INTERNATIONAL MEETING

SCIENCE & FRIENDSHIP X:

THE NORTH MEETS THE SOUTH

"International Meeting between graduate students and professors from the University of California at San Francisco and graduate students and faculty from the Fundación Ciencia & Vida"

OCTOBER 26 – NOVEMBER 1, 2015
IN CONJUNCTION WITH THE XXIX ANNUAL MEETING OF
THE CHILEAN CELL BIOLOGY SOCIETY
PTO. VARAS, CHILE

PARTICIPATION OF SCIENCE & FRIENDSHIP SYMPOSIUM GROUP

Sunday October 25th

18:45-20:15 Oral presentations I Volcanes Room	18:45 Simon Vidal (NYU-FCV)
20.15	Dinner
22:00 -23:30 Volcanes Room	Peter Walter (UCSF) From Protein folding to cognition: the serendipitous path of discovery Chair: Pablo Valenzuela

Monday October 26th

Breakfast	7:30 – 9:30
10:30-12:30 Poster Sesion I (I-87 odd numbers) Volcanes Room	Rodrigo Acuña (FCV). Poster 3. Jaime Villegas (FCV). Poster 27. Francisco Contreras (FCV). Poster 59. Kelly Crotty (UCSF). Poster 65. Kelsie Eichel (UCSF). Poster 77.
12:45-14-15	Lunch
17:30-19:30 Poster Sesion I (I-87, even numbers) Convention Center Foyer	Courney Bone (UCSF). Poster 26. Javier Cáceres-Delpiano (FCV). Poster 36. Elizabeth Costa (UCSF). Poster 64. Cristián Doñas (FCV). Poster 72.
20:30	Dinner

Tuesday October 27th

Breakfast	7:30 – 9:30
9:00-10:30 Oral Presentations III Volcanes Room	9:00 Aaron Mendez (UCSF).
12:45-14:15	Lunch
10:30-12:30 Poster Session II (88-173, odd numbers) Convention Center Foyer	Felipe Flores (FCV). Poster 91. Ignacio Fuenzalida (FCV). Poster 97. Aylin Goke (UCSF). Poster 101. Max Horlbeck (UCSF). Poster 115. Roddy Jorquera (FCV). Poster 121. Kamena Kostova (UCSF). Poster 123. Mable Lam (UCSF). Poster 125.
14:15-16:15 Tronador Room	Ciencia & Vida – UCSF Symposium on Biomedical Research Jodi Nunnari, Rodrigo Pacheco, Pamela Munster Chair: Carolina Torrealba
16:30-17:30 Tronador Room	Jonathan Weissman (UCSF) Plenary Lecture Chair: Andrés Couve
17:30-19:30 Poster Session II (88-173 even numbers) Convention Center Foyer	Arda Mizrak (UCSF). Poster 142. Andrew Murley (UCSF). Poster 152. Jaime Villegas (FCV). Poster 172.
21:00	Dinner

Wednesday October 28th

Breakfast	7:30 – 9:30
10:30-12:30 Poster Session III (174-259, odd numbers) Convention Center Foyer	Courtney Schroeder (UCSF). Poster 227. Jordan Tsai (UCSF). Poster 241. Ariadne Vlahakis (UCSF). Poster 253.
12:45-14:15	Lunch
17:30-19:30 Poster Session III (174-259, even numbers)	Vicente Muñoz (FCV). Poster 178. Grace Peng (UCSF). Poster 202. Carlos Rivera (FCV). Poster 212. Sandra Torres (UCSF). Poster 236. Ngoc-Han Tran (UCSF). Poster 238.
21:30	Dinner & Party

CONTACT INFORMATION:

Carolina Torrealba
Mobile: +56 9 9448 5036

Nicole Halcartegaray
Mobile: +56 9 9225 1888

Pablo Valenzuela
Mobile: +56 9 9444 1980

CONFERENCES

Science & Friendship 2015

“From Protein Folding to Cognition: The Serendipitous Path of Discovery”

Peter Walter
peter@walterlab.ucsf.edu
University of California at San Francisco and Howard Hughes Medical Institute

Hypothesis: Basic discovery research aiming at advancing our fundamental molecular understanding of the inner workings of the cell provides an invaluable foundation for development of new clinical therapies.
Method and Results: A screen for small, drug-like molecule modulators of the unfolded protein response yielded ISRIB, a drug-like compound that with high efficacy renders cells insensitive to translational inhibition by phosphorylation of eIF2. ISRIB acts by enhancing the activity of eIF2’s guanine nucleotide exchange factor. ISRIB proved to be a cognitive enhancer in rodents, significantly improving long-term memory of wild-type animals. This effect is exerted by blocking long-term depression (LTD) in hippocampal neurons.
Discussion: The potential application of ISRIB in diseases with associated cognitive dysfunctions will be discussed.

Relevant areas: 3- Cell Biochemistry and 5- Neurobiology

Merits, opportunities and challenges in early phase clinical trials

Pamela N Munster, MD
pamela.munster@ucsf.edu
Hellen Diller Cancer Center, University of California-San Francisco

The goals and objectives of early phase clinical trials are rapidly evolving in an era of significant advances in both hematological malignancies and solid tumors. In depth understanding of scientific principles and integration of rational drug development are ever more important.

In addition to dose and schedule determination, patient safety and pharmacokinetic assessment, most phase I trials now include the validation of molecular targets, and the assessment of pharmacodynamics effects on signaling pathways by serial tumor biopsies, liquid biopsies and molecular imaging. Biomarker selection using broad molecular profiling further tailors treatment with a novel compound to the matching pre-specified patient populations expressing select targets. The successful introduction of immunotherapy has further expanded the arsenal for specific interference with tumor targets, yet clearly point to the need to evaluate the tumor as well as its environment. Assessment of contributing host factors such as germ line mutations and gene polymorphisms will soon become an integral part in drug development.

All of these approaches contribute considerably to an early merit assessment of a novel compound but add vast complexity. The cost of current drug development mandate sophisticated guidance for timely "go - no go" decision-making and optimal clinical placement of a promising drug. An overview of merits, opportunities and challenges encountered during early phase clinical trials will be discussed.

Molecular Basis of Mitochondrial Behavior

Jodi Nunnari
jmnunnari@ucdavis.edu
University of California, Davis

Hypothesis: The endoplasmic reticulum plays integral roles in the regulation of mitochondrial behavior through region of ER-mitochondria contact.
Method and Results: Forward proteomic, as well as cytological, biochemical and genetic approaches uncovered a new conserved family of sterol transporters at ER contacts sites. One family member, termed Ltc1 (lipid transfer at membrane contact), localized at both ER-mitochondria and ER-vacuole contact sites in cells through interactions with the organelle specific partners, Tom70/71 and Vac8, respectively. At mitochondria Ltc1 is required for viability in the absence of the ER-mitochondria contact and phospholipid transport complex ERMES. At vacuoles, Ltc1 was required for sterol-enriched membrane domain formation in response to stress. Increasing the proportion of Ltc1 at vacuoles was sufficient to induce sterol-enriched vacuolar domains without stress.
Discussion: A model where Ltc1 functions as a sterol-dependent regulator of organelle and cellular homeostasis via its dual localization to ER- mitochondria and ER-vacuole contact sites will be discussed.

Relevant areas: 2- Membrane / Protein Traffic and 3- Cell Biochemistry

Teaching and Learning Workshop University of California, San Francisco

Patricia S. Caldera, Ph.D.
Emeritus Academic Coordinator
Science and Health Education Partnership

For a long time science teaching have been based on memorization of the book's material, recreating cookbook experiments and with the linear concept that the students will learn by listening to the teacher's lecture. These methods have been a barrier for many students' interest in science. The neuroscience field of research has done great contributions to better understand how we learn and the evidence suggest that it requires more than listening and reading-memorizing but that it is necessary the active participation of the learner. As science instructors we found ourselves with the dilemma of how to engage all students, how to teach more effectively, and how to demonstrate more accurately the nature of science.

The workshop will introduce and demonstrate strategies to actively engage all learners and to make learning more student-centered allowing the participants to experience first-hand their impact on student engagement and effective learning. These teaching techniques can be incorporated in formal and informal science teaching situations, allowing the learner to gain deeper understanding of the material, retain the information and enjoy science more.

This session is applicable to those involved or interested in teaching science from junior high school to the undergraduate level.

Monitoring translation in space and time with ribosome profiling

Jonathan Weissman, PhD
University of California-San Francisco/Howard Hughes Medical Institute

Introduction:

Efforts to globally monitor gene expression have historically focused on measuring mRNA levels (for example, using microarrays or RNA-seq), although we know that translational control is an essential and regulated step in determining protein expression. Until recently, precisely monitoring protein translation was far more challenging than measuring mRNA levels.

Methods and Results:

We have developed a suite of techniques based on ribosome profiling (deep sequencing of ribosome protected fragments) that dramatically expand our ability to follow translation *in vivo*. I will present recent applications of our ribosome profiling approach including the following: (1) Development of ribosome profiling protocols for a wide variety of eukaryotic and prokaryotic organisms. (2) Uses of ribosome profiling to globally monitor when chaperones, targeting factors or processing enzymes engage nascent chains. (3) Deciphering the driving force and biological consequences underlying the choice of synonymous codons. (4) The use of ribosome profiling to experimentally define the full complement of proteins encoded within complex genomes. (5) The development of a proximity-specific ribosome profiling assay for monitoring subcellular localized translation and its use to follow translation on the surface of the endoplasmic reticulum and mitochondria.

Discussion:

Ribosome profiling now makes it possible to globally monitor protein translation in vivo with unprecedented speed, precision and depth. The method has enabled discovery of the gene expression regulation underlying diverse and complex biological processes, of important aspects of the mechanism of protein synthesis, and even of new proteins, by providing the first systematic approach for the experimental annotation of the full set of proteins encoded in human and other complex genomes.

Areas:

3- Cell Biochemistry

The dopaminergic regulation of T-cell mediated immunity and its involvement in inflammatory disorders

Rodrigo Pacheco
Laboratory of Neuroimmunology, Universidad Andrés Bello and Fundación Ciencia & Vida, Santiago, Chile.

Introduction. Evidence has associated chronic inflammatory disorders, including autoimmune and neurodegenerative diseases, with alterations on dopaminergic components of immune cells. Furthermore, several studies have shown a pivotal role of CD4+ T-cells and dendritic cells (DCs) in the physiopathology of inflammatory disorders. Here, we studied the basic mechanisms underlying dopaminergic regulation of T-cell mediated response, and how this regulation is involved in autoimmune and neurodegenerative disorders.

Methods. We performed adoptive transference of wild-type (WT), or dopamine receptors deficient CD4+ T-cells into T-cell-deficient recipient mice. Similarly, DCs either WT or deficient in dopamine receptors were transferred into WT recipients. Pharmacological and genetic manipulations of *ex vivo* T-cells and DCs were also carried out. Relevance of dopamine receptors of T-cells and DCs was assessed in several models of inflammatory diseases, including Parkinson's disease (PD), Multiple Sclerosis (MS), inflammatory colitis (IC) and Rheumatoid Arthritis (RA).

Results. Stimulation of dopamine receptor D5 (D5R) expressed in DCs strongly favors the production of IL-23 and IL-12 and thereby the differentiation of CD4+ T-cells into pathogenic Th17 and Th1 cells *in vivo*. Accordingly, inhibition of D5R-signaling in DCs reduced disease severity in MS and RA. Moreover, D3R-signaling in CD4+ T-cells strongly favored Th1-differentiation and attenuated suppressive function of regulatory T-cells. Accordingly, mice bearing D3R-deficient CD4+ T-cells are resistant to neurodegeneration involved in PD and display attenuated IC. Furthermore, D3R-signaling evoked infiltration of inflammatory-T-cells into the substantia nigra upon PD, and into the colonic lamina propria during IC.

Discussion. Our findings illustrate novel and relevant mechanisms involving dopaminergic regulation of T-cell-immunity associated to inflammatory disorders.

IMMUNOLOGY

Fundación Ciencia & Vida

UCSF ABSTRACTS

Science & Friendship 2015

Cytoskeletal elements function together to move larval P-cell nuclei through constricted spaces

Courtney R. Bone*, Yu-Tai Chang, Natalie Cain, Shaun Murphy, and Daniel A. Starr
Department of Molecular and Cellular Biology, University of California Davis, Davis, CA 95616
*crbone@ucdavis.edu

Introduction Nuclear migration is critical for many developmental and cellular processes including fertilization, cell polarization, and differentiation. Disruptions of migration machinery are associated with several diseases including muscular dystrophy, cancer and autism. *C. elegans* P-cell nuclear migration occurs during the first larval stage where the 3-4 µm nucleus migrates through a 150nm space between the body wall muscle and cuticle. We hypothesize dramatic cytoskeletal and nucleoskeletal rearrangements are necessary for nuclear migration through constricted spaces.

Materials and Methods We are investigating the roles of the cytoskeleton and nucleoskeleton during this nuclear migration event by visualizing components *in vivo*. We aim to characterize the interacting roles of the cytoskeleton and identify the role of the nuclear lamina in P-cell nuclear migration.

Results & Discussion Our data suggest dynein is the primary motor functioning to move nuclei during P-cell nuclear migration, while kinesin plays a minor role. Additionally, results from a genetic screen implicate actin in P-cell nuclear migration. We are therefore investigating the dynamics of the actin network in P-cell development by live cell imaging of tagged actin binding proteins. Thirdly, we hypothesize nuclear lamina breakdown is necessary to squeeze into the constricted space and are using an endogenously GFP-tagged nuclear lamin to visualize nuclear lamina.Our model postulates that dynein provides the major force for P-cell nuclear migration, while actin assists the cell through the narrow space, and the nuclear lamina disassembles to allow nuclei to squeeze through tight spaces.

Area 6

Global analysis of translation at the endoplasmic reticulum in mammalian cells using proximity specific ribosome profiling

Elizabeth A. Costa, Calvin H. Jan, Jonathan S. Weissman
Elizabeth.costa@ucsf.edu
University of California, San Francisco

Introduction Proteins destined for the secretory pathway are targeted to the endoplasmic reticulum (ER) and translocate across the membrane through the conserved translocon. ER targeting can be mediated by either the signal recognition particle (SRP) in a co-translational manner, or by the SEC complex, which is capable of mediating post-translational translocation. Although numerous studies have characterized targeting and translocation dependencies for a small number of substrates, we lack a broader knowledge of which proteins are targeted to the ER co-translationally in vivo.

Materials & Methods We have applied a recently developed approach, termed proximity-specific ribosome profiling, for globally monitoring localized translation. Using a biotin ligase at the ER and an acceptor peptide on the ribosome, we are able to specifically label ribosomes translating at the ER. This, coupled with ribosome profiling, allows us to comprehensively identify proteins that are targeted to the ER co-translationally.

Results & Discussion We show that the majority of secretory proteins are translated at the ER, while the majority of cytosolic proteins are not. Additionally, we show that ER-associated ribosomes are dynamic and can readily exchange with the pool of cytosolic ribosomes. Our data indicate that our method is able to specifically label ribosomes at the ER. We are now able to explore what defines co-translational vs. post-translational substrates in vivo, and which targeting factors are required. We are now applying our method to understand how cells dynamically change translation at the ER in response to cellular stresses.

Area 2-

Identifying how *BiP1* mRNA is uniquely stabilized after losing its polyA tail by Ire1 cleavage in *S. pombe*

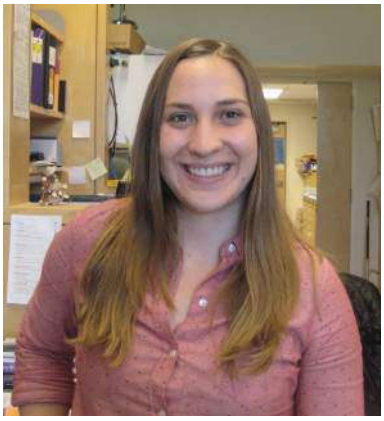
Kelly J. Crotty[1]*, Clarence Yu Cheng[2], Wipapat Kladwang[2], Rhiju Das[2], Peter Walter[1]
[1]University of California, San Francisco; [2]Stanford University, Stanford
Kelly@walterlab.ucsf.edu

Introduction The endoplasmic reticulum (ER) adjusts its capacity to fold proteins to meet its protein-folding load by activating the unfolded protein response (UPR). In *Schizosaccharomyces pombe*, the UPR is directed by ER-resident protein Inositol Requiring Enzyme 1 (Ire1), which initiates the selective decay of several mRNAs to reduce the protein-folding load. *BiP1* mRNA, which encodes the most abundant ER chaperone protein, is a substrate of Ire1 cleavage but escapes degradation. Some possible mechanisms by which this unique mRNA is stabilized despite losing its polyA tail are an RNA secondary structure, a protein binding to the mRNA, a chemical modification, or some combination of these.

Materials & Methods We used an *in vitro* biochemical technique called Multiplexed ?OH Cleavage Analysis (MOHCA-seq) to determine the structure of the *BiP1* mRNA. We have also created mutations *in vivo* to BiP1 to determine what parts of the mRNA are required for stability, which we assayed by rt-qPCR.

Results & Discussion We have found that the Ire1 cleavage site resides at the tip of a long stem-loop, reminiscent of conserved cleavage sites in other species. Additionally, we have found that the stabilization is not a switch-like mechanism, but rather progressive deletions in the 3' UTR only partially reduce the stability of the mRNA. While these data do not rule out any model yet, we are getting closer to understanding the novel mechanism by which truncated *BiP1* mRNA is stabilized.

Area 3



β-arrestin functions independent of its activating GPCR to 'arrest' endocytosis and drive MAP kinase signaling

K. Eichel[1], D. Jullié[2] and M. von Zastrow[2,3]
kelsie.eichel@ucsf.edu
[1]Program in Biochemistry and Molecular Biology, [2]Department of Psychiatry, [3]Department of Cellular & Molecular Pharmacology, University of California, San Francisco CA 94158

Introduction β-arrestins are key regulators of G protein-coupled receptors (GPCRs), which not only 'arrest' G protein signaling but also modulate endocytosis and initiate G protein-independent signaling via MAP kinase. The present dogma for how these events occur is based on key two assumptions: (1) β-arrestin traffics to clathrin-coated pits (CCPs) in obligate physical complex with its activating GPCR, and (2) the MAP kinase signal emanates from the GPCR/β-arrestin complex after endocytosis. Our understanding of how these events occur in cells remains rudimentary and based largely on inference from study of isolated proteins.

Materials and methods We tested both assumptions using real-time imaging combined with direct manipulation of surface protein dynamics in living mammalian cells. We also used western blot analysis to measure arrestin-dependent MAP kinase activation.

Results and discussion Refuting the first assumption, we show that ligand-activated GPCRs can drive robust trafficking of β-arrestin to CCPs without themselves moving there and does not require an obligate physical complex with a receptor. Refuting the second assumption, we show that β-arrestin promotes downstream MAP kinase activation not through endocytosis, but by prolonging the surface lifetime of CCPs on the plasma membrane before endocytosis. This endocytic delay, and not endocytosis itself, determines the magnitude of downstream MAP kinase signaling. These results redefine the cellular basis of β-arrestin function and demonstrate a discrete, non-endocytic role of CCPs as dynamic signaling stations on the plasma membrane.

Area 2, 7

Mechanism of homeostatic control of mitochondrial DNA copy number

Aylin Goke, Christof Osman, Peter Walter.
aylin@walterlab.ucsf.edu
University of California, San Francisco, Howard Hughes Medical Institute

Introduction Mitochondria contain a separate genome that encodes proteins essential for oxidative phosphorylation and respiratory growth. The copy number of mitochondrial DNA (mtDNA) is maintained in a narrow range over multiple generations and adjusted in response to changing metabolic needs and maintained at that level. This study aims to identify cellular pathways that coordinate metabolic state with mtDNA levels and act on the level of regulating the activity of the mtDNA replication and/or degradation machinery.

Materials & Methods We are using budding yeast *S. cerevisiae* as a model organism to understand mtDNA copy number regulation. We screened yeast deletion library mutants by using colony blot hybridization approach to identify mutants that have abnormal mtDNA levels.

Results & Discussion Majority of the mutants have WT like mtDNA levels. 200 mutants lost their mtDNA and 90 mutants have at least 50% more mtDNA than WT. mtDNA levels of 90 mutants that have more mtDNA are verified by quantitative PCR and normalized to cell volume. Currently we are investigating why these mutants affect mtDNA levels. We aim to understand the molecular mechanisms underlying mtDNA copy number regulation. Elucidating the molecular mechanism will also help us to understand pathologies arising from abnormalities in mtDNA copy number in disease models and promise to aid development of new therapeutic tools.

Area
3-Cell Biochemistry

Genome-Scale Control of Gene Repression and Activation

Max A Horlbeck, Luke A Gilbert, Chong Y Park, Jacqueline E Villalta, Hao Shao, Jason E Gestwicki, Jonathan S Weissman
Howard Hughes Medical Institute; University of California, San Francisco
max.horlbeck@ucsf.edu

Introduction Massive sequencing projects have uncovered a universe of protein coding and non-coding transcripts encoded by mammalian genomes. However, the function of many of these transcripts is poorly understood. In order to systematically interrogate their function in human cells, we recently developed a set of technologies based on the CRISPR system that enable interference (CRISPRi) or activation (CRISPRa) of gene expression for any gene.
Materials & Methods We created pooled genome-scale s(ingle)g(uide)RNA libraries, and demonstrated that screens using these tools provide highly rich, complementary, and specific information. Using this technology, we recently conducted a series of genome-scale chemical genetics screens using a novel class of chaperone inhibitors.
Results & Discussion These screens revealed critical differences in both the on- and off-target activities of these molecules, and showed that the sub-cellular distribution of small molecule inhibitors can have a profound influence on their mechanism of action. In addition, leveraging the sgRNA activity information present in these screens, we took a machine learning approach to derive critical parameters for selecting highly active sgRNAs for CRISPRi and CRISPRa. These parameters include nucleosome positioning data and other factors that may be broadly valuable for the prediction of active sgRNAs for other CRISPR-based applications. Finally, we applied this metric to construct next-generation libraries, which will enable even more robust identification of transcript function at genome scale and serve as a powerful discovery tool for cell biology.

Area 2

Investigating the fate of damaged eukaryotic ribosomes stalled during translation

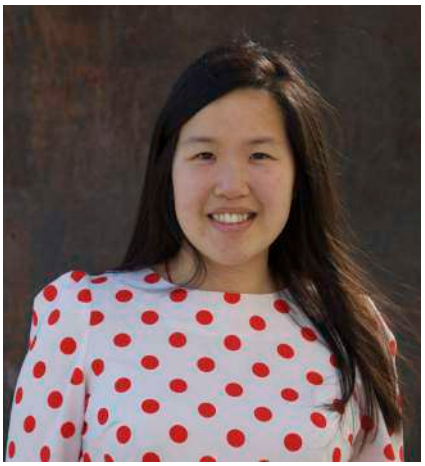
Kamena Kostova, Jonathan Weissman
Kamena.Kostova@ucsf.edu
University of California, San Francisco

Introduction Protein biosynthesis is by far the most energy-consuming process during cellular proliferation. Therefore, any event that interferes with protein production can jeopardize the viability of the cell. Of particular concern is ribosome stalling - a phenomenon in which the ribosome gets trapped on a message and can neither proceed with translation, nor get released. There are two major causes of translational stalls – faulty mRNAs and translation-incompetent ribosomes. Although stalls caused by malformed mRNAs have been extensively studied, very little is known about the quality control pathways that resolve translational blocks caused by dysfunctional ribosomes. Our research aims to determine how cells cope with ribosomes that stall on functional mRNAs due to damage or chemical inhibition.

Materials and Methods In order to study ribosome stalling in a controlled and physiologically relevant setting, we have developed a yeast system that models stochastic failure of the ribosome.

Results & Discussion We have discovered that stalled ribosomes are rapidly detected and removed from the pool of actively translating ribosomes. Importantly, the quality control mechanism that copes with stalled ribosomes is distinct from the no-go and non-stop decay pathways that have been implicated in translational stalls caused by faulty mRNAs. Currently, we are applying high throughput approaches to identify the factors that are involved in the detection and disassembly of stalled ribosomes. Understanding how eukaryotic cells cope with damaged ribosomes can not only enhance our understanding of normal cellular processes, but also shed light on human disorders that have been previously associated with ribosome dysfunction.

Area: 10



Towards Reconstituting the ER Stress Sensing Mechanism of IRE1

Mable Lam, Peter Walter
Email: mable@walterlab.ucsf.edu
UCSF, HHMI

Introduction: Detecting and regulating the protein folding capacity of the ER lumen is critical for processing secretory and transmembrane proteins. The unfolded protein response (UPR) is activated when unfolded proteins accumulate in the ER lumen. IRE1, the most conserved transducer of the UPR, senses a folding burden in the ER through dual mechanisms: 1) dissociation from the ER chaperone BiP and 2) direct binding of unfolded proteins. IRE1 then oligomerizes to activate its cytosolic domains, which execute a non-conventional splicing mechanism that upregulates the ER folding capacity. Previous work has shown that IRE1 activity is tuned to the level of ER stress, and we hypothesize that coordinating stress to activity across the ER membrane occurs through modulating the degree of IRE1 oligomerization.

Materials & Methods: To decouple IRE1 oligomerization from ER stress, I have replaced the IRE1 lumenal domain *in vivo* with FKBP domains, which multimerize in the presence of a rapamycin analogue. To study the dynamics of IRE1 oligomerization, I have reconstituted the lumenal domain of IRE1 onto supported lipid bilayers.

Results: Modulating the degree of IRE1 oligomerization *in vivo* is sufficient to tune its activity. However, how the association of BiP and unfolded protein coordinate to modulate the oligomerization and activation of IRE1 is still unclear.

Discussion: Mounting the UPR, although essential for survival, consumes a significant amount of cellular energy and resources, emphasizing the importance of coordinating ER stress to IRE1 signaling activity. Identifying the aspects of ER stress that modulate IRE1 oligomerization will advance our understanding of maintaining quality control in the ER.

Area: 7

ER stress-independent activation of unfolded protein response kinases by a small molecule ATP-mimic

Aaron S. Mendez[1†], Jennifer Alfaro[2†], Marisol A. Morales Soto[2†], Arvin C. Dar[1], Emma McCullagh[2], Katja Gotthardt[1], Han Li[1], Diego Acosta-Alvear[1], Carmela Sidrauski[1], Alexei V. Korennykh[1], Sebastian Bernales[2], Kevan M. Shokat[1], Peter Walter[1]

[1]Howard Hughes Medical Institute and University of California, San Francisco, [2]Fundación Ciencia & Vida, Santiago, [†]Co-first Authors

peter.walter@ucsf.edu ,kevan.shokat@ucsf.edu

Introduction Two ER membrane-resident transmembrane kinases, IRE1 and PERK, function as stress sensors in the unfolded protein response (UPR). IRE1 also has an endoribonuclease activity, which initiates a non-conventional mRNA splicing reaction, while PERK phosphorylates eIF2a. Chemical modulators were generated to gain better insight into the mechanistic outputs of these two UPR stress sensors in absences of stress.

Materials & Methods Structure activity relationship (SAR) was used to generate a small molecule (SM) ATP-mimetic library targeting IRE1α. Using biochemical and cellular assays we determined on target effects of the SM's against IRE1α and off target effects against PERK.

Results & Discussion We engineered a potent small molecule, IPA, that binds to IRE1's ATP-binding pocket and predisposes the kinase domain to oligomerization, activating its RNase. IPA also inhibits PERK but, paradoxically, activates it at low concentrations, resulting in a bell-shaped activation profile. We reconstituted IPA-activation of PERK–mediated eIF2a phosphorylation from purified components. We estimate that under conditions of maximal activation less than 15% of PERK molecules in the reaction are occupied by IPA. We propose that IPA binding biases the PERK kinase towards its active conformation, which *trans*-activates apo-PERK molecules. The mechanism by which partial occupancy with an inhibitor can activate kinases may be wide-spread and carries major implications for design and therapeutic application of kinase inhibitors.

Area: 2, 3

Dynamics of the Anaphase Promoting Complex Activator Subunits during Mitosis

Arda Mizrak and David Morgan
Arda.mizrak@ucsf.edu
University of California, San Francisco

Introduction Segregation of the duplicated chromosomes is tightly regulated to ensure that each daughter cell receives a complete genome. An essential ubiquitin ligase called the Anaphase Promoting Complex (APC) promotes chromosome separation and the completion of mitosis by targeting several key regulatory proteins for destruction. Its activity depends on the sequential binding of two different activator subunits. Despite their high affinity, activator subunits dissociate rapidly from the APC, resulting inhibition and reactivation of the APC at different cell cycle stages. I aim understand the mechanisms that control the timing of activator exchange.

Materials & Methods To address this problem, I used biochemical chromatography methods to purify an activity that removes activators from the APC in budding yeast *S. cerevisiae*. Using *in vitro* activator binding assays, we can monitor dissociation of radiolabelled activator subunits. Additionally, measuring *in vivo* half-lives of the activator subunits helps us to better understand the dynamics of the APC activity during mitosis.

Results & Discussion *In vitro* APC activator subunit dissociation assays suggest an ATP dependent activity that removes activator subunits. Furthermore, this biochemical activity can be regulated with APC substrates. After several chromatography steps, the activity can be restored and protein identities will be analyzed with Mass Spectrometry. Rapid removal of activator subunits from the APC is critical for the maintenance and timing of a proper cell cycle. Mechanisms that regulate the dynamics of APC activator subunits are important for expanding our knowledge on how each mitotic event is controlled.

Area: 3

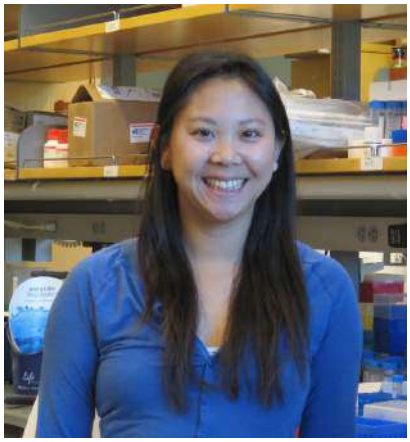
Ltc1 is a conserved sterol transfer protein and a component of ER-mitochondria and ER-vacuoles contacts in yeast

Andrew Murley[1]*, Reta D. Sarsam[1], Alexandre Toulmay[2], Justin Yamada[1], William A. Prinz[2], Jodi Nunnari[1]
*acmurley@ucdavis.edu
1 Dept. of Molecular and Cellular Biology, University of California - Davis, Davis, CA, USA, 2 National Institutes of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, USA

Introduction: Organelle contact sites perform fundamental functions in cells, including lipid and ion homeostasis, membrane dynamics and signaling. Using a forward proteomics approach in yeast, we identified new ER-mitochondria and ER-vacuole contacts specified by an uncharacterized protein, Ylr072w, which is a conserved protein with GRAM and VASt domains that selectively transports sterols and is thus termed Ltc1, for Lipid Transfer at Contact site. Ltc1 is part of a larger protein family, conserved throughout eukaryotes.
Materials and Methods: Using an interdisciplinary cell biological approach in yeast, we characterized the subcellular localization and function of Ltc1. We also tested its lipid transfer activity with different lipid species *in vitro*.
Results: Ltc1 localized to ER-mitochondria and ER-vacuole contacts via the mitochondrial import receptors Tom70/71 and the vacuolar protein Vac8, respectively. At mitochondria, Ltc1 was required for cell viability in the absence of Mdm34, a subunit of the ER-Mitochondria Encounter Structure. At vacuoles, Ltc1 was required for sterol-enriched membrane domain formation in response to stress. Increasing the proportion of Ltc1 at vacuoles was sufficient to induce sterol-enriched vacuolar domains without stress.
Discussion: Our data support a model where Ltc1 is a sterol-dependent regulator of organelle and cellular homeostasis via its dual localization to ER- mitochondria and ER-vacuole contact sites.

Research Areas:

2 Membrane/Protein Traffic



Regulation of Cardiac G-Protein Coupled Receptors from Endosomes

Grace E. Peng[1] and Mark von Zastrow[1,2]
[1]Program in Cell Biology, [2]Departments of Psychiatry and Cellular and Molecular Pharmacology. University of California, San Francisco. San Francisco, CA 94158 USA
grace.peng@ucsf.edu

Introduction: β2-adrenergic receptors (β2ARs) and M2 muscarinic acetylcholine receptors (M2Rs) are G protein-coupled receptors (GPCRs) that operate to exert largely opposing signaling and physiological effects important in heart function. β2ARs stimulate cardiac excitability through agonist-dependent coupling primarily to the heterotrimeric G protein G_s, and M2Rs reduce excitability through agonist-dependent coupling to G_i. Both GPCRs internalize robustly after agonist-induced activation. Classically, internalized GPCRs are thought to be incapable of coupling to cognate G proteins. However, recent work has shown that β2ARs initiate a second phase of signaling by coupling to G_s on endosomes. It remains unknown if other GPCRs such as G_i-coupled receptors are capable of endosomal signaling. We hypothesize that M2Rs signal from the endosome, in addition to the plasma membrane, to contribute to the overall cellular signaling response to modulate the β2AR-G_s signaling response.

Materials and methods: Spinning disk confocal microscopy and nanobody biosensors were used to detect localization of receptors and their activation states. Live cell cAMP assays and qRT-PCR for *PCK1* induction were performed to detect β2AR and M2R signaling.

Results: M2Rs localize to the same endosomes as β2ARs, and can exist in an active conformation on endosomes. An M2R internalization mutant inhibits β2AR-stimulated cAMP, but does not inhibit β2AR-stimulated *PCK1* induction *(*an endosomal cAMP transcriptional target).

Discussion: These results suggest that M2R may be able to modulate β2AR-cAMP signaling on endosomes in HEK293 cells.

Area: 7

Structural Insight into Cargo Transport by the Molecular Motor Dynein

Courtney M. Schroeder, Jonathan M.I. Ostrem, Nicholas T. Hertz, Ronald D. Vale
Courtney.Schroeder@ucsf.edu, vale@cmp.ucsf.edu
Department of Cellular and Molecular Pharmacology, Howard Hughes Medical Institute, University of California, San Francisco

Introduction The concentrated cytoplasm of the cell must obtain spatial and temporal organization, and molecular motors are one means such organization can be achieved. Dynein is a multi-subunit microtubule motor complex that is implicated in a large range of functions, including cargo transport and cell division. The mechanisms of cargo selection and attachment are unclear, yet it is known that the dynein light intermediate chain (LIC) is an accessory protein in the dynein complex that is crucial for cargo transport. We investigated how the LIC carries out intracellular transport and sought structural information about the LIC.

Materials & Methods X-ray crystallography, reverse-phase HPLC and pulldowns

Results & Discussion I have determined the crystal structure of the conserved N-terminal domain of a fungal LIC, which revealed a structure similar to small GTPases. However, despite having a G protein fold, the fungal LIC did not co-crystallize with guanosine nucleotide and revealed an unusual binding pocket that excludes nucleotide. Unlike fungal LIC, the amino acid sequence of human LIC1 contains nucleotide-binding motifs found in GTPases. I discovered human LIC1 can bind nucleotide but has a strong preference for GDP over GTP, which is unusual for GTPases. I have also shown that the LIC G domain binds the dynein motor using a conserved patch of aromatic residues, whereas the less conserved C-terminal domain binds several cargo adaptors involved in membrane transport. These findings suggest that the LIC evolved from the GTPase superfamily but no longer acts as a GTPase switch. This study provides the first structural information and insight into the evolutionary origin of the LIC as well as revealing how this critical subunit connects the dynein motor to cargo.

Area: #2 (Membrane/Protein Traffic); and/or #3 (Cell Biochemistry)

Identification of the ATF6 activation and trafficking machinery

Sandra Torres, Martin Kampmann, Jonathan Weissman and Peter Walter
storres@ucsf.edu
University of California, San Francisco, Howard Hughes Medical Institute

Introduction Most secreted and transmembrane proteins are folded and assembled in the endoplasmic reticulum. When ER homeostasis is disturbed, misfolded proteins accumulate and activate the unfolded protein response (UPR). The ER transmembrane sensors, IRE1, PERK, and ATF6, mediate the UPR signaling cascade to decrease the ER protein influx, increase ER volume and protein folding capacity, and activate the ER-associated degradation (ERAD) pathway. ATF6, an ER-tethered transcription factor, plays a key role in the UPR by transcriptionally upregulating ER-resident chaperones and folding enzymes. ATF6 is necessary for cell survival in the presence of stress. Upon ER stress, ATF6 is packaged into COPII transport vesicles that deliver it to the Golgi apparatus, where it undergoes intramembrane proteolysis. ATF6 plays an essential role in allowing cells to adapt to stress, but the events leading to its activation and trafficking are not understood.

Materials & Methods I have carried out a genome-wide CRISPRi genetic screen in the presence and absence of ER stress to identify the machinery mediating ATF6 trafficking and regulation. Next, I will characterize these candidates using an ATF6 transcriptional reporter and measuring nuclear translocation.

Results & Discussion We have identified a set of genes that are sensitizing to ER stress.

ATF6 plays a key role in allowing cells to adapt to stress, but the events leading to its activation and trafficking are not understood. Ultimately, our work will provide insight into the regulation of a key player in the UPR.

Area 2- Membrane / Protein Traffic

An unstructured linker is required for human IRE1α activation during the unfolded protein response

Ngoc-Han Tran,[1,2,3] Diego Acosta-Alvear, [1, 2] Jirka Peschek, [1, 2] Aaron Mendez, [1, 2] Gülsün Elif Karagöz,[1, 2] Shawn Douglas[3] and Peter Walter[1, 2]
Email: Han@walterlab.ucsf.edu
[1]Department of Biochemistry and Biophysics, UCSF
[2]Howard Hughes Medical Institute, UCSF
[3]Department of Cellular and Molecular Pharmacology, UCSF

Introduction: Conserved signaling pathways in eukaryotes collectively termed the unfolded protein response (UPR) are essential in sensing and responding to ER stress. The most conserved UPR effector IRE1 detects unfolded protein via its lumenal domain, oligomerizes and allosterically activates its cytoplasmic kinase and RNase domains, resulting in non-canonical splicing of its mRNA substrate. The human IRE1α (hIRE1α) is emerging as different from its better-studied yeast counterpart, and mechanistic details of its activation are not fully understood. This work focuses on the role of a previously overlooked unstructured cytoplasmic linker in modulating hIRE1α oligomerization and RNase activation.

Materials & Methods: Variants of hIRE1α with deletions or mutations within the unstructured cytoplasmic linker are transduced and inducibly expressed in mouse embryonic fibroblasts. Upon UPR induction, hIRE1α oligomerization, phosphorylation status and RNase activity were monitored by fluorescent microscopy, immunoblots, mass spectrometry and RT-PCR. In vitro biochemical and biophysical assays were performed to further characterize the effect of linker deletions and mutations.

Results: A region of the hIRE1α flexible linker enriched in Serine residues is required for activity. Its deletion and mutation impairs hIRE1α oligomerization and abolishes phosphorylation and RNase activity.

Discussion: The linker is likely functionally phosphorylated during the UPR. Initial attempts to template hIRE1α oligomerization using DNA nanostructures to further dissect the role of the linker will be discussed.

Area: 3-Cell Biochemistry

Pharmacological dimerization and activation of the exchange factor eIF2B antagonizes the integrated stress response

*Jordan C. Tsai[1,2], *Carmela Sidrauski[1,2], Martin Kampmann[2,3], Brian Hearn[4], Punitha Vedantham[4], Priyadarshini Jaishankar[4] , Masaaki Sokabe[5], Aaron Mendez[1,2], Billy Newton[6], Edward Tang[6.7], Erik Verschueren[6], Jeffrey Johnson[6,7], Nevan J. Krogan[6,7,], Christopher S. Fraser[5], Jonathan S. Weissman[2,3], Adam R. Renslo[4,] and Peter Walter [1,2]

Email: Jordan@walterlab.ucsf.edu

* Both authors contributed equally

[1]Department of Biochemistry and Biophysics, UCSF

[2]Howard Hughes Medical Institute, UCSF

[3]Department of Cellular and Molecular Pharmacology, UCSF

[4]Department of Pharmaceutical Chemistry and the Small Molecule Discovery Center, UCSF

[5]Department of Molecular and Cellular Biology, College of Biological Sciences, UC Davis

[6]QB3, California Institute for Quantitative Biosciences, UCSF

[7]Gladstone Institutes, San Francisco

Introduction The integrated stress response is a major translational control point that converges on the phosphorylation of the eIF2α which renders eIF2 an inhibitor of its GEF eIF2B. The small molecule ISRIB reverses the effects of eIF2α-phosphorylation. This work focuses on the identification of the target and mechanism of ISRIB.

Materials & Methods shRNA screening was conducted in cell lines expressing a fluorescent reporter. Modulators of reporter expression were identified by deep sequencing. eIF2B dimers were identified by velocity sedimentation and LC-MS/MS. GEF activity was measured using a filter binding assay followed by liquid scintillation.

Results & Discussion screening suggested eIF2B as the target of ISRIB. This notion was confirmed by the observations that ISRIB stabilizes eIF2B dimers and stimulates GEF activity. This work identifies eIF2B as the target of ISRIB, and provides the first correlative evidence that eIF2B dimers are more active than their monomeric counterparts. ISRIB potentially escapes the effects of eIF2α-P by stabilizing the formation of active eIF2B dimers.

Area: 3

Cell type-specific requirements for highly efficient and synchronous reprogramming to pluripotency

Simon Vidal, Bhishma Amlani, Taotao Chen, Aristotelis Tsirigos and Matthias Stadtfeld
Skirball Institute, NYU School of Medicine, USA
simon.vidal@med.nyu.edu

Introduction In a revolutionary experiment, Kazutoshi Takahashi and Shinya Yamanaka discovered that the overexpression in fibroblasts of four defined factors (Oct4, Klf4, Sox2, Klf4, and c-Myc (OKSM)) can convert them into so-called induced pluripotent stem (or iPS) cells. Since reprogramming of fibroblasts into iPS cells occurs at low frequencies (0.1-1% of input cells) and typically after a considerable lag phase (> 12 days), researchers in the field have been proposed that reprogramming is a stochastic process in which several epigenetic roadblocks have to be overcome. The goal of this study was to determine why some somatic cell types reprogram more efficiently than others.

Material & methods Mouse embryonic fibroblasts, hepatic and blood progenitor cells with dox-inducible OKSM were used in this study. Reprogramming was induced by doxycycline (1ug/ml) for 6 days. A screen with known small molecules that enhance reprogramming was done with the goal of finding chemical combinations that synergistically increase the synchronization and efficiency of iPS cell generation.

Results & Discussion Inhibition of TGF-β signaling pathway together with activation of Wnt signaling in the presence of ascorbic acid allows >80% of murine fibroblasts to acquire pluripotency after 1 week of reprogramming factor expression. In contrast, hepatic and blood progenitors predominantly required only TGF-β inhibition or canonical Wnt activation, respectively, to reprogram at efficiencies approaching 100%. The proof-of-principle that highly efficient and synchronous reprogramming can be achieved without genetic manipulation will greatly facilitate reprogramming experiments.

Area: 6 Development / Cell Differentiation

Autophagy regulation by the TOR Complex 2 (TORC2) - Mitochondria signaling axis

Ariadne Vlahakis[1], Nerea L. Muniozguren, Ted Powers[2]
University of California, Davis
avlahakis@ucdavis.edu, tpowers@ucdavis.edu

Introduction The Target Of Rapamycin (TOR) kinase forms two complexes TORC1 and TORC2 to regulate cell growth. Through TORC1, TOR negatively regulates autophagy, a process that recycles cytoplasmic components to promote cellular homeostasis and adaptation upon nutrient deprivation. We demonstrated that upon amino acid starvation TOR also operates as a positive regulator of autophagy through TORC2 by promoting the conserved General Amino Acid Control (GAAC) response. In addition to TORC2, mitochondria are implicated in regulating autophagy specifically during amino acid starvation. Here we determined the extent to which mitochondria influence the regulation of autophagy by TORC2.

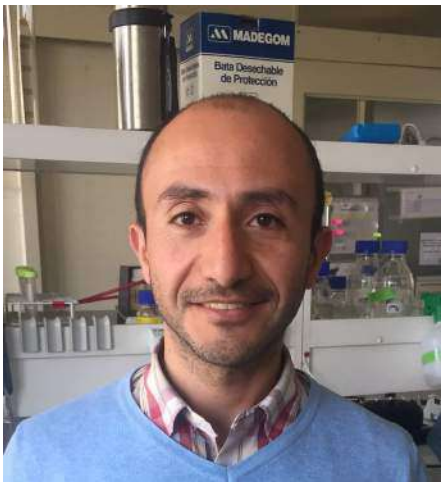
Materials & Methods Using the yeast model S. *cerevisiae,* we examined autophagy in genetic mutants in TORC2 signaling and mitochondrial respiration. We utilized the fusion autophagy protein GFP-Atg8 to examine two aspects of autophagy during amino acid starvation: 1) autophagosome mediated protein turnover termed “autophagy flux” and 2) transcriptional induction of autophagy proteins.

Results & Discussion We demonstrate a link between TORC2 signaling and mitochondrial integrity in promoting autophagy by inhibiting the Ca2+ regulated phosphatase, calcineurin, which inhibits the GAAC response and autophagy flux. Furthermore, we demonstrate that mitochondria function downstream of TORC2 to regulate the induction of a key autophagosome biogenesis protein, Atg8, by modulating the transcriptional regulators Msn2/4. Dysfunctional TORC2, mitochondria, and autophagy signaling is highly linked to metabolic and neurodegenerative diseases. A detailed understanding of the cellular mechanisms regulating autophagy is requisite to uncovering the pathogenesis of such disease states and will facilitate the discovery of novel therapeutics.

Area: 3

NATIONAL ABSTRACTS

Science & Friendship 2015



Low pH as trigger for Andes hantavirus fusion activation

Rodrigo Acuña[1], Roberta Mancini[2], Piere Yves-Lozach[3] and Nicole Tischler[1,4]

[1]Laboratorio de Virología Molecular, Fundación Ciencia & Vida, [2]Institute of Biochemistry, ETH Zurich, Switzerland, [3]Departament of Infectious Disease, Virologie, University Hospital Heidelberg, Germany, [4]Facultad de Ciencias Biológicas, Universidad Andres Bello

E-mail: ntischler@cienciavida.org

Introduction Currently little is known about the entry pathway of hantaviruses into cells. Once uptaken into endosomes, hantaviruses fuse with cellular membranes to deliver their genome into the cell cytosol. Thereby, the pH of the endosomes seems to play a crucial role. Here, we analyzed if low pH was sufficient to trigger Andes virus-membrane fusion.

Materials & Methods Different cell-free *in vitro* assays were developed to determine intermediate steps of fusion. Either ANDV or our previously developed virus-like particle (VLP) system was used for this aim. Virus particle interaction with membranes was analyzed by a liposome coflotation assays. The multimerization changes of the viral fusion protein Gc were determined by sucrose gradient sedimentation.

Results & Discussion Acidification of the VLP-liposome mixture was sufficient to trigger their interaction. Low pH also induced Gc multimerization rearrangements leading to highly resistant Gc homotrimers that were resistant to trypsine digestion, detergent and high temperatures. No acid-dependent oligomerization changes were detected for the trypsin-sensitive Gn companion protein. Low pH is sufficient to trigger ANDV fusion activation and hence, no cellular factors seem to be required for the fusion priming of mature virus particles. This work is the first report on a stable fusion protein homotrimer in the *Bunyaviridae* family. The results strongly support the notion that hantavirus Gc follows a non-reversible fusion process upon low pH exposure, leading to a stable post-fusion conformation.

Area: 2

Discovery of novel anti-osteoclastogenic small molecules

Belmar S[1], Stack K[1], *Alfaro IE [1]
[1]Fundacion Ciencia & Vida, Chile
E-mail: alfobioq@gmail.com

Introduction. Osteolytic diseases involve the excessive resorption of bone matrix by osteoclasts (OC) caused by genetic disorders, hormone deregulation, chronic inflammation or cancer. Low tolerability of bisphosphonates, the first-line therapy for bone lytic diseases, highlights the development of new anti-osteolytic treatments. Recently, the non-canonical Wnt5a/Ror2 signaling has been involved in the promotion of osteoclastogenesis in normal and disease conditions, making this pathway a promising target for the treatment of osteolytic lesions. We constructed a library of compounds by derivatization of the structure of known type-II inhibitors targeting receptor tyrosine kinases (RTKs) with high similarity to the Ror2 kinase domain to screen for inhibitors of osteoclastogenesis.

Material and Methods. Primary cultures of osteoclast precursors (OP) isolated from mouse bone marrow were used in RANKL- induced osteoclastogenesis assays. Enzymatic activity of TRAP, a marker of mature OC, was used as a relative measurement of osteoclast differentiation. Cell viability assays and kinase inhibition profiles of hit compounds (Reaction Biology Corp, USA) were also performed.

Results & Discussion. We discover a set of molecules able to inhibit osteoclastogenesis without affecting OP viability. Structure-activity analysis of the library compounds identified a novel scaffold represented in hit molecules with potent anti-osteoclastogenic activity. Kinase inhibitor profiles of hit compounds unveil a serendipitous discovery of a new class of highly selective inhibitors of specific kinases that along with Ror2 form a triad of RTKs crucial for osteoclast differentiation. A novel class of small molecule inhibitors of osteoclastogenesis was discovered. Contribution of specific kinases and Ror2 in this process is actually under study.

Area: 9-Cell Biology of Disease
(Socio Patrocinante: Pablo Valenzuela)

Targeted inhibition of ASncmtRNA promote apoptosis and inhibit tumorigenic properties in genitourinary cancer *in vitro* and *in vivo*.

Borgna, V[1]., Lobos-Gonzales, L[1]., Silva, V[1]., Araya, M[1]., Rivas, A[1]., Ávila, R[1]., López, C[1]., Socias, T[1]., Burzio, V[1,2] Burzio, L.O[1,2] and Villegas, J[1,2]

[1]Andes Biotechnologies SpA.; Fundación Ciencia & Vida, Av. Zañartu 1482; [2]Facultad de Ciencias Biológicas, Universidad Andrés Bello, República 252

E-mail: jvillegas@bioschile.cl

Introduction: We previously described a novel family of long non coding mitochondrial RNAs (ncmtRNA), transcribed from the L-Strand of the mtDNA (ASncmtRNA). These transcripts are express in normal proliferating cells, but are down regulated in tumor cells. We found that knocking down these transcripts *in vitro* with antisense oligonucleotide (ASO) induces massive death of human and murine cell lines. We evaluated the effect of ASO treatment in genitourinaries cancer, renal and bladder in vitro and in vivo.

Material and Methods: For renal (RenCa) and bladder (UMUC-3), cells were treated in *vitro* with 100nM ASO by 48h and the effect over cell viability and characterization of death cell was performed. A renal orthotopic model was established in syngenic mice and used as model for *in vivo* therapy. For bladder a sub cutaneus model was established in xenografts mice and used for *in vivo* therapy.

Results: For renal model, ASO treatment *in vitro* induce massive cell death mediated by down-regulation of survivin and *in vivo* therapy practically abolish the tumor growth and metastasis. For bladder, a strong inhibition of migration is observed, mediated by down-regulation of N-cadherin and MMP-9 *in vitro*. *In vivo*, a strong delay in tumor growth is induced by ASO.

Discussion: The ASncmtRNA is and effective target for the development of targeting therapy in genitourinaries cancer

Area 8: Cell growth/death and cancer

Expression of a dopamine receptor favors Th1 and Th17 immunity by avoiding Th2 differentiation and impairing regulatory function

Francisco Contreras, Carolina Prado, Hugo González, Dafne Franz, Francisco Osorio-Barrios, Daniela Elgueta, Alicia Figueroa, Fabiola Osorio, Rodrigo Pacheco
Laboratorio de Neuroinmunología, Fundación Ciencia & Vida, Santiago.
E-mail: crenovich@gmail.com

Introduction: Dopamine receptor (DR) expressed on lymphocytes is required to promote neuroinflammation on a murine model of Parkinson's disease. However, how DR-signaling affects the balance between effector and regulatory immunity remains unknown.
Materials & Methods: Differentiation of naïve lymphocytes from wild-type and DR-deficient mice towards different helper (Th) phenotypes and their effector/regulatory function was assessed using both *in vitro* as well as inflammatory *in vivo* settings, such as chronic and acute colitis models and an allergic asthma paradigm.
Results & Discussion: We report that activation of lymphocytes induces DR expression, regardless of the lineage specification. Furthermore, DR-signaling simultaneously favors Th1 phenotype and limits Th2 generation, using both *in vitro* and *in vivo* inflammation settings. Mechanistic analysis indicates that DR-signaling favors SOCS5 expression, a negative regulator of Th2 development. Interestingly, DR expression also contributes to Th17 differentiation under chronic inflammatory conditions, pointing towards a role for DR in favoring inflammatory Th17 expansion. Moreover, DR-stimulation reduces the suppressive potential of Treg, improving Th17-mediated immunity. These findings demonstrate that DR-signaling on lymphocytes shifts the balance towards inflammatory phenotypes and unveils DR expressed on lymphocytes as highly relevant for development of several inflammatory conditions.

Area: 4

Caveolin-1 enables dendritic cells to generate tumor-protective cytotoxic CD8 T cell responses by promoting migration and antigen cross-presentation

Sebastián Cruz[1], Cesar Oyarce[1], Felipe Gálvez-Cancino[1], Jorge Díaz[2], Natalia Díaz[2], Andrew F.G. Quest[2] and Alvaro Lladser[1].

[1]Laboratory of Gene Immunotherapy, Fundación Ciencia & Vida, Chile [2]Laboratory of Cellular Communication, Advanced Center for Chronic Diseases (ACCDiS), Facultad de Medicina, Universidad de Chile, Chile.

E-mail: alladser@cienciavida.org

Introduction Caveolin-1 is a membrane protein involved in endocytosis, cell signaling and migration. However, whether caveolin-1 is involved dendritic cell (DC) function remains unknown. In this study, the participation of caveolin-1 in the ability of DCs to generate cytotoxic CD8 T cell responses was addressed.

Material and methods Bone marrow-derived DCs obtained from WT or Cav-$1^{-/-}$ mice were analyzed by flow cytometry, western blotting and ELISA. $OVA_{257-265}$-pulsed DCs were used to induce CD8 T cell responses and tumor protection *in vivo*. DCs pulsed with ovalbumin or $OVA_{257-265}$ peptide were co-cultured with CFSE-stained $OVA_{257-265}$–specific CD8 T cells. WT and Cav-$1^{-/-}$ DCs were transferred into naïve mice to evaluate migration *in vivo*. Filopodia formation was determined by confocal microscopy. Rac1 activation was determined in pull-down assays.

Results & Discussion Caveolin-1 expression was upregulated in DCs after LPS- and TNF-α-induced maturation. Caveolin-1 promoted the ability of DCs to generate antigen-specific CD8 T cell responses and suppression of tumor growth. Although no changes in maturation and cytokine production were observed, caveolin-1 promoted DC migration to lymph nodes, filopodia formation and Rac1 activation. Also, caveolin-1 promoted antigen cross-presentation and CD8 T cell proliferation *in vitro*. Our results suggest that caveolin-1 expression in DCs is required to generate efficient CD8 T cell responses by promoting migration and antigen cross-presentation.

Area 4

Discovery of neuroprotective small molecules that induce alpha-synuclein degradation and activate lysosomal-dependent degradation.

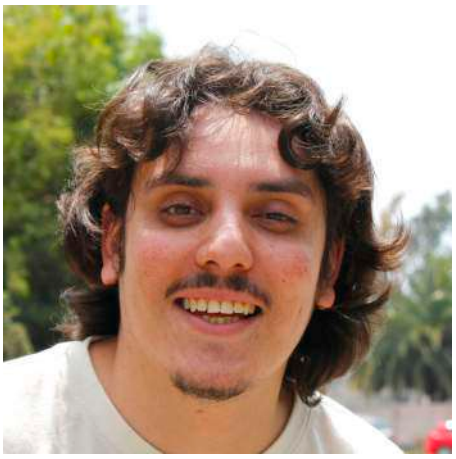
Delgado L[1], De la Cruz J[1,] Olivares F[1,] Stack K[1], Cerda C[1], Díaz-Carretero J[2], Cuervo AM[2], *Alfaro IE [1]
[1]Fundacion Ciencia & Vida, Chile; [2]Albert Einstein Col. of Med., New York, NY
E-mail: ialfaro@medivation.cl

Introduction Accumulation of α-synuclein (α-syn) contributes to neurodegeneration in synucleinopathies like Parkinson´s disease (PD). α-syn is degraded by the ubiquitin proteasome system, macroautophagy and chaperone mediated autophagy (CMA). Pharmacological modulation of these pathways could be a therapeutic strategy for the treatment of synucleinopathies.

Material & Methods SH-SY5Y cells expressing wild-type α-syn were used to screen for neuroprotective molecules against α-syn overexpression. We also used lymphoblast cells derived from PD patients carrying SNCA triplication. Additionally, NIH3T3 cells expressing a specific fluorescent reporter were used to study lysosomal degradation of the reporter. Effects of hit compounds were evaluated in transgenic mice overexpressing human α-syn.

Results & Discussion We discover a set of small molecules that reduce the cytotoxicity induced by α-syn overexpression in neuroblastoma cells and decrease the total levels of α-syn protein through a lysosomal dependent mechanism. α-syn degrading effect was reproduced in lymphoblast derived from PD patients, but not in cells expressing a α-syn mutant. Four selected α-syn degraders were able to increase significantly the levels of lysosomal degradation. In transgenic mice, two-month oral treatment with one small molecule induced a 43% reduction in α-syn levels in the cortex of transgenic mice in comparison with control animals. We discovered a series of small-molecules that induce the degradation of α-syn protein with therapeutic potential for the treatment of synucleinopathies.

Area: 9
(Socio Patrocinante: Pablo Valenzuela)



The selective jumonji H3K27 demethylase inhibitor GSK-J4 limits inflammation through dendritic cell modulation

[1]Cristian Doñas, [1]Macarena Fritz, [1]Macarena Carrasco, [1]Carolina Prado, [2]Gabriela Tejón, [2]Valeria Manríquez, [1]Rodrigo Pacheco, [2]María Rosa Bono, [1]Alejandra Loyola and [1]Mario Rosemblatt.

[1]Laboratorio de Epigenética y Cromatina, Fundación Ciencia & Vida, Santiago, Chile, [2]Departamento de Biología, Facultad de Ciencias, Universidad de Chile.

E-mail: c.donas@gmail.com

Introduction Epigenetic modifications on immune cells have been implicated in the development of several inflammatory diseases. Histone H3 lysine 27 demethylase JMJD3 plays an important role in the inflammatory response and appears as an interesting target for the treatment of inflammation. Recently, a selective and potent JMJD3 inhibitor was synthesized, however, its efficacy in an inflammatory settings or its effect on T cells and dendritic cells *in vivo* has not been studied.

Materials and Methods DCs purified from C57BL/6 mice and activated with LPS in the presence or absence of the drug were co-cultured with purified naïve CD4+ T cells from Foxp3-GFP mice under different polarizing conditions. Maturation and tolerogenic markers were evaluated. Also C57BL/6 WT mice were treated with MOG to induce EAE and then treated with the drug directly or with the dendritic cells pre-treated with the drug.

Results & Discussion *In vitro*, the drug induced a tolerogenic phenotype on dendritic cells promoting the generation, stability and suppressive functions of Tregs, without affecting the generation of Th1 and Th17 cells. Administration of the drug *in vivo* ameliorates the severity of EAE. Moreover, adoptive transfer of drug-treated DC into EAE mice reduced the clinical manifestation of the disease. Our data indicate that this drug modulates inflammation indirectly though an effect on DCs. This drug may be a promising approach for the treatment of inflammatory diseases.

Area 4

Exploring the membrane potential of simple dual-membrane systems as models for Gap-junction channels.

Yerko Escalona, Jose A Garate, Tomas Perez-Acle
Laboratorio de Biología Computacional (DLab), Fundación Ciencia & Vida, Santiago, Chile. Centro Interdisciplinario de Neurociencias de Valparaíso (CINV), Universidad de Valparaíso, Valparaíso, Chile
E-mail: yescalona@ug.uchile.cl

Introduction The conductance of ion channels can be modulated by a potential difference across the membrane due to alterations on ion-mobility and also by changes in the pore structure. Despite the vast knowledge regarding the influence of voltage on the transport properties of ion channels, little attention has been paid to describe, with atomic detail, the modulation of the ionic transport properties of gap-junction channels (GJCs) under the influence of the transjunctional voltage

Material and Methods Molecular dynamics simulations were performed to explore simple dual-membrane systems that account for the very basic features of GJCs. In doing so, we have studied a set of different model systems under the influence of an external electric field, paying attention to the behavior of the electrostatic potential, ion density, ion currents and equilibrium properties.

Results & Discussion A charge distribution akin GJCs proved to inhibit anionic currents, favoring cationic currents, in agreement with experimental evidence. As a whole, our results provide insights on the effects of charge distributions over ionic transport of these model systems, constituting a step forward into the understanding of voltage regulation in GJCs.

Area: Other / Miscelaneous

CD73-mediated adenosine production induces Wnt signaling in Tc17 cells

Felipe Flores-Santibáñez[1, 4], Dominique Fernández[1], Daniel Meza[1], Gabriela Tejón[1], Leonardo Vargas[1], Lorena Varela-Nallar[2], Sebastián Arredondo[2], Victoria Guixé[1], Mario Rosemblatt[1, 2, 3], María Rosa Bono[1], Daniela Sauma[1].

[1]Departamento de Biología, Facultad de Ciencias, Universidad de Chile, [2]Facultad de Ciencias Biológicas, Universidad Andres Bello, [3]Fundacion Ciencia & Vida

E-mail: felipeflores.uchile@gmail.com

Introduction: The CD73 ectonucleotidase catalyzes the hydrolysis of AMP to adenosine, a known immunosuppressive molecule. Altough it has been reported that TGF- induces the expression of CD73 on CD8+ T cells its function on these cells is still unknown.

Methods: We analyzed the phenotype and properties of Tc17 cells *in vitro* and in a murine model of melanoma (B16) *in vivo* using flow cytometry, westernblot, and q-PCR.

Results: We show that Tc17 cells present high levels of the CD73 ectonucleotidase and are able to produce adenosine; however, they do not suppress the proliferation of CD4+ T cells. Interestingly, adenosine is able to upregulate Wnt signaling in CD8+ T cells and to induce a stem cell-like phenotype on Tc17 cells. On the other hand, CD73 is highly expressed on memory T cells (CD62L+CD127+ CD8+) within the tumor microenvironment and is downregulated on terminally differentiated T cells (GZMB+KLRG1+ CD8+), indicating that CD73 is expressed in memory/naive T cells and is downregulated during differentiation.

Discussion: These data reveal a novel function of the CD73 ectonucleotidase and put forward the idea that CD73-driven adenosine production by Tc17 cells is not involved in immunosuppression, but instead may promote stem cell-like properties in CD8+ T cells through the activation of Wnt signaling.

Área: 4.-Immunology

In vivo distribution of candidate receptors for Andes hantavirus entry into susceptible cells

María Pía García[1], Amelina Albornoz[1], Maruquel Salamin[2], Paula Padula[3], Carola Otth[2] and Nicole Tischler[1,4]

[1]Molecular Virology Laboratory, Fundación Ciencia & Vida, [2]Instituto de Microbiología Clínica, Universidad Austral de Chile, [3]Departamento de Virología, Instituto Nacional de Enfermedades Infecciosas, A.N.L.I.S. "Dr. C.G. Malbrán", Buenos Aires, Argentina, [4]Facultad de Ciencias Biológicas, Universidad Andres Bello, Santiago, Chile,

E-mail: ntischler@cienciavida.org

Introduction Hantaviruses can produce lethal disease in humans. Among them, Andes virus (ANDV) causes hantavirus pulmonary syndrome. While the natural host of ANDV is *Oligoryzomys longicaudatus* which does not disease despite its chronic infection, Syrian hamsters serve as animal model for the disease. Yet, little is known about the receptors used by this virus *in vivo*. *In vitro* studies suggest $\alpha_V\beta_3$ integrin, DAF/CD55 and gC1qR/p32 as candidate receptors. The aim of the present work is to assess the distribution of these candidate receptors *in vivo* in rodents and hamsters in order to compare their localization with that of cells infected with ANDV.

Materials & Methods Tissues from lungs and kidneys of ANDV-infected rodents and hamsters were analyzed by immunohistochemistry using specific antibodies.

Results & Discussion In kidneys of infected rodents all three candidate receptors were present in the renal medulla; however in the renal cortex, only β_3 integrin was detected. In contrast, β_3 integrin was only found in the renal cortex of Syrian hamster. On the other hand, all three candidate receptors were present in lungs of hamsters, while in rodents β_3 integrin was not detected. The presence of some candidate receptors correlated with viral infection. The absence of β_3 integrin in lungs of rodents provides novel insights for the understanding of viral pathogenesis.

Area: 1

Unfolding of a Knotted Protein

Sebastian E. Gutierrez-Maldonado[1,2], Jose Antonio Garate[1], Maira Rivera[3], Andres Bustamante[3], Mauricio Baez[3], Tomas Perez-Acle[1,2]

[1]Computational Biology Lab, Fundación Ciencia & Vida, Santiago, Chile [2]Centro Interdisciplinario de Neurociencias de Valparaíso. Universidad de Valparaíso, Valparaíso, Chile [3]Laboratorio de Bioquímica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile.

E-mail: sebastian@dlab.cl

Introduction

Knotted proteins present different types of knots in their structure. It has been suggested that these knots confer structural stabilization. Mechanical and chemical-driven unfolding has been studied using optical tweezers and denaturing agents, but the details of the process remain unknown. By using molecular dynamics (MD) simulations, we have been able to replicate experimental procedures and delve into the details of the unfolding process.

Material and Methods

Simulations were performed using NAMD v2.10. Mechanical unfolding was simulated via steered MD, using several pulling rates, both *in vacuo* and in water, to explore how secondary structure is lost as unfolding occurs. Chemical unfolding is being explored using a 6M Guanidinium Chloride solution, although longer simulation times are required to unfold knotted proteins.

Results

High pulling rates exert forces on the protein of up to 4,000 pN, while low pulling rates decrease the force needed to extend the protein. As the protein is pulled, there is a decrease in intramolecular hydrogen bonds and an increase in the solvent-accessible surface area.

Discussion

Pulling the protein causes its secondary structure to break by disrupting the intramolecular hydrogen bonds. This allows water to solvate previously inaccessible areas of the protein, further driving this process towards unfolding.

Area: 10

(Socio Patrocinante: Pablo Valenzuela)

Predicted functions and evolution of single-exon genes in mammalian genomes

Jorquera R., Ortiz R. and Holmes D.S.
Center for Bioinformatics and Genome Biology, Fundación Ciencia & Vida and Facultad de Ciencias Biológicas, Universidad Andres Bello, Santiago, Chile.
E-mail: roddy.jorquera@gmail.com

Introduction Eukaryotic genes are typically interrupted by intragenic sequences termed introns. However, an increasing number of functional single-exon genes (SEGs) are being discovered, raising questions about their evolution and function. To date, a large scale multiple-genome comparison of predicted SEG functions in mammals has not been carried out. In order to address this issue, SEGs derived from ten completely sequenced mammalian genomes including human, were studied.

Material & Methods Ten mammalian genomes were downloaded from NCBI. Using Perl scripts. Genes were parsed into single-exon (SEGs) and multi-exon genes (MEGs). High dimensional analysis of the SEGs included classification of their function (KOG) and an examination of the relative distribution of these functions between species. Statistical analyses were carried out to determine if any KOG category was significantly enriched in SEGs.

Results & Discussion Compared to MEGs, SEGs are enriched in: i) chromatin structure and dynamics including histones; ii) signal transduction mechanisms including G protein-coupled receptors (GPCRs) and iii) translation related proteins including ribosomal proteins ($p<0.05$). We hypothesize that the enrichment of some of these functions in SEGs is related to high levels of gene expression in these categories. On the other hand, SEGs are predicted to be impoverished in functions related to: i) membrane biogenesis; ii) amino acid, nucleotide, and lipid metabolism and iii) secondary metabolites biosynthesis and catabolism ($p<0.05$), suggesting that introns play an important role in the evolution of diversity and function of genes in these latter categories.

Area 10
(Socio Patrocinante: Pablo Valenzuela)

OX40 signaling confers resistance to lactic acid in T cells transduced with chimeric antigen receptors

Ernesto López, Eduardo Durán, Elizabeth Torres, Paola Murgas, Alvaro Lladser
Laboratory of Gene Immunotherapy, Fundación Ciencia & Vida, Santiago, Chile.
E-mail: alladser@cienciavida.org

Introduction Genetic modification with chimeric antigen receptors (CAR) has been developed as a powerful strategy to redirect T cell immunity against cancer. However, tumor-derived immunosuppressive metabolites can impair T cell activation. In this work, the immunosuppressive effects of lactic acid and adenosine on the activation of T cells transduced with carcinoembryonic antigen (CEA)-specific CARs expressing either CD28 or OX40 costimulatory molecules were evaluated.

Material & Methods Phoenix-Ampho cell lines stably producing the two different CAR-encoding retroviral particles were obtained. Purified T cells were transduced using retroviral supernatants and retronectin after activation with CD3- and CD28-engaging antibodies. CEA-specific T cell activation and tumor cell cytotoxicity was evaluated *in vitro* by intracellular staining and flow cytometry. Mice bearing CEA-expressing CT26 tumors were i.v. injected with 250.000 CAR-transduced T cells and tumor growth was measured.

Results & Discussion Mouse and human T cells were transduced with nearly 50% efficiency. CAR-transduced T cells specifically produced IFN-γ, up-regulated granzyme and CD69, and induced tumor cell killing *in vitro*. CAR-transduced T cells suppressed the growth of CT26 tumors *in vivo.* Both lactic acid and adenosine reduced IFN-γ production in a dose-dependent manner. A high concentration (10 mM) of lactic acid drastically reduced the viability of CD28-CAR T cells, whereas OX40-CAR T cells were able to resist lactic acid-induced stress. This work suggests that OX40-signaling confers resistance to lactic acid-induced dysfunction and current efforts are focused on defining the mechanisms of OX40-mediated protection.

Area 4

A selective H3K27 demethylase inhibitor ameliorates experimental acute colitis.

Jocelyn C. Neira1, Dominique Fernández$_2$, Paz Reyes1, Daniela Sauma2, Alejandra Loyola1, María Rosa Bono2 and, Mario Rosemblatt1,2,3.
1Fundación Ciencia & Vida, 2Laboratorio de Inmunología, Facultad de Ciencias, Universidad de Chile and 3Facultad de Ciencias Biológicas, Universidad Andrés Bello.
E-mail: jocelyn.neira@gmail.com

Introduction The ratio of Th17/Treg cells plays a key role in intestinal homeostasis. An increase in this ratio has been implicated in the pathogenesis of inflammatory bowel diseases and thus, strategies to decrease the Th17/Treg ratio are presently considered a suitable approximation for therapeutic applications. Recent studies indicate that an inhibitor of JMJ, a demethylase of lysine 27 of histone H3, suppressed Th17 cell differentiation *in vitro* and reduced the severity of experimental autoimmune encephalomyelitis (EAE). Here, we investigated the influence of this drug in the function and balance of Treg and Th17 cells and its effect on inflammatory bowel disease.
Material & Methods The effect of the drug on the phenotype and function of *in vitro* generated Treg and Th17 cells as well as in the conversion of Treg to Th17 were evaluated by flow cytometry. In addition, we evaluated the effect of the drug in an experimental model of colitis *in vivo*.
Results & Discussion The drug improves Treg cells suppressive capacity and decreases Treg to Th17 conversion. More importantly, it ameliorates experimental colitis. Our study shows that this drug represents a new therapeutic approach to modulate the balance of Th17/Treg cells and thus it may be used to attenuate gastrointestinal inflammatory disorders.

Area 4

Characterization of human thymic B cells and their antibody-producing function

Sarah Nuñez[1,2], Carolina Moore[3], María Rosa Bono[1] Mario Rosemblatt[4] and Emmanuel Zorn[2]

[1] Departamento de Biología, Facultad de Ciencias, Universidad de Chile, [2] Columbia Center for Translational Immunology, New York NY, [3] Massachusetts General Hospital, Boston MA, [4] Fundación Ciencia & Vida, Santiago, Chile.

E-mail: sarah.nunez.c@gmail.com

Introduction The thymus, an organ where T cell maturation occurs, contains a small population of resident B cells. In mice, thymic B cells have an activated phenotype and a fraction produces antibodies spontaneously. In contrast, very little is known regarding human thymic B cells. Moreover, the functional relevance of thymic-produced antibodies has not been addressed.

Materials & Methods Human thymus tissue from infants and adults was obtained during cardiac surgery. Thymic B cell phenotype and localization was characterized by flow cytometry and immunohistochemistry, respectively. Secretion of IgM/IgG/IgA by thymic B cells was determined by ELISA and ELISpot.

Results & Discussion The human thymic B cell population displays an activated phenotype and a fraction of these cells secrete antibodies spontaneously. Importantly, this population undergoes significant changes throughout aging: the neonatal and infant thymus contains mostly naive IgM^+ B cells as well as IgM-secreting cells. During aging, there is a gradual accumulation of memory B cells that have switched Ig class and express IgG together with an increase in IgG-secreting cells. Our data reveal that human thymic B cells spontaneously produce antibodies and are a dynamic population that is significantly affected by age. Currently, we are addressing the function of thymic B cell antibodies by determining their reactivity profile.

AREA 4

SETDB1 associates to ribosomes and methylates ribosome-bound lysine 9 of histone H3.

Carlos Rivera[1], Francisco Saavedra[1], Francisca Alvarez[1], César Díaz-Celis[1], Valentina Ugalde[1], Jianhua Li[2], Ignasi Forné[2], Zachary A. Gurard-Levin[3,4,5,6,7], Geneviève Almouzni[3,4,5,6,7], Axel Imhof[2], and Alejandra Loyola[1].

[1]Fundación Ciencia & Vida, Santiago, Chile. [2]Munich Center of Integrated Protein Science and Adolf-Butenandt Institute, Munchen, Germany. [3]Institut Curie, Centre de Recherche, Paris, France. [4]CNRS, Paris, France. [5]Equipe Labellisee Ligue contre le Cancer, Paris, France. [6]UPMC, Paris, France. [7]Sorbonne University, PSL.

E-mail: fco.saavedra.uchile@gmail.com

Introduction The processing of the newly synthesized histone H3 includes the correct folding and establishment of its post-translational modifications. This maturation cascade implies the association of H3 with different chaperones and enzymes in, at least, four protein complexes. In contrast to the highly modified nucleosomal histone H3, the newly synthesized soluble histone H3 has only a few modifications, including the monomethylation of lysine 9 (H3K9), which is already present in the earliest maturation complex.

Materials & Methods Ribosomes stalled at translation were purified from cycloheximide treated HeLa cells in asynchronous cultures. Cells were lysed and ribosomes purified by loading cell extracts on a 35% sucrose cushion. Purified ribosomes were analyzed by Western-blot and H3K9-methylation assays.

Results & Discussion We detected ribosome-bound histone H3, which was K9me1 and K9me2. We detected H3K9-methyltransferase activity associated to ribosome, which was SetDB1 dependent. We found that this activity establishes K9me2 by a non-processive mechanism. Ribosome-associated SetDB1 catalyzes the mono- and dimethylation of the ribosome-bound histone H3. We propose a mechanism where SetDB1 establishes H3K9-methylation at ribosomes to prime a population of H3 for its use in defined deposition pathways to mark distinct chromatin domains.

Area: 3

Knockdown of the mitochondrial antisense ncRNAs induces complete inhibition of murine melanoma tumor growth and metastasis

Lorena Lobos-González[1], Verónica Silva[1], Mariela Araya[1], Luciana Oliveira-Cruz[1], Christopher Fitzpatrick[1], Macarena Briones[1], Jaime Villegas[1,2], Claudio Villota[1,2], Soledad Vidaurre[1,3], Vincenzo Borgna[1], Constanza Lopez[1], Teresa Socias[1], Luis O. Burzio[1,2] and Verónica A. Burzio[1]

[1]Andes Biotechnologies SpA.; Fundación Ciencia & Vida, Av. Zañartu 1482; [2]Facultad de Ciencias Biológicas, Universidad Andrés Bello, República 252

[3]Facultad de Salud, Deporte y Recreación, Universidad Bernardo O'Higgins, Gral. Gana 1702

E-mail: vburzio@gmail.com

Introduction: The mitochondrial non-coding RNAs (ncmtRNAs) are differentially expressed in normal and tumor human cells. The sense transcript (SncmtRNA) is expressed in normal proliferating cells and tumor cells. Remarkably, however, the ASncmtRNAs are down-regulated in all tumor cells. Here, we reported that knocking down the ASncmtRNAs induces apoptotic cell death of murine melanoma B16F10, potentiated by down-regulation of survivin and *in vivo* studies revealed complete inhibition of tumor growth and metastasis.

Material and Methods: B16F10 melanoma cell line were transfected *in vitro* with antisense oligonucleotides (ASO) complementary to ASncmtRNAs. After 48h post treatment cell viability, tumorigenic property and level of survivin was evaluated by western blot. For *in vivo* assays, C57BL6 mice 10^5 cells were injected subcutaneously. We evaluated the effect of ASO treatment over tumor growth and metastasis comparing two groups: the ASO-C group and the ASO-1560S group.

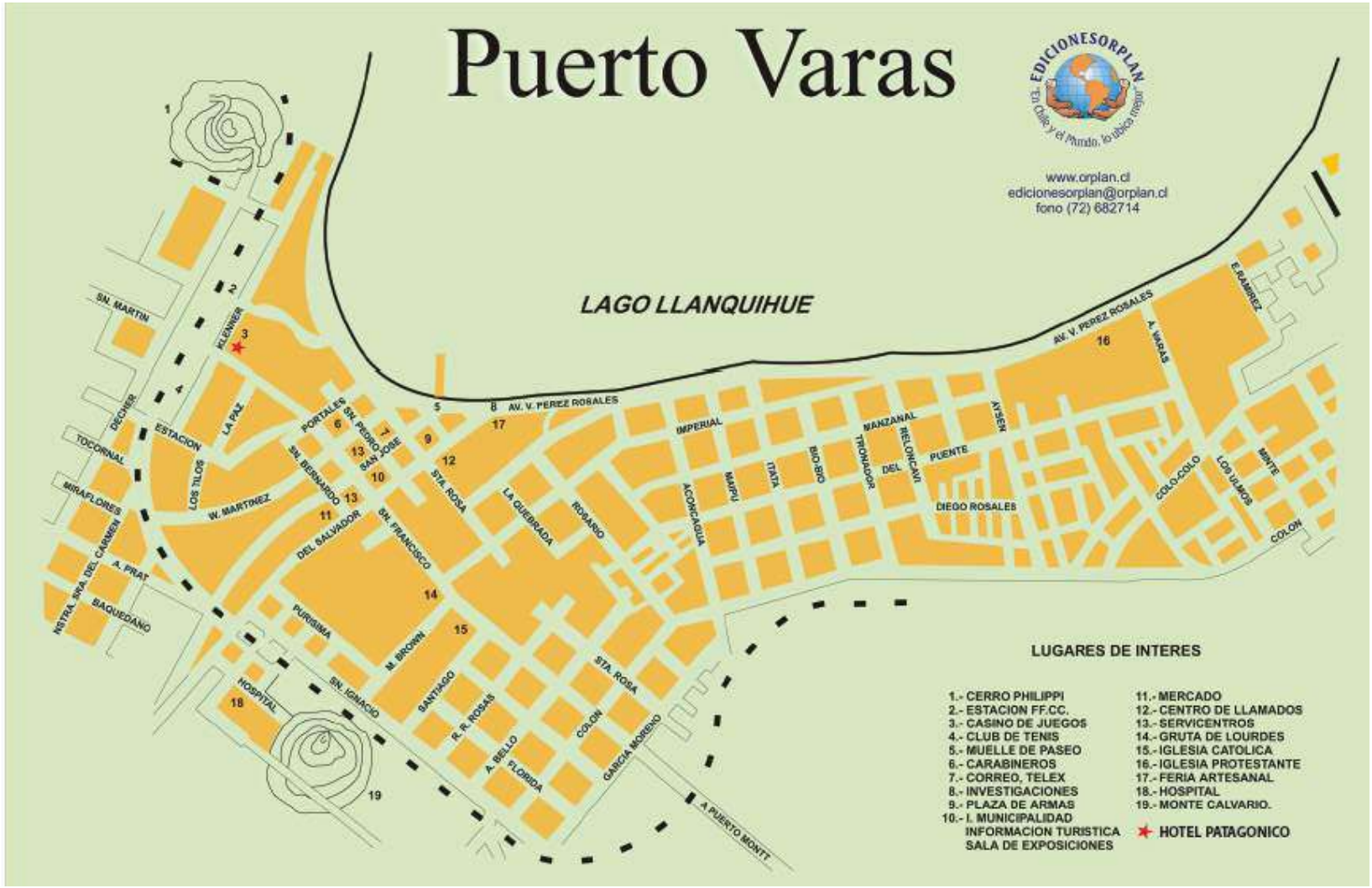
Results: Knocking down the ASncmtRNAs markedly inhibits cell proliferation, induces apoptosis, tumorigenicity, and stemness of melanoma in vitro. In addition, remarkable, ASO treatment *in vivo* induces inhibition of melanoma tumor growth and metastasis

Discussion: Melanoma is one of the most aggressive and treatment-refractory type of cancer and the present results suggest a new and potent therapeutic approach for the disease.

Area 8: Cell growth/death and cancer

Fundación Ciencia & Vida

MAPS


Puerto Varas
EDICIONESORPLAN
www.orplan.cl
edicionesorplan@orplan.cl
fono (72) 682714
LAGO LLANQUIHUE
AV. V. PEREZ ROSALES
SN. MARTIN
KLENNER
DECHER
ESTACION
LA PAZ
TOCORNAL
MIRAFLORES
LOS TILOS
W. MARTINEZ
A. PRAT
NSTRA. SRA. DEL CARMEN
BAQUEDANO
PORTALES
SN. PEDRO
SAN JOSE
SN. BERNARDO
DEL SALVADOR
SN. FRANCISCO
STA. ROSA
LA QUEBRADA
ROSARIO
IMPERIAL
ACONCAGUA
MAPU
ITATA
BIO BIO
TRONADOR
MANZANAL
RELONCAVI
DEL
PUENTE
AYSEN
DIEGO ROSALES
E. RAMIREZ
A. VARAS
COLO-COLO
LOS ULMOS
MONTE
COLON
PURISIMA
M. BROWN
SANTIAGO
R. R. ROSAS
A. BELLO
FLORIDA
COLON
STA. ROSA
GARCIA MORENO
SN. IGNACIO
HOSPITAL
A PUERTO MONTT
LUGARES DE INTERES
1.- CERRO PHILIPPI
2.- ESTACION FF.CC.
3.- CASINO DE JUEGOS
4.- CLUB DE TENIS
5.- MUELLE DE PASEO
6.- CARABINEROS
7.- CORREO, TELEX
8.- INVESTIGACIONES
9.- PLAZA DE ARMAS
10.- I. MUNICIPALIDAD
INFORMACION TURISTICA
SALA DE EXPOSICIONES
11.- MERCADO
12.- CENTRO DE LLAMADOS
13.- SERVICENTROS
14.- GRUTA DE LOURDES
15.- IGLESIA CATOLICA
16.- IGLESIA PROTESTANTE
17.- FERIA ARTESANAL
18.- HOSPITAL
19.- MONTE CALVARIO.
HOTEL PATAGONICO


Lago Puyehue
Entre Lagos
Lago Rupanco
Puerto Octay
Las Cascadas
Lago Todos Los Santos
Frutillar
Frutillar Bajo
Lago Llanquihue
Ensenada
Llanquihue
Puerto Varas
Reserva Nacional Llanquihue
Alerce
Monumento Natural Lahuen Nadi
PUERTO MONTT
Cochamó
Parque Nacional Alerce Andino
Seno de Reloncaví
A Chiloé
73° 00'
72° 45'
72° 30'