute on Immunology and Immunotherapy. [2]Facultad de Ciencias Biológicas Universidad Andrés Bello. [3]Departamento de Genética Molecular y Microbiología, Facultad Ciencias Biológicas. Pontificia Universidad Católica de Chile. [4]Departamento de Reumatología, Facultad de Medicina. Pontificia Universidad Católica de Chile. *Authors contributed equally to this work. pab.cisternas@unab.cl

(227) IP3 DEPENDENT Ca^{2+} SIGNALS IN SKELETAL MUSCLE ARE TRIGGERED AT DIFFERENT MEMBRANE POTENTIALS THAN RYR DEPENDENT ONES. Casas, M. Jorquera, G. and **Jaimovich, E**. Centro de Estudios Moleculares de la Célula. ICBM, Facultad de Medicina, Universidad de Chile. mcasas@med.uchile.cl

(228) INVOLVEMENT OF P2 RECEPTORS IN THE MYOGENIC COMMITMENT ACQUISITION OF C_2C_{12} RESERVE CELLS. **Vega JL**[1,2], Cea L[2], Riquelme M[2], Sáez JC[2]. [1]Laboratorio de Fisiología Experimental (EPhyL), Universidad de Antofagasta. [2]Departamento de Fisiología, P. Universidad Católica de Chile. jlvegapi@gmail.com

(229) ROLE OF CONNECTIVE TISSUE GROWTH FACTOR IN THE DEVELOPMENT OF FIBROSIS IN SKELETAL MUSCLE DYSTROPHY. [1]**Morales MG**, [1,2]Cabello-Verrugio C, [3]Goldschmeding R, [1]Brandan E. [1]Department of Cell and Molecular Biology, CARE, P. Universidad Católica de Chile. [2]Center for Human Genetics, Facultad de Medicina, Clínica Alemana Universidad del Desarrollo. [3]Department of Pathology, University Medical Center Utrecht, The Netherlands. mgmorales@gmail.com

(230) ATP RELEASE AND IP_3 PRODUCTION ARE IMPORTANT REGULATORS OF SKELETAL MUSCLE PLASTICITY. **Jorquera G**., Almarza G., Jaimovich E., Casas M. Centro de Estudios Moleculares de la Célula, ICBM, Facultad de Medicina, Universidad de Chile, Chile. gjorquera@med.uchile.cl

(231) MUSCLE-SPECIFIC UBIQUITIN LIGASES INCREASE IN SKELETAL MUSCLE ATROPHY WAS PREVENTED BY ANGIOTENSIN 1-7. **Cabello-Verrugio, C.** Center for Human Genetics, Facultad de Medicina, Clínica Alemana Universidad del Desarrollo. ccabello@udd.cl

(232) THE Na^+ PUMP IS NOT PREFERENTIALLY FUELED BY GLYCOLYTIC ATP. [1,2]**Ignacio Fernández** and [1]L. Felipe Barros. [1]Centro de Estudios Científicos (CECs), Valdivia, & [2]P. Universidad Católica de Valparaíso. ifernandez@cecs.cl

(233) CHOLESTEROL INVOLVEMENT IN SKELETAL MUSCLE GLUCOSE TRANSPORT. **Llanos P.,** Contreras-Ferrat AE., Osorio-Fuentealba C., Espinosa, A, Hidalgo C. and Jaimovich E. CEMC & ICMB, Faculty of Medicine, Universidad de Chile. pllanos@med.uchile.cl

(234) APOER2 POLARIZED DISTRIBUTION AND THE ROLE OF A PROLINE-RICH INSERT IN ITS CYTOPLASMIC DOMAIN. Catalina Grabowski, Pamela Farfán, María Luisa Benítez, Patricia Burgos[&], Alfredo Cáceres* and **María Paz Marzolo**. Depto. Biología Celular y Molecular, Fac. Ciencias Biológicas y MINREB, P. Universidad Católica de Chile. &Universidad Austral de Chile, *Instituto Investigación Médica Mercedes y Martin Ferreyra Córdoba, Argentina. mmarzolo@bio.puc.cl

(235) STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF CARGO-BINDING SITES ON THE μ4-SUBUNIT OF ADAPTOR PROTEIN COMPLEX 4. **Breyan Ross[1],** Yimo Lin[1], Juan Bonifacino[2], James Hurley[3], Patricia Burgos[1], and Gonzalo Mardones[1]. [1]Instituto de Fisiología, Facultad de Medicina, Universidad Austral de Chile, Valdivia, Chile, and [2]Cell Biology and Metabolism Program, NICHD, and [3]Laboratory of Molecular Biology, NIDDK, NIH, Bethesda, MD, USA. gamardon@gmail.com

(236) POST-TGN BASOLATERAL SORTING INVOLVES AN INTERMEDIATE SUB-APICAL COMPARTMENT IN POLARIZED MDCK EPITHELIAL CELLS. **Claudio Retamal** and Alfonso González**.** Departamento de Inmunología Clínica y Reumatología, Fac. Medicina. Centro de Envejecimiento y Regeneración (CARE). Fac. Cs. Biológicas. Pontificia Universidad Católica de Chile.

(237) THE C-ABL TYROSINE KINASE PHOSPHORYLATES HDAC2 REGULATING ITS LEVELS: A NOVEL MECHANISM OF EPIGENETIC CONTROL. **González-Zúñiga M.[1]**, Contreras P.[1], Loyola A.[2], Álvarez A.R.[1]. 1.Cell Signaling Laboratory. Biological Sciences Faculty.Pontificia Universidad Católica de Chile. 2.Fundación Ciencia para la Vida. magonza1@uc.cl

(238) EPIGENETIC CONTROL THROUGH HISTONE AND DNA MODIFICATIONS OF THE OSTERIX GENE EXPRESSION DURING OSTEOBLAST DIFFERENTIATION. **Sepúlveda, H.,** Pihan, P. and Montecino, M. Center for Biomedical Research and FONDAP Center for Genome Regulation, Faculty of Biological Sciences and Faculty of Medicine, Universidad Andres Bello.

(239) BISPHOSPHONATES REGULATE OSTEOGENIC GENE EXPRESSION AND β-CATENIN ACCUMULATION IN OSTEOBLAST PRECURSOR CELLS. **Nicolás Méndez**, Ana María Pino, Juan Pablo Rodríguez, Mireya Fernández. Laboratorio de Biología Celular, INTA, Universidad de Chile. nmendezd@gmail.com

(240) OSTEOBLASTIC DIFFERENTIATION IS ENHANCED BY TOPOGRAPHICAL AND CHEMICAL MODIFICATION OF AN SCAFFOLD SURFACE. **Eduardo Roa,** Juan Reyes and Nelson Osses. Instituto de Química, Pontificia Universidad Católica de Valparaíso. nelson.osses@ucv.cl

(241) RELATIONSHIP BETWEEN APOPTOSIS AND INSERTION SITE IN ROTATOR CUFF COMPLETE TEARS. **Juan Pablo Ramírez[1]**, Francesca Bonati[1], Rodrigo Liendo[2], Francisco Soza[2] and Rodolfo Paredes[1]. [1]Laboratorio Salud de Ecosistemas, Escuela de Medicina Veterinaria, Facultad de Ecología y Recursos Naturales, Universidad Andrés Bello. [2]Centro de Investigaciones Médicas, Instituto Traumatológico CIMIT. rparedes@unab.cl

(242) SKELETAL MUSCLE OF INSULIN RESISTANCE MICE HAS HIGHER INSULIN-DEPENDENT H_2O_2 PRODUCTION DUE TO HIGHER NOX2 EXPRESSION. **[1]Espinosa A**, [2]Juretić N, [1]Contardo C and [2]Jaimovich E. [1]Escuela de Tecnología Médica and [2]Centro de Estudios Moleculares de la Célula, Facultad de Medicina, Universidad de Chile, Chile. bespinosa@med.uchile.cl

(243) DIABETIC NEPHROPATHY INDUCES OVEREXPRESSION OF MUSCLE GLYCOGEN SYNTHASE IN HUMAN AND RAT KIDNEY. **Rodrigo Gatica[*# ¥],** Romina Bertinat[#], Carme Caelles[¥], Felipe Slebe[¥], Joan Guinovart[¥], Juan Carlos Slebe[£] and Alejandro Yañez[#]. [*]Escuela de Graduados Facultad de Ciencias Veterinarias, [#]Laboratorio de Metabolismo y Biotecnología, [£]Laboratorio de Enzimología, Universidad Austral de Chile; Universidad San Sebastián, Sede Puerto Montt; [¥]Institute for Research in Biomedicine, Barcelona, España. rodrigogaticaguti@gmail.com

(244) GAP JUNCTIONS FORMED BY CONNEXINS AND PANNEXINS IN ADIPOSE TISSUE. **Benvenuto MA,** Fernández PE, Sáez JC. Departamento de Fisiología, Pontificia Universidad Católica de Chile. mabenvenuto@gmail.com

(245) CONNEXIN 39 FORMS FUNCTIONAL HEMICHANNELS, BUT NOT GAP JUNCTION CHANNELS, IN TRANSFECTED HELA CELLS. **Vargas A**[1], Cea L.A[1]., Vielma A[2]., Schmachtenberg O.[2], Sáez J.C.[1,2]. [1]Departamento de Fisiología, P. Universidad Católica, Santiago, Chile and [2]Centro Interdisciplinario de Neurociencias de Valparaíso, Universidad de Valparaíso, Valparaíso, Chile. anvargas@uc.cl

(246) BNP INHIBITS HUMAN MYOMETRIAL CONTRACTION VIA NPR-C THAT IN TURN TRIGGERS THE cAMP/PKA PATHWAY. **Delpiano AM**, Garmendia LR, Poblete JA, Cuello MA, Carvajal JA. Laboratorio de Medicina Materno Fetal. Departamento de Obstetricia y Ginecología, Escuela de Medicina. Pontificia Universidad Católica de Chile. delpiano@med.puc.cl

(247) RISK OF PREECLAMPSIA (PE) AND PRESENCE OF DIFFERENT POLYMORPHISM IN ENZIMES INVOLVED IN THE METHIONINE-HOMOCYSTEINE METABOLISM (MHM). **Valenzuela FJ**, Larrain R, Perez-Sepulveda A, Torres MJ, Guzman A, Ahumada V, Figueroa-Diesel H, Illanes S. Departamento de Obstetricia & Ginecología y Laboratorio de Biología de la Reproducción. Facultad de Medicina, Universidad de los Andes, Santiago. sillanes@uandes.cl (Sponsor: U. Wyneken)

(248) CHARACTERIZATION OF OLEOSOMES FROM *Gevuina avellana* AND *Madia sativa* SEEDS IN THE HUVEC CELL LINE. **Patricia Navarrete**[1,5], Francisca Acevedo[2,5], Oscar Valerio[3,5], Jorge Parodi[4], Fernando Romero[1]. [1]Center of Neurosciences and Peptides Biology- CEBIOR, BIOREN, University of La Frontera. [2]Center of Food Biotechnology and Bioseparations, BIOREN, University of La Frontera. [3]Center of Waste Management and Bioenergy, BIOREN, University of La Frontera. [4]Laboratory of Molecular Neurobiology, CARE, P. Universidad Católica de Chile. [5]Supported by PIA DI11-7001 of University of La Frontera. patricia.bq@gmail.com (Sponsor: E.O. Campos)

(249) UNRAVELING THE MYSTERIES OF SUPERRESOLUTION OPTICAL FLUCTUATION IMAGING (SOFI). **Felipe Santibáñez**[1,2], Omar Ramírez[1], Dirk Haehnel[3], Jörg Enderlein[3] & Steffen Härtel[1,2]. [1]SCIAN-Lab, [2]BNI, ICBM, Faculty of Medicine, U. of Chile. [3]III. Physikalisches Institut, Universität Göttingen, Germany. fsantibanez@med.uchile.cl

(250) CHARACTERIZATION OF THE HANTAVIRUS GC TRANSMEMBRANE DOMAIN. **[1]Sarno-Orellana, A.V.** [1]Carrasco, M. and [1,2]Tischler N.D. [1]Fundación Ciencia para la Vida and [2]Universidad San Sebastián. victoria.sarno@gmail.com

(251) ESSENTIAL RESIDUES AND FUNCTION OF THE HANTAVIRUS GC STEM REGION. **[1]Villalón, F.,** [1]Carrasco, M., [2]Monasterio, O. and [1,3]Tischler N.D. [1]Fundación Ciencia para la Vida, [2]Universidad de Chile, [3]Universidad San Sebastián. fvillalon@ug.uchile.cl

(252) *Trypanosoma cruzi* CALRETICULIN INHIBITS ENDOTHELIAL CELL MIGRATION *in vivo* IN A MAMMAL MODEL. **Leonora Duaso***, Juan Duaso , Francisca Coddou*, Katherine Weinberger*, Ismael Maldonado*, Galia Ramírez*, Ulrike Kemmerling , Arturo Ferreira*. *Immunology Disciplinary Program, Anatomy and Developmental Biology Program, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, Santiago, Chile. aferreir@med.uchile.cl

(253) *T. cruzi* DNA REPAIR ENZYMES TCAP1 AND TCAP2 ARE LOCALIZED IN THE NUCLEUS AND THEIR OVEREXPRESSION INCREASES EPIMASTIGOTE VIABILITY WHEN SUBMITTED TO OXIDATIVE STRESS. **Sofia Sepúlveda**, Lucía Valenzuela, Iván Ponce, José Delgadillo, Santiago Ramírez, Soledad Sierra, Paula Bahamondes, Natalia Muñoz, Ulrike Kemmerling, Norbel Galanti, Gonzalo Cabrera. Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile. se_sepulveda@med.uchile.cl

(254) ASSOCIATION BETWEEN MUC1 SPLICE VARIANTS FROM SALIVARY GLANDS AND MOUTH DRYNESS. **Sung HH,** González S, Aguilera S, Bahamondes V, Castro I, Barrera MJ, Leyton C, Urzúa U y González MJ. ICBM,Facultad de Medicina, Universidad de Chile. sunghsieh@gmail.com

(255) PRESENCE OF CALRETICULIN IN *Triatoma infestans* SALIVARY GLAND. **Weinberger, K**.[*], Coddou, F.[*], Duaso, L.[*], Valck, C.[*], Ramírez, G.[*], Aguilar L.[*], Maldonado I.[*], Zulantay I. , Apt W. , Ferreira A.[*]. Disciplinary Immunology[*] and Cellular Biology Programs, Biomedical Sciences Institute, Faculty of Medicine, University of Chile. aferreir@med.uchile.cl

FRIDAY, NOVEMBER 4th, 2011

09:00 – 10:30 SYMPOSIUM
"MEMBRANE TRAFFICKING AT SYNAPSES: FROM NEURONAL TRANSMISSION TO IMMUNITY (II)"
Calbuco Room - Chair: Ana Maria Lennon

ROLE AND CONTROL OF THE FORMATION OF THE IMMUNOLOGICAL SYNAPSE IN T LYMPHOCYTES. Armelle Bohineust[1,2], Karine Chemin[1,2], Julien Husson[1,3], Marie Tourret[1,2], Stéphanie Dogniaux[1,2], Paola Larghi[1,2], Nelly Henry[1,3] and **Claire Hivroz[1,2].** [1]Institut Curie, Centre de Recherche, Paris F-75248, France. [2]INSERM Unité 932, Immunité et Cancer, Paris, France. [3]CNRS UMR 168, Paris, France.

Sec22b CONTROLS THE RECRUITMENT OF ENDOPLASMIC RETICULUM TO PHAGOSOMES IN DENDRITIC CELLS: FUNCTIONAL CONSEQUENCES FOR CROSS PRESENTATION AND PHAGOSOME MATURATION. Ignacio Cebrian[1], Geraldine Visentin[1], Nicolas Blanchard[2], Mabel Jouve[5], Alexandre Bobard[3], Catarina Moita[4], Jost Enninga[3], Luis F. Moita[4], Ariel Savina and **Sebastian Amigorena[1].** [1]Institut Curie, INSERM U932, Immunité et Cancer, 26 rue d'Ulm, 75248 Paris, Cedex 05, France. [2]Centre de Physiopathologie de Toulouse-Purpan INSERM UMR1043-CNRS UMR5282, Université de Toulouse, France. [3]Institut Pasteur; Dynamique des Interactions Hôte-Pathogène, Paris, France. [4]Cell Biology of the Immune System Unit, Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, 1649-028 Lisboa, Portugal. [5]Institut Jacques Monod, UMR7592 CNRS/Université Paris Diderot, ImagoSeine-Plateforme de Microscopie Electronique, France.

FROM HIV-1 INFECTION, TO PRODUCTION OF VIRAL PARTICLES AND CELL-TO-CELL TRANSMISSION VIA VIROLOGICAL SYNAPSES. Raphaël Gaudin[1,2], Stefano Berre[1,2], Bruna Cunha de Alencar[1,2], François-Xavier Gobert[1,2], Mabel Jouve[3] and **Philippe Benaroch[1,2].** [1]Institut Curie, Centre de Recherche, Paris F-75248, France. [2]INSERM Unité 932, Immunité et Cancer, Paris, France. [3]Institut Jacques Monod, UMR 7592, CNRS /Université Paris Diderot, 75013, Paris, France.

SYMPOSIUM NUCLEO MILENIO EN BIOLOGIA REGENERATIVA (MINREB)
P. UNIVERSIDAD CATOLICA DE CHILE
Tronador Room - Chair: Francisca Bronfman

"AXONAL CELL BIOLOGY AND REGENERATION"

NEUROTROPHIN TRAFFICKING IN AXON GROWTH. Rejji Kuruvilla, Daniel Bodmer, and Maria Ascano. Department of Biology, Johns Hopkins University, Baltimore, MD 21218.

AXONAL RETROGRADE KILLING BY NEUROTROPHINS. Francisca C Bronfman and Claudia Escudero. Millennium Nucleus in Regenerative Biology (MINREB), Facultad de Ciencias Biológicas. Pontificia Universidad Católica de Chile, Chile.

MICROTUBULE TETHERING BY DYNEIN AND NCAM FACILITATES SYNAPTIC STABILIZATION. Eran Perlson[1,2], Adam G. Hendricks[1], Jacob E. Lazarus[1], Mariko Tokito[1], Yale E. Goldman[1] & Erika L. F. Holzbaur[1]. [1]University of Pennsylvania School of Medicine, [2]Tel-Aviv University Sackler Faculty of Medicine.

10:30 – 11:30 Coffee Break – Exhibitors
Convention Center Foyer

11:30 – 13:30 Oral Presentations VI
Volcanes Room - Chair: Ariel Reyes and Co-Chair: Manuel Kukuljan

SEARCHING FOR PHYSIOLOGICAL STIMULI TO ENHANCE ADULT HIPPOCAMPAL NEUROGENESIS IN A MICE MODEL OF ALZHEIMER'S DISEASE. Lorena Varela-Nallar, Ana C. Abbott, Macarena Rojas-Abalos, Florencia C. Aranguiz, Cheril Tapia-Rojas and Nibaldo C.

Inestrosa. Centro de Envejecimiento y Regeneración (CARE), Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, P. Universidad Católica de Chile, Santiago, Chile. lpvarela@uc.cl

NEURAL PROGENITORS IN THE OLFACTORY SENSORY SYSTEM. Maegan V. Harden[1], Luisa Pereiro[4], Mirana Ramialison[2], Joachim Wittbrodt[2], Megana K. Prasad[3], Andrew McCallion[3], Jorge Torres4, and **Kathleen E. Whitlock[4]**. 1.Department of Molecular Biology & Genetics, Cornell University, NY; 2.Institute of Zoology, Heidelberg University, Germany; 3.McKusick-Nathans Institute of Genetic Medicine, Maryland, USA; 4. Interdisciplinary Center for Neuroscience, Universidad de Valparaiso, Valparaíso, Chile. kathleen.whitlock@gmail.com

XtRic-8A, A PROTEIN REQUIRED FOR PROPER NEURAL CREST FORMATION. Jaime Fuentealba, Juan Olate and **Marcela Torrejón.** Laboratory of Molecular Genetics, Biochemistry and Molecular Biology Department, University of Concepción, Chile. matorrejon@udec.cl

CoREST/LSD1 CONTROL THE DEVELOPMENT OF PYRAMIDAL CORTICAL NEURONS. **Kukuljan, M**., Cánovas J., Berndt F.A., Fuentes, P. Programa de Fisiología y Biofísica, ICBM, e Instituto Milenio de Neurociencias Biomédicas, Facultad de Medicina, Universidad de Chile. kukuljan@med.uchile.cl

ANTI-ANGIOGENIC PROPERTIES OF COAGULATION RELATED PROTEASES. **Lange S**[1,2], Cautivo K[2], Elliot M[3] , Kalergis A[1,2], Palma V[3] & Owen GI[1,2]. [1]Faculty of Biological Sciences, PUC, [2]The Biomedical Research Consortium (BMRC) and [3]Laboratory of Stem Cells and Development, Fac. of Science, U. de Chile. mslange@uc.cl

THE HYPOXIA FACTOR Hif-1α IS ESSENTIAL FOR NEURAL CREST MIGRATION. **Elías H. Barriga[1,2]**, Roberto Mayor[2] y Ariel E. Reyes[1]. [1]Laboratorio de Biología del Desarrollo, Departamento Ciencias Biologicas, UNAB, Chile. [2]Department of Cell and Developmental Biology, UCL, London, UK. arielreyes@unab.cl

GAP JUNCTIONS PROMOTES THE COMMUNICATION BETWEEN HUMAN NATURAL KILLER CELLS WITH DENDRITIC AND TARGET CELLS. **Andrés Tittarelli**[(1)], Marcela Farias[(2)], Ariadna Mendoza-Naranjo[(3)], Benedict Chambers[(4)], Andreas Lundqvist[(5)], Flavio Salazar-Onfray[(1)]. 1:Millennium Institute on Immunology and Immunotherapy. 2:Faculty of Odontology, University of Chile. 3:UCL Cancer Institute, UK. 4:Center for Infectious Medicine, Karolinska Institute. 5:Cancer Center, Karolinska Institute, Sweden. tittarelli@gmail.com

CAVEOLIN-1 INHIBITS TRANSCRIPTION BY HYPOXIA INDUCIBLE FACTOR-1A IN TUMOR CELLS. **Sanhueza C.**,[1] Lladser, A.,[2] Valenzuela M.,[1] Nuñez S.,[1] Diaz M.I.,[1] Leyton L.,[1] Quest A.F.G.[1] [1]Laboratorio de Comunicaciones Celulares, Centro de Estudios Moleculares de la Célula, Facultad de Medicina, Universidad de Chile. [2]Laboratory of Gene Immunotherapy, Fundación Ciencia para la Vida, Santiago. aquest@med.uchile.cl

13:30 – 15:30 Lunch

16:30 – 18:00 SYMPOSIUM FODECYT (1100027 AND 1100896)
UNIVERSIDAD AUSTRAL DE CHILE
Calbuco Room - Chair: Patricia Burgos

"A BUMPING TOUR UNRAVELING THE MAGICAL MYSTERIES OF THE SECRETORY PATHWAY"

MEMBRANE PROTEIN BIOSYNTHESIS AND QUALITY CONTROL. **Ramanujan Hegde,** MRC Laboratory of Molecular Biology Cambridge, United Kingdom, CB2 0QH.

SIGNAL-ADAPTOR INTERACTIONS THAT MEDIATE POLARIZED SORTING IN NEURONS. **Juan S. Bonifacino.** Cell Biology and Metabolism Program, Eunice Kennedy Shriver, National Institute of Child Health and Human Development, National Institute of Health, Bethesda, Maryland, USA.

NIPPED IN THE BUD: THE STRANGE WAYS THAT ESCRTS SEVER MEMBRANES. **James H. Hurley**, Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, U. S. Department of Health and Human Services, Bethesda, MD 20892.

SYMPOSIUM INSTITUTO MILENIO DE INMUNOLOGIA E INMUNOTERAPIA
UNIVERSIDAD NACIONAL ANDRES BELLO
Tronador Room - Chair: Claudia Riedel

"IMMUNOLOGICAL BASES OF THE INFLAMMATION AT THE CENTRAL NERVOUS SYSTEM"

NOVEL CELLULAR COMMUNICATION SYSTEMS IN HIV INDUCED NEUROINFLAMMATION AND TOXICITY. **Eugenin, Eliseo**. Department of Pathology, Albert Einstein College of Medicine, Bronx, New York, NY, 10461, USA.

MATERNAL THYROID HORMONE DEFICIENCY INCREASES CENTRAL NERVOUS SYSTEM DAMAGE IN THE OFFSPRING SUFFERING EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS. **Claudia Riedel**, Universidad Andrés Bello.

IMMUNE SIGNALING IN NEURONAL DEVELOPMENT. **Helene Boudin**, Inserm 643, Nantes, France.

MECHANISMS OF LEUKOCYTE TRANSMIGRATION ACROSS THE BLOOD BRAIN BARRIER: IMPLICATIONS FOR NEUROAIDS AND OTHER CNS INFLAMMATORY PROCESSES. **Joan W. Berman**, Ph.D., Departments of Pathology and Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY, USA.

18:00 – 19:00 **Coffee Break – Exhibitors**
Convention Center Foyer

19:00 – 20:00 **PLENARY LECTURE SOCIEDAD DE BIOLOGIA CELULAR DE CHILE**
Volcanes Room - Chair: Maria Rosa Bono

HOW SCIENCE IMPACTS SOCIETY: THE ROLE OF KNOWLEDGE DRIVEN RESEARCH AND ENTREPRENEURSHIP. **Pablo Valenzuela.** Fundación Ciencia para la Vida, Santiago, Chile.

20:00 **AWARDS CEREMONY**
Volcanes Room
Nikon - Loncotec: **Best Images in Cell Biology**
Genexpress: **Best Presentations in Oral and Poster Communications**

21:00 **Closing Dinner**

PLENARY LECTURES

PLENARY LECTURE DR. LUIS IZQUIERDO FERNANDEZ

FROM RIBOSOMES TO PSYCHOSIS. **Alfonso González.** Departamento de Inmunología Clínica y Reumatología, Fac. Medicina. Centro de Envejecimiento y Regeneración (CARE). Fac. Ciencias Biológicas. Pontificia Universidad Católica de Chile.

In this conference I will show how working on the biogenesis of the cell surface we became interested in the role of ribosomes in the neuropsychiatric manifestations of lupus patients. Poly-ribosomes that synthesize plasma membrane or secretory proteins attach to membranes of the endoplasmic reticulum (ER) by a signal-peptide mediated process that determines entry into the classical secretory pathway. After translocation and folding in the ER, these proteins are transported in vesicular carriers first to the Golgi complex and then to the cell surface or extracellular medium. We currently work on the mechanism that sort selected proteins to either the apical or basolateral domain of polarized epithelial cells, which involve the trans-Golgi network (TGN) and recycling endosomes. Our recent work defined a crossroad between post-TGN and endocytic recycling routes crucial for polarized sorting, and also discovered a signaling pathway that regulates endocytic recycling of specific proteins. Searching for a model of non-conventional secretion we considered the current belief in rheumatology that a ribosomal protein constitutes a cell surface target of certain autoantibodies associated with lupus psychosis and mood disorders. Unexpectedly, we found no evidence for such non-conventional secretion, but instead discovered a true cell surface target of anti-ribosomal antibodies. This is a novel surface protein that in the brain is expressed by neurons at specific regions, including areas involved in cognition, memory and emotion, and mediates neuropathogenic effects of anti-ribosomal antibodies, potentially leading to psychiatric or cognitive disorders.

(Financed by CONICYT grant #PFB12/2007 and FONDECYT grant #1110849).

PLENARY LECTURE CENTER FOR AGING AND REGENERATION (CARE)

A NANOSCALE VIEW INTO THE DYNAMIC OF AMPA RECEPTOR ORGANIZATION IN SYNAPSES. **Daniel Choquet.** Institut Interdisciplinaire de Neuroscience, UMR 5297 CNRS-Université de Bordeaux.

Ionotropic AMPA glutamate receptors (AMPAR) mediate fast excitatory synaptic transmission in the central nervous system. Using a combination of high resolution single molecule imaging techniques and video-microscopy, we have previously established that AMPARs are not stable in the synapse as thought initially, but undergo continuous entry and exit to and from the post-synaptic density through lateral diffusion.

Single molecule approaches give access to the full distribution of molecule behaviors and overcome the averaging intrinsic to bulk measurement methods. They allow access to complex processes where a given molecule can have heterogeneous properties over time. We will present recent developments in single molecule imaging technologies and their application to track single molecules in live neurons and determine the organization of AMPA receptors at the nanometer scale.

We have recently found a new function for this fast diffusion in controlling fast synaptic transmission. Upon consecutive synaptic stimulation at high frequency, synaptic transmission is depressed. This depression shapes the frequency dependent adaptation of individual synapses. AMPAR lateral diffusion allows fast exchange of desensitised receptors with naïve functional ones within or nearby the post-synaptic density. This participates to the recovery from depression in the tens of millisecond time range, in parallel with recovery from desensitization.

In addition, we show that the Ca^{2+}/Calmodulin-dependent protein kinase II (CaMKII), which is critically required for the synaptic recruitment of AMPA-type glutamate receptors (AMPARs) during both development and plasticity, induces the synaptic trapping of AMPARs diffusing in the membrane. Furthermore, this CaMKII dependent AMPAR immobilization regulates short term plasticity. Thus, NMDA dependent Ca2+ influx in the post-synapse trigger a CaMKII and Stargazin dependent decrease in AMPAR diffusional exchange at synapses that controls synaptic function.

PLENARY LECTURE FUNDACION CIENCIA PARA LA VIDA AND MIFAB

NAVIGATING THE CELLULAR LANDSCAPE WITH NEW OPTICAL PROBES, IMAGING STRATEGIES AND TECHNICAL INNOVATIONS. **Jennifer Lippincott-Schwartz**. Eugene Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892.

Emerging visualization technologies are playing an increasingly important role in the study of numerous aspects of cell biology, capturing processes at the level of whole organisms down to single molecules. Recent developments in probes, techniques, microscopes and quantification are dramatically expanding the areas of productive imaging. Photoactivatable fluorescent proteins (PA-FPs) have been particular fruitful in this regard. They become bright and visible upon being exposed to a pulse of UV light. This allows selected populations of proteins to be pulse-labeled and tracked over time. Used for *in cellulo* pulse chase experiments, the PA-FPs have helped clarify mechanisms for biogenesis, targeting, and maintenance of organelles as separate identities within cells. PA-FPs have further permitted the development of single

molecule-based superresolution (SR) imaging, which dramatically improves the spatial resolution of light microscopy by over an order of magnitude (~10-20 nm resolution). Involving the controlled activation and sampling of sparse subsets of photoconvertible fluorescent molecules, single molecule SR imaging offers exciting possibilities for obtaining molecule scale information on biological events occurring at variable time scales. Here, I discuss the new fluorescent imaging techniques and the ways they are helping researchers navigate through the cell to unravel long-standing biological questions.

PLENARY LECTURE CENTRO FONDAP DE REGULACION DEL GENOMA

IMMUNE MODULATION OF MAMMALIAN REGENERATION. **Nadia Rosenthal.** Mouse Biology Unit, EMBL-Monterotondo Outstation, Rome**.** Heart Science Centre, Imperial College London**.** Australian Regenerative Medicine Institute, Monash University, Melbourne.

The limited restorative capacity of many adult human tissues has been attributed to the loss of adequate cell replacement coupled with persistent inflammation with increasing age. Using mouse genetics we have investigated the role of growth factors and resident immune cells in the resolution of tissue injury. We have uncovered a complex interaction between local repair mechanisms and cells of the innate immune system, which participate in the removal of necrotic cells, secrete growth factors that limit inflammation and promote tissue replacement, and also act as tissue stem cells in the repair process. Our work supports the feasibility of improving human regenerative capacity by modulating key signaling pathways controlled by these cells, providing new targets for clinical intervention and improving prospects for molecular and cellular combination therapies.

PLENARY LECTURE COOPERACION INTERNACIONAL FONDECYT

REGULATION OF PLASMA CELL DIFFERENTIATION AND SURVIVAL IN AUTOIMMUNITY. **Loren D. Erickson**, Ph.D. Department of Microbiology, University of Virginia, Charlottesville, Virginia, USA.

Systemic lupus erythematosus (SLE) and its preclinical lupus-prone mouse models are autoimmune disorders involving the production of pathogenic autoantibodies by B cells of the immune system. Genetic predisposition to SLE results in B cell hyperactivity, survival of autoreactive B cells, and differentiation of autoreactive B cells into specialized cells known as plasma cells, which secrete large amounts of autoantibodies. These corrupt B cell responses are, in part, controlled by abnormally high levels of the cytokine BAFF. In contrast, in healthy hosts, physiologic low levels of BAFF maintains B cell homeostasis and self tolerance, eliminating autoreactive B cells from the repertoire of peripheral B cells. The mechanisms by which excess BAFF levels inappropriately control B cells are unknown. Recent work in my laboratory, and the focus of this presentation, identified the BAFF-binding receptor B cell maturation antigen (BCMA) on B cells to be important in controlling disease susceptibility. In lupus-prone mice, BCMA deficiency causes dramatic B cell lymphoproliferation, a loss of B cell tolerance, and enhanced differentiation of B cells into autoantibody-secreting plasma cells. These observations suggest that, in autoimmune-prone mice, signals through BCMA on B cells help control B cell homeostasis and the stringent elimination of autoreactive B cells. We further hypothesize that BCMA signaling controls the levels of BAFF in autoimmune-prone mice by modulating the frequency of BAFF-producing innate immune cell types, establishing an inflammatory loop between innate immune cells and B cells that exacerbates autoimmunity when BCMA signaling is disrupted.

PLENARY LECTURE I. NUCLEO MILENIO EN BIOLOGIA REGENERATIVA (MINREB)

HUNTINGTIN FROM EVOLUTION TO STEM CELL BIOLOGY AND TRANSPLANTATION. **Elena Cattaneo**, Coordinator NeuroStemcell (www.neurostemcell.org). Department of Pharmacological Sciences and Centre for Stem Cell Research (UniStem), University of Milano, Italy. Laboratory web site: www.cattaneolab.it - UniStem web site: www.unistem.it

Huntingtin (htt) is the 3144 amino acid protein product of the Huntington's disease (HD) gene. The gene contains a polymorphic tri-nucleotide CAG repeat that is translated into polyglutamine amino acid (polyQ) residues in the protein. When this polyQ stretch at the 18 aminoacid position of the protein expands to over 39 residues, HD occurs, a fatal, genetically dominant, neurodegenerative disease leading to progressive disability and early death. There are currently no disease-modifying treatments available. Research efforts aim at understanding and interfering with the toxic gained activity acquired by the mutant protein. Our strategy is focused on the understanding of the biology and evolution of the normal htt protein in order to identify activities of the protein in the adult brain which may be affected by the CAG expansion. A further approach in HD aims at replacing the striatal medium spiny neurons (MSNs) lost in the disease with new cells that

are able to take over their function and reconnect the circuitry. A very promising source of these neurons are the embryonic stem cells. Here I will review our studies on Huntingtin's biology and will present our attempts at developing protocols allowing the generation of progenitors of human MSNs suitable for experimental transplantation approaches in HD.

PLENARY LECTURE SOCIEDAD DE BIOLOGIA CELULAR DE CHILE

HOW SCIENCE IMPACTS SOCIETY: THE ROLE OF KNOWLEDGE DRIVEN RESEARCH AND ENTREPRENEURSHIP. **Pablo Valenzuela.** Fundación Ciencia para la Vida, Santiago, Chile.

The cultural and economical impact of science in society is today increasingly stronger. Probably that is one of the reasons why some of us have chosen to be scientist. As biologist we are driven by the intellectual satisfaction of impacting human health, environment, food supply, energy supply, etc. As human we are also driven by recognition and in some cases by economical gains derived by discoveries. I would like to discuss the importance of basic research, entrepreneurship and lucro in achieving these targets.

SYMPOSIUMS

SYMPOSIUM "NEURONAL CYTOSKELETON: PHYSIOLOGICAL AND PATHOLOGICAL ASPECTS" INSTITUTE FOR CELL DYNAMICS AND BIOTECHNOLOGY (ICDB), UNIVERSIDAD DE CHILE - COOPERACION INTERNACIONAL FONDECYT 1095089

MICROTUBULE ASSOCIATED PROTEIN (MAP1B) IN INVOLVED IN DENDRITIC SPINE DEVELOPMENT AND NEUROTRANSMISSION. **Christian González-Billault,** Laboratory of Neuronal and Cell Dynamics, Department of Biology, Universidad de Chile and Institute for Cell Dynamics and Biotechnology (ICDB) Santiago, Chile.
Microtubule- associated protein 1B (MAP1B) is predominantly expressed during the early stages of neuronal development. Later, its expression becomes developmentally down-regulated until it almost disappears, however it expression remain increased in adult brain areas of high synaptic plasticity such as the hippocampus and cerebellum. For such a reason we hypothesized that it may have a role in the control of synapses. MAP1B can be found in the pre- and postsynaptic compartment, opening the possibility that it can have differential roles in the control of synaptic transmission. Using the yeast two hybrid (Y2H) approach we were able to pull down LC1 interacting partners from an adult mice brain genomic library. In this work we described the interaction between MAP1B and Snapin, a soluble protein associated with synaptic vesicles, which has a role in mediating exocytosis of synaptic vesicles. Additionally we determine that the kinetic of vesicle release is decreased in MAP1B-/- neurons, suggesting a possible role for MAP1B at the pre-synaptic level. On the other hand, we observed the presence of MAP1B in dendritic spines and synaptosomal fractions of wild type adult mice. Neurons derived from MAP1B-deficient mice showed a decrease in the density of mature dendritic spines and an increase of immature filopodia-like protrusions. In addition, these neurons show a significant decrease in their synaptic currents mediated by AMPA receptors. Moreover, using post-synaptic densities from adult MAP1B +/- mice, we show a significant decrease in Rac1 activity and an increase in RhoA activity. These MAP1B +/- fractions present a decrease in phosphorylated cofilin. Taken together, these results define a new and important function of MAP1B in dendritic spine formation and maturation through the regulation of actin cytoskeleton at the postsynaptic level. In conclusion, MAP1B could regulate neural transmission at both the pre- or/and post-synaptic level.
(Supported by Fondecyt 1095089 and ICM P05-001-F).

ACUTE EFFECTS OF ACTIN ON THE POSTSYNAPTIC DENSITY. **Thomas A. Blanpied**, School of Medicine, University of Maryland.
AMPA receptors (AMPARs) mediate synaptic transmission and plasticity during learning, development, and disease. Mechanisms determining subsynaptic receptor position are poorly understood but are key determinants of quantal size. By photobleaching synapse subdomains, we found most AMPARs do not freely diffuse within the synapse, indicating they are embedded in a matrix that determines their subsynaptic position. However, confocal and photoactivated localization microscopy (PALM) revealed that synaptic AMPARs are continuously repositioned by plasticity of this scaffold matrix rather than by free diffusion. Given this organization, we found that the actin cytoskeleton regulated PSD architecture in two ways. First, the overall structure of the postsynaptic receptor cluster was dynamically modulated by actin treadmilling. Second, across the matrix, the component scaffold protein families were differentially distributed, and this distribution was continually adjusted during actin dynamics. Nevertheless, subsynaptic immobility of both AMPARs and scaffold molecules remained essentially intact even after loss of actin filaments, highlighting the novel, acute roles of actin in driving synapse internal plasticity. We conclude that receptors are actively repositioned at the synapse by treadmilling of the actin cytoskeleton, an influence which is transmitted only indirectly to receptors via the pliable and dynamic internal structure of the PSD.

Thy-1/INTEGRIN-MEDIATED BIDIRECTIONAL SIGNALING BETWEEN NEURONS AND ASTROCYTES. **Lisette Leyton.** Laboratorio de Comunicaciones Celulares, Centro de Estudios Moleculares de la Célula (CEMC), Biomedical Neuroscience Institute (BNI), ICBM, Facultad de Medicina, Universidad de Chile.
Lack of functional repair in the central nervous system (CNS) is a consequence of the unfavorable environment present at the site of lesion, due to the presence of a redundant number of proteins with axonal growth-inhibitory effects that exist in the brain. Thy-1 is a neuronal membrane protein suggested to possess such inhibitory properties; however, its function has remained obscure, because the endogenous ligand for this protein was unknown. Additionally, astrocytes form a barrier to regeneration and upon injury they undergo astrogliosis changing their shape and migrating to form the glial scar. We have shown that neuronal Thy-1 binds to $\alpha_V\beta_3$ integrin and the heparan sulfate proteoglycan, Syndecan-4, *in trans* eliciting responses in astrocytes that include the modulation of cell shape, adhesion and migration. Thy-1-induced effects occur via signaling pathways emanating downstream of $\alpha_V\beta_3$ integrin and Syndecan-4 in a sequential mode, with orchestrated activation of the cytoskeleton-controlling GTPases, RhoA and Rac1. On the other hand, the possibility that an astrocyte receptor, such as $\alpha_V\beta_3$ integrin, might also act as a Thy-1-ligand triggering responses in neurons was explored. Thy-1-mediated effects of $\alpha_V\beta_3$ integrin on growth and retraction of neuronal processes was tested. $\alpha_V\beta_3$-Fc was sufficient to

inhibit neurite extension in Thy-1(+), but not in Thy-1(-) cells. In differentiating neurons exposed to $\alpha_V\beta_3$-Fc, fewer and shorter dendrites were detected. Moreover, $\alpha_V\beta_3$-Fc induced retraction of Thy-1(+)-neurites in differentiated neurons. Therefore, $\alpha_V\beta_3$ integrin is a ligand for Thy-1 that upon binding triggers inhibition of neurite outgrowth and retraction of existing processes. At the same time, Thy-1 is a ligand for $\alpha_V\beta_3$ integrin and upon ligation, changes in astrocyte morphology as well as increased adhesion and migration are induced.
These results provide molecular insight to mechanisms involved in astrogliosis and how this process might induce neurite retraction in the CNS, both of which are likely to be relevant to inhibition of neuronal regeneration.
LL is supported by FIRCA-NIH 5R03TW007810-3, FONDECYT 1110149, Iniciativas Científicas Milenio: BNI P09-015-F, FONDECYT-FONDAP 1510006.

SYMPOSIUM "MEMBRANE TRAFFICKING AT SYNAPSES: FROM NEURONAL TRANSMISSION TO IMMUNITY (I)"

THE VESICULAR SNARE SYNAPTOBREVIN IS REQUIRED FOR SEMAPHORIN 3A AXONAL REPULSION. Kathleen ZYLBERSZTEJN[1,2], Maja PETKOVIC[1,2], Andrea BURGO[1,2], Marie DECK[3], Sonia GAREL[3], Séverine MARCOS[4], Evelyne BLOCH-GALLEGO[4], Fatiha NOTHIAS[5], Guido SERINI[6], Dominique BAGNARD[7], Thomas BINZ[8] and **Thierry GALLI[1,2].** [1]University Paris Diderot, Sorbonne Paris Cité, Jacques Monod Institute, CNRS UMR7592, Program in Development & Neurobiology, Paris, 75013 France. [2]INSERM ERL U950, 'Membrane Traffic in Neuronal & Epithelial Morphogenesis', Paris, 75013 France. [3]Ecole Normale Supérieure, IBENS, INSERM U1024, CNRS UMR8197, Paris, 75005 France. [4]Cochin Institute, University Paris Descartes, CNRS UMR8104, Department in Genetic and Development, INSERM U567, Paris, 75005 France. [5]'Axon Regeneration and Growth', PMSNC, INSERM U952, CNRS UMR 7224, University Pierre and Marie Curie, Paris, 75005 France. [6]University of Torino School of Medicine, Candiolo, Torino, 10100 Italy. [7]INSERM U682, Strasbourg, 67000 France. [8]Institute of Biochemistry, Medizinische Hochschule Hannover, Hannover, 30001 Germany.
Attractive and repulsive molecules such as Semaphorins (Sema) trigger rapid responses which control the navigation of axonal growth cones. The role of vesicular traffic in axonal guidance is still largely unknown. The exocytic vesicular SNARE Synaptobrevin 2 (Syb2) is known for mediating neurotransmitter release in mature neurons but its potential role in axonal guidance remains elusive. Here we show that Syb2 is required for Sema3A-dependent repulsion but not Sema3C-dependent attraction in cultured neurons and in the mouse brain. Syb2 associates with Neuropilin 1 and Plexin A1, two essential components of the Sema3A receptor, *via* its juxta-transmembrane domain. Sema3A receptor and Syb2 colocalize in endosomal membranes. Moreover, upon Sema3A treatment, Syb2 deficient neurons fail to collapse and transport PlexinA1 to cell bodies. Reconstitution of Sema3A receptor in non-neuronal cells revealed that Sema3A further inhibits the exocytosis of Syb2. Therefore, Sema3A-mediated signalling and axonal repulsion require Syb2-dependent vesicular traffic.

Cdc42 CONTROLS THE BALANCE BETWEEN KISS-AND-RUN AND FULL FUSION OF SECRETORY VESICLES BY REGULATING MEMBRANE TENSION. Marine Bretou[1,2], Ouardane Jouannot[1], Claire Desnos[1], Paolo Pierobon[2], Isabelle Fanget[1], Nathanaël Larochette[1], Pierre Gestraud[3,4,5], Marc Guillon[6], Valentina Emiliani[6], Stéphane Gasman[7], Ana-Maria Lennon-Duménil[2] and **François Darchen[1].** [1]Centre National de la Recherche Scientifique, Université Paris Descartes, Sorbonne Paris Cité, UMR8192, 45 rue des Saints-Pères, 75270 Paris cedex 06, France. [2]Inserm U932, Institut Curie, 12 rue Lhomond, 75005, Paris, France. [3]Institut Curie, Paris F-75248, France. [4]Inserm, U900, Paris F-75248, France. [5]Ecole des Mines ParisTech, Fontainebleau, F-77300 France. [6]INSERM U603, CNRS UMR 8154, Université Paris Descartes, Sorbonne Paris Cité, 45 rue des Saints-Pères, 75270 Paris cedex 06, France. [7]CNRS/UPR3212, INCI, Université Strasbourg,
Membrane fusion underlies several physiological processes including exocytosis of hormones and neurotransmitters. Fusion pore dynamics controls the release of vesicle content but is poorly understood. Here we show that the small GTPase Cdc42 controls the dilation of the fusion pore during exocytosis. We found that Cdc42 silencing in neuroendocrine cells had no effect on the probability of vesicle exocytosis but compromised the enlargement of the fusion pore. As a consequence, the mode of vesicle exocytosis was shifted from full-collapse fusion to kiss-and-run in Cdc42 knockdown cells. Remarkably, Cdc42 silencing reduced membrane tension and the artificial increase of membrane tension restored fusion pore enlargement in these cells. We conclude that membrane tension is the driving force for fusion pore dilation and that Cdc42 is a key regulator of this force.

POLARIZED SECRETION OF LYSOSOMES AT THE B CELL SYNAPSE COUPLES ANTIGEN EXTRACTION TO PROCESSING AND PRESENTATION. Maria-Isabel Yuseff[1,2], Anne Reversat[1,2], Danielle Lankar[1,2], Jheimmy Diaz[1,2], Isabelle Fanget[3], Paolo Pierobon[1,2], Stéphane Gasman[4], François Darchen[3], Claire Desnos[3] and **Ana-Maria Lennon-Duménil[1,2].** [1]Institut Curie, Centre de Recherche, Paris F-75248, France. [2]INSERM Unité 932, Immunité et

Cancer, Paris, France. [3]CNRS/Université Paris Descartes UMR8192, 45 rue des Saints-Pères, 75006, Paris, France. [4]CNRS/UPR3212, INCI, Université Strasbourg, France.

Engagement of the B Cell Receptor (BCR) by surface-tethered antigens (Ag) leads to formation of a synapse that promotes Ag uptake for presentation onto MHCII molecules. Here, we highlight the membrane trafficking events and associated molecular mechanisms involved in Ag extraction and processing at the B cell synapse. We show that MHCII-containing lysosomes are recruited at the synapse where they locally undergo exocytosis, allowing synapse acidification and the extracellular release of hydrolases that promote the extraction of the immobilized Ag. Lysosome recruitment and secretion results from the polarization of the microtubule-organizing center (MTOC), which relies on the Cdc42-downstream effector atypical Protein Kinase C (aPKCζ). aPKCζ is phosphorylated upon BCR engagement, associates to lysosomal vesicles and is required for their polarized secretion at the B cell synapse. Regulation of B lymphocyte polarity therefore emerges as a central mechanism that couples Ag extraction to Ag processing and presentation.

SYMPOSIUM "TRAFFIC OF SYNAPTIC RECEPTORS"
CENTER FOR AGING AND REGENERATION, P. UNIVERSIDAD CATOLICA DE CHILE

REGULATION OF NEURONAL DENDRITOGENESIS BY NMDA RECEPTORS AND THEIR UNDERLYING SCAFFOLDING AND SIGNALLING PROTEINS. **van Zundert, B**. Centro de Investigaciones Biomédicas (CIB), Fac. Ciencias Biológicas y Fac. Medicina, Universidad Andrés Bello, Santiago. bvanzundert@unab.cl

We study the basic cellular and molecular mechanisms underlying neuronal plasticity in an attempt to reactivate dendrite branch development in the adult injured brain to compensate for lost neuronal connections. Data from our laboratory indicate that dendritogenesis is dependent on the specific composition of the NMDA receptors and their interaction with particular scaffolding and signaling proteins located within the synapse. Thus, using a multidisciplinary approach that includes electrophysiology, molecular and cellular biology, and fluorescence techniques we discuss here recent data showing that during early development strong branch development is induced when NR2B-containing NMDA receptors are coupled to the signaling proteins RasGRF1 and CaMKII and maintained at the synaptic membrane by the scaffold protein SAP102. On the contrary, our data indicate that the dominated expression of the scaffold protein PSD95 (which act as a stop signaling) and the NR2A subunit (which has limited interaction with the signaling proteins RasGRF1 and CAMKII) in mature synapses both contribute to the restricted dendritogenesis in the adult CNS. We also discuss recent studies in which we are assessing which epigenetic mechanisms regulate PSD95 gene transcription with the aim to specifically suppress its expression in mature neurons and reactivate their dendrite branch development.

Acknowledgement: Funded by Fondecyt 1101012.

THE ROLE OF TROPHIC FACTORS AND TRAFFICKING MOLECULES IN ANTIDEPRESSANT ACTION. **Ursula Wyneken.** Laboratorio de Neurociencias, Universidad de los Andes, uwyneken@uandes.cl

Major Depressive Disorder (MDD) is a complex neuropsychiatric syndrome that affects ~16% of the population during their lifetime. MDD is treated primarily by antidepressant drugs (ADDs), that acutely elevate brain norepinephrine and serotonin levels. Even though decreased levels of such neurotransmitters may underlie MDD, compelling evidence suggests that antidepressant action is mediated by trophic factors that induce adaptive mechanisms in extensive neuronal forebrain networks involved in the control of depressive symptoms. Fundamental questions persist regarding the identity of trophic factors and the cellular mechanisms triggered by them. We had first focused on the ADD-induced signaling of brain-derived neurotrophic factor (BDNF) at glutamatergic synapses, where the BDNF/TrkB complex becomes endocytosed and potentially trafficks to the cell body, an idea that is supported by our observation of TrkB-containing multivesicular bodies in dendrites by electron microscopy. To further characterize signaling events, we have used proteomic analysis of several subcellular fractions. Surprisingly, protein networks that respond to other trophic molecules, such as Insulin-like-Growth Factor-1, have been identified in addition to a list of proteins involved in the trafficking and incorporation of glutamate receptors to the dendritic spine, such as SNAP-25. Indeed, the ADD fluoxetine induced significant changes in the subunit composition of glutamate receptors that favored stable, but less plastic spines. The mechanisms participating in the incorporation of specific subunits are being addressed by *in-utero* mediated gene transfer. In conclusion, ADDs induce the release of a growing list of neurotrophins, growth factors and signaling molecules that regulate glutamate receptor trafficking and incorporation to the synapse, thereby remodeling synaptic structure and efficacy.

Acknowledgements:DFG-Conicyt.

CaMKII TRIGGERS THE DIFFUSIONAL TRAPPING OF SURFACE AMPARs THROUGH PHOSPHORYLATION OF STARGAZIN. **Patricio Opazo** and Daniel Choquet. Institut Interdisciplinaire de NeuroScience IINS, Bordeaux, France.

The Ca^{2+}/Calmodulin-dependent protein kinase II (CaMKII) is critically required for the synaptic recruitment of AMPA-type glutamate receptors (AMPARs) during long-term potentiation (LTP) and memory. However, the underlying

mechanism is unknown. Using a single particle tracking approach, we found that CaMKII activation promote the phosphorylation of Stargazin, an AMPAR auxiliatory subunit, and the subsequent immobilization of the Stargazin-AMPAR complex. Using Fluorescence Lifetime Microspcopy (FRET-FLIM), we found that CaMKII phosphorylation trigger a conformational change in Stargazin that both release the c-tail of stargazin from a membrane-bound state and thus facilitate the interaction with PSD95, a synaptic scaffolding protein. We believe that this novel function of CaMKII in regulating the lateral diffusion of the Stargazin-AMPAR complex can fully account for its role in LTP and memory.

REGULATION OF KAR PLASTICITY AND TRAFFICKING BY CaMKII AND INTERACTION WITH PSD95. Mario Carta, Patrizio Opazo, Julien Veran, Daniel Choquet, Christophe Mulle and **Françoise Coussen.** Institut Interdisciplinaire de Neuroscience, UMR 5297 CNRS-Université de Bordeaux.
CaMKII is key for long-term potentiation of synaptic AMPA receptors. Whether CaMKII is involved in activity-dependent plasticity of other ionotropic glutamate receptors is unknown. We identify a novel form of spike-timing dependent depression (STDP-LTD) of kainate receptor-mediated responses at hippocampal mossy fiber synapses which depends on Ca^{2+} influx, activation of CaMKII, and on the GluK5, kainate receptor subunit (KAR). CaMKII phosphorylation of three residues in the C-terminal domain of GluK5 subunit promotes surface expression of KARs, but concomitantly decreases its synaptic content. CaMKII activation also markedly increases lateral mobility of KARs, possibly by decreasing the binding of GluK5 to PSD-95. We demonstrate that the direct phosphorylation of GluK5 by CaMKII is necessary for STDP-LTD. We propose that CaMKII-dependent phosphorylation of GluK5 is responsible for synaptic depression by untrapping of KARs from the PSD and increased diffusion away from synaptic sites.

SYMPOSIUM "FOCUS ON GASTRIC CANCER"
CENTRO DE ESTUDIOS MOLECULARES DE LA CELULA (CEMC), UNIVERSIDAD DE CHILE

TRANSCENDING CLASSIC PARADIGMS IN CANCER – E-CADHERIN IS A MOLECULAR SWITCH THAT DEFINES CAVEOLIN-1 FUNCTION *IN VIVO*. Andrew F. G. Quest. Laboratorio de Comunicaciones Celulares, Centro de Estudios Moleculares de la Célula (CEMC), Facultad de Medicina, Universidad de Chile. aquest@med.uchile.cl
Caveolin-1 is a scaffold protein that functions in some cancer cells as a tumor suppressor, yet promotes metastasis in others. The mechanisms explaining this ability of caveolin-1 remain largely undefined. Enhanced expression of cyclooxygenase2 (COX2) and survivin, a member of the Inhibitor of Apoptosis Protein (IAP) family, are frequently observed in human cancers where their presence is associated with increased tumor cell survival. Our previous studies revealed that caveolin-1 reduces COX2 and survivin expression via a b-catenin/Tcf-Lef-dependent transcriptional mechanism in a manner that requires the presence of E-cadherin. In cancer cells in culture, co-expression of caveolin-1 with E-cadherin reduces tumor cell proliferation and enhances apoptosis. In a pre-clinical C57BL6 mouse model, expression of caveolin-1 alone in B16F10 melanomas reduces subcutaneous tumor growth but dramatically increases metastasis to the lung, highlighting *in vivo* the ambiguity of caveolin-1 function in this model of cancer. Intriguingly, co-expression of E-cadherin with Caveolin-1 completely suppresses both tumor formation and metastasis to the lung. Thus E-cadherin functions as a "molecular switch" that determines Caveolin-1 function *in vivo*. These studies identify caveolin-1 as a member of a still rather select group of proteins that may function either as a tumor suppressor or promote metastasis depending on the cellular context, a view that contrasts dramatically with the classic vision of cancer as a disease dominated by either oncogenes or tumor suppressors. Understanding the underlying patterns in such functional ambiguity is likely to aid in our ability to develop more efficient treatments for cancer.
Acknowledgements: FONDECYT-FONDAP15010006, FONDECYT1090071 (AFGQ).

Stat3 PROVIDES A DRUGABLE SIGNALING NODE LINKING INFLAMMATION TO GASTROINTESTINAL CANCER. Matthias ERNST. Ludwig Institute for Cancer Research, Melbourne, VIC 3055, Australia.
Tumour promotion is often facilitated though interactions between neoplastic cells and the microenvironment that is characterized by abundance of inflammatory mediators, including those of the interleukin (IL-)6 family. While the molecular mechanisms linking inflammation and cancer may vary between different malignancies, ultimately most soluble mediators converge on NFkB and Stat3 in cells comprising the tumour and its microenvironment to enhance survival, and in the case of the Stat3, to also promote proliferation, invasion, and mediate an angiogenic switch.
IL-6 family cytokine are readily detected in various inflammation-associated pathologies, including gastrointestinal cancers, and mediate pleiotropic activities through a receptor complex comprising the shared, ubiquitously expressed signal-transducing gp130 receptor β-subunit. Using various combinations of loss- and gain-of-function alleles in mouse models of spontaneous, inflammation-associated gastric cancer (GC) and colitis-associated cancer (CAC), we established a novel link between IL-6 family cytokines, the gp130/Stat3 signaling axis, and tumour progression. GC and CAC lesions of gp130(Y757F) mutant mice, where ligand-dependent Stat3 hyper-activation mimics the smoldering inflammation observed

in corresponding human cancers (Nature Med 11:845). Tumour growth is attenuated in gp130(Y757F) mice lacking expression components of this gp130 signaling cascade. Meanwhile, tumour maintenance is susceptible to systemic administration of Stat3 or IL-6 cytokine family antagonists and GC formation is susceptible to therapeutic inhibition of excessive mTorc signaling that coincides with the aberrant Stat3 activation in the neoplastic epithelium of gp130(Y757F) mice.
The close physical proximity between IL-6 family cytokine producing cells in the tumour microenvironment and the responsive neoplastic epithelium suggests that inhibition of this signaling axis represents a novel therapeutic target for the treatment of inflammation-associated (gastrointestinal) cancers.

REPRIMO, A NOVEL BIOMARKER AND POTENTIAL TUMOR SUPPRESSOR GENE IN GASTRIC CANCER. **Alejandro Corvalán**, Dept. Hematology-Oncology, P. Universidad Católica de Chile.
Gastric cancer (GC) accounts for 8% and 10% of new cases and deaths, respectively by cancer worldwide. The combination of bacterial and environmental factors drives the dynamic pre-cancerous process. Non-invasive biomarkers to document this process are required. We identified differences in the methylation of circulating free-cell DNA (cfDNA) of the promoter region of Reprimo (RPRM), a p53-dependent G2 arrest mediator candidate, between GC vs healthy donors (HD). More recently, an in-house assay to quantify cfDNA of RPRM by MethyLight (Taqman), demonstrate 91.3% sensitivity and 92.9% specificity to distinguished GC vs HD. Next we tested cfDNA in GC patients under adjuvant chemotherapy finding 100% of correlation with clinical response. To investigate the functional role of RPRM we performed RT-PCR and sequence of coding as well as promoter region in five GC cell lines. Downregulation of RPRM and dense methylation in the promoter region was found in AGS & MKN-45 cell lines. No coding mutations were found in any cell line. Stable transfections of pCMV6 vector containing RPRM full-length coding region demonstrate 50% decrease in cellular proliferation and anchorage independent growth in both negative RPRM cell lines. Ability to migrate of both cell lines was also decreased. Taken together, RPRM not only should be considered a non-invasive biomarker for documenting precancerous process and monitoring clinical response but also a potential tumor suppressor gene in GC.
Grants Fondecyt 1111014 & Fondef D09i1137.

SYMPOSIUM "MEMBRANE TRAFFICKING AT SYNAPSES: FROM NEURONAL TRANSMISSION TO IMMUNITY (II)"

ROLE AND CONTROL OF THE FORMATION OF THE IMMUNOLOGICAL SYNAPSE IN T LYMPHOCYTES. Armelle Bohineust[1,2], Karine Chemin[1,2], Julien Husson[1,3], Marie Tourret[1,2], Stéphanie Dogniaux[1,2], Paola Larghi[1,2], Nelly Henry[1,3] and **Claire Hivroz[1,2].** [1]Institut Curie, Centre de Recherche, Paris F-75248, France. [2]INSERM Unité 932, Immunité et Cancer, Paris, France. [3]CNRS UMR 168, Paris, France.
Setting up an adaptive immune response requires the recognition by the TCR of MHC-peptides on the surface of antigen presenting cells (APC). This recognition requires a direct interaction between the two cell types and is accompanied by activation of the T lymphocyte and the APC, which are both necessary to mounting an efficient immune response. The interaction zone between T lymphocyte and APC is organized in time and space and has been called: the immunological synapse (IS). The formation of this zone favors the interaction and exchange of information between the two cell types. It is characterized by remodeling of the actin and microtubule cytoskeletons that leads to the polarization of the T lymphocyte toward the APC. We are studying the control of this polarity by signaling molecules, its role in cytokine secretion and activation of T lymphocytes and APC as well as the biophysical cues accompanying the formation of the IS. We will discuss the role of TCR signaling in the formation of the IS, the exquisite control by cytoskeleton remodeling of secretion events and how these latter control APC activation by T lymphocytes. We will finally discuss, how mechanical forces that build up during the formation of the IS might contribute to cross-talk between T lymphocytes and APCs.

Sec22b CONTROLS THE RECRUITMENT OF ENDOPLASMIC RETICULUM TO PHAGOSOMES IN DENDRITIC CELLS: FUNCTIONAL CONSEQUENCES FOR CROSS PRESENTATION AND PHAGOSOME MATURATION. Ignacio Cebrian[1], Geraldine Visentin[1], Nicolas Blanchard[2], Mabel Jouve[5], Alexandre Bobard[3], Catarina Moita[4], Jost Enninga[3], Luis F. Moita[4], Ariel Savina and **Sebastian Amigorena[1].** [1]Institut Curie, INSERM U932, Immunité et Cancer, 26 rue d'Ulm, 75248 Paris, Cedex 05, France. [2]Centre de Physiopathologie de Toulouse-Purpan INSERM UMR1043-CNRS UMR5282, Université de Toulouse, France. [3]Institut Pasteur; Dynamique des Interactions Hôte-Pathogène, Paris, France. [4]Cell Biology of the Immune System Unit, Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, 1649-028 Lisboa, Portugal. [5]Institut Jacques Monod, UMR7592 CNRS/Université Paris Diderot, ImagoSeine-Plateforme de Microscopie Electronique, France.
The role of the endoplasmic reticulum (ER) in phagocytosis and in antigen cross presentation has been a matter of sustained debate, at least in part because the molecular mechanisms underlying the proposed interactions between the two compartments were unclear. We now show that, in dendritic cells (DCs), the knock down of the ER-SNARE Sec22b -a

member of a wide family of proteins involved in intracellular membrane fusion- inhibits the recruitment of ER resident proteins to phagosomes and to the *Toxoplasma gondii* vacuole. In Sec22b knocked down cells, the cross presentation of ovalbumin (OVA) coated on latex beads, of soluble OVA or of OVA expressed in *Toxoplasma gondii* was compromised. In contrast, MHC class II and endogenous MHC class I-restricted presentations of the same antigen were not affected. The knock down of Sec22b also inhibited the export of endosomal cargo to the cytosol and caused a marked increase of phagosomal proteolysis. We conclude that Sec22b plays a critical role in the recruitment of ER membrane proteins to phagosomes in dendritic cells and that ER recruitment modifies phagosomal functions to promote cross presentation.

FROM HIV-1 INFECTION, TO PRODUCTION OF VIRAL PARTICLES AND CELL-TO-CELL TRANSMISSION VIA VIROLOGICAL SYNAPSES. Raphaël Gaudin[1,2], Stefano Berre[1,2], Bruna Cunha de Alencar[1,2], François-Xavier Gobert[1,2], Mabel Jouve[3] and **Philippe Benaroch[1,2].** [1]Institut Curie, Centre de Recherche, Paris F-75248, France. [2]INSERM Unité 932, Immunité et Cancer, Paris, France. [3]Institut Jacques Monod, UMR 7592, CNRS /Université Paris Diderot, 75013, Paris, France.

Macrophages are versatile cells present in most tissues and endowed with a variety of different functions, including in innate and adaptive immunity against pathogens. Macrophages are targets of HIV and implicated in HIV-1 pathogenesis. They are also long-lived reservoir of virus that appear resistant to drug treatments and to attacks from the immune system. Infected macrophages accumulate large internal vacuoles containing virus, the Virus-Containing Compartments or VCCs that are not found in infected T cells. Newly formed viral particles bud and pinch off at the limiting membrane of the VCC, thus accumulating virions in the lumen of the compartment. The exact nature of this compartment remains obscure and very little is known about the late stages of the viral cycle in macrophages.We will discuss our recent results on the biogenesis, the maturation, the transport and the probable function of the VCC in infected primary macrophages. We will also review the way macrophages and other HIV-infected cells, dendritic cells and T lymphocytes, can perform cell-to-cell transfer of viral particles through the so-called Virological Synapse.

SYMPOSIUM "AXONAL CELL BIOLOGY AND REGENERATION" NUCLEO MILENIO EN BIOLOGIA REGENERATIVA (MINREB), P. UNIVERSIDAD CATOLICA DE CHILE

NEUROTROPHIN TRAFFICKING IN AXON GROWTH. Rejji Kuruvilla, Daniel Bodmer, and Maria Ascano. Department of Biology, Johns Hopkins University, Baltimore, MD 21218.

Neurotrophins are target-derived growth factors that regulate neuronal survival and differentiation during development. Despite considerable progress in understanding functions of neurotrophins, how a limited repertoire of neurotrophins and their Trk receptors elicit a diverse array of neuronal responses remains a fundamental question. We previously showed that two distinct neurotrophins, Nerve Growth Factor (NGF) and Neurotrophin-3 (NT-3), activate the same TrkA receptor to promote sequential stages of axon growth in sympathetic neurons. NT-3 promotes early axon outgrowth, whereas NGF is required for innervation of final target tissues. How does a common TrkA receptor respond to two distinct neurotrophins to facilitate different phases of axon growth? We now show that NGF and NT-3 differentially employ the Ca^{2+}-responsive phosphatase, calcineurin, to promote axonal growth. Conditional deletion of *calcineurin* in mice disrupts NGF-dependent innervation of peripheral target tissues, while NT-3-mediated stages of proximal axon growth remain normal. We identified the endocytic GTPase, dynamin1, as a local target of calcineurin signaling in axons that is critical for NGF-mediated axonal growth, in a manner independent of transcription. Calcineurin interacts specifically with dynamin1 splice variants containing a PxIxIT interaction motif. Dephosphorylation of PxIxIT-containing dynamin isoforms is selectively required for NGF-dependent TrkA internalization and axon growth. Thus, our results point to critical differences in the mechanisms by which two neurotrophins, NT-3 and NGF, act on a common TrkA receptor to promote axonal growth during sympathetic nervous system development.

AXONAL RETROGRADE KILLING BY NEUROTROPHINS. Francisca C Bronfman and Claudia Escudero. Millennium Nucleus in Regenerative Biology (MINREB), Facultad de Ciencias Biológicas. Pontificia Universidad Católica de Chile, Chile.

During development of the sympathetic nervous system, it is well established that NGF/TrkA survival signaling competes with BDNF/p75 death signaling to modulate the number of sympathetic neurons and their target innervation. The functional significance of intracellular trafficking of NGF/TrkA in this process is well established; however, less is known about the functional significance of the internalization and trafficking of p75 in the cell body and in axons. The aim of our work was to study the mechanism of internalization and the post-endocytic trafficking of p75 in sympathetic neurons where p75 plays a key role in developmental-regulated cell death. We will discuss our resent finding related to the interesting particularities of p75 trafficking in neurons, the role of the c-Jun animo terminal kinase (JNK) in this process and the cellular mechanism that govern the retrograde killing of neurons after p75 activation in distal axons.

FONDECYT (1885273), MINREB (P07/011-F), FONDAP-Biomedicine 13980001 and CARE PFB 12/2007.

MICROTUBULE TETHERING BY DYNEIN AND NCAM FACILITATES SYNAPTIC STABILIZATION. **Eran Perlson[1,2]**, Adam G. Hendricks[1], Jacob E. Lazarus[1], Mariko Tokito[1], Yale E. Goldman[1] & Erika L. F. Holzbaur[1]. [1]University of Pennsylvania School of Medicine, [2]Tel-Aviv University Sackler Faculty of Medicine.

The intracellular motor protein cytoplasmic dynein drives the long-distance transport of vesicles and organelles along neuronal axons. Defects in the function of dynein or its activator dynactin lead to neurodegenerative disease in human patients and mouse models. Here, we identify a novel interaction between the neuronal cell adhesion molecule NCAM180 and dynein, suggesting an analogous and potentially conserved mechanism in eukaryotes whereby dynein, anchored to the cell cortex by NCAM180, transiently tethers microtubule plus ends. In vitro modeling of this interaction using an optical trap to position dynein-bound beads near microtubule plus-ends shows dynein-specific tethering leads to enhanced microtubule stability. In live cell assays, microtubule plus-ends are transiently tethered by dynein recruited to the cortex by NCAM180, leading to enhanced cell-cell adhesion. In vivo, NCAM180 is localized to sites of cell-cell interaction, notably the synapse. Disruption of the dynein-NCAM180 interaction in primary neurons leads to synaptic loss. A mouse model expressing a mutation in the dynein heavy chain exhibits a reduced interaction between NCAM180 and dynein that correlates with synaptic destabilization at the neuromuscular junction. These data support a model in which the dynamic, dynein-mediated tethering of microtubules at the cortex facilitates cell-cell interactions leading to enhanced synaptic stability, and delineates a mechanistic interaction between the microtubule cytoskeleton and the extracellular environment that can facilitate directed intracellular trafficking or enhance intercellular associations.

SYMPOSIUM "A BUMPING TOUR UNRAVELING THE MAGICAL MYSTERIES OF THE SECRETORY PATHWAY" FONDECYT (1100027 AND 1100896), UNIVERSIDAD AUSTRAL DE CHILE

MEMBRANE PROTEIN BIOSYNTHESIS AND QUALITY CONTROL. **Ramanujan Hegde,** MRC Laboratory of Molecular Biology Cambridge, United Kingdom, CB2 0QH.

Insertion of proteins into biological membranes is a fundamental process vital to all organisms. To understand this process in molecular detail, we have been studying the insertion pathway of tail anchored (TA) membrane proteins. These are relatively simple membrane proteins defined by a cytosolic-facing N-terminal domain followed by a single C-terminal transmembrane domain (TMD). Examples of TA proteins are found on essentially all cellular membranes in every organism and have diverse functional roles ranging from intracellular trafficking to regulation of cell death. Despite this widespread importance, the machinery and mechanisms underlying the recognition, targeting, and insertion of TA proteins into the correct organellar membrane long remained obscure. This talk will discuss recent studies from my and other labs describing the discovery and functional analysis of the tail-anchored insertion machinery. These studies have revealed in considerable detail how membrane proteins are recognised, how they are shielded in the cytosol during their targeting to the membrane, and how they are then inserted into the lipid bilayer. More recent studies have revealed that when these targeting and insertion reactions fail, quality control mechanisms rapidly ubiquitinate and degrade the failed insertion products. The general principles that emerge from these studies of membrane protein insertion and quality control will be discussed.

SIGNAL-ADAPTOR INTERACTIONS THAT MEDIATE POLARIZED SORTING IN NEURONS. **Juan S. Bonifacino.** Cell Biology and Metabolism Program, Eunice Kennedy Shriver, National Institute of Child Health and Human Development, National Institute of Health, Bethesda, Maryland, USA

Neurons are anatomically and functionally polarized cells that conduct nerve impulses in a vectorial fashion. Impulses are received by dendrites, propagated through the soma, and eventually transmitted to other cells by axons. The plasma membrane of each of these neuronal domains has a distinct protein composition, but the mechanisms responsible for this differential distribution remain poorly understood. By analogy to other protein sorting processes, we hypothesized that biosynthetic delivery of transmembrane proteins to different neuronal domains could be mediated by interaction of sorting signals in the cargo proteins with adaptor proteins that are components of protein coats. Indeed, we found that a tyrosine-based sorting signal in the cytosolic domain of the transferrin receptor (TfR) mediates sorting of this protein to the somatodendritic domain in both hippocampal and cortical neurons. This signal binds to the m1A subunit of the clathrin-associated, heterotetrameric adaptor protein 1 (AP-1) complex. Overexpression of a dominant-negative m1A mutant incapable of binding signals, or RNAi-mediated depletion of another subunit of the AP-1 complex, g-adaptin, resulted in missorting of the TfR to the axon. Similar means of interference with clathrin or other components of the AP-1 interactome caused axonal missorting of the TfR. Various microscopic techniques revealed that sorting of the TfR occurs at AP-1-coated areas of the TGN through exclusion of the TfR from transport carriers bound for the axon. These findings demonstrate that interactions of sorting signals with AP-1 mediate clathrin-dependent sorting of the TfR, and likely other cargo, to the

neuronal somatodendritic domain. Together with recent observations in epithelial cells, our findings support the notion that AP-1 is a global regulator of polarized sorting.

NIPPED IN THE BUD: THE STRANGE WAYS THAT ESCRTS SEVER MEMBRANES. **James H. Hurley**, Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, U. S. Department of Health and Human Services, Bethesda, MD 20892.
The ESCRT complexes catalyze one of the most unusual membrane remodeling events in cell biology. ESCRT-mediated membrane neck cleavage is critical for the biogenesis of multivesicular bodies (MVBs), key intermediates in lysosomal sorting; cytokinesis; and the detachment of HIV-1 and other enveloped viruses from the plasma membrane of infected cells. Moreover, ESCRTs are responsible for ubiquitinated cargo sorting and membrane budding into multivesicular bodies. The biogenesis of MVBs was reconstituted and visualized using giant unilamellar vesicles, fluorescent ESCRT-0, I, II, and III complexes, and a membrane-tethered fluorescent ubiquitin fusion as a model cargo. ESCRT-0 forms domains of clustered cargo but does not deform membranes. ESCRT-I and II in combination deform the membrane into buds, in which cargo is confined. ESCRT-I and II localize to the bud necks, and recruit ESCRT-0-ubiquitin domains to the buds. ESCRT-III subunits localize to the bud neck and efficiently cleave the buds to form intralumenal vesicles. Intralumenal vesicles produced in this reaction contain the model cargo but are devoid of ESCRTs. The observations explain how the ESCRTs direct membrane budding and scission from the cytoplasmic side of the bud without being consumed in the reaction. Among the upstream human ESCRT complexes 0-II, ESCRT-I is unique in its ability to function at both endosomes and the plasma membrane. We have characterized a novel domain in ESCRT-I responsible for binding acidic lipids in both endosomes and the plasma membrane. This domain appears to confer on ESCRT-I the ability to localize to and function at distinct membrane with different lipid compositions.

SYMPOSIUM "IMMUNOLOGICAL BASES OF THE INFLAMMATION AT THE CENTRAL NERVOUS SYSTEM"
INSTITUTO MILENIO DE INMUNOLOGIA E INMUNOTERAPIA, UNIVERSIDAD NACIONAL ANDRES BELLO

NOVEL CELLULAR COMMUNICATION SYSTEMS IN HIV INDUCED NEUROINFLAMMATION AND TOXICITY. **Eugenin, Eliseo**. Department of Pathology, Albert Einstein College of Medicine, Bronx, New York, NY, 10461, USA.
HIV entry into the central nervous system (CNS) is an early event after peripheral infection, resulting in neurological dysfunction in a significant number of individuals. As people with AIDS live longer, the prevalence of cognitive impairment is increasing, despite antiretroviral therapy. The mechanisms that mediate CNS dysfunction are still not well understood. However, this damage includes inflammation, viral reservoirs and unknown mechanism of amplification. We demonstrated that gap junctions in astrocytes play a key role in amplification of inflammation and toxicity within the CNS, damage to BBB integrity and neuronal function. Our findings describe a novel mechanism of toxicity within the brain, triggered by low numbers of HIV-infected astrocytes and amplified by gap junction contributing to the pathogenesis of NeuroAIDS.

MATERNAL THYROID HORMONE DEFICIENCY INCREASES CENTRAL NERVOUS SYSTEM DAMAGE IN THE OFFSPRING SUFFERING EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS. **Claudia Riedel**, Universidad Andrés Bello.
Thyroid hormone deficiency is a highly prevalent condition in women. It has been shown that the human offspring gestated in mothers that suffer from thyroid hormone deficiency have low intellectual coefficient (IQ). Animal models that resemble this condition have shown impairment in learning, reduction in neuronal branch and myelin. Moreover, it has shown that these mice have altered the number of T and B lymphocytes. These facts rouse us to propose that thyroid hormone deficiency during gestation will imprint in their offspring a higher susceptible to acquire inflammation at the central nervous system (CNS). We will show that the experimental autoimmune encephalomyelitis (EAE), a model for multiple sclerosis, induces a more severe disease in those mice gestated in mothers with thyroid hormone deficiency.

IMMUNE SIGNALING IN NEURONAL DEVELOPMENT. **Helene Boudin**, Inserm 643, Nantes, France.
Molecules originally characterized in the immune system constitute a novel class of brain molecules that have distinct nonimmune functions in the central nervous system (CNS). In particular, members of the major histocompatibility complex class I (MHC I) and their related receptors and signaling molecules are expressed in neurons and have been shown to play critical roles in the development and plasticity of the CNS. These findings have led to the hypothesis that in the brain immunoreceptors serve a related, but evolutionary older function in the formation and maturation of neuronal network and in neuronal synaptic signaling. We have focused our work on one such molecule, CD3ζ. CD3ζ , a transmembrane adaptor

signaling protein first characterized in T cells as a component of the CD3ζ complex. Our data suggest a non-immune function of CD3ζ seems to be related with dendritic arborization, controlling neuronal outgrowth and synapsis establishment.

MECHANISMS OF LEUKOCYTE TRANSMIGRATION ACROSS THE BLOOD BRAIN BARRIER: IMPLICATIONS FOR NEUROAIDS AND OTHER CNS INFLAMMATORY PROCESSES. **Joan W. Berman**, Ph.D., Departments of Pathology and Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY, USA.

HIV enters the CNS early after peripheral infection, resulting in damage that causes cognitive impairment and other neurological manifestations in a large number of infected individuals. The virus gains access to the CNS, in part, by the transmigration of HIV infected monocytes across the blood brain barrier (BBB). This results in infection of CNS macrophages/microglia and of some astrocytes, as well as in ongoing inflammation. Despite successful combined antiretroviral therapy (cART), low level virus and inflammatory cells persist within the CNS and this chronic inflammation results in CNS dysfunction. Thus, prevalence of the resultant cognitive impairment is increasing, and NeuroAIDS is among the most urgent public health concerns worldwide.

Our laboratory identified and characterized a subpopulation of monocytes that preferentially transmigrates in very large numbers across the BBB. We also demonstrated that these cells are highly susceptible to HIV infection, and that HIV infection of monocytes results in dysregulation of surface protein expression that facilitates transmigration. We showed that the chemokine CCL2 is critical to transmigration of HIV infected cells across the BBB. We also identified a biomarker of CNS cognitive impairment that is shed from these infected cells. Our findings will be discussed in the context of potential sites of therapeutic intervention to target CNS inflammation in the HIV infected population in the current ART era.

ORAL PRESENTATIONS

ORAL PRESENTATIONS I

***In vivo* ANALYSIS OF CELLULAR TOXICITY MECHANISMS OF ALPHA-SYNUCLEIN AGGREGATES AND POTENTIAL THERAPEUTIC DRUGS IN *Caenorhabditis elegans*.** A. Minniti[4], I. Alfaro[2], S. Bernales[2,3] and **R. Aldunate[1]**. [1]Escuela de Biotecnología, Universidad Santo Tomás, [2]Fundación Ciencia para la Vida, [3]Medivation Inc., [4]Fac. Ciencias Biológicas, P. U. Católica de Chile.

Introduction: *C. elegans* models of age-associated neurodegenerative diseases have provided several meaningful insights into the understanding of age-associated disease conditions. We employed transgenic nematode models to understand the mechanistic aspects involved in the prolonged time course of aggregation of alpha-synuclein (a-S) as one of the pathological hallmarks of Parkinson's disease.

Material and Methods: In this study, we used two transgenic *C. elegans* models, one expressing the a-synuclein::YFP human peptide in muscle cells, and a second one that expresses the Ab_{1-42} human peptide in muscle cells. We characterized the density and morphology of (a-S) aggregates, the morphological effects in the muscle cell structure, the locomotory behavior and neuromuscular synaptic transmission, and evaluated the effects of an experimental neuroprotective drug for Alzheimer´s (AD) and Parkinson´s diseases (PD), FCVM100.

Results: The *C. elegans* strain expressing the a-synuclein::YFP human protein forms protein aggregates in body-wall muscle cells and presents severe locomotory impairments and defective neuromuscular synaptic transmission as has also been observed in Ab strains. We developed an assay to evaluate the effects of a drug candidate for AD and PD in the corresponding transgenic strains by means of the analysis in locomotory behavior. The results showed that the tested drug was able to reduce the toxicity of the peptide aggregation only in a-synuclein transgenic animals.

Discussion: The use of *C. elegans* models allow us to integrate pharmacological information and examine the mechanisms involved in the drug action in different neurodegenerative disease.

Financial Support Internal Proyect UST to RA.

UNDERSTANDING THE NETWORK BETWEEN Th17 CELLS, REGULATORY T CELLS AND MYELOID DERIVED SUPPRESSOR CELLS IN THE TUMOR MICROENVIRONMENT. **Sarah Núñez[1]**, Juan José Sáez[1], Mario Rosemblatt[1,2], María Rosa Bono[1] and Daniela Sauma[1,2]. [1]Departamento de Biología, Facultad de Ciencias, Universidad de Chile; [2]Fundación Ciencia para la Vida. sarah.nunez.c@gmail.com

Introduction: Tumor evasion of effector T cell responses is mediated in part through tumor-infiltrating regulatory T cells (Treg) and myeloid derived suppressor cells (MDSC) which possess immunosuppressive functions. The T helper subset Th17 has been found in human and mouse tumors and it has been proposed that this subset could promote tumor progression. A possible role for Th17 cells in the recruitment and function of Treg and MDSC in the tumor environment has not been fully studied.

Materials and Methods: We addressed Th17 function using the knock-in mouse strain $ROR\gamma t^{GFP/GFP}$ lacking Th17 cells, and also blockade of signature Th17 effector cytokine IL-17 using antibodies. Mice were injected with B16.F10 melanoma cells and tumor growth was measured. Treg and MDSC cells present in the tumor, and their immunosuppresive activity, were analyzed by flow cytometry.

Results: $ROR\gamma t^{GFP/GFP}$ mice as well as anti-IL-17 treated mice have enhanced tumor growth compared with control C57BL/6 mice. However, the percentage of Treg and MDSC in the tumor is not altered. Presently, we are evaluating the effect of Th17 cells on Treg and MDSC function. We have observed that Treg from $ROR\gamma t^{GFP/GFP}$ mice have similar expression levels of functional markers as in C57BL/6 mice, and are now analyzing the ability of Treg and MDSC from $ROR\gamma t^{GFP/GFP}$ mice to suppress T cell proliferation.

Discussion: Our results suggest that Th17 cell function contributes to antitumor immunity.

FONDECYT 3100077, 1100557 and 1100448.

MEGALIN PROTEOLYTIC PROCESSING AND PHOSPHORYLATION IS AFFECTED BY OCRL1 DOWN REGULATION. **Lisette Sandoval[1]**, Antonella De Matteis[2] and María Paz Marzolo[1]. [1]Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile. [2]Telethon Institute of Genetics and Medicine, 80131 Naples, Italy. mmarzolo@bio.puc.cl

Introduction: Megalin is an endocytic receptor for several ligands expressed at the apical side of epithelial renal cells. Megalin´s function is controlled in different ways, including phosphorylation and proteolytic processing. This receptor is phosphorylated regulating the receptor cell surface levels. Megalin experiences ectodomain shedding and this has been suggested as responsible for the presence of soluble Megalin in the urine. Both modifications in Megalin have functional consequences in kidney and are reflected by defects in the endocytosis and degradation of the receptor´s ligands, producing low molecular weight proteinuria. In Lowe Syndrome, a disease in which there are mutations in the gene encoding a 5-phosphatidil inositol phosphatase (OCRL1), the patients' urine have proteinuria of low-molecular weight proteins and selectively reduced presence of Megalin. So, our interest is to evaluate the role of OCRL1 in the regulation of Megalin function.

Material and Methods: We have evaluated Megalin secretion by western blot and phosphorylation by metabolic labelling, in kidney epithelial cells knock-down (KD) for OCRL1.
Results: We report that cells KD for OCRL1 show decreased Megalin phosphorylation. Moreover we observed that secreted Megalin, lacking its C-terminal domain, is significantly decreased in OCRL1 KD cells.
Discussion: These results suggest an important role of OCRL1 in the function of Megalin that could explain the loss of ligands of this receptor in the urine of Lowe Syndrome patients concomitantly with a decrease of soluble Megalin in this fluid.
Supported by CONICYT AT24100200, MINREB and Fondecyt 1110382.

REPROGRAMMING OF A HUMAN CARCINOMA CELL LINE BY ZEBRAFISH EMBRYONIC MICROENVIRONMENTS. **Leonel Muñoz,** Germán Reig, and Miguel Concha. Laboratory of Experimental Ontogeny - LEO, ICBM, Faculty of Medicine, University of Chile; and Biomedical Neuroscience Institute, Santiago, Chile. lamunoz@med.uchile.cl; mconcha@med.uchile.cl
Introduction: Reprogramming is the reacquisition of the differentiation potentiality by a differentiated cell. In this process, cell stemness can be acquired when induced to an epithelial-to-mesenchymal transition (EMT), giving the ability to go through a new ontogenic process. In this study, we explored whether zebrafish embryonic microenvironments are able to reprogram human carcinoma cells, and if this process is associated to EMTs.
Material and Methods: Approximately 100 cells of the human colon cancer cell line SW-620 were implanted into zebrafish blastula embryos. The expression of representative transcription factors of undifferentiated (Oct4, Sox2), differentiated (Cdx2), and EMT (E-Cadherin, Snail and Slug) states were quantified through qPCR with human-specific primers, at 24 and 48 hours post implantation (hpi), and normalized to the expression in culture.
Results: Oct4 showed 20 and 30 fold increase at 24 and 48 hpi respectively. Expression of sox2 did no significantly change at 24 hpi but decreased in 50% at 48 hpi. Cdx2 showed a 50% decrease at 24 hpi, and was almost undetectable at 48 hpi. E-Cadherin showed 1.5 fold increase at 24 hpi, whereas Snail and Slug had no variation compared with the relatively high levels in culture.
Discussion: It was intriguing that Oct4 increase was not paralleled by Sox2, as both form part of an auto-regulatory loop. Decrease in Cdx2 favours a process of reprogramming. Null variation of the EMT inductors, and the increase of E-cadherin, blurs the association to EMTs in this model.
Grant sponsors: HHMI, ICM (P07-048-F; P09-015-F).

LACONIC, A GENETICALLY ENCODED FRET NANOSENSOR FOR LACTATE. [1,2]**Alejandro San Martín**, [1,2]Sebastián Ceballo, [3]Wolf B. Frommer and [1]L. Felipe Barros. [1]Centro de Estudios Científicos (CECs), Valdivia, [2]Universidad Austral de Chile, Valdivia, & [3]Carnegie Institution of Wahington. aalejo@cecs.cl
Introduction: Lactate is an intermediate metabolite of great interest for biomedicine, biotechnology and industry. Existing methods to measure lactate require destruction of the sample and/or need to consume lactate for the measurement. None is capable of detecting lactate with cellular resolution.
Materials and Methods: Fusion proteins were generated by homologous recombination, including a bacterial template and two fluorescent proteins.
Results: Studied *in vitro*, several constructs displayed sizable changes in FRET efficiency upon exposure to lactate. The best one showed a maximum FRET change of 13%. The sensor did not respond to millimolar levels of pyruvate, acetate or glutamate. Expressed in mammalian cells, the sensor allowed single cell measurement of lactate concentration.
Discussion: The concentration, production and transport of lactate are parameters of relevance for various areas of biomedicine, including body weight control, brain energy metabolism, ischemic disease, cancer metabolism and infectious disease. Lactate is also of interest for food and detergent industry. Used *in vitro*, Laconic was 10-times more sensitive than the best commercially-available lactate kit. Expressed in living cells, Laconic represents a new tool for monitoring intracellular or subcellular lactate in real-time.

RUNX2 PROMOTES MIGRATION AND INVASION IN HUMAN OSTEOSARCOMA CELL LINES. **Óscar Vega[1,2],** Claudia Lucero[1,2], Julio Tapia[2], Marcelo Antonelli[2], Mercedes Lopez[1,3], Flavio Salazar-Onfray[1,3], Gary S Stein[4], Andre van Wijnen[4], Mario Galindo[1,2]. [1]Millennium Institute on Immunology and Immunotherapy, [2]Programa de Biología Celular y Molecular, [3]Prode Inmunología, ICBM, Facultad de Medicina, Universidad de Chile. [4]Department of Cell Biology and Cancer Center, University of Massachusetts Medical School, USA. mgalindo@med.uchile.cl
Introduction: Runx2 transcription factor regulates expression of bone phenotypic genes which are essential for normal osteoblast differentiation. In the other hand, osteosarcoma (OS) cells exhibit an aberrantly increased expression of Runx2. Also, there is a positive correlation between high Runx2 levels and metastasis in OS. We propose that Runx2 promotes tumor progression in osteosarcoma by enhance cell migration and invasion.
Material and Methods: mRNA and protein levels for Runx2 and markers related to tumor progression were assessed by RT-PCR and western blot in six human osteosarcoma cell lines which exhibit differents metastatic potential. We tested the

functional role of Runx2 to enhance cell migration and invasion of OS cells by modulated Runx2 expression using adenoviral vectors and siRNA.
Results: Our results reveal that Runx2 depletion in OS cell lines significantly diminishes cellular migration and invasion. Consequently, over-expression of Runx2 in OS cell lines results in an increase cellular migration and invasiveness.
Discussion: We proposed that Runx2 promotes tumor progression by enhancing cellular migration and invasion in OS.
Grant sponsors: Fondecyt 1095234 and Iniciativa Científico Milenio P09-016-F.

ROLE OF SYNDECAN-4 IN WNT/PLANAR CELL POLARITY PATHWAY DURING MOUSE DEVELOPMENT. **Noelia Escobedo[1],** Marjorie Farías[1], Osvaldo Contreras[1], Hector Carrasco[1], Uyen Tran[2], Oliver Wessely[2], Andrew Copp[3] and Juan Larraín[1]. [1]Center for Aging and Regeneration, Millennium Nucleus for Regenerative Biology, Faculty of Biological Sciences, P. Universidad Católica de Chile; [2]Department of Cell Biology, Louisiana State University, Health Sciences Center, EEUU; [3]Institute of Child Health, University College London,UK. jlarrain@bio.puc.cl
Introduction: Non-Canonical Wnt/Planar Cell Polarity (PCP) pathway in mammals regulates cell polarity, convergent-extension movements and oriented cell division. Analyses of mice mutants for PCP core genes have revealed a growing list of new PCP phenotypes as neural tube formation, polarization of the sensory epithelia in the cochlea, among others. Previously, we demonstrated that Syndecan-4 (Sdc4), a cell-surface heparan sulfate proteoglycan, regulates convergent-extension and neural tube closure in *Xenopus*. This suggests interaction between Sdc4 and PCP pathway.
Methods: By *in-situ-hybridization,* immunofluorescence, scanning electron microscopy and Sdc4-null/LacZ *knock-in* mice we studied the expression and function of Sdc4 during mouse development. To study genetic interaction between Sdc4 and PCP we used the $Vangl2^{Lp/+}$ mice.
Results: Sdc4 has a dynamic expression pattern including tissues that requires PCP pathway, overlapping temporally and spatially with Vangl2 and Frizzled-3 in neurulation. The cochlea phenotype in $Sdc4^{LacZ/LacZ}$ embryos shows asymmetry in the hair cells of the organ of Corti and additional sensory hair cells outside of the sensory epithelia, a classical PCP phenotype. Genetic interaction with Vangl2 suggests that Sdc4 cooperates with PCP during neurulation. Additionally, Syndecan -1 and -3 are up-regulated in Sdc4-/- MEFs suggesting redundancy between syndecans in Sdc4-/- mice.
Discussion: Our studies will contribute to the understanding of the interaction between extracellular matrix components and PCP pathway signaling.
Grant Sponsors: Beca Apoyo de Tesis (AT24090074) CONICYT; FONDECYT (1100471).

TESTOSTERONE INCREASES GLUCOSE UPTAKE THROUGH GLUT4 AND CaMKII/AMPK PATHWAY IN RAT CARDIOMYOCYTES. **Carlos Wilson,** Ariel Contreras, Katherine Montoya, Rodrigo Maass and Manuel Estrada. Facultad de Medicina, ICBM, Universidad de Chile. carloswr@gmail.com
Introduction: Metabolic changes are crucial adaptations during the development of cardiac hypertrophy, mainly to supply high contractile and growth demands under this condition. Testosterone induces cardiac hypertrophy; however, their metabolic effects in the heart are unknown. In this work, we study the effect of testosterone on glucose uptake through glucose transporter 4 (GLUT4) and the CaMKII/AMPK pathway in cardiomyocytes.
Materials and Methods: All experiments were performed in primary cultures of rat cardiomyocytes. Glucose uptake was determined after testosterone stimulation (100nM) by both fluorescent glucose 2–NBDG and ^{3}H-glucose. Epifluorescence microscopy and liquid scintillation counter were used, respectively. Cardiomyocytes were transfected with the GLUT4myc-eGFP plasmid to determine GLUT4 cell distribution, after stimuli. GLUT4myc-eGFP migration was visualized by confocal microscopy. Total and phospho-protein levels were determined by western blots. Images were analyzed with ImageJ. For statistical analyses ANOVA and Bonferroni post-test were used.
Results: Testosterone increased glucose uptake in cardiomyocytes, which was blocked by indinavir, a specific GLUT4 blocker. Testosterone increased phosphorylation of both CaMKII and AMPK in time-course manner. KN-93 and compound C (CaMKII and AMPK inhibitors, respectively) blocked both glucose uptake and GLUT4 cell surface increase induced by testosterone. Moreover, CaMKII inhibition blocked AMPK phosphorylation, suggesting that CaMKII upregulates AMPK activation.
Discussion: These results show that testosterone increase glucose uptake through GLUT4 and CaMKII/AMPK pathway in cardiomyocytes. Glucose uptake through these intermediaries could represent a mechanism by which testosterone increases energy production for cell growth, hypertrophy or other anabolic processes in cardiomyocytes,
FONDECYT 1090276.

ORAL PRESENTATIONS II

DEVELOPMENT OF NEW BIOINFORMATICS TOOLS FOR THE ACCURATE PREDICTION OF TRANSCRIPTION FACTOR BINDING SITES. Tomás Norambuena, Alex W. Slater and **Francisco Melo.** [1]Molecular Bioinformatics Laboratory, Millennium Institute on Immunology and Immunotherapy, Alameda 340, Santiago, Chile. [2]Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Alameda 340, Santiago, Chile. fmelo@bio.puc.cl

Introduction. All currently existing computer software to predict transcription factor (TF) binding sites in whole genomes is based on sequence motifs and rely on sequence alignment. The major drawback of these methods is their poor sensitivity/specificity trade off. In practical terms, such software gives as a result either few true positives or many false positive predictions. Our working hypothesis is the following: "To improve the accuracy in the prediction of TF binding sites, structural information at the atomic level is required".

Material and Methods. We have developed a series of new bioinformatics tools to build full atom 3D models of protein-DNA complexes; 2) estimate the binding constant of protein-DNA complexes; 3) identify the target DNA sequences that would be recognized by a given TF; and 4) perform an efficient full genome search of TF binding sites.

Results. The accuracy of the predictions obtained with these new bioinformatics tools will be demonstrated by a comparison with known experimental data for different TFs that include Helix-Turn-Helix, Leucine Zippers and Zinc Fingers domains.

Discussion. The advance in the development of accurate structure-based bioinformatics tools for the prediction of TF binding sites may have a large impact in understanding how gene expression is regulated, the development of new cancer therapies and the diagnosis of several diseases.

Acknowledgements. This work was funded by grants from FONDECYT (1110400) and ICM (P09-016-F).

ACUTE TREATMENT WITH ANTI-NEOPLASTIC DRUGS INDUCED CAVEOLIN-1 UP-REGULATION AND INCREASED MIGRATION VIA A MEK/ERK-DEPENDENT PATHWAY IN COLON CANCER CELLS. **Díaz-Valdivia, N.,** Leyton, L., Quest, AFG. CEMC, Facultad de Medicina, Universidad de Chile. aquest@med.uchile.cl

Introduction: Chemotherapy is the main treatment of cáncer. Often, tumor cells develop multidurg resistance, wich reduce the effectivity of the treatment. Caveolin-1 (Cav-1) is a membrane protein that plays an ambiguous role in tumor development and progression. Consistent with function as a tumor suppressor, reduction of Cav-1 levels in normal cells promotes cell transformation and re-establishing levels of Cav-1 is sufficient to revert the transformed phenotype. Alternatively, in later stages of cancer, augmented Cav-1 levels are associated with metastasis and multidrug resistance. However the mechanisms that regulate the re-expression of Cav-1 in cancer cells remain unclear. Here we investigated mechanisms responsible for Cav-1 up-regulation induced by the anti-neoplastic drugs Methotrexate and Etoposide and how these changes affected cell migration.

Methodology: DLD1 colon adenocarcinoma cells were treated with Methotrexate (MTX) or Etoposide (ET). Expression of Cav-1 was analyzed by RT-PCR and Western blotting. Cells pre-treated with the MEK inhibitor (PD98059) or the anti-oxidant Trolox prior to anti-neoplastic drugs exposure were also analyzed. MAPK/ERK phosphorylation and ROS levels were determined by Western blotting and flow cytometry, respectively. DLD1 migration was evaluated in transwell assays.

Results: In DLD1 cells, MTX and ET treatment augmented Cav-1 expression and their migratory capacity via pathways requiring formation of ROS and MAPK/ERK activation.

Conclusions: MTX and ET promote Cav-1 expression, which is associated with augmented migratory capacity, a property characteristic of metastatic cells.

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TMBIM3/GRINA IS A CONSERVED UNFOLDED PROTEIN RESPONSE (UPR) TARGET GENE THAT CONTROLS APOPTOSIS THROUGH THE MODULATION OF ER CALCIUM HOMEOSTASIS. **Diego Rojas-Rivera,[1,2,3]** Ricardo Armisén,[2] Alicia Colombo,[3] Gabriela Martínez,[1,2,3] Diego Rodríguez,[1,2,3] Rosario Rizzuto,[5] Geert Bultynck,[4] Miguel L. Concha,[3] Jimena Sierralta,[3] Andrés Stutzin,[1,2] Claudio Hetz [1,2,3]. [1]Neurounion Biomedical Foundation, Santiago, Chile, [2]Center for Molecular Studies of the Cell, Institute of Biomedical Sciences, University of Chile, [3]Biomedical Neuroscience Institute, Faculty of Medicine, University of Chile, [4]Katholieke Universiteit ***Leuven,*** Belgium and [5]University of Padova, Italy. diegorojasrivera@gmail.com

Introduction: TMBIM6, also known as BAX-inhibitor 1 (BI-1), is an anti-apoptotic protein that belongs to a putative family of highly conserved and poorly characterized genes. Here we report that TMBIM3/GRINA expression specifically control cell death by ER stress-related stimuli *in vitro* and *in vivo*.

Material and Methods: *tmbim3* mRNA levels were evaluated by qPCR. ER Calcium levels were evaluated by $^{45}Ca^{2+}$ and ER-aquorine measurement. RNAi system expression and morpholine were used in *D. melanogaster* and zebrafish, respectively.

Results: *tmbim3* mRNA levels are strongly upregulated in cellular and animal models of ER stress, controlled by PERK signaling branch of the UPR. TMBIM3/GRINA synergies with TMBIM6/BI-1 in the control of ER calcium homeostasis and apoptosis, associated with physical interactions with IP3-R. Loss-of-function studies in *D. melanogaster* demonstrated that TMBIM3/GRINA and TMBIM6/BI-1 have functionally-related activities against ER stress *in vivo*. Similarly, manipulation of TMBIM3/GRINA levels in zebrafish embryos revealed an essential role in the control of apoptosis during neuronal development and in experimental models of ER stress.
Discussion: These findings suggest the existence of a conserved group of functionally-related cell death regulators across species beyond the BCL-2 family of proteins.
FONDECYT1100176, FONDAP15010006, Millennium Institute P09-015-F, ICGEB and Alzheimer's Association.

BONE MORPHOGENETIC PROTEIN 2 INHIBITS NEURITE OUTGROWTH OF MOTOR NEURON-LIKE NSC-34 CELLS AND UP-REGULATES ITS TYPE II RECEPTOR. Francisca Benavente[1], Margarita Parada[1], Cristina Pinto[2], Juan Pablo Henríquez[2], and **Nelson Osses[1].** [1]Instituto de Química, Pontificia Universidad Católica de Valparaíso. [2]Departamento de Biología Celular, Universidad de Concepción. nelson.osses@ucv.cl
Introduction: Bone morphogenetic proteins (BMPs) regulate several aspects of neuronal behavior. In *Drosophila* motor neurons, the expression of the BMP type II receptor (BMPRII) homolog *wishful thinking* is crucial for proper neuromuscular synaptogenesis. However, a role for BMP signaling at the vertebrate neuromuscular junction has not been addressed.
Materials and Methods: We have analyzed the expression of BMPRII and the effect of BMP-2 during the morphological differentiation of motor neuron-like NSC-34 cells, a mouse model of motor neurons.
Results: Morphological differentiation of these motor neuron-like cells was characterized by the protrusion of neurites in most cells, which number and length increase as differentiation proceeds for at least 48h. Our data indicate that BMPRII is up-regulated upon motor neuronal differentiation and localized in the developing growth cone tips of differentiated cells. Conversely, BMP-2 inhibits the differentiation of NSC-34 cells, an effect that correlates with activation of a Smad-dependent pathway, induction of the inhibitory Id1 transcription factor, and down-regulation of the neurogenic factor Mash1. Importantly, BMP-2 treatment also increases the expression of BMPRII.
Discussion: Our results raise the possibility that BMP-2 activates Smad-dependent pathway to inhibit axonal extensions of motor neurons but at the same time, it could provide cells the required proteins for proper synaptogenesis. One such protein is the BMPRII, as signaling through this receptor could play key roles for the assembly, maintenance and/or regeneration of the neuromuscular synapse.
FONDECYT 11060513, 1100326, VRIEA-PUCV.

RESVERATROL INHIBITS Cdk5 ACTIVITY THROUGH REGULATION OF p35 EXPRESSION. **Elias Utreras**, Anita Terse, Jason Keller, Michael Iadarola and Ashok Kulkarni. Functional Genomics Section, NIDCR, NIH, USA. elias.utreras@uchile.cl
Introduction: We have previously reported that cyclin-dependent kinase 5 (Cdk5) participates in the regulation of nociceptive signaling. Through activation of the ERK1/2 pathway, Tumor Necrosis Factor-a (TNF-a) induces expression of Egr-1. This results in the sustained and robust expression of p35, a co-activator of Cdk5, in PC12 cells, thereby increasing Cdk5 kinase activity. The aim of our present study was to test whether resveratrol, a polyphenolic compound with known analgesic activity, can regulate Cdk5/p35 activity.
Material and Methods: Here we used a cell-based assay in which a p35-luciferase construct was stably transfected in PC12 cells and treat it which resveratrol. Moreover, we analyze mRNA and protein levels of Cdk5/p35, and Cdk5 kinase activity in PC12 cells and DRG neuronal culture.
Results: we found that resveratrol inhibits p35 promoter activity and locks the TNF-a mediated increase in Cdk5 activity. In the presence of resveratrol, the MEK inhibitor decreased p35 promoter activity, whereas the inhibitors of p38 MAPK, JNK and NF-kB increased p35 promoter activity, indicating that these pathways regulate p35 expression differently. The TNF-a-mediated increase in Egr-1 expression was decreased by resveratrol with a concomitant reduction in p35 levels, resulting in reduced Cdk5 kinase activity.
Discussion: We demonstrate here that resveratrol regulates p35 promoter activity in PC12 cells and DRG neurons. Most importantly, resveratrol blocks the TNF-a-mediated increase in p35 promoter activity, thereby reducing p35 expression and subsequent Cdk5 kinase activity. This new molecular mechanism adds to the known analgesic effects of resveratrol and confirms the need for identifying new analgesics based on their ability to inhibit Cdk5 activity for effective treatment of pain.

DETERMINATION OF THE ROLE OF p53-RELATED PROTEIN KINASE (PRPK) IN AXON ELONGATION. **Villarroel D**.[1], Henriquez D.[1], Glavic A.[2] and González-Billault C.[1]. [1]Cellular and Neuronal Dynamics Laboratory and [2]Center for Genome Regulation, Department of Biology, Faculty of Sciences, Universidad de Chile, Santiago, Chile. davidbiovilla@gmail.com

Introduction. Axonal elongation precedes the molecular events underlying axonal guidance. In the last few years we showed the importance of MAP1B for axonal outgrowth. In a Y2H screen we identify PRPK as a MAP1B interactor. In this work we present the expression and subcellular localization of PRPK in rat neurons. We also show PRPK gain- and loss-of-function experiments in mice neurons and *Drosophila* photoreceptors.
Material and Methods. Embryonic rat hippocampal neurons were used to analyze PRPK expression by immunoblot and immunofluorescence. Functional assays were performed in *D. melanogaster* using the Gal4/UAS system and the results evaluated at 95% pupal development through confocal microscopy.
Results. We found no significant changes neither in the expression levels or localization of PRPK in rat neurons between 18 hrs and 3 days *in vitro*. However, the heterologous overexpression of *Drosophila* PRPK generated neurons displaying shorter axons. Conversely, the expression of a dead kinase construct (DN-PRPK) or a PRPK interference RNA showed longer projections in *Drosophila*.
Discussion. Gain- and loss-of-function experiments in rat and fly suggest that PRPK regulates axonal elongation. The localization and levels of PRPK observed in this study may not reflect PRPK activity, since PRPK needs an activating phosphorylation. Ongoing work will address the molecular mechanisms for PRPK-dependent axonal elongation.
Supported by Fondecyt-089095 and ICM P05-001-F (to CG-B).

C/EBPβ-MEDIATED RECRUITMENT OF SWI/SNF IS A MECHANISM FOR RIC-8B GENE REPRESSION DURING OSTEOBLAST DIFFERENTIATION. **Rodrigo Aguilar**, Aníbal Arce, Berta Henríquez, Hugo Sepúlveda, Martín Montecino. Center for Biomedical Research and FONDAP Center for Genome Regulation, Universidad Andres Bello, Santiago, Chile. raguilarmaureira@hotmail.com
Introduction: The Ric-8B gene is essential for cell division and is down-regulated during osteoblast differentiation by a mechanism involving C/EBPβ-LAP*-mediated repression and SWI/SNF-dependent chromatin remodeling. Interaction of SWI/SNF and C/EBPβ has been previously demonstrated in myeloid gene promoters. Here, we present evidence that supports C/EBPb-LAP*-mediated recruitment of SWI/SNF during Ric-8B gene repression in osteoblastic cells.
Methods: C/EBPβ silencing in osteoblastic cells was achieved by specific siRNAs or lentiviral-mediated expression of shRNAs. Binding of SWI/SNF and C/EBPb to the Ric-8B gene promoter was determined by ChIP. Changes in gene transcription were evaluated by QPCR. The analyses were also carried out in stable cell lines expressing dominant negative forms of SWI/SNF subunits. Undifferentiated pre-osteoblastic cells were co-transfected with constructs carrying the Ric-8B promoter fused to a Luciferase reporter gene together with vectors coding for mutated C/EBPβ proteins that impair interaction with SWI/SNF. Interaction between C/EBPβ and SWI/SNF was evaluated by co-immunoprecipitation.
Results: Silencing of C/EBPβ impairs SWI/SNF binding to the Ric-8B promoter and increases Ric-8B expression levels. This increase is also observed in cell lines expressing dominant negative forms of SWI/SNF. Over-expression of C/EBPβ-LAP* carrying a mutation that impairs SWI/SNF recruitment is unable to repress the Ric-8B promoter activity. Co-immunoprecipitation studies show that C/EBPβ and SWI/SNF interact.
Discussion: Our data support a model where C/EBPβ recruits SWI/SNF to the Ric-8B promoter leading to Ric-8B gene repression in differentiated osteoblasts.
CONICYT Fellow (R.A,H.S), FONDAP 15090007, FONDECYT 1095075 (MM), Post-doctoral FONDECYT 3110138 (B.H).

WNT/b-CATENIN SIGNALING ENHANCES RUNX1 TRANSCRIPTIONAL ACTIVITY IN HEMATOPOIETIC CELLS. **Medina M**, Pérez E, Gajardo I, Ugarte GD, De Ferrari GV. Center for Biomedical Research, Faculty of Biological Sciences and Faculty of Medicine, Andres Bello University, Chile. gdeferrari@unab.cl.
Introduction: The transcription factor RUNX1 modulates the expression of genes involved in hematopoiesis, a process which may involve the activity of the Wnt/b-catenin signaling cascade. Since, up-regulation of Wnt/b-catenin signaling has been associated with leukemia, here we have examined whether Wnt/□-catenin participates in the transcription of the human RUNX1 gene.
Material and Methods: RUNX1 expression (through alternative promoters P1 and/or P2) was studied in human hematopoietics cells lines, HL60 and Jurkat, in the absence/presence of lithium or Wnt3a. mRNA and protein isoforms were determined by quantitative-PCR and WB, respectively. A 1,5 Kb region of RUNX1-P1 distal-promoter containing 7 putative TCF/LEF binding elements (TBE) was cloned and their activity, in response to Wnt/b-catenin signaling, measured through serial deletions (reporter assays in HEK293 cells) or ChIP assays.
Results: Wnt/b-catenin stimulation of HL60 and Jurkat cells induced a dose and time-dependent increase in the expression of RUNX1 transcripts and protein isoforms. Likewise, through reporter assays mimicking active Wnt/b-catenin signaling, we observed that there was an enhancement in the promoter activity of the RUNX1-P1 constructs. Through serial-deletions we identified a minimal RUNX1-P1 promoter (ca. 500 pb), which includes a TBE upstream of the RUNX1 transcription start site, that positively responds to Wnt/b-catenin signaling. This observation was further confirmed through ChIP assays.
Discussion: Our results link the transcription of the RUNX1 gene to Wnt/b-catenin signaling and may open a novel window to understand the development or the deregulation of the hematopoietic process.
FONDECYT 1100942 and UNAB DI-41-11/R.

ORAL PRESENTATIONS III

TOXIC FACTORS RELEASED BY ALS-LINKED MUTATED SOD1 ASTROCYTES INDUCE MOTONEURON PATHOLOGY AND DEATH BY TARGETING SODIUM CHANNELS. **Elsa Fritz**[1,4], Fabiola Rojas[1,4], Constanza Riquelme[4], Camila Segura[4], Rodolfo Madrid[2], Felipe Court[3], and Brigitte van Zundert[4]. [1]Universidad de Concepción; [2]Universidad de Santiago de Chile; [3]Pontificia Universidad Católica de Chile. [4]Universidad Andrés Bello. bvanzundert@unab.cl

Introduction: Astrocytes expressing mutated human superoxide dismutase (hSOD1) cause amyotrophic lateral sclerosis (ALS) by releasing unknown toxic factor(s) that specifically kill motoneurons. The mechanism(s) responsible for this toxic effect is still unknown. We recently found that short-term exposure of ventral spinal cord cultures (VSCCs) with astrocyte conditioned media (ACM) derived from $hSOD1^{G93A}$ transgenic mice increases repetitive firing. Here we analyzed the effect of ACM-$hSOD1^{G93A}$ on the activity of voltage-sensitive Na^+ channels (Nav), intracellular Ca^{2+} transients and mitochondrial morphology.

Material and Methods: VSCCs (3 DIV) were co-incubated with ACM-$hSOD1^{G93A}$ and different Nav blockers, fixed at 7 DIV, and motoneuron survival determined by performing immunofluorescence using specific antibodies (MAP2 and SMI-32). Ca^{2+} transients of ACM-$hSOD1^{G93A}$-treated cultures were measured using Fura-2. The ultrastructure of mitochondria of ACM-$hSOD1^{G93A}$-treated VSCCs was analyzed by electron microscopy.

Results: We found that chronic treatment of VSCCs with ACM-$hSOD1^{G93A}$ killed 50% of the motoneurons. Application of the Nav blockers riluzole, mexiletine, and spermidine prevented this ACM-$hSOD1^{G93A}$-induced cell death. We also found that short-term exposure of VSCCs with ACM-$hSOD1^{G93A}$ was sufficient to increase Ca^{2+} transients and produce alterations in mitochondria's circularity.

Discussion: Our data suggest that the Nav is a primary target of toxic factors in ACM-$hSOD1^{G93A}$, and that its activation triggers a cascade of events which increase intracellular Ca^{2+} levels causing mitochondria dysfunction that finally leads to motoneuron death.

Financed by Fondecyt 1101012 and ATA.

MMP-14 PRODUCED BY BONE MARROW-DERIVED CELLS SHEDS EPITHELIAL ENDOGLIN MODULATING THE MIGRATORY PROPERTIES OF HUMAN BREAST CANCER CELLS. **Tobar N[1].,** Toyos M[1]., Quintanilla M[2]., Bernabeu C.[3] and Martínez J.[1]. Laboratorio de Biología Celular y Molecular, INTA, Universidad de Chile. [2]Instituto de Investigaciones Biomédicas and [3]Centro de Investigaciones Biológicas, CSIC, Madrid, Spain. nicolastobar@gmail.com

Introduction. Besides the pro-angiogenic role of endothelial Endoglin (Eng), epithelial Eng plays a crucial role in carcinogenesis. Here, we addressed the stromal-dependent mechanism by which soluble endoglin (sEng) is released from the membrane of a breast epithelial cell line. We analyzed the consequences that this loss of surface Eng has on cell signaling and migratory behavior.

Material and Methods. We used media conditioned (MC) by the HS-5 bone marrow cell line as a source of stromal factors and MCF-7 a weakly migratory human breast cancer cell line. Migration was assessed by Transwell assay, overexpression of Eng was achieved transfecting cells with a plasmid codifing the wild type form of Eng, MMP14 activity was measured by zimography and enzyme immunoprecipitation was done using an anti MMP-14 antibody bound to Sepharose.

Results. MCF-7 cells pretreated with conditioned media of CM HS-5 suffered a proteolytic processing of Eng which resulted in an enhancement of their motile properties, a phenomenon that was reversed by the forced expression of membrane bound Eng. Pretreatment of MCF-7 cells with CM HS-5 also induced changes in TGF-β1 signaling. The stimulus on MCF-7 migration was abolished when the CM HS-5 was collected in the presence of GM 6001 (Ilomastat), a broad range inhibitor of metaloproteinases and by immunoprecipitating MMP-14 from CM HS-5. We also found that TGF-β1 present in CM MCF-7 constitute a stimulus for the stromal MMP-14 expression.

Conclusions. These results suggest the existence of a cross-talk between mammary epithelia and bone stroma that modulates epithelial migration through changes in the abundance of epithelial Eng.

Funding: FONDECYT 1080196.

CROSS-TALK BETWEEN Ca^{+2}, NO AND H_2O_2 IN THE MARINE ALGA *Ulva compressa* (*Chlorophyta*) IN RESPONSE TO COPPER EXCESS. **Alberto González**, M. Josefa Henríquez, Rodrigo A. Contreras and Alejandra Moenne. alejandra.moenne@usach.cl (Sponsor: M. Imarai)

Introduction. The marine alga *Ulva compressa* is a heavy metal-tolerant species that dominates copper-enriched coastal areas in northern Chile and other parts of the world. The alga cultivated with copper excess showed increases in intracellular Ca^{+2} level at 2, 3 and 12 h, H_2O_2 level at 2, 3 and 12 h and nitric oxide (NO) level at 2 and 12 h of copper exposure. The co-occurrence of Ca^{+2}, H_2O_2 and NO increases suggests that there is a cross-talk between these intracellular signals.

Materials and Methods. To analyze the influence of calcium on H_2O_2 and NO production, the alga was treated with inhibitors of calcium channels, ryanodine (rya), xestospongin C (xes) and ned-19, and cultivated with 10 μM copper for 2, 3

and 12 h. To analyze the influence of NO and H_2O_2 levels on calcium release, the alga was treated with cPTIO, a NO scavenger, and ascorbate, a H_2O_2 scavenger, and cultivated with 10 µM copper for 2, 3 and 12 h.
Results. The level of H_2O_2 at 2, 3 and 12 h decreased with ned-19, rya and xes, and rya, respectively, indicating that calcium release activates H_2O_2 production. The level of NO at 2 and 12 h decreased with ned-19 indicating that calcium release activates NO synthesis. The level of calcium decreased with cPTIO and ascorbate indicating that calcium release is activated by H_2O_2 and NO.
Discussion. Thus, there is a cross-talk between Ca^{+2}, H_2O_2 and NO in *U. compressa* in response to copper excess.
Financed by Fondecyt 1085041.

INFECTIOUS PANCREATIC NECROSIS VIRUS IN SALMONIDS: PERSISTENCE AND IMMUNOLOGICAL RESPONSE. **Reyes-Cerpa S[1]**, Toro-Ascuy D[1], Montero R[1], Cottet L[2], Reyes-López FE[1], Sandino AM [2], Imarai M[1]. [1]Laboratorio de Inmunología, [2]Laboratorio de Virología. Centro de Biotecnología Acuícola. Facultad de Química y Biología. Universidad de Santiago de Chile. sebastian.reyesc@usach.cl
Introduction: The infectious pancreatic necrosis virus (IPNV) causes a persistent infection in salmonids. In mammals, viral persistence seems to be related to an increase of immunoregulatory cytokines that can attenuate the inflammatory immune response.
Material and Methods: In the current work, we have challenged one hundred Rainbow trout with IPNV by immersion and then maintained during 70 days. Fish were collected when they showed signs of disease or at the end of the experiment (without signs of disease); this latter group was classified as persistent because they show a detectable viral load. Later we have determined the level of expression of pro-inflammatory and anti-inflammatory cytokines by qPCR and we have evaluated the levels of IgM and IgT by western blot and ELISA and viral neutralization activity of serum.
Results: Results revealed that IL-1β, IL-12, IL-10 and TGF-β1 were up-regulated and IL-8 was down-regulated in head kidney during a persistent infection respect to an acute infection. In addition a diminished production of total and specific antibodies in serum and intestine was observed in all infected fish.
Discussion: The preferential induction of anti-inflammatory cytokines IL-10 and TGF-β1 may be partially related to viral persistence and the apparent absence of immune response reflected in a diminished production of antibodies.
INNOVA-Chile 07CN13PBT90, INNOVA-Chile 09MCSS6698, CONICYT AT-24100133, VRID-USACH, MECESUP UCH0604.

COPPER OVERLOAD IN THE ERYTHROPOIETIC CELL LINE K562 GOVERNS CELL FATE ALONG WITH CHANGES IN MITOCHONDRIAL PHYSIOLOGY. **Lina Ruiz,** Yancing Rossel, Rodrigo Bustos, Alvaro Elorza. Universidad Andrés Bello. aelorza@unab.cl
Introduction: Mitochondria play a key role in erythropoiesis. They synthesize heme and ATP along with reactive radicals. Copper is essential for complex IV and SOD1 function. It has been reported copper overload is toxic due to an increase in reactive radicals which might produce anemia. We hypothesize that copper overload alters mitochondrial physiology which in turn will affect cell proliferation, differentiation or death.
Materials and methods: K562 cells were treated with increasing doses of $CuCl_2$ with or without the differentiation agent BA. Cell proliferation, differentiation and death were assessed by trypan blue and benzidine staining. Cell respiration and glycolysis were measured with the XF24 technology. Mitochondrial membrane potential and superoxide production were measured by confocal microscopy and both total and mitochondrial protein oxidation by oxyblot.
Results: Copper induces differential cellular responses according to its concentration. Mild copper overload (95uM) increases proliferation and decreases BA-induced differentiation. High copper overload (200uM) induces cell death. The increased K562 proliferation is associated with uncoupled mitochondria and augmented glycolysis. Increased mitochondrial protein oxidation and mitochondria- generated superoxide were also detected.
Discussion: Mild copper overload increases mitochondrial oxidation affecting mitochondrial ATP production. Due to this, cells are forced to switch to glycolysis which in turns might favor cell proliferation as well as inhibition of differentiation. Copper toxicity has been associated to the development of cancer and anemia. Thus the study of mitochondrial function in erythropoiesis provides new insights to prevent or treat those diseases.
COCHILCO-FONDECYT 1100995; DI 10-10R.

PANNEXIN HEMICHANNELS CONTRIBUTE TO Ca^{2+} DYNAMICS DURING ATP-INDUCED MIGRATION IN CONFINEMENT. **Sáez PJ[1],** Vargas P[2], Lennon-Duménil AM[2] and Sáez JC[1]. [1]Departamento de Fisiología, Santiago, Chile and [2]INSERM U932, Institut Curie, Paris, France. pjsaez@uc.cl
Introduction. Immature dendritic cells (DCs) sense the extracellular ATP through purinergic receptors (P2), which increase the intracellular free Ca^{2+} concentration $[Ca^{2+}]_i$. The activation of P2 receptors induces opening of pannexin hemichannels (Px HCs), which are membrane channels that communicate the cytoplasm with the extracellular medium and contributes to neutrophil, but no macrophage, migration. In this work we study the contribution of Px HCs to Ca^{2+} dynamics during ATP-induced migration in DCs.

Methods. Murine bone marrow-derived DCs (BMDCs) and spleen-DCs (sDCs) were used. The activity of HCs was evaluated through time-lapse dye uptake measurements. Changes in the $[Ca^{2+}]_i$ were studied using fluorescent Ca^{2+} probes. To study migration in confinement we used microchannels, a microfluidic device that provides a three-dimensional model.
Results. Under control conditions sDCs and BMDCs presented low HCs activity and low $[Ca^{2+}]_i$. Both parameters rapidly increased upon addition of ATP (>500 μM) in both 2D or into microchannels. These responses were partially impaired by Px HCs blockers (probenecid, ^{10}Panx1). In microchannels experiments, ATP increased DC migration in a concentration-dependent manner (>500 μM) which were partially prevented with Px HCs and $P2X_7$ receptor inhibitors. Immunofluorescence analysis in microchannels showed that Px1 and $P2X_7$ receptors co-localize at the leading edge of DCs.
Discussion. DCs express functional Px HCs that contribute to purinergic signaling and to the rise in $[Ca^{2+}]_i$ observed during ATP-induced migration. These results provide a new target to modulate the DCs migration for therapy.
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ANTI-P AUTOANTIBODIES ENHANCE CYTOSOLIC CALCIUM AND NEUROTRANSMISSION IN HIPPOCAMPAL NEURONS BY CROSS-REACTING WITH CELL SURFACE NSPA. **Fabian Segovia-Miranda**[1,2], Jorge Parodi[1], Felipe Serrano[1], Noelia Escobedo[1], Marcela Bravo-Zehnder[1,2], Pedro Zamorano[3], Juan Larraín[1], David Valenzuela[4], Loreto Massardo[2], Nibaldo C. Inestrosa[1], Alfonso González[1,2]. Centro de Envejecimiento y Regeneración (CARE), Fac. Ciencias Biológicas[1]. Departamento de Inmunología Clínica y Reumatología, Facultad Medicina[2]. Pontificia Universidad Católica de Chile. Departamento Biomédico, Universidad de Antofagasta de Chile[3]. Regeneron Pharmaceuticals Inc[4].
Introduction: Autoantibodies against the carboxyterminal region of three ribosomal phosphorylated proteins (anti-P) have been associated with psychosis and depression in systemic lupus erythematosus. We recently showed that anti-P antibodies induce calcium influx followed by apoptosis in cortical neurons and gave evidence of cross-reaction with a high molecular mass protein of unknown function, called NSPA (Neuronal Surface P Antigen) (Matus et al., 2007).
Material and methods: To test more directly the role of NSPA as an anti-P target we cloned the NSPA cDNA linked to GFP at its N-terminal region to transfect HEK293 cells, and also produced an NSPA-null/LacZ knock-in mice for studying NSPA expression in the brain and calcium and neurotransmission assays in the hippocampus.
Results: Cell surface immunocapture and biotinylation assays in HEK293 cells expressing NSPA-GFP point to NSPA as anti-P cell surface target. Beta-Gal staining in mice shows an expression mostly in hippocampus. Anti-P autoantibodies increase cytosolic calcium levels in hippocampal neurons in culture and neurotransmission assessed in hippocampal slices, but only from wild type and not from NSPA-null mice.
Discussion: These results provide evidence that NSPA, and not other antigens, display a P epitope at the cell surface and may mediate deleterious effects of anti-P in hippocampal neurons.
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INCREASED RESTING CALCIUM LEVELS ACTIVATE NF-kB IN DYSTROPHIC (*MDX*) MYOTUBES. **Altamirano F.[1],** López JR.[2], Allen PD[2] and Jaimovich E[1]. [1]Centro de Estudios Moleculares de la Célula, ICBM, Facultad de Medicina, Universidad de Chile, Santiago, Chile and [2]Department of Anesthesia, Brigham and Women's Hospital, Harvard Medical School, Boston, USA. fcoaltamirano@gmail.com
Introduction: Duchenne muscular dystrophy (DMD) is a genetic disorder characterized by severe muscle wasting. Dystrophic muscle consists of activated immune cells infiltrates with up-regulated inflammatory gene expression and increased NF-kB activity, but the contribution of the skeletal muscle cell in this event was still unclear. The aim of this work was to study the resting calcium ($[Ca^{2+}]_i$) dysregulation and the possible link with NF-kB up-regulation in mdx myotubes.
Materials and Methods: $[Ca^{2+}]_i$ was measured with Ca^{2+}-selective microelectrodes and NF-kB transcriptional activity was studied using luciferase reporter and its sub cellular distribution by immunofluorescence in *Wt* and *mdx* myotubes.
Results: $[Ca^{2+}]_i$ was increased in *mdx* compared with *Wt* myotubes (308±6 *vs* 113±2 nM) Both the inhibition of calcium entry (Gd^{3+} and low calcium solutions) and blockage of either one of the calcium release channels: RyR (ryanodine) and IP_3R (XestosponginB), reduced $[Ca^{2+}]_i$ in *mdx* myotubes. Basal activity of NF-kB was significantly up-regulated in *mdx* myotubes, showing increased p65 nuclear localization and increased transcriptional activity, which could be reversed using inhibitors that reduced $[Ca^{2+}]_i$.
Discussion: We propose that NF-kB is constitutively active in *mdx* myotubes, modulated by increased $[Ca^{2+}]_i$ in resting conditions. We hypothesize that the differences in NFκB activity between normal and dystrophic muscle cells may help to understand the mechanisms of pro-inflammatory gene expression in DMD.
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ORAL PRESENTATIONS IV

NEURONAL ENDOPLASMIC RETICULUM (ER) IMAGING WITH SUPER-RESOLUTION OPTICAL FLUCTUATION IMAGING (SOFI). **Omar Ramírez[1],** Felipe Santibáñez[1], Dirk Haehnel[3], Andrés Couve[2], Jörg Enderlein[3] & Steffen Härtel[1]. [1]SCIAN-Lab, [2]Laboratory of Cellular and Molecular Neurobiology, BNI, ICBM, Facultad de Medicina, U-Chile. [3]III. Physikalisches Institut, Universität Göttingen, Germany. oramirez@med.uchile.cl

Introduction: The ER plays a fundamental rol in membrane and secretory protein synthesis, quality control, transport, and Ca^{2+} management. The neuronal ER follows the extremely polarized shape of this cell type, being present throughout the soma, axons, and dendrites. So far, little is known about structural-function interrelationships within this highly dynamic network, due to the limited resolution of optical microscopy (~200 nm). Diffraction limited techniques restrict our capacity to combine detailed structural measurements with functional studies at molecular level. SOFI is a novel technique based on the statistical analysis of the blinking fluorophores that fluctuates between on/off states. We present a first approach to resolve the morpho-topological properties of the ER with SOFI beyond the diffraction limit.

Material and Methods: Cultured hippocampal neurons were grown for 1-3 weeks and processed for immunofluorescence to detect ER specific proteins using Qdot-conjugated secondary antibodies. ~2000 fluorescence images were taken for every focal plane, processed with cross-cumulant analysis, deconvolved, and analyzed with SCIAN-software for 3D feature extraction.

Results: We show an improved spatial resolution compared with conventional fluorescence microscopy. In addition, SOFI images are intrinsically noise free which allows an improved segmentation and reconstruction of the 3D ER structure.

Discussion: The resolution of ER features beyond the diffraction limit will improve our understanding of subcellular processes. The combination with blinking proteins like Dropa will further enable *in vivo* observations of structural-function properties in the future.

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SYNERGISTIC EFFECT OF PLASMID pcDNA-SURV IN CONJUNCTION WITH THE PARASITIC CALRETICULIN ON B16F10 TUMOR MODEL *IN VIVO*. **Aguilar L.**[1,2], Lobos L.[2], Quest A.F.G.[2], Ferreira A.[1]. [1]Laboratorio de Inmunología de la Agresión Microbiana, [2]Laboratorio de Comunicaciones Celulares, ICBM, Universidad de Chile. aferreir@med.uchile.cl

Introduction: Calreticulin (CRT), a pleiotropic protein, displays anti-angiogenic capacity and participates in the phagocytosis of apoptotic cells. *Trypanosoma cruzi* CRT (TcCRT), shares many structural and functional characteristics with other CRTs. Recently we demonstrated TcCRT anti-angiogenic capacity in *in vivo, in vitro* and *ex vivo* models, by its interaction with endothelial cells, interfering with their proliferation, motility and capillary morphogenic capacity. On the other hand, Survivin (Surv), a tumor-associated antigen, belongs to the inhibitor of apoptosis family of proteins, absent in normal adult cells but over-expressed in almost all solid tumors. Surv is required for tumor cell survival, and is considered an ideal target for tumor therapy. Our previous results indicate that immunization with a plasmid encoding Surv induces cytotoxic response, leading to a partial protection against tumor development. We propose to evaluate the combined effect of a DNA vaccine encoding Surv and the inoculation of rTcCRT in an experimental melanoma.

Methodology: C57BL/6 mice were immunized with pcDNA-Surv plasmid twice, and after that were inoculated subcutaneously with B16F10 cells, previous incubation with TcCRT. Finally, subcutaneous inoculations were performed with rTcCRT.

Results: In the animals immunized with pcDNA-Surv and inoculated with TcCRT a delay in tumor growth was observed ($p=0.03$).

Discussion: Although rTcCRT and immunization with pcDNA-Surv focused their actions on endothelial cells, the molecular targets are probably different, thus explaining the observed results.

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ADAM17, AS A NEW CANDIDATE IN THE MECHANISM OF XENOESTROGENS-INDUCED APOPTOSIS IN TESTICULAR GERM CELLS. **Raúl Lagos-Cabré**, Paulina Urriola-Muñoz, Pablo Sáez, Juan C. Sáez and Ricardo D. Moreno. Pontificia Universidad Católica de Chile, Facultad de Ciencias Biológicas, Departamento de Fisiología. rclagos@uc.cl

Introduction: Plasticizers with oestrogenic activity (xenoestrogens) such as Bisphenol A (BPA) and 4-nonilphenol (NP), induce germ cell apoptosis, but the mechanism is unknown. Our aim was to study the participation of ADAM metalloproteases and the ROS/ASK1/$p38^{MAPK}$ pathway in rat germ cell apoptosis during spermatogenesis.

Materials and Methods: 21 day-old rats were intraperitoneally injected with 50 mg/kg BPA or NP at different times, alone or with ADAM17 inhibitor, GW280264X (GW), ADAM10 inhibitor, GI254023X (GI) or $p38^{MAPK}$ inhibitor PD169316 (PD). Testes samples were analyzed by Western Blot, RT-PCR, TUNEL, immunohistochemistry and flow cytometry.

Results: BPA and NP significantly increased germ cells apoptosis 24h after treatment, which was prevented by GW but not GI inhibitor. Total ADAM17 levels did not change, whereas ADAM10 levels increased after BPA and NP. Nevertheless, ADAM17 levels increased at the cell surface. In addition we observed an increased in protein levels of p-p38^{MAPK} and a reduction in p-ASK-1. Injection of PD prevented ADAM17 exposure at the cell surface induced by BPA and NP, along with a reduction in p-p38^{MAPK} and an increase in p-ASK-1. Even more, PD prevented apoptosis induced by BPA. BPA and NP treatment increased lipid peroxidation and proteins nitration, suggesting reactive oxygen species (ROS) production.
Discussion: ADAM17 is involved in germ cell apoptosis induced by BPA and NP. **T**he ROS/ASK-1/p38^{MAPK} pathway is activated upon BPA and NP treatment and it is upstream of ADAM17 activation.
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THE ROLE OF MATRIX METALLOPROTEINASE 9 (MMP9) AND PI3K/AKT SIGNALING IN INFAMMATORY RESPONSE IN ZEBRAFISH LARVAE. **Oscar Peña,** Mario Sánchez, Nicole Reynaert, José Moya, Miguel Allende. FONDAP Center for Genome Regulation, Facultad de Ciencias, Universidad de Chile. oscarpena@ug.uchile.cl; allende@uchile.cl
Introduction. Leukocyte recruitment to wounds is essential during the innate immune response. Given the transparency of the zebrafish during early developmental stages and the availability of fluorescently labeled cells and tissues, it is now possible to study the innate immune system by following the behavior of infiltrating cells in the live animal. Matrix metalloproteinase 9 (MMP9) and PI3K/Akt signaling have been implicated in the pathogenesis of several inflammatory conditions.
Materials and Methods. Inflammation was induced by exposure of zebrafish larvae to sublethal concentrations of copper sulphate or by physical ablation with a tungsten electrode, a method described here for the first time. Leukocyte behavior was followed by using transgenic larvae harboring labeled leukocytes. *In situ* hybridization of *mmp9*, immunohistochemistry of MMP9 and phosphorylated Akt, and inflammation assays using the MMP9-inhibitor LY294002 and Wortmannin, were performed.
Results. Using a described chemical damage protocol and electroablation of single neuromast we induced a strong inflammatory response. We provide evidence for *mmp9* upregulation in neuromast-damaged zebrafish larvae. MMP9 induction and Akt phosphorylation occurs in cells surrounding damaged neuromasts, while inhibition of MMP9 and PI3K activity leads to retarded leukocyte migration to damaged lateral line neuromasts.
Discussion. We provide evidence for MMP9 upregulation in cells surrounding damaged neuromasts, possibly in response to cell death, as well as decreased neutrophil recruitment after inhibition of MMP9 and PI3K activity. This study suggests an important role for MMP9 in leukocyte motility during inflammation.
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THE DENDRITIC ENDOPLASMIC RETICULUM AND CONVENTIONAL KINESIN DEFINE A NON-CANONICAL TRAFFICKING MODALITY FOR GABA$_B$ RECEPTORS. **José Ignacio Valenzuela**[1,3], Matías Jaureguiberry[1,3,4], Omar Ramírez[2,3], Thomas Blanpied[1], and Andrés Couve[1,3]. [1]Physiology and Biophysics, [2]Anatomy and Development, ICBM and [3]Biomedical Neuroscience Institute (BNI), Facultad de Medicina, Universidad de Chile, Santiago, Chile. [4]School of Biochemistry, Faculty of Biological Science, Universidad Andrés Bello, Santiago, Chile. [5]Department of Physiology, University of Maryland School of Medicine, Baltimore, MD. joseignacio.v@gmail.com
Introduction: The availability of neurotransmitter receptors is regulated by coordinated mechanisms that deliver newly synthesized receptors to the plasma membrane and remove them for storage, recycling or degradation. In neurons, a complex spatial distribution of secretory organelles defines local trafficking pathways that comprise the dendritic endoplasmic reticulum and satellite Golgi outposts. Metabotropic GABA$_B$Rs regulate the efficacy of synaptic transmission throughout the brain, but the mechanisms controlling their biosynthetic route and plasma membrane delivery remain unclear.
Material and Methods: The localization, colocalization and mobility of GABA$_B$Rs were evaluated using fixed and live-cell imaging in primary cultures of rat hippocampal neurons under control conditions or after blockade of intracellular trafficking. *De novo* receptor insertion was determined using a-bungarotoxin labeling and single molecule tracking was performed using photoactivated localization microscopy (PALM).
Results: GABA$_B$Rs insert throughout the somatodenritic plasma membrane and traffic through satellite Golgi outposts. GABA$_B$R1, the subunit that contains an ER retention motif, colocalizes with the dendritic ER. The transport of dendritic GABA$_B$R1 has a modal velocity of 200 nm/sec, is kinesin-I and microtubule acetylation-dependent.
Discussion: GABA$_B$Rs utilize a non-canonical secretory route in dendrites uncovering a novel role for kinesin-I in the transport of cargo along the dendritic ER.
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UBIQUITIN-DEPENDENT PROTEASOMAL DEGRADATION AND CLEAVAGE BY g-SECRETASE COMPETE FOR THE AMYLOID PRECURSOR PROTEIN CARBOXY TERMINAL FRAGMENT BETA (C99).

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Introduction. Accumulation of the amyloid-β (Aβ) peptide is widely believed to play a causal role in Alzheimer's disease (AD). Proteolytic cleavage of APP by the β-site APP cleaving enzyme (BACE1) near the C-terminus results in the formation of the APP C-terminal fragment beta (C99), a substrate for subsequent cleavage by γ-secretase that ultimately generates Aβ. Currently the inhibition of γ-secretase activity is an important target in the developing of AD therapeutics, although serious concerns remain mainly due to a growing list of cellular substrates. Our aim was to investigate whether C99 can be reduced by a degradation mechanism independent of γ-secretase activity.

Material and Methods: H4 neuroglioma cells stably expressing C99-EGFP were treated with drugs that inhibit endoplasmic reticulum associated protein degradation (ERAD) in the absence or presence of γ-secretase inhibitors. After the treatment cells were analyzed by microscopy and/or biochemical assays to study C99 turnover and/or proteolytic processing.

Results: Treatment with proteasomal inhibitors and/or endoplasmic reticulum (ER) stressors strongly accumulated C99 in ER and Golgi apparatus leading to an increase in γ-secretase cleavage. This accumulation is dependent on ubiquitination of lysine residues within the C99 cytosolic tail.

Discussion: Our results suggest that C99 can be actively degraded at the ER reducing Aβ generation.

FONDECYT 1100027 and DID-UACH.

***In vivo* AND *in vitro* FUNCTIONAL CHARACTERIZATION OF RhoGEF3, A NEW GUANINE NUCLEOTIDE EXCHANGE FACTOR (GEF) OF *Drosophila melanogaster*.** **Alejandro Zúñiga**, Leandro Farías and Verónica Cambiazo. Laboratorio de Bioinformática y Expresión Génica, INTA-Universidad de Chile & Center for Genome Regulation (CRG). jano@inta.uchile.cl

Introduction: Actin cytoskeleton dynamics is responsible of morphogenetic processes where RhoGTPases have a fundamental regulatory role. GTPases are temporal and spatially activated in a specific manner by guanine nucleotide exchange factors proteins (GEF). In *Drosophila* genome has been detected 22 genes which codify for putatives GEF proteins, of which only a reduced number has been well characterized in development. Here we show the molecular and functional characterization of a novel GEF protein, RhoGEF3.

Materials and Methods: In this study we used: 1) S2R+ cells as a model of *in vitro* assays, 2) *in situ* hybridization, 3) antiserum against RhoGEF3 protein, 4) expression of lacZ (wild type), dsRNA against *rhogef3*, and dominant negative forms of GTPases, under the control of Gal4-UAS system. Cells and embryos were analyzed by indirect immunofluorescence assays.

Results: RhoGEF3 protein promote the remodeling of the actin cytoskeleton in S2R+ cells in a Rac1 dependent manner. The *rhogef3* transcript and RhoGEF3 protein were detected in tubular tissues during embryogenesis, here we focused in salivary glands (SG) expression. RhoGEF3 protein was detected in apical region of cells of secretory portion of the SG. Loss of function of *rhogef3 in vivo* shows defects in SG lumen expansion very similar those of loss of function of Rac1 and Cdc42 GTPases.

Discussion: Our results suggest that RhoGEF3 is involved in the regulation of actin cytoskeletal remodeling in cells undergoing morphogenetic events during salivary gland development.

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AUXIN-INDEPENDENT LATERAL DEVELOPMENT INDUCED BY A CELLULAR TRAFFICKING DISRUPTING-DRUG. **Perez P[1,2],** Norambuena L[1,2]. [1]Plant Molecular Biology Laboratory, Faculty of Science, University of Chile. [2]Plant Cell Biotechnology Millennium Nucleus. lnorambuena@uchile.cl

Introduction: Plant roots are vital underground organs as they allow water and nutrients uptake. Nutrient adaptation depends upon postembryonic lateral root formation (LRF) for increasing uptake surface area. LRF is thought to be triggered by the plant hormone auxin. Mechanical stimulus and primary root disruption are also LRF triggers, suggesting an auxin-independent LRF mechanism. Our lab has described Sortin2, a chemical genomics approach-described drug that provokes endomembrane system trafficking disruption. Sortin2 inhibits auxin response at transcriptional level and it is a potent inhibitor of primary root growth. Therefore, we explore the putative effects of Sortin2 upon LRF in its auxin-independent action mechanism context.

Material and Methods: 1.Lateral root inducible system was used to compare LRF between auxin- and Sortin2-treated *A. thaliana* seedlings. 2.Auxin dependency in Sortin2-induced LRF was evaluated using auxin-signaling mutants. 3.Early stage lateral root development was examined using the mitotic activity marker CYCB1:GUS 4. Cellular trafficking was analyzed by confocal microscopy combining Sortin2 with other known trafficking disrupting-drug such as Brefeldin A.

Results: Sortin2 increases lateral root occurrence regardless plant auxin signaling. Coherently with primary root growth Sortin2 inhibition, mitotic activity was inhibited in primary but not lateral root tips. Strikingly, Sortin2 increases mitotic

activity and lateral root primordia, suggesting a novel auxin-independent LRF mechanism. In addition, Sortin2 discriminates molecular features between lateral and primary root since disaggregates Brefeldin A bodies differentially.
Discussion: Sortin2 arises as a potent tool to study auxin-independent lateral root formation and to describe lateral root molecular components important for cellular trafficking.
FONDECYT-11080240 and ICM-P06-065-F.

ORAL PRESENTATIONS V

ANGIOTENSIN-(1-7) REDUCES FIBROSIS AND IMPROVES FUNCTION IN DYSTROPHIC SKELETAL MUSCLE. **Acuña MJ**[1], Vío CP[2], Cabello-Verrugio C[1,3] and Brandan E[1]. [1]Laboratorio de Diferenciación Celular y Patología; [2]Laboratorio de Fisiología, CARE, P. Universidad Católica de Chile. [3]Centrode Genética Humana, Clínica Alemana-Universidad del Desarrollo. ebrandan@bio.puc.cl
Introduction: Duchenne muscular dystrophy (DMD) is a pathology characterized by loss of muscle function and development of skeletal muscle fibrosis. Transforming Growth Factor Type Beta (TGFβ) and Renin Angiotensin System, whose fibrotic exponent is Angiotensin-II, are involved in the fibrotic progression of DMD. Since opposite function among Angiotensin-II and Angiotensin-(1-7) (Ang-(1-7)) has been described we evaluated Ang-(1-7) effect on skeletal muscle fibrosis and strength.
Materials and Methods: *mdx* mice, DMD murine model, were treated with Ang-(1-7) by systemic infusion, and levels of fibrotic proteins were determined. To assess the muscle function we measured skeletal muscle strength. TGFβ signaling activity was evaluated by phosphorylation of Smad3. To study the Ang-(1-7) Mas receptor participation in Ang-(1-7) effects, *mdx* mice were treated with the Mas receptor antagonist A-779.
Results: Ang-(1-7) reduced the exercise-induced fibrosis in *mdx* muscle, determined by decrease in collagen and fibronectin levels. This reduction in fibrosis was accompanied by an improvement of skeletal muscle strength. Ang-(1-7) also reduces TGFβ signaling evaluated by the decrease in Smad3 phosphorylation. These effects were mediated by its transducer receptor Mas, since infusion of A-779, resulted in exacerbated fibrosis and TGFβ signaling together with a decrease in muscle strength.
Discussion: Ang-(1-7) reduces skeletal muscle fibrosis by reducing TGFβ signaling activation and improves dystrophic skeletal muscle function. Ang-(1-7) could be a novel agent for the treatment of skeletal muscle fibrosis associated to DMD.
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DEHYDROASCORBIC ACID MODULATES GLYCOLITIC, PENTOSE-PHOSPHATE PATHWAY AND GLUTATHION ACTIVITY IN ASTROCYTES. **Pedro Cisternas,** Carmen Silva-Alvarez, Karina Oyarce, Paula Llanos and Francisco Nualart. Department of Cell Biology, University of Concepcion. pecister@udec.cl
Introduction: Specialized cells transport vitamin C in its reduced form using sodium-dependent cotransporters (SVCTs). Additionally, different cells transport the oxidized form of vitamin C, dehydroascorbic acid, through glucose transporters (GLUTs). Vitamin C recycling between neurons and astrocytes regulates the levels of vitamin C in the brain. However, the mechanism and metabolic activity associated to the vitamin C recycling in normal and activated astrocytes have not been defined.
Materials and Methods: Astrocytes isolated from 3 days-old rat brains were cultured in MEM by 15 days (normal astrocytes) and 30 days (activated astrocytes). First, we analyzed the uptake of vitamin C, mainly dehydroascorbic acid (DHA). The cells were incubated with DHA and the glycolytic flux (GF) was measured by the rate of ^3H_2O production from 3-3H-glucose. Additionally, we measured the activity of the pentose-phosphate pathway (PPP) by the rate of $^{14}CO_2$ production from 1- 14C-glucose or 6- ^{14}C-glucose. Glutathion (GSH) concentration was determined in microtiter plates according to the colorimetric method.
Results: Kinetic data indicate that astrocytes transport mainly DHA. Inside the cells, DHA is reduced to AA and the accumulation of vitamin C does not affect the glycolytic flux. However, DHA stimulates PPP activity in 15 DIV astrocytes also increasing GSH biosynthesis. Activated astrocytes (30 DIV) increase DHA uptake and decrease PPP activity and GSH concentration. Additionally, activated astrocytes increase SVCT2 expression and AA uptake.
Discussion: Normal and activated astrocytes are mainly involved in vitamin C recycling, however, these cells show differential mechanisms to regulate glycolytic flux, PPP activity and GSH biosinthesis.
Grants Support by FONDECYT 1100396.

REGULATION OF DENDRITIC BRANCHING BY BDNF-INDUCED INCREASE OF RAB11 ACTIVITY IN HIPPOCAMPAL NEURONS. **Oscar M. Lazo**[1], Maria Ascano[2], Rejji Kuruvilla[2], Andrés Couve[3] and Francisca C. Bronfman[1]. [1]Millennium Nucleus in Regenerative Biology (MINREB), Facultad de Ciencias Biológicas. Pontificia Universidad Católica de Chile. [2]Department of Biology. Johns Hopkins University. USA. [3]ICBM, Universidad de Chile. omlazo@uc.cl

Introduction. Dendritic branching is a source of neuronal plasticity regulated by extrinsic cues as neurotrophic factors. BDNF and its receptor TrkB are the most widely expressed neurotrophic system in the brain and their signaling increases dendritic branching. Neurotrophic signaling is modulated by post-endocytic trafficking of the receptor, which is controlled by the Rab family of monomeric GTPases. We have hypothesized that the activity of Rab11, that regulates the recycling pathway, is required for BDNF-dependent dendritic arborization.
Methods. We analyzed BDNF-dependent branching in hippocampal neurons expressing EGFP or constitutively active (CA) and dominant negative (DN) mutants of Rab11-EGFP, and then stimulated with BDNF. We analyzed the effect of BDNF in Rab11-positive endosomes performing live-cell imaging. Activity of Rab11 was measured with the effector FIP3 that was used as a probe for detection of GTP-bound Rab11 in dendrites.
Results. BDNF-dependent dendritic branching is blocked in neurons expressing Rab11DN. Moreover, expression of Rab11CA increases arborization, even without exogenous BDNF. Live-cell imaging shows that BDNF induces an actin-dependent mobilization of Rab11 into dendrites. Additionally, BDNF significantly increases the GST-FIP3 signal in dendrites.
Discussion. Our results suggest that BDNF increases the availability of GTP-bound Rab11, which interacts with effector proteins and mobilizes Rab11-positive endosomes to dendrites, probably through an actin-dependent molecular motor, to induce dendritic branching.
Financial support of MINREB, CARE-Chile and FONDECYT 1085273.

MCT1 KNOCKDOWN IN HYPOTHALAMIC GLIAL CELLS AND THEIR EFFECT IN THE EXPRESSION OF NEUROPEPTIDES THAT CONTROL FOOD INTAKE. **Cortés-Campos C**, Elizondo R, Nualart F, García MA. Departamento de Biología Celular, Facultad de Ciencias Biológicas, Universidad de Concepción. mgarcia@udec.cl
Introduction: It is well known that food intake is mainly controlled in the hypothalamus by several inputs such as glucose. We postulate that tanycytes, transfer lactate into neighboring orexigenic an anorexigenic neurons via monocarboxylate transporters (MCTs), lactate released could modulates the neuropeptide secretion and consequently controlling food intake. Previously we demonstrated the MCT1and MCT4 localization in the hypothalamus. Here we assess the effect of glial MCT1 knockdown over the expression of orexigenic and anorexigenic neuropetides in rats.
Material and Methods: 250-300g male rats were stereotaxically cannulated to third ventricle and injected with adenoviruses encoding shRNA against MCT1 (inhibitor) or E. coli b-galactosidase (control). Ninety-six hours later the animals were challenged intracerebroventricular with 50mM glucose or saline previous a fasting of 48. After 2h were sacrificed, the hypothalamic area dissected and qRT-PCR analysis were performed.
Results: The MCT1 knockdown produced two different outcomes. In the orexigenic neurons there was an inhibition of a classical response to hyperglycemia, which is the reduction of the expression in NPY and AgRP neuropeptides. On the other hand, in the anorexigenic neurons the MCT1 knockdown altered response to hypoglycemia, the animals did not show the reduction in the expression of the POMC neuropeptide.
Discussion: Knocking down of MCT1 in tanycytes showed that this cells might be involved in the inhibition of hunger feeling in hyperglycemic conditions through lactate release and not by direct action of glucose. *Fondecyt 1100705.*

INHERENT GROWTH HORMONE RESISTANCE IN FISH SKELETAL MUSCLE IS MODULATED BY THE NUTRITIONAL STATUS AND IS CHARACTERIZED BY HIGH CONTENTS OF TRUNCATED GHR, IMPAIRMENT IN THE JAK2/STAT5 SIGNALING PATHWAY AND LOW IGF-I EXPRESSION. **Eduardo N. Fuentes**[a], Ingibjörg Eir Einarsdottir[b], Juan Antonio Valdes[a], Marco Alvarez[c], Alfredo Molina[a], Björn Thrandur Björnsson[b]. [a]Laboratorio de Biotecnología Molecular, Universidad Andrés Bello, Chile, [b]Fish Endocrinology Laboratory, University of Gothenburg, Sweden. edua.fuentes@gmail.com
Introduction: The mechanisms by which IGF-I is synthesized largely depend on the interaction of growth hormone (GH) with specific membrane-bound GH receptors (GHRs) and the subsequent activation of the JAK2/ STAT5 signaling pathway. The impairment of this system, a phenomenon termed GH resistance, will significantly impair somatic growth and is often observed under various catabolic or pathologic conditions, such as fasting or muscle waste.
Materials and Methods: We study the influence of nutritional status on GH and IGF-I plasmatic levels by radioimmunoassay and qPCR, also the activation of JAK/STAT pathway. In addition, it was mesured the GHBPs and GHR contents by western blotting in fine flounder juveniles undergoing compensatory growth trials.
Results: The study revealed an inherent systemic and local (muscle) GH resistance, characterized by high GH and low IGF-I plasmatic levels, higher contents of truncated GHR than full-length GHR, together with impaired activation of the JAK2-STAT5 signaling pathway. Fasting causes further impairment of the GH system, which is reversed when fish are returned to nutritionally favorable, anabolic conditions.
Discussion: This study reveals a unique model of inherent GH resistance in the skeletal muscle of a vertebrate species and therefore contributes to novel insights of the endocrine and molecular basis of growth regulation in earlier vertebrates.
FONDECYT 1090416.

ROLE OF PK17E AND RECS1 IN THE REDISTRIBUTION OF CADHERIN DURING *Drosophila* WING EPITHELIAL REMODELING. **Alvaro Glavic**. Center for Genome Regulation, Facultad de Ciencias, Universidad de Chile.

Introduction. Morphogenetic processes are based on the coordinated changes in cell adhesion, migration and shape. In the Drosophila wing, the progression of epithelial remodeling involves the core planar cell polarity elements (Fz/PCP), independently of Fat/Hippo pathway, to regulate the distribution of the exocyst complex, targeting cadherin-containing vesicles to the sites of reinsertion. We have identified two uncharacterized genes involved in cadherin trafficking that impact on epithelial remodeling and therefore on wing morphogenesis.

Materials and Methods. Using gain and loss of function approaches combined with confocal microscopy and flow cytometry analysis we have addressed the function of these proteins in epithelial dynamics.

Results. PK17E and RECS1 regulate the shape and size of the wing. This is achieved controlling cell packing during wing morphogenesis, particularly activating Ral GTPase and through it the exocyst complex. This function has important effects in the balance between cadherin recycling and degradation by the lysosome. Additionally, we show that PK17E locates in different intracellular domains depending on the activity of Ds/Fat signaling pathway and that both proteins modulate the respond of cells to autophagy induction, which suggests a restrictive input along the degradative pathway.

Discussion. Our results suggest that Fat/Ds and core Fz/PCP are coupled through these two proteins, which balance the recycling/degradation behaviors and therefore regulate the delivery of cadherin during epithelial remodeling. Thus the balance between cadherin recycling and degradation is fundamental in the control of wing morphogenesis. These observations suggest that both proteins could have a more general role in pathology as well.

Founding: FONDECYT 1100366.

DENDRITIC AND STROMAL CELLS FROM THE SPLEEN OF LUPIC MICE PRESENT PHENOTYPIC AND FUNCTIONAL ABNORMALITIES. **A. Gleisner**[1], P.A. Reyes[2,3], M. Rosemblatt[1,2,3], M.R. Bono[1]. [1]Facultad de Ciencias, Universidad de Chile, [2]Universidad Andrés Bello, [3]Fundación Ciencia para la Vida. alejandra.gleisner@gmail.com

Introduction. Systemic Lupus Erythematosus is an autoimmune disease of unknown etiology and characterized by autoreactive T and B cells. In this work we analyzed the maturation/activation state and the migratory capacity of spleen dendritic cells (DCs) in a murine lupus model to further elucidate their role in autoimmunity.

Material and Methods. We used the BWF1 mouse model that develops lupus spontaneously. We analyzed the distribution and phenotype of DCs by flow cytometry in spleen from BWF1 and control mice at different time points during disease development. Using PCR Arrays, we evaluated the expression levels of chemokines and their receptors on stromal cells and DCs from spleens of lupic and control mice.

Results. We demonstrate that lupic mice present an increased percentage of DCs in spleen compared with control mice. Moreover, lupic DCs show an altered activation state and expression of signaling lymphocyte activation molecule (SLAM) family receptors. Furthermore, lupic DCs migrate preferentially to the spleen compared with control DCs. In agreement with this, we found statistical differences in gene expression of several chemokines and chemokine receptor genes when comparing spleen DCs and stromal cells from lupic and control mice**.**

Discussion. Our results show that the phenotypic and migratory alterations observed in lupic DCs could result in altered distribution of DCs in lymphoid organs, contributing in this way to the pathogenesis of lupus.

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SCHWANN CELL TO AXON TRANSFER OF EXOSOMES PROMOTES AXONAL GROWTH AND REGENERATION. **M. Alejandra Lopez-Verrilli**, Felipe A. Court. Millennium Nucleus in Regenerative Biology, Catholic University of Chile and Neurounion Biomedical Foundation. alejanddra@gmail.com, fcourt@bio.puc.cl.

Introduction. We have previously shown that in the peripheral nervous system, ribosomes are transferred from Schwann cells (SC) to axons, but the transfer mechanism was not investigated. Here, we explored the possibility that SC uses nanometre-sized vesicles known as exosomes for cargo transfer to axons. It has been shown that exosomes mediate cellular communication by transferring proteins, mRNA and/or miRNA between cells. We propose that exosomes mediates macromolecular transfer between SC and axons during neuronal growth and regeneration.

Material and Methods. SC primary cultures were produced from newborn rat sciatic nerves. Exosomes were purified from SC culture supernatants by ultracentrifugation and characterized by Western-blot. Axonal growth and regeneration assays were performed in rat dorsal root ganglia (DRG). Exosome internalization in DRG axons was observed using vital dyes, immunofluorescence and confocal microscopy. Ultrastructural analyses were performed by electron microscopy.

Results. Exosome markers CD63, Tsg101 and flotillin were observed in exosomes secreted by SC. Internalization of SC-derived exosomes by axons increased after 2 and 4 hours of incubation (0.13 ± 0.02 and 0.49 ± 0.06 internalization/$10*nm^2$ of axonal area, respectively). Axonal growth *in vitro* increased after two days of exosome treatment respect to controls. When axons were cut, SC-derived exosomes increased axonal regeneration by 40% at 3 days-post injury.

Discussion. We demonstrate for the first time that SCs secrete exosomes and that transfer of SC-derived exosomes to axons increases axonal growth in basal conditions and axonal regeneration after injury.

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ORAL PRESENTATIONS VI

SEARCHING FOR PHYSIOLOGICAL STIMULI TO ENHANCE ADULT HIPPOCAMPAL NEUROGENESIS IN A MICE MODEL OF ALZHEIMER'S DISEASE. **Lorena Varela-Nallar**, Ana C. Abbott, Macarena Rojas-Abalos, Florencia C. Aranguiz, Cheril Tapia-Rojas and Nibaldo C. Inestrosa. Centro de Envejecimiento y Regeneración (CARE), Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, P. Universidad Católica de Chile, Santiago, Chile. lpvarela@uc.cl

Introduction: The generation of new neurons in the adult hippocampus has been related to hippocampal plasticity and memory. This process is impaired in different pathological conditions such as Alzheimer's disease (AD). Here we aim to stimulate adult neurogenesis in this neurodegenerative disease by physiological conditions that stimulate the Wnt/b-catenin signaling, a known positive regulator of adult neurogenesis that is affected in AD brains.

Material and Methods: Double transgenic mice (APPswe-PS1) model of AD were exposed to chronic hypoxia or voluntary wheel running. Neurogenesis was analyzed by incorporation of the nucleotide analog BrdU and double labeling with neuronal markers. Morris water maze task was used to evaluate spatial memory.

Results: Increased cell proliferation and generation of new neurons was observed in the hippocampus of APPswe-PS1 mice exposed to hypoxic conditions and voluntary running. In addition, running improved memory and decreased histopathological markers of AD. Mice were also exposed to the hyperforin derivative IDN5706. Interestingly, IDN5706 was able to increased neurogenesis and improved memory in APP-PS1 mice as well as wild-type animals.

Discussion: We have identified three different physiological stimuli (chronic hypoxia, voluntary running and IDN5706 treatment) that increase neurogenesis in an *in vivo* model of AD, and may have therapeutic value. Currently we are evaluating the role of the Wnt/β-catenin cascade in these effects.

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NEURAL PROGENITORS IN THE OLFACTORY SENSORY SYSTEM. Maegan V. Harden[1], Luisa Pereiro[4], Mirana Ramialison[2], Joachim Wittbrodt[2], Megana K. Prasad[3], Andrew McCallion[3], Jorge Torres4, and **Kathleen E. Whitlock[4]**. 1.Department of Molecular Biology & Genetics, Cornell University, NY; 2.Institute of Zoology, Heidelberg University, Germany; 3.McKusick-Nathans Institute of Genetic Medicine, Maryland, USA; 4. Interdisciplinary Center for Neuroscience, Universidad de Valparaiso, Valparaíso, Chile. kathleen.whitlock@gmail.com

Introduction. Vertebrate sensory organs originate from both cranial neural crest (CNC) and neurectodermal placodes. The developmental events determining the fate of neural and non-neural precursors in the olfactory sensory system are poorly understood. We followed the development of the forming olfactory placodes (OP) and the dorsally migrating CNC cells in the zebrafish embryo, as well as the subsequent division of neuronal precursor domains within the OP.

Material & Methods. Using zebrafish as a model system we analyzed gene expression by *in situ* hybridization. We generated a transgenic zebrafish expressing the fluorescent reporter protein mCherry under the control of a *six4.1* promoter. We combined the *six4.1:mCherry* line with existing fluorescent reporter lines to analyze OP differentiation in living embryos.

Results. We show that during migration, the CNC cells associate with and eventually surround the forming OP. In spite of the close association between the CNC and OP fields, little cell mixing occurs during this process. The pre-placodal transcription factors *dlx3b* and *six4.1* are localized to different domains of the developing OP. The cells expressing *six4.1:mCherry* condense away from the anterior midline and designate neural precursors.

Discussion. The OP appears to contain two separate domains: *six4.1* and *dlx3* expressing. These domains may delineate neural precursors giving rise to microvillous and ciliated olfactory sensory neurons.

XtRic-8A, A PROTEIN REQUIRED FOR PROPER NEURAL CREST FORMATION. Jaime Fuentealba, Juan Olate and **Marcela Torrejón.** Laboratory of Molecular Genetics, Biochemistry and Molecular Biology Department, University of Concepción, Chile. matorrejon@udec.cl

Introduction. The neural crest is a transient embryonic cell population that migrates extensively to various parts of the embryo, where it differentiates into diverse derivatives, including most of the craniofacial skeleton and peripheral nervous system. Ric-8 has been characterized as a GEF for heterotrimeric G proteins. We have determined that in *Xenopus tropicalis* embryos, XtRic-8A is expressed in neural crest cells before and after the migration step takes place and later in neural crest derivatives. In this work we have carried out functional studies in order to analyzed XtRic-8A participation during neural crest formation.

Material and Methods. Loss-of-function experiments in two-cell stage with antisense morpholino were carried out. To analyze induction/specification we used *in situ* hybridization assays of neural crest markers. Transplantation and explants assays were carried out in order to analyze XtRic-8A participation during migration and alcian blue staining to analyze craniofacial cartilage formation.

Results. Loss-of-function experiments resulted in an altered expression of neural crest markers during induction/specification and migration of neural crest cells. In addition, transplantation and explants assays showed migration defects and cartilage staining assays resulted in craniofacial defects.
Discussion. These results suggest that XtRic-8A is necessary for proper neural crest specification and migration. Loss-of-function experiments effects in neural crest formation could be due to the fact that XtRic-8A is also probably required for normal development during previous stages. On the other hand, transplantation assays, resulting in migration defects and cartilage staining assays, resulting in craniofacial defects, confirmed that XtRic-8A is required for proper neural crest formation.

CoREST/LSD1 CONTROL THE DEVELOPMENT OF PYRAMIDAL CORTICAL NEURONS. **Kukuljan, M**., Cánovas J., Berndt F.A., Fuentes, P. Programa de Fisiología y Biofísica, ICBM, e Instituto Milenio de Neurociencias Biomédicas, Facultad de Medicina, Universidad de Chile. kukuljan@med.uchile.cl
Introduction: the transition from proliferating neural progenitors to neurons and the maintenance of neuronal identity relies on genetic and epigenetic programs that coordinate the exit of the proliferative cycle, migration and differentiation into mature neurons. REST corepressor-1 (CoREST) has been proposed, on *in vitro* grounds, to be a key factor in the epigenetic regulation of neurogenesis. We set to test this proposal in vivo in the context of the development of the cerebral cortex.
Methods: we conducted manipulation of gene expression by in utero electroporation of the cerebral cortex of mouse embryos and analyzed proliferation, migration and neuronal fate at different developmental stages.
Results: the transition between multipolar and bipolar stages of migration for newborn cortical pyramidal neurons is markedly delayed by depletion of CoREST. This profoundly affects the onset of their radial migration. The effect of loss of CoREST function also perturbs the dynamics of neuronal precursor cell populations, transiently increasing the fraction of cells remaining in progenitor states. The function of CoREST in these processes appears to be independent of its best known interactor, REST/NRSF, and requires the histone demethylase LSD1. Further, loss of CoREST function leads to morphological defects of mature neurons.
Discussion: CoREST function, in association with LSD1, but not with REST/NRSF is required for the expression of the physiological developmental program of cortical projections neurons. One step possibly regulated by CoREST/LSD1 is the choice of division type of neuronal precursor cells.
FONDECYT 1090281; ICM P09-015-F; CONICYT Fellowships (PF, JC).

ANTI-ANGIOGENIC PROPERTIES OF COAGULATION RELATED PROTEASES. **Lange S**[1,2], Cautivo K[2], Elliot M[3] , Kalergis A[1,2], Palma V[3] & Owen GI[1,2]. [1]Faculty of Biological Sciences, PUC, [2]The Biomedical Research Consortium (BMRC) and [3]Laboratory of Stem Cells and Development, Fac. of Science, U. de Chile. mslange@uc.cl
Introduction. Angiogenesis plays a key role in physiological and pathological processes. Coagulation related proteases are capable of triggering cell signaling pathways. The aim of this study was to evaluate these coagulation proteases in the processes involved in angiogenesis.
Materials and Methods. Angiogenesis was evaluated using HUVECs and the EA.hy926 endothelial cell line. Capillary–like structure formation and migration were determined by matrigel and transwell assays, respectively. The involvement of protease activated receptors -1 and -2 was evaluated by using peptide agonists or antagonists. The capacity of proteases to induce regression of the capillary–like structures was also evaluated. Biological relevance of our findings was confirmed in the chick chorioallantoic membrane (CAM) model and a mouse xenograft model of endometrial cancer.
Results. We demonstrate that the active and inactive form of a coagulation protease can inhibit capillary formation in a concentration-dependent manner; however dissociation of endothelial cell organization and subsequent contraction occurs only with the active form. The mechanism involves a reduction in endothelial cell migratory potential and activation of PAR1. The administration of the active form of this protease was antiangiogenic in the physiological CAM model, yet surprisingly increased tumor growth in the mouse.
Discussion. We demonstrate a previously unreported anti-angiogenic function of a coagulation related protease. Further understanding of these different effects observed *in vitro* and *in vivo* of this protease may, in the future, give rise to a new class of anti-angiogenic drug.

THE HYPOXIA FACTOR Hif-1α IS ESSENTIAL FOR NEURAL CREST MIGRATION. **Elías H. Barriga[1,2]**, Roberto Mayor[2] y Ariel E. Reyes[1]. [1]Laboratorio de Biología del Desarrollo, Departamento Ciencias Biologicas, UNAB, Chile. [2]Department of Cell and Developmental Biology, UCL, London, UK. arielreyes@unab.cl
Introduction. Hypoxia-inducible factors (HIFs) are a key regulator of gene expression in response to Hypoxia. *In vitro* experiments have shown that Hif-1a regulates cancer cell migration during metastasis. However, little is known about the role of hypoxia and Hif-1a on NCC migration.
Materials and Methods. We used zebrafish and *Xenopus* embryos to assay the effect of Hif-1a loss and gain of function, on the NCC development. We performed *in situ* hybridization to *foxd3*, *ap2* and *slug* (NCC induction markers) and *crestin,*

slug, twsit and cxcr4b (NCC migration markers). Also, using *Xenopus* NCC explants, we analyzed the cell dispersion (EMT) and chemotaxis.

Results. We observed that the *knockdown* of Hif-1a doesn't induce a variation in the expression of *foxd3* and *ap2*. However, the expression of *crestin,* was strongly affected. Also we observed disorders in pharyngeal cartilage and melanocites. Consistently, Hif-1α protein is present in migrating NCC, as determined by Western blot and immunofluorescence. Also, the loss and gain of function of Hif-1α affects the normal NCC dissociation (EMT), and chemotaxis. Finally, we ob serve a decreased expression of *Twist* (EMT regulator) and *Cxcr4* (chemotaxis regulator), markers.

Discussion. Here we show that Hif-1α is essential for neural crest migration in zebrafish and *Xenopus* embryos. The next experiments targets to reveal the molecular mechanism by Hif-1 is regulating *Cxcr4* and *Twist's*.

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GAP JUNCTIONS PROMOTES THE COMMUNICATION BETWEEN HUMAN NATURAL KILLER CELLS WITH DENDRITIC AND TARGET CELLS. **Andrés Tittarelli**(1), Marcela Farias(2), Ariadna Mendoza-Naranjo(3), Benedict Chambers(4), Andreas Lundqvist(5), Flavio Salazar-Onfray(1). 1:Millennium Institute on Immunology and Immunotherapy. 2:Faculty of Odontology, University of Chile. 3:UCL Cancer Institute, UK. 4:Center for Infectious Medicine, Karolinska Institute. 5:Cancer Center, Karolinska Institute, Sweden. tittarelli@gmail.com

Introduction: Gap junction (GJ) mediates inter-cellular communication through linked multichannel from each of two adjacent cells. Functional GJs channels are composed by connexins (Cxs). We are recently published that Cx43- and GJ-mediated inter-cellular communication (GJIC) participates in key immunological processes, such as cross-presentation, and dendritic cell (DC)-mediated T cell activation. Because DC-mediated natural killer (NK) cells activation and NK cytotoxicity are cell-to-cell contact dependent mechanisms, in this study we evaluated the role of GJ in these process.

Material and Methods: We used Cx43-specific and GJ chemical inhibitors, on NK-DC and NK-K562 co-cultures. We evaluated the functional GJ formation by calcein transference assays, and assessed the NK and DC activation by surface markers expression and cytokine secretion. Also, we evaluated the NK cytotoxicity by Cr^{51} release, degranulation and granzyme-B activity assays.

Results: We show that Cx43 allow a bidirectional communication between NK cells and DCs. This intercellular cross-talk contributed to NK cell activation, as neutralization of Cx43 impaired CD69 induction and IFN-g release, but have almost no effect in the DC activation. Also, we proved that NK cells form GJs communication with the target cell line K562, contributing to mediate the granzyme B-dependent cytotoxicity.

Discussion: These findings identify for first time a fundamental role of GJs in regulate human NK cells functions.

CAVEOLIN-1 INHIBITS TRANSCRIPTION BY HYPOXIA INDUCIBLE FACTOR-1A IN TUMOR CELLS. **Sanhueza C.**,[1] Lladser, A.,[2] Valenzuela M.,[1] Nuñez S.,[1] Diaz M.I.,[1] Leyton L.,[1] Quest A.F.G.[1] [1]Laboratorio de Comunicaciones Celulares, Centro de Estudios Moleculares de la Célula, Facultad de Medicina, Universidad de Chile. [2]Laboratory of Gene Immunotherapy, Fundación Ciencia para la Vida, Santiago. aquest@med.uchile.cl

Introduction: Stabilization and activation of the Hypoxia Inducible transcription Factor-1α (HIF-1α) is required for tumor neovascularization in response to reduced oxygen availability in solid tumors. Caveolin-1 interacts with and inhibits many signaling proteins possibly explaining its tumor suppressor role. Given the importance of HIF-1α in tumor development, we investigated whether caveolin-1 modulates HIF-1α transcriptional activity and whether this correlated with the possibility of interaction between both proteins.

Methodology: HT29(US) colon and MDA-MB-231 breast cancer cells expressing or not Caveolin-1 were exposed to hypoxia (O_2 1%) for 24 h. HIF-1α transcriptional activity, target-gene expression and HIF-1α protein levels were detected by gene-reporter asssays, RT-PCR and Western-blotting, respectively. HIF-1α/Caveolin-1 colocalization and interaction were assessed by immunofluorescence and co-immunoprecipitation. Cell viability was evaluated by FACS.

Results: Caveolin-1 over-expression reduced, while caveolin-1 silencing increased HIF-1α transcriptional activity in hypoxia, without affecting HIF-1α protein levels. Reduced HIF-1α-dependent transcriptional activity was associated with decrease HIF-1α target-gene expression. Hypoxia induced colocalization between caveolin-1 and HIF-1α as well as co-immunoprecipitation of both proteins. Importantly, cell viability during hypoxia was not affected by caveolin-1 presence.

Discussion: The results suggest that caveolin-1 reduces HIF-1α activity and target-gene expression in hypoxia, presumably via sequestration-mechanism. Such inhibition may explain how caveolin-1 act as a tumor suppressor.

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POSTER PRESENTATIONS I

(1) HEMICHANNELS FORMED BY PANNEXIN-1 ARE ACTIVATED BY EXTRACELLULAR ATP IN ADIPOCYTES. **Fernández PE,** Benvenuto MA, Sáez JC. Departamento de Fisiología, Pontificia Universidad Católica de Chile. paolafernandez.bq@gmail.com

Introduction: Adipose tissue is the primary site of inflammation in obesity and releases several factors involved in immune responses. It is known that interaction between channels formed by pannexins (Panxs) and different P2 receptors play a crucial role in the coordination of inflammatory responses. Here, we tested if differentiated adipocytes from visceral adipose tissue express functional Panx1 hemichannels (HCs) linked to activation of P2 receptors.

Material and Methods: We used adipocyte primary cultures derived from visceral adipose tissue obtained from Sprague Dawley rats (male, 230-260g). The presence of Panx1 was evaluated with western blot and immunofluorescence analyses. The presence of functional HCs was studied in cells treated with 500 μM ATP and ethidium (5 mM) uptake measurements in real time.

Results: Panx1 was detected in undifferentiated and differentiated rat adipocytes. Moreover, the application of extracellular ATP induced ethidium uptake, which was blocked by 18-bglycyrrhetinic acid (β-GA, 50μM), a connexin/Panx HC blocker, and carbonexolone (5 μM) or probenecid (1mM), two Panx1 hemichannel blockers.

Discussion: Extracellular ATP associated with Panx1 HCs could be part of the mechanisms underlying chronic inflammatory processes present in obesity.

(2) ROLE OF mTOR PATHWAY IN POST-MITOTIC TRANSLATION OF INHERITED RUNX2 mRNA IN PRE-OSTEOBLASTS CELLS. **Alejandra Aránguiz[1,2]**, Nelson Varela[1,2], Marcelo Antonelli[2], Carlos Lizama[3], Ricardo Moreno[3], Hugo Sepúlveda[4], Martin Montecino[4], Zhang Ying[5], Gary Stein[5], Andre van Wijnen[5] y Mario Galindo[1,2]. [1]Programa de Biología Celular y Molecular, ICBM, Facultad de Medicina, Universidad de Chile. [2]Millennium Institute on Immunology and Immunotherapy. [3]Departamento de Ciencias Fisiológicas, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, [4]Center for Biomedical Research, Faculty of Biological Science and Faculty of Medicina, Andres Bello University. [5]Department of Cell Biology and Cancer Center, University of Massachutsetts Medical School, USA. mgalindo@med.uchile.cl

Introduction: Osteoblast lineage commitment is stringently controlled by the cell-fate–determining Runx2 transcription factor. Previously, we had shown that Runx2 mRNA accumulates during mitosis and then inherited and translated by daughter cells. This mechanism ensures the early restitution of this factor after cell division to maintaining lineage commitment in pre-osteoblasts. In the current study, we investigated the mechanisms related to post-mitotic translation of inherited Runx2 mRNA.

Material and Methods: We evaluate the role of mTOR pathway in post-mitotic translation of inherited Runx2 mRNA. MC3T3 pre-osteoblasts cells were arrested in mitosis and then were stimulated to progress synchronically into G1 phase. We analyze resumption of Runx2 protein levels and functional association to promoter region of target gene by western blot, immunocytochemistry and ChIP during the M/G1 phase transition. We also analyzed translational control of mRNA Runx2 in presence or absence of α-amanitin (RNAII polymerase inhibitor), cycloheximide (general translational inhibitor) and/or rapamycin (mTOR inhibition).

Results: Runx2 protein levels were post-mitotically up-regulated during G1 phase in both cells treated with or without α-amanitin. However, Runx2 protein was not up-regulated during M/G1 transition in cells treated with α-amanitin plus cycloheximide or α-amanitin plus rapamycin.

Discussion: Our results suggest that Runx2 mRNA post-mitotically inherited is translated by mTOR pathway in daughter cells during G1 phase. We have shown clear evidence supporting a major role of mTOR pathway in Runx2 protein synthesis during G1 phase in proliferative pre-osteoblasts**.**

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(3) ACTIVATION OF THE UNFOLDED PROTEIN RESPONSE (UPR) ENHANCES MOTOR RECOVERY AFTER SPINAL CORD INJURY. **Vicente Valenzuela[1,2,3],** Eileen Collyer[3], Donna Armentano[4], Geoffrey Parsons[4], Felipe A. Court[3,5] and Claudio Hetz[1,2,5]. [1]Biomedical Neuroscience Institute and [2]Center for Molecular Studies of the Cell, Institute of Biomedical Sciences, University of Chile. [3]Millennium Nucleus in Regenerative Biology (MINREB), Catholic University of Chile. [4]Department of Molecular Biology, Genzyme Corporation, USA. [5]Neurounion Biomedical Foundation. chetz@med.uchile.cl, fcourt@bio.puc.cl

Introduction: Spinal cord injury (SCI) triggers many cellular and tissular responses. Here, we used knockout mic and adeno-associated virus (AAV) mediated gene therapy to assess the function of the UPR in SCI and then observed the locomotion recovery pattern after SCI. Other tissular parameters were also analyzed, including cellularity and oligodendrocyte density at 35 days post-SCI.

Material and Methods: C57BL/6 wild-type mice were lateral spinal cord hemisected and immediately injected with 2μl (10^{12} DRP/mL) of AAV2 expressing either GFP alone or the XBP1s transgene in the injured region. Basso-Mouse-Scale open-field test was scored at different times after SCI to monitor locomotor performance. Spinal cord tissue was collected 35 days post-lesion and processed to monitor nuclear and Olig2-positive cells distribution.

Results: Local expression of active XBP1 into the spinal cord using AAVs immediately after SCI improved locomotor recovery, decreased general nuclear density and augmented Olig2-positive cells 35 days after SCI.
Discussion: We demonstrated a functional role of UPR in locomotor recovery after SCI. We speculate that the UPR may be involved in controlling oligodendrocyte-dependent protective functions in the injury site that could contribute to locomotion recovery after SCI.
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(4) PHOSPHOENOLPYRUVATE CARBOXYKINASE EXPRESSION IN A CELLS FROM HUMAN AND MOUSE PANCREAS. **Romina Bertinat**[1], Fabián Pardo[1], Cristian Carrasco[2], Karen Jaramillo[1], Juan Carlos Slebe[1] and Alejandro J. Yáñez[1]. Instituto de Bioquímica y Microbiología, Universidad Austral de Chile[1]; Unidad Anatomía Patológica, Hospital Base Valdivia[2], Valdivia, Chile. romibert@gmail.com
Introduction: Glucose homeostasis is mainly regulated by the coordinated secretion of glucagon and insulin from α- and β-pancreatic cells. Glucose induced an opposite physiological response in these cells, but the exact mechanisms that induce glucagon release are not known. It has been shown that β-cells express the mitochondrial isoform of phosphoenolpyruvate carboxykinase (PEPCK), a gluconeogenic enzyme with a broader metabolic function in the cataplerosis of citric acid cycle intermediates The aim of this study was to analyze PEPCK expression in α-cells.
Materials and Methods: Total pancreata from healthy and streptozotocin-induced diabetic C57BL/J mice were analyzed by Western blot and immunohistochemical (IHC) assays for the expression and localization of cytosolic (C) and mitochondrial (M) isoforms of PEPCK. Pancreas biopsies from control and autopsies from diabetic humans were analyzed by IHC.
Results: Western blot analysis showed that both cytosolic (C) and mitochondrial (M) PEPCK isoforms are expressed in control mice pancreas, and that an increase of PEPCK-C and a decrease of PEPCK-M expression occurs after induction of diabetes. IHC studies showed that PEPCK-C is mainly expressed in α-cells and PEPCK-M in β-cells, in human and mouse pancreas.
Discussion: These data suggest that the segregated expression of PEPCK-C and -M in α- and β-cells is responsably, at least in part, for the differential response of these cells to metabolic stimuli. Moreover, PEPCK-C overexpression in diabetic α-cells could be involved in glucagon hypersecretion in response to gluconeogenic substrates.
(FONDECyT 1090694)

(5) ANALYSIS OF THE EPIGENETIC RESPONSE UNDER CONDITIONS OF CELLULAR ENERGY RESTRICTION DURING THE ACCLIMATIZATION OF *C. carpio*: THE eNosC COMPLEX. **Fernández de la Reguera C.,** Nardocci G., Morales J., Molina A., Vera MI., Alvarez M. L. de Biología Celular y Molecular, F. de Ciencias Biológicas, U. Andrés Bello, Viña del Mar, Chile. malvarez@unab.cl; c.fernandezde.c@uandresbello.edu
Introduction: Regulation and transcriptional reprogramming of ribosomal genes is one of the most striking events during the carp seasonal acclimatization. Recently, in mammals, it has been described a novel nucleolar complex named eNosC (Sirt-1, SUV39H1 and NML subunits), which is activated according to cellular energy status. Moreover, eNosC is suggested to contribute in the epigenetic regulation of ribosome biogenesis. Consequently, we propose that eNosC participates to ribosomal gene reprogramming in response to cellular energy levels during the carp acclimatization.
Materials and Methods: Coding sequences of eNosC complex components were amplified and cloned. We carried out the MTT cell viability assay in different culture media using *Epithelioma Papulosum Cyprini* cells (EPC) acclimated to 22 °C and 8 °C. We evaluated the NML content and transcriptional levels of eNosC components by RT-PCR in carp acclimatized tissues (n = 3) and also in acclimated EPC cells grown in different conditions.
Results: We obtain partial coding sequences of eNosC components from carp. Tissue protein levels of NML revealed no significantly variations between seasons. However, transcriptional expression of eNosC components varied significantly in the conditions studied.
Discussion: Our results show that the activity of eNosC displays a compensatory effect, which could contribute to prevent a drastic cellular energy imbalance during carp acclimatization. This epigenetic mechanism is for the first time described in a fish and related to natural adaptation process.
DI-4511/R.

(6) TISSUE FACTOR PATHWAY INHIBITOR (TFPI) AND TISSUE FACTOR (TF) CO-LOCALIZE IN LIPID RAFTS (LR) OF PLATELET MEMBRANES: MECHANISM TO INHIBIT PLATELET PROCOAGULANT (PCA) ACTIVITY. **González César**, Matus Valeria, Pereira Jaime, Mezzano Diego, Panes Olga. Department of Hematology-Oncology, School of Medicine, P. Catholic University of Chile. cesar.gonzalezg@usach.cl (Sponsor: V. Velarde)
Introduction: TFPI, a natural inhibitor of blood clotting initiated by TF is bound to endothelial cells through its affinity with glycosyl-phosphatidil-di-inositol (GPI), which is associated with cholesterol-rich domains in cell membranes (LR).

Human platelets contain TFPI, but also functional TF, a fraction of which is localized in LR. However, the TF procoagulant activity (PCA) in LR fraction has not been demonstrated.
Aims: To show that TFPI co-localizes with TF in LR of human platelets, and this association inhibits the TF-induced PCA.
Methods and Results: LR-rich fractions were obtained from washed, stimulated (Collagen or VWF-Ristocetin) platelets lysed with 1% Triton X-100 at 4°C, and subjected to differential centrifugation to obtain the insoluble fraction, enriched in LR. We detected TFPI in Western blots of LR fractions, which disappeared after membrane cholesterol removal by methyl-:-cyclodextrin (M:CD). By immunoprecipitation (IP) of LR fractions we found co-precipitation of TFPI with TF. Lastly, no PCA was detected in LR fractions.
Conclusions: Platelet TF and TFPI localize and co-precipitate in LR, indicating that platelets are able to assemble the TFPI-TF complex and participate in the inhibition of clotting amplification. These results show that platelet function is pivotal in the whole haemostatic process.
Fondecyt: 1080369, 1090170, 1110404.

(7) INVOLVEMENT OF CONNEXIN-26 HEMICHANNELS IN PURINERGIC SIGNALING AND THEIR POSSIBLE CONTRIBUTION IN OTOTOXICITY. **Figueroa VA.[1,3],** Jara O.[1], Martínez AD.[1], Fiori M.[2], Altenberg GA.[2], and Sáez JC.[1,3]. [1]Centro Interdisciplinario de Neurociencias de Valparaíso (CINV), Universidad de Valparaíso, [2] Department of Cell Physiology and Molecular Biophysics and Center for Membrane Protein Research, Texas Tech University Health Sciences Center, [3]Departamento de Fisiología, P. Universidad Católica de Chile. vaniafigueroa@gmail.com
Introduction: Connexin26 **(**Cx26) is widely expressed in the mammalian cochlea and over 100 human Cx26 mutations have been associated with deafness. Moreover, it has been proposed that activation of Cx hemichannels (HCs) leads to increases in $[Ca^{2+}]_i$ through ATP release to the extracellular milieu, initiating a purinergic signaling cascade and thus, dismissing a possible HC contribution as a normal route for Ca^{2+} influx and its possible dysfunction in deafness.
Materials and Methods: HeLa cells transfected with wild type Cx26 fused to green fluorescent protein (Cx26WT-GFP) or its deafness-related mutant Cx26V37I-GFP, were used. $[Ca^{2+}]_i$ changes were monitored in cells loaded with Fura-2AM and the Ca^{2+} permeability of HCs was determined in Cx26 HCs reconstituted in liposomes loaded with Fluo-5N.
Results: HeLa-Cx26WT-GFP cells responded to extracellular ATP with oscillations in the $[Ca^{2+}]_i$, that were almost lost in cells transfected with Cx26V37I-GFP that did not form functional HCs. Calcium oscillations in HeLa-Cx26WT-GFP cells were also completely abrogated by gentamicin, an ototoxic agent that causes hearing loss; gentamicin blocked Cx26WT-GFP HCs. Finally, Cx26 HCs reconstituted into liposomes were permeable to Ca^{2+}.
Discussion: Cx26 HCs are permeable to Ca^{2+} and blocked by gentamicin or mutation V37I. Low Cx26 HC activity reduces oscillations of the $[Ca^{2+}]_i$, which could avoid a Ca^{2+}-mediated intracellular signaling that might be required for normal audition.

(8) INCREASE COPPER LEVELS IN AN HEPATOMA CELL LINE MIMICKING NPC1 PHENOTYPE. **Mary Carmen Vázquez[1]**, Mauricio González[2] and Silvana Zanlungo[1]. [1]Departamento de Gastroenterología, Facultad de Medicina, P. Universidad Católica de Chile. [2]Laboratorio de Bioinformática y Expresión Génica, INTA, Universidad de Chile. mc.vazquez.rodriguez@gmail.com
Introduction: Niemann-Pick type C disease (NPC) is a neurovisceral atypical lipid storage disorder characterized by unesterified cholesterol accumulation in lysosomal/late endosome compartments and oxidative stress. We have previously reported an increase in hepatic copper levels in a NPC mouse model. In NPC disease, there are two main organs affected; the liver and the cerebellum.
Materials and Methods: Hipocampal neurons primary cultures and a human hepatoma cell line, HepG2, were treated with the lysotropic compound U18666A (U18), that has been widely used as a pharmacological model of NPC disease, or vehicle for 24 h, in the presence or absence of 5 mM Cu (Cu:His=1:10). Mouse neuroblastoma cell line, Neuro2A, stably transfected with shRNA against NPC1 protein or shRNA mock were incubated with or without 5 mM Cu for 24 h. After treatments media was collected and cells were either fixed on cover slips for Filipin dye or lysed for copper content determination by Atomic Absorption Spectroscopy (AAS).
Results: In both NPC neuronal type models we did not find an increase in copper content whereas we did find this effect in the hepatic cell model. . Filipin dye corroborates the NPC phenotype in all the three cellular models analyzed.
Discussion: These results shows a cellular type dependence for copper accumulation suggesting that copper transport imbalance may be more relevant in liver than in cerebellum in NPC disease.
FONDECYT 3100026, 1071083 and 1110310 to MCV, MG and SZ. Proyecto FONDAP 15090007, Centro de Regulación del Genoma (CRG).

(9) EVALUATION OF THE CYTOTOXIC EFFECT OF A HYDROALCOHOLIC EXTRACT OF *Ruta graveolens* IN THE HEK 293 AND HUVEC CELL LINES. **Ignacio Jofré[1,3]**, Fernando Romero[1], Jenny Rudlinger[1], Karina Mansilla[1], Jorge Parodi[2], Raúl Salvatici[1], Patricia Navarrete[1]. [1]Center of Neurosciences and Peptides Biology- BIOREN,

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Introduction. We have obtained an IC_{50} value of a hydroalcoholic extract of *Ruta graveolens* with vasodilating properties in aorta of rat mean isolated organ. This study aims to determine possible cytotoxic effects and intracellular calcium responses of a hydroalcoholic extract of *Ruta graveolens* in the HEK 293 and HUVEC cell lines.

Materials and Methods. The HEK 293 and HUVEC cell lines were exposured to concentrations differents of a hydroalcoholic extract of *Ruta graveolens* and was to procedure measure cytotoxicity mean propidium iodide, test TUNEL and luminol for to determine viability, DNA integrity and reactive oxygen species extracellular production, respectively. Moreover, was determined the intracellular calcium response by fluorescent calcium indicator FLUO-4.

Results. The results obtained shows that the concentration used as IC_{50} of the hydroalcoholic extract of *Ruta graveolens* is not produce cytotoxicity, DNA fragmentation neither ROS extracelullar production, not thus to majors concentrations. Moreover, the microfluorometry tests showed that calcium do not enter in cells endothelial (HUVEC), while there is a flow in epithelial (HEK 293).

Discussion. These findings show that the hydroalcoholic extract of *Ruta graveolens* is cytotoxic at high concentrations, while the concentration used as the IC_{50} is not toxic. It follows that this concentration can probably be used as drug in future medical applications of this extract.

(10) HIGH LEVELS OF LEPTIN DECREASES CILIARY ACTIVITY, THROUGH THE ACTIVATION OF NOS IN CILIATED CELLS OF THE RAT OVIDUCT. **Carolina Oses[1]**, Maria Paz Hernández[1], Daniela Careño[1], Carmen Llados[1], Manuel Villalón[1]. [1]Department of Physiology, Faculty of Biological Sciences. Pontificia Universidad Católica de Chile, Santiago, Chile. cdoses@uc.cl

Introduction: Recent studies have shown a correlation between the increase in adipose tissue and high concentrations of plasmatic leptin, suggesting a possible cause for decrease fertility. Ciliary activity in oviduct plays a key role in the transport of gametes in the female reproductive tract. In the cardiovascular system it is known that leptin increases nitric oxide plasma levels. We hypothesized that leptin can affect the ciliary beat frequency through the activation of nitric oxide production in the rat oviduct.

Materials and Methods: We used primary cultures of rat (Sprague Dawley) oviductal epithelium grown in cultures chambers. We incubated the cultures with 3 different concentrations of leptin (1, 10 and 100 ng/ml) and measured the ciliary beat frequency, using a microphotodensitometric technique ($n > 3$). We also tested the effect of L-NAME (nitric oxide synthase inhibitor) upon the effect of leptin on the CBF. The experiments were analyzed with ANOVA and post-test Tukey.

Results: The results show a decrease of the ciliary beat frequency that is dependent on the leptin concentration. This effect was blocked by the previous incubation of the ciliated cells culture with L-NAME. Furthermore ciliary beat frequency increases in a leptin concentration dependent manner in the presence of NOs inhibitor.

Discussion: Elevated levels of leptin can affect oviductal physiology through the activation and local production of nitric oxide and suggest that abnormal levels of leptin associated to obesity can alter female fertility.

(ACT-79 and FONDECYT 1080679).

(11) NEURONAL ENDOPLASMIC RETICULUM ORGANIZATION AND MODULATION OF CALCIUM SIGNALS. **Figueroa C[1]**, San Martín C[3], Couve A[2], Härtel S[1], Ramírez O[1]. [1]SCIAN-Lab, [2]Laboratory of Cellular and Molecular Neurobiology, [3]Molecular Studies Center of the Cell, ICBM, Faculty of Medicine, U-Chile. carolina.figueroa.a@gmail.com

Introduction: The endoplasmic reticulum (ER) is a membranous organelle present in all eukaryotic cells. The ER stores and controls the entry and release of Ca^{2+}, secondary messenger that regulates fundamental physiological processes such as cell cycle, synaptic transmission, and cell death. In neurons, the generation of specific patterns of Ca^{2+} signal plays a major role in key processes such as neuronal excitability and synaptic plasticity. We hipothezise that the ER integrity is important for maintaining normal spreading of Ca^{2+} signals. We subjected neurons to glutamate treatments to modify the ER continuity and evaluate the Ca^{2+} signals induced after IP_3Rs activation.

Material and Methods: Cultured rat hippocampal neurons were transfected with mRFP-KDEL. 24h later neurons were loaded with Fluo-4, left unstimulated or treated with glutamate 100 µM. Neurons were transferred to a chamber for live cell fluorescence imaging and stimulated with ATP to induce Ca^{2+} signals. Images were acquired with confocal microscopy and analyzed with SCIAN-Software.

Results: ER fragments after glutamate receptor activation. ATP treatment leads to modified dendritic Ca^{2+} signals concomitant to ER fragmentation.

Discussion: Ca^{2+} imaging combined with image analysis enables the study of shape changes in dendritic ER and opens future venues to understand the relationship between Ca^{2+} modulation and ER morphology. In the future, we will test if dendritic ER recovers its shape and restores Ca^{2+} homeostasis.

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(12) NEOGENIN 1 (Neo1): A TARGET OF SONIC HEDGEHOG SIGNALLING PATHWAY IN HUMAN NEUROBLASTOMA CELLS. **Natalie Espinoza G.**[1], Luis A. Milla[1] and Verónica Palma[1]. [1]Laboratory of Stem Cells and Development. Faculty of Sciences, University of Chile, Santiago, Chile. nat.espinozagiacomozzi@gmail.com

Introduction: Sonic Hedgehog signaling pathway plays a critical role in development of many organs and cells types and its deregulation has been implicated in appearance, grow and maintenance of several cancers. We have demonstrated in a Shh driven cerebellar cancer mouse model that the receptor Neo1 is dramatically overexpressed. Neo1 has been proposed as a death dependence factor acting as a tumor suppressor gene. The aim of this work is to evaluate the Shh/Neo1 relationship in human neuroblastoma, a malignancy due to deregulated Shh signaling, that arises from neural crest cells.

Methodology: We took advantage of the human neuroblastoma cell line SH-SY5Y to evaluate the relationship of the Shh/Gli pathway and Neo1 expression using RT-PCR, immunoflurescences and western blots. We used Chromatin Immunoprecipitation and luciferase reporter assays to ascertain if this regulation is direct.

Results: We demonstrated that the Shh pathway controls *neo1* expression in our neuroblastoma model. Using an *in silico* approach, we identified several putative Gli binding sites in the neo1 promoter that are being tested for their direct regulation by Gli transcription factors.

Discussion: Neo1 is highly expressed in SH-SY5Y cells maintaining their tumorigenicity leaving us to propose Neo1 as a novel cancer progression marker. The requirement of Neo1 mediated hedgehog signaling for survival of SH-SY5Y cells is currently being tested.

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(13) A FAILURE IN ASCORBIC ACID HOMEOSTASIS IS RESPONSIBLE FOR THE METABOLIC IMPAIRMENT IN HUNTINGTON'S DISEASE. [1]**Felipe Beltran,** [1]Macarena Solis, [2]Rene Vidal, [13]Carlos Cepeda, [1]Ilona I. Concha, [2]Claudio Hetz, [3]Michael Levine, [1]Maite A. Castro. [3]Semel, UCLA; I. Ciencias Biomedicas, U. Chile; I. Bioquímica y Microbiología, UACh.

Introduction: Huntington's disease (HD) is an neurodegenerative disorder characterised by progressive abnormalities in cognitive and motor control. Several studies have demonstrated an impairment in glucose metabolism in the basal ganglia and in the cerebral cortex of symptomatic HD patients. When HD animal models become behaviourally active, the level of ascorbic acid in striatal extracellular fluid is abnormally low in relation to that of littermate controls. We have demonstrated that intracellular ascorbic acid inhibits glucose transport and stimulates lactate transport in synaptically active neurons. In this work we studied the ability of ascorbic acid to modulate neuronal glucose consumption in HD mice (R6/2 mice).

Materials and Methods: Synaptic activity was measured as recordings from striatal medium spiny neurons, using whole cell configuration of the voltage-clamp technique. Excitatory post synaptic currents were evoked by stimulating cortico-striatal pathway. Using immunofluoresence, qPCR and Western blot analyses we explored protein and mRNA levels of proteins involved in neuronal metabolism modulation.

Results: Ascorbic acid was not able to modulate the ability of glucose to serve as an energetic fuel sustaining glutamatergic synaptic activity in presymptomatic and symptomatic HD animals. The presynaptic component was not affected, suggesting a failure on postsynaptic neuron metabolism modulation.

Discussion: Abnormalities observed in the ascorbic acid-dependent modulation of neuronal metabolism of presymptomatic R6/2 mice, suggests that ascorbic acid homeostasis failure could be important in the development of HD.

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(14) DIMEBON PROTECTS FROM CELL DEATH-INDUCED BY OXIDATIVE STRESS AND ALPHA-SYNUCLEIN OVER-EXPRESSION, AND DECREASES ALPHA-SYNUCLEIN PROTEIN LEVELS IN A PARKINSON´S DISEASE CELL MODEL. **Iván E. Alfaro**[1], Luz Delgado[1], Dania Valdovinos[1], Andrew Protter[1,2], Sebastián Bernales[1,2]. [1]Fundación Ciencias Para la Vida, Santiago, Chile. [2]Medivation Inc., CA, USA. alfobioq@gmail.com

Introduction: Aggregation of alpha-synuclein (α-syn) and mitochondrial dysfunction are important intermediaries in the development of Parkinson's disease (PD). Dimebon is a drug with cognition-enhancing, pro-neurogenic and neuroprotective effects in rodents that acts through a mitochondrial-related mechanism and is currently in clinical trials for the treatment of Alzheimer´s Disease. In this work we evaluated the neuroprotective properties of Dimebon in a cell model of PD.

Materials and Methods: Neuroprotective properties of Dimebon was evaluated in SH-SY5Y neuroblastoma cells treated with hydrogen peroxide and rotenone and in cells conditionally overexpressing α-syn. Cell viability was measured by LDH release and MTS assays. Changes in expression of α-syn protein were evaluated by western blotting and by immunocytochemistry and levels of α-syn mRNA was by qPCR.

Results: Dimebon was associated with a statistically significant reduction in LDH release in SH-SY5Y cells treated with hydrogen peroxide and rotenone. Dimebon, at nanomolar concentrations, significantly improves cell viability in SH-SY5Y cells overexpressing α-synuclein. Chronic treatments with Dimebon decreases total levels of Triton X-100 soluble and insoluble α-syn protein in α-syn overexpressing cells and in SH-SY5Y wild type cells treated with rotenone. No changes in α-syn mRNA levels were observed in response to Dimebon.

Discussion: These results indicate that in cell cultures, Dimebon enhances the viability of neuronal cells under oxidative stress and high α-syn protein levels. Dimebon reduces α-syn protein but not mRNA, suggesting an effect on protein turnover rather than synthesis.

(15) REDUCTION OF Aβ-OLIGOMERS IN PLASMA OF ALZHEIMER TRANSGENIC MICE TREATED WITH A c-Abl INHIBITOR. **Estrada LE[1]**, Chamorro D[1], Inestrosa NC[2], and Alvarez AR[1]. [1]Laboratorio de Señalización Celular, [2]CARE, Depto. de Biología Celular y Molecular, FCB, P. Universidad Católica de Chile. lestrada.biotech@gmail.com

Introduction: Alzheimer´s disease (AD) is a degenerative disorder characterized by memory loss and cognitive disabilities. The main component of AD amyloid plaques is the Aβ-peptide, which is part of the amyloid-precursor-protein (APP). Interestingly, our group previously reported the clearance of amyloid plaques in AD-transgenic mice treated with the c-Abl kinase inhibitor: STI571.

In this work we showed that STI571 decreases plasma levels of Aβ-oligomers *in vivo*. Besides, we observed that STI571 changes the Ab secretion in hippocampal neurons and HT22-cells overexpressing APP_{Swe}.

Material and Methods: APPsw/PSEN1ΔE9 mice were treated with STI571 or vehicle for 14-days by intraperitoneal injection. Plasma from cardiac blood fractions were isolated using a Ficoll-gradient and subjected to an anti-Ab ELISA. Additionally, primary hippocampal neurons and HT22-hippocampal cells lines transfected with $APP_{Swe,}$ were treated with STI571.

Results: The peripheral administration of STI571 decreased the plasmatic levels of Ab-oligomers in APPswe/PSEN1ΔE9 mice. In addition, we observed a decrease in the Aβ burden of treated AD-mice, while the levels of microglia activation, measured by GFAP staining, remain similar in control and STI571-mice. Likewise, in both hippocampal neurons and HT22-APP_{swe} cells we observed a decrease of Ab and changes in the distribution of APP cleavage fragments.

Discussion: It has been shown that c-Abl participates in endosomal regulation and since APP amyloidogenic proteolytic cleavage occurs in endosomal compartments, we believe that this process may be regulated by inhibiting c-Abl by STI571, thus decreasing the Ab oligomers production.

Fondecyt-Postoctorado-3110052/Fondecyt-1080221.

(16) THE cJUN-AMINO TERMINAL KINASE (JNK) REGULATES INTERNALIZATION AND RETROGRADE APOPTOTIC KILLING OF THE p75 NEUROTROPHIN RECEPTOR (p75) IN SYMPATHETIC NEURONS (SCGS). Escudero CA[1], **Cabeza C[1]**, Galleguillos C, Uzma S[2], Maloney M[3], Carter BD[2], Mobley W[3,4], Bronfman FC[1]. 1Millennium Nucleus in Regenerative Biology (MINREB), Facultad de Ciencias Biológicas. Pontificia Universidad Catolica de Chile, Chile. 2Department of Biochemistry, Vanderbilt University, USA. 3Department of Neurology, Stanford University, USA. 4Department of Neurosciences, UCSD, USA. caescude@gmail.com

Introduction: The functional significance of intracellular trafficking of NGF/TrkA in the neuronal development of sympathetic neurons is well established; however, less is known for p75 signaling that triggers apoptosis of SCGs during development. The aim of our work was to unravel the functional significance of internalization and the retrograde transport of BDNF/p75 in SCGs.

Materials and Methods: The model used was SCGs in normal and compartimentalized culture were the cell body is cultured in a separately chamber compared to the distal axons. To study the endocytosis and trafficking we used immunoendocytosis, and electron, real-time and fluorescence microscopy.

Results: In the cell body, the internalization of p75 is JNK-dependent and is necessary for signaling, since the overexpression of a mutant of p75 with reduced internalization, abolish the NRIF nuclear translocation, necessary for p75-induced apoptosis. In compartmentalized cultures of SCGs we found that p75 is retrogradely transported, in a ligand-dependent fashion inducing apoptosis in the cell body. Dynein and JNK inhibitors reduce the retrograde transport of p75 and the retrograde killing.

Discussion: Our results suggest that p75 activation regulates p75 trafficking in the cell body and in axons triggers the retrograde transport of an apoptotic endosome.

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(17) CHARACTERIZATION CDNF EXPRESSION BY LENTIVIRAL VECTORS AS A VEHICLE FOR THE POTENTIAL TREATMENT OF PARKINSON'S DISEASE. **Sheyla Guzmán**, Jorge Escobar, Pedro Zamorano. Laboratorio de Neurobiología, Facultad de Ciencias de la Salud, Universidad de Antofagasta. zamorano@uantof.cl

Introduction: Parkinson's disease is the second neurodegenerative disease with a high incidence in elder human population that is in needs for new therapies. A potential treatment for this disease is the recent and important discovery of a protein called CDNF (cerebral dopamine neurotrophic factor) belonging to the family of neurotrophic factors MANF/CDNF, which has been shown to promote the survival, protection and regeneration of dopaminergic neurons (Nature 448, 73). Due to the importance of this protein as a possible therapy for Parkinson's disease here we characterize the expression of CDNF by lentiviral vectors.

Material and Methods: The expression of CDNF by monocistronic (CDNF) and bicistronic (CDNF/EGFP) lentiviral vectors was characterized by Western blot, inmunofluorescence and their neuroprotective activity was assessed by MTT determined as cell viability in HEK293 cells after a H_20_2 challenge.
Results: Heterologous expression of lentiviral vectors in HEK293T cells show that in both lentiviral vectors, mono and bicistronic, expressed CDNF properly and the expression of this factor in either case appears to be secreted by the canonical cellular secretion pathway, due to the presence of inmunoreactive protein in secretory granules determined by immunofluorescence. Protection experiments against oxidative stress with H_2O_2 in HEK293T cells suggest that the protein expressed by the bicistronic vector is functional.
Discussion: The results show that lentiviral vectors are suitable vehicles for the expression of CDNF to the study of CDNF biology and possibly for the treatment of Parkinson's disease.
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(18) cAMP-EPAC SIGNALING IS INVOLVED IN THE DEVELOPMENT OF THE AXON. **Pablo Muñoz-Llancao[1]**, Daniel R. Henriquez[1], Martina Schmidt[2] and Christian Gonzalez-Billault[1]. (1)Laboratory of Cell and Neuronal Dynamics, Department of Biology, Faculty of Sciences and Institute for Cell Dynamics and Biotechnology (ICDB), Universidad de Chile, Santiago, Chile. (2)Department of Molecular Pharmacology, University of Groningen, The Netherlands
Introduction. Acquisition of neuronal polarity is a complex process involving several cellular and molecular changes, including vectorial cytoplasmic flux, differential molecular sorting, local protein degradation and cytoskeleton dynamics. Most of these processes are finely regulated by signaling molecules, such as kinases and phosphatases, phosphoinositides, small GTPases and second messengers. Amongst second messengers, cAMP is important for the outgrowth and elongation of the axon. CAMP-dependent signaling had been most of the times related with changes in the activity of the protein kinase A. However, there is an alternate cAMP-dependent mechanism which involves the participation of the exchange proteins directed activated by cAMP (EPAC). The role of EPAC and their effector Rap1B in the development of the axon will be discuss in this work.
Materials and Methods. Primary hippocampal neurons were treated with either the specific agonist (8-CPT) or transfected with constitutively active construct for EPAC, and the morphology of neurons was assessed by confocal microscopy.
Results. EPAC1 and EPAC2 are differentially expressed during the development of axons in culture. Primary culture of neurons treated with the specific agonist of Epac (8-CPT) and a constitutively active EPAC vector, showed the presence of supernumerary axons.
Discussion. EPAC increased activity (pharmacological and genetic) induces exacerbated neuronal polarity and their function should be related with the activation of RAP1B.
(Supported by Fondecyt 1095089, ICM P05-001-F and CONICYT Doctorate Scholarship).

(19) CHARACTERIZATION OF AN *in vitro* MODEL OF MOTOR NEURON DISEASE. Cristina Pinto[1], Nelson Osses[2], **Juan Pablo Henríquez[1]**. [1]Department of Cell Biology, University of Concepcion, and [2]Institute of Chemistry, Catholic University of Valparaiso, Chile. jhenriquez@udec.cl
Introduction: NSC-34 cells are a well known *in vitro* model to study motor neuron behavior, either in normal or pathological conditions. Amyotrophic lateral sclerosis is a neurodegenerative disease characterized by motor neuron death. Mutations in the Cu/Zn superoxide dismutase-1 (SOD1) are associated to hereditary forms of the disease. Indeed, mice and neuronal cells carrying the G93A mutation of human SOD1 are extensively used as model systems. Here, we characterized cellular properties of NSC-34 cells stably expressing wild-type (NSC-hSOD1^{WT}) or mutated (NSC-hSOD1^{G93A}) forms of SOD1.
Materials and Methods: Cell adhesion and differentiation were primarily analyzed in nitrocellulose-coated plates containing different substrates. Morphological properties were quantified in MAP1B stained cells using ImageJ. Cell viability and death were determined with Alamar blue staining and lactate dehydrogenase release in growing, differentiating, and H_2O_2-treated cells.
Results: Whereas a higher proportion of cells, particularly NSC-hSOD1^{G93A}, bound to poly-L-lysine and laminin, cells seeded on poly-L-lysine plus gelatin had a spread and flattened appearance. NSC-hSOD1^{WT} cells display better morphological differentiation than NSC-hSOD1^{G93A} cells, evaluated as cells bearing neurites, and as the number and length of neuron-like projections. Even though the number of cells remained similar in growing conditions, a significantly lower fraction of NSC-hSOD1^{G93A} cells were quantified under differentiating and oxidative conditions than control NSC-hSOD1^{WT} cells, a feature likely related to increased cell death.
Discussion: NSC-hSOD1 cell lines represent an adequate model to approach the pathophysiological mechanisms underlying motor disease and to test potential therapeutic strategies.
Funded by FONDECYT 1100326 (JPH), PUCV 037.361 (NO).

(20) WNT-5A LIGAND INDUCES FISSION-FUSION OF MITOCHONDRIA AND PROTECTS HIPPOCAMPAL NEURONS FROM Ab OLIGOMER NEUROTOXICITY. Juan A. Godoy**, Macarena S. Arrázola** and Nibaldo C. Inestrosa. Centro de Envejecimiento y Regeneración (CARE). Departamento de Biología Celular y Molecular. Facultad de Ciencias Biológicas. Pontificia Universidad Católica de Chile, Santiago, Chile. msarrazo@puc.cl

Introduction: The *Wnt-5a* signaling pathway plays an important role in different aspects of the central nervous system function. A tight relationship between endoplasmic reticulum (ER) and mitochondria mediated Ca^{2+} signaling is well established. We report here, that the *Wnt-5a* ligand induces an increase in intracellular calcium, and initiates fission-fusion processes in mitochondria.

Material and Methods: The effect of *Wnt-5a* was followed in time, studying mitochondrial morphology (Mitotracker) and protein staining for Fis-1 (fission) and Mfn-2 (fusion) by immunofluorescense. In addition we carried out experiments with ryanodine receptor agonist-antagonist and smooth endoplasmic reticulum calcium pump blockers.

Results: *Wnt-5a* increases intracellular calcium and triggers fission-fusion of mitochondria in a short time period and in a reversible manner. After damage induced by Aβ oligomers, a sustained imbalance in intracellular calcium as well as mitochondrial damage is observed in cultured hippocampal neurons. These changes can be reversed by acute application of *Wnt-5a*.

Discussion: *Wnt-5a* regulates physiological changes in mitochondrial dynamics (fission-fusion), and protects this organelle from Aβ oligomers neurotoxicity. We conclude that *Wnt-5a* ligand and therefore the non-canonical (*Wnt*/Ca^{2+}) signaling, generates a physiological signal that regulates intracellular calcium and protects mitochondria from Aβ oligomers damage.

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(21) TREHALOSE TREATMENTS ALLEVIATE AND ATTENUATE ALS PROGRESSION POSSIBLY THROUGH AUTOPHAGY ACTIVATION. **Castillo K.**[1,2,3], Lopez E [1,2,3] , Nassif M [1,2,3], Matus S [1,2,3] and Hetz C. [1,2,3]. [1]Biomedical Neuroscience Institute, Faculty of Medicine, [2]Center for Molecular Studies of the Cell, Institute of Biomedical Sciences, University of Chile, Santiago, Chile. [3]Neurounion Biomedical Foundation, Santiago, Chile. karencitakc@gmail.com

Introduction: Amyotrophic Lateral Sclerosis (ALS) is a progressive and fatal neurodegenerative disease with no effective treatment. Mutations in superoxide dismutase (SOD1) causes familial ALS (fALS) characterized by its abnormal folding and aggregation specifically in motoneurons. Autophagy is a catabolic process by which cellular contents are degraded and recycled maintaining cellular balance and has been emerged as key process involved in maintains neuronal homeostasis. Trehalose is a disaccharide that potently induces autophagy. Triggering autophagy could decrease protein aggregates typical of ALS progression ameliorating symptoms and animal survival.

Materials and Methods: We generated a transgenic mice harboring ^{G86R}SOD1 mutation and monitored, in pre-sympotomatic animals, the effects of intraperitoneal injection (2g of sugar/Kg of animal weight) of trehalose, glucose and controls with non treatment on disease progression, rotarod performance, loss of weight and survival.

Results: Induction of mTOR-independent autophagy by trehalose prolonged animal survival, delayed onset disease and dramatically decreased the progression of ALS compared to control animals. In order to establish a correlation with autophagy induction, we are measuring autophagy markers and levels of SOD1 aggregates in animal tissues.

Discussion: Administration of trehalose to our animal model of ALS suggest that induction of autophagy ameliorate the pathology, extending the healthy period of the animal, reducing the aggressiveness of symptoms and prolong life span, suggesting that autophagy modulation may have therapeutic benefits to treat ALS.

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(22) ROLE OF ApoER2 IN REGENERATION OF THE PERIPHERAL NERVOUS SYSTEM (PNS) AFTER NERVE DAMAGE. **Joaquín Cerda**, María Luisa Benítez, Felipe A. Court[1] and María Paz Marzolo[2]. Millennium Nucleus in Regenerative Biology (MINREB), P. Universidad Católica de Chile, Santiago, Chile. [1]fcourt@bio.puc.cl, [2]mmarzolo@bio.puc.cl

Introduction: ApoER2 is an endocytic and signaling receptor, member of the low-density lipoprotein receptor family. ApoER2, along with its ligand reelin, has a relevant role in the central nervous system, both during brain and spinal cord development as well as in adult tissues. ApoER2 is found in several splicing variants and specifically the presence of the exon 19, encoding a proline-rich insert in the cytoplasmic domain, has been linked to neuronal survival in normal conditions but to apoptosis upon injury. Reelin has been associated to regeneration in the PNS but the presence of ApoER2 has not been determined. Considering that reelin also binds to other receptors, we are interested to know if ApoER2 has a role in peripheral nerve regeneration.

Methods: Evaluation of the basal and post-injury expression of ApoER2 and reelin (WB, IF and qPCR) in sciatic nerves (SN) of rats and mice. The ApoER2 KO mice were used as negative controls.

Results: ApoER2 is expressed at low levels in SN in control conditions, and its expression was upregulated after injury. IF showed staining associated to Schwann cells and by WB, a band corresponding to a specific receptor isoform was present after injury.
Discussion: Overall these results indicate that ApoER2 could be the receptor mediating the regenerative effects of reelin in the PNS, aspects that will be evaluated by analyzing PNS regeneration in ApoER2 KO mice.
Supported by: Millennium Nucleus no. P-07-011-F, Fondecyt 1110382 and 1110987.

(23) MOTOR IMPAIRMENT OF A BRAIN SPECIFIC ERp57 KNOCKOUT MOUSE MODEL. **Andreu C.**[1,2],Valenzuela V.[1,2],Woehlbier U.[1,2],Irrazábal T.[1,2] and Hetz C[1,2,3]. [1]Biomedical Neuroscience Institute, [2]Center for Molecular Studies of the Cell, Institute of Biomedical Sciences, University of Chile, [3]Neurounion Biomedical Foundation, Santiago, Chile. chetz@med.uhile.cl
Introduction: ERp57 is a 58 kDa protein of the endoplasmic reticulum (ER), belonging to the protein disulfide isomerase family. ERp57 catalyzes disulfide bond formation, isomerization and is a component of the Calreticulin/Calnexin cycle, playing an important role in protein quality control in the ER. Protein misfolding disorders such as ALS and prion diseases are associated with a high upregulation of ERp57. However, the function of ERp57 in the nervous system is unknown because its complete deletion in mice is embryonic lethal. The aim of this work is to generate and characterize a brain specific ERp57 knockout mouse model.
Material and Methods: To generate the brain specific deletion, we crossbred mice with a lox-flanked *erp57* gene with Cre recombinase transgenic mice under the control of nestin promoter. The litters were genotyped by PCR and the specific ERp57 deficiency was confirmed by western blot. Visual observation, weight measurements and rotarod performed were evaluated every week in 7 knockout (flox/flox-cre) and 13 litter mate control animals (flox/flox-wt and wt/wt-wt). In addition, histological analysis of spinal cords was performed.
Results: Knockout mice present ~40% reduction in body weight compared to controls and show a ~85% decrease in motor abilities.
Discussion: The conditional knockout model was viable. Our results suggest that ERp57 deficiency causes motor impairment which could be a relevant phenotype to explore in diseases such as ALS.
FONDECYT 1100176, FONDAP 15010006, Millennium Institute P09-015-F, ICGEB and Alzheimer's Association.

(24) BIPHASIC EFFECTS OF COPPER ON NEUROTRANSMISSION IN RAT HIPPOCAMPAL NEURONS. **Christian Peters**, Braulio Muñoz, Fernando Sepúlveda, Juan Urrutia, Mauricio Quiroz, Sandra Luza, Giancarlo V. De Ferrari, Luis G. Aguayo, Carlos Opazo. Laboratorio de Neurobiometales, Departamento de Fisiología, Facultad de Ciencias Biológicas, Universidad de Concepción, Chile. cpeters@udec.cl.
Introduction: Copper is released at the synaptic cleft, where it may modulate neurotransmission by direct or indirect mechanisms.
Material and Methods: We have analyzed the synaptic activity of primary rat hippocampal neurons by using whole cell patch clamp, recording of calcium transients, immunofluorescence and Western blot.
Results: When copper is acutely applied to the plate it blocks neurotransmission. Interestingly, when it is applied for 3 h to hippocampal neurons mainly increases the frequency and amplitude of AMPAergic currents (Control: 0.21 ± 0.05 Hz/ 22.9 ± 1.3 pA; Copper: 0.68 ± 0.16 Hz/ 30.5 ± 2.5 pA), intracellular calcium transients (Control: 0.05 ± 0.013 Hz; Copper: 0.11 ± 0.02 Hz) and evoked AMPA currents (Control: EC50 8.3 ± 0.5 μM; Copper: EC50 2.9 ± 0.2 μM). These correlated with an increase in GluA1 subunit of AMPA receptor and PSD95. After 24 h of treatment, changes in neurotransmission induced by copper at 3 h of incubation are homeostatically regulated, showing no differences on neurotransmission.
Discussion: The data reveals an unexpected biphasic effect of copper on neurotransmission. Our results also suggest that copper increases GluA1 subunit levels of the AMPA receptor through the anchorage of AMPA receptors to the plasma membrane as a result of PSD-95 accumulation.
Acknowledgments: This work was supported by the Chilean Government (Anillo-PBCT ACT-04 project to LGA, GVD and CO), FONDECYT 1100502 (LGA), FONDECYT 1100942 (GDV) and DIUC grant N° 205.033.101-1.0 (CO).

(25) αVβ3 INTEGRIN EXPRESSION IN RAT BRAIN UPON INJURY. **Soto, C.**[1,2], Rojas-Mancilla, E.[1,2], Alvarez, A.[1,2], Hermosilla, T.[1], Díaz, E.[2], Herrera-Marschitz, M.[2] and Leyton, L.[1,2]. [1]Centro de Estudios Moleculares de la Célula (CEMC), [2]Biomedical Neuroscience Institute, ICBM-Facultad de Medicina, U de Chile.
Introduction. Interactions between neuronal Thy-1 and αvβ3 integrin in DI-TNC1 astrocytes increase astrocyte adhesion, inducing migration and morphological changes resembling *in vivo* events of astrogliosis. However, primary astrocytes lack β3 integrin, and consequently, are unresponsive to Thy-1. Reportedly, β3 integrin is expressed in reactive astrocytes. Thus, here we investigated whether proinflammatory cytokines induce the expression of β3 integrin in astrocytes allowing them to respond to Thy-1. The expression of β3 integrin in a proinflammatory condition *in vivo* was also tested.
Methods. Rat primary astrocytes from neonates were stimulated 48h with proinflammatory cytokines, prior to Thy-1 stimulation. Focal adhesion formation and migration were monitored by microscopy. β3 integrin levels were assessed by immunoblotting. Wounded-fixed tissues obtained 7 days after brain injury in rats were analyzed for GFAP and β3 integrin by immunofluorescence.

Results. proinflammatory cytokines increased β3 integrin expression levels in primary astrocytes, and only upon this treatment, astrocytes responded to Thy-1 increasing adhesion to the extracellular matrix and migratory capacity. *In vivo*, levels of β3 integrin and GFAP, a marker of reactive astrocytes, increased in astrocytes near the injury zone.
Discussion. Insights obtained with these *in vitro* and *in vivo* models should improve our understanding of astrogliosis, a process that alters astrocyte physiology by changing their protein expression, structure and supportive role, thereby modulating neuronal function and survival.
Acknowledgements: FIRCA-NIH 5R03TW007810-3 (LL), FONDECYT 1110149 (LL), 1080447 (MH-M); Iniciativas Científicas Milenio: BNI P09-015-F (LL, MH-M), FONDECYT-FONDAP 1510006 (LL), CONICYT (ER-M, AA).

(26) GLIAL CELL TYPE DEFINES MORPHOLOGICAL AND TEMPORAL CHARACTERISTICS OF AXONAL DEGENERATION. **Alejandra Catenaccio**, Jaime Alvarez and Felipe A. Court. Millennium Nucleus in Regenerative Biology (MINREB), Catholic University of Chile and Neurounion Biomedical Foundation. fcourt@bio.puc.cl, alecatenaccio@gmail.com
Introduction. Axonal degeneration is an early feature of neurodegenerative conditions leading to loss of neuronal function. It has been long assumed that axonal degeneration takes place by autonomous mechanisms, nevertheless participation of other cells has been suggested. We explored whether Schwann cells in the PNS and oligodendrocytes in the CNS participates in axonal degeneration.
Material and Methods. Sciatic or optic nerves of C57BL/6 or Thy1-YFP mice were crushed. Drugs were administered by intraneural injection. Nerves were collected at different times post-lesion. Single teased fiber and cryosections were analyzed by inmunofluorescence, and morphometry was done at light and electron microscope levels. For *in vitro* degeneration, DRG neurons were grown in compartmentalized Campenot chambers.
Results. We found that glial participation in axonal degeneration takes place at early stages, overlapping with axonal intrinsic mechanisms. In the same organism, the morphological characteristics of axonal degeneration is defined by its associated glia: Peripheral axons degenerate by fragmentation (AxF type), process determined by Schwann cells, while CNS axons, associated to oligodendrocytes, degenerate at a slower rate by an autonomous process characterized by beading (AxB type). Importantly, pharmacological modification of glial cells, can alter the axonal degeneration type.
Discussion. We showed that axonal degeneration is modulated by glial cells. Furthermore, the cell type associated to axons defines morphology and dynamics of degeneration. We speculate that the glial complement of axons modifies the axonal regenerative capabilities at different nervous system territories by modulating axonal degeneration.
FONDECYT no. 1110987 and Millennium Nucleus no. P-07-011-F.

(27) GLIAL SUBVENTRICULAR TUMORS INDUCED IN THE NEUROGENIC NICHE INCREASE GENERATION OF NEUROBLASTS. **Nery Jara**, Federico Rodríguez and Francisco Nualart. Neurobiology and Stem Cells Laboratory, Department of Cell Biology, University of Concepción. neryalejara@udec.cl
Introduction: The adult mammalian brain maintains the generation of new neurons or neuroblasts by neural stem cells (NSC), which can be found mainly in the subventricular zone (SVZ) of the lateral ventricles (LV), zone that is also called "neurogenic niche". It has been reported that NSC can proliferate, self-renew and migrate, however these capabilities give them the potential to cause tumors in the SVZ.
Material and Methods: To analyze the effect of this type of tumors on adult neurogenesis, we induced SVZ tumors in adult guinea pigs by stereotaxic injection of U87 cells (human glioblastoma multiforme cells) in one ventricle, leaving the contralateral ventricle as a control. The animals were mantained for 3 weeks before sacrifice, and we performed immunohistochemical analysis of brain tumors, using glial, neuronal and microglial markers. Additionally, BrdU labeling was used for proliferation analysis.
Results: In the tumor area, we observed a different extent of damage in the ependymal cell layer (ILB4 +), a reactive gliosis around the tumor (GFAP + and vimentin +) and a microglial infiltration (ILB4 +). Furthermore, the average number of precursor cells (BrdU +) in the SVZ next to the tumor was two times greater than in the control SVZ, whereas the average number of neuroblasts (tuj 1 +) generated was nearly five times greater in the SVZ next to the tumor.
Discussion: The tumor cells induce gliosis, increase neurogenesis and microglial infiltration.
Grant support by FONDECYT 1100396.

(28) NH_4^+ AS A POSSIBLE SIGNAL LINKING NEUROTRANSMITTER RECYCLING WITH FAST GLYCOLYTIC STIMULATION IN ASTROCYTES. [1,2]**Rodrigo Lerchundi** and [1]L. Felipe Barros. [1]Centro de Estudios Científicos (CECs), Valdivia, Chile & [2]Universidad Austral de Chile, Valdivia, Chile. rlerchundi@cecs.cl
Introduction: Ammonia (NH_4^+) is released by presynaptic neurons along glutamate during excitatory synaptic activity. Astrocytes swiftly capture and conjugate both molecules into glutamine, which is shuttled back into neurons, thus closing the glutamate/glutamine cycle. Test tube measurements with purified enzyme have shown that NH_4^+ can activate phosphofructokinase. More recent studies reported that the brain content of NH_4^+ may double after electrical stimulation and that hyperammonemic animals present enhanced brain tissue glycolysis. Thus, we investigated whether NH_4^+ may serve as a fast modulator of glycolysis in astrocytes.

Materials and Methods: Glucose consumption by mice astrocytes in culture and in organotypical hippocampal slices was monitored with a genetically encoded FRET glucose nanosensor (*Bittner et al. Front Neuroenergetics 2010*). Intracellular lactate was measured in real-time with the newly-developed FRET lactate nanosensor Laconic *(San Martín et al., this meeting*).
Results: Exposure of astrocytes in culture and in slices to NH_4^+concentrations as low as 0.5 mM were able to induce an acute, reversible activation of glycolysis, evidenced by a fall in intracellular glucose and a simultaneous rise in intracellular lactate.
Discussion: These findings suggest that NH_4^+ may serve to link presynaptic activity with fast stimulation of glycolysis in astrocytes, complementing the glycolytic effect of K^+, which is a postsynaptic signal (*Bittner et al., J. Neurosci. 2011; Ruminot et al., J. Neurosci. 2011*).

(29) SUBCELLULAR LOCALIZATION ANALYSIS OF XTRIC-8A AND ITS PARTICIPATION IN CELL POLARITY IN *X. tropicalis* EMBRYOS. **Cecilia Arriagada[1],** Maria V. Hinrich[1] and Marcela Torrejón [1]. [1]Laboratory of Genetic and Molecular Biology, Department of Biochemistry and Molecular Biology, University of Concepción, Chile**.** ceciarriagada@udec.cl
Introduction. Ric-8 a GEF protein has been related with asymmetric cell division in *C. elegans* and *Drosophila* neuroblast. In addition, Ric-8 loss of function assay in *X. tropicalis* generates a delay in cell division and pigmentation defect during early stages of embryonic development. Similar phenotype has been also observed in embryos with polarity defects in animal cap cells. In this work, we analyzed the subcellular localization of XtRic-8A *in vivo* and the effect of depletion on cell polarity.
Material and Methods. Subcellular localization of XtRic-8A during cell cycle was analyzed in HeLa cells and in early embryo sections from *X. tropicalis* by overexpression of the XtRic-8A in fusion with fluorescent proteins and immunocitochemistry. To study the effect of XtRic-8A loss of function in cell polarity, we depleted this protein with a specific morpholino, and analyzed different polarity markers in animal cap cells by immunocitochemistry.
Results. During the interphase XtRic-8A is located at the plasma membrane, perinuclear region and cytoplasm. In addition, XtRic-8A loss of function assays generates changes in the distribution of polarity markers during early stages of embryogenesis.
Discussion. The different subcellular location observed for XtRic-8A may reflect its multiple functions mediated by different protein partner. Changes at the cell polarity by depletion of XtRic-8A suggest that XtRic-8A could be required during cell polarization events, an important step during different processes such as asymmetric cell division and cell migration.

(30) HETEROCHRONY AND HETEROTOPY IN THE EVOLUTION OF BRAIN ASYMMETRY DEVELOPMENT BETWEEN ZEBRAFISH AND MEDAKA. Iskra Signore[1,2], **Geraldine Vásquez**[1,2], Javiera Ríos[1,2], Alexander Jares[3], Alicia Colombo[1], and Miguel Concha[1,2]. [1]Laboratory of Experimental Ontogeny - LEO, ICBM, Faculty of Medicine, University of Chile; [2]Biomedical Neuroscience Institute - BNI, Santiago, Chile; [3] Yale University, USA. iskra.signore@gmail.com; mconcha@med.uchile.cl
Introduction: Comparison between related species is a successful approach to uncover conserved and divergent principles of development. Here we studied the pattern of epithalamic asymmetry in zebrafish (*Danio rerio*) and medaka (*Oryzias latipes*), two related teleost species with 115-200 million-year of independent evolution.
Material and Methods: Zebrafish used in this study are wild type Tübingen and Tg(foxD3::GFP). Medaka lines are wild type Cab and Tg(fRx2::GFP). The transgenic lines express green fluorescent protein (GFP) in the epithalamus. Comparison of the parapineal-habenula connectivity ontogeny will be performed using *in vivo* confocal microscopy. The organisation of habenular domains will be analized by whole mount *in situ* hibridisation.
Results: We found that these species share a strikingly conserved overall pattern of asymmetry in the parapineal-habenular-interpeduncular system. Despite the overall conservation of asymmetry, we observed heterotopic changes in the topology of parapineal efferent connectivity and heterochronic shifts in the timing of developmental events underlying the establishment of asymmetry. We are currently testing hypotheses of inter-species variation that link the topology of parapineal efferent connectivity to the underlying organisation of domains within the left habenula, and are also comparing the ontogeny of this connectivity.
Discussion: Together, these findings highlight the usefulness of zebrafish and medaka as tools to study the developmental mechanisms of epithalamic asymmetry, and provide support to the utility of a normalisation method for comparison of developmental time among related species.
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(31) ETHANOL EXPOSURE DISRUPTS CELL MIGRATION AND PRIMARY CILIA STRUCTURE IN DEVELOPING EMBRYOS. **Katica Boric[1]**, [2]Eduardo Couve[2], Patricio Orio[1] & Kathleen Whitlock[1,2]. [1]CINV, Universidad de Valparaíso, Chile. [2]Universidad de Valparaíso. kboric@gmail.com
Introduction: Ethanol (EtOH) exposure during early development can cause Fetal Alcohol Syndrome (FAS) which affects

craniofacial (CF) structures and parts of the nervous system that are derived from cranial neural crest cells (CNCC). These migratory precursors are susceptible to EtOH exposure. Premigratory CNCCs share a common border with the cells of the olfactory placode precursor (OPP) field; therefore we propose that OPP as well as CNCC progenitor migration is disrupted by EtOH exposure. Primary cilia (PC), important for transduction of morphogens, in CNCCs are important in cell migration. Thus EtOH exposure may disrupt PC structure/function in the CNCCs, resulting in cell migration defects.
Materials & Methods: Zebrafish embryos were exposed to increasing EtOH concentrations for 20 hours. Expression of chemokine-signaling genes, necessary for both OP formation and CNCC migration was studied using *in situ* hybridization. *In vivo* analysis of CNCC migration was performed and a novel motion quantification method "Optical Flow" (OF) was applied. PC in CNCC were studied using immunocytochemistry and electron microscopy.
Results: EtOH induces phenotypes similar to FAS patients; embryos show a series of craniofacial and axial malformations. EtOH disrupts the expression of chemokine-signaling genes, altering OP formation and CNCC migration. We show that EtOH affects migration of CNCC populations in developing embryos. Also EtOH disrupts ultrastructure of PC in CNCC and apparently reduces PC numbers.
Discussion: These novel findings suggest that EtOH-induced alteration of PC in CNCCs, may underlie the structural/neural defects observed in FAS as well as other environmentally induced CF and NS malformations.

(32) GENERATION A MODEL OF OXIDATIVE STRESS INDUCTION USING ZEBRAFISH AND EVALUATION OF ANTI-OXIDANT, PROTECTIVE AND REGENERATIVE ACTIVITY OF RESVERATROL.
Marjorie Alvarez[1], Tomas Egaña[2], Miguel L. Allende[1]. 1.FONDAP Center for Genome Regulation, Facultad de Ciencias, Universidad de Chile. 2.Department of Plastic Surgery and Hand Surgery, Faculty of Medicine, Technical University of Munich. Munich, Germany. marjorie.alvarez@ug.uchile.cl
Introduction: Oxidation is biochemical process of electron loss always associated with another of uptake called reduction. Oxidation is essential for life, but an excess of oxidation in cells leads to oxidative stress, with toxic effects on different biological systems. We seek to generate an animal model of oxidative stress induction and to link oxidative status with regenerative capacity.
Material and Methods: We use larval and adult zebrafish of diverse transgenic strains to monitor cell survival and behavior. Oxidative stress is induced by exposure to cigarette smoke derivatives or by exposure to copper dissolved in water. As an antioxidant, we use resveratrol treatments in larval fish.
Results: Using different transgenic strains, we have evaluated the effects of cigarette smoke on cell death, angiogenesis and regeneration, we have shown cigarette smoke induces oxidative stress. Using a cell death and regeneration protocol by CuSO4, we have used another transgenic line to evaluate the protective effect of the polyphenolic molecule resveratrol (3,5,4 '-trihydroxy-trans-stilbene), known to promote cell survival and regeneration. Finally, we have measured the effect of cigarette smoke exposure on regeneration speed in adult fish.
Discussion: Our strategy will improve our ability to evaluate the effects of oxidative stress as well as to measure the antioxidant activity of different molecules *in vivo* using zebrafish as a bioassay.
Grant sponsors: FONDECYT 1110275; FONDAP 15090007; CORFO-Innova (09MCSS-6705).

(33) SYNDECAN-4 AND FIBRONECTIN, FOCAL ADHESION COMPONENTS, REGULATE WNT/b-CATENIN SIGNALING.
Pablo Astudillo, Héctor Carrasco and Juan Larraín. Center for Aging and Regeneration (CARE), P. Universidad Católica de Chile. prastudi@uc.cl
Introduction: Syndecan-4 is a membrane-associated heparan sulfate proteoglycan and an essential component of focal adhesions (FA). Our laboratory has demonstrated that Syndecan-4 regulates non-canonical Wnt signaling during *Xenopus laevis* development; also, Syndecan-4 interacts with Dishevelled, a common component of both canonical and non-canonical Wnt pathways, and this interaction is enhanced by Fibronectin. Here, we hypothesized that FA components Syndecan-4, Fibronectin and b1-integrin can regulate the canonical Wnt pathway.
Materials and Methods: Standard techniques were used, including reporter assays and Western Blot to evaluate b-catenin stabilization and LRP6 phosphorylation. Experiments were performed using *Xenopus laevis* embryos and mouse embryonic fibroblasts from wild-type (MEF Sdc4 +/+) and Syndecan-4 null (MEF Sdc4 -/-) mice.
Results: Syndecan-4, b1-integrin and Fibronectin knockdown upregulates canonical Wnt signaling. MEF Sdc4 -/- exhibit enhanced canonical Wnt signaling, and Fibronectin treatment and the rescue of Syndecan-4 expression inhibits canonical Wnt signaling. Epistasis experiments suggest that Syndecan-4 acts at the level of the LRP6 co-receptor, since it decreases the activity from a constitutive active form of LRP6 but failed to block the activity from a stabilized form of b-catenin in *Xenopus* embryos. MEFs lacking Syndecan-4 show increased levels of phoshorylated LRP6. Syndecan-4 interaction with Dishevelled depends on Dishevelled DEP/PDZ domains.
Discussion: These results show that Syndecan-4 and Fibronectin inhibit the canonical Wnt pathway, suggesting an inhibitory function of FAs and cell-extracellular matrix adhesion.
FUNDING: CARE; Fondecyt #1100471; Beca de Apoyo a Tesis Doctoral AT-24100095.

(34) AVERSIVE MEMORY IN *Drosophila melanogaster* LARVA CAN BE MODIFIED BY LIGHT ACTIVATION OF CHANNELRHODOPSIN-2. **Paula Burgos**, Karina Palma & Jorge M Campusano. Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad de Chile. paulaburgos.s@gmail.com, jcampusano@bio.puc.cl (Sponsor: E. Aliaga).

Introduction. The olfactory system of fly larvae is able to detect, discriminate and generate specific responses to different odors. Thus, larvae learn to avoid an odor that is aversive and crawl towards odors that recognize as appetitive. Several evidence show that aversive memory depends on activation of dopamine pathways. Here we used optogenetics to generate and modify aversive memory in fly larvae, by activation of channelrhodopsin2 (Chr2) expressed in dopaminergic cells.

Materials and Methods. We expressed Chr2 in dopaminergic cells by using the Gal4-UAS technique. As odorants we used ethyl acetate and n-amyl acetate. Whenever needed, we used agar plates containing 2M NaCl (aversive reinforcer). Fly larvae (3^{rd} instar), were exposed to one odor in presence of salt, and to the second odor in normal agar plates. This training was repeated three times. When light conditioning was carried out, blue (470 nm) or red (600 nm) light stimulation were used as reinforcer or neutral stimulus, respectively. At different time points, memory was evaluated by measuring larval odor preference.

Results. By using salt as reinforcer, we induced an aversive memory in larvae that lasts 30 min. Larva stimulation with blue light is effective at modifying this aversive memory, while red light is not.

Conclusion. Our data suggests that aversive memory can be modified by optogenetic activation of dopaminergic neurons in fly larvae. This could be associated to an increase in dopamine levels in association areas of larval brain.

Fondecyt 1100965; MSI P10-063-F.

(35) INFLAMMATION RESOLUTION BY RETROGRADE MIGRATION OF NEUTROPHILS AFTER LOCALIZED TISSUE DAMAGE IN ZEBRAFISH LARVAE. Oscar Peña, **Nicole Reynaert,** Miguel Allende. FONDAP Center for Genome Regulation, Facultad de Ciencias, Universidad de Chile. nicole.reynaert@googlemail.com; allende@uchile.cl

Introduction. Recruitment of neutrophils to sites of tissue injury is a hallmark of inflammation, but persistent inflammation may lead to tissue damage. Resolution of inflammation is critical to the restoration of normal tissue function. In zebrafish, $CuSO_4$ exposure induces damage to lateral line neuromast cells. This is followed by leukocyte infiltration to neuromasts within minutes, but the mechanisms of neutrophil clearance during inflammation resolution in this inflammation model remain unexplored.

Materials and Methods. We performed *in vivo* time-lapse imaging of transgenic zebrafish larvae harboring labeled leukocytes (*BACmpx::GFP*) after $CuSO_4$ exposure. Cell tracking of resolving neutrophils, statistical analysis of leukocyte migration and assays using immunomodulatory molecules were also performed.

Results. Copper induced infiltration of neutrophils to posterior lateral line neuromasts is followed by inflammation resolution in which the neutrophil count in the vicinity of damaged tissue diminishes within the first 8 hours. *In vivo* time-lapse imaging of $CuSO_4$ exposed larvae shows active retrograde migration of resolving neutrophils to caudal hematopoietic tissue. Cell tracking of resolving neutrophils migrating away from the posterior lateral line shows a highly directional migration.

Discussion. Neutrophil clearance following $CuSO_4$ induced inflammation is characterized. We provide evidence for active and highly directional retrograde migration of neutrophils during inflammation resolution toward the caudal hematopoietic tissue while no apparent apoptosis of neutrophils was observed as has been reported in mammals. Our results introduce a useful assay to detect the effects of immunomodulatory compounds on inflammation resolution.

Grant sponsors: FONDAP 15090007, FONDECYT 1110275.

(36) PHENOTYPIC CHANGES INDUCED BY LOW-OF- FUNCTION OF PATCHED-RELATED DURING EMBRYOGENESIS OF *Drosophila melanogaster*. **Carmen Bolatto** y Verónica Cambiazo. Laboratorio de Bioinformática y Expresión Génica INTA-Universidad de Chile. Depto. de Histología y Embriología, Facultad de Medicina- UdelaR, Uruguay. cbolatto@fmed.edu.uy, vcambiaz@inta.cl

Introduction. The aim of this work is to provoke a reduction in the expression of Patched-related (Ptr) to evaluate its role during the development of *Drosophila melanogaster*. The observation and characterization of morphological changes helped us to estimate the role of Ptr within the pathway triggered by the Hedgehog (Hh) morphogen.

Material and Methods. Production of *Drosophila* fly lines expressing double strand RNA (dsRNA) complementary to the first *ptr* exon under the control of GAL4/UAS system (UAS-*ptr*-dsRNA). Cuticular preparations and QPCR assays. Hh cultured assay using cl-8 cells transfected with a control reporter and a Hh-responsive luciferase reporter**.**

Results. The 20-50% of the embryos from a cross between UAS-*ptr*-females and males expressing GAL4 ubiquitously in the embryo (GAL4-nanos or GAL4-MATalpha) did not hatch. Cuticular preparations revealed alterations at the level of ventral denticles (loss and / or fusion) and a correlation between these changes and the decline in the abundance of *ptr* mRNA in embryos expressing *ptr* dsRNA. Furthermore, the use of *ptr* dsRNA in Hh cultured assay shown an enhanced responsiveness to Hh suggesting that Ptr has a role as a negative regulatory component of Hh-signaling pathway.

Conclusions. Our results suggest that a decrease in mRNA abundance of Ptr causes changes in the development of *Drosophila* that are morphologically evident at later stages of development and are compatible with an involvement of that Ptr in the Hh signaling pathway.
PEDECIBA and CSIC I+D 2010 (CB). Fondecyt 1090211 (VC).

(37) HYPOXIA-INDUCIBLE FACTOR-1 ALPHA IS NECESSARY FOR THE POSTERIOR LATERAL LINE CELL-PROLIFERATION ON ZEBRAFISH. **Barros M,** Reyes A.E. Facultad de Ciencias Biológicas. Universidad Andrés Bello. Avda. República 217, piso 4. Santiago, Chile. arielreyes@unab.cl
Introduction. Hif-1 is the master gene in response to low oxygen tension. In zebrafish, the Hif-1α transcript is expressed maternally and thereafter remains evenly distributed up to 10 h post-fertilization (hpf) and later on, at 72 hpf, expression could be detected in neuromasts of the posterior lateral line (PPL). The PPL is a mechanosensory system present in fish and amphibians to detect water pressure changes, presence of predators, among others. In this study we determined the effect of Hif-1 in the cell proliferation of the PLL development.
Materials and Methods. By morpholino injection experiments in zebrafish embryos, we studied the loss-of-function of HIF-1α. The gain-of-function of HIF-1α was studied by injection of dominant-active of HIF-1α mRNA, that lacks the oxygen-dependent degradation domain. We used bromodeoxyuridine (BrdU) incorporation and Phospho-Histone-H3 immunohistochemistry to assay the effect of Hif-1 loss- and gain-of-function on prolifferation during PLL development. The phenotypes on neuromasts were analyzed using transgenic lines, expressing GFP in the primordium cells *Tg*(*claudinB:GFP)*, hair cells *Tg(ET4:EGFP)* and mantle cells *Tg(ET20:GFP)*.
Results. Hif-1α morphant embryos showed a smaller primordium than matched control embryos. Also, we observed that Hif-1a morphant embryos show impaired cell proliferation in the PLL.
Discussion. The development of the posterior lateral line system involves cell proliferation, migration, and differentiation. These results suggest that Hif-1 alpha is neecssary for cell proliferation in the PLL. This data unveil a new role for the transcription factor Hif-1α during zebrafish embryonic development.
FONDECYT #1095128.

(38) CHARACTERIZATION OF PELADO DURING ZEBRAFISH DEVELOPMENT. **Solís C[1]**, Feijóo CG[1] and Glavic A[2]. (1) Facultad de Ciencias Biológicas, Universidad Andrés Bello, Chile. (2) Facultad de Ciencias, Universidad de Chile, Chile.
Introduction: Pelado was first discovered in *Drosophila*, but it is also present in the genome of other vertebrates such as zebrafish, and mammals. This gene encodes a large protein with a zinc finger like domain, the SWIN domain and a FH2 domain. Its function has not been determined yet, but preliminary studies in *Drosophila* indicate that is involved in cytoskeleton dynamics and neurogenesis. In this work we characterize its function during zebrafish development.
Materials and Methods: We have defined the temporal and spatial expression pattern by RT-PCR and *in situ* hybridization. Also, we performed loss of function analysis by the injection of Pelado mopholino. To evaluate the phenotype we injected different transgenic line, utilized the vital stain Di-asp, *in situ* hybridization and immunohistochemistry.
Results: Pelado is expressed during embryonic and larval stages. Loss of function analysis indicates that morphant embryos lack hair cells in both the anterior and posterior lateral line as a result of impaired differentiation.
Discussion: Our results indicate that this gene has an important role in the differentiation of the sensory neurons in the lateral line.
Founding: Fondecyt 1100366, Conicyt 7909006.

(39) CALCIUM AND cAMP SIGNALING IN THE PROTHORACIC GLAND AND ITS ROLE IN THE CIRCADIAN TIMING OF *Drosophila* ECLOSION. **Angelina Palacios-Muñoz** and John Ewer. Laboratory of Neurogenetics and Development, Interdisciplinary Center of Neuroscience of Valparaíso, University of Valparaíso, Chile. angelina.palacios@cinv.cl
Introduction. In *Drosophila melanogaster* the circadian clock regulates the timing of behavior and physiology. Yet, how the activity of the clock causes these outputs to be rhythmic is poorly understood. The prothoracic gland (PG) is a peripheral clock that synthesizes the molting hormones, which control metamorphosis. The PG, together with the circadian clock present in the fly's brain, also imposes a circadian rhythmicity to the timing of adult emergence. We investigated the pathway through which the PG causes the pattern of *Drosophila* emergence to be rhythmic by determining whether calcium or cAMP levels vary in the PG during the course of the day prior to eclosion.
Material and Methods. We monitored calcium and cAMP levels by measuring the intensity of fluorescence of sensors expressed in the PG using the GAL4/UAS system. Animals were collected at the start of metamorphosis and their calcium and cAMP levels measured at different ages and times of day in wildtype and arrhythmic mutant animals.
Results. The levels of calcium and cAMP in the PG vary during the course of the day, and differ between animals whose clock is in opposite phase but which are of the same chronological age.

Discussion. Our results suggest that calcium and cAMP signaling expresses a circadian rhythm and may contribute to the circadian regulation of adult emergence.
CONICYT, #24110076 to Angelina Palacios-Muñoz. FONDECYT 1071079 and 1111023, and NIH to John Ewer.

(40) TESTOSTERONE INDUCES HYPERTROPHY IN SKELETAL MUSCLE CELLS BY ACTIVATING BOTH mTOR/p70S6K PATHWAY AND THE CLASSIC ANDROGEN RECEPTOR. **Basualto-Alarcón C**., Jorquera G., Estrada M. and Jaimovich E. Centro de Estudios Moleculares de la Célula, ICBM, Facultad de Medicina, Universidad de Chile, Santiago, Chile. carlabasualto@gmail.com
Introduction: The mechanisms of action of testosterone in skeletal muscle are not completely known. Rapid non genomic effects of this hormone could be involved in the known hypertrophy response. We studied whether classic hypertrophy pathways are activated by testosterone and the possible role of the androgen receptor in these pathways.
Materials and Methods: five to seven day old rat myotubes were used. Hypertrophy was measured by microscopy, western blot and qPCR after 100nM testosterone, a dose that produces oscillatory calcium transients and induces activation of ERK1/2. Pathways activation was studied using western blot and inhibitors. Androgen Receptor action was studied using siRNA.
Results: 12 hours of testosterone induced increases in myotube cross sectional area and α-actin (a sarcomeric protein) at transcriptional and translational levels. ERK1/2 and Akt showed increased phosphorylation at 5 and 15 minutes, respectively. p70S6K was phosphorilated after 60 minutes. This response was inhibited by Ly294002, Akt-inhibitor-VIII and Rapamycin but not by PD98059. Similarly, the hypertrophy response was abolished both by these inhibitors and by siRNA against androgen receptor but not by PD98059.
Discussion: we demonstrated that testosterone activates a known hypertrophy pathway (Akt/mTOR pathway) at short times, while three selected parameters of hypertrophy showed an increase at 12 hours. Knockdown of the androgen receptor also unraveled an important role for this receptor in the hypertrophy response, a crosstalk between genomic and non genomic effects being plausible.
CONICYT:AT-24091020, FONDAP 15010006, FONDECYT 1110467.

(41) CTGF INDUCES INFLAMMATION IN THE SKELETAL MUSCLE. **Cabrera D.,** Morales MG., Cabello-Verrugio C. and Brandan E. Laboratory of Cell Differentiation and Pathology, CARE. Department of Cell and Molecular Biology, Catholic University of Chile. dacabrer@puc.cl
Introduction: Connective tissue growth factor (CTGF) is involved in the induction of extracellular matrix, in a process called fibrosis. The levels of CTGF correlate with the degree and severity of fibrosis in many tissues, including dystrophic muscles. M1 macrophages play a major role in worsening muscle injury in the *mdx* mouse model of Duchenne muscular dystrophy (DMD). However, *mdx* muscle also contains M2 macrophages that can promote tissue repair, indicating that factors regulating the balance between M1 and M2 phenotypes could influence the severity of the disease.
Materials and Methods: We evaluated the inflammatory effect of CTGF overexpression in Tibialis anterior muscle of wild-type mice treated or not with an antinflammatory drug (D07I1051), using an adenovirus containing the CTGF mouse sequence (Ad-mCTGF). Also we isolate muscle macrophages to evaluate the CTGF effect on M1 macrophages phenotype.
Results: CTGF overexpression induced an acute inflammatory response in skeletal muscle tissue, featured by an early increase of M1 macrophages. Treating muscle macrophages with CTGF induced activation of the M1 phenotype, assessed by iNOS expression. All these effects were inhibited using D07I1051.
Discussion: Together, the results show that CTGF plays a significant regulatory role in muscular dystrophy that may be caused by inducing an inflammatory response increasing M1 macrophage activation. These observations underscore the importance of CTGF in the patho-physiology of muscular dystrophies and suggest that targeting CTGF might have significant potential in development of novel therapies for DMD and related diseases *(Supported by CARE PFB-12/2007, MDA-89419, Fondecyt-11080212, Conicyt AT-24091098).*

(42) ISOLATION OF RAT SKELETAL MYOFIBERS AND DENERVATION, BUT NOT IMMOBILIZATION OF A FAST SKELETAL MUSCLE PROMOTES AN INCREASE IN CONNEXINS LEVELS. **L.A. Cea,** M.A. Riquelme, A. Vargas J.C. Sáez. Departamento de Fisiología, Pontificia Universidad Católica de Chile, Luisceapisani@gmail.com
Introduction. Connexins (Cxs) are not detected in rat adult skeletal muscles. However, during myogenesis or regeneration Cxs39, 43 and 45 are transiently expressed and become undetectable upon differentiation. The possible role of innervation and activity remain unknown. Adult skeletal muscles are known to release different autocrine/paracrine factors under normal condition. One of them is interleukin-6 (IL-6), which is released preferentially during exercise. The aims of this work were to study in adult rat skeletal muscles 1) if levels of Cxs are affected by denervation and/or immobilization; 2) if these Cxs form functional hemichannels and 3) if IL-6 plays any regulatory role.
Material and Methods. Myofibers were isolated from flexor digitorium brevis muscles (Sprague-Dawley rats, males~300g) by collagenase digestion at 37°C. Muscle denervations were performed by sectioning the sciatic nerve and left hind-limb immobilization was performed with metal staples. Cx levels were evaluated by immunoblotting and

immunofluorescence. Functional Cx HCs in skeletal myofibers were evaluated through ethidium uptake sensitive to Cx HC blockers in divalent cation-free solution (DCFS).
Results. Levels of Cxs evaluated increased progressively over the first 2 and 6 days reaching maximal values, in cultured myofibers and denervated muscle, respectively, but did not change in immobilized muscles. Treatment with IL-6 prevented the increase in Cx levels. In myofibers cultured, DCFS increased the HC activity by ~3-fold, which was prevented by IL-6. The Etd uptake was blocked with La^{3+}.
Discussion. In adult skeletal muscle innervation and IL-6 downregulate Cxs39, 43 and 45.

(43) MOLECULAR TOOLS TO CHARACTERIZE MULTIPROTEIN COMPLEXES CONTAINING $P2Y_2$ RECEPTORS AND PANNEXIN-1 CHANNELS. **Buvinic, S**[1,2] and Jaimovich, E[2]. [1]Facultad de Odontología; [2]Centro de Estudios Moleculares de la Célula, ICBM, Facultad de Medicina. Universidad de Chile, Santiago, Chile. sbuvinic@u.uchile.cl
Introduction: Extracellular ATP is a relevant mediator between membrane depolarization and signaling involved in gene expression in skeletal muscles. We propose that this signaling would require the proper coordination between the voltage sensor, pannexin hemichanels (ATP releaser), nucleotide receptors such as $P2Y_2$ and several signaling molecules, forming a multiprotein complex. Our aim was to produce molecular tools to characterize multiprotein complexes containing either $P2Y_2$ or pannexin-1 (PnX1), in order to unveil a putative signaling complex in skeletal muscles.
Materials and Methods: We constructed cDNA for fused $P2Y_2$- or PnX1-proteins with the One-STrEP-tag, to allow rapid one-step purification of protein complexes on Strep-tactin Superflow. $P2Y_2$ and PnX1 were sublcloned from SPORT-6 to the pEXPR-IBA103 vector. Positive clones were selected by restriction maps and sequencing. $P2Y_2$-STrEP and PnX1-STrEP were transient and stable transfected into HEK293T cells to assess the proper expression by immunoblot (IB) and immunofluorescence (IF).
Results: We obtained $P2Y_2$-STrEP and PnX1-STrEP cDNAs. These constructions were transient or stable expressed in HEK293, with proper expression levels detected by IB and IF. With these tools we can now test for multiprotein complex intractors that co-purify with $P2Y_2$-STrEP and PnX1-STrEP, which will be analyzed by IB or MALDI-TOF.
Discussion: We have constructed a powerful molecular tool to study multiprotein complexes associated to $P2Y_2$ and PnX1 hemichannels. After characterization in HEK293T cells, we are ready to test them in skeletal muscle cells to purify and characterize the multiprotein complex involved in excitation-transcription coupling.
Fondecyt-1110467-11100454, Conicyt-79090021, FONDAP-15010006.

(44) EARLY UPTAKE OF VITAMIN C UP-REGULATES ITS SVCT2 TRANSPORTER AND STIMULATES MYOGENESIS. **Jorge Ojeda**, Daniel Sandoval, Marcela Low, Juan Pablo Henriquez**.** Department of Cell Biology, Faculty of Biological Sciences, University of Concepcion, Concepcion, Chile. jhenriquez@udec.cl
Introduction: Cumulative evidence reveals that vitamin C stimulates differentiation of several cell lineages. Besides, its SVCT2 transporter is up-regulated upon differentiation. We have previously shown that SVCT2 expression increases during muscle differentiation *in vitro*. Although vitamin C stimulates myoblasts differentiation, the molecular mechanisms involved, including the potential participation of SVCT2, are unknown. Here, we have explored the role of SVCT2-mediated uptake of vitamin C on early myogenesis.
Matherials and Methods: C2C12 myoblasts were supplemented with ascorbic acid (AA), as well as with the structural analogues ascorbic acid 2-phosphate (AA2P) and ethyl-3,4-dihydroxybenzoate (EDHB) 24h prior to trigger differentiation. Myogenesis was evaluated as myotube surface, fusion index, and myogenin-positive nuclei. The effect on SVCT2 expression was addressed by qRT-PCR.
Results: Treatment of myoblasts with AA up-regulates their fusion into myotubes, an effect mimicked by the stable analogue AA2P. Besides, both molecules significantly increased the number of nuclei expressing myogenin, a master gene of myogenesis. In turn, the competitive analogue EDHB impaired all parameters of myogenesis. None of the observed effects were related to the proliferation rate of myoblasts. In addition, AA and its analogues increased *SVCT2* transcript expression, a finding related to the ability of the transporter to trigger myogenin transcription.
Discussion: Our findings suggest that the uptake of AA exerts positive roles on the early differentiation of myoblasts. We believe that these effects could be mediated by collagen secretion as well as by the early activation of signaling pathway(s).
Funded by FONDECYT 1100326 grant to JPH.

(45) ATP REDUCES THE UP-REGULATION OF CONNEXINS 39, 43, AND 45 IN CULTURED ADULT SKELETAL MYOFIBERS. **Cisterna B.A.,** Cea L.A., Riquelme M.A., and Sáez J.C. Departamento de Fisiología, P. Universidad Católica de Chile. bcisterna@uc.cl
Introduction. Connexins (Cxs) are not detected in adult rat skeletal muscles. However, Cxs 39, 43, and 45 are transiently expressed during myogenesis or regeneration. Skeletal muscles release autocrine and/or paracrine molecules such as ATP, and its release increases during exercise, potentiation of muscle contraction and damage. We tested if ATP down-regulates the abundance of connexins in fibers of a fast skeletal muscle.
Material and Methods. Myofibers were isolated from *flexor digitorium brevis* muscle of adult Sprague-Dawley rats (males of ~300g). The myofibers were cultured under absence or presence of ATP (300 μM) during 48h. The abundance of Cxs was evaluated by western blot analyses, and the functional activity of Cx HCs was tested through the ethidium uptake in the absence of extracellular divalent cations.

Results. The abundance of Cxs 39, 43, and 45 increased at 48h in culture and functional Cx HCs were recorded. In contrast, treatment with 300 mM ATP prevented the increase in levels of Cxs 39, 43, and 45 and drastically decreased the functional activity of Cx HCs.
Discussion. Extracellualr ATP prevents the up-regulation of Cxs 39, 43, and 45 expressed by cultured adult skeletal (fast) myofibers.
Acknowledgment: Supported by Anillo ACT-71, AT 24091065 to Luis Cea, and CONICYT fellowship.

(46) UNVEILING A MULTIPROTEIN COMPLEX INVOLVED IN EXCITATION-TRANSCRIPTION COUPLING IN NORMAL AND DYSTROPHIC SKELETAL MUSCLE. **Almarza, G.[1]**, Valladares D.[1], Jaimovich E.[1] and Buvinic, S[1,2]. [1]Centro de Estudios Moleculares de la Célula, ICBM, Facultad de Medicina, [2]Facultad de Odontología. Universidad de Chile, Santiago, Chile. gonzalo.almarza@gmail.com
Introduction: We recently reported that nucleotides released through pannexin1 (PnX1) hemi-channels during skeletal muscle depolarization activate P2X and P2Y receptors to lead gene expression. We have suggested a novel multiprotein complex involving physical interaction between dihydropyridine receptor (DHPR), PnX1, $P2Y_2$ receptor and dystrophin in skeletal muscle models. The aim of this study is to unveil the components of this complex at the T-tubule membrane of skeletal muscles and its differences in dystrophic (*mdx*) vs wt (C57) mice.
Material and Methods: We used triads-enriched preparations from adult skeletal muscles derived from Rat and BalbC-, C57- and *mdx*-mice. The complex components were assessed by co-precipitation assays; whole complexes were resolved using 2D Blue Native-SDS/PAGE.
Results: Characterization of triad-enriched fractions showed higher amounts of PnX1, $P2Y_2$ and ryanodine receptor, but lower amount of caveolin-3 in *mdx* compared to C57 triads. Immunoprecipitation revealed that DHPR association with $P2Y_2$ and PnX1 is strongly augmented in the *mdx* model. The isolation of the complex by Blue Native-SDS/PAGE showed some differences in the distribution of the complex proteins.
Discussion: We strongly suggest that there is a multiprotein complex in skeletal muscle triads involving DHPR, PnX1, and $P2Y_2$ receptor. Expression levels and interactions between them are altered in dystrophic *mdx* skeletal muscles, opening new insights into the role of dystrophin as a scaffold protein in this complex.
Fondecyt-1110467-11100454, Conicyt-79090021, FONDAP-15010006.

(47) AMP-ACTIVATED PROTEIN KINASE (AMPK) PATHWAY IS INVOLVED IN TESTOSTERONE-INDUCED CARDIOMYOCYTE HYPERTROPHY. **Katherine Montoya,** Carlos Wilson, Rodrigo Maass and Manuel Estrada. ICBM, Facultad de Medicina, Universidad de Chile. iestrada@med.uchile.cl
Introduction: Testosterone induces cardiomyocyte hypertrophy by activation of the mTOR (mammalian target of rapamycin). The AMP-activated protein kinase (AMPK) inhibits mTOR signaling, suggesting AMPK as a key regulator of cardiomyocyte hypertrophy. However, the involvement of AMPK in cardiomyocyte hypertrophy induced by testosterone is unknown. In this study we examined the effects of AMPK activation on cardiomyocyte hypertrophy induced by this hormone.
Materials and Methods: Cultured neonatal rat cardiomyocytes were first treated with specific AMPK activators (AICAR and metformin) or with the mTOR antagonist (rapamycin), then stimulated with 100 nM testosterone. The expression of β-myosin heavy chain (β-MHC), skeletal actin (SKA) and the phosphorylation levels of AMPK and S6K1 (a downstream target for mTOR), were measured by Western blot. Cell size was determined by confocal microscopy in cardiomyocytes loaded with the fluorescent dye cell tracker green.
Results: Testosterone increased the AMPK phosphorylation (Thr^{172}) at 60 min. Cyproterone (an inhibitor of intracellular androgen receptor) had no effect on AMPK phosphorylation induced by testosterone. The AMPK activators AICAR and metformin stimulated AMPK phosphorylation and inhibited testosterone-stimulated phosphorylation of S6K1. Rapamycin also inhibited S6K1 phosphorylation and increased the AMPK phosphorylation mediated by the hormone. Testosterone (24 h) increased both cell size as well as the expression of hypertrophy markers (β-MHC and SKA), which were inhibited by AMPK activation.
Discussion: These results suggest that AMPK inhibits cardiomyocyte hypertrophy induced by testosterone through the mTOR pathway, independently of the intracellular androgen receptor.
FONDECYT Grant 1090276.

(48) VASOPRESSIN-INDUCED PROLIFERATION OF VASCULAR SMOOTH MUSCLE CELLS INVOLVED THE EGFR TRANSACTIVATION AND ACTIVATION OF ERK SIGNALING PATHWAY. **Marianne Brenet R.** Carolina Villanueva M., Pamela Carmona R., Carlos B. González. Instituto de Fisiología. Facultad de Medicina. Universidad Austral de Chile. Marianne.brenet@alumnos.uach.cl.
Introduction: Vascular smooth muscle cell (VSMC) proliferation and remodeling are associated with many vascular diseases. AVP induces proliferation in VSMC; however, the pathways involved in AVP-induced proliferation of VSMCs

leading to vascular remodeling remain unclear. We investigated the signaling event triggered by AVP and its participation in the proliferation of VSMCs.
Material and Methods: A-10 cells which endogenously express both the V1 vasopressin receptor and the EGFR were stimulated with AVP. We studied the ERK (Extracellular Regulated Kinase) activation by Western blotting using phospho-specific antibodies. To determine proliferation, cells were seeded onto 96 well plates and after 24 without FCS cells were stimulated with AVP and labeling with BrdU. In some cases cells were pre-incubated with the inhibitor AG1478 and PD98059.
Results: AVP induced the proliferation of A-10 cells after 48 to 96 hours. AVP induced proliferation in the range of 1 to 100 nM. The proliferation was inhibited by AG1478 and PD98059 suggesting that the AVP-induced proliferation involved the EGFR transactivation and ERK activation. The AVP-induced ERK phosphorylation showed a biphasic kinetic with an early peak and a late peak response. The vasopressin-induced early peak of ERK phosphorylation involves the transactivation of the EGFR.
Discussion: Our results suggest that AVP is able to induce the ERK phosphorylation by EGFR-dependent. The EGFR transactivation and ERK activation are essential for AVP-induced VSMCs proliferation.
Supported by CONICYT and FONDECYT 1100871.

(49) ATP-DEPENDENT GLUCOSE UPTAKE IN SKELETAL MUSCLE CELLS. [*†]**Osorio-Fuentealba C**., [†]Contreras-Ferrat AE., [†]Altamirano. F., [†]Espinosa A. and [†]Jaimovich E. [*]Facultad de Medicina, Universidad Finis Terrae, [†]Centro de Estudios Moleculares de la Célula, ICBM, Facultad de Medicina, Universidad de Chile, Santiago.
Introduction Skeletal muscle is the main target of insulin in the organism. Once insulin binds to its specific receptor, several downstream signaling pathways such as PI3K and Akt are activated. On the other hand, ATP is released by contractile activity in skeletal muscle cells. Our aim was to investigate whether ATP released by electrical stimulation induces glucose uptake in myotubes and adult mouse muscle fibers and the possible links with insulin induced glucose uptake.
Material and Methods Both **r**at myotubes in primary culture and cultured adult mouse muscle fibers were exposed to either tetanic electric field stimulation (400 1 ms pulses, 45 Hz), 100 nM insulin, 100 μM ATP or nothing. The uptake of the fluorescent hexoses 2-NBDG was assayed at room temperature (23°C) in real time. Cultures were excited at 488 nm; 2-NBDG and 6-NBDG were imaged at 505–550 nm emission. Intracellular hexose concentration was estimated by comparing intracellular fluorescence with the signal outside the cells.
Results Tetanic electrical stimulation, insulin and ATP were able induces glucose uptake in myotubes and adult fiber; ATP before insulin addition, increased glucose uptake by insulin over insulin alone values. Akt VIII inhibitor and LY 294002 (10 μM), Akt Dominant negative isoform, inhibited the glucose uptake in myotubes.
Discussion Ours results indicates that electrical stimulation potenciates insulin signaling pathways in skeletal muscle and ATP plays an important role as extracellular mediator of glucose uptake during muscle activity.
FONDAP 15010006, Fondecyt 11090301, FONDECYT 3110170, AT-24100067.

(50) CROSS TALK BETWEEN ApoER2 AND THE NEUROTROPHINS RECEPTORS: EFFECT OF NEUROTROPHINS AND REELIN ON PROTEOLYTIC PROCESSING OF ApoER2. **Jorge Larios Y.**, Francisca Bronfman C., María Paz Marzolo C. Millennium Nucleus in Regenerative Biology (MINREB), Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile. jalarios@uc.cl, fbronfman@bio.puc.cl, mmarzolo@bio.puc.cl
Introduction: ApoER2 and neurotrophins receptors TrkA and p75 neurotrophin receptor (p75) are expressed in the central nervous system regulating key aspects of neuronal function such as survival and neuronal morphology. It is know that both ApoER2 and p75 are processed by metalloproteinases followed by regulated-intramembrane proteolysis (RIP); however whether there is a common mechanism of regulation it is not known. We have previously shown that TrkA activation increases the proteolytic processing of p75 mediated by ADAM 17. The aim of our work was to study if TrkA activation also regulates the proteolysis of ApoER2.
Methods: Studies were performed in PC12 clones that endogenously express p75 and TrkA, and transfected ApoER2. PC12 cells were treated with NGF (activating TrkA and p75) and reelin, a ligand for ApoER2, and the processing of ApoER2 was analyzed by western blotting. Different pharmacological inhibitors were used to achieve specificity of signaling pathways and proteases involved in the regulated-processing of ApoER2.
Results: Activation of TrkA by NGF induces the proteolytic processing of ApoER2, a process that is dependent on TrkA signaling pathways as well as metalloproteinases. In addition, reelinalso regulates ApoER2 processing independent of ApoER2 downstream signaling.
Discussion: Our results suggest that there are complex connections between neuronal signaling systems, which could integrate two pathways related to brain development and to neuronal survival.
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(51) COX-2 EXPRESSION IS REGULATED BY CK2 THROUGH THE WNT/b-CATENIN PATHWAY IN HUMAN EMBRYONIC CELLS. **Pablo Cabello**[1], Roger Yefi[1], Ignacio Niechi[1], Eduardo Silva[1], Diego A. Rodriguez[2], Daniela P. Ponce[1],

Katherine Marcelain[2], Ricardo Armisen[2], Andrew F.G. Quest[2] & Julio C. Tapia[1,2]. [1]Cell Transformation Laboratory, [2]Institute of Biomedical Sciences (ICBM), Faculty of Medicine, University of Chile. jtapia@med.uchile.cl

Introduction: CK2 is an important component in the activation of the Wnt/β-catenin pathway. This pathway promotes cell viability by regulating specific target genes in normal and cancer cells. Here we show that CK2 through the Wnt/β-catenin pathway regulates COX-2 expression and increases viability of human embryonic kidney HEK-293T cells.

Methods: In HEK-293T cells, we analyzed COX-2, β-catenin and survivin levels by RT-PCR and western blot. COX-2 transcription was evaluated with a *cox-2* promoter-specific reporter. CK2 activity was inhibited with DMAT. To up-regulate the Wnt/β-catenin pathway, we overexpressed a constitutively active form of Akt/PKB, wild-type forms of COX-2, CK2a and β-catenin, while to down-regulate the pathway we used dominant negative forms of CK2α, β-catenin and Akt. Cell proliferation and apoptosis were measured with MTS® and Caspase-Glo® kits, respectively. We treated cells with PGE2 to determine its effect on COX-2 expression, cell proliferation and apoptosis.

Results: DMAT decreased COX-2 expression in a dose-dependent fashion, similarly to the inhibition of the Wnt/β-catenin pathway. The effects in COX-2 expression as well as in cell viability were plecluded by Wnt/β-catenin pathway activation, COX-2 overexpression or treatment with PGE2.

Discussion: Regulation of COX-2 expression by CK2 does occurr via the Wnt/β-catenin pathway and is important for cell viability.

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(52) CO-INCUBATION WITH TOLL LIKE RECEPTORS ACTIVATORS, LPS AND DNA-CPG, INDUCED A SYNERGISTIC INCREASE OF CILIARY BEAT FREQUENCY IN RESPIRATORY CILIATED CELLS. **Daniela Carreño[1]**, Claudia González[2], Carolina Oses[1], María Paz Hernández[1], Carmen Llados[1] and Manuel Villalón[1]. [1]Faculty of Biological Sciences; [2]Hospital Clínico. Pontificia Universidad Católica de Chile. dvcarren@uc.cl

Introduction: Mucociliary clearence constitute a barrier against contaminants and microorganisms inhaled from the environment and its efficiency depends mainly on the frequency of ciliary beat of the airways epithelium. Airways contaminants include components of the bacteria wall like lyposaccharide (LPS) and segments of nucleic acid DNA (DNA-CpG), both activators of specific toll like receptors (TLR) of the immune system. Activation of TLR4 and TLR9 by LPS and DNA-CpG respectively produce an inflammatory response associated to the production of cytokines by the airway epithelium, however in not known if these contaminants can affect the frequency of ciliary beat and the effectiveness of mucociliary clearence.

Materials and Methods: Primary cultures of mouse (Balbc) tracheal epithelium grown in culture chambers were incubated with different concentrations of LPS (1ng/ml, 10ng/ml, 1µg/ml y 10µg/ml) or DNA-CpG (0,1µM, 0,5µM y 1µM). Cultures were also incubated with low concentration of both activators simultaneously, while the CBF was measured. All these experiments were statistically analyzed with ANOVA, and post- test Tukey.

Results: The results show an increase of the CBF in response to LPS or DNA-CpG with a minimum concentration effect at 1µg/ml for LPS and 1µM for DNA-CpG ($p<0.05$). Simultaneous incubation with both activators produced a synergistic increase in CBF.

Discussion: Both bacterial components, LPS and DNA-CpG, activators of TLR receptors affect ciliary activity in the airways epithelium and provide evidence of the relation between the immune system activation and the inflammatory response of the airways. *(FONDECYT 1080679).*

(53) FUNCTIONAL CHARACTERIZATION OF CARGO-BINDING SITES ON MU-SUBUNITS OF ADAPTOR PROTEIN COMPLEXES. **Esteban Corales[1],** Yimo Lin[1], Juan Bonifacino[2], James Hurley[3], Patricia Burgos[1], and Gonzalo Mardones[1]. [1]Instituto de Fisiología, Facultad de Medicina, Universidad Austral de Chile, Valdivia, Chile, and [2]Cell Biology and Metabolism Program, NICHD, and [3]Laboratory of Molecular Biology, NIDDK, NIH, Bethesda, MD, USA. gamardon@gmail.com

Introduction: Protein trafficking in the secretory and endocytic pathways is mediated by vesicular transport. The four heterotetrameric adaptor protein (AP) complexes (AP-1 to AP-4) play key roles in cargo selection previous to vesicle formation. The functions of AP complexes are essential for many physiological processes, as emphasized by the embryonic lethality of mutations in several AP subunits and the occurrence of genetic disorders due to AP subunit defects. The µ subunit of AP complexes recognizes sorting signals fitting the YXXØ consensus motif (Ø is a bulky hydrophobic residue) by a conserved, µ2-binding site. Recently we discovered that the µ4 subunit of AP-4 recognizes a different, non-canonical YXXØ motif by a distinct µ4-binding site. The aim of this study was to determine which of these mechanisms is used by the other AP complexes.

Materials and Methods: we used site-directed mutagenesis, yeast-two hybrid (Y2H) analyses, X-ray crystallography, and confocal microscopy of cultured cells.

Results: Substitutions in either of both binding sites on each µ subunit abrogated binding to reporter proteins in Y2H experiments. Overexpression of µ subunits with substitutions in either of both binding sites produced varied levels of effects on the normal transport of reporter proteins.

Discussion: Our data demonstrate the functionality of both binding sites on the recognition of the corresponding specific cargo molecules.

FONDECYT 1100896.

(54) INHIBITION OF PHOSPHATIDIC ACID HYDROLISIS CHANGES THE ENDOCYTIC TRAFFICKING AND SIGNALING OF ONCOGENIC EGFRvIII. **Apud M.,** Shaughnessy R.[1,2], Otero C. [1,2], Metz C, Soza A.[1,2], González A.Departamento de Inmunología Clínica y Reumatología, Fac.Medicina, Centro de Envejecimiento y Regeneración (CARE), Fac.Ciencias Biológicas, Pontificia Universidad Católica de Chile. apud.mari@gmail.com

Introduction: Endocytosis is a crucial player in the mechanisms that regulate receptor signaling.We described that increments in phosphatidic acid leading to down regulation of PKA, (PA/PKA pathway), induce ligand-independent endocytosis and accumulation in recycling endosomes of empty/inactive EGFR(Norambuena et al Mol. Biol Cell.-2010). Here westudiedthe effect of this PA/PKA pathway on endocytic and signaling properties of the oncogenic mutant EGFRvIII that lacks exons 2 to 7 and is constitutively active.

Materials and Methods: We produced neuroblastoma (N2a-EGFRvIII) and glioblastoma (U87-EGFRvIII) cells permanently transfected with EGFRvIII.Cell surface versus intracellular distribution was assessed by cell surface biotinylation assays. ERK1/2 and AKT pathways were analyzed by immunoblot. Propranolol was used as an inhibitor of the phosphatidic acid phosphohydrolase (PAP) that triggers the PA/PKA pathway.

Results: N2a-EGFRvIII and U87-EGFRvIII cells show higher proliferation and migrationthan the correspondinguntransfected cells. EGFRvIII expression does not change ERK1/2 activity but has different effects on AKT, increasing its activity in N2a cells and decreasing its mass and activity in U87 cells. Under propranolol treatment, ERK1/2 becomes activated while the mass of EGFRvIII at the cell surfacedecreases within 15 min to thenrecovers and decreases again, indicating recycling. AKT does not change.

Conclusion: PAP inhibition induces endocytosis and recycling of EGFRvIII accompanied by increases in the activity of ERK1/2 but not AKT.

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(55) KININ B2 RECEPTOR STIMULATION AMELIORATES EPITHELIAL MESENCHYMAL TRANSITION INDUCED BY ALBUMIN IN RENAL EPITELIAL CELLS. **Cárdenas A,** Campos J, Ehrenfeld P, Ardiles L, Figueroa CD. Laboratorio de Nefrología e Instituto de Anatomía, Histología y Patología, Universidad Austral de Chile. arelicardenas0@gmail.com

Introduction: Albumin overload has been shown to induce inflammation and epithelial mesenchymal transition (EMT) together with TGF-β expression in tubular cells to end with renal fibrosis. On the other hand, stimulation of the kallikrein-kinin system and activation of the kinin B2 receptor (B2R) reduces renal fibrosis in several animal models by a mechanism still unknown. Our aim was to investigate the effect of B2R stimulation on tubular EMT *in vivo* and *in vitro*, to shed light on the mechanism by which the kinin system protects tubular cells from EMT and TGF-β1.

Material and methods: For *in vivo* experiments, rats were exposed to albumin overload and fibrosis was evaluated by Masson's trichrome stain. *In vitro* studies were carried out on HK-2 proximal tubule cells exposed to albumin to induce EMT. Cell markers associated to EMT were evaluated by Western blotting and immunofluorescence and TGF-β expression was determined by qPCR and ELISA.

Results: B2R stimulation reduced fibrosis *in vivo* and EMT induced by albumin overload *in vitro*. Thus, downregulation of vimentin, α-SMA and upregulation of E-cadherin and cytokeratin-18 was observed in HK-2 cells pretreated with the B2R ligand previous to albumin overload. Further, B2R stimulation decreases both mRNA and active TGF-β in HK-2 cells exposed to albumin.

Discussion: Upregulation of the kinin system and the B2R may become a potential target to downregulate TGF-β activity and reduce EMT and fibrosis in the injured kidney.

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(56) NSPA AND NOT THE RIBOSOMAL P0 PROTEIN IS THE CELL SURFACE TARGET OF ANTI-P AUTOANTIBODIES ASSOCIATED WITH LUPUS PSYCHOSIS. **Espinoza S**.[1,2], Segovia F.[1,2], Bravo-Zehnder[1,2] M., Massardo L.[1], González A.[1,2]. Departamento de Inmunología Clínica y Reumatología, Facultad Medicina[1], Centro de Envejecimiento y Regeneración (CARE), Facultad Ciencias Biológicas[2], Pontificia Universidad Católica de Chile. csespino@gmail.com

Introduction: Patients with systemic lupus erythematosus (SLE) produce anti-ribosomal P proteins autoantibodies (anti-P) that have been associated with psychosis, depression, hepatitis and nephritis. In a variety of cell lines, including the human hepatoma HepG2 cells, anti-P interacts with a cell surface component, for a long time assumed to be the P0 ribosomal protein. It has been reported that anti-P antibodies become endocytosed causing deleterious effects in HepG2 cells. In neurons, we have identified another cell surface target, which we called NSPA (Neuronal Surface P Antigen). Here we tested whether NSPA is expressed in HepG2 cells and whether it is target of anti-P antibodies in addition or instead of P0.

Material and Methods: RT-PCR, biotinylation and cell surface immuno-capture assays were used to asses the expression and cell surface distribution of NSPA. I^{125}-anti-P was used for cell surface binding and internalization assays.

Results: RT-PCR showed NSPA expression in HepG2 cells, which was detectable at the surface by immunoprecipitation with anti-P followed by streptavidin-HRP analysis of blotted proteins. Immuno-capture of the P antigen from the cell

surface of metabolically labelled cells also revealed NSPA as the anti-P target. Overexpression of NSPA increased the internalization of I^{125}-anti-P. None of the assays showed the P0 at the cell surface.
Conclusion: HepG2 cells express NSPA, but not P0, at their surface as the crossreacting antigen recognized by anti-P autoantibodies.
Programa de Financiamiento Basal (PFB12/2007), Fondecyt N°1110849.

(57) LEPTIN INCREASES CILIARY ACTIVITY AND AFFECT THE RESPONSE TO CHEMICAL SIGNALS IN THE RESPIRATORY EPITHELIUM. **Maria Paz Hernández**, Carolina Oses, Daniela Carreño, Carmen Llados, Manuel Villalón. Department of Physiology, Faculty of Biological Sciences. Pontificia Universidad Católica de Chile, Santiago, Chile. mpherna2@uc.cl
Introduction: There is evidence that obesity and respiratory diseases are related. Mucociliary clearance is an important host defense mechanism of the airway epithelia determine by different factors, including the ciliary beat frequency (CBF) that is modified by ATP and lipopolysaccharide (LPS), a component of the Gram-negative bacteria cell wall. It has been reported that leptin, a protein associated with obesity, can affect the action mechanisms of both ATP and LPS in several tissues. Based on this, we hypothesized that leptin can affect the CBF and the response to chemicals signals (ATP or LPS) in the mouse tracheal epithelium.
Materials and Methods: Primary cultures of mouse (Balbc) tracheal epithelium grown in Rose chambers, were incubated with different concentrations of leptin (5, 50 and 100 ng/ml). CBF was measured, using the microphotodensitometric technique ($n > 3$). Cultures were also preincubated with leptin (5, 50, 100 ng/ml) for 48 hours and the CBF changes were measured in response to ATP (0,1 uM), and LPS (1 µg). All these experiments were statistically analyzed with ANOVA, and post-test Tukey.
Results: The result shows an increase of the CBF concentration dependent in response to leptin. Furthermore preincubation of cultures with leptin for 48 hours significantly increased (~20%) the changes of the CBF induced by ATP and LPS.
Discussion: These results indicate that high levels of leptin affect ciliary activity in the airways and provide evidence of a possible correlation between respiratory physiology and obesity.
(FONDECYT 1080679).

(58) CK2 UP-REGULATES COX-2 EXPRESSION AND THEREBY INCREASES VIABILITY AND INVASIVENESS OF COLON CANCER CELLS. **Roger Yefi**[1], Ignacio Niechi[1], Eduardo Silva[1], Pablo Cabello[1], Diego Rodriguez[2], Daniela P. Ponce[1], Katherine Marcelain[2], Ricardo Armisen[2], Andrew F.G. Quest[2] & Julio C. Tapia[1,2]. [1]Cell Transformation Laboratory, [2]Institute of Biomedical Sciences (ICBM), Faculty of Medicine, University of Chile. jtapia@med.uchile.cl
Introduction: CK2 and COX-2 expression increase in colon cancer, as well as have been associated with hyperproliferation, resistance to apoptosis and tumor progression. We investigated whether CK2 up-regulates COX-2 expression and thereby increases viability and invasiveness of colon cancer cells.
Methods: Changes in COX-2 expression were analyzed by RT-PCR and Western blotting in colon cancer cells. COX-2 activity was measured using a commercial kit. CK2 and COX-2 activities were inhibited using DMAT and SC-791, respectively. Proliferation was measured by the MTS® assay and apoptosis using the Caspase-Glo® kit. Cell invasion was assessed for colon cancer cells using a matrigel-based assay. The effect of PGE2 on proliferation, apoptosis and cell invasion was also evaluated.
Results: In colon cancer cells elevated levels of COX-2 expression and activity were detected. Inhibition of either COX-2 or CK2 significantly decreased viability. CK2 inhibition reduced COX-2 mRNA and protein levels, as well as increased apoptosis. Supplementation with PGE2 prevented DMAT-induced loss of COX-2 protein, increased proliferation and invasiveness, as well as decreased apoptosis.
Discussion: These results identify the COX-2/PGE2 axis downstream of CK2 as relevant to tumor cell viability and malignancy. Moreover, they point towards CK2 as a potentially interesting pharmacological target in colon cancer.
Supported by CONICYT Ph.D. fellowship (R.Y. & P.C.); FONDAP 15010006 (A.F.G.Q.); FONDECYT 11070116 and ICGEB CRP/CHI10-01 (J.C.T.).

(59) THE ACTIVATION OF KEY SIGNALING PATHWAYS IN BREAST CANCER CELLS INVOLVES TRANSACTIVATION OF THE EGFR BY THE KININ B1 RECEPTOR. **Andrade Y,** Ehrenfeld P, Plasencia M, Cardenas A, Matus CE, Pavicic F, Figueroa CD**.** Institute of Anatomy, Histology and Pathology. Universidad Austral de Chile. ingridehrenfeld@uach.cl
Introduction: We have demonstrated that the kinin B1 receptor (B1R), a GPCR, transactivates the epidermal growth factor receptor (EGFR), activates ERK1/2 mitogen-activated protein kinases (MAPKs) and induces cell proliferation in breast cancer cells. Based on this evidence we investigated some of the tyrosine residues of the EGFR molecule phosphorylated after B1R stimulation and signaling pathways such as p38 and JNK MAPKs and AKT/mTOR, traditionally associated with cell survival and metastasis.

Material and Methods: MCF-7 and MDA-MB-231 breast cancer cells were stimulated at different times with various concentrations of the B1R ligand and involvement of each pathway was evaluated by Western blotting and antibodies that recognize specific phosphorylated proteins. Transactivation of EGFR was evaluated using AG1478, an inhibitor of the EGFR tyrosine kinase activity and antibodies directed to phosphorylated tyrosines 845, 992 and 1068.
Results: The B1R activation induces transactivation of the EGFR confirmed by phosphorylation of Tyr^{845}, Tyr^{992} and Tyr^{1068} residues present in the EGFR molecule and subsequently activates p38 and JNK MAPKs, and AKT and mTOR signaling pathways.
Discussion: Stimulation of the kinin B1R triggers transactivation of the EGFR to subsequently activate important signaling cascades which are key regulators of cell proliferation, progression and metastasis.
Supported by Fondecyt Nº 11090292 and DID-UACh.

(60) IL-10 DECREASES MICA CELL SURFACE EXPRESSION IN GASTRIC ADENOCARCINOMA CELL LINES. **Garrido-Tapia M**, Hernández CJ, Ribeiro CH, Kramm K, Molina MC. Laboratorio de Evasión Inmune. Programa Disciplinario de Inmunología (ICBM). Facultad de Medicina, Universidad de Chile. macagarrido@gmail.com
Introduction: Natural-killer group 2, member D (NKG2D) binds to a variety of ligands (NKG2DL), including the major histocompatibility complex class I chain-related proteins (MIC) and UL16-binding proteins (ULBP). NKG2D is a co-activating receptor on NK cells, having an important role in the cell-mediated immune response to tumors. Tumor cells can interfere with NKG2D-mediated activity by different mechanisms, such as the release of soluble forms of NKG2DL by cell surface shedding. Interleukin 10 (IL-10) is a cytokine expressed in many tumors, including gastric cancer. In this work, we aimed to describe the role of IL-10 on the expression and shedding of MICA.
Material and Methods: Cell lines from gastric adenocarcinoma, AGS and MKN-45, were used. Cells were stimulated with different doses of exogenous IL-10 for 48 h, with or without the metalloprotease inhibitor TAPI-1 and cell surface expression of MICA and ADAM17 was measured by flow cytometry. Additionally, it was analyzed the induction of p-STAT3 by western blot.
Results: We observed that IL-10 downregulates MICA cell surface expression on MKN45 cells, while ADAM17 expression does not vary. IL-10 activates STAT3. The use of TAPI-1 does not change the effect of IL-10.
Discussion: As we have previously reported in melanoma cell lines, IL-10 also downregulates MICA on the cell surface of gastric cancer cells. The results observed with the use of a metalloprotease inhibitor suggest that IL-10 decreases MICA on the cell surface in a metalloprotease-independent way, but possibly in a STAT3-dependent way.
Financial support: Becas de Apoyo de Tesis 24110228, CONICYT. Proyecto ENL 2010/12.

(61) *REPRIMO*, A CANDIDATE BIOMARKER FOR EARLY DIAGNOSIS AND RESPONSE TO TREATMENT OF HUMAN GASTRIC CANCER. **Maturana MJ.**[1], Torres V.[1], Olivares W.[1], Cerda E.[1], Padilla O.[1], Garrido M.[1], Aguayo F.[3], Calvo A.[2], Ferreccio C.[1], Corvalán AH.[1]. [1]Hematology-Oncology Pontificia Universidad Católica de Chile, [2]CRS-San Rafael, SSMSO, [3]Virology Department, Universidad Chile. mariaj.maturana@gmail.cl (Sponsor: G. Owen)
Introduction: Gastric cancer (GC) is the second leading cause of cancer death worldwide. There are currently no clinically useful biomarkers for early detection and prediction for treatment response. We have identified one such biomarker, the DNA methylation of the promoter region of Reprimo (RPRM), a p53-dependent G2 arrest mediator candidate. Since RPRM has been found methylated in more than >90% of GC cases, we also evaluate RPRM for prediction for treatment response.
Material and Methods: We developed an in-house MethyLight assay for absolute quantitative detection of RPRM DNA methylation. This assay was tested in cell-free DNA (cfDNA) from plasma of 23 GC *vs* 42 healthy donors (HD) and 14 GC treated with chemotherapy (CMT).
Results: By MethyLight assay and using a cut-off of 2.5 copies/ml, we correctly distinguish GC *vs* HD with 92.9% specificity and 91.3% sensitivity by ROC curve analysis. Among patients with CMT and at least three detections of RPRM in plasma, we observed an agreement of 100% with RESIST clinical criteria. Progression of the disease was found in 2 cases, both anticipated by RPRM by 1-2 months.
Discussion: Detection of RPRM by MethyLight is able to correctly distinguish GC *vs* HD. Levels of RPRM anticipated clinical progression in patients under treatment. These findings should be evaluated in a large and prospective clinical trial.
Fondecyt 1111014, Fondef D09I1137 and PG/10-PUC.

(62) LACTATE EFFECT ON MITOCHONDRIA AND GLUCOSE UPTAKE IN HUMAN CANCER CELLS. **Viviana Ahumada**[1,2], María Cabrera[1], Claudio Acuña-Castillo[1], Dante Miranda[2], Margarita Montoya[1]. 1.Department of Biology. Faculty of Chemistry and Biology. University of Santiago of Chile. 2.Faculty of Chemical and Pharmaceutical Sciences. University of Chile. margarita.montoya@usach.cl
Introduction: Cancer cells show an increase in glycolytic rate and in the production of lactate which correlate with poor prognosis. Recently, it was shown that lactate can be used as an energy molecule by aerobic cancer cells. Previously, we showed that mitochondria of cancer cell express proteins necessary for lactate oxidation, suggesting that lactate can be metabolized in this organelle. We postulate that lactate can modulate glucose metabolism of tumor cells, decreasing glucose uptake and increasing mitochondrial mass and/or membrane potential and ROS generation.

Materials and Methods: MDA-MB-231 and Caco-2 cells were incubated with 0, 5, 10, 15 and 20 nM of lactate during 96 hours. Cells were further analyzed for mitochondrial mass (MitoTracker® Green FM), mitochondrial membrane potential (JC1) and ROS (CM-H_2DCFDA) by flow cytometry. Glucose concentration was measure spectrophotometrically (glucose oxidase kit). Mitochondrial morphology was observed by transmission electron microscopy.
Result: In MDA-MB-231 and Caco-2 cells, high concentration of lactate decreased glucose uptake and induced an increased on mitochondrial mass and ROS generation. Moreover, 20 mM of lactate also induced change in mitochondrial morphology. However, mitochondrial membrane potential was not altered in our experimental conditions.
Discussion: The results show that lactate treatment decrease glucose uptake and increase mitochondrial mass, ROS generation and induced changes in mitochondrial morphology. We could speculate that lactate induce change in cancer cells metabolism, increasing mitochondrial activity.
Financial support: Proyecto Bicentenario PDA-20 (Conicyt) and DICYT-USACH.

(63) DEVELOPING AN RNAi-BASED GENETIC ADJUVANT SILENCING STAT3 THAT COUNTERACTS TUMORASSOCIATED TOLERANCE. **Nicole Rojas-Colonelli**, Jonathan Roco, Andrés Herrada, Octavio Aravena, Manuel Varas, Alvaro Lladser. Laboratory of Gene Immunotherapy, Fundación Ciencia Para la Vida. alvaro.lladser@bionova.cl
Introduction: The signal transducer and activator of transcription (STAT) 3 has been identified as an intrinsic negative regulator of dendritic cells (DCs) that inhibits the induction of antitumor T cell immunity. Therefore, developing an RNAi-based adjuvant targeting STAT3 represents an attractive strategy to enhance the potency of DNA vaccines encoding the tumor antigens.
Material and Methods: Constructs encoding shRNAs targeting STAT3 (shSTAT3) or luciferase (shLuc) were generated into the pLL3.7 vector. The shRNAs under de control of a U6 promoter were transferred into the pVAX vector encoding the antigen tyrosinase-related protein 2 (TRP2). STAT3 knock-down was evaluated by transfecting B16F10 and DC2.4 cell lines followed by RT-qPCR and western blot analysis. DC phenotype was evaluated by measuring surface levels of CD40, CD80, CD86, MHC class II by flow cytometry and the secretion of IL-6, IL-10 and IL-12 by ELISA.
Results: A bicistronic DNA vaccine pTRP2-shSTAT3 encoding the melanomaassociated antigen TRP2 and the shSTAT3 was generated. Transfection experiments showed decreased STAT3 mRNA and protein levels. Knock-down of STAT3 in bone marrow-derived DCs and DC2.4 showed an enhanced maturation phenotype as measured by different surface marker levels and cytokine secretion and displayed an enhanced resistance to B16-mediated immunosuppression.
Discussion: We developed an shSTAT3-based genetic adjuvant that enhances DC function, thereby future work will focus on testing the ability of this adjuvant to boost the induction of antitumor T cell immunity elicited by DNA vaccines.
Funding: CONICYT Program PFB-16.

(64) AN ASSAY TO PERSONALIZE CHEMOTHERAPY IN OVARIAN CANCER PATIENTS. **M.L. Bravo**[1,5], P. González[1,5], S. Kato[2,5], M. Garrido[2], S. González[3], J. Pizarro[3], M.I. Barriga[3], H. Leon[4], E. Bustamante[4], M.A Cuello[2,5], G. I. Owen[1,5]. [1]Facultad de Ciencias Biológicas, [2]Facultad de Medicina, Pontificia Universidad Católica de Chile, [3]Hospital Sotero del Rio, [3]Fundacion Arturo López Pérez [5]Biomedical Research Consortium of Chile (BRMC)
Introduction: Ovarian cancer patients respond well to first line chemotherapy but consistently relapse within 18 months. Despite every cancer being unique, in general all cancer patients are treated by the same chemotherapy regimes. Recent advances have demonstrated that the metastatic cancer cell is distinct to that found in the primary tumor and is thus the true target of chemotherapy. Our objective was to develop an assay, based on cancer cells present in circulation and using revised drug concentrations, to predict the clinical response to chemotherapy in ovarian cancer.
Materials and Methods: We determined the response to chemotherapies currently used in clinic in primary cultures of ovarian cancer ascites (peritoneal fluid) by the MTT cytotoxicity assay. These data were correlated with clinical response (CA125 levels, imaging and medical criteria).
Results: Assay responses were classified into 3 groups: Response (R), Intermediate (I) No response (N). To date we have been able to predict the response in 21/22 cancer patients analyzed. Preliminary results show a clear distinction in time to relapse between patients classified R as opposed to N.
Discussion: We have established an assay to correlate in vitro chemotherapy toxicity with clinical response in patients with ovarian cancer. We confirm that every cancer is unique and believe that with this additional information the oncologist can personalize chemotherapy, minimizing treatment costs and improving patient survival.
FONDECYT 1080163.

(65) SALMONID SELENOTRANSCRIPTOME: *in silico* AND *in vivo* CHARACTERIZATION. **Francisco Altimiras,** Rodrigo Pulgar y Verónica Cambiazo. Laboratorio de Bioinformática y Expresión Génica, INTA, Universidad de Chile and Center for Genome Regulation (CRG). fjaltimiras@gmail.com
Introduction: The selenotranscriptome is the set of selenoprotein transcripts. Selenoproteins are a diverse group of proteins implicated in immune and oxidative responses that contain selenium (Se) in the form of the amino acid selenocysteine (Sec). Sec is encoded by the STOP codon (UGA). The presence of an RNA stem-loop structure, the Sec insertion sequence

(SECIS) element, in the 3' untranslated region (UTR) of eukaryotic mRNAs, differentiates the Sec or stop function of UGA codons. Because the dual meaning of the UGA codon, selenoprotein genes are often miss-predicted by standard annotation pipelines. Here we applied a series of bioinformatics tools to predict the *S. salar* and *O. mikiss* selenoproteomas from large collections of EST sequences.
Materials and Methods: We identified SECIS elements and UGA codon in frame in a set of 121.569 contigs and analyzed their expression in several tissues of *S. salar*. We characterized the mRNA 3' UTR using RACE-PCR and sequencing. For *in vivo* characterization, we used the Head Kidney cell line (SHK1) of *S. salar* exposed to increased concentration of Se and measured transcript abundance by qPCR.
Results: Salmonid selenotranscriptome is formed by 31 transcripts with UGA in-frame codon. The *in silico* prediction was verified by measuring transcript abundance in five tissues of *S. salar*. In current experiments we are characterizing the expression pattern of predicted the selenotranscriptome in the SHK1 cell line and its selenium-dependent expression.
Discussion: The non-conventional method for selenoprotein gene prediction was successful to identify the salmonid selenotranscriptome.
Fondecyt 109021, Fondap 15090007.

(66) PROTEOMIC ANALYSIS OF PROTOSCOLEX PROTEINS FROM *Echinococcus granulosus*. **María Pía García**[a], Christian Hidalgo[a], Henrique Bunselmeyer Ferreira[b], Ulf Hellman[c], Norbel Galanti[d] and Rodolfo Paredes[a,*]. [a]Escuela de Medicina Veterinaria, Facultad de Ecología y Recursos Naturales, Universidad Andrés Bello. [b]Centro de Biotecnologia, UFRGS, Brazil. [c]Ludwig Institute for Cancer Research Ltd., Sweden. [d]Programa de Biología Celular y Molecular, ICBM, Facultad de Medicina, Universidad de Chile. rparedes@unab.cl
Introduction: Cystic echinococcosis is a zoonotic parasitary disease, caused by the metacestode stage of *Echinococcus granulosus*. This disease is characterized by the growth of hydatid cysts, these can be either fertile or infertile. Fertile cysts have protoscolex floating in the hydatid fluid and attached to the inner wall of the germinal layer.
Methodology: Spots from 2D-Electrophoresis were excised, destained and digested by Trypsine, for posterior analysis through of Peptide mass fingerprint (PMF) or MS/MS by MALDI-TOF-TOF. Data analyses were made with Flex Analysis v3.3; MS/MS analyses were made with Biotools v3.0. PMF and MS/MS data were searched against databases Swissprot, NCBInr and LophDB using MASCOT software.
Results: Data from 178 spots that underwent MALDI-TOF mass spectrometry was collected. This data was paired with public protein databases using MASCOT Mass Fingerprint found 52 protein matches. The same data was then paired with our closed database, LophDB and found 30 additional protein matches.
Conclusions: Several proteins identified in protoscolex could be a potential target for diagnosis, treatment or vaccine for control of this disease.
Financing: FONDECYT Iniciación N° 11070082, Project DI 19-09/R-UNAB and Cooperación Internacional Conicyt/CNPq.

(67) MITOCHONDRIAL DYNAMICS IN ERYTHROPOIETIC CELLS IS MODULATED BY COPPER. **Rodrigo Bustos,** Yancing Rossel, Alvaro Elorza. Universidad Andrés Bello**.** aelorza@unab.cl
Introduction. Copper is essential in cell physiology, functioning as an enzyme cofactor in numerous redox reactions. In mitochondria, copper is part of two important enzymes, respiratory complex IV and SOD1. Copper deprivation was reported to cause anemia and elongated mitochondria in erythropoietic cells. We hypothesize that copper may modulate mitochondrial dynamics (MtDy) i.e. fusion and fission events therefore playing a major role during erythrocyte differentiation.
Materials and Methods. K562 cells were treated with $CuCl_2$ for copper overload and with BCS for copper chelation. Differentiation was induced with BA and quantified by benzidine staining. MtDy proteins were detected by immunoblot with antibodies against mitofusin 2, DRP-1 and FIS-1. Mitochondrial morphology and membrane potential were analyzed by confocal microscopy.
Results. Copper overload treatments although increase cell proliferation, inhibits differentiation and decrease mitochondrial membrane potential, do not alter mitochondrial morphology. Western blot analysis of MtDy proteins showed both fusion MFN2 and fission DRP1 were up-regulated, keeping MtDy balanced. On the other hand, copper chelation with BCS had little effect on cell proliferation while decreased differentiation. At protein level, chelation highly increased MFN2 and decreased FIS-1. In terms of morphology, mitochondria appeared hyper-fused.
Discussion. Both copper overload and removal decrease cell differentiation, indicating a need for tight balance of copper levels. Copper deficiency causes enlarged mitochondria, mainly due to an increase in fusion. Fusion is usually associated with a good health cell status. Thus, this approach might be used to improve mitochondrial function.
COCHILCO-FONDECYT 1100995; DI 10-10R.

(68) HANTAVIRUS LIKE-PARTICLES: A TOOL FOR DISEASE PREVENTION AND VIRUS-CELL ENTRY STUDIES. **[1,2]Acuña, R.,** [1]Cifuentes, N., [1]Bulling, M. and [1]Tischler, N.D. [1]Fundación Ciencia para la Vida and [2]Universidad Andrés Bello. rodrigo.acuna.bq@gmail.com

Introduction. Hantaviruses cause worldwide hemorrhagic fevers with renal or pulmonary syndrome in humans. Currently, no antiviral drugs are available and little is known about how hantaviruses enter into cells. In this work, hantavirus-like particles (HVLPs) consisting in an envelope membrane in which the viral glycoproteins Gn and Gc are anchored have been developed and characterized. These particles are being used as antigens for the immunization of animals and to characterize essential steps during viral cell entry.
Materials and Methods. Antigenicity of HVLPs was analyzed by ELISA of HVLPs with antibodies present in sera of infected patients. Immunogenicity of HVLPs was measured by ELISA of sera obtained from HVLP-immunized mice. Functional incorporation of Gc was examined by its multimerization state through sucrose gradient sedimentation and membrane interaction was measured by liposome co-flotation assays.
Results. Patient serum showed strong recognition of HVLPs and HVLP-immunized mice developed specific antibodies. HVLPs included trimeric Gc complexes after their activation through low pH which further interacted with artificial membranes. Currently, studies of HVLPs including fusion inactive mutant Gc proteins are under way in order to determine at which state of the fusion process the mutants are retained.
Discussion. The results confirm that antigenicity and immunogenicity of HVLPs resemble characteristics of the native virus. These results demonstrate that the HVLP system is a powerful tool for antiviral drug development and studying viral fusion mechanism in absence of other viral components.
Funding: FONDECYT 1100756 and CONICYT PFB-16. RA is supported by a CONICYT doctoral fellowship.

(69) EFFECT OF EGG YOLK IgY ANTIBODIES AGAINST *Piscirickettsia salmonis* INFECTION IN ATLANTIC SALMON SHK-1 CELLS. **Oliver C[1].,** Valenzuela K[1]., Silva H[1]., Oyarzún F[1]., Pontigo JP[1]., Álvarez C[1]., Olavarría VH[1]., Amthauer R[1]., Romero A[2] and Yáñez AJ[1]. [1]Instituto de Bioquímica y Microbiología, [2]Instituto de Patología Animal. Universidad Austral de Chile, Valdivia, Chile. cristianoliver7@gmail.com
Introduction: *Piscirickettsia salmonis* is the etiologic agent of the salmonid rickettsial septicaemia (SRS), which causes significant mortality and economic losses in salmon industry in Chile. This facultative intracellular bacterium is normally cultured in salmonid cell lines and during the infection induces a cytopathic effect (CPE). In the present study, we evaluated the effect of IgY against *P. salmonis* in Atlantic salmon SHK-1 cells.
Materials and Methods: SHK-1 cells were cultured in L15 media containing 2% FBS. The cells were incubated with IgY anti-*P. salmonis* and then, infected with *P. salmonis*. The CPE was evaluated up to 12 days post-infection by inverted microscope. The release of Lactate dehydrogenase (LDH) was quantified from the cell culture supernatant.
Results: The results showed an evident CPE with an increase of vacuoles in infected cells. The CPE correlates with measuring the activity of LDH in the supernatant of cultured cells infected with *P. salmonis*. Interestingly, we detect that the preincubation with antibodies against *P. salmonis* protected bacterial infection in SHK-1 cells leading to a large decrease of CPE post-infection compared with control IgY.
Discussion: The inhibitory effect of antibodies against *Piscirickettsia salmonis* on the infection in SHK-1 cells indicates that IgY anti-*P. salmonis* may be a preventive treatment for SRS. This tool may be used in the salmon industry as an alternative innocuous drug to improve the quality of aquaculture products.
Supported by: INNOVA-CORFO 07CN13PPT-256.

(70) MODEL SYSTEMS FOR ACCURACY TESTING OF SKELETON METHODS FOR BIOLOGICAL STRUCTURES. **Alcayaga L[13]**, Santibáñez F[1], Ramírez O[1], Palma K[12], Jara J[13], Concha M[1], Hitschfeld N[3] and Härtel S[1]. [1]SCIAN-Lab, [2]LEO, BNI, ICBM, Faculty of Medicine, [3]DCC, FCFM, U-Chile. lalcayag@dcc.uchile.cl
Introduction: The quantitative analysis of tree-like biological structures in 3D presents a challenge for mathematical, computational and biological sciences. Skeletons are one-dimensional representations of 2D/3D objects that retain topological and geometrical properties, allowing to extract relevant information of graph features. So far, no robust method exists for skeletonization of highly ramified systems observed at the resolution limit of confocal microscopy. Several approaches have been developed, however, they lack rigorous testing. We propose a model system that allows the analysis of the accuracy of skeletonization methods. Two skeletonization approaches are compared within complementing biological systems.
Material and Methods: The model system employs a randomized generation of graph structures and region growing of tubular edges, concomitant to registration of skeleton parameters (gold standard). Skeletonization methods based on Surface Mesh Contraction and Point Clouds were tested to match the gold standard, quantifying path length, connectivity, junctions and orientation. Also, 3D biological structures were used for expert validation: endoplasmic reticulum in COS-7 cells, neuronal arborization, and habenular neuropil in zebrafish epithalamus.
Results: We test the suitability of both methods to quantify highly ramified objects, by comparing the resulting graph features against the model system. The optimal results are obtained through interactive adjustment of the skeletonization parameters.
Discussion: Our model system presents a novel tool for accuracy testing of 3D skeletons. We will apply the tools for quantification of graph features on subcellular, cellular, and supracellular levels.
Funding: Fondecyt 1090246/1090242, HHMI, ICM P07-048-F/P09-015-F.

(71) GENERATION OF *Escherichia coli* K12 Xth NULL MUTANT. **José Delgadillo**, Sofia Sepúlveda, Ulrike Kemmerling, Norbel Galanti, Gonzalo Cabrera. Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile. gcabrera@med.uchile.cl

Introduction: The BER (Base Excision Repair) pathway is the most important mechanism to repair oxidative DNA damage. Previously, we have identified two *Trypanosoma cruzi* DNA BER repair apurinic/apyrimidinic enzymes (TcAP1, TcAP2). In the present work, we generate an *E. coli* that lacks the AP endonuclease Xth gene in order to perform future complementation studies.

Materials and Methods: PCR product**:** The pkD4 plasmid containing the kanamycin resistance gene was used as DNA template to amplify a PCR product. The primers contain sequences that are homologous to regions adjacent to the Xth and to the kanamycin genes.

Bacterial transformation: *E. coli* K12 strain carrying pkD 46 plasmid expressing phage ׀ Red recombinase were transformed with the PCR product described above to promote gene replacement. Bacteria were selected by kanamycin antibiotic resistance. The recombination efficiency was tested by PCR using different primer combinations:

1. Forward and backward internal Xth coding sequence gene.
2. Forward external upstream Xth coding sequence gene and backward internal Xth coding sequence gene.
3. Forward external upstream Xth coding sequence gene and backward internal kanamycin coding sequence gene.

Results: *E. coli* K12 Xth null mutant did not amply PCR products with primer combination 1 and 2, but amply PCR products with primer combination 3. Those results indicate that the Xth gene was replaced by the kanamycin gen in this bacterium.

Acknowledgements: FONDECYT 11100053 (GC), 11080166 (UK) and CONICYT Fellowship for PhD Thesis 24110156 (SS).

(72) SHORT TOLEROGENIC DENDRITIC CELLS PROTOCOL REPRESSES T CELLS PROLIFERATION AND EFFECTOR T HELPER PROFILES INDUCTION. **Falcón-Beas C[1,2]**, Tempio F[1,2], Pesce B[1,2], Aguillón J[1,2], Pereda C[1,2], Saffie C[1,2], Salazar-Onfray F[1,2], López M. N[1-3]. [1]Millennium Nucleus on Immunology and Immunotherapy; [2]Disciplinary Program of Immunology, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile; [3]Research Support Office, University of Chile Clinical Hospital. cfalcon@med.uchile.cl

Introduction: Tolerogenic dendritic cells (tolDCs) are known to induce tolerance by displaying a semimature state phenotype. We have previously developed mature DCs protocol from monocytes in a shor-term culture. Here we show a new tolDCs protocol generated in a short-term culture.

Materials and Methods: Monocytes were incubated for 3 days in the presence of rhIL-4 and rhGM-CSF, stimulated at day 2 with the tumor lysate TRIMEL or LPS and harvested at day 3. For the TolDCs protocol, dexamethasone was given twice (day 1 and 2). Nine hours later maturation stimuli were given. Maturation markers were tested by flowcytometry. Cytokines determination was performed by ELISA and by FlowCytomix® Technology. The phagocytosis capacity was determined by a dextran-FITC endocytosis assay. The proliferation induction was evaluated by CFSE dilution assay. T_H1 (CD4+IFN-γ+) and T_H17 (IL-17+CD4+) populations were determined by flow cytometry.

Results: We here show that a short 3 days tolDCs protocol with an early suppressor stimulus display a semimature phenotype between the maDCs and the iDCs, secreting lower levels of proinflammatory and high levels of regulatory cytokine. They are also able to repress T cell proliferation and differentiation to effector T helper profiles.

Discussion: This new biotechnological development could be eventually used in clinical approaches like autoimmune diseases and allo-transplants inducing tolerance to autoantigens and alloantigens respectively.

Support Fondecyt 1090243.

(73) ANTIBODIES GENERATED AGAINST *Trypanosoma cruzi* CALRETICULIN S-DOMAIN DETECTS PUTATIVE MURINE CALRETICULIN. [1]**González, A.,** [1]Valck, C., [1]Maldonado, I., [1]Ramirez, G., [2]Galanti, N., [1]Ferreira, A. [1]Immunology Disciplinary Program, [2]Program of Cellular and Molecular Biology, Faculty of Medicine, University of Chile, Chile. aferreir@med.uchile.cl

Introduction: Calreticulin (CRT) is a conserved and pleiotropic protein. It functions as chaperone for protein folding and regulate intracellular calcium. It locates mainly in the endoplasmic reticulum (ER). Most CRT's have N, amino terminus, P, proline-rich and C, carboxi-terminus domains. In *T.cruzi* calreticulin (TcCRT), an S-domain (TcS) binds C1q. Proteins in mammals with similar domains should be interesting to investigate.

Materials and Methods: Confluent RAW 264.7 macrophages were harvasted and sonicated to generate a whole murine cell extract. Immunowesternblot (IWB) with extract and rTcCRT was developed with an anti TcS polyclonal serum. Mass espectrometry of an immunoreactive band was performed. Peptides obtained were aligned with Clustal W program.

Results: IWB showed an antigenic band at 46 kDa, that was analyzed by Mass Spectrometry, which shows 3 peptides in 2 spectra, in agreement with the presence of murine calreticulin (MmCRT) on the sample. These peptides were aligned with clustal W program, showing 100% identity with murine calreticulin. In total, 47 aminoacids (11.3%) were sequenced.

Conclusion: MmCRT is partially identified on sample from whole murine macrophage cell extracts.

Discussion: A mammalian CRT is detected by cross-reactivity with a polyclonal serum generated against a domain of a parasite molecule. Final identification will be made by 2D electrophoresis, followed by Mass Spectrometry.
Suported by Proyecto Bicentenario de Anillos Investigación ACT112(AF), FONDECYT 1095095(AF), Beca de Doctorado CONICYT (AG), Beca de Doctorado CONICYT de Apoyo de Tesis (AG).

(74) TUMOR NECROSIS FACTOR DIFFERENTIALLY MODULATES HUMAN Th1 AND Th17 CELLS. **B. Pesce,** J.C. Aguillón, M. Cuchacovich, D. Catalán. Immune Regulation and Tolerance Research Group, Programa Disciplinario de Inmunología, ICBM, Facultad de Medicina, Universidad de Chile. www.irtgroup.cl**,** bpesce@gmail.com
Introduction: A recently described subset of T helper lymphocytes, called Th17, which are characterized for secreting IL-17, has been associated to inflammatory conditions such as rheumatoid arthritis (RA). These cells differentiate from näive T cells in the presence of pro-inflammatory cytokines such as IL-1 and IL-6. The aim of this study was to evaluate the effect of other pro-inflammatory cytokine relevant in RA, tumor necrosis factor (TNF), on Th17 cells and on IFNg-secreting Th1 cells.
Materials and methods: We isolated Th1 and Th17 cells from peripheral blood of healthy donors by cell sorting, according to the secretion of IFN-g and IL-17, respectively. We cultured these cells in the presence of TNF, and evaluated apoptosis, cytokine production, and TNF receptors expression by flow cytometry.
Results: Th1 and Th17 cells were isolated from peripheral blood, obtaining a 97% of purity. TNF was able to rescue cultured Th1 cells from apoptosis. Additionally, we found that TNF induced the double secretion of IFNg and IL-17 by Th17 cells. Finally, we detected an increased expression of the TNF receptor II on Th1 cells, in comparison to Th17 cells.
Discussion: IFNg has been described as a modulator of Th17 cells development. In this study, we describe the ability of TNF to rescue Th1 cells from apoptosis, and to induce IFNg production by Th17 cells. Hence, our results suggest that TNF may play a regulatory effect on Th17 cells, both directly and indirectly via IFN-g.
Support: FONDECYT 1090174.

(75) STUDY OF THE FUNCTIONAL PLASTICITY IN TUMOR-SPECIFIC MEMORY T CELLS STIMULATED WITH DIFFERENT TYPES OF DENDRITIC CELLS. **Mora Gabriela**, Tittarelli A., Ramirez M., Pereda C., Ortiz C., Tempio F., Falcon C., López, M.Salazar-Onfray F. Tumor Immunology Laboratory, University of Chile.
Introduction: Based on the fact that the immune system has the ability to recognize and kill tumor cells, several immunotherapies have been developed to enhance anti-tumor immune responses. In dendritic cell (DC)-based immunotherapies, a proper DC-mediated activation of T cells is necessary. It has been observed that different types of DCs are able to generate different effectors profiles in naïve lymphocytes. Our laboratory has developed a therapy for metastatic melanoma patients, based on the *in vitro* generation of autologous DCs (TAPCells) stimulated with a melanoma lysate. TAPCells have the capacity to generate melanoma-specific T cells responses *in vitro* and induce cellular immune response *in vivo*, both associated with a prolonged patients survival. Taking into account the existence of tumor-specific memory T cells in cancer patients, in this study we evaluated the capacity of different DCs to modulate the memory T cell response.
Materials and Methods: Tumor infiltrating lymphocytes (TILs) were expanded and characterized. TILs were co-cultured with TAPCells, TAPCells+IFN-a or tolerogenic DCs. Cytokine secretion and cell proliferation were measured by flow cytometry. Additionally, cytotoxic activity was measured by calcein release assay.
Results: We observed a higher accumulation of Th1/Tc1 cytokines in lymphocytes co-cultured with TAPCells and TAPCells+IFN-a compared to tolerogenic DCs. Furthermore, TAPCells and TAPCells+IFN- have a higher capability to induce T cell proliferation and cytotoxicity than tolerogenic DCs.
Discussion: These results suggest that melanoma-specific memory T lymphocytes can be reactivated differentially, depending on the type of DC that is used to stimulate them.

(76) MODULATION OF SIGNALING PATHWAYS BY DOPAMINE RECEPTORS D3 AND D5 IN CD4+ T-CELLS. **Dafne Franz**[1], Hugo González[1], Carolina Prado[1] and Rodrigo Pacheco[1,2]. [1]Fundación Ciencia para la Vida and [2]Universidad San Sebastián. Santiago, Chile. dafne.franz@gmail.com
Introduction: Dopamine, through the stimulation of its receptors (DARs), regulates the adenylate cyclase activity, and some others important signaling pathways such as MAPKs. Despite the expression of DARs, termed D1R-D5R, has been described in T-cells, signaling pathways coupled to these receptors remain unclear. Since cAMP and ERKs play key roles modulating T-cell activation/function, the aim of this work was to determine how cAMP levels and ERK1/2-phosphorylation can be modulated by stimulation of D3R and D5R.
Material and Methods: Purified CD4+ T-cells from wild-type and D3R- or D5R-deficient mice were treated with forskolin and D3R-agonist (PD128907) or D1/D5-agonist (SKF38393), and cAMP levels were quantified. TCR-stimulated CD4+ T-cells were co-stimulated with dopamine or PD128907, and ERK1/2-phosphorylation levels were determined by immunoblot.
Results: Dopamine regulates TCR-triggered ERK1/2-phosphorylation in CD4+ T-cell in a doses-dependent way. D3R-stimulation by PD128907 reduced significantly forskolin-induced cAMP levels and ERK1/2-phosphorylation in CD4+ T-

cell. Simultaneous stimulation of D3R and D5R by DA 500 nM abrogated D3R-mediated decrease on ERK1/2-phosphorylation. D5R-stimulation by SKF38393 diminished forskolin-induced cAMP levels. Moreover, D5R expression was required to allow efficient TCR-triggered ERK1/2-phosphorylation and IL-2 secretion by CD4+ T-cells.
Discussion: D3R and D5R expressed in murine CD4+ T-cells are functional and, upon stimulation, they can modulate signaling pathways involving cAMP synthesis and TCR-triggered ERK1/2-activation and, consequently, they can regulate CD4+ T-cell activation.
Supporting Grants: FONDECYT-1095114, CONICYT PFB-16. HG and CP are CONICYT fellows.

(77) INVOLVEMENT OF P2X7R IN ANTIGEN CROSS-PRIMING, *in vivo*. **Ximena López**, Bélgica Villegas, Yohana Labra, Javier Mena, Víctor Lazo, Ronny Hernández, Alejandro Torres, Alejandro Escobar, Margarita Montoya, Mónica Imarai, Claudio Acuña-Castillo. Universidad de Santiago de Chile. claudio.acuna@usach.cl
Introduction: Antigen cross-priming is a dendritic cells (DCs) mechanism necessary to induce response against tumours and virus-infected cells. The molecular basis of how a phagocytosed antigen is presented on MHC-class I pathway has not been solved. We postulated that for membrane-enclosed antigens, this process is mediated by P2X7 receptor (P2X7R) inducing fusion between the membranes of the antigen donor cells and phagolysosome of DCs. Our objective was to corroborate this by using an immunological model with ovalbumin (OVA) and apoptotic bodies, *in-vivo*.
Materials and Methods: We immunized C57BL/6 mice 3 times with apoptotic bodies obtained from, either wild type HEK293 cells, OVA expressing cells (controls) and OVA plus P2X7R expressing cells (treatment group), or directly immunized with OVA CFA (immunized group). Then, splenocytes were purified, stained with CFSE and proliferation of T cells was evaluated after challenge them with OVA.
Results: *In vitro*, P2X7R mediated the transference of various molecules to DCs cytoplasm. *In vivo*, CD8 and CD4 lymphocytes from treatment group and OVA CFA group were induced to proliferate in response to OVA. Similar results were obtained for CTLs in response to a specific CD8 peptide challenge. No significant differences were found in splenocytes challenged with OVA from mice used as controls, which were immunized with HEK293 cells expressing or not OVA.
Discussion: Results are in agreement with the fact that P2X7R could induce the fusion between apoptotic bodies and DCs phagolysosome, which would result in cross-priming. Several other experiments are required to confirm this hypothesis.
FONDECYT 11070177 and 1110734.

(78) DEVELOPMENT OF AN ANTIBODY AGAINST CD28 TO IDENTIFY T LYMPHOCYTES OF RAINBOW TROUT *(Oncorhynchus mykiss)*. **Soto-Aguilera S**, Valenzuela B, Maisey K, and Imarai M. Laboratorio de Inmunología, Centro de Biotecnología Acuícola (CBA), Facultad de Química y Biología, Universidad de Santiago de Chile. sarita.soto@usach.cl
Introduction: CD28 is a co-stimulatory molecule and a cell surface marker of T lymphocytes. In fish, and particularly in Rainbow trout *(Oncorhynchus mykiss)*, the characterization of immunocompetent cells positive for CD28 expression is limited due to the absence of specific antibodies directed against this cell surface marker. Then the aim of this study was to produce antibodies to identify and characterize the CD28+ cells in lymphoid organs of the Rainbow trout.
Materials and Methods: Using RT-PCR, the gene segment corresponding to the extracellular domain of CD28 was amplified and cloned into the pET expression system. The recombinant polypeptide was used to immunize New Zealand rabbits and the anti-serum was collected and characterized by ELISA. Expression of CD28 was analyzed in spleen, head kidney and gills of control and zymosan-treated trout by flow cytometry.
Results: The results show that the polyclonal antibodies recognize the recombinant CD28 polypeptide in ELISA assay. In addition, the antiserum was useful to identify immunocompetent populations in the gills and spleen of Rainbow trout treated with immunostimulants.
Discussion: These results suggest that the polyclonal antibody allows the identification and localization of immunocompetent populations CD28+ in organs of Rainbow trout.
CONICYT, INNOVA-CHILE 09CN13PBT90, 09MCSS6698, VRID-USACH.

(79) *Trypanosoma cruzi* CALRETICULIN INHIBITS THE CLASSICAL PATHWAY OF COMPLEMENT ACTIVATION IN *Gallus gallus*. **Paula Abello,** Carolina Valck, Ismael Maldonado, Hector Hidalgo, Arturo Ferreira. Disciplinary Immunology Program, ICBM. University of Chile. aferreir@med.uchile.cl
Introduction: Bird resistance to *T. cruzi*, has been attributed to efficient activation of their alternative complement pathway. However, it is unknown why the classical pathway is not operative to eliminate trypomastigotes. On the other hand, *T. cruzi* Calreticulin (TcCRT) inhibits the human complement system classic pathway, because it binds to the first complement component C1, thus interfering with the function of the serine-protease C1s. In this work we propose that, similar to humans, TcCRT is responsible, at least partially, for the inability of the classic pathway to lyse bird trypomastigotes.
Material and Methods: In ELISA, putative serum chicken C1 activates human C4, to the same extent as human C1 does it, in a calcium dependent fashion and in a non species-specific way.

Results: TcCRT inhibits the activation of putative C1 in chicken serum, in similar way as the parasite molecule inhibits human C1, and this effect is reversed by $F(ab)'_2$ immunoglobulin fragments anti-TcCRT. Moreover, in this *in vitro* assay chicken C1 is also antigenically detected by cross reactive anti-human C1 antibodies.
Discussion: This preliminary work indicates that, in *G. gallus*, there exists a molecule that functionally and antigenically behaves as human C1 and that TcCRT inhibits its C4 converting capacity, thus explaining, at least partly, why the chicken complement classical pathway is unable to control *T. cruzi* infection.
Supported by Proyecto Bicentenario de Anillos de Investigación ACT29 y ACT112(AF), FONDECYT 1095095(AF), Beca de Doctorado CONICYT(PA).

(80) ASSESSMENT OF THE VIABILITY OF OLIGODENDROCYTES FROM THE PROGENY GESTATED IN MOTHERS WITH THYROID HORMONE DEFICIENCY IN RESPONSE TO INFLAMMATORY MOLECULES. **Eduardo Albornoz**[1,3], Claudia Cortes[1,3], Pablo Cisternas[1,3], Pablo Gonzalez[1,3], Carlos Pizarro[1,3], Leandro Carreño[2,3], Susan Bueno[2,3], Eliseo Eugenin[4], Joan Berman[4], Alexis Kalergis[2,3] and Claudia Riedel[1,3]. [1]Facultad de Ciencias Biológicas, Universidad Andrés Bello. [2]Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, PUC. [3]Instituto Milenio de Inmunología e Inmunoterapia. [4]Albert Einstein College of Medicine of New York. edua.albornoz@uandresbello.edu
Introduction: Thyroid hormone deficiency (THD) during pregnancy, affects the central nervous system development. This condition increases clinical score intensity for experimental autoimmune encephalomyelitis (EAE). We proposed that THD during pregnancy alters oligodendrocytes, astrocytes and the blood brain barrier of their offspring.
Methodology: Mice gestated in mothers with THD were induced with EAE, after 21 days induction, spinal cord and brain slices were analyzed for myelin expression and GFAP by immunofluorescence and western blot. $CD4^+$, blood brain barrier markers and TUNEL by immunofluorescence. To analyze the effects of THD during pregnancy on oligodendrocyte differentiation and viability after tumor necrosis factor α (TNFα), primary oligodendrocytes cultures were performed from rats gestated in THD.
Results: Mice gestated in mothers with THD and induced with EAE in the adulthood showed an alteration in myelin, astrogliosis, BBB markers and an increase in positive oligodendrocyte for TUNEL compared to control mice. The primary oligodendrocytes cultures from mice gestated in THD were less viable in the presence of TNFα compared to control.
Discussion: Our data indicates that oligodendrocytes, astrocytes and endothelial cells from the BBB are affected by THD during pregnancy, suggesting that these alterations could contribute to the fact that these mice have strongest EAE clinical score.
Fondecyt 1100926. Proyecto Interno UNAB DI-11-11/R. Millennium Institute on Immunology and Immunotherapy (MIII) P09-016-F.

(81) SOLUBLE TOLL LIKE RECEPTOR 2 (sTLR2) IS PRODUCED BY METALLOPROTEASE ECTODOMAIN TLR2 SHEDDING. **Langjahr, P.E**.; Rubio, E.; Díaz-Jiménez, D.; Hermoso, M.A. Laboratorio de Inmunidad Innata, Programa D. de Inmunología, ICBM, Facultad de Medicina, Universidad de Chile. plangjhar@med.uchile.cl
Introduction: Toll like receptors (TLRs) participate in the initial immune response against pathogens and endogenous damage associated molecules. TLRs activation is tightly regulated through several negative effectors to avoid over-inflammation and autoimmune reactions. Human TLR2 is produced either as a transmembrane or soluble forms (sTLR2) mainly by monocytes/macrophages and dendritic cells. sTLR2 negatively regulates TLR2 activation, however, the molecular mechanism implicated in its generation has not been described. Aim: Determine the mechanism of constitutive and ligand-induced generation of sTLR2.
Materials and Methods: sTLR2 variants were characterized by ultracentrifugation and western blot of human monocytic and stably transfected HEK293-TLR2-YFP cell supernatants. sTLR2 production was quantified by ELISA in cells exposed to $Pam3CSK_4$, a specific TLR2 ligand, phorbol esters, lipopolysaccharide and/or pharmacologic inhibitors. Membrane-associated TLR2 was evaluated by flow cytometry and western blot.
Results: We demonstrated that sTLR2 variants are constitutively produced and induced by $Pam3CSK_4$ or other inflammatory stimuli by monocytic cells through a post-translational mechanism that involves metalloproteases.
Discussion: sTLR2 production occurs by metalloprotease TLR2 ectodomain shedding and increases during inflammatory conditions. This mechanism regulates the level of membrane-associated TLR2, participating in down-regulation of the receptor signaling pathway. Understanding the mechanisms involve in extracellular receptor variants generation will contribute to solve the role of TLR2 in pathological conditions.
FONDECYT 1080290.

(82) CHARACTERIZING THE DNA-SENSING SIGNALING PATHWAYS OF THE INNATE IMMUNITY THAT MEDIATE THE INDUCTION OF ANTITUMOR T CELL IMMUNITY ELICITED BY DNA VACCINES: THE ROLE OF NF-κB. **Jonathan Roco**[1], Maarten Ligtenberg[2], Andrés Herrada[1], Rolf Kiessling[2], Alvaro Lladser[1]. [1]Laboratory of Gene Immunotherapy, Fundación Ciencia para la Vida, Santiago, Chile. [2]Immune and Gene Therapy Unit, CancerCentrum Karolinska, Karolinska Institutet, Stockholm, Sweden. alvaro.lladser@bionova.cl (Sponsor: P. Valenzuela)

Introduction: DNA vaccination is an attractive approach to induce antigen-specific cytotoxic T lymphocyte (CTL) responses that can mediate long-lasting protective immunity against cancer. The early activation of innate immunity is essential to promote effective adaptive immunity. Consequently, understanding the DNA-sensing signaling pathways that dictate the immunogenicity of DNA vaccines represents a unique opportunity to develop novel adjuvant strategies to enhance vaccine potency.
Material and Methods: Mice were intradermally electroporated with plasmids encoding either ovalbumin (pOVA) or tyrosinase-related protein 2 (pTRP2). A plasmid encoding a degradation resistant mutant of IκBα (pIκBα-SR) was used to block NF-κB activation. CD8+ T cell responses were analyzed after two vaccinations by intracellular IFN-γ staining and *in vivo* cytotoxicity assay. Tumor protection was evaluated by i.v. injection of B16 melanoma cells and counting of metastatic foci in lungs.
Results: Using different knock-out models, we found that functional CTL responses were elicited by *in vivo* intradermal DNA electroporation regardless the presence of TLR9, MyD88, IRF3 or IFN-α/β-receptor. However, blocking NF-κB activation at the site of electroporation impaired both TAA-specific CTL responses and protection against B16 melanoma.
Discussion: This study validates NF-κB as a master regulator of DNA vaccine immunogenicity. Based on this knowledge, we are developing RNAi-based adjuvants silencing IκBα to harness DNA-mediated NF-κB activation to enhance gene vaccine potency.
Funding: CONICYT Program PFB-16.

(83) ALLOGENEIC PLGA PHAGOSOMES DECREASE THE ALLOIMMUNE RESPONSE *IN VIVO*. **Yessia Hidalgo**[1], Paulina Ruiz[1], Paula Maldonado[1], Cinthia Silva[1], Mario Rosemblatt[1,2], María Rosa Bono[1]. Laboratorio de Inmunología, Facultad de Ciencias, Universidad de Chile[1]. Fundación Ciencia para la Vida[2]. yhidalgo@ug.uchile.cl
Introduction: Immunosuppressive drugs are commonly used to induce long-term acceptance to organ transplantation. These drugs are non-specific and have associated side effects such as increased risk of infection, and are ultimately unable to prevent long-term organ rejection. A mechanism to decrease the alloimmune response is the presentation of donor antigens in a tolerogenic context. We developed a protocol to administer alloantigens in a tolerogenic fashion through vesicles, termed phagosomes, that are generated from immature dendritic cells (iDC) charged with donor alloantigens.
Materials and Methods: iDC were generated from bone marrow cells and differentiated with supernatant from J558L cell line that secretes GM-CSF. iDC were incubated with PLGA microspheres (lactic-co-glycolic acid), and vesicles were obtained through rupture with osmotic shock. C57BL/6 mice were pre-treated with allogeneic BALB/c phagosomes and immunized with BALB/c splenocytes. Levels of alloantibodies were measured through flow cytometry cross-match.
Results: Allogeneic phagosomes were analized by flow cytometry and found that they were surrounded by plasmatic membrane that carry MHC-II molecules among others. Most importantly, mice treated with allogeneic phagosomes show a significant decrease in alloantibody levels compared with control mice after alloimmunization.
Discussion: Allogeneic phagosomes are a suitable approach to deliver alloantigens in a tolerogenic fashion generating alloantigen tolerance measured by a decrease of the humoral response. This method could be used as a novel treatment to induce allogeneic immune tolerance.
FONDECYT 1100557, 1100448.

(84) LOW PLASMA LEVELS OF 2-METHOXYESTRADIOL (2-ME) IN EARLY PREGNANCY OF PATIENTS THAT WILL DEVELOP PREECLAMPSIA (PE)
Pérez-Sepúlveda A, Torres MJ, Valenzuela FJ, Larraín R, Galaz J, Valenzuela I, Soto MJ, Figueroa-Diesel H, Illanes S. Departamento de Obstetricia & Ginecología y Laboratorio de Biología de la Reproducción. Facultad de Medicina, Universidad de los Andes, Santiago. sillanes@uandes.cl (Sponsor: U. Wyneken).
Introduction: PE is a main cause of maternal/fetal morbi-mortality, and is characterized by an abnormal trophoblastic invasion and a reduction of utero-placental flow, which results in hypoxia and HIF-1α expression. Significantly lower levels of 2-ME are observed in animal models of PE and have been associated to an abnormal function of Catechol-O-Methyltransferase (COMT). The Val158Met polymorphism of COMT sequence leads to 4-fold decrease in its activity. We hypothesize that 2-ME levels are decreased at early pregnancy in women that will develop PE and associated to the presence of the Val158Met polymorphism in COMT sequence.
Methods: Plasma levels of 2-ME were assessed by HPLC MS/MS in samples from 15 PE and 13 healthy pregnant women, at 11-14 weeks of gestation. The presence of COMT polymorphism Val158Met was assessed by RFLP-PCR in the placenta of 18 PE and in 31 control patients.
Results: Plasma levels of 2-ME were significantly lower in women who subsequently developed PE (Control: 53.5pg/ml; PE: 0.62pg/ml t-Test p=0.0477). COMT Val158Met polymorphism has a frequency of 35,7% but is not related with PE development (X2=2.51 p=0.15; O.R.=0.44).
Discussion: Low levels of 2-ME in the first trimester of pregnancy are observed in patients that will develop PE. Our results those no suggest an association of this low levels with Val158Met polymorphism of placental COMT sequence. More studies are needed to establish the causes of this interesting finding.

(85) REVISITING GLUCOSE TRANSPORTER 1 AND 3 THROUGH LIVE CELL FLUORESCENT MICROSCOPY. **Aníbal I. Acuña**, Ilona I. Concha, Maite A. Castro. Instituto de Bioquímica y Microbiología, UACh. macastro@uach.cl

Introduction: Facilitative glucose transporters (GLUTs) are expressed in all mammalian cells. GLUT1 is ubiquitously expressed, while GLUT3 is present in organs with high energetic requirements. It has been described the presence of GLUT1,3 in membrane domains. This compartmentalization could be related to functional changes in these proteins. However there are no extensive studies on GLUT1,3 dynamics at plasma membrane in live cells. In this work we analyzed GLUT1,3 behavior through live cell microscopy techniques.

Methods: Total internal reflection microscopy, fluorescence recovery after photobleaching and confocal microcopy real-time uptake assays using fluorescent glucose analogs (NBDG) were used. These experiments were complemented by biotinylation, immunofluorescence and immunoprecipitation assays.

Results: We observed mild changes at plasma membrane availability and mobile fraction of GLUT1,3 in response to changes of extracellular glucose. Interestingly, phosphorylable NBDG (2-NBDG) uptake assays revealed the presence of two components and non-phosphorylable NBDG (6-NBDG) only displayed one component. The first component corresponds to 2-NBDG uptake and the second component corresponds to 2-NBDG phosphorylation. Disrupting cholesterol rich domains only decreased the rate of 2-NBDG phosphorylation suggesting a close relationship between hexokinase and GLUTs. To examine this we performed 2NBDG uptake assays in cells treated with shRNA to knock down GLUT1 or GLUT3 expression, colocalization and immunoprecipitation assays using specific antibodies against GLUT1,3 and HKI,II,III.

Discussion: Glucose transport and phosphorylation appear to be closely related. Alterations in compartmentalization could drive changes in glucose uptake and utilization.

FONDECYT1110571&1110508.

POSTER PRESENTATIONS II

(86) TACE/ADAM17 PROMOTER IS DIFFERENTIALLY REGULATE BY RUNX2 TRANSCRIPTION FACTOR DURING OSTEOBLAST DIFFERENTIATION. **Héctor Araya**[1,2], Oscar Vega[1,2], Nelson Varela[1,2], Marcelo Antonelli[2], Julio Tapia[2], Hugo Sepúlveda[3], Martin Montecino[3], Gary S Stein[4], Andre van Wijnen[4], Mario Galindo[1,2]. [1]Millennium Institute on Immunology and Immunotherapy, [2]Programa de Biología Celular y Molecular, ICBM, Facultad de Medicina, Universidad de Chile. [3]Center for Biomedical Research, Faculty of Biological Science and Faculty of Medicina, Andres Bello University. [4]Department of Cell Biology and Cancer Center, University of Massachusetts Medical School, USA. mgalindo@med.uchile.cl

Introduction: Runx2 transcription factor regulates bone differentiation by activation or repression of bone phenotypic genes. In this way TACE/Adam17 expression was down regulated during osteogenic differentiation. TACE/Adam17 promoter (-2.3Kpb) has several canonic and non-canonic Runx2 binding sites. We propoused that TACE/Adam17 promoter is differentially regulated by Runx2 during lineage commitment and osteoblast differentiation.

Methods: We evaluated the activity of a mouse TACE/Adam17 promoter associated to the luciferase reporter gen. A series of deletion mutants spanning the mouse TACE/Adam17 gene promoter were co- transfected with Runx2 or Runx2 carboxy terminal deleted mutant in mesenchimal (C2C12 and C3H10T1/2) and pre-osteoblas (MC3T3, Runx2-/-) cell lines. We analysed expression of different ADAM and ADAMTS genes during osteogenic lineage commitment and also in pre-osteoblast cells by Affymetrix. Runx2 binding to the TACE/Adam17 promoter was evaluated in vivo by ChIP assay.

Results: Runx2 positively regulated TACE/Adam17 promoter (-2.3Kpb) but not the proximal promoter (-0.4Kpb) in mesenchymal C2C12 cells which correlated with up regulation of TACE/Adam17 gene expression in C2C12 stimulated with BMP2. In pre-osteoblast Runx2 negatively regulated TACE/Adam17 proximal promoter (-0.4Kpb) which is correlated with a down-regulation of TACE/Adam17 gene expression during osteogenic differentiation.

Discussion: Our results suggest that TACE/Adam17 promoter has different Runx2 binding sites which are differentially regulated (positively or negatively) by Runx2 during lineage commitment and osteoblast differentiation.

Grant sponsors: Fondecyt 1095234 and Iniciativa Científico Milenio P09-016-F.

(87) REGULATION OF N-TYPE Ca^{2+} CHANNELS BY THE INTERACTION WITH THE LIGHT CHAIN 1 (LC1) OF MICROTUBULE ASSOCIATED PROTEIN B (MAP1B). **Henríquez, D.R.**[1], Gandini, M.A.[2], Sandoval, A.[2], Felix, R.[2] and González-Billault, C.[1]. [1]Laboratory of Cell and Neuronal Dynamics, Department of Biology, Universidad de Chile, [2]Departmento de Biología Celular, Cinvestav-IPN, México. darohenga@gmail.com

Introduction. Voltage-gated Calcium (Ca_V) channels are transmembrane proteins that regulate the Ca^{2+} entry into the cell in response to membrane depolarization, coupling the electrical event in intracellular processes which result in physiological events like muscle contraction, hormone secretion and neurotransmission. Distinct Ca_V-channels have been described based on their biophysical properties including low voltage-activated (LVA or T-type) and high voltage-activated (HVA). At the molecular level, Ca_V-channels are complexes of at least 3 subunits: the pore-forming (α_1) subunit and the auxiliary $\alpha_2\delta$ and β subunits. Interestingly, recent studies have found that LC1, interacts with proteins like-NMDA receptors regulating their trafficking or the AMPA-receptors modifying their role in exocytosis.

Materials and Methods. Using patch-clamp recordings with biochemical and molecular biology techniques, we investigated whether LC1 regulates recombinant N-type Ca_V-channels heterologously expressed in HEK-293 cells. Moreover, by using confocal immunofluorescence in neurons kept in culture, we looked for their co-localization, as their interaction using mouse brain total proteins by pulldown assays.

Results. Co-expression of N-type Ca_V-channels with LC1 in HEK-293 cells revealed a significant decrease in whole cell current density without affecting the time constant of inactivation, voltage dependence or the steady-state inactivation, which strongly suggests a decrease in the number of functional Ca_V-channels on the cell surface. Furthermore, pulldown experiments and confocal microscopy showed an interaction between LC1 and the channel.

Discussion. These results reveal a functional relationship between the LC1 protein and the N-type Ca_V-channels.

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(88) HEMICHANNELS PARTICIPATE IN MAST CELLS DEGRANULATION INDUCED BY ANTIGEN RECOGNITION. **Harcha PA,** Sáez JC. Departamento de Fisiología, P. Universidad Católica de Chile. paharcha@puc.cl

Introduction: During allergic responses, antigen recognition, through antigen-specific immunoglobulin E (IgE) receptors in mast cells (MCs), promotes the release of proinflammatory mediators from vesicular storages. This degranulation process depends on Ca^{2+} entry from the extracellular medium. The CRAC channels, molecular entities responsible for this Ca^{2+} entry identified so far, do not explain the membrane permeability changes described during antigen recognition. We studied if hemichannels (HCs) formed by connexin (Cx) or pannexin (Panx) proteins, which are permeable to Ca^{2+} and small organic molecules, are necessary for the degranulation response induced by antigen recognition through IgE receptors in MCs.

Materials and Methods: Primary naïve MCs were derived from bone marrow precursors (BMMCs) using WEHI3 conditioned medium. Panx1 and Cx43 proteins were evaluated by immunofluorescence and western blot analyses. The HC activity was assessed by ethidium (Etd) uptake (fluorescence intensity) recorded in time-lapse measurements. BMMCs were

sensitized with monoclonal anti-ovalbumin (OVA) IgE before antigen stimulation. BMMCs degranulation was determined by time-lapse measurements of toluidine blue (TB) released from secretory granules.
Results: Panx1 and Cx43 were detected in BMMCs. OVA recognition induced BMMCs degranulation and increased Etd uptake, which were inhibited by 5μM carbenoxolone and suramine, a Panx-HCs and purinergic receptor wide spectrum blocker respectively.
Discussion: BMMCs express functional Panx HCs, but since carbenoxolone is an unspecific Panx HC blocker, we cannot discard the possible contribution of Panx2 or Panx3 HCs during antigen-induced degranulation responses. Inhibition of HCs might avoid the spread of the inflammatory responses induced by antigen recognition during allergic reactions.

(89) ROLE OF α6β1 INTEGRIN IN SECRETION, ORGANIZATION, ADHESION AND SURVIVAL OF DIFFERENTIATED 3D ACINI OF SALIVARY GLANDS CELLS: IMPLICATIONS IN SJÖGREN'S SYNDROME. **Urra H,** Cortés J, Bahamondes V, Castro I, Barrera MJ, Aguilera S, Molina C, Leyton C, Alliende C, González S, and González MJ. ICBM Facultad de Medicina Universidad de Chile. hery.urra@gmail.com
Introduction: Sjögren syndrome (SS) is a frequent autoimmune disease associated with dysfunction of the exocrine glands. Previous work from our laboratory demonstrates a down-regulation of active α6β1 integrin in SS-patients; however the importance of this integrin in the pathogenesis of this disease is unknown. Our hypothesis is that the loss of α6β1 integrin is associated with the disorganization of acini of salivary gland cells.
Materials and Methods: Human salivary gland (HSG) cells were grown on Matrigel for 5 days and differentiated. The effect of α6β1 integrin blocking antibody on secretion, survival, morphology and adhesion was evaluated by indirect immunofluorescence and cell adhesion assays.
Results: We demonstrated that the integrin blockade induces the disorganization of the 3D acinar structure, associated with decreased secretion of amylase and loose of adhesion to the substrate. Additionally, an increase of active caspase-3 was observed, indicating induction apoptotic cell death.
Discussion: Our data support the hypothesis that α6β1 integrin is important for the organization and survival of 3D acini of salivary cells. The culture of HSG cells on matrigel and downregulation of activity or expression of α6β1 integrin recapitulates several changes observed in SS-patients. This study reveals a novel mechanism of α6β1 integrin in the pathogenesis of Sjögren's syndrome. The specificity of the role of α6β1 integrin is currently been studied by inducible shRNA targeting.
FONDECYT 1080006 (MJG, SA, CM).

(90) THE HEDGEHOG (Hh) PATHWAY MODULATES MATURATION OF CHONDROCYTES *in vitro* THROUGH NEOGENIN 1. **João Francisco Botelho,** Cynthia Villarroel, Pablo Lois and Verónica Palma. Laboratory of Stem Cells and Development, Facultad de Ciencias, Universidad de Chile chicobotelho@gmail.com
Introduction. Endochondreal ossification (EO) gives rise to most of skeletal elements and is initiated by a cartilage precursor that is used for subsequent ossification. Hh is a key component of EO controlling the maturation of chondrocytes to hypertrophy and also maintaining a pool of proliferating non-chondrocytes. Recently it has been reported that Neogenin 1, a death dependence receptor, also regulates EO. We have shown that Neogenin-1 is a direct target of the Hh pathway. Here, we set out to determine what role, if any, Neogenin-1 as a mediator of Hh signaling plays in EO.
Methodology. Primary cultures of chondrocytes were obtained from the ribs of chicken embryos (E14). Chondrocytes were grown on collagen gels in standard osteogenic media allowing differentiation to hypertrophic chondrocytes and osteoblasts. In order to study Hh influence on Neogenin-1 mediated EO cells were transfected with Hh gain of function constructs, or treated pharmacologically with agonists or antagonists of the hedgehog pathway. The phenotypes were analyzed by specific dyes and immunohistochemistry after 2, 4 and 7 days of culture.
Results. We were able to establish a successful *in vitro* culture of chondrocytes. Expression of collagens II, collagen IX, sox-9 verified differentiation of chondrocytes. Chondrocytes also express Neogenin-1 and this expression is regulated by Hh signaling.
Discussion. Our results indicate that events related to chondrocyte differentiation, including increased ALP activity and bone-like nodule formation, are enhanced by Hh signaling and that this effect is likely to occur through Neogenin-1.
Funding: Fondecyt 1110237.

(91) EXPRESSION OF AKT/PKB IN STREPTOZOTOCIN-INDUCED DIABETIC RAT KIDNEY. **Marcos Soto**, Romina Bertinat, Pamela Silva, Pamela Kairath and Alejandro Yañez. Instituto de Bioquímica y Microbiología, Universidad Austral de Chile (UACH). marcossoto85@hotmail.com
Introduction: Diabetic Nephropathy (DN) is a renal chronic disease. There is strong evidence that alterations in Akt activity in insulin-sensitive tissues participate in the development of type I and II diabetes. Here, we studied the Akt expression, activation and localization in renal tissue of streptozotocin-induced diabetic rats, to elucidate its implication in the DN progression.

Materials and Methods: To induce diabetes, a single intravenous dose of 65mg/Kg streptozotocin was administered to male Sprague Dawley rats. After 2-8 months, kidneys were processed for qRT-PCR, Western blot and immunohistochemical assays.
Results: qRT-PCR assays showed that Akt isoform 1 has the highest expression level in normal kidneys, compared to the other two isoforms (2 y 3), and it is overexpressed in both short and long term diabetic rat kidney. However, Western blot analysis did not show significant difference in the protein expression of total Akt1/2 between both groups. Immunohistochemical assays did not show any difference in the signal intensity and localization either. The phosphorylated form of Akt (pAkt) was mainly detected in renal outer medulla, and a significant increase was observed in diabetic rats.
Discussion: These results suggest that the increased activation of Akt in the kidney from this type I diabetic rat model is not dependent on insulin signaling, and might be the consequence of an adaptive mechanism to counteract hyperglycemia.
(FONDECyT 1090694).

(92) RUNX2 EXPRESSION IS DIFFERENTIALY REGULATED DURING CELL CYCLE IN HUMAN OSTEOSARCOMA CELL LINES. **Claudia Lucero**[1,2], Oscar Vega[1,2], Julio Tapia[2], Marcelo Antonelli[2], Gary S Stein[3], Andre van Wijnen[3] and Mario Galindo[1,2]. [1]Millennium Institute on Immunology and Immunotherapy, [2]Programa de Biología Celular y Molecular, [3]Programa Disciplinario de Inmunología, ICBM, Facultad de Medicina, Universidad de Chile. [4]Department of Cell Biology and Cancer Center, University of Massachusetts Medical School, USA. mgalindo@med.uchile.cl
Introduction: Runx2 transcription factor regulates lineage commitment, proliferative expansion of osteoprogenitors and osteogenic differentiation. Runx2 levels are negatively modulated during cell cycle progression into S and G2/M phases in proliferative pre-osteoblasts whereas Runx2 over-expression attenuates cell growth (in G1 phase) and induces osteblast differentiation. Osteosarcoma (OS) is the most prevalent malignant bone tumor in infants and adolescents. Recently, we reported that wnt-canonical pathway modulates positively Runx2 expression. Moreover, Runx2 and β-catenin expression are linked to tumor progression and metastasis in OS. We proposed that Runx2 expression is differentially regulated during cell cycle which correlated with high proliferative rates in human OS cells.
Material and Methods: Six different human OS cell lines were arrest in G1 phase with mimosine and stimulated to progress synchronically through the cell cycle. Cell synchronization was monitored by FACS analysis. We determined Runx2, β-catenin and cell cycle markers levels by RT-PCR and western blot. Additionally, were tested the anti-proliferative effect of Runx2 in OS cell lines.
Results: Runx2 and β-catenin were expressed during the cell cycle. However, only Runx2 protein levels were cell cycle modulated in three OS cell lines. Consecuently, these OS cell lines had a high proliferatives rates and a short cell cycle time. Runx2 over-expression had been a differential effect in OS cell lines proliferation.
Discussion: These results suggest that modulation of Runx2 expression through the cell cycle may contribute to the pathogenesis of this bone cancer.
Grant sponsors: Fondecyt 1095234 and Iniciativa Científico Milenio P09-016-F.

PANNEXIN MEDIATED COUPLING OF OLIGODENDROCYTES AND OF A CELL LINE DERIVED FROM HUMAN OLIGODENDROGLIOMA. **Paola A. Soto**[1], Paola Fernández[1], Maximiliano Rovegno[1], Agustín D. Martínez[2], Bruno Cisternas[1], Felipe Court[1], Alex Vielma[2], Oliver Schmachtenberg[2], Michael V.L. Bennett[3] and Juan C. Sáez[1,2]. [1]Departamento de Fisiología, Pontificia Universidad Católica de Chile, Santiago, Chile and [2]Instituto milenio, CINV, Valparaíso, Chile. [3]Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY, USA.
Introduction. Oligodendrocytes express connexins and functional gap junctions. They also express pannexin1 (Panx1), but there is no evidence of Panx1 gap junction channels (GJCs).
Material and Methods. Cultured rat oligodendrocyte, TC620 cells, an oligodendroglioma cell line, and HeLa cells transfected with Panx1, Cx29, Cx32 or Cx43 were used. The expression of these connexins and Panx1 was studied by immunoblotting and immunofluorescence. Intercellular coupling was assessed by cell-cell transfer of different permeability tracers microinjected into one cell. Electrical coupling was assessed with the dual patch voltage clamp technique.
Results. Panx1 was detected in oligodendrocytes and TC620 cells. The latter also expressed Cx32 but not Cx29. Confluent cultures of oligodendrocytes, TC620 cells or HeLa-Panx1 cells showed intercellular transfer of DAPI. This coupling as well as electrical coupling was resistant to octanol, but blocked by low concentrations of carbenoxolone, which blocks Panx1 hemichannels and not connexin GJCs. In re-aggregating TC620 or HeLa-Panx1 cells, dye coupling was prevented by the Panx1 mimetic peptide, ^{10}Panx1, but not by connexin nimetic peptide, Gap26. In TC620 cells, dye coupling was blocked by Panx1 siRNA, which markedly reduced Panx1 levels assayed by immunoblotting.
Discussion. These results provide the first evidence of Panx1 GJCs in endogenous expressing cells and do not exclude co-expression with connexin gap junctions.

(94) ANALYSIS OF A FRACTION OF HUMAN RECOMBINANT FSH THAT INDUCES CELL DEATH. **Orellana RF**, Ríos M, Ortiz ME, Owen GI and Velásquez EV. Faculty of Biological Sciences, Pontificia Universidad Católica de Chile and Chilean Institute of Reproductive Medicine (ICMER), Santiago, Chile.

Introduction: Follicular maturation and development are processes regulated by the Follicle Stimulating Hormone (FSH). Commercial human recombinant preparations of this glycosylated hormone act upon follicular granulosa cells (GC) inducing proliferation and oestradiol production. Our laboratory has separated a recombinant FSH, used for clinic treatment, into fractions which differ in their biological properties. One of these fractions (Fraction 4) induces death in granulosa cells and cancer cells of GC origin.

Aim: Herein, our objective was to determine the composition of Fraction 4 and the means in which cell death occurred.

Methods and Results: Using western blot analysis we detected FSH in each fraction obtained and the presence of an additional 26kD band in fraction 4. Analysis of this band by LC MS/MS showed the presence of FSH and Concanavalin-A (ConA). Primary cultures of rat GCs and a cancer cell line of GC origin showed that ConA reduced cellular viability in a concentration dependent manner. Interestingly, cell death only occurred in FSH-Receptor expressing cells of the ovarian follicle and thus future experimentation will reveal if the presence of the FSHR is required for this action of ConA.

Conclusion: We demonstrate the presence of a ConA contaminant in a commercial preparation of FSH that mediates cell death. Elimination of this contaminant using our fractionation characteristics may improve the clinical efficiency of commercial FSH.

Fondecyt 1100870 y 3090066.

(95) QUANTIFICATION OF ACTIVE ORGANIZATION AND DIFFUSION WITHIN THE ENDOPLASMIC RETICULUM (ER). **Briones L**[1], Ramírez O[1], Scheer J[1], Moraga H[1], Jara J[13], Osorio-Reich M[1], Asahi T[4], Ortega J[4], Couve A[2], and Härtel S[1]. [1]SCIAN-Lab, [2]Couve-Lab, BNI, Faculty of Medicine, [3]DCC, [4]CMM, FCFM, U. of Chile. lbriones@med.uchile.cl

Introduction: ER imaging with spinning disk microscopy (SDM) reveals a constant reorganization of the network. Fusions and fissions are observed concomitant to migrating fluorescent patches along tubular paths. In addition, diffusion processes are suspected to play an important role for protein trafficking. No method exists which discriminates between active and passive transport processes in SDM data. We applied optical flow (OF) algorithms for motion estimation of fluorescent point signals and implement image processing techniques to calculate diffusion coefficients through FRAP experiments taking into account the morpho-topological structure of the ER.

Material and Methods: Different OF methods were implemented and calibrated. Diffusion coefficients were determined through (i) estimation of the bleaching function, (ii) segmentation of the ER network, (iii) determination of path-length within the ER towards the border of the bleaching frontier, and (iv) solving the diffusion equation for each pixel within the ER.

Results: OF determines the movement of point signals up to a distance of 160 nm with errors below 3%. In addition, our FRAP approach leads to a more robust calculation of diffusion coefficients when compared to previously published methods.

Discussion: Our approach enables us to identify and determine active transport processes within the ER network. At the same time, we can estimate the diffusion coupled transport. This balance enables us to differentiate between both processes and leads to a better understanding of transport processes within the ER.

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(96) GLUCOSE INCREASES INTRACELLULAR FREE Ca^{2+} IN TANYCYTES VIA ATP RELEASED THROUGH CONNEXIN 43 HEMICHANNELS. **Juan A. Orellana[1]**, Pablo J. Sáez[1], Christian Cortés-Campos[2], Roberto J. Elizondo[2], Kenji F. Shoji[1], Susana Contreras-Duarte[1] Vania Figueroa[1], Victoria Velarde[1], Jean X. Jiang[3], Francisco Nualart[2], Juan C. Sáez[1] and María A. García[2]. [1]Departamento de Fisiología, Pontificia Universidad Católica de Chile, Santiago, Chile; [2]Departamento de Biología Celular, Universidad de Concepción, Concepción, Chile; [3]Department of Biochemistry, University of Texas Health Science Center, San Antonio, TX, USA.

Introduction: The ventromedial hypothalamus is involved in regulating feeding and satiety behavior, and its neurons interact with specialized ependymal-glial cells, termed tanycytes. The latter express glucose-sensing proteins, including glucose transporter 2, glucokinase and ATP-sensitive K^+ (K_{ATP}) channels, suggesting their involvement in hypothalamic glucosensing. Here, the transduction mechanism involved in the glucose-induced rise of intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) in cultured β-tanycytes was examined.

Material and Methods: Primary cultures of rat tanycytes were exposed to acute concentration of glucose (10 mM) and then intracellular Ca^{2+} signal and functional state of hemichannels were measures by Fura-2AM time-lapse fluorescence images and ethidium uptake, respectively.

Results: Fura-2AM time-lapse fluorescence images revealed that glucose increases the intracellular Ca^{2+} signal in a concentration-dependent manner. Glucose transportation, primarily via glucose transporters, and metabolism via anaerobic glycolysis increased connexin 43 (Cx43) hemichannel activity through a K_{ATP} channel-dependent pathway. Consequently,

ATP export to the extracellular milieu was enhanced, resulting in activation of purinergic $P2Y_1$ receptors followed by inositol trisphosphate receptor activation and Ca^{2+} release from intracellular stores.
Discussion: The present study identifies the mechanism by which glucose increases $[Ca^{2+}]_i$ in tanycytes. It also establishes that Cx43 hemichannels can be rapidly activated by the sequential activation of glucosensing proteins in normal tanycytes.

(97) AT EARLY STAGE OF DIABETIC NEPHROPATHY (DN), RAF KINASE INHIBITOR PROTEIN (RKIP) DOWNREGULATION PRODUCE AN UNUSUAL THICKNESS OF GLOMERULAR BASEMENT MEMBRANE (GBM). **Fabian Pardo**, Romina Bertinat, Juan Carlos Slebe and Alejandro Yáñez. Instituto de Bioquímica y Microbiología, Universidad Austral de Chile. fabian.pardo@gmail.com
Introduction: Diabetes is a group of metabolic disorder of multiple etiologies characterized by chronic hyperglycaemia. In diabetic patients, one of the most devastating effects of this disease is the diabetic nephropathy (DN). In DN has been determinate a peptide (RKIP) that is overexpressed. Moreover, RKIP participate in several signalling pathways, but its role in DN is not understood. The aim of this study was to determinate the expression of RKIP during DN and its consequences in a KO model mouse.
Materials and Methods: Generation of ND in adult transgenic mouse that has null expression of RKIP (KO) treated 8-weeks with high fat diet. Renal function, morphologic and ultraestructural analisys were performed.
Results: No difference in renal function and morphologic characteristics in basal group both WT and KO were seen. At 8-weeks of treatment there is not overexpression of RKIP in WT mouse, however it begin to appear a minor increase in microalbuminury and an alteration in glomerular filtration rate (GFR). Ultraestructural analysis showed renal damage (GBM thickness, podocyte foot process effacement) characteristics of an early state in DN. In KO treated, the GBM presented irregular and unusual thickness without increment in glomerular cells number, compare with WT.
Discussion: Rkip is not overexpressed at early stage in DN, however seem to be involved in the response to control the thickness of GBM. Further investigation, in glomeruli, is necessary to determinate the role in DN.
(FONDECyT 1090694).

(98) BMP-2 ON MESENCHYMAL STEM CELLS (MSCS) ADIPOGENIC DIFFERENTIATION. **Oscar Donoso**, Nelson Osses*, Ana María Pino, Mireya Fernández, Juan Pablo Rodríguez. Laboratorio de Biología Celular y Molecular, INTA, Universidad de Chile. *Facultad de Ciencias. P. Universidad Católica de Valparaíso. oscardonosomora@gmail.com
Introduction. MSCs are pluripotent cells able to differentiate along different pathways, including the osteogenic and adipogenic lineages. MSCs from osteoporotic donors characterize by decreased cell commitment into osteoblast, going together with increased commitment towards adipogenesis. BMP-2 modulates the osteoblast/adipocyte balance by binding to different type 1 BMP receptors. In experimental models, BMPR1A is related to enhanced adipogenesis; while BMP-2 binding to BMPR1B promotes osteogenesis. The aim of this study was to analyze BMP-2 action through its BMPR1s in MSCs along adipogenic differentiation.
Materials and Methods. Cells were isolated from bone marrow obtained from women donors. We analyzed the level of both BMPR1, RUNX2, PPARγ and p-PPARγ by RT-PCR and Western Blot, in MSCs in basal (BM) and adipogenic medium (AD), in the presence or absence of BMP-2 (100 ng/ml) for 2 days.
Results. RT-PCR and western blot analysis showed the expression of BMPRIA by MSCs, while BMPR1B expression was almost undetected. In AD, the expression level of mRNA and protein decreased in 80, 40 and 70 %, for BMPR1A, the pPPARγ/PPARγ ratio and RUNX2, respectively, compared to cells in BM. On the other hand, MSCs in AD in the presence of BMP-2 increased the expression of BMPR1A (five fold), pPPARγ/PPARγ by 46 %, and RUNX2 (2-3 fold), compared with BM.
Discussion. BMP-2 does not enhance adipogenesis in human MSCs. Unlike observations in mice and cell line models, the increased level of BMPRIA could be involved in the BMP-2 mediated enhanced expression of RUNX2.
Fondecyt N°1090093.

(99) COTRANSIN INHIBITS p58 AND ERdj3 TRANSLOCATION AND DOWNREGULATES THE UNFOLDED PROTEIN RESPONSE. **Ureta, G.[1,2],** Amoroso, A.[3], McCullahg, E.[1], Taunton,J.[4], Snapp,E.[5], Bernales,S.[1]. 1.Fundación Ciencia para la Vida. 2.Universidad Andrés Bello. 3.Universidad San Sebastián. 4.University of California. 5.Albert Einstein College of Medicine. g.ureta.diaz@gmail.com
Introduction: When secreted and membrane proteins enter the endoplasmic reticulum (ER) are rapidly recognized by ER quality control (QC) proteins that perform their correct folding and assembly. Chronic perturbation in the ER folding capacity leads to the activation of the unfolded protein response (UPR) that can triggers cell death in several ER stress-associated human diseases. A selective translocon inhibitor small molecule, termed "cotransin", has been proposed to modulate the UPR by inhibiting the translocation of some ER proteins. Understanding the mechanism by which cotransin modulates the UPR could help the developing of a new class of drugs to cope with ER stress-associated human diseases.
Material and methods: HEK293 cells were treated with cotransin, tunicamycin and/or thapsigargin for different times. UPR-associated, QC-related, and secreted protein levels were evaluated by Western blot. Newly synthesized proteins were

pulse-labeled with [^{35}S]met after treatments. BiP mobility assay was performed using BiP-GFP construct and measured via FRAP. Cell viability was measured by FACS.
Results: Cotransin blocks the activation of UPR induced by tunicamycin and blocks the expression of p58 and ERdj3 by preventing its translocation. It also stimulates a potential increase in protein secretion and prevents the tunicamycin-induced decrease in BiP mobility.
Discussion: Here we shown that cotransin can modulate the UPR activation by inhibiting the translocation of the BiP regulators p58 and ERdj3, mechanism that could promote cell viability under stress conditions. Some evidences suggest that this could be achieved by increasing protein secretion.

(100) ROLE OF CHOLESTEROL AND MLN64 IN MITOCHONDRIAL DYSFUNCTION AND OXIDATIVE STRESS IN NIEMANN-PICK TYPE C DISEASE MODELS. **Elisa Balboa**, Nuria Matías, Carlos Fernandez-Checa, Silvana Zanlungo. Departamento de Gastroenterología, Facultad de Medicina, Pontificia Universidad Católica de Chile. ebalboa@gmail.com
Introduction: The biochemical hallmarks of Niemann-Pick Type C (NPC) disease are the abnormal accumulation of unesterified cholesterol in lysosomes, the presence of oxidative stress and cell death. The mechanism that produces oxidative stress is not yet determined.
We propose that in NPC cells there is an increase of mitochondrial cholesterol mediated by the cholesterol-carrying protein MLN64 and consequently mitochondrial dysfunction and decreased input of reduced glutathione (GSH), an important cellular antioxidant, into the mitochondria. Our aim was to evaluate if there are alterations in the mitochondrial function associated with increased transport of cholesterol into mitochondria in NPC cells.
Material and Methods: In cellular and mice NPC models and mice overexpressing MLN64 we evaluated: MLN64 expression, O_2 consumption, oxidative stress markers, mitochondrial membrane potential, and mitochondrial cholesterol and GSH contents.
Results: In cells and mice hepatic tissue from NPC models we found a higher content of cholesterol in the mitochondrial fraction, mitochondrial dysfunction and increased expression of MLN64. Congruently, in livers of animals overexpressing MLN64 we found a decrease in mitochondrial GSH levels related to increased cholesterol in the mitochondrial fraction.
Discussion: increased cholesterol transport to mitochondria mediated by MLN64 could be one of the pathogenic mechanisms causing mitochondrial dysfunction and increased oxidative stress in Niemann Pick Type C disease.
FONDECYT: 1110310.

(101) MOLECULAR CHARACTERIZATION AND DEVELOPMENTAL EXPRESSION OF CG6234 PROTEIN FROM *Drosophila melanogaster*. **Carlos Chacón,** Christian Hodar and Verónica Cambiazo. Laboratorio Bioinformatica y Expresión Génica, INTA-Universidad de Chile and Center for Genome Regulation (CRG). chacon.biotec@gmail.com
Introduction. CG6234 encodes a predicted transmembrane protein that is presumably involved in the development of the amnioserosa (AS). Only the mRNA of the gene has been characterized, and the cellular localization of the encoded protein is unknown. This gene is regulated by the Dpp signaling pathway and gain-of-function CG6234 mutants display developmental defects due to a failure of proper AS morphogenesis.
Materials y Methods. Production of recombinant CG6234 protein and CG6234 antiserum. Indirect immunofluorescence assays in wild type embryos and CG6234 gain-of-function mutants using antibodies against CG6234 and cell surface markers. Subcellular fractionation of S2R+ cells stably transfected with CG6234 and Western blot analysis using the anti-CG6234 antibody.
Results. During early stages of embryogenesis, CG6234 protein was detected in the apical regions of epithelial cells at the presumptive AS territory. During germ-band extension and retraction CG6234 was mainly localized in the AS squamous epithelium and traqueal primordium. At the beginning of dorsal closure CG6234 was restricted to the leading edge cells. Western blot analysis of cell fractions revealed that CG6234 is associated to particulate components of cells, including cellular membranes. In current experiments, we are analyzing the distribution of CG6234 to specific membrane domains and its co-distribution with known cell surface markers.
Discussion. CG6234 protein is an early marker of AS development putatively involved in AS differentiation.
Fondecyt-1090211.

(102) ROLE OF THE NEUROTROPHIN BRAIN DERIVED NEUROTROPHIC FACTOR (BDNF) IN LATERAL LINE NERVE REGENERATION IN ZEBRAFISH LARVAE. **Moya J.**, Villegas R., Sánchez M., Peña O. and Allende M. FONDAP Center for Genome Regulation, Facultad de Ciencias, Universidad de Chile. allende@uchile.cl
Introduction. Brain-Derived Neurotrophic Factor (BDNF) has been involved in morphology maintenance, regeneration and trophic support of neurons. This secreted growth factor has also been implicated in recovery of axons after injury. In this work, we analyzed the participation of this neurotrophin in zebrafish lateral line nerve regeneration after axotomy.
Materials and Methods. We used transgenic fish of the *neuroD::GFP* line, in which the lateral line nerve is labeled with GFP. For nerve axotomy we applied current pulses at 70 μA/s with a tungsten microelectrode in the lateral line nerve in of

72 hours post fertilization larvae. The study and characterization of the role of BDNF in nerve regeneration was done by *in situ* hybridization, quantitative PCR analysis, and loss of function methods using morpholino oligonucleotides.
Results. We determined that there are 3 phases of nerve regeneration after axotomy: there is a lag phase, a high-speed growth phase and a stationary growth phase. In BDNF loss of function larvae, the rate of regeneration is significantly slower compared to control conditions between 7 y 10 hours post-injury. Also, preliminary analysis by RT-PCR showed an increase in BDNF expression after axotomy, suggesting that the gene is upregulated by injury.
Conclusions. We have established a new axotomy method for the study of axonal guidance and regeneration in the zebrafish lateral line nerve. Also, we show a role for BDNF in this process.
Funding: FONDAP 15090007, FONDECYT 1110275, CONICYT.

(103) SPINAL CORD INJURY AND REGENERATION IN *Xenopus laevis*. **Dasfne Lee-Liu[1,3],** Mauricio Moreno[1], Leonardo Almonacid[2], Ricardo Tampe[1], Marcia Gaete[1], Francisco Melo[2], Juan Larrain[1]. [1]Center for Aging and Regeneration and Millenium Nucleus in Regenerative Biology, Pontificia Universidad Catolica, Santiago, Chile; [2]Molecular Bioinformatics Laboratory, Millennium Institute on Immunology and Immunotherapy, Santiago, Chile; [3]Faculty of Chemical and Pharmaceutical Sciences, Universidad de Chile, Santiago, Chile. dasfne@gmail.com
Introduction. Spinal cord injury (SCI) results in motor and sensory loss- usually irreversible, as mammals lose their limited regenerative faculty shortly after birth. *Xenopus laevis* presents an interesting model for studying SCI, because its regenerative capacity is also limited to an, albeit longer, defined period. Regeneration occurs during larval stages before metamorphosis, but not after its conclusion. Our hypothesis is: SCI induces a regenerative-permissive transcriptome in the *X. laevis* spinal cord, which is no longer induced after metamorphosis has concluded.
Material and Methods. We evaluated transcript (RT-PCR) and protein levels (immunoblot) of neural stem cell and progenitor markers (*e.g. sox2*, *nestin*), present in *X. laevis* spinal cord through metamorphosis and/or after SCI, in regenerative and non-regenerative stages. Furthermore, we performed an RNA-Seq study of the spinal cord transcriptome in regenerative and non-regenerative stages.
Results. We found Sox2, Nestin and GFAP protein levels significantly reduced after metamorphosis, when compared to pre-metamorphic stages. Instead, while *nestin* mRNA levels were modestly upregulated after SCI in pre-metamorphic stages, we observed it more markedly in post-metamorphic stages. Furthermore, our RNA-Seq study shows several transcripts differentially expressed after SCI.
Discussion. The differential transcript and protein levels may account for the regenerative abilities of pre-metamorphic stages, and the lack thereof after metamorphosis. These will help us find a way to enhance mammalian regenerative capacity.
Funding: Center for Aging and Regeneration (CARE) and Millenium Nucleus in Regenerative Biology (MINREB) (P07/011-F).

(104) TRANSIENT INACTIVATION OF MYOSTATIN IN DIFFERENT DEVELOPMENTAL STAGES REGULATES DIFFERENTIALLY MYOGENESYS IN ZEBRAFISH. **Navarro C.,** Valdés J.A. and Molina A. amolina@unab.cl. Universidad Andrés Bello. Chile.
Introduction. Myostatin negatively regulates the formation and growth of muscle tissue in vertebrates. In contrast, the myogenesis is regulated positively by several genes, such as *myf5*, *myod*, *myogenin*, *pax7*, among others. The aim of this work was to study how the significant increase in the size of muscle fibers generated as a consequence of transient inactivation of myostatin in different developmental stages of zebrafish, affect the regulation of the expression of genes involved in myogenesis.
Materials and Methods. The transient inactivation of myostatin was performed in several developmental stages of *Danio rerio*. Embryos were treated with a myostatin dominant negative in transient expression experiments. Larvae and juveniles were treated with immersion baths with the dominant negative recombinant protein. Subsequently was analyzed by RT-PCR its effect on the expression of transcripts that regulate myogenesis. In addition, immunoassays were performed to analyze MyoD myogenic factor and histological analysis were performed for fiber morphometric analysis.
Results. The embryos showed an increase in the expression of *pax 7*, and earlier expression of *myod*; this result is corroborated in the content of this protein at 24 hpf. The juvenile fish treated showed a significant increase in the expression of *myogenin* and MyoD content. In addition, treatment with recombinant protein caused a significant increase in both weight and height, compared with treated control fish.
Discussion. Transient inactivation of myostatin in zebrafish embryos induces a differential regulation of genes involved in the formation of muscle tissue by promoting hypertrophy.
FONDECYT 1090416.

(105) GENETIC ANALYSIS OF ECLOSION HORMONE FUNCTION IN *Drosophila* ECDYSIS BEHAVIOR. **Eileen Kruger**, Wilson Mena & John Ewer. Centro Interdisciplinario de Neurociencia de Valparaíso. Universidad de Valparaíso. eileen.kruger@cinv.cl

Introduction: In insects, the molting process ends with the elimination of the old cuticle using a stereotyped behavior called ecdysis. Ecdysis behavior is controlled by several interacting neuropeptides, and is triggered by Ecdysis Triggering hormone (ETH) and Eclosion Hormone (EH) via an endocrine positive feedback loop that results in the complete release of ETH and EH. These two hormones then activate downstream targets that drive pre-ecdysis and ecdysis behavior. We isolated a null EH mutant to understand the exact role of this neuropeptide in the control of ecdysis.
Material and Methods: We characterized the behavioral and endocrine defects of EH null mutants as well as of mutants injected with synthetic EH and ETH. We also ectopically expressed EH and ETH and evaluated ecdysis in these individuals. Finally, we analyzed the activation of downstream targets using immuno-histochemistry and by visualizing neural activation using calcium sensitive GFP in transgenic *Drosophila*.
Results: Most *eh* mutant animals die at the larval ecdyses, show no pre-ecdysis behavior and express an extremely protracted ecdysis. Although *eh* mutants show no significant release of ETH at ecdysis, the behavioral defects are not due to the lack of ETH release, as they cannot be rescued by injection of ETH. EH injections as well as ectopic expression of EH in ETH or CCAP cells rescues the mutant phenotype.
Discussion: EH appears to have an important role in activating pre-ecdysis and ecdysis behaviors. None of these functions are subserved by ETH, suggesting that ETH acts mostly as a trigger to cause EH release.

(106) TARGET GENES OF DPP/BMP SIGNALING PATHWAY REVEALED BY TRANSCRIPTOME PROFILING IN THE EARLY *D. melanogaster* EMBRYO. **Calixto Domínguez,** Alejandro Zúñiga, Carlos Chacón, Michael Pino y Verónica Cambiazo. Laboratorio Bioinformatica y Expresión Génica, INTA-Universidad de Chile and Center for Genome Regulation (CRG). cdportilla@yahoo.com
Introduction. *Drosophila* dorsal patterning is controlled by an extracellular gradient of the morphogen Dpp. Dpp signaling is transduced to the nucleus by the transcription factors, Mad/Medea, which elicit at least three threshold responses: high, intermediate and low. However, the mechanism underlying differential response to Dpp is poorly understood, due in part to the insufficient number of well-studied target genes. Here, we combined genetic transcriptome perturbations and *in silico*/*in vivo* prediction of TFBSs to identify a set of potential new target genes of Dpp pathway.
Materials y Methods. *Drosophila* microarrays were screened with RNAs extracted from wild type and homozygous dpp^{hr92} embryos. The *Drosophila* genome was scanned to identify relevant TFBSs using HMMER. *In situ* hybridizations of wild type and Dpp signaling pathway mutants. Cloning of predicted gene regulatory regions in reporter vector and generation of transgenic lines of flies.
Results. Genes down-regulated in dpp^{hr92} embryos included known dorsal patterning genes and several novel genes. Bioinformatics methods allowed us to predict 20 direct Mad target genes. For some of these genes, *in situ* hybridizations assays in wild type and mutant backgrounds suggested that they are under the control of Dpp pathway. Putative regulatory regions of selected genes are being analyzed by *in situ* hybridization in transgenic reporter lines.
Discussion. We identified a set of potential new target genes of Dpp/Mad pathway. The analysis of TFBSs features will be used to decipher the underlying *cis*-code controlling dorsal ectoderm patterning.
Fondecyt-1090211.

(107) MAGUK PROTEINS PARTICIPATES IN *Drosophila* OLFACTORY LEARNING. **Claudia Molina** and Jimena Sierralta. ICBM, Faculty of Medicine, Universidad de Chile and BNI. cmolina@med.uchile.cl
Introduction: The synaptic membrane associated guanylate kinase (MAGUK) protein family is thought to play a key role in synaptic plasticity. However the *in vivo* role of this family of proteins is still unclear. Previously we have shown that *Drosophila* mutants lacking DlgS97 protein (homologue to mammals SAP97 protein) display no alterations in locomotion or olfaction, but show defects in complex behaviors without obvious developmental defects. Evidence in larval neuromuscular junction (NMJ), demonstrate that DlgS97 is part of a protein complex, with Metro, another MAGUK protein. The aim of this study was to assess the *in vivo* role of DlgS97 and Metro in the larval locomotion, and in the olfactory associative learning, which involves neuromuscular and central synapses respectively.
Methods: locomotion tests were performed in second instar larvae of *dlgS97* and *metro* null mutants at control (21°C) and high (29°C) environmental temperature. The olfactory learning tests were performed in adult flies, matching an odorant (3-OCT or 4-MCH) with electric-shock and assessing the immediate ability of the flies to avoid the electroshock-mated odorant.
Results: *dlgS97* mutant larvae display normal locomotion at 21°C but defective at 29°C, instead *metro* mutant show defective locomotor activity only at 21°C. In the learning paradigm, both mutant strains show significantly decreased learning index compared with the control strain.
Discussion: Our results indicate that both DlgS97 and Metro proteins display synaptic defects observed as an altered locomotion in larvae mutants, as well as defects in synaptic plasticity observed as an impaired learning ability in mutant adult flies.
Financed by FONDECYT 1090272 and ICM P09-015-F.

(108) Daam1 CONTROLS MORPHOGENESIS OF THE HABENULO-INTERPEDUNCULAR CIRCUIT IN ZEBRAFISH. Alicia Colombo, **Álvaro Díaz-Briceño**, Lorena Armijo, and Miguel Concha. Anatomy and Developmental Biology Program, Institute of Biomedical Sciences, University of Chile, Santiago, Chile. acolombo@med.uchile.cl; mconcha@med.uchile.cl

Introduction: Proteins that regulate cytoskeletal dynamics play a critical role in controlling the growth and directionality of axons and dendrites. Daam1, a formin homology protein, participates in this process through modulation of actin remodelling. In zebrafish, Daam1 is expressed asymmetrically primarily on the left side of the diencephalic habenular complex, and its expression matches in time and space with morphogenesis of the habenular-interpenducular circuit. Here we explored the role of Daam1 during this process.

Material and Methods: We used transgenic zebrafish that label the habenular-interpenducular circuit with GFP, and locally electroporated the habenular region with either morpholino antisense oligonucleotides against Daam1 or pCDNA-HA-Cdaam1, to achieve local loss or gain of gene function, respectively. For rescue experiments, embryos were injected with Daam1 morpholino followed by local electroporation of pCDNA-HA-daam1. Phenotypes were analysed through spinning disc microscopy of samples inmunostained against GFP and acetylated alfa-tubulin.

Results: Local loss of Daam1 function led to decreased left-sided habenular neuropil, inducing a bilaterally symmetric habenular complex. The same manipulation also induced a thinning and disorganisation of the habenulo-interpeduncular tract. In contrast, local gain of Daam1 function led to an increase of left-sided habenular neuropil.

Discussion: The results suggest that Daam1 plays is involved in development of the habenulo-interpeduncular circuit in zebrafish, specifically controlling axonal and dendritic morphogenesis of habenular neurons.

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(109) ODOR-MEDIATED REGULATION OF OLFACTORY RECEPTOR EXPRESSION IN ZEBRAFISH. **Cristian Calfún[1,2],** Maegan Rivard[3], Juanita Astudillo[1,2], & Kathleen Whitlock[1,2]. 1.CINV, Universidad de Valparaiso, Chile. 2.Centro de Genómica de la Célula, Núcleo Milenio. Universidad de Valparaíso. 3.MBG, Cornell University USA. cristian.calfun@gmail.com

Introduction. In order to test our hypothesis that the odor environment can alter olfactory receptor (OR) expression we have examined OR expression in animals exposed to specific odors during early development. Previously we have shown that zebrafish make an olfactory memory of the artificial odorant phenyl ethyl alcohol (PEA). This memory is correlated with an increase in *otx2* expression in the olfactory epithelium (OE). After identifying ORs containing Otx2 binding sites we examined the effects of PEA on OR expression.

Materials and Methods. We performed an *in silico* analysis to identify Otx2 binding-sites upstream of zebrafish ORs. We exposed zebrafish embryos to two isoforms of PEA and analyzed the quantitative expression of eight ORs using qRT-PCR and expression of *otx2* in the OE using in situ hybridization.

Results. We show that αPEA does not affect the quantitative expression of the eight ORs nor the *otx2* expression in the OE. In contrast, exposure to βPEA resulted in down regulation of five ORs, and also increased *otx2* expression in the OE.

Discussion. We have shown a correlation between the down-regulation expression of five ORs and theincreased expression of *otx2* in βPEA exposed embryos. These data are in agreement with our model where the environment can modulate gene expression during early development and suggest a role for the ORs in the memory generation.

(110) HIF-1α IS REQUIRED FOR THE NORMAL FORMATION AND ARBORIZATION OF THE TRIGEMINAL GANGLION. **Santander L**. and Reyes A.E. Laboratorio Biología del Desarrollo, Facultad de Ciencias Biológicas, Universidad Andrés Bello, Santiago, Chile. arielreyes@unab.cl

Introduction: Hypoxia-induced factor-1 (HIF-1) is a key regulator for the induction of genes in response to low oxygen tension. In zebrafish Hif-1α transcript have maternal expression and later during embryonic development is expressed in the nervous system. The aim of this work is study the role of Hif-1α on the trigeminal ganglion (TG) during the zebrafish development.

Materials and Methods: To study the role of Hif-1α on the TG development and arborization, we performed experiments of loss and gain of function of Hif-1α on zebrafish embryos, by injecting antisense oligonucleotides (morpholinos) (MOATG-*hif*-1α) and a dominant-active form of Hif-1α, respectively. The phenotypes were analyzed by *in situ* hybridization to *islet1* and *islet2* (expressed in post-mitotic neurons), *ngn1* (expressed in sensory neurons), also we used double immunofluorescence against both, Phospho-HistoneH3, to detect cell proliferation, and GFP in transgenic embryos *Tg*(*sensory:eGFP*), that express eGFP in post-mitotic neurons .

Results: The loss of function of Hif-1α decreased the cell number and the arborization of TG, on the other hand, the over expression of Hif-1α increases the arborization and cell number of the trigeminal ganglion neurons.

Discussion: Our results suggest an undescribed role for Hif-1α on TG formation by regulate the cell number and arborization of the trigeminal neurons, fulfilling a key role in the neurogenesis.

FONDECYT 1095128 to A.R.

(111) CTIP1/Bcl11a PARTICIPATES IN THE DETERMINATION OF NEURONAL IDENTITY IN THE DEVELOPING NEOCORTEX. Cánovas J., Berndt F.A., Oliva C., Sierralta J., **Kukuljan M**. Programa de Fisiología y Biofísica, ICBM, e Instituto Milenio de Neurociencias Biomédicas, Facultad de Medicina, Universidad de Chile. kukuljan@med.uchile.cl

Introduction: the mammalian neocortex is organized into six layers; projection neurons in each layer display similar morphology, connectivity and gene expression profiles, although diversity within a layer is being recognized. We aim to understand how these common and divergent features are established, starting with the identification of transcriptional regulators of neuronal fate. We used *Drosophila* as a model to identify genes involved in neuronal differentiation and found CTIP1/Bcl11a, which is expressed in the mouse cerebral cortex. We set to explore its role in mammalian corticogenesis.

Methods: we characterized the expression of CTIP/Bcl11a by immunofluorescence, conducted shRNA-mediated loss of its function by in utero electroporation of mouse embryos at E13.5-E14.5 and analyzed neuronal fate at different developmental stages.

Results: CTIP1/Bcl11a is expressed in the developing cortex, particularly in layers 2, 3 and 5. Loss of CTIP1/Bcl11a function associates to loss of layer identity and ectopic expression of layer specific markers. Further, loss of CTIP1/Bcl11a leads to increased development of axonal branches by newborn neurons before reaching the cortical plate (E17.5) and to marked defects in axon navigation evidenced at later stages (P3).

Discussion: CTIP1/Bcl11a function is required for the establishment layer-specific properties in the neocortex as inferred by its restricted expression pattern and results of loss of function experiments. Besides this, and based in results from our and others laboratories, CTIP1/Bcl11a may act as a more general determinant of neuronal morphogenesis.

FONDECYT 1090281; ICM P09-015-F; CONICYT Fellowship (JC).

(112) THE ROLE OF THE UNFOLDED PROTEIN RESPONSE IN AXONAL REGENERATION AFTER SCIATIC NERVE INJURY. **Oñate M.**[1,2,3], Court F.A.[3,4] and Hetz C.[1,2,4]. [1]Biomedical Neuroscience Institute and [2]Center for Molecular Studies of the Cell, Institute of Biomedical Sciences, University of Chile. [3]Millennium Nucleus in Regenerative Biology (MINREB), Catholic University of Chile. [4]Neurounion Biomedical Foundation. chetz@med.uchile.cl, fcourt@bio.puc.cl

Introduction. Injury to peripheral nerves triggers a mechanism termed Wallerian degeneration at the distal portion, and a regenerative process in the proximal region. It has been proposed that the endoplasmic reticulum (ER) may be one of the main organelles that sense this stress, leading to the activation of the unfolded protein response (UPR) and autophagy in injured neurons. Here, we characterized the UPR and autophagy components after sciatic nerve injury in a mouse model.

Material and Methods. C57BL/6 wild-type and Beclin1+/- mice were crush injured in the sciatic nerve and used at different time intervals for analysis of UPR markers. Locomotor recovery of the injured hindlimb was evaluated using the sciatic functional index (SFI).

Results. Sciatic nerve crush increased levels of UPR markers, including the expression of the chaperon BiP and ERp57 in the injury and distal region 8-days after surgery, which return to basal level from day 21th in wild-type mice. Massive accumulation of LC3(+) vacuoles were observed in Schwann cells associated to degenerating axons.

Discussion. Sciatic nerve injury leads to the activation of UPR components and accumulation of LC3(+) vacuoles during the degeneration/regeneration process. These events could be involved in the process of myelin removal after nerve injury, suggesting a role of UPR-mediated autophagy in axonal regeneration.

FONDECYT-1100176, FONDAP-15010006, Millennium Institute P09-015-F, and ICGEB, Alzheimer's Association (C.H); FONDECYT-1110987 and Millennium Nucleus P-07-011-F (F.C).

(113) BMP SIGNALLING MODULATES PROLIFERATIVE POTENTIAL OF ADULT SPINAL CORD DERIVED NEURAL STEM CELLS. **Emilio Méndez**, Alejandro Erices. Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile. eemendez@uc.cl

Introduction. Spinal cord (SC) neurogenesis is restricted to specific neurogenic niches for neural stem cells (NSC) during adulthood. However, the role of specific signals involved in SC-NSC homeostasis has not been elucidated. Considering BMP roles on encephalic NSC proliferation and fate determination, our aim is to analyze de putative role of BMP signalling on the SC-derived NSC function.

Materials and Methods. Thoracic SC cells were dissociated from fetal and adult c57bl6 mice, grown as neurospheres and characterized by NSC gene expression and trilineage differentiation potential. BMP family ligands (BMP-2, -4, -6, -7) and receptors (BMPR-IA, -IB, -II) were analyzed by RT-PCR, and their role on SC-NSC proliferation and differentiation was evaluated in presence of BMP-pathway inhibitor DMH1 by 5BrdU incorporation and specific cell lineage markers inmunofluorescence respectively.

Results. Isolated adult SC-derived neurospheres were validated as NSC based on proliferative capacity (self-renewing), gene expression (nestin and sox2), and the differentiation to neural (βIII-tubulin, BMP2), glial (GFAP) and oligodendroglial (Olig2, MAG) lineages. All BMP analyzed ligands, and also all different BMP receptors, were expressed in neurospheres, suggesting their involvement in NSC homeostatic regulation. Functional assays demonstrated that a general BMP signalling inhibition, as confirmed by p-smad-1/5/8 translocation, increased NSC proliferation favoring glial differentiation.

Discussion. Our results suggest an autocrine/paracrine role for BMP signalling on the adult SC-NSC proliferation/differentiation balance. Discerning specific BMPs contribution to this process could provide useful information in order to modulate SC neurogenesis.
Funding Fondecyt 1090427, Millennium Nucleus in Regenerative Biology MINREB.

(114) META-ANALYSIS OF GENOME-WIDE ASSOCIATION STUDIES (GWAS) IN ALZHEIMER'S DISEASE. **Bustos B**, Pérez E and De Ferrari GV. Center for Biomedical Research. Faculty of Biological Sciences and Faculty of Medicine, Universidad Andres Bello, Chile. gdeferrari@unab.cl
Introduction: GWAS have successfully identified several susceptibility loci for Alzheimer's disease (AD), including the apolipoprotein E (APOE) locus as the major risk factor for the disease. Here, to improve the statistical power to detect risk loci for AD we combined data from publicly available GWAS.
Materials and Methods: We used the inverse variance method implemented in PLINK and R to perform a meta-analysis on four GWAS in AD (5,101 cases and 4,516 controls). We imputed 2.3 millions of single nucleotide polymorphisms (SNPs) from the HapMap CEU population to maximize information on linkage disequilibrium structure between the studies. We finally used ONTOLOGIZER to detect biological processes, molecular functions or cellular components, which might be overrepresented in AD.
Results: We observed a strong genetic association in the PVRL2 gene (rs2075650, $P=1.94x10^{-86}$), surrounding the APOE locus. We likewise identified a novel genome-wide association signal on the MECOM locus (MECOM: MDS1 and EVI1 complex locus), which is located in chromosome 3q26 (rs9809961, $P=4.17x10^{-7}$), and detected marginally significant associations on PRKCQ (rs943451, $P=5.29x10^{-7}$) and MS4A3 (rs474951, $P=4.89x10^{-6}$). The ontological analysis revealed that genetic variants within synaptic, cytoesqueletal and cellular adhesion categories are overrepresented in AD.
Discussion: Adding support to the previous signal on the APOE locus, we demonstrate that genetic variation within MECOM represents a novel susceptibility locus for AD. Overrepresentation of these variants in three ontological categories related to normal brain function may help us to understand their roles in the etiology of AD.
Funding: FONDECYT 1100942.

(115) *ANDROGRAPHOLIDE* PREVENT THE DECREASE OF SYNAPTIC PROTEINS AND INDUCES LTP IN BRAINS OF A DOUBLE TRANSGENIC MODEL OF ALZHEIMER'S DISEASE, POSSIBLY BY A MECHANISM INVOLVING CANONICAL *WNT* PATHWAY. **Cheril Tapia-Rojas**, Felipe G. Serrano and Nibaldo C. Inestrosa. Centro de Envejecimiento y Regeneración (CARE), Departamento de Biología Celular y Molecular, Pontificia Universidad Católica de Chile. cctapia1@uc.cl
Introduction: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the accumulation of senile plaques, neurofibrillary tangles and a decrease in the levels of synaptic proteins. Here, we carry out a detailed analysis of the effect of Andrographolide (ANDRO), the major component of *Andrographis paniculata,* on the levels of synaptic proteins, synaptic plasticity and Aβ neurotoxicity *in vivo* in the double transgenic APPswe/PSEN1ΔE9 mouse model of AD.
Material and Methods: Twelve-month-old mice were treated with xx ANDRO for 4 weeks via i.p. injection. The brains were used for biochemical and electrophysiological analysis.
Results: APPswe/PSEN1ΔE9 animals show reduced levels of synaptic proteins in the hippocampus and cortex as compared with age-matched wild-type animals. Treatment with ANDRO significantly prevents the decrease of the post-synaptic proteins PSD95, NMDAR (NR2B), AMPAR (GluR2) and Shank. Pre-synaptic proteins were not affected. ANDRO also induced long-term potentiation (LTP) by (TBS). Interestingly, ANDRO modulates the Wnt/β-catenin signaling components increasing β-catenin levels and inducing the transcription of Wnt target genes.
Discussion: We conclude that ANDRO, possibly by a mechanism involving canonical Wnt signaling, prevents the decrease of post-synaptic proteins and increases synaptic plasticity in an *in vivo* model of AD, suggesting a therapeutic potential of this drug.
Supported by BASAL Project (CONICYT-PFB12/2007), Pre-doctoral Fellowship from CONICYT to CTP.

(116) INTERNALIZATION AND TRAFFICKING OF THE p75 NEUROTROPHIN RECEPTOR (p75). EVADING THE LATE ENDOSOMAL ROUTE TOWARDS MULTIVESICULAR BODIES SPECIALIZED FOR EXOSOMAL RELEASE. **Galleguillos C[1]**, Escudero CA[1], Parraguez JI[1], Uzma S[2], Lopez-Verrilli MA[1], Carter BD[2], Court FA[1], Bronfman FC[1]. 1Millennium Nucleus in Regenerative Biology (MINREB), Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile. 2Department of Biochemistry and Center for Molecular Neurosciences, Vanderbilt University Medical School, Tennessee, USA. carolinapgd@gmail.com
Introduction: During development of the sympathetic nervous system, it is well established that NGF/TrkA survival signaling competes with BDNF/p75 death signaling to modulate the number of sympathetic neurons (SCGs) and their target innervation. The functional significance of intracellular trafficking of NGF/TrkA in this process is well established; however, less is known about the functional significance of p75 internalization and trafficking. The aim of our work was to study the mechanism of internalization and the post-endocytic trafficking of p75 in PC12 and SCGs.

Materials and Methods: To study the endocytosis and trafficking of p75 we used immunoendocytosis, confocal, electron and real-time fluorescence microscopy and exosome purification by ultracentrifugation.
Results: p75 internalization is ligand-dependent, dynamin and AP2 clathrin adaptor-dependent. After internalization, p75 pass quickly through early endosomes and evades the late endocytic route accumulating in recycling endosomes positive for Rab11 and transferrin, as well as in multivesicular bodies positive for the exosomal marker CD63 and negative for Rab7 a late endocytic marker. In addition, full-length p75 is secreted in exosomes.
Discussion: Our results suggest that after ligand-dependent internalization, there is an intercellular transfer of p75 signaling complexes that may regulate neurotrophin signaling in SCGs.
Funds, FONDECYT (1085273 a FCB, 1110987 a FC, 3110014 a MALV), MINREB (P07/011-F), FONDAP-Biomedicine 13980001 and CARE PFB 12/2007.

(117) EPIGENETIC MECHANSIMS THAT CONTROL PSD95 GENE EXPRESSION IN DEVELOPING HIPPOCAMPAL NEURONS. Fernando Bustos[1,2], Berta Henríquez[2], Rodrigo Aguilar[2,3], David González[2], **Martín Montecino[2,3], Brigitte van Zundert[2]**. [1]University of Concepción; [2]Center for Biomedical Research, Andres Bello University, Santiago; [3]FONDAP Center for Genome Regulation. fbustosf@gmail.com.
Objectives: Our recent data indicate that increased expression of the NMDA receptor anchoring protein PSD95 is primordial in the restricted dendritogenesis observed in the adult CNS. Here we analyzed during development whether epigenetic mechanisms involving specific covalent histone tail modifications are associated with PSD95 promoter activity in hippocampal tissue. We also studied how inhibition of histone deacetylases (HDACs) affects PSD95 gene expression in hippocampal cultures.
Methods: Chromatin was extracted at distinct development stages from rat hippocampal tissue (E18, P10, P30 and Adult). Chromatin immunoprecipitation (ChIP) was performed using antibodies that recognize HDACs and activation or repression marks in histone tails. ChIPs were quantified by qPCR with primers for the PSD95 promoter. Hippocampal neurons (14 DIV) were treated with HDACs inhibitors TSA and Na-butyrate. After 24 hrs, RNA was extracted and cDNAs for PSD95 and GAPDH were amplified using qRT-PCR.
Results: Histone modifications interacting with the PSD95 promotor associated with activation (H3K4me3 and H3K9Ac) increase with hippocampal development, while repression marks (H3K27me3 and H3K9me3) decrease with maturation. Binding of HDAC1 and HDAC2 to the PSD95 promoter is apparent during early development and disappears with maturation. Exposure of hippocampal neurons to HDAC inhibitors increases PSD95 mRNA levels.
Conclusions: We found a specific pattern of histone tail modifications that are related with PSD95 expression during development of the hippocampus. HDACs1/2 bind to the PSD95 promoter only in early development. PSD95 expression can be manipulated using broad range HDAC inhibitors in hippocampal neurons.
Acknowledgement: Funded by Conicyt 24110099 (F.B.), Fondecyt 1101012 (B.vZ.), FONDAP 15090007 (M.M.).

(118) METABOLIC MODULATION BY ASCORBIC ACID IN NEURONS UNDER GLUTAMATERGIC ACTIVITY DOES NOT RELY ON THE ANTIOXIDANT PROPERTIES OF THIS MOLECULE. **[1]María Paz Miró,** [1]Felipe Beltran, [1]Aníbal I. Acuña, [1]Ilona I. Concha, [2]Michael Levine, [1]Maite A. Castro. [2]Semel Institute, UCLA; [1]Instituto de Bioquímica y Microbiología, UACh. macastro@uach.cl
Introduction: Ascorbic acid is an important cofactor in various enzyme systems and it is a powerful water-soluble antioxidant. We have demonstrated that intracellular ascorbic acid inhibits glucose transport and stimulates lactate uptake in cells expressing facilitative glucose transporter isoform 3 (GLUT3). In brain, ascorbic acid works as metabolic switch inhibiting glucose consumption and stimulating lactate transport in neuronal cells (which express GLUT3) under glutamatergic synaptic acitvity. In this work we analyzed wether ascorbic acid exerts its inhibitory effect by acting as an electron donor.
Materials and Methods: Using radioactive tracers and fluorescent glucose analogs we evaluated the effect of ascorbic acid, an unrelated electron donor (glutathione, GSH), and the non-electron donor phospho-ascorbate on the transport of lactate and glucose analogues (DOG or 2NBDG) in cultured cells. Also, we studied phospho-ascorbate effect by means of electrophysiology assays on acute striatal slices from mouse brain.
Results: We observed a mild and comparable DOG transport inhibition by GSH in cells expressing or not GLUT3. In the opposite way, phospho-ascorbate only inhibited glucose utilization in cells expressing GLUT3. Also, phospho-ascorbate, as we demosntrated for ascorbic acid previously, was able to inhibit glucose consumption in neurons under glutamatergic synaptic acitvity. Discussion: We conclude that metabolic modulation by ascorbic acid in neurons under glutamatergic activity does not rely on the antioxidant properties of this molecule.
FONDECYT 1110571 & 1110508.

(119) ATF-4 DEFICIENCY PROTECTS AGAINST AMYOTROPHIC LATERAL SCLEROSIS ASSOCIATED TO AN ALTERATION OF THE PROTEIN DISULPHIDE ISOMERASE (PDI) FAMILY EXPRESSION PATTERN AND A CHANGE IN CELLULAR REDOX STATE. **Matus S**.[1,2,3], Lopez E.[1,2,3], Hetz C.[1,2,3]. [1]Biomedical Neuroscience

Institute, Faculty of Medicine. [2]Center for Molecular Studies of the Cell, Institute of Biomedical Sciences, University of Chile, Santiago, Chile. [3]Neurounion Biomedical Foundation, Santiago, Chile. mmatus@med.uchile.cl

Introduction: Familial amyotrophic lateral sclerosis (fALS) is a neurodegenerative disease that could be caused by mutations in Superoxide Dismutase-1 (SOD1). Recent evidence implicates adaptive responses to endoplasmic reticulum (ER) stress in the disease process, via a pathway known as the unfolded protein response (UPR). Here we have investigated the contribution of the activating transcription factor 4 (ATF-4), a transcription factor that regulates genes involved in redox homeostasis, amino acid metabolism, apoptosis and autophagy, to mutant SOD1 pathogenesis *in vivo*.

Material and Methods: We generated mutant SOD1 transgenic mice with a deletion of the ATF-4 gene.

Results: The experimental SOD1 transgenic and deficient for ATF-4 mice develops the disease later than the controls with a similar time of disease duration. They also present an alteration in chaperon expression pattern. Cellular experiments also demonstrated an alteration of cellular redox state.

Discussion: Our results reveal a protective critical role of ATF-4 in the develop of the ALS. The lack of ATF-4 factor could have a prosurvival effect in vivo. These results identify ATF-4 as a possible therapeutic target for the treatment of ALS.

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(120) ASSESSMENT OF THE AGGREGATION PROPERTIES OF PICCOLO AND BASSOON POLY-GLUTAMINE (PQ) DOMAINS: TOWARDS A PRION-LIKE MECHANISM FOR PROTEIN RECRUITMENT TO THE SYNAPSE. **Jaime Villalobos**, Yocelin Cruz, Viviana Torres, Pedro Zamorano. Laboratorio de Neurobiología, Facultad de Ciencias de la Salud, Universidad de Antofagasta. zamorano@uantof.cl

Introduction: The molecular mechanism of protein recruitment to the synapse is unknown. The PQ domain of the dendritic protein CPEB shows prion-like behavior and its aggregation is important to its function. The presynaptic proteins Piccolo/Bassoon contain several PQ domains. We hypothesize that these domains show similar aggregation properties and the clustering of these molecules may result in a mechanism of molecular trapping of these proteins to the synapse. Here, we assessed which of the domains of Piccolo/Bassoon aggregates using a heterologous expression system.

Material and Methods: The PQ1,2,3 domains of Piccolo and PQ1,2 of Bassoon were constructed with EGFP/mRFP tags in lentiviral vectors and expressed in HEK293 cells. The intrinsic order of these domains was also assess by FoldIndex©.

Results: Aggregation was observed in the PQ1,3 domains of Piccolo and PQ 1 domain of Bassoon. Nuclear localization was observed for PQ2,3 domains of Piccolo and PQ1,2 domain of Bassoon. The PQ3 domain of Piccolo showed a large aggregation after 48 h. The FoldIndex© that identified intrinsic disordered regions to be present in prion-like proteins predict strongly this type of structure for the PQ 1, 2 of Piccolo/Bassoon but much less for the PQ 3 domain of Piccolo.

Discussion: The PQ domains of Piccolo/Bassoon showed some degree of aggregation suggesting that these domains may interact with each other as homodimers to form these clusters. PQ3 showed a strong aggregation pattern despite a higher intrinsic order predicted by FoldIndex©.

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(121) COPPER REDUCES Aβ OLIGOMERIC SPECIES AND AMELIORATES NEUROMUSCULAR SYNAPTIC DEFECTS IN A *C. elegans* MODEL OF IBM. **Daniela L. Rebolledo[1]**, Rebeca Aldunate[1,2], Alicia N. Minniti[1], Nibaldo C. Inestrosa[1]. [1]CARE, P. Universidad Católica de Chile. [2]Escuela de Biotecnología. Universidad Santo Tomás. dvreboll@uc.cl

Introduction: Alzheimer's disease (AD) and Inclusion Body Myositis (IBM) are disorders frequently found in the elderly. The amyloid-β (Aβ) peptide is the main constituent of both senile plaques (SPs) in AD and intracellular amyloid aggregates in skeletal muscle cells.

Methods: We used transgenic C. *elegans* that express Aβ in muscle cells as a model of IBM. Motility and paralysis behaviors were analyzed. Neuromuscular Junction (NMJ) synaptic transmission was evaluated using agonists of Acetylcholine Receptors (AChRs). Localization of AChRs was analyzed by visualization of GFP-coupled proteins. Histological (ThS and BSB staining) and biochemical (dot and western blot) assays were used to evaluate Aβ species and aggregation.

Results: We tested if the altered motility we observed in Aβ transgenic strains could be the result of a compromised neuromuscular synapse. Synaptic transmission is defective in our model and suggest a specific defect on nicotine sensitive AChRs. Synaptic dysfunction correlates with misslocalization of ACR-16, the AChR subunit essential for nicotine triggered currents in *C. elegans*. Copper treatment increases the amyloid deposits and decreases Aβ oligomers in this model. Furthermore, copper treatment improves motility, ACR-16 localization, synaptic function, and delays Aβ-induced paralysis.

Discussion: Copper modulates Aβ-induced pathology and suggest that Aβ oligomers are triggering neuromuscular dysfunction. Our findings emphasize the importance of neuromuscular synaptic dysfunction and the relevance of modulating the amyloidogenic component as an alternative therapeutic approach for IBM.

Supported by CONICYT through the Basal Center of Excellence (PFB 12/2007).

(122) CLONING AND EXPRESION OF A NOVEL NEURON SURFACE PROTEIN, NSPA, INVOLVED IN PSYCHIATRIC LUPUS. **Bravo-Zehnder M**[1,2], Segovia F.[1,2], Jurado A[2], Zamorano P[3], Massardo L.[1,2], González A.[1,2]. Departamento de Inmunología Clínica y Reumatología, Facultad Medicina[1], Centro de Envejecimiento y Regeneración, Fac. Ciencias Biológicas[2], Pontificia Universidad Católica de Chile. Departamento Biomédico, Universidad de Antofagasta[3]. mbravo@med.puc.cl

Introduction: Autoantibodies against ribosomal P proteins (anti-P) have been clinically associated with lupus psychosis and depression. We recently showed that anti-P autoantibodies induce calcium influx followed by apoptosis in cortical neurons and identified a novel protein exposing a P epitope at the neuronal cell surface, called NSPA (Neuronal Surface P Antigen) (Matus et al. J Exp Med-2007). Here we present a new cellular model that allows a more direct assessment of NSPA as target of anti-P antibodies, as well as an eventual target of other lupus autoantibodies, which might be directed against an epitope distinct from the P epitope shared by ribosomal P proteins.

Methods: We cloned NSPA tagged with GFP at its N-terminus and produced permanently transfected HEK-293 cells. To detect NSPA at the cell surface we used biotinylation assays and surface immunocapture in combination with metabolic labeling and cross-immunoprecipitation. Immunoblot was used to search for anti-NSPA reactivity in the sera of 84 patients with SLE.

Results: GFP-NSPA ectopically expressed in HEK-293 cells, reaches the cell surface exposing a P epitope. Using GFP-NSPA/HEK-293 cells we detected anti-NSPA autoantibodies distinct from anti-P antibodies at a frequency of 4% in patient with SLE.

Discussion: NSPA definitively account for the interaction of anti-P antibodies with the cell surface. In addition to anti-P(+) /anti-NSPA(+) autoantibodies, SLE patients also produce (4%) anti-NSPA(+)/anti-P(-).antibodies. The pathogenic potential of these new autoantibodies remains to be defined.

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(123) A ROLE OF Beclin1 IN THE REGULATION OF AUTOPHAGY IN AMYOTROPHIC LATERAL SCLEROSIS. **Nassif M**. and Hetz C. Biomedical Neuroscience Institute, Center for Molecular Studies of the Cell, Institute of Biomedical Sciences, University of Chile, Neurounion Biomedical Foundation, Santiago, Chile. chetz@med.uhile.cl

Introduction: Mutations in superoxide dismutase-1 (SOD1) that triggers its misfolding and aggregation cause familial amyotrophic lateral sclerosis (fALS), a motor adult-onset neurodegenerative disease characterized by progressive loss of motoneurons. Strategies to clear out abnormal protein aggregates may have therapeutic benefits to treat ALS. Autophagy is the main cellular pathway for degradation of long-lived proteins and organelles activated under nutrient deprivation conditions. We and others had described increased levels of autophagy markers in cellular and animal models of fALS expressing mutants SOD1. However, the direct contribution of autophagy to fALS remains less explored.

Materials and Methods: We have investigated the contribution of Beclin1 to fALS using iRNA and overexpression strategies in NSC34 motoneuron cellular model. For *in vivo* model, we employed the mutant $SOD1^{G86R}$ mice heterozygote to Beclin1.

Results: Our results revealed a critical role of Beclin1-dependent autophagy and its interaction with BCL-2 in the control of mutant SOD1 clearance. We monitored the evolution of the disease in SOD1 transgenic mice, and in an unexpected result, we observed a tendency to increased survival in SOD1 transgenic mice heterozygote for Beclin1 (n=10).

Discussion: Beclin1-dependent autophagy is essential for mutant SOD1 clearance. However, our *in vivo* results suggest compensation pathways in SOD1 transgenic mice heterozygote for Beclin1 that must to be explored.

Financial support: CONICYT Doctoral Fellowship, Supported by FONDECYT no. 1100176, FONDAP grant no. 15010006, Millennium Institute No. P09-015-F, and ICGEB, and Alzheimer's Association.

(124) MYELIN ASSOCIATED PROTEINS BLOCK MIGRATION OF OLFACTORY ENSHEATHING CELLS: AN IN VITRO STUDY USING SINGLE CELL MIGRATION AND TRACTION FORCE MICROSCOPY ANALYSIS. Sara Nocentini*, **Diego Reginensi***, Simón Garcia, Patricia Carulla, María Teresa Moreno-Flores, Francisco Wandosell, Xavier Trepat, Ana Bribian, José A. del Río. Molecular and Cellular Neurobiotechnology. Institute for Bioengineering of Catalonia (IBEC), Spain. *These authors contribute equally to this study. diego.reginensi@gmail.com

Introduction. Newly generated olfactory receptor axons grow between the peripheral to central nervous system aided by the olfactory ensheathing cells (OECs). Thus, OECs transplantation has emerged as a promising therapy for spinal cord injuries as well as for other neural diseases. Some studies reported that the migratory properties of OECs can be compromised by inhibitory molecules or potentiated by chemical gradients. In the study, we determined that OECs express all the components of the Nogo Receptor complex and that their migration is blocked by Myelin. We used cell tracking and traction force microscopy to analyze OECs migration

Material and Methods. Our project includes the use of tools such as: TEG3 cultures, western blotting techniques, adhesion stripes assay, immunocytochemical methods, time lapse analysis of cell migration and traction force measurement.

Results. Our data relates the absence of traction force of OECs with lower migratory capability that correlates with changes in F-Actin cytoskeleton and Focal adhesion distribution. These effects are recovered by the incubation with the Nogo Receptor blocker NEP1-40.

Discussion. Our data suggest that a cell based strategy using OECs that may also overcome the inhibitory action of MAIs is needed in other to enhance the migratory properties and the persistence of the OECs in parallel axon regrowth and the functional recovery in lesioned CNS.

(125) BDNF INDUCES DENDRITIC BRANCHING OF HIPPOCAMPAL NEURONS THROUGH A RAB11-DEPENDENT MECHANISM: DOES THE SAME MECHANISM ACCOUNT FOR AXONAL OUTGROWTH?
Andrés González, Oscar Lazo, Carlos Flores and Francisca C Bronfman. Millennium Nucleus in Regenerative Biology (MINREB). Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile. augonzal@uc.cl
Introduction. The neurotrophin BDNF activates TrkB receptors increasing dendritic arborization of neurons; however, the underlying mechanisms are not well understood. Post-endocytic trafficking of receptors is an important aspect for regulating the intensity and duration of BDNF/TrkB signaling. We have found that Rab11, a regulator of trafficking along the recycling pathway, modulates dendritic branching induced by BDNF in hippocampal neurons and that this mechanism may also be involved in BDNF-induced axonal branching and outgrowth.
Materials and Methods. Dendritic arborization of 7DIV hippocampal neurons was evaluated after 48hrs of stimulation with BDNF. The role of Rab11 was assessed by overexpressing mutants of Rab11 and by silencing Rab11 expression. BDNF/TrkB signaling was evaluated by measuring phosphoCREB nuclear accumulation. The effect of BDNF in axonal growth and retrograde propagation of signaling was assessed stimulating only axons of compartmentalized hippocampal cultures. In this last experiment, we quantified density of axons and nuclear pCREB in neurons.
Results. The expression of Rab11DN inhibited the increase of both BDNF-induced dendritic branching and nuclear pCREB. Expression of Rab11CA increases dendritic arborization even in the absence of BDNF. The silencing of Rab11 expression also inhibits BDNF-induced dendritic branching. Finally, BDNF stimulation of axons induced axonal growth and nuclear pCREB accumulation.
Discussion. Our results suggest that Rab11 mediates the BDNF-induced dendritic branching and is needed for CREB activation. BDNF increases both axonal growth and nuclear pCREB in compartmentalized cultures.

(126) A RETINOIC ACID-DEPENDENT CHECKPOINT IN THE DEVELOPMENT OF $CD4^+$ T CELL-MEDIATED IMMUNITY.
Karina Pino-Lagos[1], Yanxia Guo[1], Chrysothemis Brown[3], Matthew P. Alexander[1], Raul Elgueta[3], Kathryn A Bennett[1], Victor De Vries[1], Elizabeth Nowak[1], Rune Blomhoff[4], Shanthini Sockanathan[5], Roshantha A Chandraratna[6], Ethan Dmitrovsky[2] and Randolph J Noelle[1, 3*]. 1.Department of Microbiology and Immunology, Dartmouth Medical School and Norris Cotton Cancer Center, Lebanon, NH 03756, USA. 2. Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, NH 03755, USA. 3.King's College London, King's Health Partners, Medical research Council (MRC) Centre of Transplantation, Guy's Hospital, London SE1 9RT, UK. 4.Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo. Oslo, Norway. 5.The Solomon H. Snyder Department of Neuroscience, Johns Hopkins University, School of Medicine, Baltimore, MD 21205, USA. 6.IO Therapeutics, Santa Ana, CA 92705, USA.
For nearly a century, it has been known that vitamin A and its metabolite, retinoic acid (RA) are essential for host defense. However, the mechanisms for how RA controls inflammation are incompletely understood. The findings presented in this study show that robust RA signaling occurs concurrent with the development of inflammation.
In models of vaccination and allogeneic graft rejection, whole body imaging revealed that RA signaling was temporally- and spacially-restricted within the site of inflammation. Specifically, robust RA signaling was detected preferentially on $CD4^+$ T cells. Conditional ablation of RA signaling in T cells, driven by the restricted expression of a dominant negative form of the RARα, significantly interfered with $CD4^+$ T cell effector function, migration and polarity.
In this regard, $CD4^+$ T cells that were unable to sense RA produced less Th1/Th17 cytokines and chemokines, but conversely, an enhanced production of Th2-type cytokines was detected. These findings provide a fundamentally new perspective of the role of RA as a mediator directly controlling $CD4^+$ T cell differentiation and immunity.

(127) THE AXONAL ENDOPLASMIC RETICULUM AND $GABA_B$1a TRAFFICKING.
Viviana Valdés[1,2], Christoph Schmidt[3] and Andrés Couve[1,2]. [1]Physiology and Biophysics, ICBM and [2]Biomedical Neuroscience Institute (BNI), Facultad de Medicina, Universidad de Chile, Santiago, Chile. [3]Georg-August-Universität Fakultät für Physik, Göttingen, Germany. vivianavaldes@gmail.com
Introduction. Intracellular trafficking of neurotransmitter receptors is crucial for proper control of synaptic function. In a canonical trafficking modality synaptic proteins move long distances in post-Golgi vesicles before plasma membrane delivery. In a non-canonical modality transport occurs in pre-Golgi secretory compartments. GABA is the major inhibitory neurotransmitter in the central nervous system and $GABA_B$ receptors ($GABA_B$Rs) control the long and prolonged phase of inhibitory post-synaptic potentials. $GABA_B$Rs are composed of two related subunits, $GABA_B$R1a/b and $GABA_B$R2. The $GABA_B$R1a subunit is selectively localized to pre-synaptic terminals in a sushi domain-dependent manner. Despite its crucial role in regulating presynaptic function, key aspects of the intracellular trafficking and surface availability of $GABA_B$R1a in axons remain unclear. In particular, it has not been established whether $GABA_B$R1a traffics to the axon terminal utilizing a canonical or non-canonical modality.

Materials and Methods. The localization and mobility of secretory organelles and $GABA_BRs$ were evaluated using fixed and live-cell imaging in primary cultures of rat hippocampal neurons.
Results. $GABA_BR1a$ localizes to the axon after blockade of Golgi transport. $GABA_BR1a$ colocalizes with the endoplasmic reticulum (ER) in axons. The mobilities of $GABA_BR1a$ and the ER are microtubule-dependent and are in agreement with a kinesin dependent transport. 10% of mobile events correspond to $GABA_BR1a$/ER synchronous packets.
Discussion. We suggest that $GABA_BR1a$ reaches the axon following a non-canonical modality using the local ER. The relevance of axonal ER transport for pre-synaptic delivery is currently under investigation.
Funded by Fondecyt 1100137 and ICM P-09-015F.

(128) EXERCISE MODEL CHARACTERIZATION TO ASSESS MUSCLE ADAPTATIONS TO ATP-MEDIATED INTERLEUKIN-6 EXPRESSION IN MICE. **Fernández, R.**[1], Galgani, J.[2], Jaimovich, E.[1] and Buvinic, S.[1,3]. [1]Centro de Estudios Moleculares de la Célula, ICBM, Facultad de Medicina, [2]Departamento de Nutrición, Facultad de Medicina and [3]Facultad de Odontología. Universidad de Chile, Santiago, Chile. rodrigolicaf@gmail.com
Introduction: Slow calcium signaling pathway (SCSP) increases IL-6 mRNA in skeletal myotubes through an ATP-mediated process, but this has not been studied in adult muscle fibers. Also, effects of regular exercise over this SCSP response are unknown. The aims of this study were to evaluate if SCSP in adult muscle fibers increases IL-6 mRNA, and characterize an exercise training model to study its effects on SCSP response.
Materials and Methods: Concentration- and time-dependent ATP stimulations were done in muscle fibers of 6-8 week-old Balbc mice, and IL-6 mRNA was measured by qPCR.
Mice (5-6 week-old) were divided in two groups: VA, with full-time access to a wheel; and SED, without wheel. Distance and speed were monitored in VA. After five weeks, metabolic (muscle glycogen and palmitate oxidation) and macroscopic (body and organs masses) parameters were determined.
Results: ATP stimulation increased IL-6 mRNA in a concentration- and time-dependent manner.
Distance and speed progressively increased in VA, however, this was not associated neither with differences in glycogen nor in macroscopic parameters v/s SED. There was a lower palmitate oxidation rate in the soleous muscles of VA.
Discussion: Voluntary activity improved exercise performance, as shown by activity measures. However, except for palmitate oxidation, it did not produce measurable physiological adaptations. SCSP increased IL-6 mRNA in adult muscle fibers, so now the model can be used to test associations between improved exercise performance and modifications in the SCSP.
FONDAP-15010006, Fondecyt-1110467-11100454-11090007, Conicyt-7909002.

(129) MUSCLE STEM-CELL THERAPY IS IMPROVED BY REDUCING THE FIBROSIS ASSOCIATED TO MUSCULAR DYSTROPHIES. **Jaime Gutiérrez**, Cabrera D., Morales MG., Brandan E. Laboratory of Cell Differentiation and Pathology, CARE. Department of Cell and Molecular Biology, Catholic University of Chile. jagutiep@puc.cl
Introduction: The Duchenne muscular dystrophy (DMD) is a genetic disorder characterized by the absence of the protein dystrophin, which cause myofiber degeneration, necrosis and subsequently accumulation of extracellular matrix proteins (ECM) in a process called fibrosis.
The therapy with progenitor muscle cells is a promissory treatment for the DMD. Grafted cells must migrate and fuse with the regenerating fibers, restoring the expression of dystrophin. Previous attempts have shown low efficiency, but the exact causes remain unknown.
Our aim was to study whether the success of the cell therapy is enhanced if the muscle fibrosis is diminished.
Material and Methods: Satellite cells (muscle stem-cells) were obtained from freshly isolated skeletal muscle fibers from wild-type mice and transplanted in the Tibialis anterior muscles (TA) of *mdx* mice, a mouse model for DMD. To diminish fibrosis we use genetic and pharmacological approaches, *mdx* mice heterozygous for CTGF, a potent pro-fibrotic factor, or *mdx* mice treated with an antinflammatory drug (D07I1051). One month after cell transplantation the number of myofibers expressing dystrophin as well as the levels of ECM proteins were determined by immunofluorescence in TA cross-sections.
Results: The number of dystrophin-positive myofibers compared to control *mdx* mice was significantly increased in both experimental models where fibrosis was diminished.
Discussion: These results indicate that the muscle cell therapy can be improved under conditions where fibrosis is diminished, opening new approaches for the successful treatment of individuals with DMD.
(Supported by Conicyt-79090027, CARE-PFB-12/2007, MDA-89419 and Fondecyt-1110426).

(130) THE TRANSCRIPTION FACTORS NFAT, CREB AND SMAD2/3 ARE DIFFERENTIALLY REGULATED BY MYOSTATIN/IGF-1 DURING MYOBLAST DIFFERENTIATION. Sylvia Flores, Rodrigo Zuloaga, Andrea Retamales, Alfredo Molina, **Juan Antonio Valdés**. Universidad Andrés Bello, Facultad de Ciencias Biológicas, Laboratorio de Biotecnología Molecular. Santiago, Chile. jvaldes@unab.cl
Introduction: Skeletal muscle growth and regeneration are regulated by a variety of endogenous growth factors, most notably IGF-1 and myostatin. IGF-1 is a positive regulator in proliferation and differentiation of skeletal muscle cells, while

myostatin acts as a negative regulator of skeletal muscle mass. Previously we showed that IGF-1 induces calcium release from intracellular stores that activates calcium dependent signaling pathways and induces myostatin mRNA expression suggesting a negative feedback in the pathways that regulates myoblast proliferation and differentiation.
Materials and Methods: Myoblast primary culture was stimulated with physiological concentrations of IGF-1 and/or myostatin in the presence or absence of pharmacological inhibitors of the transduction pathways Calcineurin/NFAT, ERK1/2-CREB, Smad2/3. Activation of the signaling pathways were evaluated by western blot, reporter vector and immunocytochemistry protocols.
Results: IGF-1 and myostatin differentially regulates in a dose and time dependent manner the activation of the signaling pathways Calcineurin/NFAT, ERK1/2-CREB and Smad2/3.
Discussion: During myogenesis, IGF-1 and myostatin regulates myoblast differentiation through differential activation of the signaling pathways Calcineurin/NFAT, ERK1/2-CREB, Smad2/3. Our data support the hypothesis of a negative feedback in the IGF-1 and myostatin signaling pathways.
Financed by Fondecyt 11090274 and UNAB DI- 31/11R.

(131) ALTERATION OF IL-6 SIGNALING IN UREMIC SKELETAL MUSCLE. **Dünner N**., Venegas F., Peña JP., Coronado F., Michea L. and Jaimovich E. Center for Molecular Studies of the Cell. ICBM, Facultad de Medicina, Universidad de Chile. dailea_nd@yahoo.com.
Introduction: Chronic renal failure (CRF) causes decreased exercise endurance. Interleukin-6 (IL-6) is a cytokine upregulated by exercise in skeletal muscle with autocrine effects on skeletal muscle metabolism. Several studies show that IL-6 mRNA is altered in CRF, but whether CRF affects the response to exercise is unknown. We hypothesized that the regulation of muscle IL-6 pathway is impaired in CRF in response to exercise.
Materials and Methods: Male Sprague-Dawley rats were given 5/6 nephrectomy (NPX) or sham surgery and pair-fed. The response to exercise was measured in *extensor digitorum longus* (EDL) fatigued by *in situ* electrical stimulation through the sciatic nerve, or after swimming. EDL was dissected at the end of protocols for mRNA and protein evaluation.
Results: After *in situ* stimulation we observed 85±8 fold induction of IL-6 mRNA in the EDL of Sham rats; however, in EDL from NPX rats, IL-6 mRNA increased only 22±7 fold ($n=6$, $P<0.01$). NPX did not affect mRNA of IL-6 receptors. JAK2 protein decreased to 63±4.5% of control in EDL of NPX rats ($n=5$, $P<0.01$). We observed reduced activation of Stat3 and reduced induction of SOCS-3 mRNA in EDL of NPX rats after exercise ($n=3$-4, $P<0.05$). Basal P-AMPK levels were reduced in NPX and this difference was maintained after exercise.
Discussion: These data shows reduced activation of IL-6 pathway in EDL of NPX rat in response to exercise that could contribute to low endurance to exercise in CRF.
CONICYT AT-24080078, FONDECYT 1090223, 1110467, FONDAP 15010006.

(132) THE VITAMIN C TRANSPORTER SVCT2 IS DOWN-REGULATED DURING EARLY POST-NATAL DEVELOPMENT OF SLOW SKELETAL MUSCLE FIBERS. **Daniel Sandoval,** Marcela Low, Jorge Ojeda, Jaime Teneb, Francisco Nualart and Juan Pablo Henríquez. Department of Cell Biology, Faculty of Biological Sciences, Universidad de Concepcion, Concepcion, Chile. jhenriquez@udec.cl
Introduction: We have demonstrated that the vitamin C transporter SVCT2 is preferentially expressed in oxidative slow skeletal muscle fibers. In primary myotubes, SVCT2 is up-regulated upon differentiation and depolarization. To gain further insights into the role of vitamin C during skeletal muscle physiology, we determined SVCT2 expression throughout post-natal development of chick skeletal muscles.
Materials and methods: Criosections, protein and RNA samples were obtained from slow *anterior* (ALD) and fast *posterior latissimus dorsi* (PLD) muscles of post-hatched Leghorn chicks ranging from day 7 to adulthood. Samples were analyzed for NADH□thioreductase activity by histochemistry, slow C protein content by Western blot, and *SVCT2* transcript expression by RT-PCR.
Results: NADH-TR staining was observed in most ALD fibers. In turn, even though several NADH□TR positive fibers were detected at day 7 in PLD muscles, these fibers are scarce in the adult PLD. Similarly, both muscles express slow C protein at day 7 but PLD fibers drastically down-regulate this slow-specific protein later. *SVCT2* transcript expression was barely detected in PLD fibers in all stages, in spite of decreasing levels of slow fibres. Remarkably, even though most ALD fibres are slow in all stages, *SVCT2* expression decreases after hatching.
Conclusions: Our findings suggest that the requirement of intracellular vitamin C in slow muscles is higher at embryonic and early post-natal stages, possibly due to a role for SVCT2 mediated uptake of ascorbate during adult myogenesis.
Funded by FONDECYT 1100326 grant to JPH.

(133) TRANSFORMING GROWTH FACTOR-β STIMULATES MAMMARY MYOFIBROBLASTS DIFFERENTIATION THROUGH NOX4 INDUCTION AND JNK ACTIVATION. **Toyos M**. Tobar N., and Martínez J. Laboratorio de Biología Celular y Molecular, INTA, Universidad de Chile. marcetoyos@gmail.com

Introduction. Breast tumors belong to a group of neoplastic lesions which, under the influence of tumoral cell products, originate a fibrous structure responsible for hard consistency of the tumoral mass. Myofibroblasts have been identified as major players in this phenomenon acting either as producers of extracellular matrix (ECM) or as elastic components of tumor structure. In the present work we present evidences supporting the hypothesis that a redox stromal environment favour the Transforming Growth Factor-β (TGF-β1)-dependent myofibroblasts differentiation.
Material and Methods. As a cellular model, we used the fibroblastic RMF-EG cell line derived from normal human mammary tissue. NOX-4, a-sma and CTGF were assessed by qPCR and western blot (WB). JNK activity was assayed measuring p-JNK levels by WB. Knockdown of NOX-4 was achieved using a siRNA approach.
Results. We describe that the expression of mRNA for NOX-4 in RMF-EG cells precedes the expression of TGF-β1-stimulated myofibroblasts markers. Consequently with this, the TGF-β1-stimulated protein expression of myofibroblasts markers as a-sma or Fn-EDA was abolished by DPI an inhibitor of NOX activity and the expression of a siRNA for NOX4. We discuss the role of JNK pathway in the TGF-β1-stimulated myofibroblasts differentiation.
Conclusions. Our results suggest that stromal breast cells undergo myofibroblasts differentiation under TGF-β1 stimulus. This process, in turn, operates under a facilitating effect of a pre-existent oxidative environment.
Funding: FONDECYT 1080196.

(134) DIFERENCIAL GENE EXPRESSION BETWEEN NORMAL AND MDX MOUSE FIBERS INDUCED BY ATP SIGNALING. **Valladares D.,** Almarza G., Jaimovich E. Centro de Estudios Moleculares de la Célula, ICBM, Facultad de Medicina, Universidad de Chile.
Introduction: We have demonstrated that ATP signaling is altered in *mdx* fibers and it's implicated in calcium disturbance induced by electrical stimulation. Extracellular ATP has relevant functions and has been shown to be released from muscles during exercise. The aim of this study was to determine the differences in basal extracellular levels of ATP between normal and *mdx* fibers and how these differences can induce changes in gene expression related to degeneration and apoptosis.
Materials and Methods: The experiments were performed in muscle fibers obtained from normal and *mdx* adult mice. Basal ATP was measured by luciferin-luciferase method. Gene expression was determined by qPCR and western blot.
Results: Basal extracellular ATP levels were significantly higher in *mdx* fibers than in normal fibers. Moreover every 5-7 minutes there is a burst of ATP release in fibers from both strains that is more important in *mdx*. The basal expression of pannexin (ATP release) and Bax (apoptotic cascade) was increased in *mdx* fibers. Stimulation with external ATP or electrical stimulation induced changes in gene expression of particular genes for each fiber type; in particular, the proapoptotic protein Bax is overexpressed in *mdx* fibers, while is repressed in controls after ATP addition.
Discussion: This study demonstrates that basal ATP release is altered in *mdx* fibers and could be implicated in the observed differences in gene expression. ATP signaling appears to be involved in the activation of pathways related with degeneration and apoptosis in *mdx* fibers.
FONDECYT-1110467, FONDAP-15010006, AFM-14562, CONICYT-PhD fellowship (DV).

(135) UBIQUITIN-PROTEASOME SYSTEM MEDIATED STABILITY REGULATION OF PAX7 AND ITS ROLE IN ADULT MUSCLE SATELLITE STEM CELL FUNCTION. **Francisco J. Bustos**[1], John Yates III[2] and Hugo C. Olguín[1]. [1]Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile. [2]Dept. of Chemical Physiology, The Scripps Research Institute, La Jolla, USA. fubustos@uc.cl (Sponsor: E.O. Campos).
Introduction: Pax7 is a transcription factor expressed in quiescent satellite stem cells that are located between the basal lamina and sarcolemma of adult muscle fibers and are responsible for the remarkable muscle regenerative potential. Pax7 appears to regulate satellite cell specification, survival and myogenic progression. Pax7 is rapidly downregulated during early steps of myoblast differentiation, while is maintained in self renewing satellite cells. This process appears to be regulated at the post-translational level since proteasome inhibition can partially restore Pax7 levels. Here we attempt to characterize ubiquitin proteasome system (UPS) and Nedd4 E3 ligase role in regulation of Pax7 stability during myogenesis and self-renewal of satellite cells.
Material and Methods: We have evaluated Pax7-Nedd4 biochemical and functional interaction by using *ex vivo* muscle fiber and satellite cell derived cell lines cultures. We have studied Pax7 ubiquitination and degradation by *in vitro* and *in vivo* ubiquitination assays and pharmacological proteasome inhibition.
Results: Our results indicate that Pax7 is ubiquitinated and differentially regulated by the UPS during early steps of myogenesis. Nedd4 E3 ligase is expressed during satellite cell activation and differentiation and physically interacts with Pax7 during myogenic process.
Discussion: These results suggest a novel level for Pax7 regulation during muscle regeneration and situate the UPS as a possible mechanism to regulate Pax7 function during muscle stem cells differentiation and self-renewal.
Supported by: VRAID Límite Nº 17/2010 and CONICYT.

(136) PROCESSING, RELEASE AND NUCLEAR RELOCALIZATION OF THE INTRACELLULAR TAIL DOMAIN OF BONE MORPHOGENETIC PROTEIN RECEPTOR II (BMPRII). **Margarita Parada**[1], Juan Pablo

Henríquez[2] and Nelson Osses[1]. [1]Instituto de Química, Pontificia Universidad Católica de Valparaíso. [2]Departamento de Biología Celular, Universidad de Concepción. nelson.osses@ucv.cl

Introduction: BMPRII is a transmembrane protein receptor of the BMP family characterized by a long intracellular C-terminal tail with still unknown functions. Tail truncating mutations are associated to pathological condition highlighting its essential biological function. We hypothesize that BMPRII tail is processed releasing an intracellular functional domain. Therefore, our first aim has been to provide evidence of BMPRII processing in the C-terminal domain.

Materials and Methods: We used BMPRII overexpression systems to analyze processing. Detection of BMPRII and its released tail was accomplished by western blotting using antibodies raised against C-terminal tail or N-terminal ectodomain. Endogenous BMPRII processing was analyzed in NSC-34 motoneuron-like cells.

Results: Western Blot analyses for BMPRII with anti-C-terminal antibody showed a ~150 kDa single band whereas the anti-N-terminal antibody showed the same band of ~150, and a additional at ~130 kDa. Membrane enriched fractions from cells expressing BMPRII incubated with calcium at 37º C for different times showed a decrease of the ~150 kDa band and concomitant increase of the ~130 kDa band recognized by the anti-N-terminal antibody, and a ~20 kDa band recognized by the anti-C-terminal antibody. The ~20 kDa band was also found in nuclear enriched fractions only under proteasome inhibition conditions. Besides, endogenous BMPRII processing seems to be a component of motoneuron-like cells differentiation.

Discussion: Our findings suggest that the BMPRII C-terminal tail is released as a functional domain that could be essential for motoneuron cell differentiation.

FONDECYT 11060513, 1100326, VRIEA-PUCV.

(137) ALTERED PRO-INFLAMMATORY GENE EXPRESSION IN DYSTROPHIC *MDX* SKELETAL MUSCLE CELLS. **Henriquez C.**, Altamirano F. and Jaimovich E. Centro de Estudios Moleculares de la Célula, ICBM, Facultad de Medicina. Universidad de Chile, Santiago, Chile. carlos.henriquez@ug.uchile.cl

Introduction: Duchenne muscular dystrophy (DMD) is a human lethal X-linked genetic disorder caused by mutations in the *dystrophin* gene. Like humans with DMD, *mdx* mice lack functional dystrophin, providing a good model to study the disease. Pro-inflammatory genes as TNF-a, IL-1b and IL-6 and iNOS are up-regulated in muscles from DMD patients and *mdx* mice, but the contribution of skeletal muscle cells to this anomaly was unclear. The aim of this work was to study the differences in pro-inflammatory gene expression in *wt* and *mdx* myotubes.

Materials and Methods: Myoblasts were obtained from *wt* or *mdx* neonatal mice (1-3 days). RNA was isolated from differentiated myotubes using Trizol and mRNA expression was assessed by real time PCR. iNOS protein expression was confirmed by western blot.

Results: TNF-a, IL-1b and IL-6 basal expression of mRNA was not significantly different between *wt* and *mdx* myotubes, suggesting that the dystrophic skeletal muscle cells do not differentially express this cytokines at resting conditions. However, we found that iNOS mRNA expression was five fold higher in *mdx* myotubes (5.2±2.3), and iNOS proteins levels were increased three fold in *mdx* myotubes (3.0±0.4).

Discussion: Our data provides insight on the regulation of pro-inflammatory gene expression in dystrophic skeletal muscle cells. The mechanism that promotes iNOS overexpression in *mdx* myotubes could be relevant to explain the oxidative stress and damage reported in DMD pathology.

Fondecyt 1110467, FONDAP 15010006 and AFM14562 (EJ). Beca apoyo CONICYT AT-24100066 (FA).

(138) ACTIVATION OF THE KININ B1 RECEPTOR INCREASES THE EXPRESSION AND RELEASE OF MATRIX METALLOPROTEASE-9 FROM HUMAN HaCaT KERATINOCYTES. **Matus CE,** Mejia AJ, Ehrenfeld P, Pavicic MF, Figueroa CD. Instituto de Anatomia, Histologia y Patologia, Universidad Austral de Chile. matus.carola@gmail.com

Introduction: In wound healing, keratinocytes proliferate and migrate to repair tissue damage, after they become activated by soluble factors and extracellular matrix components. To migrate they release matrix metalloproteases, MMP-2 and MMP-9 to degrade extracellular matrix components. Keratinocyte differentiation is partially regulated by the kinin B1 receptor (B1R), which is overexpresed during shock, inflammation or tissue damage. Our aim was to investigate whether B1R activation increases mRNA expression and release of MMP-9.

Material and Methods: The HaCaT keratinocytes were cultured and stimulated with the B1R agonist Lys-des[Arg^9]bradykinin (LDBK) at different periods of time. The MMP-9 expression levels were analyzed by RT-PCR and release was determined by zimography. Involvement of specific signaling pathways was investigated by pretreating the cells with inhibitors for Src kinases, PI3K/Akt, p38 and ERK 1/2 MAPK.

Results: When keratinocytes were stimulated with 1 nM LDBK the mRNA expression levels of MMP-9 increased after 3 h, whereas the highest enzyme activity was observed after 24 h. The pre-incubation with specific inhibitors revealed the participation of Src kinases, PI3K/Akt and p38 MAPK. By contrast, inhibition ERK1/2 MAPK phosphorylation partially decreased MMP-9 release.

Discussion: The expression and release of MMP-9 in HaCaT keratinocytes were dependent on B1R activation as well as Src kinases, PI3K/Akt, p38 and ERK1/2 signaling pathways, events that may be relevant for extracellular matrix degradation and keratinocyte migration.
Supported by grant CONICYT 24090029 and DID-UACh.

(139) MINIMAL DOMAIN OF WNT3A ABLE TO ACTIVATE WNT/b-CATENIN SIGNALING. **Burgos CF**[1,2], Peralta A[1], Martinez J[2] and De Ferrari GV[1,2]. [1]Center for Biomedical Research, Faculty of Biological Sciences and Faculty of Medicine, Universidad Andrés Bello, Santiago, and [2]Department of Biochemistry and Molecular Biology, Faculty of Biological Sciences, Universidad de Concepción, Chile. gdeferrari@unab.cl
Introduction: Wnt/β-catenin signaling is initiated through interaction of a Wnt protein with the cysteine-rich domain of a Frizzled receptor (Fzd-CRD). Since no structural information is available for such interaction, we studied the Wnt-Fzd-CRD interface to define a Wnt3a domain which may account for Wnt/b-catenin activation.
Material and Methods: 3D models of Wnt3a were created using I-TASSER and refined with Modeller9v4, Phyre and SCWL3. In all steps, energy profiles and structural analysis were calculated. Next, the docking of a refined model of Wnt3a onto the available structure of Fzd8-CRD (1ijy) was performed by ZDock and analyses of these complexes were made with PROTORP. Proposed regions for Wnt3a-Fzd8-CRD docking were examined through synthesis of a library of 34 Wnt3a-derived peptides in Wnt/b-catenin signaling assays on HEK293T-BAR cells.
Results: The analysis of Wnt3a-Fzd8-CRD complexes revealed 4 regions of interaction in the Wnt3a ligand: Wnt3a residues 213-221, 230-245, 258-261 and 317-322. The peptide library confirmed these regions as capable to active Wnt/b-catenin signaling in a dose-response manner. Moreover, sFRP1, a secreted Wnt inhibitor containing a CRD domain, produced a competitive inhibition of Wnt3a-derived peptides.
Discussion: We propose a model of interaction between Wnt3a and Fzd-8-CRD, identifying a group of Wnt3a residues important for this process. Moreover, peptides containing such sequence modulate positively the activity of the cascade, opening potential pharmacological applications where decreased Wnt/b-catenin signaling activity is observed.
Funding: FONDECYT 1100942.

(140) ROLE OF INTRACELLULAR CALCIUM CHANNELS RYR, IP3R AND NAD(P)H OXIDASE IN INSULIN-INDUCED GLUT4 TRANSLOCATION AND GLUCOSE UPTAKE IN SKELETAL MUSCLE CELLS. **Contreras-Ferrat A.**[1,2], Vasquez C.[1], Espinosa A.[1], Lavandero S.[1], Klip A.[2], Jaimovich E.[1]. [1]Centro de Estudios Moleculares de la Célula, Universidad de Chile, Santiago, Chile [2]Hospital for Sick Children, Research Institute, Toronto, ON, Canada. acontreras@med.uchile.cl
Introduction: Insulin-regulated GLUT4 traffic has been widely explored, but the role of calcium release and ROS signaling in this process is poorly understood.
Material and Methods: We studied insulin-induced GLUT4 traffic in GLUT4*myc* skeletal muscle cells using single cell imaging and quantization of extracellular *myc* exposure.
Results: insulin-induced exofacial exposure of *myc* epitope, glucose uptake and $AktS^{473}$ activation were independent of extracellular calcium. The intracellular calcium chelator BAPTA inhibited activation of Akt, p70S6K and ERK1 but not p-ERK2. p-ERK1/2 was inhibited by 2-APB, an IP3R inhibitor. BAPTA-AM and a cytosol directed parvalbumin reduced insulin-dependent exposure of *myc*. Moreover, 50μM ryanodine and 20 μM 2-APB partially inhibited exposure of *myc* with an additive effect. The calcium ionophore ionomycin induced an increase in extracellular exposure of *myc* and was additive to insulin. In contrast, in myotubes maintained in calcium-free media, the effect of insulin was totally inhibited by ionomycin. Insulin increased H_2O_2 production measured with the cytosolic molecular sensor HyPer and was inhibited by NAC. p47 subunit of NADP(H)ox labeled differentiated myotubes. Both antioxidant agents, NAC and trolox partly reduced the externalization of *myc* as did the NADP(H)ox inhibitor apocynin and overexpression of catalase.
Discussion: These data suggest that insulin induces an increase in NADP(H)ox activity and activation by ROS of RyR and IP_3R intracellular calcium channels to foster GLUT4 translocation.
FONDAP 15010006, FONDECYT 3110170, FONDECYT 11090301.

(141) TURNOVER OF AMYLOID PRECURSOR PROTEIN CARBOXY TERMINAL FRAGMENT BETA (C99) IN LYSOSOMAL COMPARTMENTS. **Andrés Rivera-Dictter**[1], Hianara Bustamante[1], Vanessa Muñoz[1], Viviana Cavieres[1], Juan S. Bonifacino[2], Gonzalo Mardones[1], and Patricia Burgos[1]. [1]Laboratorio de Biología Celular y Molecular, Instituto de Fisiología, Facultad de Medicina, Universidad Austral de Chile, Valdivia, and [2]Cell Biology and Metabolism Program, NICHD, National Institutes of Health, Bethesda, MD USA. burgospa@gmail.com
Introduction: Proteolytic processing of the amyloid precursor protein by β-secretase generates C99, which subsequently is cleaved by γ-secretase resulting in amyloid β peptide (Aβ) production. Several lines of evidence suggest that C99 contains within its cytosolic tail key signals for its delivery to lysosomal compartments. Our aim was to investigate lysosomal turnover of C99 in a mechanism independent of g-secretase cleavage.

Materials and Methods: H4 neuroglioma cells stably expressing C99-EGFP were analyzed by cycloheximide pulse-chase experiments, in the absence or presence of g-secretase inhibitors, testing several conditions in order to: 1) block delivery to lysosomes and 2) disrupt lysosomal function.
Results: Blocking delivery of C99 to lysosomes or disrupting lysosomal function caused an enhancement in its proteolytic processing by g-secretase. Similar conditions but in the presence of g-secretase inhibitors showed a significant delay in C99 turnover, with a strong accumulation in lysosomal compartments.
Discussion: Our results show that lysosome function play an important role in the turnover of C99 suggesting that dysfunction of these organelles might have direct consequences in Ab production and implications for Alzheimer's disease.
FONDECYT 1100027 and DID-UACH.

(142) FLUCTUATIONS IN BRAIN EXTRACELLULAR ASCORBIC ACID CONCENTRATION COULD DRIVE CHANGES IN THE AVAILABILITY OF SVCT2 AT PLASMA MEMBRANE. [1]**Magdalena Esparza**, [1]Aníbal I. Acuña, [1]Carlos Kramm, [2]Carlos Toro, [2]Sebastián Brauchi y [1]Maite A. Castro. [1]Instituto de Bioquímica y Microbiología, [2]Instituto de Fisiología, Universidad Austral de Chile. macastro@uach.cl
Introduction: Ascorbic acid is an important water-soluble antioxidant and it is concentrated in brain and other organs. Sodium vitamin C transporters (SVCTs) are able to translocate ascorbic acid across the plasma membrane. SVCT2 is the only ascorbic acid transporter expressed in brain. SVCT2 is highly expressed by neuronal cells showing an intracellular and plasma membrane localization. It has been described that synaptic activity triggers the release of ascorbic acid from intracellular reservoirs. Indeed, glutamate is able to stimulate ascorbic acid release from astrocytes. However, there is no data about the possible changes in SVCT2 localization induced by acute exposition to ascorbic acid.
Methods and Results: Immunofluorescence analyses showed SVCT2 colocalization with endosomal and plasma membrane markers. After ascorbic acid exposition we observed an increase in SVCT2 at plasma membrane. This increase was abolished in presence of an exocytosis inhibitor, citochalasin D. Using total internal reflection microscopy (TIRM) we observed an increase of SVCT2-EGFP at plasma membrane level in ascorbic acid treated cells. The same was also observed in presence of an endocytocis inhibitor (phenylarsine oxide, PAO). In the opposite way, this effect was not seen when exocytosis was inhibited. These studies were supported by biotinylation assays and kinetic assays using ^{14}C-ascorbic acid.
Discussion: Mechanisms for acute modulation of SVCT2 localization could be relevant in brain where ascorbic acid fluctuates with synaptic activity.
FONDECYT1110571&1110906.

(143) GALECTIN-8 INDUCES EGFR ACTIVATION, ENDOCYTOSIS AND CELL PROLIFERATION IN HELA CELLS. **Remziye Döger,** Ronan Shaughnessy, Andrea Soza, Alfonso Gonzalez. Departamento de Inmunología Clínica y Reumatología, Fac. Medicina. Centro de Envejecimiento y Regeneración (CARE). Fac. Ciencias Biológicas. Pontificia Universidad Católica de Chile. remziye.doger@gmail.com
Introduction: The EGFR is a tyrosine kinase receptor that regulates processes of cell proliferation, survival and migration that become altered in cancer. Ligand-binding induces activation of the tyrosine-kinase activity followed by endocytosis of the EGFR. Galectins are a family of sugar binding proteins (lectins) that can modulate similar processes interacting with cell surface and extracellular matrix glycoconjugates, and have been also implicated in cancer. In order to study possible functional relationships between Galectins and EGFR, we studied the effects of Galectin-8 (Gal-8), which was originally identified as PCTA1 [Prostate carcinoma tumor antigen 1].
Methods: HeLa cells expressing 300.000 EGFR/cell was used. Activation of the EGFR was assessed by western blot with anti-phosphotyrosine of immunoprecipitated receptor. Endocytosis was evaluated by immunofluorescence, ligand-binding and cell surface biotinylation assays, as well as by live-cell imaging in cells expressing EGFR-GFP. Proliferation was measured by ^{3}H-thymidine incorporation.
Results: Gal-8 treatment induces concentration-dependent transient activation of EGFR and its downstream signaling element ERK1/2, but seemingly enough as to increase cell proliferation. It also induces rapid EGFR endocytosis, which seems to be followed by recycling.
Discussion: These results show for the first time trans-activation of EGFR by a member of the galectin family involved in cancer, opening the possibility that other galectins might also modulate EGFR function, and perhaps not only in the absence of ligand.
(Financed by BASAL grant#PFB12/2007 and FONDECYT grant#1110849).

(144) TH1 AND TH17 PROFILES INDUCTION ARE ASSOCIATED WITH IMMUNOLOGICAL RESPONSES AND LONG-TERM PATIENT SURVIVAL ON MELANOMA PATIENTS TREATED WITH DENTRITIC CELLS BASED IMMUNOTHERAPY. Falcón-Beas C[1,2], Tempio F[1,2], Pesce B[1,2], Aguillón J[1,2], Pereda C[1,2], Saffie C[1,2], Salazar-Onfray F[1,2], **López M. N[1-3].** [1]Millennium Nucleus on Immunology and Immunotherapy; [2]ICBM, Faculty of Medicine, University of Chile; [3]Research Support Office, University of Chile Clinical Hospital. melopez@med.uchile.cl
Introduction: We have developed a protocol to generate DCs in 48 hrs (TAPCells®), to treat advance melanoma patients. Our therapy showed two distinct groups of patients, differentiated by their tumor-specific delayed type hypersensitivity

(DTH) response, correlated with an increased median survival rate in those patients that did respond immunologically (DTH+) showing a three-fold prolonged survival.
Materials and Methods: DCs were differentiated from monocytes, loaded with the melanoma cell lines lysate (TRIMEL®) + TNF-α. Serum and PBMC were extracted at each immunization time. Serum levels of citokines were determined by ELISA, and effector and regulatory T cell populations were measured by flow cytometry. At the end of the therapy, skin punch biopsies from DTH were performed and effector populations were determined by immunofluorescence. Aditionally, *in vitro* induction of TH1 and TH17 population by TAPCells was determined by flow cytometry.
Results: Patients DTH+ have soluble and cellular factors that are involved in these differential responses. We demonstrate that TH1 and TH17 populations are increased on theses patients after our immunotherapy.
Discussion: Our findings indicated that the TAPCells treatment could be inducing effectors profiles while decreasing regulatory profiles in those same patients. Moreover, *in vitro* experiments show that TAPCells are capable of inducing antitumor profiles in CD4+T cells. Thus different responses evoked by treated melanoma patients could be essential factors for more effective and personalized therapies.
Support Fondecyt 1090243.

(145) DOPAMINE RECEPTOR D5 EXPRESSED ON IMMUNE CELLS PROMOTES CD4$^+$ T-CELL-MEDIATED AUTOIMMUNITY. **Carolina Prado**[1,2], Francisco Contreras[1,2], Rodrigo Pacheco[1]. [1]Fundación Ciencia para la Vida and [2]Universidad Nacional Andrés Bello. Santiago, Chile. carolinapradot@gmail.com
Introduction: Dendritic cells (DCs) are responsible for priming naïve T-cells and for promoting their differentiation into appropriate effector cells. Emerging evidence points toward dopamine contributes to the modulation of immunity. Accordingly, we have analyzed the role of dopamine receptor D5 (D5R) in the regulation of DCs and T-cell function.
Material and Methods: Splenic or bone marrow-derived DCs and CD4$^+$ T-cells were obtained from wild-type or D5RKO C57BL/6 mice. Dopamine receptors (DARs) expression was analyzed by FACS. DCs were co-cultured with T-cells to evaluate differentiation to Th1, Th17, and Treg phenotypes by FACS. Experimental Autoimmune Encephalomyelitis (EAE) was used as autoimmunity model. Animal work was performed according to institutional guidelines. Results shown are from three independent experiments and statistical significance was determined by unpaired Student's *t*-test or Mann-Whitney *U*-test.
Results: D1R and D5R were the main DARs expressed in both DCs and CD4$^+$ T-cells. D5RKO animals developed EAE with reduced severity when compared to wild-type animals. Importantly, D5R-deficient DCs prophylactically transferred to wild-type recipients were able to reduce EAE severity. Accordingly, Tregs generation from naïve CD4$^+$ T-cells was increased in co-cultures with D5R-deficient DCs. On the other hand, lack of D5R in CD4$^+$ T-cells induced decreased Th17/Th1 ratio after stimulation with wild-type DCs in polarizing conditions.
Discussion: These results show that D5R expressed in both DCs and T-cells contributes to CD4$^+$ T-cell differentiation, increasing generation of inflammatory phenotype and attenuating differentiation toward regulatory T-cells, thereby favoring the development of autoimmunity.
Supporting Grants: FONDECYT-1095114, CONICYT PFB-16. FC and CP are CONICYT fellows.

(146) INHIBITION OF HANTAVIRUS ENTRY INTO THE CELL. [1,2]**Barriga., G.P.** and [1]Tischler, N.D. [1]Fundación Ciencia para la Vida and [2]Universidad Andrés Bello, Santiago, Chile. gonzalo.barriga@gmail.com
Introduction. To initiate viral replication, Andes virus (ANDV) fuses its membrane with host cell membranes. This process is performed by the viral Gc fusion protein. Previous studies showed that Gc shares similarity with class II fusion proteins. Their ectodomain is composed of three domains rich in beta sheets and a stem region. A crucial step during the fusion process consists in the movement of domain III (DIII) towards domain II, thereby inducing a hairpin structure. For other class II fusion proteins it has been shown that the addition of exogenous DIII with or without the stem region inhibits the membrane fusion process. Here we postulate that this strategy inhibits the ANDV Gc fusion process.
Materials and Methods. The inhibitory activity of Gc DIII and the stem region was studied in cell-cell fusion assays and in ANDV infection assays which allow virus-plasma membrane fusion by lowering the pH of the media.
Results. The cell-cell fusion assay showed that ANDV DIII produced 50 % of fusion inhibition at a 25 μM concentration. Inhibition was highly specific as this domain did not inhibit Gc of other hantaviruses. Preliminary data indicates that Gc DIII inhibits infection of cells with ANDV.
Discussion. The results suggest that ANDV Gc DIII has a specific inhibitory effect on the ANDV-cell fusion process. The data on fusion inhibition and viral infection by DIII emphasizes that Gc may resemble a class II fusion protein. This study corresponds to the first *in vitro* report on the specific inhibition of ANDV cell entry.
Fondecyt 1100756, CONICYT PFB-16.

(147) AtPRP3 BUT NOT AtPRP1 IS ACTIVELY ENDOCYTOSED FROM THE CELL WALL AT THE ROOT HAIR GROWING TIP. **Rodriguez-Furlan, Cecilia I** (A), Orellana, Ariel (A), Tierney, Mary (B). (A) Plant Biotechnology Center, Universidad Andres Bello. (B) Plant Department, Vermont University. cec.rodriguez.f@uandresbello.edu

Introduction: Root hairs are extensions of epidermal cells that elongate by tip growth using secretory and endocytic mechanisms. Current models of cell wall assembly suggest that endocytosis is important in establishing ECM structure during growth. ATPRP3 and ATPRP1, two structural Proline-Rich Proteins, are secreted into the ECM at the tip of growing hairs. Here we further characterized the role of polarized secretion and possible endocytosis in trafficking ATPRP3 and ATPRP1 to the root hair cell wall.
Material and Methods: Root hairs of 3-day-old seedlings expressing AtPRP1::ATPRP1-GFP and AtPRP3::ATPRP3-GFP were analyzed using confocal microscopy. Treatments were performed with 15uM FM4-64 (5-30 min) and 100uM BFA (30 min).
Results: Vesicles containing ATPRP1-GFP and ATPRP3-GFP moved in an anterograde to retrograde direction and were concentrated at the root hair tip. Incubation with the endocytic tracer FM4-64 showed labeling at the plasma membrane as well as punctate structures (the putative early endosomes) within root hairs. These punctate structures showed co-labeling with AtPRP3-GFP but not with AtPRP1-GFP. Brefeldin A treatment, which inhibits secretion but not endocytosis, resulted in the co-accumulation of ATPRP3-GFP and FM4-64 in BFA compartments in root hairs. This contrasted with ATPRP1-GFP labeling that was found outside of the BFA bodies.
Discussion: ATPRP1 and ATPRP3 reside in trafficking vesicles of the endomembrane system that are both secreted to the root hair tip. However, ATPRP3 localization within the root hair cell wall is regulated by both a secretory and endocytic mechanism(s).
Supported by: FONDAP CRG-15090007; Basal Program PFB-16; ICM-P006-065-F.

(148) ANTI-CD115 ANTIBODIES: TOOL FOR CHARACTERIZATION OF MACROPHAGES-LIKE CELLS OF RAINBOW TROUT. **Maisey K[1]**, Torres-Undurraga C[1], Wang T[2], Secombes CJ[2] and Imarai M[1]. [1]Laboratorio de Inmunología, Centro de Biotecnología Acuícola (CBA), Universidad de Santiago de Chile. [2]Scottish Fish Immunology Research Centre University of Aberdeen, UK. kmaisey@cba-usach.cl
Introduction: CD115, also known as macrophage colony-stimulating factor receptor (M-CSFR), is a transmembrane tyrosine kinase receptor expressed in monocytes, macrophages and their progenitor in mammals. CD115 activation controls differentiation, proliferation and functional activity of macrophages. CD115 has emerged as a potential useful marker of macrophages in fish, because the homologous genes of CD115 are available in some teleost fish, including Rainbow trout. We present the generation and characterization of antibodies recognizing CD115 in Rainbow trout and described their use to detect and characterize macrophages after vaccine and cytokine stimulation.
Materials and Methods: Polyclonal antibodies was developed using a CD115 peptide. We characterized the antibodies using macrophages-like RTS-11 cells by flow cytometry, RT-PCR and immunofluorescence. In *Yersinia ruckerii* vaccinated trout, spleen and head kidney were extracted and the cells were cultured and re-stimulated with the vaccine and/or recombinant cytokines. The effects on CD115+ cells were analyzed by FC.
Results: The anti-CD115 recognized RTS-11 cells and reacts in spleen and head kidney with macrophages while no reaction with lymphocytes or erythrocytes was detected. Macrophages in spleen and head kidney of trout injected with a vaccine against *Y. ruckerii* and/or stimulated with rtIFN□, rtIL-15 and rtIL-21 suggest a differential role of vaccine and cytokines in the macrophage response.
Discussion: We developed a useful antibody to study the macrophage/monocyte like cells from rainbow trout. This tool will be useful to investigate the involvement of trout monocytes/macrophages in immune response.
CONICYT, INNOVA-CHILE 09CN13PBT90, 09MCSS6698, VRID-USACH.

(149) CALRETICULIN DETECTION IN SALIVA OF DOMESTIC DOGS (*Canis lupus familiaris*). **Coddou, F.,** Weinberger, K., Duaso, M.L., Valck, C., Ramírez, G., Maldonado, I., Ferreira, A. Disciplinary Immunology Program, Biomedical Sciences Institute, Faculty of Medicine, University of Chile. aferreir@med.uchile.cl
Introduction: Calreticulin (CRT), a protein that resides in the endoplasmic reticulum, translocates to the extracellular environment where it mediates effects on the rate of acceleration and quality of tissue repair. CRT has been detected in the saliva from humans, ticks and fleas. Although canine CRT gene has been sequenced, the protein has not been expressed, nor defined its presence in saliva. Since CRT is highly conserved structurally and functionally, we propose its presents in domestic canine saliva using antigenic and functional criteria. Thus, in dogs, the behavioral fact of licking their wounds could generate a pro-healing effect attributable in part, to CRT.
Methodology: The presence of CRT in dog saliva was determined by: a) SDS-PAGE, followed by western-blotting and incubation with normal human serum as a source of C1q; b) its capacity to inhibit C1-mediated C4b generation in ELISA.
Results: Heterolog antibodies recognized a protein of 55 kDa, corresponding antigenically to canine CRT (CfCRT). Functionally, CfCRT, binds to C1q and participates in the inhibition of this activation pathway.
Conclusion: Dog saliva contains a molecule that has structural, antigenic and functional properties compatible with those known for CRT.
Discussion: CRT is detected in dog saliva, by functional and antigenic criteria. Efforts to clone the CfCRT gene are currently underway, as well as studies aimed at determining the effects of CfCRT on cutaneous wound healing.

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(150) INDUCIBLE REGULATORY T CELLS PRODUCE FACTORS THAT GENERATE REGULATORY T CELLS *in vitro*. **C. Fuentes[1]** C. Moore[2], M.R. Bono[1], M. Rosemblatt[1,2]. [1]Facultad de Ciencias, Universidad de Chile, [2]UNAB y Fundación Ciencias para la Vida. camifuentes@gmail.com

Introduction. Regulatory T cells (Tregs) mediate immunological self-tolerance and suppress immune responses. Natural Tregs are produced in the thymus as part of central tolerance mechanisms; however Tregs can also be induced in the periphery from naive T cells in tolerogenic environments. The aim of this work is to study the properties of soluble factors produced during the *in vitro* generation of allogeneic Tregs**.**

Materials and Methods. Splenic naive T cells from WT C57BL/6 mice were co-cultured with BALB/c splenic antigen presenting cells (APCs) in the presence of TGF-β, RA and IL-2 to generate allogeneic Tregs. On day 6, supernatants from these cocultures were collected and used in a second coculture of allogeneic naïve T cells and APCs. On day 12, the cells from the second coculture were analyzed for FoxP3 expression, characteristic Tregs markers and cytokine production or sorted for suppression assays.

Results. Surprisingly, supernatants from the first coculture where Tregs were generated induced the transcription factor FoxP3 in the second coculture of naïve T cells activated by APCs. These *de novo* generated Tregs, express several activation Tregs markers, inhibit cytokine production and proliferation of effector T cells.

Discussion. The results show that co-culturing naive T cells with allogeneic APCs in the presence of Treg supernatants generates **de novo** Tregs with suppressive function. In summary, soluble factors present in the supernatants produced during allogeneic Treg generation are capable of inducing FoxP3 on naïve T cells.

(Funding: FONDECYT 1100557, 1100448)

(151) PRODUCTION OF ANTI-CD3e ANTIBODIES: A TOOL FOR CHARACTERIZATION OF SALMONID LYMPHOCYTES. **Montero R**, Valenzuela B, Maisey K, Imarai B. Laboratorio de Inmunología, Centro de Biotecnología Acuícola. Facultad de Química y Biología, Universidad de Santiago de Chile. ruth.montero@usach.cl

Introduction. Antibodies against cell surface markers are widely used to characterize the different immunocompetent cell types. In mammals, CD3ε has been well described as a surface marker of T lymphocytes; in salmonids, T lymphocyte populations have not been characterized yet.

Material and Methods. In the present work, we have cloned the sequence of the extracellular fragment of Rainbow trout CD3ε into an expression vector. Bacteria were transformed with the recombinant vector and then induced to over-express the desired protein fragment. Subsequently, the extracellular protein fragment was purified and concentrated. Two rabbits were injected with the concentrated protein and then the polyclonal serum was obtained. The titer of the anti-CD3ε present in rabbit serum was determined by ELISA and Flow Cytometry. A Western Blot assay with the concentrated proteins was also achieved.

Results. Bacteria with the recombinant vector were obtained and the fragment protein was expressed in this prokaryotic system. The protein was purified and concentrated and a Rainbow trout CD3ε specific antiserum was obtained and titrated by both ELISA and Flow Cytometry (FC). In Western Blot, the anti-serum against CD3ε recognized two proteins bands one of which seems to be a dimer. Cells of lymphocyte morphology and size are immunoreactive as detected by FC.

Discussion. This is the first report of the production of Rainbow trout CD3ε specific antiserum that may be useful to characterize lymphoid immune response in salmonids.

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(152) DEXAMETHASONE AND MONOPHOSPHORYL LIPID A STIMULATION GENERATES TOLEROGENIC DENDRITIC CELLS WITH AN *in vitro* STABLE IMMUNOREGULATORY PHENOTYPE IN HEALTHY VOLUNTEERS. **Lorena Hoyos,** Paulina García, Rodrigo Morales, Bárbara Pesce, Karina Pino-Lagos, Diego Catalán, Juan Carlos Aguillón. Programa Disciplinario de Inmunología, ICBM, Facultad de Medicina, Universidad de Chile. www.irtgroup.cl; lorehoyosp@gmail.com

Introduction: Tolerogenic DCs (TolDCs) are capable of inducing tolerance through antigen presentation with defective co-stimulation or by secreting anti-inflammatory cytokines. TolDCs generate an immunoregulatory environment by controlling the activation of effector T cells making them a promising therapy for autoimmunity. Our goal is to generate monocyte-derived TolDCs (Mo-TolDCs) from healthy donors and RA patients and demonstrate their efficacy.

Methods: A 5-day protocol was developed for generating Mo-TolDCs from healthy donors using dexamethasone, and the lipopolysaccharide-derivative monophosphoryl lipid A (MPLA). Cells were cultured in AIM-V medium in the presence of IL-4 and GM-CSF. We evaluated DC phenotype in terms of cellular markers and cytokine profile by flow cytometry and ELISA. Tol-DC regulatory capacity was assessed through allo-stimulatory assays with CFSE labeling.

Results: Mo-TolDCs show characteristic tolerogenic properties such as reduced costimulatory and maturation molecules, anti-inflammatory cytokine secretion profile and reduced allo-stimulatory capacity, shown in co-cultures with CD4+ T cells.

Mo-TolDCs maintain their tolerogenic profile when activated with MPLA, only exhibiting higher expression of migratory marker CCR7. We were also capable of identifying TLR2 as possible TolDC marker, highly expressed only at this DC stage differentiation.
Discussion: We addressed several issues pertaining TolDC generation, such as shorter obtainment time, stability, identification of specific TolDC markers and functionality, the later which is crucial for validating this protocol for future clinical procedures in RA patients.
Support: Fondecyt-1100102, Millennium Institute on Immunology and Immunotherapy- P09-016-F.

(153) *Neisseria gonorrhoeae* EFFECTS ON THE MATURATION OF DENDRITIC CELLS. **Bélgica Villegas-Valdés**, Sebastián Reyes-Cerpa, Kevin Maisey, Alejandro Escobar, Mónica Imarai, Claudio Acuña-Castillo. Universidad de Santiago de Chile. claudio.acuna@usach.cl
Introduction: *Neisseria gonorrhoeae* (Ngo) is the causal agent of gonorrhoea. This disease frequently occurs without any clinical manifestations and immune response. The absence of response is related to several bacteria strategies used to evade the immune system. Data obtained from mice under experimental infection showed an increase of T regulatory cells in genital tract drained lymph nodes. Our objective was to evaluate the effects of Ngo in the maturation of antigen presenting cells.
Material and Methods: We challenged murine dendritic cells and the macrophage Raw cell line, with two variants of Ngo expressing both pili and opa or none of these membrane components. We assessed the production of TNF-α and IL-10 by ELISA. Additionally, we evaluated the surface expression of MHC II and CD86 by flow cytometry.
Results: Raw cells challenged with pili-opa- Ngo showed secretion of TNF-α levels similar to levels induced by LPS. The secretion of IL-10 was higher with Ngo pili+opa+. Dendritic cells challenged only with pili+opa+ Ngo showed higher levels of TNF-α and IL-10 relative to levels induced by LPS. On the other hand, dendritic cells and Raw cells challenged with pili+opa+ Ngo had a decrease in CD86 expression. In addition, expression of MHC II was diminished in the raw cells but not in dendritic cells with relation to levels induced by LPS.
Discussion: These achievements can contribute to understand the strategies used by Ngo to evade immune response and help to develop a therapy to reduce the immune evasion of Ngo, on their turn allowing the development of a new treatment and prevention of gonococcal infections.
DICYT021043IB, FONDECYT 1110734.

(154) REPRIMO REDUCES TUMORIGENIC CHARACTERISTICS OF HUMAN GASTRIC CANCER CELL LINES. **Olivares W.**[1], Torres V.[1], Leguina A.[1], Maturana MJ.[1], Montecinos V.[1], Aguayo F.[2], Corvalán AH.[1]. [1]Hematology-Oncology Department, Pontificia Universidad Católica de Chile. [2]Virology Department, Universidad Chile. wildapucv@gmail.com (Sponsor: A. Quest)
Introduction: Gastric cancer (GC) is the first leading cause of cancer death in Chile. The progress of GC is also related to epigenetic changes, like DNA methylation, that involves downregulation of tumor suppressor genes. Recently we have identified Reprimo (RPRM), a p53 dependent G2 arrest mediator candidate as a putative biomarker for early diagnosis in GC. However, the role of RPRM in GC is unknown.
Methods: Expression and sequence of the promoter and coding region of RPRM was performed in five GC cell lines. Two expression negative RPRM cell lines were stable transfected with pCMV6 vector containing the full-length coding region of RPRM (pCMV6-RPRM). Cell growth and proliferation was analyzed by MTS, colony-formation and soft-agar assays. In addition, we analyzed the ability of migration through wound/healing assay.
Results: No mutations were found in the coding region. A dense DNA methylation was found in the promoter region. Only two cell lines were negative for RPRM expression. In these cell lines, a 50% decreased cellular proliferation and anchorage indepent growth was observed after the transfection with pCMV6-RPRM. The migration ability was also decreased in both cell lines.
Discussion: Our results suggest that RPRM may be a tumor suppressor gene in GC. These findings extend our previous observation suggesting that downregulation of RPRM is not only a putative biomarker for early diagnosis but also might play a role in the pathogenesis of GC.
Fondecyt 1111014 & Fondef D09I1137.

(155) CHEMOTHERAPEUTIC NUCLEOSIDE ANALOG TRANSPORTER HUMAN ENT1 (EQUILIBRATIVE NUCLEOSIDE TRANSPORTER 1) EXPRESSION AND ACTIVITY IS MODULATED BY PEROXISOME PROLIFERATOR ACTIVATED-RECEPTORS (PPARs) IN OVARIAN CANCER CELLS. **Trinidad Montero**[1], Sumie Kato[2], Dusan Racordon[4], M[a] Loreto Bravo[4], Gareth Owen[4], Mauricio Cuello[2], Miguel Bronfman[3] and Andrea V Leisewitz[1]. [1]Hematology-Oncology Department, [2] Division of Obstetrics and Gynecology, School of Medicine,[3] Cellular and Molecular Biology Department, [4]Physiology Department, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile, Santiago, Chile.
Introduction: Several reports correlate the presence of hENT1 with chemotherapy success in solid tumors (i.e. pancreas, lung and ovary), making hENT1 regulation an important target of study. hENT1 promoter analysis showed two putative

PPAR response elements (PPRE) and preliminary data suggest a regulation through these nuclear receptors. Using A2780 ovarian-derived tumor cell line, we investigated the role of PPARs on hENT1 expression and activity.
Material and Methods: A2780 cells were treated with the PPARa agonist, Wy14,643 or PPARg agonist Rosiglitazone and transfected with PPARα or PPARg expression vectors. hENT1 expression (Western blot) and activity (^{3}H-adenosine uptake assay) were analyzed. hENT1 promoter was analyzed with MatInspector database and promoter constructs coupled to the luciferase gene were analyzed for their response to PPARα or PPARg.
Results: PPARα activation or overexpression resulted in higher hENT1 expression and activity. PPARg activation or overexpression upregulated hENT1 expression but the activity was not upregulated. Consistently, PPARα and PPARg overexpression increased luciferase activity only on hENT1 promoter constructs containing one or both PPRE.
Discussion: Our results suggest that PPARs modulate hENT1 expression and nucleoside uptake in an A2780 cells, potentially affecting nucleoside analog-dependent cell death in ovarian cancer cells.
Research funded by FONDECYT 11080206(AL), 1080163(MC), 1060495(GO) PFB12-2007(MB).

(156) DIFFERENTIAL EXPRESSION PATTERN OF STROMA CELL MARKERS AND VEGF-A IN PRIMARY CULTURED STROMA FROM BENIGN AND PROSTATE CANCER EXPLANTS. **Javier Cerda**[1], Ignacio San Francisco[2], Alejandro Godoy[3], Viviana Montecinos[1]. [1]Hematology-Oncology and [2]Urology Departments, Pontificia U. Católica de Chile. [3]Department of Urology, Roswell Park Cancer Institute, Buffalo NY. jcerda.infante@gmail.com (Sponsor: MA García).
Introduction: Tumor microenvironment plays a critical role in the progression of prostate cancer (CaP). During progression of CaP, the tumor-associated stroma becomes "activated", which is characterized by appearance of several hallmarks, including: presence of myofibroblast/fibroblast, significant decrease in the number of differentiated smooth muscle cells (SMC) and increase in the microvascular density (MVD). Previous studies suggest that vascular endothelial growth factor-A (VEGF-A) synthesized by the tumor-associated stroma could play a critical role to facilitate increase in MVD during progression of CaP.
Material and Methods: Primary cell cultures of human prostate stroma were established from benign and malignant human prostate tissue explants and analyzed by the expression of the stroma cell markers calponin, vimentin, desmin, pro-collagen, α-smooth muscle actin and VEGF-A.
Results: The analysis of human primary cultures isolated from benign and malignant prostate tissue explants revealed morphologically distinctive subpopulations of stromal cells, which presented differential expression patterns of stroma cell markers and VEGF-A.
Discussion: Tumor-associated stroma corresponds to a heterogeneous population of cells that differentially express VEGF-A and therefore may have distinct roles on regulating CaP progression and angiogenesis. Functional studies from the individual subpopulations are needed to elucidate the potential role of stromal VEGF-A in CaP.
Proyecto Inicio VRI 034/2010.

(157) ANALYSIS OF NKG2D LIGANDS EXPRESSION ON TUMOR CELLS FROM GASTRIC CANCER PATIENTS. **Kramm K.,** Ribeiro C. H., Bustamante M, Garrido Tapia M., Hernández C. J., Collazo N., Sotelo P., Zúñiga R. and Molina M.C. Laboratory of Immunosurveillance and Immune Evasion. Disciplinary Program of Immunology, Faculty of Medicine, Universidad de Chile. karinakv@ug.uchile.cl
Introduction: Gastric cancer (GC) is one of the main causes of death in Chile and worldwide. The innate immunity is considered the first line of host defense against cancer, and its most important components are the natural killer (NK) cells. Among the stimulatory receptors of NK cells, NKG2D is of great importance. Distinct NKG2D ligands (NKG2DL) have been described. The aim of this work is to analyze expressions patterns of NKG2DL in patients with CG.
Material and Methods: The expression pattern of NKG2DL was evaluated at the level of mRNA and protein cell surface expression in cells derived from tumor and adjacent normal tissues, obtained from patients with GC, through semiquantitative RT-PCR and flow cytometry, respectively.
Results: NKG2DL are expressed both, on tumor and normal adjacent tissues, at mRNA and protein cell surface levels. However, a higher expression tendency was observed in tumor tissue as compared to "normal" adjacent tissue. ULBP3 expression on adjacent tissue was exceptionally higher than in tumor tissue in half of the patients analyzed.
Discussion: This is the first work describing the expression of NKG2DL on gastric cancer cells from patients. The lower expression of ULBP3 on tumor cells could be involved in mechanisms of tumor evasion from the immune response and another NKG2DL might be used as a therapeutic target.
Financial support: FONDECYT 3100151, Proyecto Enlace ENL2010/2012 and Beca CONICYT para Estudios de Magíster en Chile, 2011.

(158) NOVEL ANDROGEN RECEPTOR PURE ANTAGONIST MDV3100 PREVENTS NUCLEAR RECEPTOR TRANSLOCATION AND INDUCE TUMOR REGRESSION IN MICE MODEL OF CASTRATE-RESISTANT

PROSTATE CANCER. J. Guerrero[1], **F. Gomez[1]**, I.E. Alfaro[1], A.A. Protter[2], S. Bernales[1,2]. [1]Fundación Ciencia para la Vida, Santiago, Chile. [2]Medivation Inc., San Francisco, CA, USA. fcogomezm@gmail.com

Introduction: Prostate cancer is one of the most commonly diagnosed cancers. Initially, prostate tumors respond to anti-androgen therapy but eventually progress to castration-resistant prostate cancer (CRPC). In this stage, androgen receptor (AR) antagonists like bicalutamide and flutamide are ineffective at controlling tumor growth. A novel anti-androgen MDV3100, suppresses AR functions including androgen binding, nuclear translocation and DNA association. In this study, we evaluated *in vitro* and *in vivo* anti-tumoral activity of MDV3100.

Material and Methods: We compare MDV3100 vs bicalutamide for AR binding, AR translocation in *in vitro* and cell assays. The agonist/antagonist activity was evaluated in LnCap cells the presence of dihydrotestosterone, using qRT-PCR method. Additionally, tumour growth in an *in vivo xenograft* model of CRPC was studied in response to the drugs.

Results: MDV3100 inhibits agonist-induced AR-target gene expression more effectively than bicalutamide, and does not display agonistic effects. In contrast to other anti-androgens, MDV3100 prevents the nuclear translocation of the AR-complex. Finally, we show that MDV3100 inhibits tumor growth and induces the regression of tumor volume in a xenograft model tumoral cell apoptosis.

Discussion: These results indicate that MDV3100, unlike classic anti-androgens, is effective in models of CRPC, and that its mechanistic advantages over other anti-androgens could be explained by its efficient block of AR nuclear translocation and subsequent induction of cell death. These pharmacological properties may contribute to the effectiveness of this compound reported in patients with castrate-resistant prostate cancer.

(159) STATINS COUNTERACT THE LEPTIN INDUCED MIGRATION AND INVASIVENESS IN HUMAN EPITHELIAL OVARIAN CANCER CELLS BY INHIBITION OF RHOA SIGNALLING PATHWAY. **Díaz D[1]**., Kato S[1]., Cuello M[1]. [1]Division of Obstetrics and Gynecology, Pontificia Universidad Católica de Chile. diazsilva.dm@gmail.com

Introduction: Epidemiologic studies support a potential relationship between obesity and cancer. Epithelial cancers associated with obesity should have a hormonal basis. Leptin, a peptidic hormone mainly produced by adyposites, induced migration and invasiveness in epithelial cancer cells through RhoA pathway. Members of this family of small GTPases are key regulators in cell motility, and cell adhesion, cell cycle progression, gene expression, and apoptosis. Previously, we have shown that statins, HMGCoA reductase inhibitors, used to reduce cholesterol synthesis in obese patients, are also able to induce cell death in epithelial cancer cells.

Objectives: In the present work we studied the effects of statins in migration, invasiveness in human epithelial ovarian cancer cells and underlying mechanisms.

Materials and Methods: Ovarian cancer cells lines, A2780 and SKOV3, were treated with leptin with or without Simvastatin, and a ROCK inhibitor (Fasudil). Matrigel invasion, and migration assays were conducted. Underlying mechanisms were explored by immunoblotting of key players involved in signalling pathway (pAKT, ERK) and pull down of activaded RhoA.

Results A significant decrease in leptin- induced migration and invasiveness was observed in both cell lines upon treatment with statins and Fasudil. Simvastatin decreases p-ERK, p-AKT and reduces the activated form of RhoA.

Conclusion: In the present work we demonstrated that statins counteract leptin effect in migration and invasiveness in ovarian cancer through inhibition of RhoA signalling pathway. This work supports the beneficial use of statins in the treatment of this disease.

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(160) THE ANTI-TUMORIGENIC ACTION OF 2-METHOXYESTRADIOL IS INHIBITED BY SULFONATION IN THE BREAST CANCER CELLS. **Vargas MF[1,3]**, Spink DC[2] y Owen GI[1,3]. [1]Facultad de Ciencias Biológicas, Universidad Católica de Chile, Chile; [2]Wadsworth Center, SUNY, USA. [3]Biomedical Research Consortium of Chile (BMRC).

Objectives. The estradiol metabolite 2-methoxyestradiol has emerged as a potential anti-tumorigenic agent due to its ability to induce apoptosis in cancer cell lines. However, not all cancer cells have the same susceptibility to cell death. A possible mechanism may be the further metabolism of 2-methoxyestradiol to the inactive forms 2-methoxyestrone, 2-methoxyestradiol-sulfate and -glucuronide.

Methodology. Cell death was measured in the breast cell lines MDA-MB-231 and MCF-7 by MTT assay. Conversion of 2-methoxyestradiol to the inactive metabolites was determined by GS-MS and MS-MS. The role of sulfonation was assessed by the inhibition and over-expression of the enzyme that brings about sulfonation, SULT1A1. Finally, the mRNA levels of sulfonation related enzymes SULT1A1, 1A2 and STS (de-sulfonation) were determined by real time RT-PCR.

Results. 2-methoxyestradiol caused cell death in MDA-MB-231 cells, but not in MCF-7. In MCF-7, 80% of this compound is metabolized to 2-methoxyestradiol-sulfate, whereas in MDA-MB-231 cells 0.01% conversion occurs. In MCF-7, the inhibition of sulfonation causes a decrease in cell death, whereas overexpression of SULT1A1 in MDA-MB-231 prevents loss of viability. In accordance with sulfonation preventing cell death in MCF-7 cells, the transcript levels of SULT1A1 and 1A2 are 5 times greater than in MDA-MB-231.

Conclusions. The ability of 2-methoxyestradiol to induce cell death these breast cell lines is dependent on sulfonation. A positive relation between sulfonation and levels of SULT enzyme may present a rapid screening technique to determine which breast cancers will respond to this drug.

(161) MOLECULAR ANALYSIS OF THE rDNA TRANSCRIPTIONAL REGULATION DURING THE SEASONAL ADAPTATION OF *Cyprinus carpio* FISH. **Fumeron R.,** Dupré G., Molina A., Vera MI., Alvarez M. Laboratorio de Biología Celular y Molecular, Facultad Ciencias Biológicas, Universidad Andrés Bello, Viña del Mar, Chile. malvarez@unab.cl

Introduction: Transcriptional regulation of the ribosomal genes (rDNA) has been described as one of the most important events during carp seasonal acclimatization. Previously, we have demonstrated that rDNA activity is direct correlated with a change of activity and structure of the nucleolus. Moreover, this trait seems to be based in substantial changes in the number of active rDNA genes during carp acclimatization.

Materials and Methods: A carp cell line (*Epithelioma Papulosum Cyprini*, EPC) was grown at 10ºC and 20°C respectively. rDNA activity was estimated by RT-qPCR. Nucleolar chromatin markers for transcriptional activation and/or inactivation were evaluated using ChIP assays. The nucleolar organization was monitored by electron microscopy. The fraction of active and inactive ribosomal genes was compared by psoralen photocrosslinking assays.

Results: In acclimated EPC cells, we observed that rDNA gene reprogramming is reflected as variations of the nucleolar components organization, in the same manner as observed in the whole animal. This phenomenon is accompanied by a dramatic alteration in the fraction of active and/or inactive genes. Additionally, we observed that this feature is consistent with nucleolar chromatin modifications.

Discussion: Our results suggest that the difference in the activity of rDNA is due to a change in the portion of active ribosomal genes. This would be modulated by a change in rDNA chromatin architecture. This work constitutes a new approach to analyze how an organism is able to reprogram its ribosomal genes in response to environmental changes.

DI-UNAB02-10/I.

(162) TWO DIMENSIONAL ELECTROPHORESIS PATTERNS FROM BOTH FERTILE AND INFERTILE *Echinococcus granulosus* CYSTS. **Christian Hidalgo**, Rodolfo Paredes. Laboratorio Salud de Ecosistemas, Escuela de Medicina Veterinaria, Facultad de Ecología y Recursos Naturales, Universidad Andrés Bello. rparedes@unab.cl

Introduction: Cystic Echinococcosis is a disease caused by the metacestode of the flatworm *Echinococcus granulosus*, which produces a cyst in the viscera of the host; these can be either fertile or infertile according to their capability to produce protoscoleces. Causes of cyst fertility have not been fully understood; a proteomic approach could be useful in identifying proteins involved in this process.

Material and Methods: Fertile and infertile cysts were obtained from abattoirs. Cysts were opened and the inner layer was gently scraped to obtain germinal layer cells. IEF was performed on 13 cm 3-10 and 4-7 pH range strips. Second dimension was performed on a 12% SDS-PAGE at 50 mA. Spots were excised and submitted to mass spectrometry.

Results: Fertile cyst patterns (FCP) yielded higher reproducibility, spot quantity and quality when compared to infertile cysts patterns (ICP). Spots in FCP clustered in the acidic range while those in ICP gathered in the basic range. Spots molecular weights ranged between 90 to 25 kDa. 492 spots were excised from gels, of which 41 were identified via mass spectrometry (MS); 15 from FCP and 21 from ICP.

Discussion: FCP and ICP were consistent in both 3-10 and 4-7 pH ranges. MS data from FCP identified several parasite proteins while ICP data matched only bovine proteins; this is a key difference that should be further investigated. MS data from 451 spots didn't match with proteins available in public protein databases.

Financing: FONDECYT Iniciación N° 11070082 and Project DI 56-11/R-UNAB.

(163) EXPRESSION AND ACTIVITY OF TcAP1, A REPAIR DNA ENDONUCLEASE, IN *Trypanosoma cruzi*. **Iván Ponce**, Sofia Sepúlveda, Lucía Valenzuela, José Delgadillo, Santiago Ramírez, Soledad Sierra, Paula Bahamondes, Natalia Muñoz, Ulrike Kemmerling, Gonzalo Cabrera, Norbel Galanti. Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile. ivponce@ciq.uchile.cl

Introduction: *T. cruzi* is able to survive reactive species present in both, the insect vector and the host mammal. Survival may be achieved by repair of the parasite DNA via the base excision repair pathway (BER). In this work we show the activity of an apurinic/apyrimidinic endonuclease (AP) in *T. cruzi* (TcAP1) and its expression in the 3 cellular forms of the parasite as well as in epimastigotes exposed to oxidative agents.

Materials and Methods: To detect endonuclease activity a 22-mer oligonucleotide was synthesized with an abasic site at position 7. The 5´ end was labeled with ^{32}P and aligned with the non labeled complementary strand. This oligo was incubated with epimastigote, amastigote and trypomastigote homogenates and the resulting fragments were evidenced by electrophoresis in denaturing conditions and autoradiography. The presence and expression levels of TcAP1 were determined in homogenates from the three cellular forms of the parasite by western blot using a specific antibody as well as in epimastigotes exposed to H_2O_2.

Results: Epimastigotes, trypomastigotes and amastigotes presented AP endonuclease activity evidenced by a 7-mer fragment. TcAP1 is expressed in the three cellular forms though it does not increase in epimastigotes exposed to H_2O_2 under the experimental conditions applied.
Conclusions: Previous results showing DNA damage and repair in *T. cruzi* epimastigotes and our present results confirm that this parasite is able to resist oxidative stress maintaining a chronic presence in the hosts.
Acknowledgements: FONDECYT 1090124 (NG) and 11080166 (UK). Bicentenary Ring Project ACT 112. CONICYT Fellowship for PhD Thesis 24110156 (SS).

(164) MULTIPONTECIALITY OF HUMAN YOLK SAC ENDODERMAL CELLS. **Sulz L,** Godoy C, Pereda J. Escuela de Medicina, Universidad de Santiago de Chile, Santiago, Chile. lorena.sulz@usach.cl, carlos.godoy@usach.cl, jaime.pereda@usach.cl (Sponsor: P. Orihuela)
Introduccion: The first erythroid cells, identified as hemangioblast, that give rise to endothelial and blood cells are generated from the mesodermal compartment of the human yolk sac wall (YS). This hematopoietic function of the YS is crucial for the human embryo growth and survival. However, how the hemangioblast generate and differentiate is still a matter of debate. Based on our results we postulate that the hemangioblast, the common precursor of hematopoietic and endothelial cells, is originated by the differentiation of multipotent endodermic cells present in the endodermal compartment of the YS walls.
Material and Methods: Human YS samples between weeks 4 and 8 of development, obtained from the Human Embryofetology Collection of the Facultad de Ciencias Médicas de la USACH were selected. Optic microscopy was used to evaluate the morphology of the YS, and in order to determinate the expression of specific markers for endoderm (AFP), mesoderm (Bry) and hemangioblast (CD31, CD34 and KDR), immunohytochemistry was performed.
Results: We showed that cells of the endodermal compartment of the YS walls co-expressed endoderm and mesoderm markers, suggesting a transitional stage of differentiation from endodermic to mesodermic cells. In addition, some endodermic cells also expressed the hematopoietic markers CD34, CD31 and KDR.
Discussion: Based on our results we postulate that in the human YS wall, the precursor cells that originate the hemangioblast, are differentiated from the endodermal epithelial cells.
Support by DICYT 021001PT.

(165) INSULIN STIMULATION OF HUMAN CATIONIC AMINO ACID TRANSPORTER 1-MEDIATED L-ARGININE TRANSPORT INVOLVES A_{2A} ADENOSINE RECEPTORS IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS. **Guzmán-Gutiérrez E**, Westermeier F, Salomón C, Leiva A, Casanello P, Sobrevia L. Cellular and Molecular Physiology Laboratory (CMPL) & Perinatology Research Laboratory (PRL), Division of Obstetrics and Gynecology, School of Medicine, Faculty of Medicine, Pontificia Universidad Católica de Chile, P.O. Box 114-D, Santiago, Chile. elguzman@uc.cl
Introduction. Insulin increases human cationic amino acid transporter-1 (hCAT-1) expression and activity, and *SLC7A1* (for hCAT-1) transcriptional activity in human umbilical vein endothelial cells (HUVEC). Adenosine also stimulates L-arginine transport and increases insulin sensitivity in mammalian cells. We assayed whether A_{2A} adenosine receptors are required for insulin effect on L-arginine transport in HUVEC.
Material and Methods. L-Arginine transport (0-1000 μM, 3 μCi/ml L-[^{3}H]arginine, 37ºC, 1 minute), hCAT-1 protein abundance (western blots) and *SCL7A1* promoter transcriptional activity (-1606 and -650 bp constructs from ATG) were measured in HUVEC primary cultures (passage 2). Cells were cultured in absence or presence (8 hours pre-incubation) of insulin (1 nM), or ZM-241385 (10 nM, A_{2A} adenosine receptor antagonist).
Results. L-Arginine transport maximal velocity increases by insulin (~2.1-fold), 5'-N-ethylcarboxamidoadenosine (NECA 30 nM, A_1/A_{2A} agonist) (~2.1-fold), insulin+NECA (~4.0-fold), without altering significantly the apparent K_m values. hCAT-1 protein abundance were also increased by insulin (~1.5-fold), NECA (~1.5-fold), insulin+NECA (~1.9-fold). hCAT-1 mRNA expression and *SLC7A1* promoter (-1606 and -650) activity were increased by insulin (~10 and ~1.3-fold, respectively). Insulin and NECA effects were abolished by ZM-241385.
Discussion. Insulin effect requires A_{2A} adenosine receptors activation to stimulate hCAT-1 expression and activity in HUVEC.
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(166) TONIC INHIBITION OF THE RhoA/ROCK PATHWAY IS NOT PART OF THE MECHANISMS UNDERLYING MYOMETRIAL QUIESCENCE DURING PREGNANCY. **Garmendia LR,** Delpiano AM, Poblete JA, Cuello MA, Carvajal JA. Laboratorio de Medicina Materno-Fetal. Departamento de Obstetricia y Ginecología, Facultad de Medicina, Pontificia. U. Católica de Chile. liliana.garmendia.c@gmail.com
Introduction: The mechanisms underlying pregnancy myometrial quiescence are poorly understood. We proposed that the tonic inhibition of the myometrial RhoA/ROCK pathway plays a main role in maintaining myometrial quiescence. We also postulate that RhoA/ROCK inhibition is produced by over-expression of Rnd proteins.

Materials and Methods: Myometrium was obtained at the time of cesarean sections from four groups of pregnant women: preterm not in labor (PT-NL), preterm in labor (PT-L), term not in labor (T-NL) and term in labor (T-L). (preterm 30-34 weeks; term 38-41 weeks). mRNA and protein levels for RhoA, ROCK (I and II) and Rnd (1, 2, 3) were evaluated by semi-quantitative RT-PCR (n=6) and Western Blot (n=8). ROCK activity was studied by phosphorylation assay of a specific substrate (MYPT1).
Results: mRNAs for RHOA, ROCK I-II, Rnd1, Rnd2 and Rnd3 were identified in all groups, in similar levels. Only Rnd2 mRNA was significantly increased during quiescence (PT-NL). The studied proteins were present in all groups. Both RhoA protein and ROCK activity increased in labor samples, either preterm (PT-L) or term (T-L).
Discussion: RhoA/ROCK pathway (protein levels or activity) was not inhibited during quiescence. Rnd proteins were not increased during quiescence. These results do not support the role of the inhibition of RhoA/ROCK pathway in the maintenance of human myometrial quiescence. The increased RhoA/ROCK activity during labor suggests a role for this pathway in the process of cell contraction.
Financial Support: FONDECYT 1090616.

(167) ASSESSMENT OF THE A357D ACID SPHINGOMYELINASE GENE MUTATION FREQUENCY IN THE CHILEAN POPULATION. **Pérez MJ**[1], Robledo F[1], Castro J[1], Martínez P[1], Acuña M[1], Schuchman E[2], Miquel JM[1], Mabe P[3], Zanlungo S[1]. [1]Gastroenterology Department, Medicine Faculty, Pontificia Universidad Católica de Chile, [2]Department of Genetics & Genomic Sciences, Mount Sinai School of Medicine, New York, [3]Neurology Unit, Dr. Exequiel González Cortés Children's Hospital, Santiago de Chile. mjperez@uc.cl
Introduction: The deficiency of acid sphingomyelinase leads to Niemann-Pick B (NPB), an autosomal recessive lysosomal disorder secondary to sphingomyelin storage. NPB is one of the most frequent lysosomal storage diseases in Chile with at least 40 confirmed cases and is intriguing for our population since only one mutation, A357D, has been identified and seems to be privative to Chilean patients. Considering this, a founder effect is likely. Our interest focuses on revealing the frequency of this mutation in the general Chilean population and in our ancestral population, the Mapuches.
Material and Methods: A DNA bank drawn from Chilean Hispanic and Mapuche cohort who have participated in a Health Survey conducted by the Gastroenterology Department, PUC, was used. We used the Taqman allelic discrimination method to detect the A357D mutation and a Student´s t–test for statistical analysis.
Results: We found a higher frequency of the A357D mutation in the Chilean Hispanic population (prevalence of 1/53) than that expected by the number of confirmed cases of NPB. Furthermore, Mapuche individuals have a mutation frequency (prevalence of 1/14) significantly higher than the Hispanic individuals.
Discussion: Our results suggest that in Chile NPB might be much frequent than expected and with an origin strongly related to the Mapuche ethnia.
Supported by Genzyme; FONDAP Genome Regulation 15090007; Fondecyt 1080325 (JFM).

(168) PHYSICAL AND FUNCTIONAL ASSOCIATION OF GLYCOPROTEIN VI (GPVI) AND TISSUE FACTOR (TF) IN HUMAN PLATELETS. **Valenzuela JG**, Panes O, Román P, Pereira J, Mezzano D, Matus V. Department of Hematology-Oncology, School of Medicine, P. Catholic University of Chile. (Sponsor: V. Velarde)
Introduction: GPVI is a transmembrane collagen receptor expressed in blood platelets and megakaryocytes. Activation by its ligand triggers signaling, strong adhesion and platelet activation. Platelets contain TF co-localized with GPIbα in membrane lipid rafts (LR), cholesterol-rich domains that facilitate protein interaction and signaling. GPIbα interacts with GPVI and is essential for platelet TF-dependent pro-coagulant activity (PCA). Although GPVI has been found in LR, its role in platelet PCA is unknown.
Aims: To evaluate the relationship among GPVI, TF and GPIbα and the role of GPVI in platelet PCA.
Methods and Results: We used normal platelets and platelets from 2 unrelated bleeding patients with a novel mutation predicting a truncated GPVI without cytoplasmic region, which were unresponsive to its natural ligand, collagen. Normal platelets expressed GPVI, TF and GPIbαmRNAs (multiplex RT-PCR). Western blots disclosed the presence of the 3 GP´s in platelet membranes. In the patients, a smaller band of GPVI was detected. GPVI with TF, but not with GPIbα, co-precipitated (immunoprecipitation assay). The activity of TF was normal in patients and controls, and was unaffected in normal platelets pre-incubated with anti-GPVI antibodies.
Conclusions: Normal GPVI and TF are physically associated. However, this association is unrelated to the expression of platelet PCA, as observed in patients with dysfunctional GPVI and in antibody-blocked GPVI. The relevance of the GPVI-TF in platelet function is being studied.
FONDECYT 1100833.

(169) INTEGRIN EXPRESSION DURING THE MENSTRUAL CYCLE IN THE FALLOPIAN TUBE. **Soto V.**[1], Solar P. [1,2], Cárdenas H.[1.2], Velásquez LA.[1,2]. [1]Laboratorio de Inmunología de la Reproducción, USACH. [2]Centro para el Desarrollo de la Nanociencia y la Nanotecnología (CEDENNA). (Sponsor: R. Moreno).

Introduction. Roles of the Falopian tube (FT) include the establishment of an appropriate environment for gamete migration and fertilization. Despite a role for integrins in those processes is not known, tubal implantation is a recurrent and important condition that may involve integrins.
Methods. The pattern of expression of 16 integrins during the menstrual cycle was determined in FTs obtained during early follicular, periovulatory and luteal phases in 4 patients each. Fallopian tubes were obtained from patients submitted to elective surgical sterilization for reasons unrelated to this study. The Ethics Committees of Hospital San José and Universidad de Santiago approbed the protocols and informed consent documents. Real time PCR for determination of integrin expression was performed according to standard protocols with primers obtained from SA Bioscience (USA). Bioinformatic analysis was performed by Gene network and KEEG Pathways Database software.
Results. Expression of all integrins but ITGB1 was periovulatory > follicular > luteal phase. The integrins identified have been involved in leucoyte migration, signal transduction and cell adhesion.
Discussion. Tubal integrins appear to be regulated by changes in sex steroids during the menstrual cycle, which suggests they are involved in tubal physiology. They included that participate in cell adhesion and therefore may have a role in tubal implantation.

(170) SURFACE ADAM17 IS RELATED TO XENOESTROGENS–INDUCE GERM CELLS APOPTOSIS. **Paulina Urriola-Muñoz**, Raúl Lagos-Cabré, Magdalena Díaz and Ricardo D Moreno. Pontificia Universidad Católica de Chile, Facultad de Ciencias Biológicas, Departamento de Fisiología. paurriol@uc.cl
Introduction: Plasticizers with estrogenic activity such as Bisphenol A (BPA) and 4-nonilphenol (NP), disrupt spermatogenesis by a still unknown mechanism. In our lab, we have showed that ADAM7 and 10 metalloproteases participate in physiological and genotoxic induced-germ cells apoptosis. The aim of our work was to determine if apoptosis induced by xenoestrogens in rat spermatogenesis is related with a cell surface localization and activation of ADAM17.
Materials and Methods: 21 day-old rats were intraperitoneally injected with 50 mg/kg of BPA or NP for different times. Apoptosis and ADAM17 localization was evaluated by western blot and immunohistochemistry. *In vitro* we set up a cell line transfected with a vector encoding a fusion protein encompass Neuregulin β1 and phosphatase alkaline.
Results: BPA and NP induced a germ cell apoptosis as evaluated by increase in cleaved PARP and caspase-3. Interestingly, spermatogonia and spermatocytes responded differentially to this treatment. Immunoisolated cell surface ADAM17–positive cells had high cleaved PARP levels and mature ADAM17 levels after BPA and NP treatment. To determinate ADAM17 activity, we set up a stable transfected cell line. We found that LnCap cells line does not express endogen Neuregulin β1 and they do express ADAM17. So far we have successfully transiently transfected this cell line and detected expression of the gene.
Discussion: Our results show that surface targeting of ADAM17 is related to its role in apoptosis after xenoestrogens treatment in rat spermatogenesis.
FONDECYT 1110778.

POSTER PRESENTATIONS III

(171) RUNX2 REGULATES MMP EXPRESSION AND MODULATES CELL MIGRATION AND INVASION IN OSTEOSARCOMA CELLS. **Karina Villegas**[1,2], Oscar Vega[1,2], Marcela Hernández[3], Jorge Gamonal[3], Gary S Stein[4], Andre van Wijnen[4] and Mario Galindo[1,2]. [1]Millennium Institute on Immunology and Immunotherapy, [2]Programa de Biología Celular y Molecular, ICBM, Facultad de Medicina, Universidad de Chile. [3]Laboratorio de Biología Periodontal, Facultad de Odontología, Universidad de Chile. [4]Departament of Cell Biology and Cancer Center, University of Massachusetts Medical School, Worcester, Massachusetts. mgalindo@med.uchile.cl

Introduction: Runx2 transcription factor is a master regulator of osteogenesis. Interestingly, Runx2 is over-expressed in osteosarcoma (OS) cells, which is associated with poor prognosis. MMPs expression has been associated with increased migratory and invasive capacity of OS cells. Here, we show that Runx2 modulates MMPs expression in osteosarcoma.

Material and Methods: We analyzed MMPs (MMP2, MMP9 and MMP13) expression in six osteosarcoma cell lines. Specifically, mRNA, protein levels and enzymatic activity were evaluated by RT-PCR, western blot and zymography or fluorimetric assay, respectively. Cell migration was analyzed by wound healing assay and cell invasion using matrigel. We tested the functional role of Runx2 to enhance MMPs expression by modulated Runx2 levels using adenoviral vectors and siRNA.

Results: Runx2 positively modulates MMPs expression in osteosarcoma. This, correlates with increased migratory and invasive potential of OS cells.

Discussion: These results suggest that Runx2 could be modulating cell migration and invasion in OS by enhancing the expression of MMPs.

Grant sponsors: Fondecyt 1095234 and Iniciativa Científico Milenio P09-016-F.

(172) DIFFERENTIAL EXPRESSION OF GENES RELATED TO AUTOPHAGY AND METASTASES IN ADVANCED OVARIAN CANCERS. [1,5]**Racordon D**, [1,5]Erices R, [1,5]Bravo M, [1,5]Gonzalez P, [3]Bustamante E, [4]Pizarro J, [2,5]Kato S, [2,5]Cuello M.A, [1,5]Owen G. I. [1]Faculty of Biological Sciences, [2]Faculty of Medicine, Pontificia Universidad Católica de Chile. [3]FALP, [4]Hospital Sotero del Rio, [5]Biomedical Research Consortium of Chile.

Introduction. Epithelial ovarian cancer is the gynecological disease with the highest rate of mortality, with a 5-year survival less than 30%. Ovarian cancer is morphologically and biologically a heterogeneous disease, making it difficult to define molecular profiles associated with early diagnosis, development and progression. In search of biomarkers and drugable targets we evaluated in ovarian tissue of healthy and oncolologic origin, the expression of genes involved in autophagy and metastasis, processes related to ovarian tumor suppression and progression respectively.

Methods. We evaluated mRNA levels of recognized autophagy associated genes, together with a panel of coagulation genes and known prognostic cancer biomarkers in fifty samples of healthy ovarian tissue obtained from hysterectomy and ovarian cancer (both tumor and ascites, peritoneal fluid) using real-time PCR. Messenger levels were normalized against constitutive genes (HPRT-1, 18s and PBGD).

Results. Four autophagy related genes were significantly downregulated in ovarian tumours. Unlike breast cancers, no increase was observed in the levels of Tissue Factor gene or EGFR in ovarian tumors. A clear differential expression pattern of mRNA levels was noted between normal and oncologic samples.

Conclusion. It is possible to establish a reliable and differential pattern of gene expression to permit distinguish between normal from oncologic ovarian tissue. Elucidation of these genes in cancer progression and metastasis may permit earlier diagnostic and identification of new therapeutic targets.

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(173) EFFECT OF IGF2 OVEREXPRESSION ON TUMORIGENICITY OF HUMAN OVARIAN CARCINOMA CELLS. **Jurriaan Brouwer-Visser**, Suzan K. Chao, Ricardo D. Moreno and Gloria S. Huang. Albert Einstein College of Medicine, Bronx, New York and Pontificia Universidad Católica de Chile. jurriaan.brouwer@einstein.yu.edu

Introduction. The purpose of this study was to determine the effect of IGF2 overexpression on the tumorigenicity of human ovarian cancer cells.

Methods. HEY cells were stably transfected with IGF2 or no insert (EV) constructs. qPCR and ELISA were performed to measure the IGF2 mRNA and protein expression levels, respectively. Tumorigenicity was evaluated in athymic nude mice by subcutaneous injection of $1*10^6$ cells with matrigel, followed by monitoring of in vivo tumor growth by caliper measurements.

Results. The IGF2 mRNA level was 5604 ±617 fold-change higher in HEY-IGF2 cells relative to HEY-EV cells ($P<0.05$). The amount of IGF2 protein secreted in 48 h was 5159 ng/100.000 HEY-IGF2 cells (51.6 pg/cell), and was not detectable in the conditioned media of HEY-EV cells. After 4 weeks, the mean tumor volume of subcutaneous xenografts following cells injected with matrigel, the mean tumor volume was 1608 ±330 mm^3 versus 705 ±95 mm^3 for HEY-IGF2 and HEY-EV xenografts, respectively ($P<0.05$, N=6 per group). In the HEY-IGF2 xenografts, overexpression of IGF2 relative to HEY-EV xenografts was maintained.

Discussion. Stable overexpression of IGF2 in HEY ovarian cancer cells resulted in increased xenograft size. Further evaluation is underway to fully characterize the mechanisms by which IGF2 overexpression promotes tumor growth.

(174) ONCOGENIC KINASE AURORA-A DECREASES STABILITY OF THE TRANSCRIPTIONAL CO-REPRESSOR SKI. **Solange Rivas[1],** Jocelyn Mosquera[1], Leandro Torres[1,4], Ricardo Armisén[2,4], Julio C. Tapia[3,4], Michael J Hayman[5], Katherine Marcelain[1,4]. [1]Programa de Genética Humana, [2]Fisiopatología, [3]Biología Celular y Molecular, ICBM, Facultad de Medicina, Universidad de Chile. [4]Centro de Estudios Moleculares de la Célula, Facultad de Medicina, Universidad de Chile. [5]Microbiology and Molecular Genetics Department, Stony Brook University, USA. kmarcelain@med.uchile.cl (Sponsor: J.C. Tapia)

Introduction: The transcriptional co-repressor Ski participates in chromosomal segregation during mitosis. At this stage, Ski is phosphorylated by the oncogenic kinase Aurora-A (AURKA), but the effect of that phosphorylation is currently unknown. In this work we investigated whether that phosphorylation had an effect on the stability of Ski.

Material and Methods: Residues phosphorylated by AURKA in Ski were identified by *in vitro* kinase assays and Mass Spectrometry. S>A and S>D mutants were generated by site-directed mutagenesis and half-life of mutated proteins was assessed by cycloheximide treatment and western blotting. Centrosomes number and mitotic spindle polarity were evaluated by immunofluorescence in *Ski -/-* MEFs and in U2OS cells expressing a Ski targeting shRNAs.

Results: We identified Ser326 and Ser383 as targeted by AURKA *in vitro*. Flag-SkiS326A and S383A showed increased stability and pharmacological inhibition of AURKA increased levels of endogenous Ski. Thus, phosphorylation of Ski by AURKA is required for Ski´s degradation. Importantly, reduced levels of Ski resulted in centrosomal amplification and clustering and multipolar mitotic spindles in MEFs and U2OS cells.

Discussion: Overexpression of AURKA (found in several types of cancer) results in centrosomal amplification and multipolar spindles, same as in cells with decreased levels of Ski. We propose that targeting Ski for degradation might be important in the oncogenic mechanism of AURKA.

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(175) PHYSICOCHEMICAL AND CYTOTOXICOLOGY CHARACTERIZATION OF POLYFUNCTIONAL POLYMERIC NANOPARTICLES DESIGNED FOR CANCER TREATMENT AND DIAGNOSIS. **Solar P**.[1,2], Vilos C.[1,2], Juica N.[1], Moreno M.[2], González G.[2], Cárdenas H.[1,2], Velásquez LA.[1,2]. [1]Laboratorio de Inmunología de la Reproducción, USACH. [2]Centro para el Desarrollo de la Nanociencia y Nanotecnología (CEDENNA)**.** paula.solar@usach.cl (Sponsor: R. Moreno).

Introduction. Some types of cancer have been associated with bacterial infections, so that elimination of those infections needs to be included in the therapeutical management of cancer. We designed nanoparticles with multifunctional properties that may allow to eliminate both the cancer cells and the associated bacteria. For this purpose we developed PHBV polymer nanoparticles functionalized with superparamagnetic iron oxide (SPION) and ceftiofur (a cephalosporine). The nanoparticles were examined to determine their structure and cytotoxicity.

Materials and Methods. The nanoparticles were synthesized by the double emulsion system phase. All nanoparticles were analyzed by spectroscopy and by Differential Scanning Calorimetry (DSC). Cytotoxicological characterization was performed in HepG2 cells, examining cell viability using the tetrazolium salt MTS (Promega), and the system Live/Dead of MolecularProbes (Invitrogen). All cells were treated with concentrations ranging from 0.1 to 10,000 ug/ml of each nanoparticle (PHBV-ceftiofur, PHBV alone, PHBV-SPION, PHBV-ceftiofur-SPION).

Results. A model for the molecular structure of the nanoparticle was built by means of spectroscopy and DSC data. We determined that the nanoparticle of PHBV-ceftiofur decreased cell viability by 50% at the highest concentration.

DiscussionThe molecular structure of these nanoparticles reveals a new composite. PHBV nanoparticles functionalized with ceftiofur and SPION are not cytotoxic at the tested concentrations, so it may now be examined for their potential usefulness in diagnosis and treatment of certain types of cancer.

(176) A LENTIVIRUS ENCODING RNAI TARGETING A NON-CODING MITOCHONDRIAL RNA INHIBITS MELANOMA TUMOR GROWTH AND METASTASIS IN A MOUSE MODEL *In vivo*. **Manuel Varas**[1,2,3], Alvaro Lladser[1], Nicole Farfan[1], Pablo D.T. Valenzuela[1,2,3], Luis O. Burzio[1,2,3]. [1]Fundación Ciencia para la Vida; [2]Andes Biotechnologies S.A; [3]Facultad de Ciencias Biológicas, Universidad Andrés Bello. manu.varas@uandresbello.edu

Introduction. We have described a family of non-coding mitochondrial RNAs (ncmtRNA) differentially expressed in normal and tumor cells. Normal proliferating cells express both sense and antisense ncmtRNAs. In contrast, tumor cells express sense ncmtRNA and down-regulate antisense ncmtRNA. Previously, we have shown that a lentivirus encoding RNAi against ncmtRNA (Lv-ncntRNA) induces apoptosis in both human and mouse cancer cells *in vitro*. The aim of this study was to determine whether *in vivo* administration of Lv-ncmtRNA could inhibit melanoma tumor growth and metastasis in a mouse model.

Material and Methods. C57BL/6 mice were injected subcutaneously with 10^6 B16F10 cells and seven days later treated every two days with five intratumoral administrations of $4x10^7$ TU of Lv-ncmtRNA or a lentivirus-RNAi targeting luciferase (Lv-control). Tumor size and weight were measured over the treatment time. For lung metastasis assay, C57BL/6 mice were injected intravenously with $4x10^5$ B16F10 cells and four days later treated with four intravenous administrations of $4x10^7$ TU Lv-ncmtRNA or Lv-control three days apart. Mice were sacrified and metastatic foci counted in the lungs.

Results. Mice treated with Lv-ncmtRNA showed a 4-fold decrease in subcutaneous tumor growth and 4-fold decrease in the number of metastatic foci as compared to mice treated with Lv-control.
Discussion. These results suggest that intratumoral and systemic administration of a ncmtRNA-targeting RNAi represents a promising strategy awards the development of potencial cancer therapies.
Financed by CONICYT PFB-16; UNAB DI-11-11/I.

(177) RAB5 ACTIVATION IS REQUIRED FOR CAVEOLIN-1 ENHANCED TUMOR CELL MIGRATION. **Jorge Díaz[1, 2],** Lisette Leyton[1], Andrew F.G. Quest[1], Vicente Torres[1,2]**.** [1]Centro de Estudios Moleculares de la Célula, Facultad de Medicina, Universidad de Chile. [2]Laboratorio de Biología Celular y Molecular, Departamento de Ciencias Básicas y Comunitarias, Facultad de Odontología, Universidad de Chile. aquest@med.uchile.cl, vatorres@med.uchile.cl
Introduction: The small GTPase Rab5 is a key regulator of the early endosome implicated in integrin trafficking and cell migration. Caveolin-1, a membrane protein involved in the invaginations of the plasma membrane called caveolae, also participates in vesicle trafficking and bind to Rab5. Additionally, Caveolin-1 has been suggested to promote tumor cell migration. Mechanisms by which Caveolin-1 and Rab5 favor migration remain unclear. In particular, whether Rab5 is a relevant intermediate in the events by which Caveolin-1 promotes migration of metastatic cancer cells is not known.
Methodology: The human colon adenocarcinoma HT-29(US) and B16F10 mouse melanoma cell lines expressing or not Caveolin-1 were infected with a lentivirus encoding shRNAs for Rab5. Rab5 activity was measured by using the GST-R5BD pull down assay and cell migration was evaluated in the wound-healing assay.
Results: Caveolin-1 expression in HT-29(US) and B16F10 cells promoted the migratory capacity of both cells. Also, Rab5 activation increased upon Caveolin-1 expression**.** Alternatively, shRNA-mediated Rab5 knock-down reduced Caveolin-1-dependent cell migration.
Conclusions: Rab5 expression and activation are required for Caveolin-1 enhanced cell migration *in vitro* in both cell lines. Future studies will elucidate the mechanism of Rab5 activation by Caveolin-1 and the extent to which these mechanisms are important for metastasis *in vivo*.
Acknowledgements. This work was supported by FONDECYT-FONDAP 15010006, FONDECYT 1090071 (AFGQ), FONDECYT 1070699 (LL), FONDECYT 11100287 (VT) and a CONICYT PhD Student Fellowship (JD).

(178) NEURAL TENEURINS ARE EXPRESSED IN HUMAN TUMORS AND TUMOR-DERIVED CELL LINES. [1]**Ziegler A.,** [1]di Capua G., [1]Oyarzún J.E., [2]Roa I.E., [3]Brañes J.A., [3]Casanello P., [1]Repetto G. [1]Center for Human Genetics, Faculty of Medicine, Clínica Alemana-Universidad del Desarrollo; [2]Division of Pathology, Clínica Alemana de Santiago; Perinatology [3]Research Laboratory (PRL) & Cellular and Molecular Physiology Laboratory (CMPL), Division of Obstetrics and Gynaecology, School of Medicine, Pontificia Universidad Católica de Chile. aziegler@udd.cl (Sponsor: C. Cabello)
Introduction: Teneurins are a family of transmembrane proteins involved in development of the central nervous system. We previously detected teneurin-2 expression in malignant mesothelioma and proposed a potential role as tumor biomarker.
Purpose: We evaluated 12 human tumor-derived cell lines to identify additional tumor types that express teneurin-2 or teneurin-4. Based on the evidence obtained, we analyzed human ovarian tumors for the presence of teneurin-2 and teneurin-4 to assess the patterns and frequency of expression.
Methods: In cell lines, expression of teneurin mRNAs was assessed by RT-PCR. Teneurin protein expression was detected by immunohistochemistry. Expression of teneurins in frozen tumors and control tissues was determined by comparative real-time RT-PCR and immunohistochemistry.
Results: In cell lines, teneurin-2 and teneurin-4 mRNAs were expressed in ovarian and breast cancer cells. Multiple teneurin transcript variants could be identified. The presence of teneurin mRNAs correlated with expression of teneurin proteins. In a preliminary set of ovarian tumors, teneurin-4 was detected in 6/8 tumors. Immunohistochemistry revealed a membrane localization pattern in tumors but not in surrounding stroma.
Discussion: We describe the expression of teneurins in ovarian and breast cancer cell lines. Seventy five percent of human ovarian tumors expressed teneurin-4. Some teneurins might harbor potential as tumor biomarkers.
Supported by Fondecyt 1100605.

(179) INHIBITION OF CAVEOLIN-1 PHOSPHORYLATION ON TYROSINE-14 IS ASSOCIATED WITH REDUCED MIGRATION, INVASION AND METASTASIS OF B16F10 MELANOMA CELLS. **Ortiz R.J*,** Urra H*, Lobos, L., Leyton L*, Quest AFG*. *Laboratorio de Comunicaciones Celulares, Centro de Estudios Moleculares de la Célula (CEMC), Facultad de Medicina, Universidad de Chile. aquest@med.uchile.cl
Introduction: Caveolin-1 is a membrane protein that reportedly functions as a tumor suppressor during early stages of tumor development and later on as a promoter of metastasis. The latter traits are related to the ability of caveolin-1 to promote cell migration, a process which is thought to depend on tyrosine-14 phosphorylation by Src family kinases. Here we evaluated the importance of tyrosine-14 phosphorylation in promoting cell migration and invasion *in vitro*, as well as metastasis in an *in vivo* melanoma model.
Methods: B16F10 mouse melanomas were stably transfected to generate B16F10(Cav-1) cells. Cell migration was evaluated in transwell assays and invasion in matrigel assays. PP2 was employed to inhibit Src-family kinases. The effect of

B16F10(Cav-1) cell pre-treatment with PP2 was tested *in vivo* in isogenic C57BL/6 mice by evaluating lung metastasis following intravenous injection of 200.000 cells.
Results: Caveolin-1 enhanced migration and invasion *in vitro* of B16F10 cells was reduced in the presence the Src-family kinase inhibitor PP2. *In vivo*, pre-treatment of B16F10 (Cav-1) cells with PP2 significantly reduced lung metastasis in C57BL/6 mice.
Conclusions: The results suggest that phosphorylation of Caveolin-1 on tyrosine-14 by Src family kinases is required for Caveolin-1 to promote characteristics associated with enhanced malignancy of B16F10 melanoma cells.
Acknowledgements. This work was supported by FONDAP 15010006 (AFGQ), FONDECYT 1090071 (AFGQ), FONDECYT 1070699 (LL) and a CONICYT PhD Student Fellowship (RJO).

(180) RUNX2 EXPRESSION MODULATES ADHESION OF OSTEOSARCOMA CELLS TO HUMAN PULMONARY ENDOTHELIAL CELLS AND POSITIVELY CORRELATES WITH METASTATIC POTENTIAL. **Francisco Villanueva[1,2],** Óscar Vega[1,2], Mercedes Lopez[1,3], Flavio Salazar-Onfray[1,3], Gary Stein[4], Andre van Wijnen[4], Mario Galindo[1,3]. [1]Millennium Institute on Immunology and Immunotherapy, [2]Programa de Biología Celular y Molecular, [3]Programa Disciplinario de Inmunología, ICBM, Facultad de Medicina, Universidad de Chile. [4]Department of Cell Biology and Cancer Center, University of Massachusetts Medical School, USA. mgalindo@med.uchile.cl
Introduction: Osteosarcoma (OS) is a malignant tumor with elevated incidence of lung metastasis. Runx2 transcription factor regulates bone differentiation and it is aberrantly expressed in tissue samples and cell lines of OS. We propose that Runx2 promotes lung metastasis by enhance cell adhesion of OS cells to pulmonary endothelial cells.
Material and Methods: We analyzed Runx2 protein and mRNA levels in six human OS cell lines. Capacity of human OS cells to generate lung metastasis was evaluated in a NOD/SCID mice model. We tested the functional role of Runx2 to enhance cell adhesion of OS cells to human pulmonary endothelial cells (HPMECs) by modulated Runx2 expression using adenoviral vectors and siRNA.
Results: Runx2 expression was positively correlated with capability of OS cells to generated lung metastasis. Consistently, over-expression of Runx2 increase adhesion of OS cells to HPMECs.
Discussion: These finding show that Runx2 plays a key role in OS tumor progression by modulate cells adhesion to endothelial cells, which is a key step in metastasis process.
Grant sponsors: Fondecyt 1095234 and Iniciativa Científico Milenio P09-016-F.

(181) REGULATION OF ENDOTHELIN CONVERTING ENZYME-1 (ECE-1) EXPRESSION BY THE CYCLOOXYGENASE-2/PROSTAGLANDIN E2/β-CATENIN AXIS IN COLON CANCER CELLS. **Eduardo Silva[1]**, Pablo Cabello[1], Luis R. Cataldo[1], Roger Yefi[1], Ignacio Niechi[1], Daniela P. Ponce[1], Ricardo Armisen[2], Cristina Fernandez[3] & Julio C. Tapia[1,2]. [1]Cell Transformation Laboratory, [2]Institute of Biomedical Sciences (ICBM), Faculty of Medicine, and [3]Department of Anatomopathology, HCUCH, University of Chile. jtapia@med.uchile.cl
Introduction: Overexpression of cyclooxygenase-2 (COX-2) in colon cancer cells leads to increased production of prostaglandin E2 (PGE2), which subsequently enhances the β-catenin activity and ultimately increases the expression of genes promoting tumor development. One interesting putative gene is ECE-1, which is crucial in the endothelin-1 production and displays known cancer-related effects in several cell models. Thus, we studied whether the COX-2/PGE2/β-catenin axis up-regulates the ECE-1 expression.
Methods: Positive modulation of β-catenin activity in both HEK-293T and DLD-1 colon cancer cells was performed by ectopically expressing β-catenin, CK2α, AKT-CA (constitutively active) and COX-2, or alternatively incubating with PGE2 and a GSK3β inhibitor. Negative modulation of the pathway was performed by expressing AKT-DN (dominant negative) and siRNA$^{ck2\alpha}$, or incubating with specific CK2 (TBB) and COX-2 (SC-791) inhibitors. ECE-1, COX-2, survivin and β-catenin mRNA and protein levels were analyzed by RT-PCR and western-blot, respectively.
Results: As anticipated, ECE-1 mRNA and protein levels did change following modulation of β-catenin activity in HEK-293T and DLD-1 cells.
Discussion: Our results suggest that the COX-2/PGE2/β-catenin axis effectively up-regulates the expression of ECE-1. This should have a deep impact on the malignant potential of colon cancer cells.
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(182) REGULATORY T CELLS FROM PATIENTS WITH GASTRIC CANCER PRODUCE IL-10, A CYTOKINE THAT MODULATES NK CELL FUNCTION. **Ribeiro CH**, Kramm K, Bustamante M, Garrido-Tapia M, Hernández C, and Molina MC. Laboratorio de Inmunovigilancia y Evasión Inmune, Programa Disciplinario de Inmunología, Facultad de Medicina, Universidad de Chile. chager@med.uchile.cl
Introduction: Gastric cancer (GC) is the second most common cause of cancer death worldwide. Natural killer (NK) cells play an important role against transformed cells; they express the activating receptor NKG2D, whose ligands are generally expressed on tumors. Interleukin (IL)-10 is an immunoregulatory cytokine involved in malignant diseases. Regulatory T

cells (Tregs) are $CD4^+$ T cells that participate in the suppression of immune responses. High levels of IL-10 and increased frequency of Tregs have been demonstrated in patients with GC, which could favor tumor evasion.
Materials and Methods: We studied the expression of NKG2D ligands (MICA) on tumor tissue cells of gastrectomized GC patients and the expression of IL-10 by Tregs from their peripheral blood. We also evaluated the effect of IL-10 on the expression of MICA by the gastric adenocarcinoma cell line MKN-45 and on its capacity to activate NK cells. All the analyses were performed by flow cytometry.
Results: A subpopulation of $CD4^+CD25^+$ T cells from GC patients significantly expressed higher levels of IL-10 than healthy controls, which correlated with low MICA expression on cells from the tumor microenvironment. MKN-45 cells cultured with IL-10 presented decreased cell surface expression of MICA. NK cell activation was low when challenged with IL-10-treated cells.
Discussion: The immunosuppressive effects of IL-10 on NKG2D ligands, associated with reduced NK cell function, may favor immune evasion by malignant cells.
Grants: Proyecto Fondecyt 3100151 and Proyecto Enlace ENL2010/2012.

(183) STUDY OF THE MIGRATORY CAPABILITY OF TUMOR ANTIGEN PRESENTING CELLS (TAPCELLS) UNDER DIFFERENTIAL EXPRESSION OF CCR7. **Ortiz Carolina**, González F, Mora Gabriela, Salazar-Onfray F. Programa Disciplinario de Inmunología, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile.
Introduction. Recently, a vaccine based on dendritic cells loaded with an allogeneic tumor lysate TRIMEL (TAPCells) has been developed for the treatment of patients with malignant melanoma. These TAPCells are highly efficient in presenting melanoma-associated antigens (MAAs) and therefore generating an anti-tumor immune response. One of the properties that these cells must have is the ability to migrate to lymph nodes. It has been shown that one of the molecules involved in the migration process is the chemokine receptor CCR7, upregulated during the maturation of dendritic cells.
Materials and Methods. In this work we measured the expression of CCR7 in TAPCells, TAPCells + IFN-α and activated monocytes by flow cytometry. We also studied the migration ability through *in vitro* and *in vivo* assays. The *in vitro* assays were performed in *Transwell* double chamber in response to the CCR7 ligand, CCL19. The *in vivo* assays instead were performed in C57/BL6J mice previously depleted of natural killer cells by evaluating the presence of TAPCells and TAPCells + IFN-α in the mice lymph nodes by flow cytometry and immunohistochemistry.
Results. We found that CCR7 is upregulated the during the maturation process induced by TRIMEL. We also showed that both TAPCells and TAPCells + INF-α are able to migrate *in vivo* to lymph nodes and *in vitro* in response to CCL19.
Discussion. All these results, suggest the migratory ability of TAPCells used in immunotherapy, which is critical for the development of an effective anti-tumor immune response.

(184) THE ANTI-INFLAMMATORY SOLUBLE ST2 PROTEIN IS INDUCED BY GLUCOCORTICOIDS IN ULCERATIVE COLITIS. Lucía Núñez-Aguilera, **David Díaz-Jiménez**, Marjorie de la Fuente, Karen Dubois, Francisco López-Kostner, Rodrigo Quera, Marcela Hermoso. Innate Inmunity Lab, Immunology Program, ICBM, Universidad de Chile. lenunez@med.uchile.cl
Introduction: The soluble protein, ST2s, expressed by mast cells, negatively regulates the pro-inflammatory ST2L/IL-33 pathway and is increased in active ulcerative colitis (UC) patients. In atopic dermatitis (AD) patients, plasmatic SST2 levels were associated with polymorphisms in the distal promoter of the *st2* gene (DP-*st2*).Putative consensus glucocorticoid (GC) response elements (GRE) are present in DP-*st2*. Aim: to determine the relation between sST2 to genetic variants of *st2* and GC treatment in UC patients.
Material and Methods: Plasmatic sST2 content and SNPs in DP-*st2* were determined in UC patients receiving or not GC treatment (15 and 28) and controls (76) by ELISA and PCR-sequencing, respectively. GC effect on ST2 was analyzed in mast cells and reporter assays. GC receptor (GR) participation was determined by pharmacological inhibition or hGRa-D428-490 over-expression.
Results: In all patients, a new DP-*st2* was found: -27.076 A/G, besides -27.084 G/C, and -26.999 G/A associated to AD. GC-treated UC patients showed increased plasmatic sST2 that was related to -27.084C/-27.076A/-26.999A haplotype. This haplotypes was related to a higher DP-*st2* GC-dependent transcriptional activity. GC-induced sST2 content decreased with RU-486 or hGRa-D428-490 over-expression *in vitro*.
Discussion: sST2 is a potencial factor contributing to attenuate inflammatory responses in UC and induction by GC might play a role in anti-inflammatory mechanisms involving GR gene regulation. Presence of putative GREs in proximity to SNPs in DP-*st2* suggests that GC treatment contribute to the increase in sST2 depending on patient's genetic background.
FONDECYT 1110381/CLC-DA2011.

(185) *Helicobacter pylori* INCREASES MICA AND ULBP-2 EXPRESSION ON GASTRIC ADENOCARCINOMA CELLS. **Hernández CJ**, Garrido-Tapia M, Kramm K, Ribeiro CH, Molina MC. Laboratorio de Evasión Inmune. Programa Disciplinario de Inmunología (ICBM), Facultad de Medicina, Universidad de Chile. carojimena@gmail.com

Introduction: NKG2D ligands (NKG2DL) MHC related proteins (MIC) and UL-16 binding protein (ULBP) are inducible molecules that respond to cellular stress that are highly expressed in tumors. Their recognition allows damage cells to be eliminated by Natural Killer (NK) cells. Some tumors can modify the expression and evade cytotoxicity. The influence of Helicobacter pylori (HP) infection on gastric cancer (GC) development has not been associated, but its influence to chronic inflammation has been well established. HP and Lipopolysaccharide (LPS) activate Toll like receptor (TLR) 2 and 4, inducing inflammatory response. Our aim was to describe the effect of HP and LPS on NKG2DL expression on gastric adenocarcinoma cells.
Materials and Methods: Gastric adenocarcinoma cells were treated with LPS or heat killed HP (HKHP). NKG2DL expression was analyzed by flow cytometry, while MICA and ULBP2 expression was also analyzed by RT-PCR. Results: We observed that LPS and HKHP induce MICA and ULBP2 surface expression. Both bacterial molecules are synergic in their effect. Other NKG2DL have not been affected by both treatments.
Discussion: Bacterial molecules, such as LPS, are potent immune activators, but its influence in NK response to tumor has not been yet described. HP infection is highly prevalent and it has been associated to GC. In this work we demonstrate that HKHP induces MICA and ULBP-2 surface expression and this could compromise NK cell cytotoxic response, since chronic stimulation of NKG2D induces anergy of cytotoxic cells, helping tumor establishment.
Financial support: Apoyo a la realización de tesis doctoral 2411032. Enlace ENL2010/12.

(186) NPC2 PROTEIN INCREASES CHOLESTEROL CRYSTALLIZATION IN MODEL BILE. González L, Castro J, Amigo L, Miquel JF, Zanlungo S. Departamento de Gastroenterología, Facultad de Medicina, Pontificia Universidad Católica de Chile. ldgonzal@uc.cl
Introduction: NPC2 is a soluble glycoprotein localized in lysosomes that binds cholesterol. It is secreted and it's present in fluids like bile. Although NPC2 participation in cholesterol transport inside the cell is well known, its function in bile is not clear. Cholesterol gallstone disease is characterized by the formation of cholesterol crystals, followed by crystal aggregation and growth in the gallbladder. Several evidence showed that some biliary proteins affect cholesterol crystallization. The hypothesis of this work is that NPC2 promotes cholesterol crystallization and gallstone formation in bile.
Material and Methods: NPC2 concentration in human gallbladder bile was determined through an ELISA essay. HEK-293 cells were transfected with myc-6xHis-tagged constructs containing cDNAs of NPC2 wild-type and cholesterol binding mutants. NPC2 was purified from the medium through a Ni-Affinity Chromatography. The activity of the wild-type NPC2 protein was analyzed through complementation of NPC2-deficient cells. Model bile was used for the cholesterol nucleation essays, adding NPC2 wild-type and mutant protein to bile. As negative controls we used BSA and Ni-Affinity proteins obtained from cells transfected with an empty vector.
Results: NPC2 concentration in human gallbladder bile is in the range of 5-20 ng/μL. The wild-type recombinant NPC2 purified protein was capable to complement NPC2-deficient cells. The nucleation essays using NPC2 in a physiologic concentration range show that wild-type and mutants accelerate cholesterol nucleation time and increase crystal mass in model bile in comparison with controls.
Discussion: These results suggest that NPC2 accelerates biliary cholesterol crystallization, independently of its cholesterol binding pocket structure.
Fondecyt 1070622 and 1110310 to SZ; 1080325 to JFM.

ACTIVATING THE b–CATENIN PATHWAY MODULATES THE PHENOTYPE OF DENDRITIC CELLS. César Oyarce[1], Andrés Herrada[1], Andrew F.G. Quest[2] and Alvaro Lladser[1]. [1]Laboratory of Gene Immunotherapy, Fundación Ciencia para la Vida, Santiago, Chile. [2]Laboratory of Cellular Communication, Center for Molecular Studies of the Cell (CEMC), Facultad de Medicina, Universidad de Chile, Santiago, Chile. alvaro.lladser@bionova.cl
Introduction: Dendritic cells (DC) play a crucial role in the maintenance of self-tolerance as well as the induction of immunity. A possible candidate involved in regulating the balance between tolerance and immunity is the β-catenin pathway. Here, we studied whether activation of the Wnt/β-catenin pathway modulates the DC phenotype.
Material and Methods: The dendritic cell line DC2.4 and bone-marrow-derived DCs were stimulated with LiCl (1, 10 and 15 mM) in the presence or absence of LPS (10ng/ml), or with different dilutions of a Wnt3a or Wnt5a ligand-containing medium. Changes in the expression of CD40, CD80, CD86, MHC class II was evaluated by flow cytometry whereas secretion of IL-6, IL-10 and IL-12 was assessed by ELISA. Additionally, we also evaluated changes in STAT3 activation and expression by Western blot analysis, luciferase-based reporter assays and real time qPCR.
Results: Activation of the β-catenin pathway in DCs using either LiCl or Wnt ligand-containing medium increased the expression of CD86 and IL-6 and decreased IL-10 secretion. Moreover, stimulation of the β-catenin pathway decreased the expression and transcriptional activity of STAT3.
Discussion: These results suggest that activation of the Wnt/β-catenin pathway modulates the phenotype of DCs by regulating the expression of STAT3. These observations will potentially be useful in developing more effective immunotherapeutic strategies.
Funding: CONICYT Program PFB-16, FONDECYT-FONDAP 15010006, CONICYT for Postgraduate studies (to CO).

(188) *Pseudomonas aeruginosa* INDUCES DIAPAUSE FORMATION IN *Caenorhabditis elegans*: A LINK BETWEEN ANTIBACTERIAL IMMUNITY AND RNAi. Chavez F[1], Pollak B[2], Ortiz J[1] and **Calixto A**[2]. [1]Department of Biology, Faculty of Sciences, University of Chile. [2]Center of Aging and Regeneration (CARE), P. Catholic University of Chile. acalixto@bio.puc.cl

I**ntroduction.** Pathogen infection awakens responses in an organism that will determine its survival or demise. These responses ought to be systemic, as pathogens spread and colonize far from their entry place. RNA interference (RNAi) is a mechanism for defense against viruses while its link to bacterial pathogenesis is unknown. *C. elegans* has been previously used to identify host responses to infection related to mechanisms of bacterial virulence. Here we report a novel response to *P. aeruginosa* infection: entry into diapause (dauer) in an RNAi-pathway dependent manner. We hypothesized that RNAi signaling constitutes a mechanism of systemic cellular communication in response to bacterial pathogenesis.

Materials and Methods. *C. elegans* strains were fed with *P. aeruginosa* for two generations. Larvae in diapause were isolated by SDS treatment, their percentage was obtained from total counts.

Results. Worms formed dauer in response to *P. aeruginosa* infection. *P. aeruginosa* grown in high phosphate conditions were less virulent than those on low phosphate, allowing natural mechanisms of defense to be expressed and therefore a larger amount of dauer to be formed. Worms with mutations in the Argonauts (endogenous RNAi) and *sid-1* (exogenous RNAi) did not form dauer under any condition.

Discussion. Here we report a novel defense mechanism of *C. elegans* against pathogenic bacteria: Entering a diapause state. This novel type of natural immunity requires an intact RNAi machinery, linking for the first time RNAi to bacterial pathogenesis.

Acknowledgements. This research was supported by the Fondecyt grant 31000099 to AC.

(189) GENE EXPRESSION PROFILE IN MELANOMA PATIENTS TREATED WITH IMMUNOTHERAPY BASED IN DENDRITIC CELLS. **Garcia T**, Tittarelli A, Villablanca A, Pereda C, Matthäus F, López M, Hoheisel J, Gebicke-Haerter, Salazar F. Tumor Immunology Laboratory, Faculty of Medicine, University of Chile. tgarcia@med.uchile.cl

Introduction: Clinical trials used dendritic-like cells named Tumor Antigen Presenting Cells (TAPCells®), loaded with a melanoma cell lysate (TRIMEL), showed 61% of treated patients develop a Delayed Type Hypersensitivity reaction (DTH) against melanoma, associated with a prolonged median survival in stage IV responder patients (DTH+) of 35 months compared to the non-responders (DTH-) which reported 11 months. Based on the differences detected in patient's blood (T-cell populations and *tlr4* polymorphism) in this study our aim was to evaluate differences in gene expression patterns by microarray analysis in these two group of patients, and identify molecular prognostic markers which can ultimate be of value to predict the clinical outcome.

Material and Methods: Blood samples were collected from 12 melanoma patients at different time points throughout the vaccination protocol. Total RNA was isolated and cDNA was generated for subsequent microarray analysis and real-time PCR.

Results: Three groups of patients were identified by performing statistical and mathematical analyses; one group of responder and two of non-responders – of these, one showed a poor reaction to the treatment, and the other react in the opposite way to the responders group. We also identified 17 genes up-regulated in responder patients, most of them related with the immune response.

Discussion: Our preliminary results showed the feasibility of our approach to obtain a group of genes differentially expressed between responder and non-responder patients, to design therapeutic interventions that may improve the clinical response rate.

(190) ISA VIRUS INFECTION ALTERS THE CELLULAR REDOX BALANCE IN PRIMARY SALMON KIDNEY CELLS. **Víctor Olavarría** and Alejandro Yáñez. Laboratorio de Metabolismo y Biotecnología, Instituto de Bioquímica y microbiología, Universidad Austral de Chile. Campus Isla Teja, Valdivia, Chile. volavarria@gmail.com

Introduction: The virus of infectious salmon anemia (ISAV) is the etiologic pathogen responsible for high economic losses in the salmon industry. ISAV is an RNA virus of the Orthomyxoviridae family like the influenza virus is characterized by generating mortality ranges between 0 and 50% in infected fish. In particular, it has been reported that ISAV infection activates apoptosis in infected cells, apparently as a mechanism for multiplication and survival.

Material and Methods: In vitro, the viral infection was studied by measurement of cytopathic effect (CPE). Respiratory-burst activity was measured as the luminol-dependent chemiluminescence produced by *S. salar* head kidney leukocytes.

Results: We demonstrated that ISA virus stimulates the expression of the subunit p47phox of the NADPH oxidase complex and antioxidant enzymes in cells infected. Additionally, we demonstrate that viral infection triggers the production of superoxide anion in kidney cells of *S. salar*. Interestingly, when infected cells were co-incubated with MAPK pathways inhibitors, superoxide anion production decreased significantly in the same way that the phosphorylated MAPK protein level.

Discussion: The results suggest that the ISA virus alters the redox balance ISAV-infected cell. The increased of ROS production a key steps that precede apoptosis let as to postulate as one of the first event of the mechanism of ISAV opening

new opportunities for developing new antiviral drugs or therapies targeting against ISAV and other Orthomyxoviridae viruses.
This work was supported by FONDEF D08I1055.

(191) IDENTIFICATION OF CD8+ CELLS IN LYMPHOID ORGANS OF SALMONIDS. **Valenzuela B**., Rodríguez F.E., Maisey K., Imarai M. Laboratorio de Inmunología, Centro de Biotecnología Acuícola. Facultad de Química y Biología, Universidad de Santiago de Chile. beatriz.valenzuelam@usach.cl
Introduction: In higher vertebrates, CD8α is a cell surface marker of cytotoxic T lymphocytes (CTL), natural killers, monocytes and dendritic cells. In fish, and particulary in salmon and trout, the characterization of immunocompetent cells which are positive for CD8α expression has been limited due to the absence of specific antibodies directed against this cell surface marker. The aim of this study was then to produce antibodies to identify and study the CD8+ cells in lymphoid organs of Rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar)*.
Materials and Methods: Using RT-PCR, the CD8 gene was amplified, sequenced and cloned into the pET expression system. The recombinant polypeptide was used to immunize New Zealand rabbits and the anti-serum was collected and characterized.
Results: The results obtained so far indicated that the polyclonal antibodies recognize the recombinant CD8α polypeptide in ELISA and Western Blott assays. In addition, antiserum was useful to identify immunocompetent populations in the lymphoid organs (spleen, kidney and gills) of trout and salmon.
Discussion: These results suggest that the polyclonal antibody allows the identification and localization of immunocompetent populations CD8+ in different lymphoid organs of salmonid fish.
Acknowledges: CONICYT, VRID-USACH, Funded by grants: INNOVA-CORFO 07CN13 PBT-90 and 09-MCSS-6698.

(192) PRODUCTION OF VLPs OF INFECTIOUS PANCREATIC NECROSIS VIRUS (IPNV) BY USING BACULOVIRUS SYSTEM. René A. Manríquez, Melina V. Villalba, Freddy A. Calderón, **Juan G. Cárcamo**. Laboratorio de Bioquímica Farmacológica, Instituto de Bioquímica y Microbiología, Universidad Austral de Chile. gcarcamo@uach.cl
Introduction. The baculovirus system has been widely used for the production of a variety of recombinant proteins in insect cells. This technology allows express viral proteins with post-translational modifications, characteristic of higher organisms. The infectious pancreatic necrosis virus (IPNV) is the causal agent of a highly contagious disease in salmonids, which generates large economic losses. IPNV contains two segments of double-stranded RNA (A and B). Segment A encodes the structural proteins VP2 and VP3 and protease NS. We prepared recombinant constructs of the A segment to generate recombinant baculovirus, and from the latter produce empty viral particles (VLPs) of IPNV in insect cells.
Materials and Methods. Invitrogen kit M-MLV was used for cDNA synthesis. The PCR of segment A was performed according to the Promega kit GoTaq. Segment A was cloned into the vector pFastBacDual, *E.coli* cultures were transformed, and recombinant bacmid was purified and transfected into Sf9 cells. The baculovirus generated were used to transfect Sf9 cells and produce VLPs. Western blot and electron microscopy (EM) were used for detect the VLPs.
Results. Recombinant baculovirus was generated. Insect cells Sf9 were infected with recombinant baculovirus, and the VLPs generated were isolated and detected by Western blot and EM.
Discussion. We achieved to generate VLPs of Chilean isolates of IPNV by using baculovirus technology. These results demonstrate functional establishing of a versatile and scalable platform for the expression of proteins and VLPs with scientific and biotechnological importance.
(Financed by Fondef D08i1096).

(193) REGULATION OF CYTOKINE EXPRESSION IN SALMONIDS SUBJECTED TO OXIDATIVE STRESS BY OVERCROWDING. **Rodríguez FE**., Cappelli, C., Valenzuela B., Imarai M. Laboratorio de Inmunología, Centro de Biotecnología Acuícola. Facultad de Química y Biología, Universidad de Santiago de Chile. felipe.rodriguezti@usach.cl
Introduction: Growing conditions and output of salmonids in aquaculture have been suggested as inducers of oxidative stress. Previous studies in mice have shown that intracellular oxidized milieu reduces the expression and production of pro-inflammatory cytokines such as IL-12 and IFN-g, while increases IL-4 and the anti-inflammatory cytokine IL-10. Cloning of several cytokine genes and the evaluation of oxidative stress has been successful achieved in salmonids. However, studies to understand the association between cytokine expression and oxidative stress have not been done yet.
Materials and Methods: Oxidative stress was induced in Atlantic salmon (*Salmo salar*) and Rainbow trout (*Oncorhynchus mykiss*) by overcrowding during 7 and 14 days. Oxidative stress was addressed by the determination of membrane lipid peroxidation (TBARS quantification). Expression of IL-12, IFN-g, and TGF-b1 was evaluated by qPCR at the indicated times.
Results: Significant increase in lipid peroxidation was observed in fish submitted to overcrowding for 14 days. Interestingly, at the same time, expression of IL-12 and IFN-g mRNAs significantly decreased in the spleen of fish kept for 14 days in overcrowding conditions.

Discussion: Changes in cytokine expression profiles under stress conditions might determine the kind of immune response probably by diminishing Th1 response.
Acknowledges: MECESUP USA0701, VRID USACH, INNOVA-CORFO 07CN13 PBT-90 and 09-MCSS-6698.

(194) GENOME-WIDE SURVEY OF GENE EXPRESSION RESPONSE TO *Piscirickettsia salmonis* IN RESISTANT AND SUSCEPTIBLE FAMILIES OF *Salmo salar*. **Rodrigo Pulgar,** Christian Hodar, Verónica Cambiazo. Laboratorio de Bioinformática y Expresión Génica, INTA, Universidad de Chile and Center for Genome Regulation (CRG). rpulgar@gmail.com
Introduction. *Piscirickettsia salmonis* is the etiological agent of the salmonid rickettsial septicemia, an endemic disease that causes significant losses in Chilean salmon production.
The aim of this study was to determine whether the differences in mortality rates between families of Atlantic salmon (*Salmo salar*) infected with *P. salmonis* correlate with changes in the pattern of transcriptional response among families.
Materials and Methods. To determine the levels of mortality, 30 full-sib families of Atlantic salmon were challenged for 40 days with *P. salmonis*. Subsequently, we conducted a second challenge for 14 days using representatives of high resistance (HR) and low resistance (LR) families. Microarray-based experiments were employed to identify in hematopoietic kidney, genes that were differentially expressed in infected HR and LR families. A cluster analysis was used to compare the transcriptional response patterns among infected families and qPCR to validate some indicators.
Results. There is a differential distribution of mortality among families, which is correlated with differences in the transcriptional response patterns. Also, results show that fish are able to trigger a broad response to fight the infection. Finally, potential indicators of resistance were identified from the transcriptional patterns of HR and LR families. Processes such as ATP synthesis, oxide reduction and iron binding seem to be important for resistance to *P. salmonis*.
Discussion. These results provide insights into the specific mechanisms underlying *S. salar* response to the infection and contribute to identify transcriptional biomarkers of resistance to *P. salmonis*.
Supported by: INNOVA-Consorcio 206-5047, FONDAP (15090007), Aquainnovo graduate fellowship.

(195) IN SEARCH OF MARKERS FOR THE IDENTIFICATION OF LYMPHOID CELLS IN ZEBRAFISH. **Rubio S.,** Wittamer V., Bertrand J., Menares E., Allende M., Traver D., Rosemblatt M. Fundación Ciencia para la Vida, Universidad Andrés Bello; Facultad de Ciencias, Universidad de Chile; Santiago Chile; University of California, San Diego, USA. solangerubio@gmail.com
Introduction. The zebrafish as model offers many advantages for the study of immune system in vertebrates. But a major downside in this field is the lack of tools such as monoclonal antibodies that would allow to isolate different lymphoid lineages.
Currently, 4 types of Igs have been described in zebrafish: IgM, IgD, IgZ. Among these, the IgM tetramer is the only Ig that has been reported to play a role in the teleost humoral immune response.
Material and Methods. Our objective is to characterize B lymphocytes in zebrafish, using an anti-IgM monoclonal antibody generated against salmon IgM, in combination with transgenic zebrafish lines that mark different lymphoid cell.
We labeled the kidney of adult zebrafish with the monoclonal antibody and we analized the IgM+ cells by FACS and their genetic profile expression trough Real Time PCR
Results. By cytometry, 8% of lymphoid cells showed expression of cell surface IgM. These cells constitute a subpopulation of B lymphocytes, as indicated by gene expression analyses and co-expression of the mhc2dab:GFP transgene. In addition, staining of kidney cells isolated from the rag2:GFP transgenic line that mark lymphoid progenitor cells allowed discriminating discrete subsets of B cell development.
Discussion. This work should prove an important contribution to our understanding of fish adaptive immune system focusing on the role of the B cells in humoral immunity in zebrafish.
Financed by CONICYT PFB-16; UNAB DI-04-10/I; ICM P06-039F.

(196) GALECTIN-8 ENHANCES B CELL ANTIGEN PRESENTATION. Yuseff MI.[3], Soza A.[1,2] **Pardo E.**[1,2], Lennon-Duménil A.M.[3] and González A.[1,2]. Centro de Envejecimiento y Regeneración (CARE), Fac. Ciencias Biológicas[1]. Departamento de Inmunología Clínica y Reumatología, Fac. Medicina[2]. Pontificia Universidad Católica de Chile. Institut Curie, Paris, France[3].
Introduction: During onset of adaptive immune response B cells must present antigen to primed CD4 T cells. The process involves formation of an immunologic synapse followed by degradation of B cell receptor (BCR)-antigen complexes into Ag peptides that are then loaded into MHCII molecules, to be finally exposed at the cell surface for recognition by T cell receptor. Gal-8 interacts with glycoproteins at the cell surface, including integrins, and therefore has the potential to modulate a variety of cell surface-dependent processes. Here, we analyze the function of Gal-8 on the B cell immunological synapse involved in Ag-presentation.
Material and Methods: IIA1.6 B cells expressing BCR for IgG were incubated with beads containing IgG or IgM, antigen (LACK or OVA) and either Gal-8 or BSA. Ag-extraction was assessed by FACS. Ag-presentation to primed CD4+T lymphocytes was analyzed by IL-2 secretion.

Results: Gal-8 increases BCR-dependent antigen (Ag) extraction, as well as the T cell response to Ag-presentation.
Discussion: Gal-8 contributes to the process of Ag-presentation, enhancing the ability of B cells to extract Ag at the immunological synapse.
(Financed by CONICYT PFB12/2007, Fondecyt Nº 1100921 and ISERM/CONICYT program).

(197) A KININ B1 RECEPTOR AGONIST INDUCES THE EXPRESSION AND RELEASE OF IL-4 FROM HUMAN HaCaT KERATINOCYTES. **Mejia AJ,** Matus CE, Pavicic F, Ehrenfeld P, Figueroa CD. Instituto de Anatomía, Histología y Patología. Facultad de Medicina. Universidad Austral de Chile. ajmejiam@gmail.com
Introduction: Skin inflammatory disorders have been associated with states of suppressed cellular immunity in combination with enhanced humoral response and their associated cytokines such as IL-4. In addition, the kinin B1 receptor (B1R), which is upregulated by cytokines such as IL-1b and TNF-a, has also been implicated in inflammation, metalloprotease secretion and skin damage. However, the release of IL-4 from human keratinocytes, as a consequence of B1R stimulation, as well as the signaling pathways that are involved in this event has not been described.
Material and Methods: The HaCaT cell line was subcultured and stimulated with the B1R agonist Lys-des[Arg^9]bradykinin (LDBK). IL-4 levels were evaluated by Western blotting in cellular extracts and media. Activation of the JNK-MAPK was assessed by a specific inhibitor.
Results: Time-course and ligand concentration experiments showed that 100 nM LDBK increase IL-4 levels and induce its release after 24 h post-stimulation, an effect that was blocked by preincubation with an excess of the B1R antagonist des-Arg^9-[Leu^8]-bradykinin. Cellular levels of IL-4 were reduced when the cells were preincubated with the JNK inhibitor II for 1 h. In addition, the B1R agonist produced the nuclear translocation of JunB, transcription factor that may bind to the promoter region of IL-4 gene.
Discussion: Activation of the B1R in HaCaT keratinocytes stimulates the synthesis and release of IL-4 through a mechanism that involves activation of the JNK-MAPK pathway and the transcription factor JunB.
Supported by grant D-2010-02, DID-UACh.

(198) RhoGEF3, A NEW GUANINE EXCHANGE FACTOR OF *Drosophila melanogaster*, IS INVOLVED IN TRACHEAL SYSTEM DEVELOPMENT. **Leandro Farías,** Alejandro Zúñiga and Verónica Cambiazo. Laboratorio de Bioinformática y Expresión Génica, INTA-Universidad de Chile and Center for Genome Regulation (CRG). leafariasag@gmail.com
Introduction: RhoGEF3 is a Guanine Nucleotide Exchange Factor (GEF) that regulates Rac1 GTPase in *Drosophila* cell lines. In a previous work, we detected the presence of RhoGEF3 in tubular organs, such as salivary glands and the tracheal system of *Drosophila melanogaster*. Knowing that different GTPases regulate distinct aspect of cell morphology during tracheal system development, in the present study we evaluated the participation of RhoGEF3 in cell morphogenesis during tracheal development.
Materials and Methods: The GAL4/UAS system was used to address the *in vivo* function of RhoGEF3. We examined tracheal development in embryos that express dsRNA against *rhogef3*, Cdc42L89 or Rac1N17 by using the trachea specific driver *btl*-GAL4-UAS-*actin-gfp*. The embryos were analyzed by indirect inmunofluorescence assays.
Results: The tracheal system of *rhogef3* loss-of function mutants displayed several defects in primary and secondary branches, suggesting that cell migration and/or cell adhesion processes might be altered. In current experiments we are characterizing with detail *rhogef3* loss-of function phenotypes and comparing with that of dominant negative forms of Cdc42 and Rac1 GTPases.
Discussion: Our results suggest that the protein RhoGEF3 is involved in morphogenetic events during tracheal system development. By identifying the specific GTPase activated by RhoGEF3 we expect to gain insight into the molecular mechanisms involved in branched tubular organ differentiation.
Fondecyt 1090211.

(199) PARTICIPATION OF dp115 IN CELL PROLIFERATION IN *Drosophila melanogaster*. **Consuelo Ibar[1]** and Álvaro Glavic[1]. [1]Laboratorio de Biología del Desarrollo, Faculty of Science, University of Chile. con.ibar@gmail.com
Introduction. Golgi ribbon/stack unlinking has been proposed as a cell cycle checkpoint by allowing an equal partitioning of the Golgi during cell division or releasing Golgi associated signaling molecules necessary to cycle progression. Golgins (p115, Grasp65, GM130) have been suggested to participate in this process being phosphorylated by Cdk1. Here, we analyze the phenotypes of the loss of function of dp115 and their relation with this process.
Materials and Methods. A UAS-RNAi construction against dp115 is expressed (UAS-dp115i) in the posterior compartment of the wing (en>dp115i and hh>dcr, dp115i) to analyze proliferation and cell death by immunofluorescence and flow cytometry. We blocked apoptosis co-expressing p35 and probe the genetic interactions between dp115 with genes related with cell cycle and other golgins.
Results. dp115i expression caused a decrease in tissue size. This is reflected in imaginal discs with an increase for proliferation markers (BrdU, phosphohistone H3) and also apoptosis markers (cleaved Caspase 3) in the zone of RNAi

expression, suggesting a role in proliferation of dp115. This role appears to independent of secretion, because the destination of E-cadherin is not affected by the loss of function. Flow cytometry analysis resemble a partial loss of function of Cdk1. Genetic interactions showed the phenotype is rescued with a gain of function of Cdk1 and enhanced with a loss of Grasp65 function.
Discussion. These results suggest that dp115 has effects in cell proliferation that are conserved despite of the differences in Golgi structure between Drosophila and mammals.
Grant sponsors: FONDECYT 1100366, ICM P06-039F, Beca apoyo a la tesis doctoral 24091047.

(200) TISSUE DYNAMICS AND TENSILE PROPERTIES UNDERLYING THE FORMATION OF CELLULAR ROSETTES IN AN *in vivo* MODEL OF EPITHELIAL ORGANOGENESIS IN ZEBRAFISH. Eduardo Pulgar[1,2,3], Felipe Santibañez[2,3], **Ricardo Figueroa**[1,3], Justin Steinfeld[1], Luis Briones[2,3], Steffen Hartel[l2,3], Miguel Concha[1,3]. [1]Laboratory of Experimental Ontogeny – LEO and [2]SCIAN-Lab, ICBM, Faculty of Medicine, University of Chile; [3]Biomedical Neuroscience Institute, Santiago, Chile. eduardopulgar.bioq@gmail.com; mconcha@med.uchile.cl
Introduction: The assembly of multicellular epithelial structures during development requires the precise coordination of cell adhesion, shape changes, polarisation and epithelialisation of progenitor cells. How this is achieved *in vivo* remains unclear. In this study we have addressed this issue using the Kupffer's vesicle (KV) of zebrafish, a recently developed *in vivo* model of epithelial organogenesis.
Material and Methods: We used Tg(*sox17::GFP*) and Tg(*ß-actikn:HRAS-EGFP*) zebrafish embryos; injection of morpholino-antisense oligonucleotides; *in vivo* 4D spinning disc microscopy; morpho-topological analysis; and physical / chemical manipulation of mechanical cellular properties.
Results: KV precursors, known as dorsal forerunner cells (DFCs), initially form a mesenchymal-like cell group. At early stages of KV formation, DFCs become increasingly compact while remaining coupled to the surface epithelium (EVL). Later, the DFC cluster separates from the EVL, arranges into a compact structure and generates a ciliated organ (the KV). During the DFC to KV transformation, cellular rosette-like structures form in relation to DFC-EVL cell contacts. During rosette formation, DFCs behaviour and organisation becomes polarised along the anterior-posterior axis. Impairment of tissue surface tensile properties in DFCs leads to disruption of rosettes formation.
Discussion: These results indicate that rosette formation depend on mechanical tensile forces exerted by epiboly and the tight interaction established between DFCs and EVL, a process that seem to polarise epithelial behaviour along the anterior-posterior axis.
Grant sponsors: HHMI, ICM (P07-048-F; P09-015-F), Fondecyt 1090246.

(201) PERSISTENT EXPOSURE OF ZEBRAFISH LARVA TO OXYTETRCYCLINE INDUCES CHRONIC INFLAMMATION AND DECREASES THE CELL REGENERATION CAPACITY. **Barros-Becker F[1].,** Pulgar A[1]., Romero J[2]., Feijóo CG[1]. 1Departamento de Ciencias Biológicas, Universidad Andrés Bello. 2INTA, Universidad de Chile. fbarros86@gmail.com
Introduction: The use of antimicrobial drugs in aquaculture has well-now positive effects, however, several side effects on fish are associated with their excessive use. If one takes into account that 70 to 80% of the antibiotics administered to fish as medicated pelleted feed are released into the aquatic environment as unused medicated food, it is not hard to imagine the degree of impairment that antibiotics can produce in the aquatic habitat. There is evidence that some antibiotics can induce alterations on the immune system, but depending on the route of administration, the fish species and the dose, it could generate contradictory results. In this work we analyze the effect of chronic exposure to oxyteracycline on the zebrafish immune system.
Material and Methods: We monitor immune function by three different approach. To analyze neutrophils migration, we monitor the transgenic line BacMpo::GFP by stereoscope. To quantify total neutrophils number we use flow cytometry. Finally, to characterized immune response at a molecular level we analyze different markers (Il1, Il10, Lys, Mpo and Cyp1a) by qPCR.
Results: Our results suggest, that oxytetracycline unleash an inflammation process that cannot by resolve and progress to a chronic inflammation after 48 hours post incubation, moreover in this condition the regeneration capacity is clearly decrease.
Discussion: It is important to further analyze oxytetracycline effect when treated larvae are exposed to pathogens and if these results can be extrapolated to salmonids.
Founding: Fondecyt 11090102, UNAB, DI39-11/R, Conicyt 79090006.

(202) ROLE OF THE CHEMOKINE SDF1A IN COLLECTIVE CELL MIGRATION AND AXON PATHFINDING IN ZEBRAFISH. **Mardones, Camila[1]**; Valdivia, Leonardo[1]; Young, Rodrigo[2]; Wilson, Stephen[2]; Allende, Miguel[1]. [1]FONDAP Center for Genome Regulation, Universidad de Chile. [2]University College London, UK. camilamardonesk@gmail.com
Introduction. SDF1a is a chemoattractant necessary for many functions during development that involve directed cell migration. This ligand interacts with CXC receptors and participates in guidance and homing of primordial germ cells and

immune cells, among other cell types. We aim to uncover additional biological functions for this molecule, specifically in the mechanosensory lateral line system.

Material and Methods. We utilized zebrafish carrying mutations for *sdf1a* expressing GFP/RFP in the primordium, neuromasts or nerve of the lateral line. Migratory events were monitored through timelapse microscopy. Protrusion formation, cellular adhesion, migration path and velocity were studied in mutant primordia, and were compared with sibling primordia. Immunocytochemistry allowed us to examine primordium-nerve interaction. Vital dye staining was used to evaluate neuromast functionality in mutant fish.

Results. As part of a large-scale screen to search for zebrafish mutants affecting the lateral line, we recovered a point mutation in *sdf1a*, a gene previously found mutated in *medusa* mutants. Our mutant has a primordium aberrant in form, cohesion and protrusion polarity. Remarkably, mutant primordia are able to migrate, but misguided and slower than sibling primordia. The lateral line nerve in mutant fish defasciculates and generates branches that do not innervate neuromasts. However, neuromasts of mutant fish are functional.

Discussion. The absence of SDF1 does not prevent migration of this cellular cohort, but is required for directionality of migratory behavior of primordium cells, for maintaining cell-cell adhesion and to generate proper nerve fasciculation.

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(203) *Prokineticin2* DISRUPTION IN ZEBRAFISH RESULT IN KALLMANN SYNDROME RELATED ANOMALIES. **Joaquín Letelier**[1,2] & Kathleen Whitlock[1,2]. [1]Centro Interdisciplinario de Neurociencias Valparaíso, [2]Center for Genomics of the Cell, Facultad de Ciencias, Universidad de Valparaíso. joaquin.letelier@cinv.cl

Introduction: GnRH cells play a crucial role in the establishment of the hypothalamic-pituitary-gonad axis in many species. Mutations in a variety of genes result in Kallmann Syndrome (KS) characterized by anosmia and hypogonadotrophic hypogonadism. Recent studies have found that the *prokineticin2* (*prok2*) and *prokineticin receptor2* (*prokr2*) genes are involved in the development of olfactory bulb and reproductive system and mutations in either of these genes result in a phenotype similar to KS.

Material and Methods: We cloned the zebrafish homologues of *prokineticin2* (*prok2*) and *prokineticin receptor2* (*prokr2*) genes. We used morpholino gene knock-down techniques to investigate the function of the *prok2* and *prokr2* genes in the development of hypothalamic, pituitary and olfactory tissues.

Results: We found two homologues for *prokr2* gene, *prokr2a* and *prokr2b*, having 69% and 62% identity, respectively. We found one homologue of *prok2* mouse gene with a 65% identity. These genes are expressed in zebrafish larvae and adult brain tissues. Knockdown of *prokr2a*, *prokr2b* and *prok2* proteins caused a significant reduction in the number of GnRH and *oxytocin-like* cells compared with controls. In addition, the adenohypophysis was malformed, as assayed by POMC-GFP cell lineages. Finally, *prok2*, *prokr2a* and *prokr2b* disruption result in abnormal development of the olfactory system.

Discussion: Zebrafish have homologues of the mouse *prok2* and *prokr2* genes, with an apparent duplication of *prokr2*. Knockdown of *prok2*, *prokr2a* and *prokr2b* gene function resulted in abnormal development of the olfactory system and hypothalamic cell types, a phenotype resembling the clinical manifestations of KS.

(204) MACROH2A2 AFFECTS CELL PROLIFERATION IN RETINAL PROGENITORS. **Guajardo L**. and Reyes A.E. Laboratorio de Biología del Desarrollo, Facultad de Ciencias Biológicas, Universidad Andrés Bello, Av. República 217, piso3. Santiago, Chile. arielreyes@unab.cl

Introduction: The histone variant macroH2A interacts with signalling pathways that acts in cell cycle control and cell differentiation. MacroH2A also interacts with regulator complexes of HOX genes expression, and some of these genes are involved in neurogenesis and eye development, suggesting that macroH2A may act as regulator in the fine-tuning of these developmental programs. Therefore, the objective of our work was to analyze the effect of macroH2A2 knockdown during the retinal neurogenesis.

Material and Methods: MacroH2A2 knockdown was generated by specific-morpholino injection, in wild type and transgenic embryos tg[*shh*::GFP], tg[*XOPS*::GFP]. Expression of specific markers such as *neuroD*, *cyclinD1* and *p27* was assessed by *in situ* hybridization. Moreover, markers of differentiated neurons in the retina and proliferation markers were detected by immunohistochemistry.

Results: Macroh2a2-knockdown generates morphant embryos showing microcephalia, microphthalmia and defects in retinal development. Early stages of development, brain segmentation and eye field territory specification were not affected in morphant embryos; however some markers of cell proliferation such as p-histone H3, BrdU incorporation, cyclinD1 and p27b were affected. On the other hand, Sonic hedgehog signalling, which is essential for retinal differentiation was not affected, however there is no retinal progenitor cells differentiation.

Discussion: In morphant embryos, early processes involved in retinal development are not affected by macroh2a2-knockdown. However, in the retinal neuroepithelium, proliferation markers do not reduce their level expression, preventing the cell cycle exit and cell differentiation, as is suggested by the altered expression of cyclinD1 and p27b.

FONDECYT 1095128 to A.E.R. and 24100169 to L.G.

(205) *Enterctopus megalocyathus* EYE LENS STRUCTURE: AN EVIDENCE OF GROWTH. **María José Villegas**, Erick Baqueiro and Rodolfo Paredes. Escuela de Medicina Veterinaria, Facultad de Ecología y Recursos Naturales, Universidad Andrés Bello. rparedes@unab.cl

Introduction: The need for age determination for the proper management of octopus fisheries has directed research attention to the few hard structures in the octopod soft body, beak, statoliths and the vestigial shell. The eye weight, pigment and nitrogen content have been used successfully in age determination of vertebrates. Given the structural similarity of lenses between octopus and vertebrate, we explore the possibility of using octopus eye lens weight, diameter and structural marks as age indicators.

Materials and Methods: The lenses of 228 octopuses were obtained from the commercial catch from Ancud and Queilén, in Chiloe**,** Chile. They were measured, weighed and prepared for histological analysis of the concentric lines structure.

Results: Preliminary analysis of the octopus lenses showed concentric layers, which show as dark and clear concentric bands. The pre-hatching nucleus was identified and the total number of lines counted. The lines start to form in the pre-hatching larvae, and one line equals to one day of life in each individual of *Enteroctopus megalocyathus*.

Discussion: Lens diameter and weight showed a much smaller variation than body length or weight, with an apparent correspondence of size or weight classes of body measurements to a lens diameter or weight.

The numbers of lines counted on the preliminary samples of this study suggested a corresponding number of lines to days of age, and to eye weight and diameter, but this will have to be ascertained with a significant sample size.

Financing: FONDECYT Iniciación N° 11070082 and Project DI 56-11/R-UNAB.

(206) Akt Inh-IV INDUCES UPR INDEPENDENT OF Akt CLASSICAL PHOPHORYLATIONS. **Jennifer Alfaro**[1]; Daniela Perez[1], Carolina Urrutia[1], Matías Blaustein[2], Alejandro Colman-Lerner[2] and Sebastian Bernales[1]. [1]Fundación Ciencia para la Vida. [2]IFIByNE, Universidad de Buenos Aires-CONICET. jennifer.alfaro.formas@gmail.com

Introduction: Akt kinase activation predominantly depends on the phosphorylation at Ser473 and Thr308. Activated Akt induces survival but can also induce cell death. Its deregulation has been associated with many diseases. Unfolded protein response (UPR) involves activation of three branches (IRE1, PERK and ATF6) that respond to unfolded protein accumulation. UPR can also induce survival or cell death depending on the severity of the ER stress. Therefore, both Akt and the UPR have an influence over cell fate. We use Akt Inh-IV to study the role of Akt as a regulator of the UPR.

Materials and Methods: MEF cells were treated with Akt Inh-IV and UPR activation was evaluated. Total-PERK, P-eIF2α, and total-eIF2α were evaluated by western blot (WB), translation was evaluated by incorporation of radioactive methionine; splicing of *Xbp-1* was evaluated by PCR and localization of ATF6-YFP reporter was evaluated by immunofluoresce. P-Ser473, P-Thr308 and total-Akt were evaluated by WB.

Results: Akt Inh-IV induces the phosphorylation of PERK and eIF2α, inhibits translation, induces splicing of *Xbp-1* and induces a change in the localization of ATF6. P-eIF2α induced by Akt Inh-IV does not depend on Akt P-Ser473 or P-Thr308. Translation inhibition and splicing of *Xbp1* induced by Akt Inh-IV is Akt-1/2 independent.

Discussion: Most of the reports show that UPR regulates Akt activation. We show, for the first-time, that inhibition of the Akt pathway activates the UPR, suggesting that interactions between theses pathways are even more complex. We are evaluating the consequences of UPR activation on cell fate.

(207) FUNCTIONAL ANALYSIS FROM A PROTEOMIC AND YEAST TWO-HYBRID SCREENING FOR NEW IRE1a INTERACTING PROTEINS. **Diego Rodriguez,** Hery Urra, Daniel Henriquez, Tomas Vaisar, Christian Gonzalez-Billault, Laurie Glimcher and Claudio Hetz. [1]Biomedical Neuroscience Institute, Faculty of Medicine, [2]Center for Molecular Studies of the Cell, Institute of Biomedical Sciences, University of Chile, Santiago, Chile. chetz@med.uchile.cl

Introduction: Adaptation to ER stress depends on part by the activation of the unfolded protein response (UPR) stress sensor IRE1a. Active IRE1a splices the mRNA of XBP-1 and induces the signaling of alarm pathways through the assembling of a complex termed the "*UPRosome*". Our aim is to identify new direct or indirect modulators of IRE1a signaling by mass spectrometry and yeast two hybrid coupled to an shRNA functional screening.

Methods: We performed a proteomic analysis using a Linear Ion Trap Mass Spectrometer (LTQ) and for direct protein interactors a yeast two hybrid screening. We validated major candidates using shRNAs followed by UPR signaling measurements including xbp-1 mRNA splicing and quantification of transcriptional responses.

Results: Both screenings identified clusters related with apoptosis, cytoskeleton, RNA and metabolism. We successfully validate a direct interaction of some BCL-2 family members, which maintain sustained IRE1a signaling after prolonged ER stress. Interestingly, we found a different pattern of proteins bound to IRE1a in cells in absence or presence of ER stress. Otherwise, other candidates involved in activation/inactivation of IRE1a were evaluated by XBP-1 splicing assay, as well as, XBP-1 downstream responses and Tm-induced apoptosis.

Conclusions: We conclude that the identification of *UPRosome* components will increase our understanding of the biological function and regulation of IRE1a.

Supported by FONDECYT – 1100176 (CH) - 3100033 (DRG); FONDAP – 15010006; Millennium Nucleus - P09-015-F; ICGEB and Alzheimer's Association (CH).

(208) SODIUM-DEPENDENT VITAMIN C TRANSPORTER 2 (SVCT2) AVAILABILITY AT PLASMA MEMBRANE IS IMPAIRED IN HUNTINGTON'S DISEASE MODELS. [1]**Carlos Kramm**, [1]Aníbal I. Acuña, [1]Magdalena Esparza, [2]Carlos Toro, [1]Ilona I. Concha, [2]Sebastián Brauchi y [1]Maite A. Castro. [1]I. Bioquímica y Microbiología, [2]I. Fisiología, UACh. macastro@uach.cl

Introduction: Huntington's disease (HD) is caused by an expanded polyglutamine stretch in the exon 1 of huntingtin gene. HD animals show a loss of ascorbic acid in extracellular fluid during behavioural activation. Ascorbic acid is present in high concentrations in brain. Normally, synaptic activity triggers an increase in extracellular ascorbic acid concentration. Sodium vitamin C transporter isoform 2 (SVCT2) is the only ascorbic acid transporter expressed in brain. SVCT2 translocates to plasma membrane in response to an increase of extracellular ascorbic acid concentration. Huntingtin is involved in vesicle and protein traffic. The aim of this work was to study SVCT2 translocation to plasma membrane in HD cellular models.

Methods and Results: Kinetic assays using ^{14}C-ascorbic acid showed a decrease (relative to wt STHdh^{Q7} cells, Q7) of ascorbic acid transport in immortalized knock-in mutant STHdh^{Q111} striatal cell lines (Q111). Using total internal reflection microscopy (TIRM) we observed an increase of SVCT2-EGFP at the plasma membrane in Q7 cell treated with ascorbic acid. This effect was not observed under the same condition in Q111 cells. Fluorescence Recovery After Photobleaching (FRAP) experiments in NG108 cells unveil an decrease on mobile fraction of SVCT2-EGFP in presence of extracellular ascorbic acid. Mobile fraction was unchanged NG108 cells expressing SVCT2-EGFP and mHttΔQ138-DSRED.

Discussion: Huntingtin should be involved in SVCT2 traffic and in SVCT2 availability at the plasma membrane.

FONDECYT 1110571, 1110906 & 1110508.

(209) A ROLE OF MUTANT PROTEIN DISULFIDE ISOMERASES IN AMYOTHROPIC LATERAL SCLEROSIS PATHOGENESIS. **Woehlbier U.**[1,2,3], Gonzalez-Perez P.[4], Irrazábal T.[1,2,3], Colombo A.[1], Diaz A.[1] Sagredo A.[2], Armisen R.[2], Concha M.[2], Brown R.H.[4], Hetz C.[1,2,3]. [1]Biomedical Neuroscience Institute, Faculty of Medicine, University of Chile, Santiago, Chile, [2]Center for Molecular Studies of the Cell, Institute of Biomedical Sciences, University of Chile, Santiago, Chile, [3]Neurounion Biomedical Foundation, Santiago, Chile, [4]University of Massachusetts, Worchester, USA.

Introduction: Amyotrophic lateral sclerosis (ALS) is a progressive and deadly adult-onset motoneuron disease. We identified via a large scale genetic screen of ALS patients novel point mutations in the genes of three members of the large protein disulfide isomerase (PDI) family, namely PDI, ERp57 and ERp72, as possible disease causing genes. PDIs are ER-targeted foldases responsible for the formation, reduction or isomeration of disulfide bonds.

Methods: To define the physiological role of these mutant PDIs, we transiently or stably overexpressed them in the motoneuron cell line NSC34 and investigated their localization by immunofluorescene microscopy, toxicity by PI staining and MTT assay, in vivo folding activity using a ER-targeted GFP (HyPER) specifically sensing H_2O_2 levels via live cell imaging. In addition we expressed these mutants in a zebrafish model and observed the resulting phenotypes in the nervous system using GFP transgenic animals.

Results: Differential individual localization, toxicity, folding activity and zebrafish developmental phenotypes were observed for the investigated two PDI mutants, two ERp57 mutants and one ERp72 mutant compared to their respective wildtype version.

Discussion: Overall our data suggest a pathogenic role of the mutant PDIs in the nervous system, suggesting that they may represent an unanticipated new factor driving ALS.

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(210) UP-REGULATION OF NEUROTROPHIC FACTORS AND RELATED MOLECULES IN THE CONTRALATERAL MOTOR CORTEX AFTER UNILATERAL FOCAL STROKE. **Claudia Pissani**[1,2]*, Katherine Stack[1,2]*, Macarena Vargas[1], Gareth I. Owen[1], Francisca C Bronfman[1,2]. [1]Physiology Department, Pontificia Universidad Católica de Chile. [2]Millennium Nucleus in Regenerative Biology (MINREB). *Both contributed equally. chpissan@uc.cl , stacklkatherine@gmail.com

Introduction: Focal stroke is a vascular event caused by reduced blood supply in a particular brain area. This condition results in brain damage inducing neurologic impairment that includes motor and cognitive problems. After stroke, there is a window of injury-induced plasticity. The aim of our work was to study the gene expression profile of 84 key genes related to neuronal processes, including neurotrophins and neuropeptides along with their receptors at the beginning of the plasticity window.

Material and Methods: We used a rat stroke model base in the stereotaxic injection of endothelin-1, a vasoconstriction peptide, in the primary motor cortex. Five days after the induction of stroke we analyzed the gene expression profile of the contralateral primary motor cortex by PCR Array (SABioscience).

Results: We observed an up-regulation of the neuropoietic cytokine LIF and the LIF receptor, accompanied with an up-regulation of the downstream LIF associated transcription factors STAT1 and STAT3. The FGF2 growth factor and TGFb1 were also up-regulated, as was the Neuropeptide Y Receptor 2 (NpyR2), the Chemokine (C-X3-C motif) receptor 1 (Cx3cr1) and the transcription factor Tp53.
Discussion: Our results suggest that there is a specific subset of neurotrophic factors and related molecules that are up-regulated at the beginning of the plasticity window following stroke that may present new targets for inducing brain plasticity after brain injury.
Funds, FONDECYT (1885273), BMRC CTU06, MINREB (P07/011-F), FONDAP-Biomedicine 13980001 and CARE PFB 12/2007.

(211) DOPAMINE RECEPTOR D3 FACILITATES INFILTRATION OF CD4+ T-CELLS IN THE CENTRAL NERVOUS SYSTEM AND NEURODEGENERATION OF DOPAMINERGIC NEURONS IN A MOUSE MODEL OF PARKINSON'S DISEASE. **Hugo Gonzalez**[1,2], Carolina Prado[1,2], Francisco Contreras[1,2],Rodrigo Pacheco[1]. [1]Fundación Ciencia para la Vida and [2]Universidad Nacional Andrés Bello. Santiago, Chile. h.gonzalez.v10@gmail.com
Introduction: Emerging evidence has demonstrated that CD4+ T-cells infiltrate into the substantia nigra (SN) during human Parkinson's disease (PD) and upon animal-PD models. SN-infiltrated Th1 and Th17 CD4+ T-cells promote microglial activation and strongly contribute to neurodegeneration of dopaminergic neurons. On the other hand, pharmacological evidences have suggested that dopamine receptor D3 (D3R) is involved in the production of Th1-cytoquines by CD4+ T-cells. Thus, the aim of this study was to determine the role of D3R in the brain-infiltration of CD4+ T-cells, microglial activation and neurodegeneration of dopaminergic neurons in the SN during PD using a mouse-model.
Material and Methods: PD was induced in wild-type and D3R knock-out (D3RKO) C57BL/6 mice by administration of 1-methyl-4-phenyl-1,2,3,6-tertahydropyridine-(MPTP). Degeneration of dopaminergic neurons, CD4+ T-cell infiltration and microglial activation were assessed by immunohistochemical-detection of tyrosine hidroxylase, MAC-1 and CD4 in the SN, respectively. D3R expression was determined by RT-PCR. Wild-type or D3RKO T-cells were differentiated toward Th1 and Th17 phenotypes and analyzed by FACS.
Results: D3R expression facilitated T-cell activation and favored polarization towards Th1 effector-phenotype. *In vivo* experiments showed that D3R-deficiency protects from neurodegeneration of dopaminergic neurons, microglial activation and CD4+ T-cells infiltration in the SN upon PD.
Discussion: These findings increase our understanding on the mechanism by which CD4+ T-cell contribute to neurodegeneration during PD and suggest a novel target for therapeutic intervention.
Supporting Grants: FONDECYT-1095114, CONICYT PFB-16. HG, CP and FC are CONICYT fellows.

(212) IN THE SEARCH OF NOVEL FUNCTIONS FOR CDK5. Erick Contreras-Vallejos[1], **Cristina Olmos**[1], Alex di Genova[2], Alejandro Maass[2], Elías Utreras[1,3], Ashok Kulkarni[3] and Christian Gonzalez-Billault[1]. [1]Laboratory of Cell and Neuronal Dynamics, Department of Biology and ICDB, Faculty of Sciences, and [2]Mathematical Modeling Center (CMM), Faculty of Physical and Mathematical Sciences, Universidad de Chile. [3]NIDCR, NIH, Bethesda, USA. erickcontreras@gmail.com
Introduction. Cyclin-dependent kinase 5 (Cdk5) is proline-directed serine/threonine kinase active mainly in the nervous system. Cdk5 regulates several processes such as neuronal migration, cytoskeletal dynamics, axonal guidance and synaptic plasticity. In order to identify novel functions of Cdk5 in the brain we used proteomic, bioinformatics, and phosphoproteomic high-throughput approaches.
Material and Methods. Protein extracts obtained from brain of Cdk5 knockout (KO) and wild type E18.5 mice were separated in 2D-gels and proteins differentially expressed were identified by mass spectrometry and validated by qPCR. In addition, a list of possible new targets having a consensus site (S/T)PX(K/H/R) for Cdk5 were obtained for bioinformatics analysis. Finally, differential phosphorylation patterns of protein were analyzed by using a phosphoproteomic analysis.
Results. We identified 30 differentially expressed proteins in the Cdk5-KO brain by proteomics screening, which display different functions related with cytoskeleton, metabolism, and post-translational modifications. By bioinformatics analysis we identify several proteins containing consensus site for Cdk5, and we further analyzed Rap guanine nucleotide exchange factor 1 (C3G) as new target of Cdk5 for validation process. Finally, by phosphoproteomics analysis we found >40 phosphoproteins differentially phosphorylated in Ser/Thr in brain from Cdk5-KO mice.
Discussion. Altogether these results derived from proteomic, bioinformatics, and phosphoproteomic studies, will help us to identify new possible functions of Cdk5 related with the identification of novel substrates in the brain.
Supported by Fondecyt-089095 and ICM P05-001-F (to CG-B).

(213) FUNCTIONAL ROLE OF THE UNFOLDED PROTEIN RESPONSE IN HUNTINGTON'S DISEASE. **Vidal, R.**[1,2,3], Figueroa A.[1,2,3] and Hetz, C.[1,2,3]. [1]Biomedical Neuroscience Institute, Faculty of Medicine, [2]Center for Molecular Studies of the Cell, Institute of Biomedical Sciences, University of Chile, Santiago, Chile, [3]Neurounion Biomedical Foundation, Santiago, Chile. renevidalg@gmail.com

Introduction: Huntington disease (HD) is an inherited neurodegenerative disorders caused by an extended polyglutamine stretch in the *huntingtin* gene. HD is associated with the generation of neurotoxic intracellular protein inclusions of mutant huntingtin (mHtt) in striatum. The occurrence of endoplasmic reticulum (ER) stress has been recently reported in animal models of HD, associated with the activation of the unfolded protein response (UPR). The UPR is mediated mainly by XBP1 and ATF4.
Materials and Methods: We generated mHtt transgenic mice that are deficient for XBP1 ($XBP1^{Nes-/-}$-$mHtt^{Q128}$) or ATF4 ($ATF4^{KO}$-$mHtt^{Q128}$). We monitored the effects on disease progression such as, striatal neurons loss, rotarod performance and mHtt levels *in vivo*. We used 3-6 animals in each experiment and t-student statistical analysis.
Results: XBP1 deficient mice were more resistant to developing disease features, a phenotype associated with improved neuronal survival and motor performance, and a decrease of mHtt levels. These effects were specific because ATF4 deficiency did not alter mHtt levels. Cellular studies in addition to subcellular fractionation in brain tissue indicated enhanced Huntingtin clearance through macroautophagy in XBP1 deficient animals.
Discussion: Our results provide strong evidence supporting an involvement of specific UPR components in the pathogenesis of HD *in vivo*. XBP1 deficient possibly due to the upregulation of Forkhead box O1 involve in the regulation of autophagy in neurons.
Supported by FONDECYT no. 1100176/3100039, FONDAP no. 15010006, Millennium Institute No. P09-015-F, ICGEB, and Alzheimer's Association.

(214) α-CHEMOKINES MODULATE DIFFERENTIATION OF NEURAL STEM CELLS FROM SPINAL CORD.
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Introduction. α-Chemokines, CXCL1 and CXCL12, regulate proliferation, differentiation and migration of brain neural stem cells (NSC). However, it is unknown if these chemokines participate on spinal cord (SC) neurogenesis where presence of NSC has been also recognized. Considering differential response of specific NSC populations to signalling from the neurogenic niche, our aim is to evaluate the role of α-chemokines on SC-derived NSC differentiation.
Materials and Methods. NSC were obtained from SC of E18.5 mice embryos, cultured as neurospheres, and characterized by differentiation to neural lineages. Expression of α-chemokines (CXCL1, CXCL12), and their cognate receptors was analyzed by RT-PCR and inmunofluorescence. To evaluate the role of α-chemokines on NSC differentiation, neurospheres were differentiated in presence of a recombinant form of CXCL12 and/or the CXCR4 inhibitor AMD3100, and analyzed after 4 days by immunofluorescence.
Results. Neurospheres were characterized as NSC by nestin and sox2 expression, and by displaying a trilineage differentiation potential. Gene expression analysis showed that NSC expressed CXCL12, but not CXCL1. According to this result, only CXCL12 receptors, CXCR4 and CXCR7, were detected. Interestingly, their levels changed during differentiation suggesting an eventual role during this process. CXCL12 decreased neuronal differentiation and CXCR4 inhibition increased glial differentiation, as showed by βIII-tubulin and GFAP staining respectively.
Discussion. These results suggest an endogenous regulation of SC-NSC differentiation by CXCL12. However, the involvement of specific signalling by CXCR4 and/or CXCR7 needs to be elucidated.
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(215) PPAR BETA/DELTA AGONIST INDUCES CELL PROLIFERATION AND REGULATES SOX2 LEVELS IN MOUSE ADULT NEURAL PRECURSOR CELLS.
Bernal C., Araya C., Bronfman M. Departamento de Biología Celular y Molecular. Pontificia Universidad Católica de Chile. CARE-FONDAP. mbronfman@bio.puc.cl
Introduccion: PPARs are a group of ligand-activated transcription factors. PPARbeta/delta has important functions in cell proliferation and differentiation, and most PPARs target genes are involved in fatty acid metabolism and homeostasis. PPARs acts for bind to peroxisome proliferator response element (PPRE) residing in target genes and stimulates their expression. Previously we describe a PPARgamma role in adult neural precursor cells (aNPC) self-renewal and in this work we evaluated the PPARbeta/delta role in these cells.
Material and Methods: aNPC culture was prepared from SVZ of the C57bl/6 adult mice. Expression was evaluated by RT-PCR and western blot, localization by immunofluorescence, proliferation by BrdU incorporation assays. Mice were injected with 100 mg BrdU/Kg of animal body weight and BrdU incorporation and PPARbeta/delta in SVZ cells was evaluated by confocal immunofluroscence. Ethics Commission of PUC approved animal handling procedures.
Results: PPARbeta/delta is expressed in BrdU-positive cells of the SVZ. We observed PPARbeta/delta expression in aNPC *in vitro* and treatment with agonist of this factor induces proliferation and increases Sox2 levels, antagonist decreases basal levels. In the Sox2 promoter region we found two putative PPRE.
Discussion: Our results suggested a potential role of PPARbeta/delta in phenotype maintenance of aNPC through transcriptional control of Sox2, a necessary transcription factor in the aNPC phenotype maintenance and in the self-renewal. PPARgamma has a role in proliferation and slowdown differentiation of these cells, both PPARs could be having a complementary function in self-renewal of adult neural precursor cells.
Supported by Fondecyt 1095177.

(216) PROLIFERATION OF MURINE MIDBRAIN NEURAL STEM CELLS DEPENDS UPON AN ENDOGENOUS SONIC HEDGEHOG (Shh) SOURCE. **Víctor Hugo Cornejo**, Constanza Martínez, Pablo Lois and Verónica Palma**.** Laboratory of Stem Cells and Development, Facultad de Ciencias, Universidad de Chile vcornejocorona@ug.uchile.cl

Introduction. Tissue-specific stem cell populations are established in "niches", locations with precise biochemical and cellular configurations that regulate their behavior in tissue generation, maintenance, and repair. Determining the best sources for the *in vitro* derivation and growth of Neural Stem Cells (NSCs) are still goals of stem cell research.This work first aimed to establish a biological matrix, which serves as a niche for NSCs and, secondly, to assess the functional role of Shh in experiments in which differential stem cell potencies could be evaluated.

Material and Methods. We seeded NSC from E18.5 dorsal murine mesencephalon (tectum/prospective superior colliculi in mammals) into collagen type-I matrix supplemented with Shh and/or EGF/FGF-2.In order to evaluate NSC proliferation and differentiation in the 3-D cultures, we performed immunofluorescence and subsequent confocal microscopy analysis, combined withWestern blots of NSC, glial or neuronal markers.

Results. For the first time, we reveal the pivotal endogenous influence of Shh in maintaining the stem cell potential of radial glial cells (RGC), grown in the 3-D environment. We demonstrate that Shh signaling is critical for the modulation of the number of RGC, for the proliferation of neural precursors and consequently for neurogenesis.

Conclusion. We propose that canonical Shh signaling plays a central role in the control of NSC behavior in the developing dorsal midbrain by acting as a niche factor, mediating the response of NSC to EGF/FGF-2 signaling.

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(217) SPINAL CORD REGENERATION IN *Xenopus* TADPOLES PROCEEDS THROUGH ACTIVATION OF SOX2 POSITIVE CELLS. Marcia Gaete, Rosana Muñoz, **Mauricio Moreno,** Natalia Sánchez, Ricardo Tampe, Esteban Contreras, Juan Larraín. Center for Aging and Regeneration, Millennium Nucleus in Regenerative Biology, Faculty of Biological Sciences, P. Universidad Católica de Chile. mmoreno777@gmail.com

Introduction. In contrast to mammals, amphibians such as *Xenopus* and fish can regenerate their spinal cord (SC) after injury. However, the cellular and molecular mechanisms involved in spinal cord regeneration are still poorly understood. Here we report that the transcription factor *sox2* plays an important role in this process.

Material and Methods. SC lesion was performed using either transection (stage 49/66) or tail amputation (stage 42/49). The regeneration was assessed as swimming ability recovery. Sox2 expression was obtained by IIF or RT-PCR and double labeling for sox2 and BrdU was used as an indication of neural progenitors. Sox2DN transient transgenic tadpoles were obtained using the meganuclease method.

Results.We have found that tail amputation or SC transection in *Xenopus* tadpoles resulted in a global increase of Sox2 levels accompanied by increased proliferation of $Sox2^+$ cells. Experimental reduction of Sox2 activity diminished proliferation of SC resident cells affecting SC regeneration after tail amputation. At stage 42 Sox2 was also necessary for whole tail regeneration suggesting that SC regeneration commands this process. Furthermore, Sox2 levels correlated with regenerative capabilities during metamorphosis and $Sox2^+$ cell aggregates repopulated the ablation gap in the SC transection model.

Discussion. In summary, $Sox2^+$ cells are necessary for SC regeneration and lead to a model whereby SC damage activates proliferation of $Sox2^+$ cells, contributing to SC growth in tail amputation or repairing the ablation gap generated by SC transection.

(218) STUDY OF XtRic-8A DURING THE NEURAL DEVELOPMENT OF *X. tropicalis*. **Gabriela Toro**, Lester Riquelme, Juan Olate, and Marcela Torrejón. Laboratory of Molecular Genetics, Biochemistry and Molecular Biology Department, Universidad de Concepción, Chile. gabrielatoro@udec.cl

Introduction: Ric-8 a conserved protein with GEF activity is related with G protein signaling in the nervous system, promoting the synaptic vesicle priming in *C. elegans* and during the asymmetric cell division in *C. elegans* and *Drosophila* neuroblast. In mouse, the expression of Ric-8A is observed during the neural development and knockout for Ric-8A was not viable. Studies by our group have shown an important expression of Ric-8 at the neural and neural crest development in *X. tropicalis*. In order to study the effect of XtRic-8A during the neural development, our goal is to generate an effective *in vivo* inducible expression and silencing system in *X. tropicalis* using the heat-inducible promoter.

Material and Methods: We design a shRNA to deplete the expression of Ric-8 during the development of *X. tropicalis* embryos. We also created an inducible bicistronic expression system to deplete the expression of XtRic-8A and induce EGFP expression.

Results: The loss of function studies generate a development delayed and alteration of neural markers like N-tubulin at early stages. At later stages, we also observed the reduction of the larvae movement and touch responses. We used the inducible system in a preliminary assay to test the functionality of the construct and deplete the expression of XtRic-8A.

Discussion: XtRic-8A is important for the properly neural formation, probably participating in the neural differentiation. The inducible expression system might be a great tool to discriminate between different processes in order to understand the role of XtRic-8A during development.

(219) THE NEOGENIN 1 (Neo1) RECEPTOR IS CONTROLLED IN SONIC HEDGEHOG (Shh) DRIVEN MEDULLOBLASTOMAS. **Luis A. Milla**[1], Brandon Wainwright[2] and Verónica Palma[1]. [1]Laboratory of Stem Cells and Development. Faculty of Sciences, University of Chile, Santiago, Chile; [2]Institute for Molecular Bioscience, University of Queensland, Australia. lmillab@gmail.com

Introduction. The canonical Shh/Gli pathway plays multiples roles during embryonic development and adulthood. By using different genomic approaches, we have recently uncovered Neo1 as a direct Shh target. Neo1, classically known as a Netrin1 partner, has been recently involved in many processes during Central Nervous System (CNS) development. Here, we show the positive regulation in the cells that give rise to medulloblastomas, the most common malignant pediatric brain tumors.

Results. In the developing cerebellum, Neo1 expression is located in the outer External Germinal Layer (EGL) in proliferating granule cell precursors (GCP). EGL precursors that undergo active migration and differentiation do not longer express the Neo1 marker. In Shh controlled mouse models of medulloblastomas, Neo1 is upregulated in proliferative cells, and downregulated in cells positive for neuronal differentiation markers. Additionally, Chromatin Immunoprecipitation (ChIP) from GCP and luciferase reporter assays demonstrate *in vivo* and *in vitro* direct binding of Gli transcription factors to the *neo1* promoter in cerebellum and neocortex.

Discussion. Taken together, our results show that Neo1 is regulated by the canonical Hh signaling in the developing mouse CNS, and may play a prominent role in the onset of medulloblastoma, an observation essential for improving anticancer pharmacology.

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(220) LOCAL TRAFFICKING OF VOLTAGE-GATED SODIUM CHANNELS IN PERIPHERAL AXONS. **Carolina González**[1,2], Felipe Court[3,4] and Andrés Couve[1,2]. [1]Physiology and Biophysics, ICBM and [2]Biomedical Neuroscience Institute (BNI), Facultad de Medicina, Universidad de Chile, Santiago, Chile. [3]Faculty of Biological Sciences, and [4]Millennium Nucleus for Regenerative Biology, P. Universidad Católica de Chile. carolags@gmail.com

Introduction. Voltage-gated sodium channels (NaVs) are responsible for the generation and propagation of action potentials and are mostly concentrated in the axon initial segment and the nodes of Ranvier. Despite their fundamental role, little is known about the intracellular trafficking mechanisms that govern their availability. The presence of the endoplasmic reticulum (ER) has been documented in peripheral axons, but it is unclear whether this or other local secretory organelles participate in the delivery of NaVs. In the present study, we hypothesize that trafficking through the local ER is necessary for the delivery of NaVs to the nodes of Ranvier in axons of dorsal root ganglion neurons (DRGs).

Material and Methods. The distribution of NaVs was evaluated by immunofluorescence in dissociated axons. Local protein trafficking from the ER to the Golgi apparatus was blocked *in vivo* by injecting Brefeldin-A (BFA) into a discrete region of the sciatic nerve of adult male rats.

Results. NaVs were exclusively inmunodetected at the nodes of Ranvier. Exposure to BFA produced a local redistribution of NaVs at the nodes. 3D reconstructions of confocal images revealed a change from a ring-like disposition of NaVs in control conditions to a filled appearance after BFA treatment.

Discussion. Our data suggest that local early secretory organelles have a central role in the distribution of NaVs at the nodes of Ranvier in peripheral axons.

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(221) TRANSCRIPTIONAL REGULATION OF P2X3 RECEPTORS IN NOCICEPTIVE NEURONS. **Emilio Diaz**[1,3], Rodolfo Madrid[2], Martín Montecino[3], Brigitte van Zundert[3]. [1]Universidad de Concepción; [2]Universidad de Santiago de Chile; [3]Centro de Investigaciones Biomédicas, Universidad Andrés Bello.emdiaz@udec.cl

Introduction: The molecular mechanisms responsible for increases in the expression of the nociceptive P2X3 receptor (P2X3R) induced by inflammation in somatosensory ganglia remain unclear. Previously, we found that the transcription factor Runx1 directly up-regulates P2X3R gene expression in PC12 cells. Here we investigated whether silencing of Runx1 in dorsal root ganglia (DRGs) reduces transcriptional and functional expression of P2X3Rs. In addition, we analyzed the transcriptional expression of P2X3Rs in DRGs from rats after induction of inflammation.

Material and Methods: Dissociated DRGs were nucleofected at 0 DIV with siRNA-Runx1 and cultured for 3 days to extract RNA or perform Ca^{2+}imaging experiments, using Fura-2 (Ca^{2+}-indicator) and ATP (P2X3R-agonist). Inflammation was induced by CFA injection into the hindpaw(s) of rats. The L4-L5-L6-S1 DRGs were dissected 1-5 days later and RNA was extracted. mRNAs of Runx1 and P2X3R were quantified by qPCR.

Results: siRNA-Runx1 expression in DRGs reduced Runx1 and P2X3R mRNA levels. Ca^{2+}imaging experiments also showed that siRNA-Runx1 treatment decreased the percentage of DRGs that respond to ATP. CFA injection increased P2X3R expression in the ipsilateral DRGs in only~25% of the rats. This was animal dependent, as CFA injections into both hindpaws caused similar changes in P2X3R expression in the bilateral located DRGs.

Discussion: Runx1 regulates P2X3R gene transcription in DRGs and may become an important target for pain therapy. Induction of inflammation in both hindpaws allows us to correlate P2X3R expression in left-sided DRGs with epigenetic modifications associated with gene activity in right-sided DRGs.
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(222) ETHANOL EFFECT IN GLYCINE RECEPTOR IS INHIBITED BY Gβγ BLOCKING PEPTIDES. **San Martín, L.**, Cerda, F., Aguayo, L., Guzmán, L. Department of Physiology, University of Concepcion, Concepcion, Chile. loresanmartín@udec.cl
Introduction: The activity of the Glycine Receptor (GlyR), a ligand gated ion channel, is potentiated by ethanol through the Gβγ dimer. Basic amino acids motifs in the cytoplasmic domain (CD) have demonstrated to be critical for this regulation. Peptides derived from these motifs (RQHc7, RQHc10) can interfere in a specific manner the ethanol potentiation of GlyR.
Material and Methods: GST-pull down experiments were performed to determine if the peptides could interfere with the direct binding of Gβγ and GlyR. On the other hand, electrophysiological assays were done to determine if these peptides were capable to inhibit the ethanol effect of potentiation of GlyR.
Results: GST pull down assays demonstrated that RQHc7 and RQHc10 interfere with the Gβγ–GlyR interaction, decreasing notably the binding. In addition, electrophysiological studies demonstrated that these peptide could diminish significantly the potentiation of GlyR (RQHc7: from 47,1 ± 2% to a 15,7 ± 4%) by ethanol, in a concentration dependent manner. Interestingly, the RQHc7 peptide did not interfere with Gβγ activation of a GIRK channel, which indicate a certain level of specificity to the Gβγ effects on GlyR.
Discussion: Peptides like RQHc7 are effective to diminish the potentiation of GlyR by ethanol, on the other hand Gβγ activation of GIRK channel is not affected by this peptide. Other Gβγ effectors must be assayed to complete the selectivity studies for these peptides.
Grants: FONDECYT 11080145, NIH ROIAA 15150-01.

(223) REGULATION OF ERC AGGREGATION BY SRPK2/3 IS MEDIATED THROUGH THE COILED-COIL (CC) DOMAINS. **Yocelin Cruz**, Dolly Araya, Cristina Araya, Natalia Oro, Fernanda Olivares, Jonathan Bijman, Pedro Zamorano, Viviana Torres. Laboratorio de Neurobiología, Facultad de Ciencias de la Salud, Universidad de Antofagasta. viviana.torres@gmail.com
Introduction: Aggregation of Drosophila´s Bruchpilot protein is regulated by SRPK kinase prior arrival to the synapse. We have extrapolated these results to the mammalian protein ERC that due to its multiple interactors appears to be one of the main organizer of the active zones. Here, we examine the role of ERC CC domains on its self-aggregation, modulation by SRPK2/3 and their role in the interaction with Bassoon.
Material and Methods: Co-expression by lentiviral vectors of EGFP tagged CC (1, 2, 3, 4) domains of ERC and SRPK2/3 was assessed in HEK293/COS-7 cells. pmRFP-Bassoon (95-3938) was used throughout the experiments.
Results: Expression of CC1 and CC4 resulted in intracellular aggregates in heterologous cells and this aggregation was modulated by increase expression of SRPK2/3. Interestingly, Bassoon was recruited to CC1 and CC4 aggregates, but not CC2. As expected the recruitment of Bassoon to CC aggregates was prevented by co-expression of SRPK2/3.
Discussion: This result suggests that aggregation of ERC may be mediated by CC1 and CC4 and this aggregation is modulated by SRPK2/3. Interestingly, CC1 and CC4 recruit Bassoon at the aggregates, but not CC2 as previously reported, suggesting that the interaction of Bassoon with the ERC domains may not only be mediated by CC2. These findings narrow the molecular target for the modulation of ERC by SRPKs.
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(224) TGF-β1 MODULATES MAPK AND NF-κB SIGNALING BY INCREASING MKP-1 LEVELS IN GLIAL CELL CULTURES. **Betsi Flores** and Rommy von Bernhardi. Department of Neurology, Faculty of Medicine, Pontificia Universidad Católica de Chile. rvonb@med.puc.cl
Introduction: Chronic neuroinflammation in Alzheimer's disease (AD) is associated with activation of mitogen-activated protein kinases (MAPKs) and NF-κB pathways that induce the release of inflammatory mediators such as Tumor necrosis factor α (TNF-α) and nitric oxide (NO). Transforming growth factor β1 (TGF-β1) is an inflammation modulator whose levels are increased in AD. Our previous findings indicate a key role of TGF-β1 in the pathogenesis of AD, but the molecular mechanisms underlying its anti-inflammatory effect are not completely elucidated. Here, we propose a potential role for MKP-1, a MAPK phosphatase that exerts negative regulation on MAPK signaling, in the modulatory role of TGF-β1 on activated glial cells.
Material and Methods: Rat primary glial cultures were stimulated with amyloid-β (Aβ) or Interferon-γ (IFN-γ) with or without TGF-β1 pretreatment. MAPK phosphorylation and MKP-1 levels were measured by Western blot, and nuclear translocation of NF-κB p65 was observed by immunofluorescence. MKP-1 expression was inhibited by siRNA transfection.

Results: We found that TGF-β1 prevented NO and TNF-α production. This was in concordance with an increase in MKP-1 levels, prevention of MAPKs activation and attenuation of NF-κB p65 nuclear translocation. Suppression of MKP-1 expression by siRNA affected the modulation of inflammatory response exerted by TGF-β1.
Discussion: TGF-β1 induced the expression of MKP-1 in glial cells, inhibiting MAPK and NF-κB signaling. These findings can represent a potential mechanism for the protective actions of TGF-β1 and suggest that TGF-β1 signaling and MKP-1 can be new targets for the treatment of AD.
Work supported by Fondecyt 1090353.

(225) PLASTICITY OF ASTROCYTES AND ALDOC SECRETION FOLLOWING ANTIDEPRESSANT TREATMENT. **Alejandro Luarte,** Rodrigo Herrera-Molina, Mauricio Sandoval, Ursula Wyneken. Laboratorio de Neurociencias, Universidad de los Andes. uwyneken@uandes.cl
Introduction. Major depressive disorder is a high prevalence mental illness that is treated primarily by antidepressant drugs. However, the improvement of the patients´ mood takes place only after weeks of treatment indicating that adaptive mechanisms must occur to observe a therapeutic effect. We have been analyzing plastic changes induced by repetitive fluoxetine (flx) treatment (a selective serotonin reuptake inhibitor) in the rat forebrain, focusing on excitatory synapses. Interestingly, a proteomic analysis revealed changes in a glycolytic enzyme exclusively present in forebrain astrocytes, fructose-bisphosphate aldolase C (aldoC). Whereas the co-localization of this enzyme with the astrocyte marker GFAP decreased, the content of aldoC in the cerebrospinal fluid increased by 3.8±0.7 fold, suggesting that aldoC is secreted by astrocytes by a unknown mechanism. The aim of this study was to examine the morphology of hippocampal astrocytes and the co-localization of aldoC with markers of secretory vesicles such as exosomes in control and flx-treated animals.
Material and Methods. We used antibodies against the exosome markers Alix and CD-26, and against aldoC and GFAP for immunohistochemistry of control and flx-treated rats, obtaining confocal images that were analyzed with the Imaris software.
Results. We found a marked increase in the length and complexity of astrocyte processes in flx-treated rats. In addition, aldoC was present in clusters that co-localized with both exosome markers.
Discussion. The secretion of aldoC by astrocytes suggests a yet unknown signalling function for this enzyme that should be explored in the future and reveal that astrocytes participate in flx-induced plasticity.

(226) MATERNAL THYROID HORMONE DEFICIENCY CAUSES ALTERATIONS IN NEURONS AND ASTROCYTES IN THEIR OFFSPRING. [1,2]**Pablo Cisternas*,** [1,2]Pablo Gonzalez*, [1,2]Gabriela Zuñiga*, [1,2]Claudia Cortés, [1,3]Leandro Carreño [1,3]Susan Bueno, [1,3,4]Alexis Kalergis, [1,2]Claudia Riedel. [1]Millennium Institute on Immunology and Immunotherapy. [2]Facultad de Ciencias Biológicas Universidad Andrés Bello. [3]Departamento de Genética Molecular y Microbiología, Facultad Ciencias Biológicas. Pontificia Universidad Católica de Chile. [4]Departamento de Reumatología, Facultad de Medicina. Pontificia Universidad Católica de Chile. **Authors contributed equally to this work.* pab.cisternas@unab.cl
Introduction: Maternal thyroid hormones are essential for the development of the central nervous system. It has been shown that thyroid hormone deficiency (HTD) at the beginning of pregnancy causes in their offspring difficulties in learning and memory. The effects of THD, at cellular and molecular level, haven't been explored. We hypothesized that maternal THD will affect the neuronal phenotype of the progeny through its effects on astrocytes.
Materials and Methods: Primary neuronal cultures from hippocampus were prepared from rats and kept in presence of medium derived from astrocytes in culture. Some of these primary cell cultures of astrocytes were from the progeny gestated in mothers with THD. Size and number of dendrites, establishment of the synapse, and expression of GFAP were evaluated by immunofluorescence and western blot.
Results: Numbers of astrocytes and content of GFAP were altered in cultures that were derived from rats gestated in mothers with THD. Neurons cultured with conditioned medium of astrocytes from rats gestated on mothers with THD show less and impaired synapsis establishment compared to control.
Discussion: Analysis of these results indicates that maternal THD affects neurons and astrocytes of the offspring and that these effects are mainly imprinted on the astrocytes than in neurons.
Funding: Fondecyt 1100926; DI-11-11/R; P09-016-F; Millennium Institute on Immunology and Immunotherapy (MIII) P09-016-F.

(227) IP3 DEPENDENT Ca^{2+} SIGNALS IN SKELETAL MUSCLE ARE TRIGGERED AT DIFFERENT MEMBRANE POTENTIALS THAN RYR DEPENDENT ONES. Casas, M. Jorquera, G. and **Jaimovich, E**. Centro de Estudios Moleculares de la Célula. ICBM, Facultad de Medicina, Universidad de Chile. mcasas@med.uchile.cl
Introduction: In adult muscle fibers, tetanic electrical stimulation induces two separate calcium signals, a fast one dependent on dihydropyridine receptors (DHPR) and RyRs and a slow signal, dependent on DHPR and inositol tris phosphate receptors (IP_3Rs). The amplitude of this slow signal is dependent on frequency of stimulation, having a maximum at 20 Hz. This signal participates in the activation of genes related to slow muscle fiber type phenotype.

Materials and Methods: Adult muscle fibers from mouse FDB muscles were loaded with Fluo-4 and stimulated with different concentrations of extracellular K^+.
Results: We founded the presence of calcium signals at more negative membrane potential values than those described for triggering calcium release associated with RyR.. In these conditions, we observe an increase in mRNA of the slow isoform of TroponinI. These signals have an intracellular origin, because they are still present when working in solutions with no extracellular calcium and 0.1 mM EGTA. Interestingly, they are inhibited by incubation of fibers with 20 uM XestosponginB, a specific inhibitor of IP_3.
Discussion: These results suggest strongly that the slow signal fires at membrane potential values smaller than fast signal (EC coupling related), or even at values where the fast signal threshold has not yet been reached. This opens the possibility that another part of the DHPR molecule being involved in the signal transmission of depolarization to IP_3-production machinery, independently of the process of E-C coupling.
FONDAP 15010006, FONDECYT 1080120.

(228) INVOLVEMENT OF P2 RECEPTORS IN THE MYOGENIC COMMITMENT ACQUISITION OF C_2C_{12} RESERVE CELLS. **Vega JL**[1,2], Cea L[2], Riquelme M[2], Sáez JC[2]. [1]Laboratorio de Fisiología Experimental (EPhyL), Universidad de Antofagasta. [2]Departamento de Fisiología, P. Universidad Católica de Chile. jlvegapi@gmail.com
Introduction: Reserve cells (RCs) are uncommitted myoblasts obtained from differentiated C_2C_{12} cell cultures. Myoblast commitment acquisition to the myogenic linage requires rises of intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$). Possible membrane pathways involved in these $[Ca^{2+}]_i$ increments are P2 receptors activated by extracellular ATP. Here, we tested the possible role of particular P receptors in the myogenic commitment acquisition of C_2C_{12} RCs.
Methods: RCs were isolated at day 10 of C_2C_{12} cell cultures grown in DMEM/F12 medium supplemented with 5% horse serum. Changes in MyoD levels were detected in western-blot analyses. Changes in intracellular Ca^{2+} signals (fluorescent emission ratio 340/380 nm) were determined in cells RCs loaded with fura-2.
Results: The increase in MyoD levels observed in cells committed to the myogenic lineage was drastically prevented by BBG ($P2X_7$ antangonist), MRS2179 ($P2Y_1$ antagonist), iso-PPADS (P2X antagonist), or suramin (P2 antagonist). Extracellular ATP induced a rapid and transient rise in Ca^{2+} signal followed by a plateau level higher than basal values. Treatment with MRS2179 did not prevent the transient rise, but abrogated the plateau phase. Whereas, iso-PPADS prevented most of the rapid and trasient signal leaving a delay transient rise and suramine completely prevented the ATP-elicited Ca^{2+} signal.
Discussion: In RCs the autocrine/paracrine ATP-induced increase in MyoD levels requires activation of both $P2X_7$ and $P2Y_1$ receptors. It is proposed that activation of both P2 receptors leads to the increase in intracellular Ca^{2+} concentration required to activate the expression of the master gene MyoD.

(229) ROLE OF CONNECTIVE TISSUE GROWTH FACTOR IN THE DEVELOPMENT OF FIBROSIS IN SKELETAL MUSCLE DYSTROPHY. [1]**Morales MG**, [1,2]Cabello-Verrugio C, [3]Goldschmeding R, [1]Brandan E. [1]Department of Cell and Molecular Biology, CARE, P. Universidad Católica de Chile. [2]Center for Human Genetics, Facultad de Medicina, Clínica Alemana Universidad del Desarrollo. [3]Department of Pathology, University Medical Center Utrecht, The Netherlands. mgmorales@gmail.com
Introduction: Muscular dystrophies are characterized by a decrease of muscle mass, reduced muscle force and an increase in fibrosis. Connective tissue growth factor (CTGF), is overexpressed in muscular dystrophies and correlates with the degree and severity of fibrosis in many diseases. However, the role of CTGF in Duchenne Muscular Dystrophy (DMD) and associated fibrosis remains unknown.
Material and Methods: We determined the effect of loss of CTGF function in the progression of fibrosis of *mdx* mice, a murine model of DMD, in a genetically CTGF-reduced model (*mdx*-$CTGF^{+/-}$ mice). Fibrosis was assessed in skeletal muscles by measuring ECM protein content and by histological analysis. For muscle force generation we used electrophysiological analysis.
Results: CTGF was significantly reduced in *mdx*-$CTGF^{+/-}$ mice and this correlated with a significant decrease of ECM protein levels. We detected less muscle damage in *mdx*-$CTGF^{+/-}$ mice compared to *mdx* mice, as evidenced by analyses of muscle tissue structure and reduced levels of myogenic precursor markers such as myogenin and embryonic myosin. Remarkably, the decrease of CTGF in *mdx*-$CTGF^{+/-}$ mice caused an improvement of the specific isometric contractile force.
Discussion: These observations underscore the importance of CTGF in the patho-physiology of muscular dystrophies and suggest that targeting CTGF might have significant potential in development of novel therapies for DMD and related diseases.
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(230) ATP RELEASE AND IP_3 PRODUCTION ARE IMPORTANT REGULATORS OF SKELETAL MUSCLE PLASTICITY. **Jorquera G**., Almarza G., Jaimovich E., Casas M. Centro de Estudios Moleculares de la Célula, ICBM, Facultad de Medicina, Universidad de Chile, Chile. gjorquera@med.uchile.cl

Introduction: In skeletal muscle, transcription of specific gene programs are regulated by different electrical stimulation patterns, process known as muscle plasticity. Slow isoforms of contractile proteins and oxidative enzymes are upregulated by low frequency patterns and fast isoforms and glycolytic enzymes are upregulated by high frequencies. How myofibers can discriminate among different frequencies and activate specific gene programs? We propose that this process or part of it is modulated by IP_3-dependent calcium transients, with ATP release as a key regulator.
Materials and Methods: We used adult myofibers obtained from FDB and soleus muscles. Myofibers were stimulated at 20 and 90 Hz (270 pulses, 0.3ms each) in the presence or absence of proper inhibitors. We analyzed gene expression (qPCR), ATP release (luciferase) and IP_3 production (antibody).
Results: We found that ATP release from myofibers is different depending on stimulation frequency, with an important release at 20 Hz with a peak 3-5 min after stimulation; this release is dependent on dihydropyridine receptors and pannexin channels. At 90 Hz there was no significant ATP release. We analyzed IP_3 production in 20 Hz stimulated myofibers and we found two IP_3 increments, immediately after stimulation and 5 min after stimulation. Using ATP release inhibitors (apyrase, carboxonolone) and XesponginB (IP3R inhibitor) we observed a deregulation in the expression of plasticity-related genes as troponin I, enolase and citrate synthase.
Discussion: We propose that both ATP release and IP_3 production are integrated processes that modulate muscle plasticity upon electrical stimulation.
FONDECYT-1110467, FONDAP-15010006.

(231) MUSCLE-SPECIFIC UBIQUITIN LIGASES INCREASE IN SKELETAL MUSCLE ATROPHY WAS PREVENTED BY ANGIOTENSIN 1-7. **Cabello-Verrugio, C.** Center for Human Genetics, Facultad de Medicina, Clínica Alemana Universidad del Desarrollo. ccabello@udd.cl
Introduction: skeletal muscle atrophy is characterized by a loss of muscle mass. Several events such as disuse or immobilization induce atrophy with a common program of gene expression. Among the factors involved in skeletal muscle atrophy is angiotensin II (Ang-II) belonging to classical renin-angiotensin system (RAS). In muscle wasting is observed a proteosome-mediated increase in protein catabolism with an augment in expression and activity of muscle-specific ubiquitin ligases MURF-1 and atrogin-1. Angiotensin 1-7 (Ang 1-7) belongs to non-classical RAS with opposite biological activities to Ang-II. The effect of Ang 1-7 on skeletal muscle atrophy *in vitro* and *in vivo* was evaluated.
Material and Methods: C2C12 myotubes were co-incubated with Ang-II and Ang 1-7. The levels of MURF-1 and atrogin-1 were determined. Mice were treated with Ang 1-7 during 7 days and then were unilaterally immobilizated with cast during 7 days. Levels of MURF-1 and atrogin-1, and fiber diameters were evaluated.
Results: MURF-1 and atrogin-1 levels were increased in cells after 24 h of incubation with Ang-II which were prevented by pre-incubation of the cell with Ang 1-7. In a model of immobilization-induced muscle atrophy, the induction of MURF-1 and atrogin-1 in gastrocnemius and tibialis anterior was prevented by systemic administration of Ang 1-7. The decrease of fiber diameters in these muscles, a parameter of atrophy, was prevented by treatment with Ang 1-7.
Discussion: These results from studies *in vitro* and *in vivo* suggest that Ang 1-7 can prevent skeletal muscle atrophy by, at least, a decrease of the proteosome components.

(232) THE Na^+ PUMP IS NOT PREFERENTIALLY FUELED BY GLYCOLYTIC ATP. [1,2]**Ignacio Fernández** and [1]L. Felipe Barros. [1]Centro de Estudios Científicos (CECs), Valdivia, & [2]P. Universidad Católica de Valparaíso. ifernandez@cecs.cl
Introduction: It is widely assumed that the Na^+/K^+ ATPase pump is exclusively fueled by glycolytic ATP. Applied to astrocytes, this notion was pivotal for the current model of energy cycling in the brain. However, there has been no experimental demonstration of a glycolytic ATP pool and it is not obvious how could the Na^+ pump avoid using mitochondrial ATP, which represents the largest fraction of the total ATP pool. Taking advantage of the recent availability of methods to monitor glycolysis with high temporal resolution, we have put the notion of a glycolytic ATP pool to the test.
Materials and Methods: Intracellular Na^+, ATP and glucose were monitored in astrocytes cultured from mice using real-time epifluorescence microscopy with SBFI, Mg-Green and a FRET glucose nanosensor, respectively (*Bittner et al. Front. Neuroenergetics, 2010*).
Results: Blockage of mitochondrial ATP production with azide or rotenone strongly stimulated the rate of glycolysis, with predicted several-fold increases in glycolytic ATP production and in the size of the putative glycolytic ATP pool. In response to azide and rotenone, total ATP level decreased only slightly, evidencing an almost complete compensation by glycolytic ATP. However, the intracellular concentration of Na^+ increased, evidencing a functional inhibition of the Na^+ pump, despite the increased availability of glycolytic ATP. Control experiments showed that Na^+ permeability was not affected by azide or rotenone.
Discussion: These data suggest that the Na^+ pump in astrocytes is not preferentially fueled by a glycolytic ATP pool, casting a doubt on the existence of such pool.

(233) CHOLESTEROL INVOLVEMENT IN SKELETAL MUSCLE GLUCOSE TRANSPORT. **Llanos P.,** Contreras-Ferrat AE., Osorio-Fuentealba C., Espinosa, A, Hidalgo C. and Jaimovich E. CEMC & ICMB, Faculty of Medicine, Universidad de Chile. pllanos@med.uchile.cl

Introduction. In skeletal muscle, cholesterol is highly enriched in the transverse tubule (TT) network. Insulin promotes glucose uptake into skeletal muscle fibers by promoting the translocation of glucose transporters to the TT membranes. Yet, the role of TT cholesterol content on insulin-induced glucose transport remains unknown. Accordingly, we tested the effects of feeding mice a high fat diet (HFD) on TT cholesterol content and insulin-induced signaling and glucose uptake.

Materials and Methods. Male C57BL/6J mice were fed a normal or a HFD (45% fat) diet for 8-10 weeks. Cholesterol levels were measured in TT-containing triad fractions isolated from tibialis anterior or Gastrocnemius muscle. Isolated muscle fibers from flexor digitorum brevis (FDB), obtained by enzymatic digestion were used freshly for fluorescence glucose uptake determinations. Western blot assays were used to measure AKT activity in mouse myotubes.

Results. Body weight, body fat content and metabolic variables (fasting glucose levels, plasma cholesterol, HOMA-IR) were higher in mice fed a HFD diet compared to controls, and cholesterol levels in triads from HFD-fed mice increased by 30% compared to controls. Preincubation of myotubes with Methyl-β-cyclodextrin to remove cholesterol partially inhibited AKT phosphorylation in controls, and increased both basal and insulin-induced glucose uptake in control and HFD-fed mice.

Discussion. Our findings indicate that HFD increase TT cholesterol content. The finding that partial cholesterol removal from muscle fibers isolated from normal or HFD-fed mice improves glucose uptake suggests that TT cholesterol has a physiological role in this process.

FONDECYT Postdoctoral 3110105, 11090301, FONDAP 15010006).

(234) APOER2 POLARIZED DISTRIBUTION AND THE ROLE OF A PROLINE-RICH INSERT IN ITS CYTOPLASMIC DOMAIN. Catalina Grabowski, Pamela Farfán, María Luisa Benítez, Patricia Burgos[&], Alfredo Cáceres* and **María Paz Marzolo**. Depto. Biología Celular y Molecular, Fac. Ciencias Biológicas y MINREB, P. Universidad Católica de Chile. &Universidad Austral de Chile, *Instituto Investigación Médica Mercedes y Martin Ferreyra Córdoba, Argentina. mmarzolo@bio.puc.cl

Introduction: Complex systems, such as epithelial tissues and the nervous system rely functionally on the presence of polarized cells having an asymmetric plasma membrane, apical/basolateral in epithelial cells, axonal/somatodendritic in neurons. The accurate localization of endocytic and signaling proteins is critical for the cell, tissue and organism function. ApoER2 is an endocytic and signaling receptor with essential roles during development and in the adult brain. ApoER2 has several splice variants, however there is no distinction regarding the variants' polarized distribution. One variant expresses a proline-rich insert that binds PSD95, JIP- 1/2 and X11a, adaptor proteins that could determine its localization. We are interested to determine the distribution of ApoER2 in polarized hippocampal neurons and epithelial cells and evaluate the role of the insert in this localization.

Methods: In MDCK cells expressing either the full-length ApoER2, the variant lacking the insert, the ApoER2 tailless or chimeras containing TAC ectodomain, the receptor distribution was determined by cell surface biotinylation and confocal microscopy. The same constructs were analyzed by confocal microscopy, in transfected hippocampal neurons.

Results: ApoER2 full-length is non-polarized in both epithelial and hippocampal neurons. The absence of the cytoplasmic domain or the insert changes this distribution in both cell types.

Discussion: ApoER2 polarized trafficking is complex and depends on information present in its ectodomain and cytoplasmic tail.

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(235) STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF CARGO-BINDING SITES ON THE μ4-SUBUNIT OF ADAPTOR PROTEIN COMPLEX 4. **Breyan Ross[1],** Yimo Lin[1], Juan Bonifacino[2], James Hurley[3], Patricia Burgos[1], and Gonzalo Mardones[1]. [1]Instituto de Fisiología, Facultad de Medicina, Universidad Austral de Chile, Valdivia, Chile, and [2]Cell Biology and Metabolism Program, NICHD, and [3]Laboratory of Molecular Biology, NIDDK, NIH, Bethesda, MD, USA. gamardon@gmail.com

Introduction: Adaptor protein (AP) complexes assist protein trafficking by playing key roles in the selection of cargo molecules to be sorted in post-Golgi compartments. The medium-sized subunit (μ1-μ4) of the four heterotetrameric AP complexes recognizes YXXØ-sequences (Ø is a bulky hydrophobic residue), which are sorting signals in transmembrane proteins. A conserved region, the μ2-binding site, mediates recognition of YXXØ-signals. Recently we found that a non-canonical YXXØ-signal binds to a distinct μ4-binding site of the AP-4 complex. In this study we aimed to determine the functionality of both binding sites on the recognition of this non-canonical YXXØ-signal.

Materials and Methods: We used site-directed mutagenesis, yeast-two hybrid (Y2H) analyses, isothermal titration calorimetry (ITC), and X-ray crystallography.

Results: Substitutions in either of both binding sites on μ4 abrogated binding to the APP-tail in Y2H experiments. Further characterization by ITC showed no binding only with the R283D substitution at the μ4-binding site, in contrast with a

decrease in binding affinity with the substitution D190A at the μ2-binding site. We solved the crystal structure of the C-terminal domain of the D190A mutant of the μ4 subunit bound to the non-canonical YXXØ-signal. This structure showed no significant difference compared to that of wild-type μ4 subunit.
Discussion: Our mutational, biochemical and structural analyses established the role of the μ4-binding site for the non-canonical YXXØ-signal.
FONDECYT 1100896.

(236) POST-TGN BASOLATERAL SORTING INVOLVES AN INTERMEDIATE SUB-APICAL COMPARTMENT IN POLARIZED MDCK EPITHELIAL CELLS. **Claudio Retamal** and Alfonso González**.** Departamento de Inmunología Clínica y Reumatología, Fac. Medicina. Centro de Envejecimiento y Regeneración (CARE). Fac. Cs. Biológicas. Pontificia Universidad Católica de Chile.
Introduction: In polarized epithelial cells, a distinct plasma membrane (PM) protein composition is maintained at apical (Ap) and basolateral (BL) domains by exocytic and endocytic routes. Both Pathways intersect at recycling endosomes (RE). Newly synthetized proteins en route to PM from trans-Golgi-network (TGN) can traffic through RE, this pathway remains poorly characterized and is not yet fully understood.
Material and Methods: Polarized MDCK stably expressing TGN38-CFP were intranuclearly microinjected with expression vectors for Vesicular stomatitis virus glycoprotein fused to mCherry fluorescent tag (VSVG-mCherry) and Low density lipoprotein receptor fused to GFP (LDLR-GFP). Once proteins are synthetized, they are accumulated at TGN by 20°C block and then released at 37°C in absence or presence of cell membrane permeant peptides carrying sorting signals of either VSVG (VSVGp) or LDLR (LDLRp). Live cell imaging was used to follow the traffic of these BL cargo proteins.
Results:VSVGp and LDLRp block their respective cargo VSVG or LDL at TGN. VSVG transport is also sensitive to LDLRp, while LDLR is insensitive to VSVGp. Despite this differential peptide-mediated block, both VSVG and LDLR exit TGN in the same transport vesicles. Both proteins traverse a sub-apical compartment before reaching the basolateral cell surface.
Discussion: Transport of VSVG and LDLR from the TGN to an intermediate compartment, presumably part of the endocytic route, is sorting signal dependent and involves different recognition elements, which nevertheless segregate them into the same transport carriers.
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(237) THE C-ABL TYROSINE KINASE PHOSPHORYLATES HDAC2 REGULATING ITS LEVELS: A NOVEL MECHANISM OF EPIGENETIC CONTROL. **González-Zúñiga M.**[1], Contreras P.[1], Loyola A.[2], Álvarez A.R.[1]. 1.Cell Signaling Laboratory. Biological Sciences Faculty.Pontificia Universidad Católica de Chile. 2.Fundación Ciencia para la Vida. magonza1@uc.cl
Introduction. The regulation of gene expression is a key process for the development and mantention of complex organisms. The histone acetylation is an epigenetic mechanism of regulation of gene expression. This mechanism involves the activity of the HDAC enzymes family. Although the crucial role of the HDACs in genes expression is clear, the mechanisms that control both HDACs levels and activity are not currently elucidated. The c-Abl is a tyrosine kinase that controls gene expression through the regulation of transcription factors, such as p53 and p73. However, the role that c-Abl could have in epigenetic regulation is still unknown. Because of this, focused our study in the epigenetic regulation by c-Abl, specially through HDACs regulation.
Materials and Methods. HeLa cells were transfected with the c-Abl gene or a c-Abl shRNA. Protein levels for HDAC1/2 or H3ac were evaluated by Western Blot and/or Immunofluorescence. Phospho-Tyr levels on HDAC2 were evaluated by HDAC2 immunoprecipitation and Western Blot for phospho-Tyr.
Results. The cells overexpressing c-Abl showed an increase in both HDAC1 and HDAC2 levels and a decrease H3ac levels. Consistently, the inhibition of c-Abl caused a decrease in HDAC2 levels, effect that was prevented by lactacystin. Moreover, c-Abl activity regulated the Tyr phosphorylation levels of HDAC2.
Discussion. Our data suggest that c-Abl is a novel epigenetic regulator, through the regulation of HDAC2 levels. Probably Tyr phosphorylation of HDAC2 prevents its degradation via proteasome. Besides, c-Abl activity increases HDAC1 levels, suggesting that c-Abl may regulate others members of the HDAC family.
Supported by Fondecyt 1080221, González-Zuñiga M. acknowledges CONICYT Chile for Doctoral fellowship.

(238) EPIGENETIC CONTROL THROUGH HISTONE AND DNA MODIFICATIONS OF THE OSTERIX GENE EXPRESSION DURING OSTEOBLAST DIFFERENTIATION. **Sepúlveda, H.,** Pihan, P. and Montecino, M. Center for Biomedical Research and FONDAP Center for Genome Regulation, Faculty of Biological Sciences and Faculty of Medicine, Universidad Andres Bello.
Introduction. Osterix is a key regulator during osteoblast differentiation as it controls transcription of a significant number of bone-phenotypic genes. Here, we investigate epigenetic mechanisms that govern osterix gene expression when pluripotent mesenchymal cells differentiate to either osteoblasts or non-osseous cells.

Methods. The pattern of histone modifications, binding of transcription factors and co-regulators like HDACs (histone deacetylases) was determined by chromatin immunoprecipitation and quantitative Real Time PCR (qPCR). The DNA methylation state was determined by evaluating cleavage efficiency of methylation-sensitive restriction enzymes. The changes in Osterix mRNA expression were assessed by RT-qPCR.
Results. We find that the osterix gene promoter in non-osseus cells contains nucleosomes that exhibit significant tri-methylation at lysines 9 and 27 of histone H3 (H3K9Me3 and H3K27me3, respectively), reduced H3K4Me3, and decreased histone H3 and H4 acetylation (H3Ac and H4Ac). This repressive epigenetic pattern is accompanied by DNA methylation at osterix promoter sites -313 and -114, as well as downstream the transcription start site (+81). Also, we find increased binding of HDACs and no interaction of RNA polymerase II (Pol II) and the transcription factor Runx2. In contrast, in osteoblastic cells expressing osterix, we find increased H3K4me3 and H3/H4 Ac, decreased association of HDACs, and DNA methylation mostly in the coding region (site +81).
Discussion. During commitment of mesenchymal cells to the osteoblast lineage epigenetic mechanisms contribute to control the expression of the key regulator osterix.
Becario CONICYT, FONDECYT 1095075, FONDAP 15090007.

(239) BISPHOSPHONATES REGULATE OSTEOGENIC GENE EXPRESSION AND β-CATENIN ACCUMULATION IN OSTEOBLAST PRECURSOR CELLS. **Nicolás Méndez**, Ana María Pino, Juan Pablo Rodríguez, Mireya Fernández. Laboratorio de Biología Celular, INTA, Universidad de Chile. nmendezd@gmail.com
Introduction: Bisphosphonates are stable pyrophosphate analogs widely used as therapeutic agents to avoid increased resorption in bone diseases like postmenopausal osteoporosis and multiple myeloma (MM). There is evidence that, in addition to its direct effect on osteoclasts, bisphosphonates may also act on other bone marrow (BM) cells. In this report we evaluated the effects of the bisphosphonates ibandronic and zoledronic acids on mesenchymatic lineage cells.
Materials and Methods: Studies were performed in HS-5 and SaOS-2, BM stromal and osteosarcoma human cell lines; and mesenchymal stromal cells (MSCs) isolated from control and MM donors. Cell viability was assessed by the MTS method; β-catenin levels and subcellular distribution were evaluated by western blot and immunofluorescence, respectively; while osteogenic gene expression was evaluated by RT-PCR.
Results: Both bisphosphonates activated the canonical Wnt pathway as indicated from active β-catenin accumulation. The subcellular β-catenin distribution was confirmed by immunofluorescence, showing nuclear localization. Zoledronic acid could exert its effect independent of LRP5/6 co-receptor, since active β-catenin accumulated in the presence of the specific inhibitor dickkopf-1 (DKK-1). Ibandronic acid activated osteogenic differentiation of MSCs, as measured by osteogenic marker expression and cell mineralization.
Discussion: Results allow concluding direct effects of bisphosphonates on the activity of BM stromal cells, suggesting that bisphosphonates activate the canonical Wnt pathway in undifferentiated MSCs and promote osteogenic cell differentiation. Hence, these observations support an osteogenic effect of bisphosphonates, in addition to their recognized anti-osteoclastogenic action.
FONDECYT # 1090093.

(240) OSTEOBLASTIC DIFFERENTIATION IS ENHANCED BY TOPOGRAPHICAL AND CHEMICAL MODIFICATION OF AN SCAFFOLD SURFACE. **Eduardo Roa,** Juan Reyes and Nelson Osses. Instituto de Química, Pontificia Universidad Católica de Valparaíso. nelson.osses@ucv.cl
Introduction: Osteoinductive scaffolds are important in bone tissue engineering. Various studies have shown that either surface topographies or surface chemistry composition may influence osteoblastic differentiation. Our aim is to study if both features could be used to obtain enhanced osteoblastic differentiation.
Materials and Methods: Mouse embryonic fibroblast cells were plated on filter of different porosity in the absence or presence of a hydroxyl group polymer. Adhesion, proliferation and osteoblastic differentiation were analyzed.
Results: Increase in filter porosity correlated with higher roughness detected by atomic force microscopy. Time course of cell adhesion showed that augment in roughness surfaces induce cell attachment at short times. Independently of the surface roughness the presence of hydroxyl groups reduce the rate of initial cell adhesion but not the final number of attached cells. Cell proliferation was not affected by changes in roughness or by the presence of hydroxyl groups in the different topographies. Osteoblastic differentiation in the absence of stimulating media was detected in surfaces of higher roughness that was enhanced by the presence of hydroxyl groups. In the presence of osteoblastic differentiation media the highest osteoblastic differentiation was obtained in roughest surfaces containing hydroxyl groups.
Discussion: Our studies indicate that surface properties such as topography and chemical composition could be merged to enhance osteoblastic differentiation intended for bone tissue engineering purposes.
VRIEA-PUCV.

(241) RELATIONSHIP BETWEEN APOPTOSIS AND INSERTION SITE IN ROTATOR CUFF COMPLETE TEARS. **Juan Pablo Ramírez**[1], Francesca Bonati[1], Rodrigo Liendo[2], Francisco Soza[2] and Rodolfo Paredes[1]. [1]Laboratorio

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Introduction: In complete rotator cuff tears, surgery and corticosteroid are used to rehabilitate the tendon and control pain. However, patients show spontaneous new tears after corticosteroid treatment, with tendon degeneration. This work demonstrates the relationship between tears in different insertion sites and apoptosis, like possible agent of degeneration.

Material and Methods: Samples were obtained from patients with complete rotator cuff tear with or without previous corticosteroid infiltrations through arthroscopy at the Instituto Traumatológico Dr. Teodoro Gebauer Weisser in Santiago, Chile. Samples were paraffin embedded and cut in 5 μm sections. Apoptosis was detected by TUNEL assay and expressed like Percentage of Apoptotic Cells (POAC). For analysis, patients were grouped in three categories: control, non corticosteroids and corticosteroid treated. Statistic analysis was done using one way ANOVA, and differences were accepted when $P<0.05$, considering them statistically significant.

Results: Corticosteroid and non corticosteroid treated patients show greater POAC than controls in all insertion sites, but it was significant only in the infiltrated group. Additionally, the tendency of POA in all treated patients was similar, while all non treated patients were different.

Discussion: Corticosteroids induce a reduction of collagen synthesis, an increase of ROS generation, and an increase of metalloproteinases. These differences may be related with loss of tension and uniform apoptosis of tenocytes in all insertion sites. These events may help explain the tendon degeneration and could contribute to rupture in rotator cuff pathology.

(242) SKELETAL MUSCLE OF INSULIN RESISTANCE MICE HAS HIGHER INSULIN-DEPENDENT H_2O_2 PRODUCTION DUE TO HIGHER NOX2 EXPRESSION. [1]**Espinosa A**, [2]Juretić N, [1]Contardo C and [2]Jaimovich E. [1]Escuela de Tecnología Médica and [2]Centro de Estudios Moleculares de la Célula, Facultad de Medicina, Universidad de Chile, Chile. bespinosa@med.uchile.cl

Introduction: Insulin resistance (IR) is a metabolic condition characterized by impaired insulin induced glucose incorporation. H_2O_2 produced by NOX2, is involved in signaling pathways triggered by insulin in skeletal muscle. C57BL/6 mice fed with a high fat diet (HFD) are IR model having high plasma insulin levels. Our aim was to study H_2O_2 production in HFD fed animals and to measure mRNA and protein levels of $gp91^{phox}$ and $p47^{phox}$, both NOX2 subunits, in skeletal muscle.

Materials and Methods: C57BL/6J mice were fed control or HFD, *ad libitum* (D12492, Research Diets). IR was determined by HOMA-IR. Isolated muscle fibers in culture were transfected with a plasmid that encodes for HyPer protein, a sensor for intracellular H_2O_2. mRNA levels were measured by RT-PCR and protein levels with Western blot analysis.

Results: IR mice were obtained after 2 month of treatment with HFD. HOMA-IR was 4.48±1.58 in HFD, against 2.13±0.54 in control. IR mice were able to generate higher amounts of H_2O_2 upon insulin stimulation compared to control; $p47^{phox}$ and $gp91^{phox}$ mRNA levels increased similarly 3.0 and 2.9 fold in HFD mice and control, whereas protein levels were 6.8 and 1.6 fold compared to basal values, respectively.

Discussion: Our results indicate that IR mice can generate higher levels of H_2O_2 upon stimulation with insulin compared to control animals, probably by an increase of NOX2 levels in the skeletal muscle of insulin resistance mice.

FONDECYT 11090301 and 11100267.

(243) DIABETIC NEPHROPATHY INDUCES OVEREXPRESSION OF MUSCLE GLYCOGEN SYNTHASE IN HUMAN AND RAT KIDNEY. **Rodrigo Gatica**[*# ¥], Romina Bertinat[#], Carme Caelles[¥], Felipe Slebe[¥], Joan Guinovart[¥], Juan Carlos Slebe[£] and Alejandro Yañez[#]. [*]Escuela de Graduados Facultad de Ciencias Veterinarias, [#]Laboratorio de Metabolismo y Biotecnología, [£]Laboratorio de Enzimología, Universidad Austral de Chile; Universidad San Sebastián, Sede Puerto Montt; [¥]Institute for Research in Biomedicine, Barcelona, España. rodrigogaticaguti@gmail.com

Introduction: Diabetic nephropathy is characterized by glycogen accumulation in epithelial kidney cells and apoptosis. The aim of the present study was to determine the expression of different isoform of glycogen synthase (GS) in proximal tubule cells (RPTC) in diabetic state.

Material and Methods: Male Sprague-Dawley rats of 2 months in age, weghing 150-200 g were used for the study. Diabetes was induced by a endovenous injection of a buffered (0.05 M citrate, pH 4.5) solution of STZ at a dosage of 60 mg/kg body weight. Animals were divided into three groups with five animals in each group. Control group, fasted group, STZ-induced diabetic group. Statistical analysis was performed with Student's t test.

Results: We examined RPTC from control fed, starved, and diabetic rats (16 weeks) with chronic hyperglycaemia. Real time PCR analysis revealed upregulation of the muscle but not the hepatic isoform of GS (mGS) in RPTC from diabetic rats and renal cortex biopsies from type II diabetic patients. Western blot analysis demonstrated overexpression of total GS in diabetic and control starved rats, and also in renal cortex from diabetic patients.

Discussion: This different expression of GS isoforms in the diabetic kidney suggests that muscle GS is the responsible for pathological glycogen accumulation during the development of diabetic nephropathy in rats but most importantly in humans.

(FONDECYT 1090694).

(244) GAP JUNCTIONS FORMED BY CONNEXINS AND PANNEXINS IN ADIPOSE TISSUE. Benvenuto MA, Fernández PE, Sáez JC. Departamento de Fisiología, Pontificia Universidad Católica de Chile. mabenvenuto@gmail.com
Introduction: It is known that cell precursors of adipocytes express connexin43 and form functional gap junction channels (GJCs), which is lost upon differentiation. Thus, the cell-cell communication pathway that coordinates metabolic responses of differentiated adipocytes remains unknown. Here, we evaluated if adipocytes express gap junction formed by pannexin1 (Panx1), a glycoprotein that forms hemichannels and so far remains unknown if forms GJCs.
Material and Methods: Precursors of adipocytes isolated from primary cultures of visceral adipose tissue obtained from Sprague Dawley rats (male, 230-260g) were used. Cells were induced to differentiate with insulin, IBMX and dexamethasone. The presence of Cx43 and Panx1 was evaluated with Western blot and immunofluorescence analyses. Functional GJCs were evaluated with the dye coupling technique using DAPI, ethidium and Lucifer yellow.
Results: Cx43 and Panx1 were detected preferentially in pre-adipocytes and adipocytes, respectively. Moreover pre-adipocytes showed coupling with Lucifer yellow, but not with DAPI or ethidium, which was blocked by 18-βglycyrrhetinic acid (β-GA, 50μM). Differentiated adipocytes showed coupling with DAPI and ethidium, but not with Lucifer yellow, which was blocked by carbonexolone (5 μM), a Panx1 blocker.
Discussion: Pre-adipocytes are coupled via connexin43 GJCs and adipocytes are coupled through Panx1 GJCs, which could coordinate diverse metabolic responses in differentiated adipocyte tissue.

(245) CONNEXIN 39 FORMS FUNCTIONAL HEMICHANNELS, BUT NOT GAP JUNCTION CHANNELS, IN TRANSFECTED HELA CELLS. Vargas A[1], Cea L.A[1]., Vielma A[2]., Schmachtenberg O.[2], Sáez J.C.[1,2]. [1]Departamento de Fisiología, P. Universidad Católica, Santiago, Chile and [2]Centro Interdisciplinario de Neurociencias de Valparaíso, Universidad de Valparaíso, Valparaíso, Chile. anvargas@uc.cl
Introduction: Connexins (Cxs) are integral membrane proteins expressed in most tissues of vertebrate animals. Six Cx subunits form a hemichannel (HC) and two HCs form a gap junction channel (GJC), which communicates the intra- and extracellular milieus or the cytoplasm of two adjacent cells, respectively. Cx39 is expressed in few cell types including skeletal muscle precursor cells during myogenesis and regeneration. However, the functions of Cx39 as HC and/or GJC have not yet been demonstrated.
Materials and Methods: HeLa cells stably transfected with Cx39 were used. The presence of Cx39 was evaluated by immunofluorescence. HC activity was evaluated in dye uptake (Ethidium: Etd, DAPI; 5 μM) experiments using real-time imaging in normal extracellular or divalent cation-free solution (DCFS). The functional state of GJCs was evaluated with electrical (dual voltage clamp) and dye coupling measurements (Lucifer yellow: LY; Etd, DAPI, and Alexa350; 75 mM).
Results: DCFS increased the dye uptake in HeLa-Cx39 cells, which was inhibited by La^{3+} (200μM). Cx39 formed gap junction plaques between cells. However, no electrical or dye coupling was detected.
Discussion: Under basal conditions, Cx39 expressed in HeLa cells does not form active GJCs. However, it forms HCs activated by DCFS.

(246) BNP INHIBITS HUMAN MYOMETRIAL CONTRACTION VIA NPR-C THAT IN TURN TRIGGERS THE cAMP/PKA PATHWAY. Delpiano AM, Garmendia LR, Poblete JA, Cuello MA, Carvajal JA. Laboratorio de Medicina Materno Fetal. Departamento de Obstetricia y Ginecología, Escuela de Medicina. Pontificia Universidad Católica de Chile. delpiano@med.puc.cl
Introduction: We reported that brain natriuretic peptide (BNP), produced by the fetal membranes, may mediate myometrial quiescence during pregnancy. We have shown that BNP inhibits human myometrial contractions by activation of the natriuretic peptide clearance receptor (NPR-C). We postulate BNP/NPR-C intracellular cascade is the cAMP/PKA pathway.
Material and Methods: myometrial samples were obtained during cesarean section from preterm (<34 weeks) not in labor women and used to prepare cell cultures and in vitro contractility assay. **Myometrial cultures** were stimulated with BNP (10^{-7}M) or cANP (10^{-7}M, clearance receptor agonist), either in the presence or absence of AP-811 (10^{-7}M, clearance receptor blocker). The concentration of cAMP was measure by RIA and PKA activity by ELISA. **Full thickness myometrial strips** were placed in organ baths for isometric tension measurement, after oxytocin (10^{-8}M) stimulation, the effect of BNP, cAMP and H-89 (PKA inhibitor) was analyzed.
Results: BNP and cANP significantly increased cAMP concentration and PKA activity in myometrial culture; the effect was absent in the presence of AP-811. BNP and cANP significantly inhibited (~50%) myometrial contractility; this inhibition was blocked in the presence of H-89.
Discussion: BNP and cANP (NPR-C agonist) increased cAMP content and PKA activity in human preterm cultured cells by activating NPR-C. BNP and cANP inhibited human myometrial contractions by increasing PKA activity. We concluded that BNP/NPR-C intracellular cascade leading to inhibition of myometrial contraction is the cAMP/PKA pathway.
Financial Support: FONDECYT 1090616.

(247) RISK OF PREECLAMPSIA (PE) AND PRESENCE OF DIFFERENT POLYMORPHISM IN ENZIMES INVOLVED IN THE METHIONINE-HOMOCYSTEINE METABOLISM (MHM). Valenzuela FJ, Larrain R,

Perez-Sepulveda A, Torres MJ, Guzman A, Ahumada V, Figueroa-Diesel H, Illanes S. Departamento de Obstetricia & Ginecología y Laboratorio de Biología de la Reproducción. Facultad de Medicina, Universidad de los Andes, Santiago. sillanes@uandes.cl (Sponsor: U. Wyneken).

Introduction: MHM is critical in the availability of methionine (Met), essential for placental/fetal development. Defects in methylation pathway reduce the availability of Met, needed for the production of S-adenosyl methionine (SAM), donor of CH3-groups and critical for conversion of 2-OH-estradiol to 2-methoxyoestradiol (2-ME) by catechol-O-methyltransferase (COMT). Low levels of 2-ME has been reported in animals models of PE.

Hypothesis: Alterations in MHM via SNPs of methionine synthase (MTR), methioninesynthasereductase (MTRR) and methylenetetrahydrofolate (MTHFR) are associated to PE.

Methods: 90 placentas were used from a cohort of patient's controlled at the fetal medicine unit of Hospital Parroquial SB, 11 from patients that developed PE, 59 controls and 20 with another disease. Placental DNA was obtained with DNA purification kit (Master-Pure, Epicentre). Evaluation of polymorphism of MTHFR-677C>T, MTRR-66A>G, MTR-2756 A>G was performed using RFLP-PCR. The allele distribution was analyzed using Chi-Square test and Odd ratio.

Results: The MTHFR polymorphism showed a frequency of 41% and a tendency a differential distribution between control and PE (Chi square = 0.07, P=0.7, Odds ratio=0.9; control=52 and PE n=11). The frequency for MTRR was 48% (Chi square=0.03 and P=0.9, Odds ratio=0.91; control=51 and PE=8). The frequency to MTR was 19.4% (Chi square=0.73 and P=0.42, Odds ratio=1.8; control=30 and PE=19).

Discussion: No significant associations are seen between MTR and PE. The tendency observed in the MTHFR and MTRR, suggest these could potentially be interesting to study further.

Fondecyt 1110883.

(248) CHARACTERIZATION OF OLEOSOMES FROM *Gevuina avellana* AND *Madia sativa* SEEDS IN THE HUVEC CELL LINE. **Patricia Navarrete**[1,5], Francisca Acevedo[2,5], Oscar Valerio[3,5], Jorge Parodi[4], Fernando Romero[1]. [1]Center of Neurosciences and Peptides Biology- CEBIOR, BIOREN, University of La Frontera. [2]Center of Food Biotechnology and Bioseparations, BIOREN, University of La Frontera. [3]Center of Waste Management and Bioenergy, BIOREN, University of La Frontera. [4]Laboratory of Molecular Neurobiology, CARE, P. Universidad Católica de Chile. [5]Supported by PIA DI11-7001 of University of La Frontera. patricia.bq@gmail.com (Sponsor: E.O. Campos)

Introduction: Oleosomes obtained from oilseeds have been exploited for several biotechnological applications based on their non-coalescing nature. Chilean Amerindians have used *Gevuina avellana* and *Madia sativa* seeds as oil sources since pre-Columbian times and only few scientific reports about their bioactive molecules have been already found. The objective of this study was characterizing the effect of oleosomes from Chilean native mature seeds of *Gevuina avellana* and *Madia sativa* in the HUVEC cell line.

Materials and Methods: Oleosomes from Chilean native mature seeds of *Gevuina avellana* and *Madia sativa* were stained with the probe 3,3′-dihexyloxacarbocyanine iodide (DiOC6) and were evaluated by flow cytometry and fluorescence microscopy. Moreover, the HUVEC cell line was utilize for to evaluate the cytotoxic effect of oleosomes mean confocal and optical microscopy.

Results: The analysis of oleosomes showed that these show shape spherical of heterogeneous sizes, moreover these lipidic structures are dyed with the probe DiOC6. Furthermore, oleosomes are not produce cytotoxic effect in the HUVEC cell line.

Discussion: These findings show that oleosomes are to able of dyed and moreover are not produce cytotoxic effect in the HUVEC cell line. This work may help to design a fluorescent test for to analyze the effect of oleosomes in others cell line.

(249) UNRAVELING THE MYSTERIES OF SUPERRESOLUTION OPTICAL FLUCTUATION IMAGING (SOFI). **Felipe Santibáñez**[1,2], Omar Ramírez[1], Dirk Haehnel[3], Jörg Enderlein[3] & Steffen Härtel[1,2]. [1]SCIAN-Lab, [2]BNI, ICBM, Faculty of Medicine, U. of Chile. [3]III. Physikalisches Institut, Universität Göttingen, Germany. fsantibanez@med.uchile.cl

Introduction: The diffraction limit of light microscopy has been overcome in the last decade by several superresolution techniques that rely heavily on advanced optical methodologies and mathematical-computational image processing. SOFI has recently appeared as an alternative and complementary superresolution technique based on stochastic independent temporal fluorescence fluctuations of the emitters, performing statistical post-processing analysis. We propose to extend a recent approach for SOFI based on higher-order cumulants in the Fourier domain.

Material and Methods: We compare the traditional SOFI implementations with the proposed approach with simulations based on Monte Carlo stochastic blinking generation. Practical validation considered high blinking requirements using quantum dots to obtain thousands of epi-fluorescence images and post-processing data with standard SOFI implementation and the proposed method.

Results: 2-fold to 5-fold improvement in spatial resolution using conventional wide-field microscope was obtained while the outcomes are validated between SOFI implementations.

Discussion: 3D subdimensional limit optical resolution provided by SOFI is based on blinking/intermittency properties of emitters. Diverse statistical analysis of temporal fluctuations were implemented in the last years toward improvements in efficiency and broader scope for this technique using higher-order cumulants, correlations and variances in time-space

domain and recently in Fourier domain. The iterative character of discrete steps required to obtain higher order statistics gives the possibility of analyzing ROIs at different resolution levels.
Funding: ICM (P07-048-F; P09-015-F), Fondecyt 1090246.

(250) CHARACTERIZATION OF THE HANTAVIRUS GC TRANSMEMBRANE DOMAIN. [1]**Sarno-Orellana, A.V.** [1]Carrasco, M. and [1,2]Tischler N.D. [1]Fundación Ciencia para la Vida and [2]Universidad San Sebastián. victoria.sarno@gmail.com
Introduction: Andes virus (ANDV) is a member of the *Hantavirus* genus. It is composed of a lipid envelope in which the glycoproteins Gn and Gc are anchored by a transmembrane domain (TMD). To enter cells, these proteins interact with receptors and fuse the viral membrane with a membrane of the cell. For some viral fusion proteins, it has been described that the TMD has a functional role during this process being involved in late steps of fusion. Previous work identified Gc of hantaviruses as the fusion protein. We postulate that modifications of the TMD of ANDV Gc affect the fusion process.
Materials and Methods: Using high fidelity PCR, two types of Gc TMD mutants were constructed: i) reduction in TMD lenght by consecutive deletions of the C-terminal region of the protein; ii) substitution of TMD by a signal sequence for glycosylphosphatidylinositol anchoring. Expression and surface localization of wild-type and Gc mutants was determined by Western blot of transfected and biotinylated HEK cells. Functionality of these mutants was studied in fusion assays of transfected VeroE6 cells
Results: Wild-type and TMD length Gc mutants, except the mutant lacking seven residues, were accumulated at the plasma membrane of transfected cells indicating their propper folding. Of these, only the mutant without the cytoplasmatic tail retained a reduced fusion activity. In contrast, the mutant lacking four residues of TMD was fusion inactive.
Discussion: Mutations of Gc TMD length suppressed the fusogenic activity, indicating an essential role of Gc TMD in membrane fusion.
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(251) ESSENTIAL RESIDUES AND FUNCTION OF THE HANTAVIRUS GC STEM REGION. [1]**Villalón, F.,** [1]Carrasco, M., [2]Monasterio, O. and [1,3]Tischler N.D. [1]Fundación Ciencia para la Vida, [2]Universidad de Chile, [3]Universidad San Sebastián. fvillalon@ug.uchile.cl
Introduction. Andes virus belongs to the *Hantavirus* genus. It has a lipid envelope in which two transmembrane glycoproteins are anchored: Gn and Gc. These proteins are responsible for the recognition of cellular receptors and entry into target cells by fusion of the viral with a cellular membrane. Previous research has shown that Gc is responsible for the fusion process and suggests that it contains a functional region with unique characteristics. Using peptides, it has been found that this region is formed by two α-helices, H1 and H2 which interact with liposomes. We hypothesize that the Gc stem region is essential in the membrane fusion process.
Material and Methods. Two different approaches were developed, one based on peptides spanning the Gc stem region and another on site-directed mutagenesis of this region. Using peptides, destabilization and fusion of liposomes was measured. Gc mutants of the stem region were characterized in cell-cell fusion assays and infectivity studies of Gn/Gc pseudotyped particles through the detection of GFP expression.
Results. Depending on the liposome composition, the peptide spanning Gc H2 produced pores in model membranes and also induced their fusion. Further, mutations introduced within the Gc stem region diminished or suppressed the activity of the protein. Infectivity studies of Gn/Gc mutant pseudotyped particles are underway.
Discussion. The Gc stem region is fundamental in the membrane fusion process. We propose that this region may decrease the energy required to trigger fusion through the destabilization of the viral membrane.
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(252) ***Trypanosoma cruzi*** **CALRETICULIN INHIBITS ENDOTHELIAL CELL MIGRATION** ***in vivo*** **IN A MAMMAL MODEL.** **Leonora Duaso***, Juan. Duaso , Francisca Coddou*, Katherine Weinberger*, Ismael Maldonado*, Galia Ramírez*, Ulrike Kemmerling , Arturo Ferreira*. *Immunology Disciplinary Program, Anatomy and Developmental Biology Program, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, Santiago, Chile. aferreir@med.uchile.cl
Introduction: Angiogenesis, the generation of capillaries from blood vessels, occurs exceptionally (chronic inflammation, menstrual cycle, wound healing, among others), and it is fundamental for tumor growth. Calreticulin, a multifunctional, conserved protein, is expressed in nucleated cells. Both human Calreticulin and its N-terminal domain (vasostatin), interfere with endothelial cell proliferation and migration. *Trypanosoma cruzi* Calreticulin is antiangiogenic in the *in vivo Gallus gallus* chorioallantoid membrane assay. *T. cruzi* Calreticulin, or its vasostatin-like domain, interact with venous and arterial endothelial cells from several mammal species, inhibiting capillary morphogenesis, proliferation and migration. However, whether *T. cruzi* Calreticulin interferes with angiogenesis *in vivo*, in a mammalian, model is unknown. Herein, we evaluate *T. cruzi* Calreticulin interference with endothelial cell migration in mice.
Material and Methods: Matrigel plugs, containing bFGF (an endothelial cell infiltration promoter), were implanted subcutaneously. Seven days later, the plug endothelial cell infiltration was quantified.

Results: Both *T. cruzi* and Human Calreticulins almost completely inhibited bFGF-promoted Matrigel plug cell infiltration, a pre requisite for capillary morphogenesis.
Discussion: The *T. cruzi* infection capacity to inhibit tumor growth *in vivo,* may depend, at least partly, on the translocation of the parasite chaperone molecule from the endoplasmic reticulum to the exterior, where it interferes with early angiogenesis.
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(253) *T. cruzi* DNA REPAIR ENZYMES TCAP1 AND TCAP2 ARE LOCALIZED IN THE NUCLEUS AND THEIR OVEREXPRESSION INCREASES EPIMASTIGOTE VIABILITY WHEN SUBMITTED TO OXIDATIVE STRESS. **Sofia Sepúlveda**, Lucía Valenzuela, Iván Ponce, José Delgadillo, Santiago Ramírez, Soledad Sierra, Paula Bahamondes, Natalia Muñoz, Ulrike Kemmerling, Norbel Galanti, Gonzalo Cabrera. Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile. se_sepulveda@med.uchile.cl
Introduction. The DNA repair pathway via base excision (BER) is one of the survival mechanisms that *T. cruzi* could use when submitted to oxidative stress. The apurinic apyrimidinic endonucleases (APEs) are critical in the BER pathway. In this work the subcellular localization of TcAP1 and TcAP2 (*T. cruzi* APEs) as well as the effect of their overexpression on the viability of epimastigotes submitted to oxidative stress is established.
Material and Methods. TcAP1 and TcAP2 overexpression in epimastigotes was achieved using the pTREX-GFP vector and evaluated by western blot using an anti-GFP antibody. Subcellular localization of both enzymes was determined by fluorescence microscopy. Viability of modified and control parasites was measured by MTT assays after treatment with oxidative agents.
Results. Transfected *T. cruzi* epimastigotes expressed TcAP1 and TcAP2 with molecular masses similar though not equal to the expected ones. In mammals APE1 and APE2 are present in the cytoplasm; contrarily TcAP1 and TcAP2 are found in the nucleus of *T. cruzi* epimastigotes. Overexpression of these two enzymes induces an increment in epimastigote viability when parasites are submitted to oxidative stress.
Discussion. Our results suggest that TcAP1 and TcAP2 may play a key role in the resistance of *T. cruzi* to oxidative DNA damage. Consequently, these enzymes may be considered as a drug target to control parasite survival.
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(254) ASSOCIATION BETWEEN MUC1 SPLICE VARIANTS FROM SALIVARY GLANDS AND MOUTH DRYNESS. **Sung HH,** González S, Aguilera S, Bahamondes V, Castro I, Barrera MJ, Leyton C, Urzúa U y González MJ. ICBM,Facultad de Medicina, Universidad de Chile. sunghsieh@gmail.com
Introduction. Sjögren's syndrome (SS) patients usually experience mouth dryness. Mucin type 1 (MUC1) is a large glycoprotein localized at the plasma membrane (MUC1/A and MUC1/B) or as a soluble form (MUC1/SEC) in epithelial cells. MUC1/A and MUC1/B are splice variants determined by a single nucleotide polymorphism (SNP_rs4072037) consisting of a G/A substitution in position 568 of exon 2. MUC1/A contains 27 bp of intron 1 and a higher count of variable number tandem repeats (VNTR) in exon 2 than MUC1/B. This VNTR sequence codes for 5 sites of O-glycosylation which ultimately may generate a mucin with a high capacity to hydrate the mucosa. We determined the MUC1/A and MUC1/B splice variants and their relationship with the SNP_rs4072037 genotype. MUC1/SEC mRNA and protein levels and its subcelular localization in salivary glands (SG) were also evaluated.
Methods. SG biopsies of 20 SS-patients and 24 controls were obtained. RT-PCR was used to identify MUC1 variants. SNP genotyping was performed by Taqman® probes and confirmed by sequencing. Relative MUC1/Sec mRNA levels were determined by qPCR and immunochemistry for its localization.
Results. A reduced frequency of MUC1/A splice variant in SS-patients was observed. A correlation between MUC1/A, MUC1/B and SNP_rs4072037 genotype (MUC1/AA-allele_GG, MUC1/AB-allele_GA and MUC1/BB-allele_AA) was obtained. Relative MUC1/Sec mRNA levels were significantly increased and cytoplasmic protein accumulation was observed in SS-patients.
Conclusion. Both reduced frequency of MUC1/A and cytoplasmic accumulation of MUC1/SEC could explain mouth dryness in SS-patients.
Fondecyt 1080006 (MJG, SA y CM).

(255) PRESENCE OF CALRETICULIN IN *Triatoma infestans* SALIVARY GLAND. **Weinberger, K.**[*], Coddou, F.[*], Duaso, L.[*], Valck, C.[*], Ramírez, G.[*], Aguilar L.[*], Maldonado I.[*], Zulantay I. , Apt W. , Ferreira A.[*]. Disciplinary Immunology[*] and Cellular Biology Programs, Biomedical Sciences Institute, Faculty of Medicine, University of Chile. aferreir@med.uchile.cl
Introduction: Chagas' disease is a zoonosis affecting 16 million people in Latinoamerica and is caused by the protozoan *Trypanosoma cruzi*. It is mainly transmitted by haematophagous insects of the *Triatominae* subfamily, whose saliva has vasodilating and immunomodulatory properties that favour the blood ingestion by the host, without apparent damage to the

digestive system of the insect. These properties could be partially attributed to calreticulin (CRT) from the salivary gland of the arthropod.

Materials and Methods: CRT in salivary gland extract of *Triatoma infestans* (TiCRT) without infection was determined by: a) SDS-PAGE, followed by western-blotting; b) its capacity to inhibit C1-mediated C4b generation in ELISA.

Results: Heterolog antibodies recognized putative TiCRT, which bound specifically C1q, a danger signal recognition module from the classical pathway of human complement. The salivary gland extract, containing TiCRT, did inhibit that route of complement activation. Probably, TiCRT interaction with C1q mediated a decrease in the C4b deposition onto the solid phase.

Conclusion: The salivary gland extract from *T. infestans* contains a molecule that has structural, antigenic and functional properties compatible with those known for calreticulin.

Discussion: Calreticulin is detected in salivary gland of *T. infestans* by functional and antigenic criteria. Efforts to clone the TiCRT gene are currently underway, as well as studies aimed at determining the TiCRT effects on the lectin human complement pathway.

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