Institut Pasteur de Montevideo
Annual Report 2014

PREFACE

On behalf of the Institut Pasteur de Montevideo (IP Montevideo), I would like to invite you to go over the following pages and learn more about the activities we developed during 2014.

Inaugurated in 2006, the IP Montevideo's mission is to become a "state-of the-art" research center with international projection in the field of biomedicine. We currently investigate the biological mechanisms of human and animal diseases, and eventually pave the way for new treatments and cures. As part of this mission, IP Montevideo seeks to train new human capacities and transfer technology to public or private companies, thus contributing to the development of science and biotechnology in the country.

The IP Montevideo belongs to the International Pasteur Institute Network (IPIN). It includes 32 research institutes around the world with expertise to fight against infectious diseases. The IP Montevideo is one of the youngest institutes within this network, and from its inauguration, shares the same Pasteurian principles in life sciences research and applications.

By December 2014, more than 210 people worked or studied at the IP Montevideo, including almost 100 researchers, technicians or assistants, as well as 35 support staff.

The IP Montevideo has established central core facilities with "state-of-the-art" equipments to study genomic, proteomic, structural biology, cell biology and animal research. In 2014, an ambitious investment was performed in flow cytometry and cell sorting technologies. Research is performed by 17 units with personnel from different institutions and at the end of 2014 we stimulated our Researchers to consolidate and organize their research interest into Institutional Programs focused on Genomics, Cell and Animal Technology, Metabolic and Inflammatory Related Diseases and Zoonotic diseases.

In this regards, in 2014, we launched the "Urugenome Program" funded by the Interamerican Development Bank (IDB). In collaboration with Seoul University and the company Macrogen from South Korea, this program seeks to build human capacities and knowledge to analyze the genome of Uruguayan population. Also, it is for me a pleasure to announce that a new laboratory shared with the National Institute for Agricultural Research (INIA) is now being equipped at our "space for innovation" build in 2013. It will be dedicated to study the molecular aspects of prevalent zoonotic and animal diseases.

The number and impact of the publications further increased in 2014, reaching 82 publications in the period and a cumulated average of 16,2 citations per publication. In

addition, one preliminary patent application and software copyrights applications were submitted in 2014.

Our research laboratories provide an environment for the training of advanced graduate students. In 2014, more than 80 PhD/Msc students and postdoctoral researchers were trained at IP Montevideo. We have experienced great success in the organization of 8 international courses on different topics of molecular medicine. In 2014, more than 40 distinguished professors and dozens of advanced students from abroad attended the courses. In addition, we received hundreds of school and high school students in different activities of science pop.

2014 was also the year of the consolidation to the “Bioespinn”, the Enterprise Incubator of the Pasteur Institute that is located in our "Space for Innovation". It was funded by the Uruguayan Agency for Innovation and Research (ANII for its acronym in Spanish) and our own Institute and has now accepted 5 new companies leaded by young researchers.

The 2014 annual budget of the IP Montevideo was equivalent to 6,7 million dollars, 62% coming from local governmental funding and 38% from services and dozens of grants, including those allocated by European Union (Uruguay-Innova) and FOCEM (MERCOSUR Regional Convergence Fund). Thirty-seven new grants were obtained in the period, including 12 company-driven projects.

Finally, we elaborated the “IP Montevideo 2020 Strategic Plan” seeking to consolidate research and technological capacities, create more focused and integrated research programs in molecular medicine and biotechnology, and accelerate the transfer of knowledge and technologies.

I wish to thank all of our researchers and Institute members for their dedication, continued support and commitment with our Institute.

Luis Barbeito

Executive Director

Institut Pasteur Montevideo

INDEX






- **Analytical Biochemistry and Proteomics Unit**
- **Recombinant Proteins Unit & Laboratory on Chronic Lymphocytic Leukemia**
- **Protein Crystallography & Laboratory of Molecular and Sructural Microbiology**
- **Bioinformatics Unit**
- **Molecular Biology Unit**
- **Cell Biology Unit**
- **Transgenic and Experimental Animal Unit**
- **Protein Biophysics Unit**

Analytical Biochemistry and Proteomics Unit

Interim Head: Rosario Durán, PhD (IIBCE – IP Montevideo)

Members**:**	**Rosario Durán, PhD (Interim Head,** Investigator IIBCE – IP Montevideo**)**
	Carlos Batthyány, MD, PhD (Investigator-IP Montevideo; Adjunct Professor of Biochemistry, School of Medicine, UdelaR) ***Horacio Botti, MD, PhD (***Adjunct Investigator, IP Montevideo) ***Magdalena Portela*** (Technical Assistant – School of Sciences/IP Montevideo) ***Analía Lima, MSc.*** (Technical Assistant, PhD student) ***Magdalena Gil, Biochemist*** (Technical Assistant, PhD student; ANII Fellow) ***Jessica Rosello, Biochemist*** (Technician, PhD student; ANII Fellow) ***Bernardina Rivera, Biochemist*** (Technician, Graduate student) ***María Lamas, Biochemist*** (Technical Assistant, Grant Fellow)
Students:	***Jorge Rodriguez, Biochemist*** (PhD student; ANII Fellow) ***Gonzalo Spera, M.D.*** (MSc student) ***Adriana Carlomagno, M.D.*** (MSc student) ***Rosina Dapueto,*** *M.*Sc. (PhD Student, ANII Fellow) ***Germán Galliusi*** (MSc student, ANII Fellow***)*** ***Rosina Toledo*** (MSc student, CSIC) ***Josefina Peña*** (Undergraduate student, ANII Fellow)
Associate members:	***María Noel Álvarez, PhD*** (Associate Investigator, Adjunct Professor of Biochemistry, School of Medicine, UdelaR, Uruguay) ***Leonel Malacrida, PhD*** (Associate Investigator, Assistant Profesor, Pathophysiology Department, School of Medicine, UdelaR, Uruguay) ***Virginia López, PhD*** (Adjunct Professor of Organic Chemistry, School of Chemistry and Science, UdelaR)

GOALS

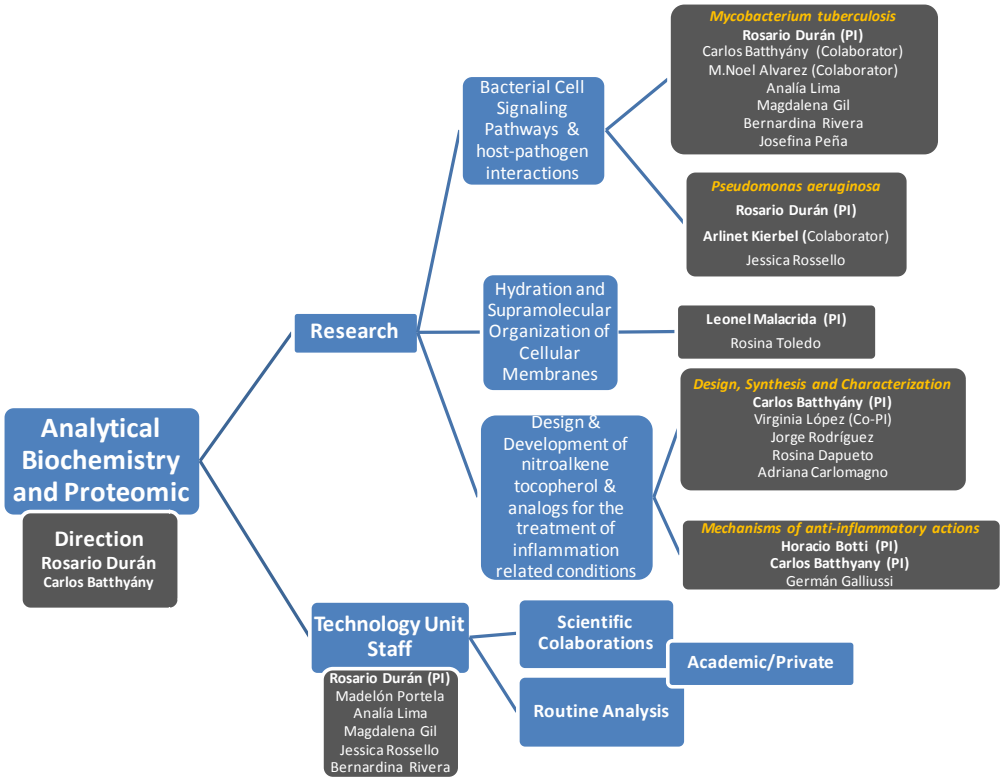
The goals of the Analytical Biochemistry and Proteomics Unit (UByPA) at the Institut Pasteur de Montevideo is to:

1. perform and support mass spectrometry (MS)/analytical biochemistry based research;
2. provide researchers with training, scientific assistance and access to MS and proteomic related technologies;
3. improve available MS & 2D-DIGE proteomic techniques for biomedical research;
4. contribute to local and regional training and education programs.

Our specific goals are:

- to provide open access to analytical biochemistry, mass spectrometry and proteomics technologies to local and regional researchers;
- to pursue biomedical research projects based on mass spectrometry and proteomics;
- to facilitate collaborative scientific projects with other national and international research groups, supporting a join-effort to seek funding;
- to train graduate students and researchers in analytical biochemistry, mass spectrometry and proteomics related technologies.

Unit Organization



Research

In the past years, members of our group have been involved in different areas of biological/biochemical research. A major contribution made by the UByPA´s scientist was the incorporation of modern mass spectrometry (MS) and 2D-DIGE to our local academy, bringing totally new analytical capabilities to perform comprehensive protein studies, including posttranslational modifications of proteins and the ability to decode cell signaling networks. Nowadays, we are involved in three main areas of research concerning protein-mediated cell signaling events:

1. **CELL SIGNALING PATHWAYS IN PATHOGENIC BACTERIA: A PROTEOMIC APPROACH**
   - **A.** Characterization of *Mycobacterium tuberculosis* Ser/Thr kinase PknG signaling pathways. PI: Rosario Durán; Graduate Thesis Project of M. Gil, PEDECIBA, UdelaR, 2011-to date.
   - **B.** Effects of *Mycobacterium tuberculosis* Ser/Thr kinase PknG on the macrophage. PIs: Rosario Durán, Carlos Batthyány and MN Álvarez; Graduate Thesis Project of MSc A.Lima, Pro.In.Bio., UdelaR, 2011-to date.
   - **C.** Proteomic characterization of c-di-GMP mediated transition from a planktonic to surface-associated *Pseudomonas aeruginosa*. PIs: R. Durán; Graduate Thesis Project of J. Rossello, PEDECIBA, UdelaR, 2013 to date).

2. **BIOLOGICAL EFFECTS OF SECOND GENERATION NITROALKENE TOCOPHEROL ANALOGS**:

Atherosclerosis is an epidemic worldwide disease and leading cause of death in developed countries. Two main pathogenic events are well recognized in the generation of the atheroma plaques: inflammation and lipid accumulation via LDL deposition/foam cells formation. Inflammation is a key event mediated by inflammasome activation by sterile signals (i.e. cholesterol crystals) and precedes massive lipid accumulation.

In our work we envision a new pharmacological strategy for the treatment and prevention of atherosclerosis. We designed a hybrid compound analog of α-tocopherol to which we added the electrophilic nitroalkene group (1). The rationale for our idea is that the nitroalkene-tocopherol analog will be selectively incorporated into the lipoprotein particles during their normal metabolism due to the presence of the chromanol structure. Once incorporated, lipoproteins will transport the compound through the body, including to the atherosclerotic lesions, were it may exert the potent anti-inflammatory and anti-atherogenic properties of the nitroalkenes.

We synthesized two series of tocopherol analogs and performed their physicochemical and biological characterization. Nitroalkene-tocopherol analogs are electrophiles that are being incorporated into lipoproteins both in vitro and in vivo and exhibit potent anti-inflammatory activity: A- inhibit pro-inflammatory cytokines (IL-6, MCP-1, TNFα) secretion controlled by NFκB factor & B- induce expression of phase 2 enzymes regulated by Nrf2/Keap-1 (HO-1, GCLM, NQO1) in murine macrophages; C- inhibit interleukin 1-β secretion by NLRP3 inflammasome in THP-1 cells. We are now evaluating their capabilities to inhibit the development of atherosclerotic plaques formation in two animal models: apo E-/- and zebrafish.

A. Design and Development of Nitroalkene Tocopherol and Analogs for Use in the Treatment and Prevention of Inflammatory Mediated Diseases. PIs: C. Batthyány and V. López; Graduate Thesis Project of J. Rodriguez, PEDECIBA, UdelaR, 2012 to date).
B. Electrophilic mediated protein modifications.
    i. "Composition and Method for Inhibition of PknG from *Mycobacterium tuberculosis*", PIs C. Batthyány & R. Durán (U.S. PCT Application No. 61/835,416; 2014).
    ii. Molecular Mechanisms of Nitroalkene mediated anti-inflammatory cell signaling events; PIs C. Batthyány & H. Botti.

3. HYDRATION AND SUPRAMOLECULAR ORGANIZATION OF CELLULAR MEMBRANES

A. "Hydration and Supramolecular Organization Studies of Lamellar Bodies in A549 Lung Cells using LAURDAN fluorescence"; PI L. Malacrida.
B. Purification and molecular characterization of lung surfactant from different species: SP-B, SP-C and lipids fractions; PI L. Malacrida.
C. Effect of tocopherol in cellular membranes; PIs L. Malacrida & C. Batthyány.
D. Characterization of phagolysosome maturation by fluorescence microscopy approaches; PIs Leonel Malacrida & R. Durán.

Services

Our Unit received in January 2007 a MALDI TOF-TOF MS instrument (AB-SCIEX, Framingham, USA) and, in December 2009, completed the MS platform with the arrival of a nano-electrospray/ion trap LTQ Velos instrument (Thermo, USA). Both instruments complement each other and expand the quality and type of mass analytical procedures we can offer to local and regional research groups.

There are two general modes to get access to the Unit facilities:

1. Routine Service

For routine analysis, users are welcome to access the UByPA as a "fee for service facility" supported by the Institut Pasteur de Montevideo. The facility offers this kind of service to researchers in the region, with priority given to users from the Institute and local academy. The analysis will be performed by members of our technical staff and will be done following standard protocols. The routine analysis includes analysis and interpretation of raw data based on routine practices only.

Routine analysis includes:

- 2-D gel electrophoresis.
- Protein sample preparation for MS analysis: in-gel digestion, in-solution digestion, desalting.
- Molecular mass determination for peptides and small proteins by MS.
- Protein identification by MALDI-TOF/TOF MS (peptide mass fingerprinting, MS/MS ion search) and database search.

In 2013 we analyzed **1178 different samples** from **83 different groups: 55%** of the samples were from our **local academy**, **42%** were from the **regional academy** (Argentine, Brazil, Chile, Colombia, and Venezuela) and **3%** from **private industry**.
In 2014 we analyzed 1238 samples from different groups from local and regional academy.

2. Non-Routine Service

Collaborative research projects, beyond routine services, are welcome. Members of the Unit are expected to significantly contribute to the conception, design of experiments and custom-design protocols, original ideas as well as data analysis and interpretation beyond routine practice.

Non routine analysis includes:

- Custom sample preparation.
- Post-translational modification analysis.
- 2-D gel electrophoresis based proteomics.
- "Shotgun" based proteomics.
- Quantitative proteomics.
- De novo peptide sequencing.
- Glycomics and glycoproteomics.

Publications

1. Turell L, **Botti H**, Bonilla L, Torres MJ, Schopfer F, Freeman BA, Armas L, Ricciardi A, Alvarez B, Radi R (**2014**) HPLC separation of human serum albumin isoforms based on their isoelectric points. J Chromatogr B Analyt Technol Biomed Life Sci 944: 144-151.
2. Trochine A, Alvarez G, Corre S, Faral-Tello P, **Duran R**, **Batthyany C**, Cerecetto H, Gonzalez M, Robello C (**2014**) Trypanosoma cruzi chemical proteomics using immobilized benznidazole. Exp Parasitol 140: 33-38.
3. Trajtenberg F, Albanesi D, Ruetalo N, **Botti H**, Mechaly AE, Nieves M, Aguilar PS, Cybulski L, Larrieux N, de Mendoza D, Buschiazzo A (**2014**) Allosteric activation of bacterial response regulators: the role of the cognate histidine kinase beyond phosphorylation. MBio 5: e02105.
4. Randall LM, Manta B, Hugo M, Gil M, **Batthyany C**, Trujillo M, Poole LB, Denicola A (**2014**) Nitration transforms a sensitive peroxiredoxin 2 into a more active and robust peroxidase. J Biol Chem 289: 15536-15543.
5. Morero NR, **Botti H**, Nitta KR, Carrion F, Obal G, Picardeau M, Buschiazzo A (**2014**) HemR is an OmpR/PhoB-like response regulator from Leptospira, which simultaneously effects transcriptional activation and repression of key haem metabolism genes. Mol Microbiol 94: 340-352.
6. Mon ML, Moyano RD, Viale MN, Colombatti Olivieri MA, Gamietea IJ, Montenegro VN, Alonso B, Santangelo Mde L, Singh M, **Duran R**, Romano MI (**2014**) Evaluation of cocktails with recombinant proteins of Mycobacterium bovis for a specific diagnosis of bovine tuberculosis. Biomed Res Int 2014: 140829.
7. Martinez A, Peluffo G, Petruk AA, Hugo M, Pineyro D, Demicheli V, Moreno DM, Lima A, **Batthyany C**, **Duran R**, Robello C, Marti MA, Larrieux N, Buschiazzo A, Trujillo M, Radi R, Piacenza L (**2014**) Structural and Molecular Basis of the Peroxynitrite-mediated Nitration and Inactivation of Trypanosoma cruzi Iron-Superoxide Dismutases (Fe-SODs) A and B: Disparate Susceptibilities Due To The Repair Of Tyr35 Radical By Cys83 In Fe-SodB Through Intramolecular Electron Transfer. J Biol Chem 289: 12760-12778.
8. Malacrida L, Reta G, Piriz H, Rocchiccioli F, **Botti H**, Denicola A, Briva A (**2014**) Sevoflurane anesthesia deteriorates pulmonary surfactant promoting alveolar collapse in male Sprague-Dawley rats. Pulm Pharmacol Ther 28: 122-129.
9. Dieterle ME, Bowman C, **Batthyany C**, Lanzarotti E, Turjanski A, Hatfull G, Piuri M (**2014**) Exposing the secrets of two well-known Lactobacillus casei phages, J-1 and PL-1, by genomic and structural analysis. Appl Environ Microbiol 80: 7107-7121.

International Patents

1. "Nitroalkene Tocopherol and Analogs for Use in the Treatment and Prevention of Inflammation Related Conditions"; (**U.S. PCT Application** No. 61/903,068; **2014**; co-inventors C.Batthyany & G.V.López).
2. "Composition and Method for Inhibition of PknG from Mycobacterium Tuberculosis"; (**U.S. PCT** No. 61/835,416; **2014**; co-inventors C.Batthyany & R. Durán).

Grants

1. **"Development of a novel class of anti-atherogenic agents: electrophilic nitroalkenes-Vitamin E (α-tocopherol) analogs"**. (2013 - 2015); CABBIO; PI Carlos Batthyány; **Amount Granted USD 30.000.**
2. **"N-Glycan fingerprint of exosomes in Chagas and Cancer diseases"**. (2014); Interdisciplinary projects IP Montevideo; Coordinator C. Batthyány, **Amount granted USD 20.000.**
3. **"Exploring the role of mosquito's saliva in the transmission of Rift Valley fever"** (2012-2014); Actions Concertées Interpasteuriennes (ACIP). Scientific coordinator: V. CHOUMET (Paris). Uruguayan PIs: C. Batthyány & R. Durán. **Amount Granted: EUR 18.000.**
4. **"Caracterización nutricional y de compuestos bioactivos del trigo en Uruguay. Variabilidad de genotipos y ambientes"**; (2014 - 2016); FPTA - INIA: Contrato de Servicio PIs C. Batthyány & L. Malacrida; **Amount Granted USD 32.000**.
5. **"Identification of tumor associated antigens"**; (2014 - 2015); Private Company: Contrato de Servicio; PIs R. Durán & C. Batthyány; **Amount Granted: USD 35.500**.
6. "**Anti-atherogenic effects and molecular mechanisms of nitroalkene tocopherol analgos: a novel pharmacological approach**" (2014-2016); PIs C. Batthyány & H. Botti; **Amount Granted: USD 30.000**.
7. **"Análisis proteómico comparativo de dos cepas de *P. aeruginosa* con distinta capacidad de adhesión a células epiteliales"** (2014-2016); J. Rossello (FCE_3_2013_1_100344); **Amount Granted: USD 25.000.**
8. "**Hacia la elucidación del mecanismo molecular utilizado por PknG para ejercer su rol como factor de virulencia**" (2014-2016). M. Gil (FCE_3_2013_1_100358); **Amount Granted: USD 20.000.**
9. "**Surfactante Pulmonar durante la Lesión Pulmonar Aguda: Abordaje estructural, dinámico y funcional**" **(**2013 – 2015) CSIC I+D 2012, **PI: L. Malacrida, Amount Granted: USD 38.000**.

Students Fellowships

1. PhD fellowship - Rosina Dapueto; 2014 (2 years); ANII
2. PhD fellowship - Jorge Rodríguez; 2013 (3 years); ANII
3. PhD Fellowship - Magdalena Gil; 2012 (3 years); ANII
4. MSc. Fellowship - Jessica Rosello; 2012 (2 years); ANII .
5. Initiation Fellowship - Josefina Peña; 2014 (1 year); ANII
6. Initiation Fellowship - German Galliuisi; 2014 (1 year); ANII

Other Activities

ORGANIZATION OF COURSES

1. **"Proteome Analysis by Mass Spectrometry"; UNU-BIOLAC & RIIP (Institut Pasteur International** Network). September 1 to 12, 2014. Organizers: Rosario Durán & C. Batthyány.

TRAINING OF STUDENTS

1. **Graduate students:**
   - **Analía Lima**. Pro.In.Bio. "Caracterización molecular del proceso de inhibición del fagosoma por una quinasa de *Mycobacterium tuberculosis*". Directores Académicos: C. Batthyány, R. Durán, MN. Álvarez.
   - **Magdalena Gil**. PEDECIBA Química, "Regulación de la actividad quinasa de PknG de *M. tuberculosis* y su rol en las primeras etapas de la infección". Director: A. Denicola; Co-Director: R. Durán.
   - **Jorge Rodríguez**. PEDECIBA Química. "I+D de análogos de la vitamina E liberadores de óxido nítrico o nitroalquenos como potenciales fármacos para prevención primaria de aterosclerosis". Director: V. López. Co-director. C. Batthyany.
   - **Rosina Dapueto**. Pro.In.Bio. "Inhibidores de CD38 y nitroalquenos derivados de la vitamina E para el tratamiento de enfermedades cardiovasculares". Director Académico Dr. Carlos Batthyány; Co-dirección: Drs. Virginia López y Carlos Escande.
2. **Magister in Science students:**
   - **Jessica Rossello**. PEDECIBA Biología. "Estudio de la adhesión y agregación de *Pseudomonas aeruginosa* en células epiteliales mediante aproximaciones proteómicas" Director: R. Durán Co-Director: A. Kierbel.
   - **Dr. Gonzalo Spera**. Pro.In.Bio. "Proteómica diferencial de líneas celulares de cáncer de mama metástasico HER2 negativo sensibles y resistentes a Docetaxel". Director: Dr. C. Batthyány, Co-Directors: Dra. C. Touriño, Dra. L. Delgado.
   - **Bernadina Rivera**. PEDECIBA Química. "Vías de señalización mediadas por PknG y su regulación en micobacterias". Director: Dra R. Durán, Co-Director: Dr. C. Batthyány.
3. **Undergraduate students:**
   - **Josefina Peña**. Lic. Bioquímica. "Modulación del proteoma del fagosoma por una quinasa de *Mycobacterium tuberuculosis*". Initiation into Research Fellowship ANII. Director: R. Durán; Co-Director: A. Lima
   - **Germán Galliusi**. Lic. de Bioquímica. "Mecanismos anti-inflamatorios de los nitroalquenos: Aparente contra regulación del Inflamasoma NLRP3 y la Apoptosis". Initiation into Research Fellowship ANII. Director: C. Batthyány; Co-Director: H. Botti.
   - **Rosina Toledo Gallo.** Lic. Bioquímica. "Purificación Analítica y Preparativa de las Proteínas hidrofóbicas del Surfactante Pulmonar". Initiation into Research Fellowship ANII.
4. **Training stages at the Unit:**
   - **Lorena Pardo, MD.** (Graduate student, Microbiology Department, School of Medicine, UdelaR); Oct 2013 to date.
   - **Germán Rosano**, **PhD**. (Instituto de Biología Molecular y Celular de Rosario, Argentina) Financial support: CONICET, September, 2014.
   - **Luciana Fleitas, Graduate Student.** (Facultad de Ciencias, UdelaR). April-May, 2014
   - **Paola Scavone, PhD.** (Instituto de Investigaciones Biológicas Clemente Estable). March-April, 2014.
   - **Adriana Martínez**, **Graduate Student**, February 2014 - to date.

Unit of Recombinant Proteins &
Research Laboratory on Chronic Lymphocytic Leukemia

Head: Pablo Oppezzo, PhD

Members: ***Pablo Morande, Ph.D.*** (Post-doctoral position)
Agustín Correa, Ph.D. (Principal technical assistant)
Claudia Ortega, Ph.D. (Technical Assistant)
Cecilia Abreu, M.Sc (Technical Assistant-Doctoral student)
Daniel Prieto, M.Sc (Doctoral student)
Noe Ramses Seija (M.Sc. student)

GOALS

Our scientific proposal is aimed at consolidating the current lines of research in Chronic Lymphocytic Leukemia (CLL), the most common adult leukemia in western countries. As a first general goal, we focus to understand how the immunologic microenvironment and subsequent cell interactions with the tumoral B-lymphocyte are involved in tumor progression of haematopoietic B-cell malignancies. In a second line, we wish to use this information to develop therapeutic and/or prognostic agents for CLL and others lymphoproliferative disorders. The development of these therapeutic tools focus to disrupt the crosstalk between malignant B cells and their microenvironment, whereas the prognosis approach focus on the characterization of new molecules isolated from tumoral proliferative subpopulations of CLL.

Research

Our work concentrates in the study of Chronic Lymphocytic Leukemia as a biological model. This haematopoietic B-cell disease follows an extremely variable course and despite the fact that treatments often induce remissions, most patients relapse and CLL remains incurable. The dissection of the molecular basis of the interactions between cancer cells and their microenvironment is leading to the development of new treatment modalities which are aimed at manipulating the communication of tumor cells with their milieu. In this regard, CLL is an instructive example of how these relationships influence the natural history of a disease. Our work is framed by the haematology and tumoral immunology. It lies on the interface between biochemistry and the molecular and cell biology fields, which in combination with protein expression approaches constitute the core of our experimental designs.

From its beginning in 2007, our group has had a double mission at the IP Montevideo: to develop original lines of research, and to set up a technological core facility for the expression of recombinant proteins. The group leader (P. Oppezzo) has background in the area of tumoral immunology and recombinant proteins production. Immuno-haematological B cell malignancies, adaptive immunity, as well as recombinant antibody production has been Oppezzo's main investigation area for the last 10 years.

The first impact work in CLL area was published in Leukemia Journal where we provided evidences that clarified the origins of the B-leukemic clone in CLL *(Oppezzo et al, Leukemia, 2002).* Next, we went deep insight in the physiological mechanism of Somatic Hypermutation (SHM) and Class Switch Recombination (CSR) events studying these processes in CLL B cells. At this time, Honjo group's described one of the most exciting molecules discovered in the last decades in B lymphocyte Immunology *(Maramatsu et al, JBC, 1999)*. Activation-Induced Cytidine Deaminase, "AID" appeared as the enzyme responsible for the origins of SHM and CSR process and is triggered after immune microenvironment signalling mainly, in secondary lymphoid organs. Our work in the field linked CSR, SHM and AID

expression in CLL disease. We demonstrated that in contrast to normal circulating B-lymphocytes, in progressive CLL cases, the leukemic cells express high levels of an active AID enzyme *(Oppezzo et al, Blood, 2003)* and *(Oppezzo et al, Blood, 2005).* These results and those from other groups suggest that, over-expression of AID could play an important role in CLL disease progression. In the last years, our group has characterized at the transcriptomic level the CLL subset expressing AID enzyme, and demonstrate that the existence of this subpopulation is associated with expression of tumor anti-apoptotic and cell proliferation markers (*Palacios and Moreno et al, Blood, 2010*). Recently, in the same line, we demonstrated that the proliferative pool in Unmutated CLL patients keep activated the PI3K signalling pathway. Our results show that this activation is triggered by up regulation of the microRNA Mir-22 which in turn down regulates the tumour suppressor *PTEN* molecule (*Palacios et al., Leukemia 2014*).

Concerning the development of prognostic markers in CLL, we described that the expression ratio of Lipoprotein Lipase (LPL) and metalloprotease ADAM29 is an important additional marker for the prognosis of CLL *(Oppezzo et al, Blood, 2005).* This data was confirmed by several groups working in CLL in the consecutive years and at present, the prognostic marker LPL is used as one of the strongest prognostic factor in a comparative analysis of RNA-based markers in CLL disease (Kaderi et al., Haematologica, 2011). Despite the usefulness of LPL for CLL prognosis, its functional role and the molecular mechanism regulating its expression remain unsolved as yet. Our recent works in this area demonstrate that an epigenetic mechanism, triggered by the microenvironment, is responsible for anomalous expression of LPL in Um CLL patients *(Moreno and Abreu et al., Leukemia 2013)*. This results lead to speculate that LPL expression on the cellular membrane of CLL B-cells could affect their biological behavior, by favoring cell spreading, and intracellular signalling in an activated tumoral microenvironment. *(Abreu et al., Leukemia & Lymphoma, 2013).*

In the context of therapeutics tools related with cancer, we also produced two chimeric anti-tumoral monoclonal antibodies (mAbs) constructed by fusion of the V_H and V_L fragments of the murine mAb 83D4. Both recombinant antibodies were found to bind breast carcinoma cells expressing the Tn antigen *(Oppezzo et al., 2000, Hybridoma)* and further demonstrated that the chimeric form IgG1κ-83D4 efficiently inhibits the growth of human carcinomas in mouse xenograft models *(Hubert et al, Cancer Research, 2011).* As the Tn antigen is widely expressed on tumor cell, this monoclonal antibody could provide a way to treat cancer based on the chi-83D4 *(Huber et al, European Patent, 2010).*

In addition to this line, our group is recently focused on the generation of new therapeutics molecules named Artificial Binding Proteins (Affitins). Compared with classical therapeutics antibodies Affitins display a broad range of advantages that could be taken into account in the development of therapeutic approaches. By example they are able to maintain high affinity constants even when their molecular weight remains small. This could be very useful in lymphoid neoplasms, in order to gain access into solid tissues as secondary lymphoid organs, where leukemic cells receive pro-survival signals through interaction with the microenvironment and acquire favourable proliferative conditions. In this line a new generation of combinatorial protein engineering technologies has been recently set up in our laboratory. The results in this line has been allowed to propose the use of Affitins as versatile selective glycosidase inhibitors and, potentially, as enzymatic inhibitors in general, that could be envisaged for futures tumor therapy strategies (*Correa et al., PLOS ONE, 2014*).

Research Lines

The dissection of the molecular basis of CLL progression focusing in the interactions between leukemic B cells and their microenvironment is becoming one of our main scientific interests. In this regard two research lines have been pursued since the establishment of our group in 2007.

A. ***Role of microenvironment interactions in CLL progression.***

At present, many questions remain unsolved concerning the role of the microenvironment interactions in the progression of tumoral diseases. We think that CLL represents an excellent model to study these relationships between the leukemic B lymphocyte and their milieu. A detailed characterization of proliferative tumoral subsets that exist in this leukemia may shed light on the association between lymphoid tumours progression and malignant transformation.

Representative publications:

1. **Palacios F, Moreno P, Morande P, Abreu C, Correa A,** Porro V, **Landoni AI, Gabus R,** Giordano M, Dighiero G, Pritsch O, **Oppezzo P.** High expression of AID and active class switch recombination might account for a more aggressive disease in unmutated CLL patients: Link with an activated microenvironment in CLL disease. **Blood, 2010**. Jun 3; 115 (22):4488-96**._** *IF: 10.55*
2. **Palacios F, Abreu C, Prieto D, Morande P,** Ruiz S, Fernández-Calero T, Naya H, Libisch G, Robello C, **Landoni AI, Gabus R,** Dighiero G**, Oppezzo P.** Activation of the PI3K/AKT pathway by microRNA-22 results in CLL B-cell proliferation**. Leukemia, 2014,** Jan;29(1):115. *_IF: 9.38*

B. ***Development of new prognostic and therapeutic tools in CLL***

Despite the fact that the experience in this area is supported by some publications as has been mentioned before, this is a recent research line that we expect to reinforce. This research project is outlined by our double profile as a research/facility group. In this context, the production of recombinant antibodies as well as recombinant proteins constitutes a very useful tool to evaluate different prognostic and/or therapeutic molecules in cancer. One of the goals of this line aim to develop new prognostic methods based on the differential expression of LPL protein in CLL B-cells. In this regard, we recently demonstrated the expression of LPL protein in CLL patients and we obtained funds to carry out the project entitled *"Red-iberoamericana de Leucemia Linfoide Crónica: hacia el desarrollo de nuevos marcadores pronósticos"* at the International call CYTED (Centro Iberoamericano de Tecnología y Desarrollo). In addition, new methodologies such as Ribosome display and Affitins production by high-throughput screening are being incorporated to our unit in order to attain the best technology to carry out this research line.

Representative publications:

1. **Moreno P, Abreu C,** Borge M, **Palacios F, Morande P, Pegazzano M,** Bianchi S, **Landoni AI,** Agrelo R, Giordano M, **Dighiero G,** Gamberale R, **Oppezzo P** et al. Lipoprotein lipase expression in unmutated CLL patients is the consequence of a demethylation process induced by the microenvironment. **Leukemia. 2013** Mar 27. _ IF: 9.38
2. **Correa A, Ortega C,** Obal G, Alzari P, Vincentelli R**, Oppezzo P.** Generation of a vector suite for protein solubility screening. **Front Microbiol. 2014** Feb 25. eCollection 2014. _ IF: 3.941
3. **Correa A,** Pacheco S, Mechaly AE, Obal G, Béhar G, Mouratou B, **Oppezzo P,** Alzari PM, Pecorari F. Potent and specific inhibition of glycosidases by small artificial binding proteins (affitins**). PLoS One. 2014** May 13;9(5):e97438. _ IF: 3.534

To accomplish the research lines mentioned above, it is mandatory to constitute a CLL network, that engages a continuous and coordinate work between our group (focused in the CLL biology) and a medical group (specialized in the management of this disease). To initiate this, in the last years our group has become a reference laboratory that performs the molecular analysis of the immunoglobulin V_H genes (IgV_H) in the leukemic clone. This method allows to separate CLL cases in two prognostic groups: Patients whose B cells express mutated V_H genes have a more indolent disease and longer overall survival those which patients express UM genes. Publications in this area have been achieved in collaboration with Pritsch group in the Biophysical Proteins Unit at IPmont *(Bianchi et al, Leuk Lymphoma, 2010).*

The establishment of this standard procedure as a routine laboratory practice allowed us to start a strong collaboration with the clinical hematologic group of Hospital Maciel in Montevideo, with the clinical hematologic group of Academy of Medicine in Buenos Aires and recently, with the hematologic group of the Haematology chair at Hospital de Clínicas in Montevideo. These collaborations resulted in the first regional CLL group as well as in the first regional cellular bank of CLL placed in IPMont.

The consolidation of this network was recently achieved after obtaining the funds supported by CYTED. Oppezzo's lab is the principal coordinator of this programme (2011-2014) devoted to join efforts from the principal experts in lymphoproliferative disorders in the Iberoamerican region and to consolidate the regional CLL groups. Presently, a number of successful events have been achieved including workshops and student training. In this context, the first international CLL meeting was carried out in November 15th to 17th, 2013 in Punta del Este, receiving 285 participants. (http://www.clliberoamericangroup.com)

Current situation and major scientific group achievements

Most significant scientific results over the last three years (2011-2014):

- ✓ Twenty peer-reviewed articles publish in the period:
  Corresponding author and/or first authors in 9 / 20
- ✓ **Five grants** obtained from national and international research agencies (120 Ku$s for this period).
  - ✓ Creation and consolidation of the LatinAmerican Group in CLL (LAG-CLL) and organization of the First IberoAmerican meeting on CLL in 2013
  - ✓ Attendance to national and international scientific meetings, among which P. Oppezzo has lectured in five international meetings as invited speaker (*International Workshop in CLL, 2011, USA - VIth Young Investigators´ Meeting on CLL, 2012, Germany - IXth International Workshop of European CLL group, 2012, Germany - Chilean Hematology Congress, 2014 and Congress of Argentinean Immunology Society, 2014*).
  - ✓ P. Oppezzo has been editor of the book entitled "Chronic Lymphocytic Leukemia" published in 2011 and served as reviewer for a number of Journals (Blood, Leukemia, Haematologica, Int. Journal of Hemat., Biotech. Journal, etc) and research agencies (ANII, Uruguay, CONICET, Argentina; Italian Cancer Research Association (AIRC) and International Union Against Cancer (UICC).

Publications 2014

1. **Palacios F, Abreu C**, **Prieto D**, **Morande P**, Ruiz S, Fernández-Calero T, Naya H, Libisch G, Robello C, Landoni AI, Gabus R, Dighiero G, **Oppezzo P**. Activation of the PI3K/AKT pathway by microRNA-22 results in CLL B-cell proliferation ***Leukemia. 2014*** Jan;29(1):115.
2. Fischer S, Echeverría N, Moratorio G, **Landoni AI, Dighiero G**, Cristina J, **Oppezzo P**, **Moreno P**. Human endogenous retrovirus np9 gene is over expressed in chronic lymphocytic leukemia patients. ***Leuk Res Rep. 2014*** Jul 25;3(2):70-2. doi: 10.1016/j.
3. **Correa A, Ortega C,** Obal G, Alzari P, Vincentelli R, **Oppezzo P.** Generation of a vector suite for protein solubility screening. ***Front Microbiol. 2014*** Feb 25. eCollection 2014.
4. **Correa A**, Pacheco S, Mechaly AE, Obal G, Béhar G, Mouratou B, **Oppezzo P**, Alzari PM, Pecorari F. Potent and specific inhibition of glycosidases by small artificial binding proteins (affitins). ***PLoS One. 2014*** May 13;9(5) eCollection 2014.
5. Borge M, Lenicov FR, Nannini PR, De Los Ríos Alicandú MM, Podaza E, Ceballos A, Grecco HF, Cabrejo M, Bezares RF, **Morande PE, Oppezzo P,** Giordano M, Gamberale R. The expression of sphingosine-1 phosphate receptor-1 in chronic lymphocytic leukemia cells is impaired by tumor microenvironmental signals and enhanced by piceatannol and R406 ***J Immunol. 2014*** Sep 15;193(6):316.
6. Alem D, Díaz-Dellavalle P, Leoni C, De-Simone SG, **Correa A, Oppezzo P**, Rizza MD. In search of topical agricultural biofungicides: Properties of the recombinant antimicrobial peptide TrxAq-AMP Obtained from Amaranthus quitensis (2014) ***Journal of Microbial and Biochemical Technology***, 6 (5), pp. 268-273.

7. Libisch MG, Casás M, Chiribao M, Moreno P, Cayota A, Osinaga E, **Oppezzo P,** Robello C. GALNT11 as a new molecular marker in chronic lymphocytic leukemia. ***Gene. 2014*** Jan 1;533(1):270.
8. Shigunov Shigunov P, Sotelo-Silveira J, Stimamiglio MA, Kuligovski C, Irigoín F, Badano JL, Munroe D, **Correa A**, Dallagiovanna B. Ribonomic analysis of human DZIP1 reveals its involvement in ribonucleoprotein complexes and stress granules (2014) BMC Molecular Biology, 15, art. no. 12.
9. **Prieto D**, Aparicio G, **Morande PE,** Zolessi FR. A fast, low cost, and highly efficient fluorescent DNA labeling method using methyl green (2014) Histochemistry and Cell Biology, 142 (3), pp. 335-345.

Grants 2011-2014

1. Fondo Clemente Estable – Dra. Cecilia Abreu – "Estudios genómicos del perfil de metilación del ADN en una población tumoral leucémica sobre-expresando la enzima AID" – 2013-2014 – ANII
2. Fondo Clemente Estable – Dr. Pablo Oppezzo – "Implicancias de la expresión anómala de la enzima mutagénica AID en los procesos leucémicos: Desarrollo de un modelo tumoral" – 2013-2015 – ANII
3. Fondo María Viñas – Dr. Pablo Oppezzo – "Expresión de la Lipoproteína Lipasa en las células B de la Leucemia Linfoide Crónica (LLC): Hacia el desarrollo de un nuevo marcador pronóstico" – 2013-2015 – ANII
4. Redes Temáticas – Dr. Pablo Oppezzo – "Red-iberoamericana de Leucemia Linfoide Crónica: hacia el desarrollo de nuevos marcadores pronósticos" – 2011-2014 – CYTED.
5. Proyectos Transversales – Dr. Pablo Oppezzo –. "Genomic landscape of the methylation pattern and the microRNAs/mRNAs expression in progressive patients with Chronic Lymphocytic Leukemia" – 2013-2014 – Institut Pasteur de Montevideo.

Other activities

TRAINING COURSES, WORKSHOPS AND CONGRESS

1. First LatinAmerican Workshop on prognosis markers in CLL: *"Fluorescence in situ hibridization (FISH) as prognosis marker in CLL"*.
   Date and place: 26-29 May 2014, Buenos Aires, Argentina
   Participants: 41
2. Second LatinAmerican Workshop on prognosis markers in CLL: *"Cytometry approaches in the prognosis of CLL"*.
   Date and place: 16-18 November 2014, Florianopolis, Brazil.
   Participants: 46

Protein Crystallography Unit &
Laboratory of Molecular and Structural Microbiology

Head: Alejandro Buschiazzo, PhD

Members: ***Juan Andrés Imelio*** (MSc student)
Nicole Larrieux (Technician)
Frank Lehmann (Technician)
Ariel Mechaly*, PhD* (Postdoctoral fellow)
Natalia Morero (Postdoctoral fellow) - *past member*
Cecilia Nieves (MSc student)
Fabiana San Martin (MSc student)
Felipe Trajtenberg*, PhD* (Research Scientist)
Leticia Zarantonelli, PhD (Associated Research Scientist)

GOALS

We wish to understand how cells sense specific signals and subsequently respond through cell regulation at the molecular level. Particular emphasis is given to these signaling pathways linked to microbial pathogenesis. To these ends we study different species, especially bacteria, both pathogenic as well as non-pathogenic, with molecular and structural approaches.

Our Unit is also committed to developing Structural Biology in Uruguay and the region. With this purpose, we have set up and currently run a Protein Crystallography facility open to users, interns and trainees. This facility now ensures all needed capacities to obtain macromolecular crystals and perform single crystal X-ray diffraction experiments, all the way to 3D structure determination. Specialized courses and workshops on protein crystallography and structural biology are organized on a regular basis to train students and research scientists in these disciplines.

Research

We are interested in studying *Leptospira spp.* (prokaryotic Spirochetes, related to *Treponema* - the agent of syphilis- and *Borrelia* -Lyme disease-), as one of the main bacterial models in our lab. We currently analyze key proteins of signaling and regulation pathways, both from *L. interrogans* (one of the principal etiologic agents of leptospirosis) and *L. biflexa* (a saprophytic model, highly related to the pathogenic relatives). Using *L. biflexa* we are also studying the motility machinery of Spirochetes, which is quite unique in many ways, aiming to understand its regulation in the long-term.
We also continue our work with *Bacillus subtilis* (Firmicutes), a well known prokaryotic model of Gram+ bacteria, to answer questions of temperature sensing and cell regulation.

A more recent line of research is now also being actively pursued, related to the study of retroviral proteins. In collaboration with the Pritsch lab here in Pasteur Montevideo, we are particularly interested in understanding the molecular bases of capsid self-assembly and uncoating. Pritsch has been studying the delta-retrovirus Bovine Leukemia Virus (BLV) with regards to immunologic aspects of the infection, which infects cattle with high specificity. Given that livestock represents one of the main economic income sources in Uruguay and the South American region, this subject has high strategic relevance for national and regional

research agencies and policy-makers. During 2014 we have focused on the capsid protein from BLV, becoming an important target to study at the molecular level with structural biology approaches.

Apart from our own main lines of research, we carry on several projects as collaborators, contributing with our expertise in protein science and structural biology. Intramural interactions with the Badano lab have been particularly active in 2014. Extramural associations with Uruguayan teams at the University Medical School (Prof R Radi) have resulted in high impact publications. Ongoing work with groups in Argentina (at IBR-Rosario) is also boosting synergic work in other bacterial systems, focused on fatty acid synthesis regulation in *Bacillus* and *Mycobacterium tuberculosis*.

In terms of the methodological approach, we intend to understand protein function at the molecular level. This is why it is essential for us to explore a diverse set of organisms, with interest in eventually extending our studies to other clinically relevant bacterial species. Our methods lie on the interface between biology, chemistry and physics: protein crystallography in combination with biochemistry, biophysics and molecular biology constitute the core of our experimental approaches. Our ongoing challenge is to incorporate Microbiology approaches into a much more integrative view of Molecular and Structural Microbiology.

Lines of Research

1. SIGNALING AND REGULATION IN MICROORGANISMS

Bacterial two-component systems (TCSs) and different kinds of regulator proteins in bacteria constitute the main protein systems that we work on. The common theme is how cells use proteins to sense extra- and intra-cellular signals in order to regulate specific functions.

a. **Signaling through TCSs in bacteria**

To understand the molecular means by which bacteria transduce signals, adapting to a changing environment, during the last few years we have been using a non-pathogenic model (*Bacillus subtilis*) focusing our efforts in elucidating the molecular mechanisms of signaling and regulation of lipid synthesis in Gram+ bacteria. Our main contribution concerns the structural studies of the TCS DesK/DesR. DesK is a trans-membrane histidine kinase that, together with its cognate response regulator DesR, regulates the membrane's fluidity in response to cold shock in B. subtilis. Previous structural and biochemical work with the entire cytoplasmic region of DesK from *B. subtilis* (Albanesi et al., *Proc Natl Acad Sci USA* 2009, 106:16185-90; Trajtenberg et al., *J Biol Chem* 2010, 285:24892-903), allowed us to propose a mechanistic model that appears to be general for histidine kinase-mediated signal transduction. More recently, we have turned our attention to the response regulator DesR. The crystal structure of full-length DesR has been obtained, in its activated state. Several crystal forms of the receiver domain were also determined in the active and inactive configurations, revealing molecular details of the activation switch (Trajtenberg et al., *mBio* 2014, 5:e02105-14).

With the aim of studying how TCS-mediated signaling regulates pathogenesis, we have launched a different line of research focused on *Leptospira spp.*, spirochetal bacteria that cause leptospirosis. This disease is the most widespread zoonosis in the world, reemerging as a major health problem. In Uruguay its prevalence as a veterinary issue is very significant, identified as the second most serious problem after brucellosis (Plan Nacional de Investigacion en Salud Animal http://www.fvet.edu.uy/planisa). A collaborative partnership has been established with Albert Ko's lab (Yale Univ) and Mathieu Picardeau's (Institut Pasteur). We have progressed in the understanding of heme sensing and metabolic regulation, which at difference with other Spirochetes, is critical for Leptospira survival. Controlled via a TCS, we have been able to solve the structure of the receiver domain of the response regulator HemR, understanding its role as a transcriptional activator and repressor of key genes in a heme regulon (Morero et al., *Mol Microbiol* 2014, 94:340-52).

Within the collaborative network that we have built in leptopsirosis, some up-stream and down-stream biological phenomena directly linked to the sensing machinery in *Leptospira*, are being studied in vivo at Albert Ko's (Yale Univ) and Mathieu Picardeau's (IPasteur) labs. In the former they have very interesting results demonstrating the relevance of the motility apparatus for *Leptospira* to be pathogenic, highlighting the fact that motility is typically regulated by the CheA-CheY TCS. On the other hand, Picardeau's lab has a long-standing interest in chemotaxis, a well known phenomenon frequently associated with initial up-stream signaling for the bacteria to change swimming behavior and directionality through Che-like systems. We are addressing the problem of motility in *Leptospira*, from a structural point of view (flagellum architecture, noting that these are periplasmic endoflagella, a quite unique feature of Spirochetes), as well as in terms of its regulation. To this aim, we are using *L. interrogans* and *L. biflexa*, as sources of native flagella, purifying the flagella filaments according to published protocols. In this way we have been comparing, using 2D gels and comparative proteomics (spot-picking and MALDI-TOF/TOF), wild-type bacteria vs mutants with impaired translational motility isolated at Albert Ko's lab (Wunder E, Trajtenberg F., et al. unpublished results). Back-complementation with identified single proteins using plasmids in vivo, resulted in rescuing the motility (and correlated virulence) phenotypes. We plan to clone specific genes encoding for flagellar proteins that we discover. We already have available in our lab two of these novel proteins (unique to *Leptospira*), denominated Flagellum-coiling protein 1 and 2 (Fcp1 and Fcp2), that have been studied by crystallography, revealing completely novel folds (San Martin at al, unpublished results).

b. **Kinases in Trypanosomatid parasites**

This is a secondary line of research. We had previously been very active in studying *Leishmania* MAPKs (mitogen-activated protein kinases), relevant in regulating stress-triggered responses and stage differentiation. At the time, a strong collaboration was established with the Spaeth lab (IP-Paris), resulting in a high impact joint publication (Horjales et al., Structure 2012). After ending a FP7 European Union multicentric project (Leishdrug) we have now decided to focus on

the bacterial models. However, we still continue a collaboration with Dr Despina Smirlis at the Helenic Pasteur Institute (Athens, Greece), together with Milena Soares (Fiocruz, Salvador, Brazil). Dr Smirlis leads an ACIP project focused on structure/function studies of a new group of *Leishmania* protein kinases, the dual specifity tyrosine-regulated kinases (DYRKs), given that a *L. major* DIRK1 knock-out is not viable. Ultimately a structure-based drug design strategy is envisaged. This project is planned to be concluded towards the end of 2015.

2. STRUCTURAL VIROLOGY

Started as a collaboration with Dr Otto Pritsch (Inst Pasteur de Montevideo), this new line of research has now become one of our central interests. We have recently submitted a manuscript to a top journal where we report our first significant set of data (Obal G, Trajtenberg F, et al, 2015). The project is focused on the structural studies and self-assembly mechanistic implications of the capsid protein from retroviruses. Working with p24 from the Bovine Leukemia Virus (BLV), we have been able to solve the crystal structure of the mature, native form of the protein, revealing an architecture that is consistent with the assembled core particle of retroviruses as observed by cryo-electron microscopy. Worth to highlight is the fact that the only high resolution crystallographic models of retroviral capsid comes from the Yeager group, who succeeded to solve the 3D structure of the capsid building blocks of HIV-1 (Pornillos O, et al. 2009 *Cell* 137:1282; Pornillos O, et al. 2011 *Nature* 469:424) only after engineering several point mutations. These mutations purposefully modify the natural behavior of the protein, leaving open questions about the observed interactions in the native capsid arrays, which we have now captured with near atomic resolution. BLV is also a pathogen causing a B-cell type of leukemia in cattle, with consequent high interest in the whole South American region, linked to very high prevalence rates, particularly in Uruguay. This aim of the project has resulted in the creation of an International Associated Laboratory ("Laboratoire International Associé" LIA) at the French Scientific Research Council (CNRS), between CNRS, IPasteur and IPMontevideo. The scientific teams integrating this LIA are, from Uruguay, the Otto Pritsch lab and my own lab both at IPMontevideo, and in France, the Felix Rey lab (Unité de Virologie Structurale) in IPasteur, and the Jean Lepault team ("Microscopie Electronique associated to the Lab of Molecular and Structural Virology) at CNRS/INRA in Gif-sur-Yvette.

Our main interest in this project focuses on the self-assembly process that p24 is spontaneously able to trigger. The 3D structure gives us novel insights as to the key interactions that mediate the intra-hexamer and inter-hexamer protein:protein contacts that ultimately hold the assembled core particle together. P24, as other retroviral capsid proteins, builds hexamers (Pornillos O, et al. 2009 *Cell* 137:1282), and these hexamers tend to pack laterally in honeycomb 2D hexagonal arrays. Our structure provides evidence as to the ability of p24 to accommodate to local variations in the packing giving rise to quasi-equivalent contacts, consistent with its capacity to form pentamers that close the pseudo-spherical core particle. These new hypotheses of assembly/disassembly will be useful in the design and optimization of antiretroviral compounds targeting these essential processes in the retrovirus life cycle.

3. COLLABORATIVE WORK

i. We continue an active collaboration with Dr Hugo Gramajo (Instituto de Biologia Molecular y Celular IBR, Rosario, Argentina) and his team, aimed at elucidating the crystal structure of two transcription factors from *Mycobacterium tuberculosis*, MabR and FasR, which are important regulators of the lipid metabolism in this pathogen. We have obtained crystals from both proteins, needing further optimization to be able to determine their 3D structures.

ii. A collaboration with Drs Gustavo Schujman and Diego de Mendoza (IBR- Rosario, Argentina) resulted in a joint publication (Trajtenberg et al., 2014, *FEBS J*), disclosing the structural bases of resistance to the antibiotic cerulenin in Gram+ bacteria, based on crystal structures of one of its target enzymes, the fatty acid condensing FabF from *Bacillus subtilis*.

iii. A collaboration with Drs Lucia Piacenza and Rafael Radi (Dept Bioquimica, UdelaR-Montevideo, Uruguay) resulted in a recent joint publication (Martinez et al., 2014, *J Biol Chem*), contributing with structural data on the differential inactivation of the mitochondrial Fe-superoxide dismutase from *Trypanosoma cruzi*, by the action of peroxynitrite.

Technological Facility

The Protein Crystallography Facility (PXF) is up and running, with a sustained number of users and trainees, from Argentina and Uruguay.
The web page (www.pasteur.edu.uy/pxf) is kept up to date, and for the last 8 years now, our facility has become fully operational to receive and process all the users' requests (mainly from IPMontevideo, from the Uruguayan community and from Argentina). The web page informs on the detailed specifics of the available equipment and ways of using the platform.

Experimental approaches currently available for users

1. Protein crystallization screenings (manual and robotic [Honeybee963® 96-well robot)
2. Follow-up and optimization of initial crystallization hits (manually and robot-assisted with an Alchemist® instrument)
3. X ray Diffraction – Testing & Crystal Characterization
4. X ray Diffraction – single crystal data collection
5. Crystal Structure Determination & Refinement

Progress 2014

1. Thirteen new structures have been determined: 9 released in the Protein Data Bank: 4LS5, 4LS6, 4LS7, 4LS8, 4Q7E, 4LDZ, 4LE0, 4LE1, 4LE2; and 4 on hold for publication: 4PH0, 4PH1, 4PH2 and 4PH3.

2. Our Unit has hosted three (3) trainees (students and postdoctoral fellows) in 2014: Jun'14 – Jul '14, Julieta Covelli (Universidad Nacional de La Plata, Argentina); Jun'14 –

Aug'14, Mathieu Cayla (Institut Pasteur, Paris, France); Sep'14 – Oct '14, Vinicius Rocha (Fiocruz, Salvador de Bahía, Brazil).

Publications (2014)

1. **Trajtenberg F**, Albanesi D, Ruétalo N, Botti H, **Mechaly AE**, Nieves M, Aguilar PS, Cybulski L, **Larrieux N**, Mendoza D, **Buschiazzo A.** Allosteric activation of bacterial response regulators: the role of the cognate histidine kinase beyond phosphorylation. (2014) *mBio* **5**:e02105-14.
2. **Morero NR**, Botti H, Nitta KR, Carrión F, Obal G, Picardeau M, **Buschiazzo A.** HemR is an OmpR/PhoB-like response regulator from *Leptospira*, which simultaneously effects transcriptional activation and repression of key haem metabolism genes. (2014) *Mol Microbiol.* **94**:340-352.
3. **Trajtenberg F**, Altabe S, **Larrieux N**, Ficarra F, de Mendoza D, **Buschiazzo A,** Schujman GE. Structural insights into bacterial resistance to cerulenin. (2014) *FEBS J.* **281**:2324-2338.
4. Martinez A, Peluffo G, Petruk AA, Hugo M, Piñeyro D, Demicheli V, Moreno DM, Lima A, Batthyány C, Durán R, Robello C, Martí MA, **Larrieux N**, **Buschiazzo A**, Trujillo M, Radi R, Piacenza L. Structural and molecular basis of the peroxynitrite-mediated nitration and inactivation of *Trypanosoma cruzi* iron-superoxide dismutases (Fe-SODs) A and B: disparate susceptibilities due to the repair of Tyr35 radical by Cys83 in Fe-SODB through intramolecular electron transfer. (2014) *J Biol Chem.* **289**:12760-12778.

Grants

1. ***"Typing and diagnosis of Leptospira spp. using molecular approaches: towards the design of recombinant vaccines"***

Uruguayan National Agency for Research and Innovation ANII, Fondo Sectorial Innovagro #FSA_1_2013_1_12557 (Uruguay)
2014-2017
Role: Principal Investigator
Partners: Vet Alejandra Suanes (Min of Agricultures, DILAVE) and Dr M Picardeau (Biology of Spirochetes Unit, IPasteur)

2. ***"Creation and characterization of a bank of Leptospira spp. strains isolated from bovine cases of leptospirosis in Uruguay"***

Uruguayan National Agency for Research and Innovation ANII, Program Alianzas # ALI_1_2014_1_4982 (Uruguay)
Granted 2015-2018
Role: Principal Investigator
Partners: Prof F Schelotto (Medical School, Univ de la Republica, Uruguay), Vet Alejandra Suanes (Min of Agricultures, DILAVE) and Vet F Riet (Uruguayan National Agency for Research in Agriculture INIA).

3. ***"Evaluation of Leishmania DYRK family of kinases as molecular targets for the development of antileishmanial drugs"***

Institut Pasteur (Paris), Actions Concertées Inter-Pasteuriennes ACIP #A13-2013 (France)
2013-2015
Role: Associate Researcher
Principal Investigator: Dr Despina Smirlis (Helenic Pasteur Institute)

Partners: A Buschiazzo (IPMontevideo, Uruguay), Dr Milena Soares (Fiocruz, Brazil) and Dr G Spaeth (IPasteur, France)

4. ***Cell signaling in bacterial pathogenesis: iron metabolism regulation in Leptospira as a working model***

Institut Pasteur, Programmes Transversaux de Recherche PTR #407 (France)
2011-2014 (including six months funded extension)
Role: Principal Investigator
Partners: Mathieu Picardeau (Biology of Spirochetes Unit, IP) and Pablo Aguilar (Lab of Cellular Membranes, IPMontevideo).

5. ***Consolidation of the "Centro de Biologia Estructural del Mercosur - CeBEM"***

Ministry of Education and Culture (MEC), Uruguay. Direccion de Innovación, Ciencia y Tecnología para el Desarrollo (DICYT)
2014-2015
Role: general coordinator; co-managed by A Buschiazzo and R Radi (heads of the 2 Uruguayan CeBEM nodes).

Other activities

WORKSHOPS, COURSES, TRAINING

1. Co-organization of the joint CeBEM-CCP4-RIIP Macromolecular Crystallography School - "From data processing to structure refinement and beyond". Venue: Instituto de Fisica de Sao Carlos, Univ de Sao Paulo, Sao Carlos, Brazil.

April 8-16, 2014.
Funded by CeBEM, CCP4, IUCr, FAPESP
This third edition is in continuity with the previous Workshops organized at IPMontevideo in 2010 and 2013. Twenty students, fourteen invited speakers and tutors (http://www.ifsc.usp.br/mx2014/).

2. Hands-on training Workshop: "Isolation of *Leptospira spp*. strains from field cases of bovine leptospirosis". Venue: Institut Pasteur de Montevideo; INIA (estacion La Estanzuela); Universidad de la Republica, Instituto de Higiene, Fac de Medicina; and DILAVE (Min de Ganaderia, Agricutlura y Pesca).
October 13- 17, 2014.
Funded by IPMontevideo and INIA.
Invited lecturers and practical tutors: Julie Collins-Emerson and Peter Wilson (Massey University, New Zealand). Participation of 20 attendees from IPMontevideo, Universidad de la Republica Medical School, Diagnostics Laboratory of the Ministry of Livestock and Agriculture, and Institute of Agronomic Research (INIA).

3. Workshop: "Modern Approaches in Drug Discovery for Neglected Infectious Diseases" November 3 - 8, 2014. Venue: Institut Pasteur de Montevideo.
Funded by UNU-Biolac, CeBEM and IPMontevideo.
Invited speakers included top-level scientists in the field: Profs Wim Hol (University of Washington, Seattle, USA), Paul Michels (University of Edinburgh, UK), Hugo Cerecetto (Universidad de la Republica, Montevideo), Celerino Abad-Zapatero (University of Illinois, Chicago, USA). Also lectured and carried hands-on practical sessions from IPMontevideo, Drs

Marcelo Comini, Sergio Pantano and Alejandro Buschiazzo. Twenty-five students were selected, coming from different South American countries.

4. Incorporation of three new members to the laboratory in 2014:

- Leticia Zarantonelli (Associated Research Scientist; in double appointment also working as Lab Manager of the newly created laboratory of the Joint Unit on Animal Health IPMontevideo/INIA)
- Frank Lehmann (staff Technician)
- Cecilia Nieves (MSc student)

NETWORKING, SCIENTIFIC MEETINGS, PRIZES AND HONORS

1. Sustained contribution of our group to the Center for Structural Biology of the Mercosur (Centro de Biologia Estructural del Mercosur, CeBEM) *www.cebem.org.ar* with nodes in Argentina, Brazil, Paraguay and Uruguay. A grant from the Ministerio de Educacion y Cultura (Uruguay) was obtained, allowing us to contribute for the first time in 4 years to the regional funds sustaining the network's training and exchange activities, extremely relevant to disseminate and consolidate Structural Biology in South America.

2. A Buschiazzo (Head of the Unit) received the prize François Jacob, Institut Pasteur, January 2014.

He has also contributed as:

- Reviewer of >5 peer-reviewed journals, including J Biol Chem, J Synchr Rad, Biochemistry, J Med Chem, Mol Microbiol, PLoS ONE, PNAS, Protein Sci, among others.

- Reviewer of scientific projects for FAPESP (CEPID program, Brazil), FONCYT (Argentina agency).

- Associate Editor of the journal PLoS Neglected Tropical Diseases.

- Member of the Advisory Board of the Software Consortium for Structural Biology (SBGrid, Harvard Medical School, https://sbgrid.org/about/advisory/)

- Chair of the National Committee of Crystallography (RUCr, Uruguay), member of the International Union of Crystallography (IUCr) http://www.iucr.org/iucr/ab.html/adhering-bodies/uruguay

- Organizer and/or attendee to several scientific meetings and activities (2014):

- January 2014 - Gordon Research Conference «Sensory Transduction in Microorganism» (Ventura CA, USA). Invited speaker
- January 2014 - Gordon Research Conference « Biology of Spirochetes » (Ventura CA, USA). Presentation of two posters.
- January 2014 - Seminar within the program «Red Top seminar series», Department of Cell Biology at Harvard Medical School (Boston, USA). Invited speaker (invited by Prof Tom Rapoport)
- May 2014 - 43rd Annual Meeting of the Brazilian Biochemistry Society SBBq (Foz do Iguaçu, Brazil). Invited speaker
- July 2014 - X Argentinian Congress of General Microbiology SAMIGE (Mar del Plata, Argentina) Invited speaker

- July 2014 - International Union of Microbiological Societies (Montreal, Canada). Invited speaker
- August 2014 - XXIII Congress and General Assembly of the International Union of Crystallography (Montreal, Canada). Invited speaker
- August 2014 - Chair of the microsymposium «Molecular Mechanisms of Therapeutics and Resistance», XXIII Congress IUCr (Montreal, Canada)
- September 2014 - International Year of Crystallography Latin American Summit on Biological Crystallography (Campinas, Brazil). Invited speaker
- November 2014 – Scientific seminar invited by Prof Alejandro Vila, Instituto de Biologia Molecular y Celular de Rosario IBR (Rosario, Argentina). Invited speaker

3. Members of the Unit attended several international scientific meetings and courses:

  - FTrajtenberg: Gordon Research Conference: Sensory Transduction in Microorganisms. January 12-17, 2014 (Ventura, USA); and Gordon Research Conference: Spirochetes, Biology of. January 19-24, 2014 (Ventura, USA);
  - AEMechaly: EMBO Global Exchange Lecture Course: Structural and biophysical methods for biological macromolecules in solution. January 19 – 26, 2014 (São Paulo, Brazil);
  - NLarrieux: Rapid Data Collection and Structure Solving at the NSLS:A Practical Course in Macromolecular X-Ray Diffraction Measurement. April 27-May 2, 2014 (Brookhaven, USA);
  - FTrajtenberg and AEMechaly: “Latin American Summit Meeting on Biological Crystallography and Complementary Methods”, September 22-24, 2014 (Campinas-SP, Brazil)

Bioinformatic Unit

Head: Hugo Naya, PhD

Members: ***Martín Graña*** (PhD, AI)
Natalia Rego (TA, MSc student in Zoology)
Lucía Spangenberg (PhD student in biology)
María Inés Fariello (PhD student in biology)
Tamara Fernandez (MSc in biology, Electrical Engineering student)
Sebastián Valenzuela (MSc student in bioinformatics)
Gregorio Iraola (MSc student in bioinformatics)
Daniela Megrian (undergrad student in biochemistry)
Gabriel Martinez (undergrad student in biology)

Research

In the past 20 years, the development of new technologies has led to amazing discoveries in biology. In particular, nano-technologies, automatization and computer science allowed a series of High-Throughput analysis in molecular and cell biology that completely changed the existent paradigm. However, these new instruments also changed unexpectedly the landscape of research conception. The promise of hypothesis-free data has conducted, in several cases, to careless experimental design that precluded full exploitation of results, increasing the experimental turnover and the storage of waste in data-repositories. Technology evolves extremely fast, but analytical methods aren't automatized enough yet, leading to the well-known effect of “Next-Generation gap”. The gap is in expansion now (with the 2nd generation sequencing) and will be enormous with 3rd generation technologies. In fact, analysis teams simply can't analyze exhaustively each dataset before a new dataset arrives, just scratching the surface and sending to the warehouse (or even garbage) tons of data.

In this context, any methodological effort towards better usage of data should be viewed as benefiting the scientific community. Our research, although diverse, is united by this underlying goal and combines the methodological strengths of bioinformatics, statistics, evolutionary genomics and quantitative genetics.

We recently proposed a method that identifies associations between amino acid changes in potentially significant sites in an alignment (taking into account several amino acid properties) with phenotypic data (Spangenberg et al., 2011), through the phylogenetic mixed model. The latter accounts for the dependency of the observations (organisms). It is known from previous studies that the pathogenic aspect of many organisms may be associated with a single or just few changes in amino acids which have a strong structural and/or functional impact on the protein. Discovering these sites is a big step towards understanding pathogenicity. Our method was able to discover such sites in proteins (RpoS) associated to the pathogenic character of a group of bacteria, highlighting several sites with significant differences in biological relevant regions. In addition, we developed a freely available R package named “bcool” (http://cran.r-project.org/web/packages/bcool/index.html). In the near future, we think to apply this strategy to search for differences in biofilm related genes.
We also assessed the question of how bacteria cause pathogenicity in humans from other perspective. Our motivation was try to give integrative information about general genome-coded signatures that explains pathogenicity for all bacterial pathogens, and not restricted to particular taxa. In this case, we explained pathogenicity based on the hypothesis that it is caused by the presence of a reduced set of virulence-related genes. To do this, we explored the presence/absence patterns of virulence genes in all available genomes of pathogenic and non-pathogenic strains. Then, this information was used to build a Support Vector Machine model that, once trained, is capable of predicting if a new sequenced genome is a human pathogen or not. This model has an average accuracy of 95%, and to the best of our

knowledge, is the statistical model with this purpose that achieves the highest accuracy reported so far. Moreover, our method can classify bacterial genomes independently of their taxonomic context, in contrast with other similar approaches that only take into account a certain part of bacterial diversity, being useful only to classify specific taxa. Our statistical learning approach is grounded on the biological meaning of the selected genes and supporting the fact that bacterial pathogenicity can be explained by the presence or absence of a set of specific genes that code for virulence determinants. Based on this, we developed "BacFier", a freely available software that may be useful for practical purposes. Beyond the implementation of our model in a program, capable to accurately classify bacteria in human pathogens or non-pathogens, we determined and discussed the biological significance of the core set of genes that mostly explains the pathogenic phenotype in bacteria. Finally, we have shown which functional categories of virulence genes (i.e: toxins, motility proteins, etc.) were likely pathogenicity signatures within each taxonomic division (i.e: Actinobacteria, Gammaproteobacteria, Firmicutes, etc.), which seems to be a completely new kind of information and could lead to important evolutionary conclusions. Nowadays, we are working in enhancing model sensitivity and exploring the possibility of developing a multiclass classifier, that could predict pathogenicity in other hosts besides human, like cattle, plant or fish.

As part of our general interest in bacterial pathogenicity, we are involved in a more specific problem; the study of biofilms formation determinants in *Leptospira*. This genus includes animal and re-emerging human pathogens, as well as non-pathogenic strains. Despite its importance for human health and animal production, genetic features that determine pathogenic phenotypes in *Leptospira* proved to be elusive. Recently, biofilms formation capability has been suggested as a key factor in pathogenesis of leptospirosis but, as mentioned above, there is a lack of knowledge regarding its genetic basis. In this ground, we are implementing comparative genomics analysis to find orthologous genes with functions associated to biofilms formation. Moreover, in the near future we plan to perform transcriptome analysis that could give data regarding expression patterns of genes involved in biofilms formation, providing with a new kind of information that could be useful to understand the pathogenesis mechanisms of these bacteria.

Services

1. NGS and microarrays data analysis.
2. Sequence alignment and phylogenetic inference software.
3. Sequence analysis software.
4. 3D molecular modeling software.
5. Database hosting and querying.
6. Tools for complex systems analysis.
7. Basic biostatistics and use of specific software advice.
8. Software development.

Publications 2014

1. Garcia-Silva MR, das Neves RF, Cabrera-Cabrera F, Sanguinetti J, Medeiros LC, Robello C., **Naya H, Fernandez-Calero T**, Souto-Padron T, de Souza W, Cayota A. Extracellular vesicles shed by Trypanosoma cruzi are linked to small RNA pathways, life cycle regulation, and susceptibility to infection of mammalian cells. Parasitol Res. **2014**;113:285-304.

Grants

1. "Investigação dos Mecanismos Genéticos e Moleculares em Biofilmes de Leptospira". Funded by CAPES – Brazil 2012/2015. A Schnadelbach/P Ristow. Special Visiting Professor H Naya. Granted R$ 100000.
2. "Análisis transcripcional en Leptospiras formadoras de biofilms". Funded by ANII 2013/2015. H Naya. Granted U$S 20000.

Other activities

We are currently involved in several teaching activities, mainly on bioinformatics-related topics. The recently created MSc in Bioinformatics is currently highly demanding, courses design and impartment being in charge of the Faculty of Sciences, School of Engineering, and our group at Pasteur. We also have punctual participations in several PEDECIBA courses, including topics in bioinformatics and quantitative genetics.

Human resources are clearly needed in this somewhat new research domain; this calls for our effort in such teaching activities, as well as for maximizing the number of graduate and undergraduate students in our lab (eight persons at the moment).

Molecular Biology Unit &
Laboratory of Host-Pathogen Interactions

Head: Carlos Robello, PhD

Members: ***Adriana Parodi-Talice*** (PhD - Associated Researcher, Facultad de Ciencias)
Dolores Piñeyro (PhD - Associated Researcher, Facultad de Medicina)
Ma. Laura Chiribao (PhD Student, Facultad de Medicina)
Paula Faral (PhD Student)
Gabriela Libisch (PhD Student)
Gonzalo Greif (PhD Student)
Cecilia Portela (Tecnician, Facultad de Ciencias)
Florencia Díaz (Master student)
Fernanda Matto (Master student)
Moira Laserre (Master student)
Luisa Berná (Postdoctoral Researcher- INNOVA II)

Research

The Unit of Molecular Biology research is focused on human and animal pathogens, in particular the protozoan parasites *T. cruzi*, *T. vivax* and *Leishmania*, and the prokariote *Mycobacterium*, with emphasis in genomics and functional genomics of those pathogens, and host-pathogen interactions.

Research Lines

A. Functional Genomics of Host-Parasite Interaction

Trypanosoma cruzi, the causative agent of Chagas disease, has the peculiarity, when compared with other intracellular parasites, that it is able to invade almost any type of cell. This property makes Chagas a complex parasitic disease in terms of profilaxis and therapeutics. The identification of key host cellular factors that play a role in the *T. cruzi* invasion, are important for understanding of disease pathogenesis. In Chagas disease most of the focus was on the response of macrophages and cardiomyocytes, since they are responsible for host defenses and cardiac lesions respectively. We studied the early response to infection of *T. cruzi* in human epithelial cells, which constitute the first barrier for establishment of infection. These studies identified up to 1700 significantly altered genes regulated by the immediate infection. The global analysis indicates that cells are literally reprogrammed by *T. cruzi*, which affects cellular stress responses (neutrophil chemotaxis, DNA damage response), a great number of transcription factors (including the majority of NFκB family members) and host metabolism (cholesterol, fatty acids and phospholipids). These results raise the possibility that early host cell reprogramming is exploited by the parasite to establishment of the initial infection and posterior systemic dissemination.

B. Benznidazole Biotransformation and Multiple Targets in Trypanosoma cruzi Revealed by Metabolomics

The first line treatment for Chagas disease involves administration of benznidazole (Bzn). Bzn is a 2-nitroimidazole pro-drug which requires nitroreduction to become active, although its mode of action is not fully understood. By using a non-targeted MS-based metabolomics approach we studied the metabolic response of *T. cruzi* to Bzn. Parasites treated with Bzn were minimally altered compared to untreated trypanosomes, although the redox active thiols trypanothione, homotrypanothione and cysteine were significantly

diminished in abundance post-treatment. In addition, multiple Bzn-derived metabolites were detected after treatment. These metabolites included reduction products, fragments and covalent adducts of reduced Bzn linked to each of the major low molecular weight thiols: trypanothione, glutathione, γ-glutamylcysteine, glutathionylspermidine, cysteine and ovothiol A. Bzn products known to be generated in vitro by the unusual trypanosomal nitroreductase, TcNTRI, were found within the parasites, but low molecular weight adducts of glyoxal, a proposed toxic end-product of NTRI Bzn metabolism, were not detected. Our data is indicative of a major role of the thiol binding capacity of Bzn reduction products in the mechanism of Bzn toxicity against *T. cruzi*.

C. Tuberculosis: Genomics and molecular typing

The incidence of tuberculosis (TB) is increasing in high-risk populations in Uruguay, possibly owing to emerging resistance. Mycobacterial interspersed repetitive units (MIRU) genotyping and katG sequence analysis of isoniazid (INH) resistance-associated mutations were performed in 45 INH-resistant *Mycobacterium tuberculosis* isolates in Uruguayan patients. The genotype distribution among INH-resistant isolates shares features of that of neighbouring countries, with a predominance of Latin American and Mediterranean, T and Haarlem genotypes, although the S genotype was particularly frequent among our isolates. Forty-four per cent of INH-resistant strains harboured the S315T mutation in katG; we found novel katG mutations (W321X, G269T, P232R and G221Wfs1) that could explain INH resistance. More recently, we reported an unusual tuberculosis (TB) outbreak centered on a professional basketball team in Montevideo. The strain, named MtURU-001, was fully sequenced: MtURU-001 has a circular chromosome of 4,378,296 bp, with an average G+C content of 65%, including 4,314 protein-encoding genes, 1 rRNA operon, and 45 tRNA genes. In comparison with **M. tuberculosis** H37Rv, 4,096 orthologous groups were defined with OrthoMCL and 1,016 polymorphisms were identified using the Burrows-Wheeler Aligner (BWA) and GATK. A subset of 849 polymorphisms (802 single-nucleotide polymorphisms [SNPs] and 47 indels) were inside coding sequences, and 480 affect protein sequences, especially 24 that introduced stop codons disrupting several hypothetical proteins, one transcriptional regulator, 2 genes for the haloacid dehalogenase (HAD) superfamily, and 3 involved in lipid metabolism. Further comparative genomics across this genome may provide genotype-phenotype associations that might explain the rapid progression of this unusual outbreak

D. Trypanosoma vivax transcriptome

Trypanosoma vivax is the earliest branching African trypanosome. This crucial phylogenetic position makes *T. vivax* a fascinating model to tackle fundamental questions concerning the origin and evolution of several features that characterize African trypanosomes, such as the Variant Surface Glycoproteins (VSGs) upon which antibody clearing and antigenic variation are based. Other features like gene content and trans-splicing patterns are worth analyzing in this species for comparative purposes. We present a RNA-seq analysis of the bloodstream stage of *T. vivax* from data obtained using two complementary sequencing technologies (454 Titanium and Illumina). Assembly of 454 reads yielded 13385 contigs corresponding to proteins coding genes (7800 of which were identified). These sequences, their annotation and other features are available through an online database presented herein. Among these sequences, about 1000 were found to be species specific and 50 exclusive of the *T. vivax* strain analyzed here. Expression patterns and levels were

determined for VSGs and the remaining genes. Interestingly, VSG expression level, although being high, is considerably lower than in Trypanosoma brucei. Indeed, the comparison of surface protein composition between both African trypanosomes (as inferred from RNA-seq data), shows that they are substantially different, being VSG absolutely predominant in *T. brucei*, while in *T. vivax* it represents only about 55%. This raises the question concerning the protective role of VSGs in T. vivax, hence their ancestral role in immune evasion.It was also found that around 600 genes have their unique (or main) trans-splice site very close (sometimes immediately before) the start codon. Gene Ontology analysis shows that this group is enriched in proteins related to the translation machinery (e.g. ribosomal proteins, elongation factors). This is the first RNA-seq data study in trypanosomes outside the model species *T. brucei*, hence it provides the possibility to conduct comparisons that allow drawing evolutionary and functional inferences. This analysis also provides several insights on the expression patterns and levels of protein coding sequences (such as VSG gene expression), trans-splicing, codon patterns and regulatory mechanisms. An online *T. vivax* RNA-seq database described herein could be a useful tool for parasitologists working with trypanosomes.

Core Facilities - Services

1. DNA sequencing (Sanger methodology)
2. Real Time PCR
3. Microarrays
4. Bioanalyzer
5. Illumina Genome Analyzer
6. Illumina MiSeq

Publications 2014

1- **Trochine A**, Alvarez G, **Corre S**, **Faral-Tello P**, Durán R, Batthyany CI, Cerecetto H, González M, **Robello C.** Trypanosoma cruzi chemical proteomics using immobilized benznidazole (2014) Experimental Parasitology, 140 (1), pp. 33-38. – *IF: 1.859*

2- **Greif G**, **Iraola G, Berná L**, Coitinho C, Rivas C, Naya H, **Robello C.** 2014. Complete genome sequence of Mycobacterium tuberculosis strain MtURU-001, isolated from a rapidly progressing outbreak in Uruguay. Genome Announcements. – *IF:* --

3- **Chiribao ML, Libisch G, Parodi-Talice A, Robello C.** Early trypanosoma cruzi infection reprograms human epithelial cells (2014) BioMed Research International, 2014, art. no. 439501. – *IF:* --

4- **Faral-Tello P,** Liang M, Mahler G, Wipf P, **Robello C**. Imidazolium compounds are active against all stages of Trypanosoma cruzi (2014) International Journal of Antimicrobial Agents, 43 (3), pp. 262-268. – *IF: 4.259*

5- **Trochine A**, Creek DJ, **Faral-Tello P**, Barrett MP, **Robello C.** Benznidazole Biotransformation and Multiple Targets in Trypanosoma cruzi Revealed by Metabolomics (2014) PLoS Neglected Tropical Diseases, 8 (5), art. no. e2844. – *IF: 4.489*

6- **Libisch MG**, Casás M, **Chiribao M**, Moreno P, Cayota A, Osinaga E, Oppezzo P, **Robello C**. GALNT11 as a new molecular marker in chronic lymphocytic leukemia. Gene. 2014 Jan 1;533(1):270-9. doi: 10.1016/j.gene.2013.09.052. Epub 2013 Sep 27. PubMed PMID: 24076351. – *IF: 2.082*

7- **Berná L, Iraola G, Greif G**, Coitinho C, Rivas C, Naya H**, Robello C**. 2014. Whole-Genome Sequencing of an Isoniazid-Resistant Clinical Isolate of Mycobacterium tuberculosis Strain MtURU-002 from Uruguay. Genome Announcements. – *IF:* --

8- **Chiribao ML, Libisch G, Parodi-Talice A, Robello C.** Early trypanosoma cruzi infection reprograms human epithelial cells (2014) BioMed Research International, 2014, art. no. 439501. – *IF:* --

9- Martinez A, Peluffo G, Petruk A A, Hugo M, **Piñeyro D**, Demicheli V, Moreno DM, Lima A, Batthyány C, Durán R, **Robello C**, Martí MA, Larrieux N, Buschiazzo A, Trujillo M, Radi R, Piacenza L. Structural and molecular basis of the peroxynitrite-mediated nitration and inactivation of trypanosoma cruzi iron-superoxide dismutases (fe-sods) a and b : Disparate susceptibilities due to the repair of tyr35 radical by cys83 in fe-sodb through intramolecular electron transfer (2014) Journal of Biological Chemistry, 289 (18), pp. 12760-12778. – *IF: 4.6*

10- Palacios F, Abreu C, Prieto D, Morande P, Ruiz S, Fernández-Calero T, Naya H, **Libisch G, Robello C**, Landoni AI, Gabus R, Dighiero G, Oppezzo P. Activation of the PI3K/AKT pathway by microRNA-22 results in CLL B-cell proliferation (2014) Leukemia. Article in Press. – *IF: 9.379*

11- Márquez VE, Arias DG, **Chiribao ML, Faral-Tello P, Robello C**, Iglesias AA, Guerrero SA. Redox metabolism in Trypanosoma cruzi. Biochemical characterization of dithiol glutaredoxin dependent cellular pathways (2014) Biochimie. – *IF: 3.123*

12- Garcia-Silva MR, das Neves RF, Cabrera-Cabrera F, Sanguinetti J, Medeiros LC, **Robello C**, Naya H, Fernandez-Calero T, Souto-Padron T, de Souza W, Cayota A. Extracellular vesicles shed by Trypanosoma cruzi are linked to small RNA pathways, life cycle regulation, and susceptibility to infection of mammalian cells. Parasitol Res. 2014 Jan;113(1):285-304. doi: 10.1007/s00436-013-3655-1. Epub 2013 Nov 17. PubMed PMID: 24241124. – *IF: 2.327*

13- Coitinho C, **Greif G, Robello C,** Laserra P, Willery E, Supply P. Rapidly progressing tuberculosis outbreak in a very low risk group. Eur Respir J. 2014 Mar;43(3):903-6. doi: 10.1183/09031936.00150413. Epub 2013 Oct 10. PubMed PMID: 24114961. – *IF: 7.125*

Grants

1. Fondo María Viñas – Identificadores de factores del hospedero necesarios para la invasión de *Trypanosoma cruzi* – Paula Faral – (2 years - 2013-2015) – ANII
2. Proyecto Transversal – Chagas disease: secreted microvesicles as predictors of persistence and pathogenicity – Carlos Robello – (1 year - 2013-2014) – IP Montevideo
3. Fondo Clemente Estable – Proteínas con motivos repetidos ricos en leucina de *Trypanosoma cruzi* y su rol en la virulencia: un abordaje bioinformático, funcional y proteómico" – Adriana Parodi – ANII (2 years – 2013-2015)

Other activities

TRAINING COURSES:

"Functional Genomics and its applications in biomedicine: Host-Pathogen interaction" RIIP/UNU-Biolac Course. November 2014

Cell Biology Unit

Head: Mariela Bollati, PhD

Members: ***Soledad Astrada, MSc*** (PhD student, Staff TA)
María Belén Harreguy (Undergraduate Intern)
Giuliana Mastropietro (Undergraduate Intern)
Romina Pagotto, PhD (Postdoctoral fellow)
Karen Perelmuter, MSc (Staff TA)
Inés Tiscornia, MSc (Staff TA)
Sabina Victoria, MSc (Staff TA)

GOALS

The mission of the Cell Biology Unit (CBU) is to:

- perform and support cell culture (CC) technology and flow cytometry (FC) based research
- provide researchers with training, scientific assistance and access to CT and FC related technologies
- contribute to local and regional training and education programs
- improve available CC and FC protocols and optimize new methodologies for biomedical research

Our specific goals are:

- to provide access and support for CC and FC technologies to local and regional scientific community
- to promote biomedical and biotechnological research projects with other national and international research groups
- to train students and researchers in FC and CC related technologies.

Research

The CBU is involved in two main areas of research:

CELL CULTURE TECHNOLOGY:

During the last years, our group has generated a variety of reporter cell lines with broader applications (NF- kB, type I IFN, redox biosensors, among others). These stable cell lines are being widely used to search and characterize substances that interfere with the type I IFN signaling pathways (Burgi *et al*, 2012 and one manuscript in preparation), for the improvement of metabolism / productivity of cells with biotechnological interest (redox biosensors in collaboration with Dr Comini from the Redox Biology of Trypanosomes Lab, IP Montevideo and we have a manuscript in preparation), or for *in vitro* models of inflammation (NF- kB, Tiscornia *et al*, 2012; Mastropietro *et al*, 2015).

ENVIRONMENTAL TOXICOLOGY:

A wide variety of anthropogenic substances in the environment, known as endocrine disruptors (EDs), are able to alter the homeostasis of the endocrine system of organisms. In the field of endocrine disruption, the working hypothesis is that the increment of certain reproductive disorders are caused, at least in part, by an increased exposure to substances

classified as EDs that are present in the environment. In this context, we focus on the design and development of *in vitro* and *in vivo* models for toxicological studies of EDs. For the *in vitro* approach, we are on the process to obtain a dual reporter cell line, to assess in a single assay the estrogenic or androgenic activity of a putative ED. For the *in vivo* studies, we proposed and validated the Oct4-GFP transgenic mouse, which mimics the endogenous expression pattern of Oct4, as a mammalian model to study the effects of EDs on the development of male germ cells. We introduced the transgenic Oct4/GFP mouse together with flow cytometry as a suitable tool to evaluate changes in male germ cells development and to identify early life exposures to EDCs (manuscript under revision and other in preparation). To accomplished the Project we have the financial support from ANII (2013-2015), one post-doctoral fellow ANII (R. Pagotto), and one initiation fellow ANII (MB. Harreguy). In addition, we are actively collaborating with Dr. H. Rodríguez (Instituto de Salud y Ambiente del Litoral, CONICET-UNL, Santa Fe, Argentina).

COLLABORATIVE PROJECT:

Since 2011 we are collaborating with MG. Vallespi, PhD, from the Pharmaceuticals Division, Center for Genetic Engineering and Biotechnology (CIGB), Habana, Cuba in the project entitled "CIGB -552: novel peptide with antitumor properties useful for cancer treatment". From this collaboration, two articles were published (Fernández Massó *et al*, 2013, J Amino Acids and Vallespí et al., 2014, J Pept Sc), one manuscript is under revision and there is one in preparation. In addition a student (S Astrada) from the Cell Biology Unit is performing her PhD thesis under the supervision of Bollati M and Vallespi M.

The work in association with Susana Etcheverry, PhD (Cequinor, UNLP, Argentina) have yielded three manuscripts and one is in preparation (Leon et al, 2013, Dalton Trans; Leon *et al*, 2014; Chem Biol Interact. and Leon *et al*, 2014, J Biol Inorg Chem).

References:

- Bürgi M, Prieto C, Etcheverrigaray M, Kratje R, Oggero M, Bollati-Fogolín M*. WISH cell line: From the antiviral system to a novel reporter gene assay to test the potency of human IFN-α and IFN-β. J Immunol Methods. 2012, 381(1-2):70-4.
- Fernández Massó JR, Oliva Argüelles B, Tejeda Y, Astrada S, Garay H, Reyes O, Delgado-Roche L, Bollati-Fogolín M, Vallespí MG. The Antitumor Peptide CIGB-552 Increases COMMD1 and Inhibits Growth of Human Lung Cancer Cells. J Amino Acids. 2013; 251398.
- Leon IE, Di Virgilio AL, Porro V, Muglia CI, Naso LG, Williams PAM, Bollati-Fogolín M and Etcheverry SB. Antitumor properties of a vanadyl(IV) complex with the flavonoid chrysin [VO(chrysin)2EtOH]2 in a human osteosarcoma model: role of the oxidative stress and apoptosis. Dalton Trans. 2013; 42(33):11868-80.
- Leon IE, Porro V, Di Virgilio AL, Naso LG, Williams PAM, Bollati-Fogolín M and Etcheverry SB. Antiproliferative and apoptosis-inducing activity of an oxidovanadium(IV) complex with the flavonoid silibinin against osteosarcoma cells. J Biol Inorg Chem. 2014, 19 (1), pp. 59-74.
- León IE, Porro V, Astrada S, Egusquiza MG, Cabello CI, Bollati-Fogolin M, Etcheverry SB. Polyoxometalates as antitumor agents: Bioactivity of a new polyoxometalate with copper on a human osteosarcoma model. Chem Biol Interact. (2014), 222C:87-96.
- Mastropietro G, Tiscornia I, Perelmuter K, Astrada S, Bollati-Fogolín M*. HT-29 and Caco-2 Reporter Cell Lines for Functional Studies of Nuclear Factor Kappa B Activation. Mediators of Inflammation, 2015, Article ID 860534, *in press*.
- Tiscornia I., Sánchez-Martins V., Hernández A., Bollati-Fogolín M*. Human monocyte-derived dendritic cells from leukoreduction system chambers after plateletpheresis are functional in an in vitro co-culture assay with intestinal epithelial cells. J Immunol Methods. 2012, 384(1-2):164-70.
- Vallespí MG, Pimentel G, Cabrales-Rico A, Garza J, Oliva B, Mendoza O, Gomez Y, Basaco T, Sánchez I, Calderón C, Rodriguez JC, Markelova MR, Fichtner I, Astrada S, Bollati-Fogolín M, Garay HE, Reyes O. Antitumor efficacy, pharmacokinetic and biodistribution studies of the anticancer peptide CIGB-552 in mouse models. J Pept Sc (2014), 20(11):850-9.

* Corresponding author

Core Facilities - Services

The CBU in 2007 has installed cell culture facilities and the flow cytometry lab (equipped with a cell sorter MoFlo and an analytical cytometer CyAn ADP, both from Beckman Coulter). By the end of 2014 we have completed the CBU platform with the arrival of a new cell sorter (FACSAria Fusion, BD) which can operate under a Class II Type A2 biosafety cabinet and a small analyser (Accuri C6, BD). Both instruments together complement each other and expand the quality and type of procedures (analytical and preparative) we can perform and offer to local and regional scientific community.

The routine services that we offer are:

1. Culture of different cell lines, cell banking.
2. Detection of mycoplasma contamination in cell culture by PCR.
3. Cell-based assays.
4. Generation of recombinant stable cell lines.
5. Flow cytometry analysis: DNA content and cell cycle analysis, fluorescent proteins detection, apoptosis, and multicolor analysis.
6. Sorting of heterogeneous cell populations into homogeneous populations: sterile sorting, single cell deposition, 4 way sorting.

Publications 2014

1. León IE, Porro V, **Astrada S**, Egusquiza MG, Cabello CI, **Bollati-Fogolin M,** Etcheverry SB. Polyoxometalates as antitumor agents: Bioactivity of a new polyoxometalate with copper on a human osteosarcoma model. Chem Biol Interact. (2014), 222C:87-96. – *IF: 2.982*
2. **Pagotto RM**, Pereyra EN, Monzón C, Mondillo C, Pignataro OP. Histamine inhibits adrenocortical cell proliferation but does not affect steroidogenesis. J Endocrinol (2014); 221(1):15-28. – *IF: 3.586.*
3. Vallespí MG, Pimentel G, Cabrales-Rico A, Garza J, Oliva B, Mendoza O, Gomez Y, Basaco T, Sánchez I, Calderón C, Rodriguez JC, Markelova MR, Fichtner I, **Astrada S, Bollati-Fogolín M**, Garay HE, Reyes O. Antitumor efficacy, pharmacokinetic and biodistribution studies of the anticancer peptide CIGB-552 in mouse models. J Pept Sc (2014), 20(11):850-9. – *IF: 1.862*
4. Domínguez MF, Koziol U, Porro V, Costábile A, Estrade S, Tort J, **Bollati-Fogolin M**, Castillo E. A new approach for the characterization of proliferative cells in cestodes (2014) Experimental Parasitology, 138 (1), pp. 25-29. – *IF: 1.859*
5. Leon IE, Porro V, Di Virgilio AL, Naso LG, Williams PAM**, Bollati-Fogolín M**, Etcheverry SB. Antiproliferative and apoptosis-inducing activity of an oxidovanadium(IV) complex with the flavonoid silibinin against osteosarcoma cells (2014) Journal of Biological Inorganic Chemistry, 19 (1), pp. 59-74. – *IF: 3.164*
6. Fernández-Calero T, **Astrada S**, Alberti Á, Horjales S, Arnal JF, Rovira C, **Bollati-Fogolín M**, Flouriot G, Marin M. The transcriptional activities and cellular localization of the human estrogen receptor alpha are affected by the synonymous Ala87 mutation (2014) Journal of Steroid Biochemistry and Molecular Biology, 143, pp. 99-104. – *IF: 4.049*
7. Beedessee G, Ramanjooloo A, **Tiscornia I**, Cresteil T, Raghothama S, Arya D, Rao S, Gowd KH, **Bollati-Fogolin M**, Marie DEP. Evaluation of hexane and ethyl acetate extracts of the sponge Jaspis diastra collected from Mauritius Waters on HeLa cells (2014) Journal of Pharmacy and Pharmacology, 66 (9), pp. 1317-1327. – *IF: 2.161*
8. Ceaglio N, Bollati-Fogolín M, Oggero M, Etcheverrigaray M, Kratje R (2014). Chapter 6.2: High cell density cultivation processes. In Animal Cell Biotechnology, Eds Wagner R and

Hauser H. De Gruyter. Berlín, Alemania (ISBN 3110278863, 9783110278866), pp. 427 – 454.

Grants and fellowships

1. Separador celular de alta velocidad, multiparamétrico y bioseguro para su utilización en biomedicina y biotecnología. PI: M Bollati, ANII (Uruguay), 2014. Granted: USD 480,000.
2. Toxicología ambiental aplicada: evaluación del riesgo por exposición a estrógenos ambientales antropogénicos en un modelo de ratones transgénicos Oct4-GFP. PI: M Bollati, ANII: FMV (Uruguay), 2013 – 2015 (2 years). Granted: USD 20,000.
3. Modulación de la respuesta innata epitelial por levaduras probióticas: determinación de los mecanismos genéticos de levaduras involucrados en esta propiedad. PI: M. Bollati (Uruguay), M. Rumbo (Argentina). Cooperación bilateral Argentina–Uruguay (AR-UR/12/03), 2013 – 2015 (2 years). Granted: USD 5,000
4. Toxicología ambiental aplicada: evaluación del riesgo por exposición a estrógenos ambientales antropogénicos en un modelo murino transgénico Oct4-GFP. PI: M. Crispo, M. Bollati (collaborative partner). Cooperación bilateral Argentina–Uruguay (AR-UR/12/02), 2013 – 2015 (2 years). Granted: USD 5,000
5. Postdoctoral fellowship CONICET-IPMontevideo, R. Pagotto, 2012-March 2014 (2 years)
6. Doctoral fellowship ANII- POS_NAC_2012_1_8523, S. Astrada, 2013-2015 (2 years)
7. Initiation fellowship ANII- INI_X_2012_1_4184, MB. Harreguy, 2013- 2014 (1 year)
8. Postdoctoral fellowship ANII- PD_NAC_2013_1_10903, R. Pagotto, 2014-2015 (2 years).

Vinculation with industry

Since 2007 the CBU has a good liaison with the private sector under different modalities: R & D contract, providing a specific service, or as consultants. During 2014 several agreements were celebrated (Biopolis from Spain, Granar from Paraguay and Biogenesis-Bago from Argentina).

Other activities

PARTICIPATION IN TRAINING COURSES

As organizers:

- ✓ Internacional **Flow cytometry and cell sorting in biotechnology and biomedicine research** course held at the IP Montevideo from April 17th to 28th. Organizing committee: **M. Bollati**, L. Groeber and JM Garcia. Supporting funds have been obtained from the Reseau International des Instituts Pasteur (RIIP), the International Centre for Genetic Engineering and Biotechnology (ICGEB), United Nations University (UNU-Biolac), and Third World Academy of Sciences (TWAS). Amount Granted: € 35,000 and USD 15,000.
- ✓ **1st Processing and Image Analysis Course Using Icy Software**. **S. Astrada** participated as organizer, December 15-17, Institut Pasteur Montevideo, Uruguay.

As lecturers and instructors:

- ✓ Animal cell culture technology course, PUCV, Valparaiso, Chile. **M. Bollati** participated as conferencist. November 2014.
- ✓ Biotecnología aplicada a la transferencia y expresión de genes recombinantes en células animales. FBCB, Santa Fe, Argentina. **M. Bollati** participated as lecturer. September 2014.
- ✓ Curso Básico de Cultivo de Células, PEDECIBA – BIOLOGIA. Montevideo, Uruguay. **I. Tiscornia** and **M. Bollati** participated in the theoretical and practical activities. May 2014.

TRAINING OF STUDENTS

In the year 2014 three students defended their thesis and graduated:

- Milagros Burgi completed her PhD studies at the FBCB, UNL, Santa Fe, Argentina. Supervisors: R. Kratje and **M. Bollati**
- **Giuliana Mastropietro** completed her diploma thesis in Biotechnology, Facultad de Ingeniería, Universidad ORT, Uruguay. Supervisors: **I. Tiscornia, K**. **Perelmuter** & **M. Bollati**
- **Ma Belén Harreguy** completed her diploma thesis in Biotechnology, Facultad de Ingeniería, Universidad ORT, Uruguay. Supervisors: **R. Pagotto**, V. Porro & **M. Bollati**

Two students are performing their PhD or diploma theis work at the Unit:

- **Soledad Astrada**. PhD student at Pro.In.Bio, Uruguay. Supervisorss: M. Bollati and M. Guerra Vallespi
- **Giuliana Mastropietro**, diploma work for Biotechnology Engineer degree, Facultad de Ingeniería, Universidad ORT, Uruguay. Supervisors: **I. Tiscornia, K**. **Perelmuter** & **M. Bollati.**

In addition, our Unit has hosted a total of four (4) traineeships for students, postdoctoral fellows and visiting scientist:

- PhD Natalia Calvo: from Universidad Nacional del Sur, Bahía Blanca, Argentina. February-March 2014 (two months).
- MSc. Brizaida Oliva: PhD student from Centro de Ingeniería Genética y Biotecnología, Habana, Cuba. April 2014 (one month).
- Bioq. Clarisa Santamaría: PhD student from Facultad de Bioquímica y Cs. Biológicas, Universidad Nacional del Litoral Santa Fe, Argentina. April 2014 (one month).
- PhD Maribel Guerra Vallespí: from Centro de Ingeniería Genética y Biotecnología, Habana, Cuba. December 2014 (one month).

RECEIVED TRAINING BY THE CELL BIOLOGY UNIT STAFF

- All members of the Cell Biology Unit were trained in the requirements of ISO 9001:2008 standard and ISO/IEC 17025:2005 standard. IP Montevideo, Uruguay. February, 2014.
- K. Perelmuter performed an intership in the Laboratorio de Investigaciones del Sistema Inmune (LISIN), Universidad Nacional de La Plata, Buenos Aires, Argentina. May 2014.
- R Pagotto accomplished one-month internship at the Facultad de Bioquímica y Cs. Biológicas, Universidad Nacional del Litoral, Santa Fe Argentina. May 2014
- K. Perelmuter attended to the "Animal Cell Technology course – 4th edition". Llafranc, Spain. September 2014.
- K. Perelmuter participated in the "Cells for compound screening course– 1st edition". Llafranc, Spain. October 2014.
- S. Victoria completed an internship at the London Research Institute (LRI) in London, UK during three weeks. The objective was to gain experience in flow cytometers and cell sorters. October 2014.

Transgenic and Experimental Animal Unit

Head: Martina Crispo, DVM, PhD

Members: ***Geraldine Schlapp, MSc*** (Full time technician)
María Noel Meikle, MSc (Technician)
Ana Paula Arévalo, TMN (Technician)
Gabriel Fernández, Biol (Technician)
Ana Paula Mulet, MSc (Research assistant)
Sergio Anchetta (Animal caretaker)
Martín Mereles (Animal caretaker)
Casandra Carrillo, (Animal caretaker)
Tali Korytnicky *(Degree student)*
Pia Jacques (Degree student)
Natalibeth Barrera, Biol *(MSc Student)*
Pedro Dos Santos, DVM, MSc *(PhD student*)
Federico Cuadro, DVM *(MSc Student)*

GOALS

Our scientific proposal is to provide high level regional support in the field of animal transgenesis including mice, zebrafish and ruminants. For that, several techniques are offered nowadays, as pronuclear microinjection, homologous recombination in embryonic stem cells, lentiviral injection, transposons and recently the revolutionary CRISPR/Cas9 system.

Research

During 2014 one full article and two abstracts in peer review journals were published, with a MSc thesis and two ANII research initiation fellowships finished and others posgraduate thesis starting and running.

One of the major achievements of the Unit during 2014 was that the firsts CRISPR/Cas9 Myostatin Knockout sheep probably in the world were born, a recent technology that promises to revolutionize the world of transgenesis. This project is carried out in collaboration with Alejo Menchaca from IRAUy and Ignacio Anegón from INSERM group in Nantes. The data led to a full article that will be submitted during 2015 and new interesting projects being discussed for 2015.

During 2014 we managed to increase the efficiencies in transgenic technologies as pronuclear DNA microinjection, improving embryo survival rate from 50 to 85% after some major modifications in the microinjection settings. This improvement will allow us to reach more clients in the region with better results.

We increased the animal facility conventional area in aprox. 90 mt^2 to attend the demand for new PI's that uses mice and also rats, introducing in 2015 a new species in the animal house. We recluted more technicians to cover the amount of work demanded.

We continue working with national and international biotechnological companies, with grants close to 100.000 USD and the services offered resulting in new projects presented at ANII Aliances for 2015, with an estimated amount of 200.000 USD shared with others facilities of IPMon.

We obtained a EUR 30.000 RIIP grant to organize during 2015 a high level International Course: **ADVANCES IN THE GENERATION OF GENETICALLY MODIFIED (GM) ANIMAL MODELS** – and an International Mini Symposium: **TRANSGENIC TECHNOLOGIES, THE LATEST TRENDS** (link). This is the first time a course with these characteristics is being dictated in our region, triggering lot of interest.

The third edition of the internal course for researchers of Institut Pasteur was organized: **Manejo, técnicas de administración de sustancias y obtención de muestras en ratones**, being mandatory for working with mice at our facility. Several lectures for undergraduated and posgraduated students were given by members of the UATE.

In summary, the efficiency of the transgenic services was improved substantially, animal house convencional area was increased, research was maintained, human resources formation continues developing and original information was published during the period.

Projects

- **2012-2016** Estudio de los mecanismos responsables de potenciales efectos probióticos de la cepa Lactobacillus rhamnosus CNCM I-3690. PhD thesis, Co-Tutor.
- **2014-2016** Evaluación por imagenología molecular del diabody anti-Tn en modelo murino de cáncer de pulmón. MSc thesis, Co- tutor.
- **2014-2016** Influencia de la progesterona en el desarrollo folicular sobre la maduración y fertilidad ovocitaria en ovinos. MSc thesis, Co-tutor.
- **2014- 2016** Efecto del contenido lipídico sobre la maduración y vitrificación de ovocitos ovinos: Mecanismo de acción y su aplicación a la producción de embriones in vitro. MSc thesis, Co-tutor.
- **2012 – 2014 C**riopreservación de embriones ovinos producidos in vitro en diferentes estadios mediante dos métodos de vitrificación. MSc thesis, Co –tutor.
- **2013-2014** Caracterización de un modelo murino inducible para el estudio de las vías de señalización de la insulina. Research Initiation fellowship, Tutor.
- **2013-2014** Derivación y caracterización de una línea de células madre embrionarias (ESC) murinas Oct4-GFP en ausencia de suero y de fibroblastos embrionarios murinos (MEF). Research Initiation fellowship, Co-tutor.
- **2011-2014** Una innovación tecnológica para la reproducción ovina en Uruguay: producción in vitro de embriones y su aplicación a gran escala. Co-responsable
- **2012-2014** Applied environmental toxicology: risk evaluation to environmental antropogenic estrogen exposition in a transgenic murine model Oct4-GFP. Collaboration.

- **2009-to date** Trypanothione biosynthesis as a basis for drugs against trypanosomes. Collaboration.

- **2008-to date** Colon anticancer immunotherapy using Trypanosoma cruzi antigens. Collaboration.

Core Facilities - Services

1. Generation of transgenic mice by pronuclear microinjection of DNA fragments (3 projects for researcher from Argentina & Uruguay).
2. Generation of transgenic mice by homologous recombination in embryonic stem cells (2 projects - mgc-1203 and Spats1).
3. Generation of transgenic mice by Sleeping Beauty Transposons (SBT 100x) technology (one project in progress – Redox GFP)
4. Embryo and sperm cryopreservation (16 projects running).
5. Rederivation of mouse lines (10 projects running).
6. In vitro fertilization: several murine lines successfully cryopreserved and rederived using CARD protocol
7. Breeding and housing of SPF and conventional mice (C57BL/6J, BALB/cJ, DBA/2J, SWISS, SJL/J, Nude, several hybrids and aprox. 30 different transgenic lines). Actual production: aprox. 2000/month.
8. Four trials of acute safety of probiotic bacteria for Biopolis Company
9. Estudio de la actividad hipolipemiante, capacidad antioxidante y actividad anti-inflamatoria de los componentes del extracto de pericarpio derivado de girasol “violeta” (EPGv)”.
10. Trials of biological activity for recombinant eritropoyetin (Lab. Clausen & LCB) (aprox. 12 per month). The animal facility is certified by the Ministry of Health.
11. Trials of toxicity for biotechnological products (EPO, Filgen, Interferon) for Lab. Clausen & LCB (10 per month).

Publications 2014

1. **Crispo M, Vilariño M**, dos Santos-Neto PC, Núñez-Olivera R, Cuadro F, **Barrera N, Mulet AP**, Nguyen TH, Anegón I, Menchaca A. Embryo development, fetal growth and postnatal phenotype of eGFP lambs generated by lentiviral transgénesis. Transgenic research 2015 Feb;24(1):31-41. doi: 10.1007/s11248-014-9816-x. Epub 2014 Jul 22.
2. **Schlapp G**, Goyeneche L, Fernández G, Menchaca A, **Crispo M**. Administration of the nonsteroidal antiinflammatory drug tolfenamic acid at embryo transfer improves maintenance of pregnancy and embryo survival in recipient mice, Journal of Assisted Reproduction and Genetics, **accepted**.
3. **Meikle MN**; **Arévalo AP**; **Schlapp G**; Fernández G., Graña M; **Crispo M.** Environmental enrichment effects on reproductive performance of foster females used in transgenesis and rederivation techniques. Transgenic research 2014, 23; 897 – 897.

4. **Schlapp G**; **Meikle MN**; **Crispo M.** Slow freezing versus vitrification of transgenic mouse embryos obtained by in vitro fertilization (IVF) intended for rederivation use. Transgenic research 2014, 23; 891 – 891.

Grants

1. "Ensayo de ingesta aguda de 4 cepas probióticas en ratones BALB/cJ". Biópolis. Responsables Mariela Bollati, Martina Crispo (2014) USD 60.000.
2. "Estudio de la actividad hipolipemiante, capacidad antioxidante y actividad anti-inflamatoria de los componentes del extracto de pericarpio derivado de girasol "violeta" (EPGv)". Igra Semillas. Responsables Mariela Bollati, Carlos Battyhany, Martina Crispo (2013-2014) USD 38.500.
3. RIIP International Course "ADVANCES IN THE GENERATION OF GENETICALLY MODIFIED (GM) ANIMAL MODELS - International Mini Symposium: TRANSGENIC TECHNOLOGIES, THE LATEST TRENDS". Organizer. EUR 30.000.

Other activities

HUMAN RESOURCES FORMATION

- ✓ Ana Paula Mulet, MSc - Estudio de los mecanismos responsables de potenciales efectos probióticos de la cepa Lactobacillus rhamnosus CNCM I-3690. **PhD Thesis** (2012-2016). UdelaR PRO.IN.BIO (Co-Tutor).
- ✓ Pedro Claudino dos Santos Neto, DMV – Criopreservacion de embriones ovinos. **MSc Thesis** (2011-2014), Facultad de Veterinaria - UDeLaR (Co-Tutor).
- ✓ Pia Jacques, student - Caracterización de un modelo murino inducible para el estudio de las vías de señalización de la insulina. **Research initiation fellowship** (2013-2014), ANII (Tutor).
- ✓ Pia Jacques, student – Obtención de herramientas moleculares para el estudio del estado redox de tripanosomátidos. **Degree thesis** (2012-2014).
- ✓ Tali Korytnicki, Biol – Derivación y caracterización de una línea de células madre embrionarias (ESC) murinas Oct4-GFP en ausencia de suero y de fibroblastos embrionarios murinos (MEF). **Research initiation fellowship** (2013-2014), ANII (Co-tutor).
- ✓ Tali Korytnicki, Biol – Derivación de Células Madre Embrionarias (ESC) murinas como alternativa a su adquisición en otros laboratorios para uso en la producción de ratones genéticamente modificados. **Degree thesis** (2012-2014) (Co-tutor).
- ✓ Natalibeth Barrera, Biol - Efecto del contenido lipídico sobre la maduración y vitrificación de ovocitos ovinos: Mecanismo de acción y su aplicación a la producción de embriones *in vitro*. MSc thesis, Co-tutor.
- ✓ Federico Cuadro, DVM - Influencia de la progesterona en el desarrollo folicular sobre la maduración y fertilidad ovocitaria en ovinos. MSc thesis, Co-tutor
- ✓ Fabricio Maschi, DVM – Tecnologías embrionarias murinas. Internship, Tutor.

MEETINGS

- **12th International Society for Transgenic Technologies Meeting – Edinburgh, UK. October 2014.**
- II Congreso FESSACAL y IV Congreso de la Asociación Argentina de Ciencia y Tecnología de Animales de Laboratorio (AACyTAL), September, Buenos Aires, Argentina. Poster presentation: Meikle MN, Schlapp G, Arevalo AP, Crispo M. **EFICIENCIA DE LA TÉCNICA DE FERTILIZACIÓN IN VITRO (FIV) MEDIANTE ESPERMA CONGELADO EN MURINOS**
- XV Jornadas de la Sociedad Uruguaya de Biociencias, September, Piriapolis, Uruguay. Poster and oral presentation: Schlapp, G; Meikle, MN; Mulet, AP; Arévalo, AP; Fernández, G; Ancheta, S; Mereles, M; Crispo, M. **Desarrollo de modelos murinos en la Unidad de Animales Transgénicos y de Experimentación (UATE).**

ORAL PRESENTATIONS IN NATIONAL AND INTERNATIONAL MEETINGS

- Banco de esperma y embriones murinos: Importancia y perspectivas. II Congreso FESSACAL y IV Congreso de la Asociación Argentina de Ciencia y Tecnología de Animales de Laboratorio (AACyTAL), September, Buenos Aires, Argentina.
- Desarrollo de modelos murinos en la Unidad de Animales Transgénicos y de Experimentación. XV Jornadas de la Sociedad Uruguaya de Biociencias, September, Piriapolis, Uruguay.
- Pautas para Fiscalizaciones 2015. III Encuentro Uruguayo de Comités de Ética en el Uso de Animales. December, Montevideo, Uruguay.

INTERNSHIPS & COURSES

- **Instituto Leloir – In vivo imaging in rodents. June, Buenos Aires, Argentina.**
- **Bioseguridad en el laboratorio,** Academia Nacional de Medicina, UBA, June, **Buenos Aires, Argentina.**
- "Curso avanzado en bioseguridad y manejo de bioterios", Universidad de los Andes y Universidad Nacional de Colombia, November, Bogota - Colombia.
- The AZA Professional Development Committee. Professional Training Course Scholarships **Les Whitt Memorial Scholarship** for participation in the Animal Training Applications in Zoo & Aquariums Settings course in April 6^{th}-11^{th} ,Disney's Animal Kingdom in Orlando, Florida, USA.
- Curso de Bioseguridad para OGMs. Instituto GENOK, September, Montevdeo, Uruguay.

TEACHING

✓ Organization of the IP Montevideo internal course 2014: *Manejo, técnicas de administración de sustancias y obtención de muestras en ratones*, for 20 researchers that uses mice at the animal facility.

✓ Several lectures in postgraduate national and regional courses.

OTHER

✓ Researcher Level 1 ANII

✓ Researcher of the Posgraduated Program of Faculty of Veterinary.

✓ Member of the International Society for Transgenic Technologies (ISTT) Council (2014-2017)

✓ Member of Scientific Comitee of Centro Multidisciplinario para Investigação Biológica (CEMIB) Universidad de Campinas (2010 - to date)

✓ Members of Comisión Nacional de Experimentación Animal (CNEA) (2010 - 2014)

✓ Members of Comisión de Evaluación del Riesgo en Bioseguridad, MGAP (2009 - to date)

✓ Members of Comite de Ética en el Uso de Animales (CEUA). Institut Pasteur de Montevideo (2009 - to date)

✓ Members of Comite de Ética en el Uso de Animales (CEUA). Facultad de Ciencias, UdelaR (2011 to date)

✓ Redaction of three chapters in the Comisión Nacional de Experimentación Animal 2010-2014 final report.

Protein Biophysics Unit

Head: Otto Pritsch, PhD

Members: ***Gonzalo Obal*** (Technical Assistant, PhD student)
Federico Carrión (Technical Assistant, MSc student)
Sergio Bianchi (MD, MSc, PhD, posdoc)
Lorena Tomé (MSc, PhD student)
Natalia Olivero (PhD student)
Andrés Addiego (MSc student)

Research

During the period 2007-2014, we started at IPMONT a project focused on the study of viral pathogenesis of human and bovine chronic lymphocytic leukemias.
We have developed two main research axes:
First, we proposed to search for a candidate virus as aetiological agent of human Chronic Lymphocytic Leukaemia (CLL), by combining high-throughput sequencing and digital subtraction. With this aim we characterized at the molecular level a group of 80 CLL patients (Bianchi et al, 2010), and thereafter, B-cell transcriptomes from CLL patients and healthy donors were sequenced by using the 454 Life Sciences pyrosequencing technology and Illumina technology. Despite an in-depth analysis of the CLL transcriptome reaching more than 100 million sequences, we have not found evidence for a putative viral candidate in CLL (Rego et al, 2012).

Second, we started to study an animal model for virus-induced Chronic Lymphocytic Leukemia. Enzootic Bovine Leukemia (EBL) is an infectious disease caused by an oncogenic member of the genus Deltaretrovirus of the family Retroviridae, the Bovine Leukemia Virus (BLV), which affects >60% of dairy cattle in Uruguay. Most infections are subclinical, but 30% of infected cattle develop persistent lymphocytosis, and 5% develop lymphosarcomas. At the moment, no vaccine against BLV is available. In order to gain insight into the degree of genetic variability of BLV in our country we have performed a phylogenetic analysis of Env sequences and revealed the presence of seven BLV genotypes in the South American region (Moratorio et al, 2010). We also performed a detailed molecular analysis of complete bovine leukemia virus genomes isolated from B-cell lymphosarcoma, and compared with other BLV full-length sequences from other clinical manifestations (Moratorio et al, 2013). In parallel we developed a rapid and sensitive real time PCR assay using SYBR green chemistry to detect and quantify BLV proviral DNA from blood obtaining an increased sensitivity over the ELISA and AGID tests (Rama, 2010).

We also initiated the characterization of the main BLV proteins at the molecular and structural levels. In particular, we analyzed the self-assembly process of the purified recombinant BLV capsid (BLV-CA) protein providing the first description of their assembly properties. On the other hand, BLV-CA full-length and separate N- and C-terminal domains were expressed and purified to homogeneity. In order to obtain insights into the detailed molecular structure and self-assembly process of a native, non-engineered retroviral CA, we solved the crystal structure of the mature BLV-CA at 2.75Å resolution, showing a 2D hexagonal lattice displaying both lateral 3-fold and 2-fold interactions between asymmetric and plastic CA hexamers. This work was done in collaboration with IPMont Protein Cristallography Unit. (Obal et al, submitted).

In the context of this project we have organized a multidisciplinary group to work on BLV, funded by the Institut Pasteur de Montevideo, the National Institute of Agronomic Research of Uruguay (INIA), the Universidad de la República de Uruguay and the Centre National de la Recherche Scientifique (CNRS, France). Moreover, we have participated in the First Latin American Workshop on EBL (2012) and created the Regional Network of EBL that integrates a diverse group of research laboratories in Latin America. In parallel, the IPMONT is engaged as a founding member of the network "Center for Structural Biology in the Mercosur" – CeBEM (http://www.cebem.org.ar). Structural virology is one of the areas that need to be developed in our region, with anticipated impact in scientific and medical terms.

Principal and specific aims

In general, retroviruses use very similar principles in their biological cycles: assembly and budding of an immature particle, proteolytic capsid maturation, entry through membrane fusion via interactions of the envelope glycoprotein complex with a cellular receptor, reverse transcription of the viral genome, mature capsid uncoating, transport of the pre-integration complex into the nucleus and integration of the provirus. The principal aim of this proposal will focus on structural and functional studies on BLV, attempting to understand the structural bases of relevant functional phenomena of the viral biology.

Our specific scientific aims are:

1. To characterize the biochemical and structural bases of BLV envelope protein: The BLV env complex plays a crucial role in determining viral infectivity, being responsible for inducing fusion of viral and cellular membranes after recognition of specfic cell-surface receptors.

To study this issue we will carry out the following specific tasks:

1.1. Cloning and expression of BLV-env glycoprotein in Drosophila S2 cells: We have optimized the expression of the soluble env ectodomain in Drosophila S2 cells, by cloning the codon-optimized env gene precursor in plasmid pT350. The env protein is truncated upstream the TM segment, and we have designed different constructs with a natural and an altered furin cleavage site. Protein expression and secretion into supernatant was induced by divalent metals, and protein purification was performed by affinity chromatography using a StrepTactin column followed by size exclusion chromatography. Protein quality control was assessed by mass spectrometry. This system should allow the production of sufficient material for crystallization trials, electron cryo-microscopy of isolated trimers, and biophysical studies of the multimeric complex formed by the recombinant proteins.

1.2. Analysis of the oligomeric structure of the expressed proteins: In order to determine the oligomeric state of the expressed BLV Env proteins, size exclusion chromatography, sedimentation analysis and light scattering we will be performed.

1.3. Crystallization trials of purified recombinant Env protein: data collection, structure determination and refinement: Crystallogenesis conditions will be screened with a robotic platform, and initial hints optimized as needed. Crystal quality will be checked with a home X ray source. Complete data sets will be collected at synchrotron facilities (Soleil, ESRF) and processed according to standard single crystal methods. Structures will be determined using Se-Met labeled proteins, and anomalous diffraction phasing techniques. Model refinement and validation will be carried out according to established methods.

1.4. Cryo-electron tomography of recombinant Env proteins. The IP has recently obtained funding through the “investissements d’avenir” (EquipEx) program to be equipped with a 300keV microscope for high resolution 3D studies on the different assemblies (capsid protein tubes, virus particles). Because the retroviral particles are pleomorphic, the studies will involve cryo-electron tomography with sub-tomogram averaging.

2. To characterize the biophysical and structural bases of BVL capsid self-assembly: Like other retroviruses, assembly of BLV virions is driven by Gag, a polyprotein precursor composed of three major domains: MA (matrix), CA (capsid), and NC (nucleocapsid). After particle budding, the virus-encoded protease PR cleaves Gag and releases the individual domains: the N-terminally myristoylated MA remains anchored at the viral envelope, NC condenses with the viral RNA, and CA spontaneously self-assembles to form a closed structure: the mature “core”or capsid. This dramatic structural rearrangement, known as maturation, is essential for infectivity, and thus constitutes an attractive target for novel antiretroviral strategies. The mechanism of viral capsid formation via self-assembly of thousands of copies of the capsid protein (CABLV) represents a key event in the retrovirus cycle.

To study this issue we will carry out the following tasks:

2.1. To perform a comprehensive characterization of the biophysical properties of the CABLV assembly process: We will explore a wide range of conditions, to obtain a complete characterization of the parameters affecting the polymerization reaction. Particularly, we will focus on analyzing the effect of compounds in near-physiological conditions mimicking the virus intra-particle environment.

2.2. To elucidate the 3D structure of the CABLV protein. X-ray crystallography will be used to determine the atomic structure of the individual N-terminal and C-terminal domains, which will provide a high resolution view of the full-length mature protein. As mentioned above, we have already advanced in this direction, although further work is needed. Crystallogenesis conditions will be screened with a robotic platform. X ray diffraction data will be collected on our home source (microsource rotating anode generator) or synchrotron facilities (Soleil, ESRF) as needed. Structures will be determined using halogen (iodide, cesium) quick-soak derivatives, or Se-Met labeled proteins, and anomalous diffraction phasing techniques. Model refinement and validation will be carried out according to established methods. The high-resolution structures of full length and individual CABLV domains will provide a substrate for testing/redirecting hypotheses.

2.3. To characterize the ultra-structural morphology of in vitro assemblies of wild-type CABLV as well as engineered mutants**.** We will use cryo-electron microscopy and image reconstruction analysis of the in vitro assemblies to obtain electron density maps onto which mapping the crystallographic structures (to be done in collaboration with IPParis).

2.4. To perform structure/function analyses of the CABLV assembly process by mapping residues/surfaces functionally relevant for polymerization via extensive site-directed mutagenesis and *in vitro* effects on assembly. Structural information will guide the design of mutants and further engineering of CABLV. In solution functional studies and ultrastructural analysis using electron microscopy, will complement these studies on selected CABLV variants.

3. To characterize the immunomodulatory activity of BLV envelope glycoprotein.

Env is one of the main targets of the antiviral immune responses, generating both humoral neutralizing antibodies and T-cell specific adaptive immunity. It has been reported for other

retrovirus that the presence of an immunosuppressive (isu) peptide in Env glycoprotein structure could be important in their ability to immunomodulate immune responses.
We propose to study the effect of amino acid modifications in the isu domain in humoral and cellular adaptive responses against challenge with modified Env glycoproteins. This will allow us to understand one of the mechanisms involved in the generation of resistance used by BLV to escape the antiviral immune response. On the other hand, we also expect to identify the modifications that reduce the immunosuppressive activity of this domain and therefore increase their immunogenicity. This result could be useful for the rational design of effective vaccines against this retrovirus.
To study this issue we will carry out the following tasks:
3.1. Expression and purification of the BLV wild-type and isu-mutant Env glycoprotein protein in Drosophila S2 cells.
3.2. Analysis of the in vitro and in vivo cytokine expression profiles in the presence of “isu mutant” versus “wild type” Env glycoprotein.
3.3 Characterize the antibody response in vivo after immunization with “isu mutant” versus “wild type” Env glycoprotein.

4. To identify the genetic characteristics associated with natural control of EBL: Given the high prevalence of EBL in Uruguay, the strategy to eradicate the disease implemented in Europe and Oceania, is impracticable in our country. An alternative control strategy by using vaccines is promising, but there are still no effective products on the market. Taking into account that recent results show that EBL has a heritable component that reaches 8%, a third strategy to control disease would involve breeding herds by increasing the frequency of genotypes associated with resistance to infection.
4.1. Identify in herds with high prevalence of BLV infection, a group of animals defined as "controllers" of the disease and characterized by low proviral load and low titers of anti-BLV antibodies. Another group defined as "non-controllers" with high proviral load and high titers of specific antibodies.
4.2. Characterize the transcriptomes of peripheral blood mononuclear cells (PBMC) by massive sequencing of mRNA (RNAseq) obtained from controller and non-controller animals.
4.3. Identify genes and isoforms differentially expressed in “controller” as compared to “non-controllers” animals. Interpret these differences in the context of biological processes, metabolic pathways ontologies sub- or overrepresented.

Services

1. Thermodynamic analysis of protein-protein and protein-ligand interaction through determination by isothermal titration microcalorimetry (ITC) of binding constants (KB), reaction stoichiometry (n), enthalpy (ΔH) and entropy (ΔS).
2. Thermodynamic analysis of conformational changes of proteins including assessment of stability and folding of recombinant proteins through differential scanning microcalorimetry (DSC) to study a wide range of thermal transitions in biological systems, to determine melting temperatures as well as thermodynamic parameters associated to these changes.
3. Kinetic analysis of protein - ligand interaction through Surface Plasmon Resonance (SPR) measurements, determination of kinetic association (kass) and dissociation (kdiss) constants.

4. Determination of the hydrodynamic radius of macromolecules or particles through dynamic light scattering measurements coupled to size exclusion chromatography SEC-HPLC.

Publications 2014

1- Tinoco LW, Fraga JL, Anobom CD, Zolessi FR, **Obal G,** Toledo A, **Pritsch O**, Arruti C. Structural characterization of a neuroblast-specific phosphorylated region of MARCKS (2014) Biochimica et Biophysica Acta - Proteins and Proteomics, 1844 (4), pp. 837-849. – *IF: 1.094*

2- Morero NR, Botti H, Nitta KR, **Carrión F, Obal G,** Picardeau M, Buschiazzo A. HemR is an OmpR/PhoB-like response regulator from Leptospira, which simultaneously effects transcriptional activation and repression of key haem metabolism genes. Mol Microbiol. 2014 Oct;94(2):340-52. Epub 2014 Sep 15. PubMed PMID: 25145397. – *IF: 5.026*

3- Correa A, Pacheco S, Mechaly AE, **Obal G**, Béhar G, Mouratou B, Oppezzo P, Alzari PM, Pecorari F. Potent and specific inhibition of glycosidases by small artificial binding proteins (affitins). PLoS One. 2014 May 13;9(5) eCollection 2014. – *IF: 3.534*

4- Correa A, Ortega C, **Obal G**, Alzari P, Vincentelli R, Oppezzo P. Generation of a vector suite for protein solubility screening. Front Microbiol. 2014 Feb 25. eCollection 2014. – *IF: 3.941*

Grants

1. **"International Associated Laboratory on Structural Virology".** Centre National de la Recherche Scientifique - IPMont-LIA. Period: January 2014 – December 20176. Felix Rey, CNRS URA 3015 Virology, Institut Pasteur, Paris - Otto Pritsch. Institut Pasteur de Montevideo. Amount granted: 15.000 euros / year.
2. **"Desarrollo de nuevos métodos para el diagnóstico del Virus de la Leucosis Bovina"**. Ministerio de Industria y Energía – Dirección Nacional de Industria, uruguay. Period: December 2012 – December 2013. PI: Otto Pritsch. Amount granted 17.000 USD.
3. Producción y Caracterización de Inmunógenos contra el Virus de la Leucosis Bovina. Proyecto CSIC I + D 2014, Universidad de la República. 2014 – 2017. PI Otto Pritsch. Amount granted 30.000 USD
4. Identificación de marcadores moleculares asociados con la resistencia a la infección por el Virus de la Leucosis Bovina mediante análisis transcriptómico de individuos controladores de la carga viral. Fondo Sectorial Innovagro 2013, 2014 – 2016. PI Otto Pritsch. Amount granted 100.000 usd
5. **Doctoral Fellowship – Lorena Tomé** – 2012 - 2014 – ANII

Other Activities

TRAINING OF STUDENTS

1. **Training of PhD degree students:**
   - Gonzalo Obal, PhD Student, PEDECIBA. "Estudios Biofísicos y Estructurales del Ensamblado de la Cápside del Virus de la Leucemia Bovina" Director: O. Pritsch. Defense date Septembre 2014.

- Lorena Tomé, PhD Student, PEDECIBA. “Estudio de la interacción entre el Virus de la Leucosis Bovina y la célula hospedera”. Directors: J. Arbiza, O. Pritsch. Defense date Septembre 2014.
- Rodrigo Puentes, PhD Student, Faculty of Veterinary. “Epidemiología molecular y efecto de la presencia del virus de la Leucosis bovina enzoótica en animales asintomáticos sobre parámetros productivos, reproductivos y sanitarios”. Directors: Silvia Llambí, Gonzalo Moratorio and Otto Pritsch.

2. Training of Master degree students:

- Federico Carrión, Master in Science, Faculty of Science. “Desarrollo de kits para genotípado de SNPs en los genes humanos, FII, FV y MTHFR , utilizando la tecnología PCR en Tiempo Real”. Directors: Andrés Abin and Otto Pritsch.
- Andrés Addiego, Master in Biomedicine, Faculty of Medicine. “Desarrollo y análisis comparativo de una nueva herramienta para el diagnóstico de la Leucosis Enzoótica Bovina; Impacto del descenso de anticuerpos anti-VLB circulantes en el periparto para el diagnóstico serológico” Directors: O. Pritsch. Defense date: March 2013.

- **Research Program in Molecular Oncology**
  - ✓ **Functional Genomics**
  - ✓ **Tumor Immunology and Glycobiology Laboratory**
- **Neurodegeneration Laboratory**
- **BioMolecular Simulation Laboratory**
- **Molecular and Human Genetics Laboratory**
- **Cellular Membranes Laboratory**
- **Redox Biology of Trypanosomes Laboratory**
- **Neuroinflammation and Gene Therapy Laboratory**
- **Cell Biology of Neural Development Laboratory**
- **Immunoregulation and Inflammation Laboratory**
- **Worm Biology Laboratory**
- **Signal Processing Laboratory**

Research Program in Molecular Oncology

Functional Genomics

Head: Alfonso Cayota, MD, PhD

Members: ***Julia Sanguinetti*** (Msc Student)
Juan Pablo Tosar (Doctoral Student)
Braulio Bonilla (MSc Student)
Fabiana Gambaro (Undergraduate Student)

GOALS

Our scientific proposal is intended to elucidate the role of small non-coding RNAs in the biology of human cancer. Additionally, we work in close collaboration with the University Hospital and the National Program for Cancer Control providing technological and experimental support for research in Clinical Oncology and the development of new biomarkers in cancer.

Research

In the last years, our main focus of research has been centered on the biology of small RNAs in the regulation of gene expression with especial emphasis in extracellular small RNAs and their role in cell-to-cell communication in human cancer. Our work is also intended to identify and validate small RNAs in different extracellular fractions as new biomarkers in human cancer

Research Lines

"THE SECRETED RNAome": AN UNEXPECTED PATHWAY OF INTERCELLULAR COMMUNICATION AND NEW SOURCE OF BIOMARKERS IN CANCER"

Cell-free DNA/RNA are normally secreted from a variety of normal and diseased cells to the extracellular media either through membrane-bound vesicles or included in ribonucleoprotein complexes. Studies over the past few years showed that these structures contain bioactive molecules, lipids, nucleic acids and proteins, which like hormones can influence normal homeostasis and many aspects of cancer progression including tumor development, invasion and metastasis.

Circulating mRNAs and microRNAs are detectable in the serum and plasma of healthy individuals and cancer patients. It is known that RNA released into the circulation is surprisingly stable in spite of the high levels of RNases in the blood. High stability and resistance to degradation is achieved through its packaging into either membrane-bound structures (i.e. exosomes, ectosomes and apoptotic blebs) or circulating ribonucleoprotein complexes.

Cancer is currently the second leading cause of death worldwide. Despite the advances in cancer therapeutic approaches during the last decades, the morbidity and mortality rates still remain high. The earliest possible diagnosis and treatment is still the best approach to improve survival. The National Cancer Institute of USA estimates that premature deaths, which may have been avoided through screening, range from 3% to 35% (www.cancer.gov). Screening for cancer is usually attempted whenever worrying symptoms arise, having as a result the diagnosis of cancer as a latest age disease. The current methods for diagnosis of the disease are usually invasive and expensive whereas the existing biological markers are not definitive and lack high sensitivity and specificity. At present, growing scientific efforts in human cancer are aimed to find and develop new, sensitive, non-invasive and inexpensive biomarkers to identify high risk individuals, detect cancer at an early stage, to predict outcome, to monitor treatment and to screen for disease recurrence. Detection of extracellular or cell-free nucleic

acids (DNA or RNA) in blood or body fluids has been recently suggested as surrogates for non-invasive and cost effective biomarkers in human cancer.
Our present work is aimed to analyze in depth the total repertoire of RNA transcripts and small RNAs secreted by tumor cells to the extracellular media and their contribution to different fractions of circulating species in normal plasma as well as in cancer patients. Methodological and conceptual results issues from this study should be useful to establish new working hypothesis in the near future and to better understand their diagnostic and predictive value in human cancer and possibly other human diseases.
Thus, vesicular and non-vesicular horizontal transfer of small RNAs could emerge as a relatively wide-spread process that may complement intercellular communication by other mechanisms. One of the most intriguing questions in this regard is how, and to what extent, is this process involved in various forms of cellular pathology. Of particular interest is the role of MVs in cancer whose exploration may both afford new avenues in cancer biology and inspire new therapeutic and diagnostic approaches in biomedicine.

"CIRCULATING SMALL RNAs AS POTENTIAL BIOMARKERS IN HUMAN LUNG CANCER"

Despite advances in diagnosis, treatment and prevention of human cancer in last years, the incidence and mortality rates remain extremely high. For this reason, cancer represents today the second leading cause of death in adults.
Lung cancer is the more frequent cancer in men and the fourth place in frequency in women and the cancer with the higher rate of mortality (rates per 100.000 of 29.2 and 10.9 in men and women respectively). Thus, lung cancer accounts for 24.3% y 7.3% of deaths by cancer in men and women respectively.
This high mortality rate of lung cancer is mainly explained by the absence of specific symptoms and signs in the initial stages which explains the high frequency of diagnosis in advanced stages of the disease. Thus advanced lung cancer (IIIa or IIIb) is associated to a poor survival and partial responses to therapy with mortality rates at 3 and 5 years of 35% and 15% respectively.
In contrast to other cancer types there is no at present biomarkers with enough sensibility to detect lung cancer at early stages when therapeutics inducing long lasting survival or disease remission are highly effectives.
This project is aimed to analyze the potential differential expression of small RNAs in tumor tissues from advanced lung cancer when compared to normal lung tissues.
Advances in this field should contribute to identify novel pathways and potential therapeutic targets in lung cancer. Additionally, correlation between tissue and plasma small RNA could have the potential to identify circulating specific small RNAs that could be used as novel biomarkers.

Publications 2014

1- **Tosar JP**, Rovira C, Naya H, **Cayota A.** Mining of public sequencing databases supports a non-dietary origin for putative foreign miRNAs: Underestimated effects of contamination in NGS (2014) RNA, 20 (6), pp. 754-757. – *IF: 5.377*
2- **Garcia-Silva MR**, das Neves RF, **Cabrera-Cabrera F, Sanguinetti J**, Medeiros LC, Robello C, Naya H, Fernandez-Calero T, Souto-Padron T, de Souza W, **Cayota A.** Extracellular vesicles shed by Trypanosoma cruzi are linked to small RNA pathways, life cycle regulation, and susceptibility to infection of mammalian cells. Parasitol Res. 2014 Jan;113(1):285-304. doi: 10.1007/s00436-013-3655-1. Epub 2013 Nov 17. PubMed PMID: 24241124. – *IF: 2.327*
3- **Garcia-Silva MR, Sanguinetti J, Cabrera-Cabrera F**, Franzén O, **Cayota A.** A particular set of small non-coding RNAs is bound to the distinctive Argonaute protein of Trypanosoma

cruzi: Insights from RNA-interference deficient organisms (2014) Gene, 538 (2), pp. 379-384. – *IF: 2.082*

4- **Garcia-Silva MR, Cabrera-Cabrera F**, Cura Das Neves RF, Souto-Padrón T, De Souza W, **Cayota A**. Gene expression changes induced by Trypanosoma cruzi shed Microvesicles in mammalian host cells: Relevance of tRNA-derived halves (2014) BioMed Research International, 2014, art. no. 305239. – *IF:* --

Grants

1. "ARNs extracelulares y cáncer: caracterización e implicancias en la modulación recíproca entre células malignas y no malignas" Juan Pablo Tosar ANII Amount Granted USD 13.000. 2013-2015
2. "Desarrollo de un servicio de genotipificación de biomarcadores de respuesta al tratamiento con bevacizumab en pacientes con cáncer colorrectal metastásico. Alfonso Cayota. Alianzas – ANII (Casmu-Ipmont) Amount Granted USD 80.000

Other activities

PARTICIPATION IN MULTICENTRIC CANCER PROGRAMS

1. Latin American Breast Cancer Pilot Project: **"Molecular Profiling of Breast Cancer Study"** NCI (United States) – Brazil – Argentina – Mexico – Chili – Uruguay - Colombia – Puerto Rico. Coordinator for Uruguay in Basic Research

Research Program in Molecular Oncology
Tumor Immunology and Glycobiology Laboratory

Head: Eduardo Osinaga, MD, PhD

Members: **Nora Berois** (MD, PhD, Associate Investigator)
Edgardo Berriel (MD, MSc, PhD student)
María Florencia Festari (MSc , PhD student)
Patricia Moerzinger (MSc student)
Diego Touyá (MD, MSc, student)
Claudia Schvartzman (MSc student)
Patricia Solari (MSc student)
Cecilia Silva (MD, MSc, student)
Guillermo Tramontín (Undergraduate student)

Research

The most abundant form of O-linked glycosylation in higher eukaryotes, termed ''mucin-type'', is characterized by the covalent linkage of an α-N-acetylgalactosamine residue (GalNAc) to the hydroxyl group of Ser/Thr residues. Mucin core O-glycosylation is catalyzed by a group of UDP-GalNAc: polypeptide N-acetylgalactosaminyl-transferases (ppGalNAc-Ts) (EC. 2.4.1.41). Subsequent elongation of *O*-linked sugar chains is achieved by the transfer of additional saccharide units, catalyzed by specific glycosyltransferases. Malignant transformation of epithelial cells is commonly associated with changes in the expression level and/or glycosylation pattern of mucins, including exposure of simple mucin-type carbohydrates, such as Tn, sialyl-Tn and TF antigens. These determinants contribute to the phenotype and biology of cancer cells and are involved in their metastatic activity. Moreover, they are considered among the most specific cancer-associated structures, and are thus being evaluated as promising targets for tumor immunotherapy. We have recently identified some apomucins and glycosyltransferases, which are abnormally expressed in certain cancer cells. One of these enzymes, ppGalNAc-T13, is probably associated to the aggressiveness of some tumors. We investigate the molecular mechanisms underlying the regulation of the initial steps of mucin-type *O*-glycosylation in human cancer, and evaluate how this abnormal process influences malignant cell behavior.

Research Lines

The Tumor Immunology and Glycobiology Laboratory research is focused on:
a) How abnormal regulation of the initial steps of mucin-type O-glycosylation in human cancer could influence malignant cell behavior. We evaluate whether the expression of GalNAc-Ts could modify cancer cell properties *in vitro* (susceptibility to apoptosis, clonogenicity, invasiveness, chemoresistance, etc.) and *in vivo* (tumor growth, metastasis). We intensify our research on the characterization of GalNAc-T isoenzymes as new tumor markers.
b) Characterization of parasite glycoproteins which induce anti-cancer immunity. We focus on the identification, purification and characterization of these molecules from *T. cruzi* and *E. granulosus*. Therapeutic experiments are performed with different fractions (enriched and depleted in specific carbohydrates).

Publications 2014

1) **Berois N**, Heard I, Fort Z, Alonso R, Sica A, **Moerzinger P**, Rodriguez G, Sancho-Garnier H, **Osinaga E**, Favre M. Prevalence of type-specific HPV infection in Uruguay (2014) Journal of Medical Virology, 86 (4), pp. 647-652. – *IF: 2.217*
2) **Berois N, Osinaga E.** Glycobiology of neuroblastoma: Impact on tumor behavior, prognosis, and therapeutic strategies (2014) Frontiers in Oncology, 4 MAY, art. no. 114. – *IF:* --
3) Libisch MG, Casás M, Chiribao M, Moreno P, Cayota A, **Osinaga E,** Oppezzo P, Robello C. GALNT11 as a new molecular marker in chronic lymphocytic leukemia. Gene. 2014 Jan 1;533(1):270-9. doi: 10.1016/j.gene.2013.09.052.
4) Ikemori R, Longo Machado C, Furuzawa K, Nonogaki S, **Osinaga E**, Umezawa K, Carvalho MA, Verinaud L and Chammas R. Galectin-3 up-regulation in hypoxic and nutrient deprived microenvironments promotes cell survival. Plos One (2014). – *IF: 3.534*

Grants

1. Producción por ingeniería genética de diabodies e inmunotoxinas anti-antígeno tumoral Tn. Aplicación en imagenología molecular y tratamiento del cáncer. ANII - Fondo María Viñas. U$S 47.000, 2013-2015
2. Grupo de Inmunología Tumoral. Proyecto Grupo I+D CSIC-UdelaR. U$S 100.000, 2011-2015

Other activities

CONGRESSES

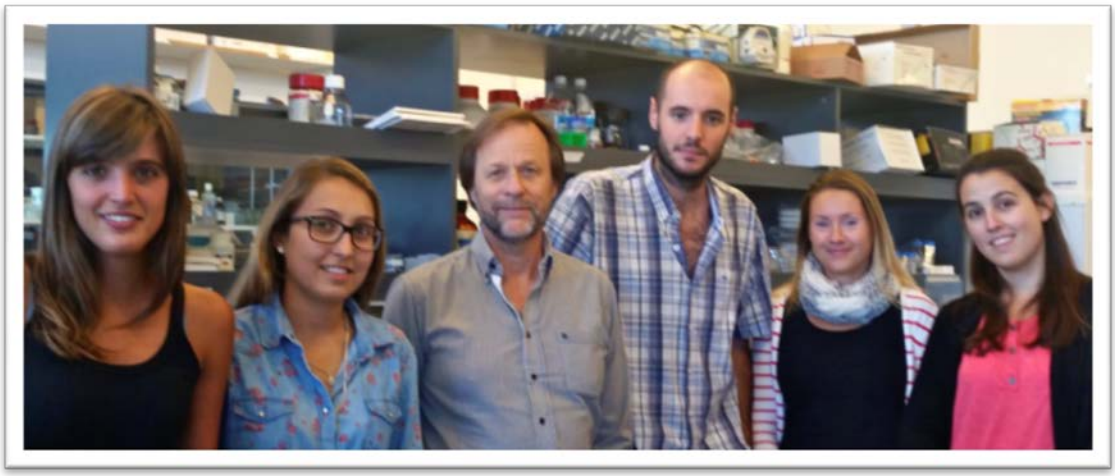
1. **Berois N**, **Touya D**, Behrens C, Alonso R, Festari MF, Pritsch O, Varangot M, Wistuba I and **Osinaga E**. Immunohistochemical analysis of GalNAc-T13 and its relationship with clinic, histologic and biomarkers profile in patients with non-small-cell lung cancer (NSCLC). GlycoT 2014, 9th International Symposium on Glycosyltransferases - 18th-21st June 2014, Porto, Portugal
2. **Festari MF**, Trajtenberg F, **Berois N**, Vester-Christensen MB, Pantano S, **Solari P**, Cabrera MJ, Freire T, Bay S, Robello C, Bénard J, Clausen H and **Osinaga E**. Identification of a complex pattern of splice variants of polypeptide-GalNAc-T13. GlycoT 2014, 9th International Symposium on Glycosyltransferases - 18th-21st June 2014, Porto, Portugal

PATENTS

1. **Berois N, Touyà D**, Varangot M and **Osinaga E.** A novel method to detect resistance to chemotherapy in patients with lung cancer.PCT International Application PCT/US2013/051904 (2013)
2. **Berois N**, Touyà D, **Varangot M** and **Osinaga E.**Use of GalNAc-T13 as a marker in breast or colon cancer diagnostics PCT International Application PCT/US14/48082 (2014)

Neurodegeneration Lab

Head: Luis Barbeito, MD



Members: ***Monique Richter*** (PhD)
Emiliano Trías (PhD student)
Valentina Varela (Msc student)
Romina Barreto (Msc student)
Sofía Ibarbouru (Msc student)

GOALS

Understanding and modeling the neurodegenerative cellular "microenvironment" and develop new therapeutics targeting glial cells.

Research Lines

1. Modulation of disease progression in Amyotrophic Lateral Sclerosis (ALS)

In most neurodegenerative diseases, neuronal death begins as a focal process that spreads contiguously along brain regions in an ineluctable manner. This implicates an acquired pathogenic mechanism involving neuronal damage and subsequent chronic inflammation. The mechanisms underlying such disease progression remain largely unknown as well as the potential therapeutic strategies to halt the process and prevent symptom aggravation. There is no cure for the neurodegenerative diseases. However, if their progression would be slowed during early symptomatic stages by interventions in glial cells, neurodegenerative diseases would become a minor chronic disabilitiy and no longer a death sentence.

We study neurodegeneration in an inherited model of ALS expressing SOD1 mutations. ALS is caused by the progressive death of motor neurons, leading to serious debility, paralysis and ultimately death within a few years. Because microglia and reactive astrocytes accumulate in the spinal cord of rats expressing the ALS-linked SOD1G93A mutation, we originally proposed that disease progression was mediated by the emergence of inflammatory glial cells. In fact, glial cells isolated from rodent models as well as ALS patients are toxic to motor neurons. In 2011, we identified a new type of glial cell referred as "AbA cells" (from aberrant astrocytes) from degenerating spinal cord from $SOD1^{G93A}$ rats, their appearance being closely associated with the progression of paralysis in $SOD1^{G93A}$ rats. Phenotypically AbA cells appear as atypical astrocytes. Functionally, AbA cellsare the most toxiccells yet identified to motor neurons. AbA cells actively proliferateafter the onset of progressive paralysis and make intimate contact with degeneratingmotor neurons. By analyzing the population of proliferating glial cells in theventral horn of symptomatic SOD1 rats, we found that microglia are the most likelycellular origin of AbA cells. Of considerable interest, AbA cells share some characteristics with glioblastoma cells, including the high proliferation rate in vivo and vitro, as well as lack of contact inhibition and predominant glycolytic metabolism.

The understanding of the mediators inducing such phenotypic transition may allow intervention to slow the progressive spread of disease in ALS patients.

One goal of our research is to develop new therapeutic agents for the treatment of ALS using mechanistic insights drawn from understanding how different cells and molecules contribute to progressive neuronal death. 20 years of animal testing have so far failed to yield an effective therapy for motor neuron disease. Such failure might be explained by the fact that, until recently, research has been mainly focused on motor neuron degeneration, the concomitant pathological changes in other cell types including glial cells being mostly neglected.

Our central hypothesis is that the spread of motor neuron disease is dependent on the formation of a neurodegenerative microenvironment surrounding damaged neurons, organized around AbA cells, with the ability to replicate and expand the inflammatory and

neurotoxic process to contiguous or distant areas of the CNS. The objective is to characterize such cellular microenvironment, unraveling the cells types involved, cell-cell interactions and the role of specific trophic factors like nitroNGF. The outcomes of these studies will strongly influence our approach to other neurodegenerative disease by providing new tools to define appropriate in vivo and in vitro models for therapeutic interventions.

2. Targeting AbA cells with the tyrosine-kinase inhibitor Masitinb.
We have recently provided evidence that Masitinib mesilate (AB1010) exerts a disease-modifying effect in ALS animal models by controlling secondary neuroinflammation that causes paralysis progression and spread. Masitinib is a selective tyrosine kinase inhibitor that targets c-Kit, platelet-derived growth factor receptors (PDGF-R), and, to a lesser extent, Lyn and Fyn pathways. By combined targeting of c-Kit and Lyn, Masitinib is particularly efficient in controlling mast cell survival, differentiation, and degranulation. Our hypothesis establishes that Masitinib also decreases the appearance of inflammatory aberrant glial cells through a specific mechanism.

3. Nitrated-NGF as a novel glial factor mediating neuronal apoptosis. We reported that activated astrocytes in ALS express increased levels of NGF, which triggers p75-dependent motor neuron apoptosis. Although adult motor neurons lack TrkA and $p75^{NTR}$ receptors, they re-express $p75^{NTR}$ following nerve injury or in ALS, thus becoming sensitive to NGF-induced apoptosis. We found that spinal cord extracts from ALS-affected SOD^{G93A} mice contained a hundred fold more active form of NGF than the mature factor. Becausereactive astrocytes and microglial cells expressing NGF also exhibit nitroxidative stress, we hypothesized that NGF could undergo post-translational nitrative modification by reaction with peroxynitrite to make it more active. This approach has allowed us to identify nitrated NGF species (nitroNGF) that are likely secreted by reactive astrocytes and exhibit apoptotic activity. In support to our hypothesis, other authors have reported the formation of nitroNGF in the brain of Alzheimer's disease patients.

Selected group's publications in ALS research:

- TRIAS et el. (2013) Phenotypic transition of microglia into astrocyte-like cells associated with disease onset in a model of inherited ALS. Front. Cell. Neurosci.
- MIQUEL et al. (2012). Modulation of astrocytic mitochondrial function by dichloroacetate improves survival and motor performance in inherited amyotrophic lateral sclerosis. PLoS One. 7:e34776.
- GANDELMAN et al (2011) P2X7 receptor-induced death of motor neurons by a peroxynitrite/FAS-dependent pathway. J Neurochem. 126:382-8.
- DIAZ-AMARILLA et al (2011) Phenotypically aberrant astrocytes that promote motoneuron damage in a model of inherited ALS. Proc Natl Acad Sci U S. 108:18126-31.
- GARRÉ et al (2010) FGF-1 induces ATP release from spinal astrocytes in culture and opens pannexin and connexin hemichannels. Proc Natl Acad Sci US107:22659-64
- GANDELMAN et al (2010) Extracellular ATP and the P2X(7) receptor in astrocyte-mediated motor neuron death: implications for amyotrophic lateral sclerosis. J. Neuroinflammation 9 :7-33
- BARBEITO et al (2010) Lead exposure stimulates VEGF expression in the spinal cord and extends survival in a mouse model of ALS. Neurobiol. of Disease 37:574-80.
- SOTELO-SILVEIRA et al (2009) Axonal mitochondrial clusters containing mutant SOD1 in transgenic models of ALS. Antioxidants & redox signaling 11:1535-1545.

- CASSINA,et al (2008) Mitochondrial dysfunction and oxidative stress in SOD1G93A –bearing astrocytes promote motoneuron degeneration. J. Neurosci. 28:4115-4122, 2008.
- DUPUIS et al (2008) Nogo receptor antagonizes p75NTR-dependent motor neuron death. Proc Natl Acad Sci 105:740-745.
- PEHAR et al (2007) Mitochondrial superoxide production and nuclear factor erythroid 2-related factor 2 activation in p75 neurotrophin receptor-induced motor neuron apoptosis. Journal of Neuroscience 29 :7777-7785.
- PEHARet al (2006) Modulation of p75-dependent motor neuron death by a small non-peptidyl mimetic of the neurotrophin loop 1 domain. European Journal of Neuroscience 6 :1575-1580.
- PEHAR et al (2006) Peroxynitrite transforms nerve growth factor into an apoptotic factor for motor neurons.. Free Radical Biology and Medicine 41:1632-1644.
- BARBEITOet al (2004) A Role for Astrocytes in Motor Neuron Loss in Amyotrophic Lateral Sclerosis. Brain Research Reviews 47:263-274.

Publications 2014

1. Isasi E, **Barbeito L**, Olivera-Bravo S. Increased blood-brain barrier permeability and alterations in perivascular astrocytes and pericytes induced by intracisternal glutaric acid. Fluids Barriers CNS. **2014** Jul 24;11:15.
2. Miquel E, Cassina A, Martínez-Palma L, Souza JM, Bolatto C, Rodríguez-Bottero S, Logan A, Smith RA, Murphy MP, **Barbeito L**, Radi R, Cassina P. Neuroprotective effects of the mitochondria-targeted antioxidant MitoQ in a model of inherited amyotrophic lateral sclerosis. Free Radic Biol Med. **2014** 70:204-13.

Grants

1. Fondo Clemente Estable ANII. FCE_1_2011_1_7342. Astrocitos fenotípicamente Aberrantes (células AbA): identificación de mecanismos y genes neurotóxicos. (2013-2014) Amount granted aprox USD 40.000
2. Movilidad Bilateral Uruguay-Brasil – DICYT (MEC) (2013-2015) Amount Granted USD 6.000.
3. Proyecto ECOS U014S02 "Mastocitos y neuroinflamación en enfermedades neurodegenerativas: caracteización de los mecanismos impllicados y nuevos blancos terapéuticos" 2014-2016.

Other activities

TRAINING COURSES

Regional Course & Symposium: "Neuron Glia Interactions in health and disease: from basic Biology to translational Neuroscience - 2nd edition" 29 september-2 october, 2014.

BioMolecular Simulation Laboratory

Head: Sergio Pantano, PhD

Members: ***Matías Machado, PhD*** (Staff Member)
Astrid Brandner (Msc. Student)
Gastón Hugo (Msc. Student)
Humberto Gonzalez (M.Sc. Student)
Steffano Silva (Undergraduate Student)

Research

The Group of BioMolecular Simulationsapplies and develops cutting-edge modeling and simulation methods to the study of problems of biomedical relevance described in the following paragraphs.

Research Lines

A. **Development of a coarse-grained force field for biomolecular simulations.**

During 2014 we finished a first stage of development of a general-purposes Coarse-Grained (CG) force field for biomolecular systems, for which we coined the name SIRAH. The force field currently includes parameters for simulating aqueous solvent and simple electrolytes, single/double stranded DNA and proteins. A substantial effort has been devoted to make the implementation of SIRAH user-friendly and straightforward in popular simulation packages. As a result, tarballs for using SIRAH on Amber and Gromacs (the two most popular molecular dynamics simulation packages) along with step-by-step tutorials and scripts for analysis and visualization were made available from our web site (www.sirahff.com). A paper describing the protein's parameterization was accepted in Dec. 2015 in J. Chem. Theory and Comput. (published in Feb. 2015).

As part of a Masters Thesis, we refined the interaction parameters for the study of protein-DNA complexes at the CG level and validated the results against a comprehensive set of data extracted from the PDB. This project was carried out in collaboration with the group of Prof. F. Melo, at the Catholic University of Chile and a manuscript and Master's Thesis are being prepared.

We also extended the development of SIRAH to improve and expand the capability of multiresolution solvation models. In particular, we created dual resolution solvation models for hybrid (CG-All Atoms) models of DNA (manuscript in preparation M. Machado and S. Pantano) and a new representation for supramolecular water that allows for three-layers of solvation. These models are currently applied to the study of the stability of the entire capsid of the Triatoma virus in collaboration with the group of Prof. M. Costabel (National University of the South, Bahia Blanca, Arg.).

Finally we also worked on the creation of CG phospholipids with the objective of simulating proteins embedded in a membrane environment. Perliminary versions of POPC showed a good representation of membrane patches in terms of area per lipid and thickness and are currently being used to study the gating mechanism of Connexin hemichannels.

B. **Cyclic Nucleotides Binding proteins and novel FRET indicators.**

In 2014we continued previous activities in the field of cyclic nucleotide binding proteins.

- In the framework of a new collaboration with the group of Dr. M. Edreira (UBA, Buenos Aires, Arg) we contributed to characterize novel cAMP binding proteins identified in *T. Cruzi* leading to propose PKA-independent cAMP signaling pathways in theparasite. These studies

resulted in a publication accepted in Molecular Biochemistry and Parasitology (published in Feb. 2015).

- Using simple geometry and structural information we elaborated a set of rules to predict the likeliness of chimeric constructs including GFP variants to become FRET sensors for cAMP concentrations in vivo. The results of this work were published in the Springer's series Methods in Molecular Biology (M.Machado and S.Pantano, published in Feb. 2015).

- In the context of an intramural transversal Project, we developed a novel architecture for a FRET biosensor to follow concentrations of cGMP base on the regulatory domain of PKG I. Preliminary results indicate that this new sensor maintain a similar affinity for cGMP as the native protein and selectivity for cGMP against cAMP. Remarkably, the dynamic range of this new construction is about 4-fold higher than those of existing cGMP sensors. An extensive characterization of this new protein will be carried out in collaboration with the groups of M. Comini, M. Bollati and F. Lecumberry at the IP Montevideo.

C. Alternative splicing of polypeptide-GalNAc-T13

We collaborated with the group of Prof. Osinaga to perform a structural characterization of the polypeptides product of alternative splicing of the polypeptide-GalNAc-T13. In this case, molecular modeling was used to predict the viability of a number of different polypeptides identified by Osinaga's group to achieve a functionally folded shape. A manuscript reporting on the Identification of a complex alternative splicing pattern in GalNAc-T13 is currently in preparation.

Publications 2014

- Sanguinetti M, Amillis S, **Pantano S**, Scazzocchio C and Ramón A. Modeling and mutational analysis of AspergillusnidulansUreA, a member of the subfamily of urea/H+ transporters in fungi and plants. Open Biology, **2014**, 4:140070

- Zecchin A, Pattarini L, Gutierrez MI, Mano M, Mai A, Valente S, Myers MP, **Pantano S**, and Giacca M. Reversible acetylation regulates vascular endothelial growth factor receptor-2 activity. Journal of Molecular Cell Biology, **2014**, 6:116.

Grants

1. Transversal Project – "Rational design of FRET sensors to monitor cyclic nucleotides concentration in vivo" – 2013-2014 – IP Montevideo
2. FOCEM – Funding to organize course in Coarse Grain modeling and meeting of south American researchers in molecular simulations
3. IUPAB– grant to support the organization of a PosLatAm course in Salto (Nov. 2015)
4. PEDECIBA – grant to support the organization of a joint meeting between the Argentinean and Uruguayan biophysical societies in Salto (Nov. 2015)

Other activities

- M.Machado and S. Pantano participated as invited scientists at the Latin-American Summit Crystallography and Complementary Methods at the CNPEM, Campinas, Brazil.

-S. Pantano participated as invited speaker at the meeting of the SBBq, Foz do Iguacu, Brazil.

-M.Machado co-organized and lectured at the course "Introducción al Análisis Estructural y Funcional de Proteínas" Institut Pasteur de Montevideo.

-A. Brandner delivered a seminar at Theoretical and Computational Biophysics Department, Max Planck Institute for Biophysical Chemistry, Goettingen, Germany.
-A. Brandner got a travel grant for the “EMBO practical Course on Biomolecular Simulations” at the Institut Pasteur, Paris.

Molecular and Human Genetics Laboratory

Head: José Badano, PhD

Members: ***Florencia Irigoín, PhD*** (Research associate)
Victoria Prieto, PhD (Postdoctoral Fellow)
Magdalena Cárdenas, MSc (Postdoctoral FellowPhD student)
Paola Lepanto (PhD student)
Rossina Novas, Bach (PhD student)
Belén Torrado (MSc student)
Matías Fabregat (MSc student)

GOALS

Since 2007, the Molecular and Human Genetics lab has been focused in the study of ciliary biology, trying to understand how cilia are formed/maintained and how do they function in order to gain insight into the role of this organelle in diverse aspects of the cell´s life. In recent years, different lines of evidence have shown that ciliary dysfunction underlies the pathogenesis of a broad group of human disorders collectively known as ciliopathies. This group of human pathologies is characterized by a number of phenotypes including retinal degeneration, cystic kidney disease, obesity, and diabetes [1-3]. One example of a ciliopathy, and the model we study in the lab, is Bardet-Biedl syndrome (BBS), a genetically heterogeneous disorder for which 19 genes have been cloned to date [4-9] and references within). The vast majority of BBS proteins tested to date localize to centrosomes, basal bodies and in some cases the ciliary compartment, and they participate in the transduction of important developmental signaling pathways such as Shh and Wnt (both canonical and the planar cell polarity "PCP" pathway) [10-23]. However, the exact biological role of these proteins is still not completely understood. In this context, we have uncovered a role of certain BBS proteins in the nucleus, and more recently, we are focusing on understanding the function of BBS proteins in intracellular trafficking[24, 25]. Therefore, on one hand we are studying individual ciliary proteins, such as several of the BBSs, and on the other hand we are tackling more general questions related to cilia biology.

Research

One protein in which we have been working is CCDC28B (coiled-coil domain containing protein 28b), a protein originally identified as a second site modifier of the BBS phenotype given that the mutation found in *CCDC28B* was not sufficient to cause BBS but did collaborate with mutations at *bona fide* BBS loci to modulate the penetrance and expressivity of the disorder [26]. Thus we started working in this protein of unknown function to both gain information regarding its role in cilia biology and to understand, at the cellular and molecular level, why it behaves as a modifier of BBS. Through a combination of bioinformatics, cellular and *in vivo* (zebrafish) studies we were able to determine that CCDC28B is a conserved protein restricted to metazoa that participates in the regulation of ciliary length. We showed that depletion of this protein both in cultured cells and zebrafish results in shortened cilia and thus *ccdc28b* morphant zebrafish embryos present with a number of cilia-associated phenotypes such as shortening of the body axis, smaller eyes, defects in the establishment of the left-right axis of symetry and hydrocephalus [27].

To understand the mechanism by which CCDC28B modulates cilia length we sought to identify proteins that physically interact with it. In a yeast two-hybrid screen we identify an interaction with the mTORC2 component SIN1. We were able to show that the CCDC28B/SIN1 interaction is relevant both in the context of cilia length regulation as well as modulating mTORC2. In the context of the mTOR complex our data showed that CCDC28B participates in its assembly and/or mediates its stability and thus, a depletion of CCDC28B results in decreased activity of the complex whereas its overexpression has the converse effect. Regarding the role of CCDC28B in cilia length regulation, we were able to show that this activity of the BBS modifier depends, at least in part, on its interaciont with SIN1 but independently of mTORC2 since i) *sin1* morphant embryos, but not other mTORC2 component (rictor), present with shortened cilia, ii) *ccdc28b* and *sin1* interact genetically and iii) overexpression of *sin1* can partially ameliorate the cilia defect in *ccdc28b* morphant embryos [28]. Interestingly, mTORC2 dysfunction resulted in cilia-related phenotypes albeit not affecting cilia directly. One possibility that we are currently exploring is that mTORC2 dysfunction could contribute to the pathogenesis of cilia-associated defects through a "PCP-like" phenotype, thus potentially providing a cellular explanation to the observed modifier effect of *CCDC28B*. Therefore, while we keep studying CCDC28B/SIN1 to gain mechanistic insight into their role in cilia regulation (CSIC Grant), the study of this particular protein has open new avenues of research in the lab.

Our initial studies on BBS7, which led to the demonstration that at least some BBS proteins play extraciliary roles in the nucleus modulating gene transcription [24], resulted in a similar process in the lab leading to a new line of research. In this project, which is being guided by Dr. Irigoín, we are interested in understanding the process of protein targeting to the cilium focusing on proteins that can localize to both the cilium and nucleus. A growing number of reports in the literature are highlighting striking similarities in the process of nuclear and cilia protein import (for example see Ref. [29]. We are focusing on a number of proteins that shuttle between these two cellular compartments, including some of the BBSs, to understand whether they used similar mechanisms and if so, identify the signals that allow them to choose between destinations. To this end, we are working on an interdisciplinary collaboration with another unit at the IPMon (UByPA) where we plan to use a combination of cell/molecular biology and mass spectrometry to explore this cilia-nucleus connection (Intramural IPMon Grant).

More recently we have started working on another BBS protein, BBS4, since we have identified interesting protein interactors potentially linked to at least some of the BBS typical phenotypes. Our results have highlighted a role of BBS4 and other BBS proteins on intracellular trafficking, an area of research in which we became especially interested through a collaboration with Dr. NorannZaghloul at University of Maryland, Baltimore, USA, who has shown that the BBS proteins participate in the intracellular trafficking of the Notch receptor[25]. Lastly, through collaboration with Dr. Flavio Zolessi, we are studying the role of cilia during the formation and differentiation of neuronal cell types, in particular retinal ganglion cells (FCE Grant).

1. Badano, J.L., et al., *The Ciliopathies: An Emerging Class of Human Genetic Disorders.* Annu Rev Genomics Hum Genet, 2006. **22**: p. 125-148.
2. Cardenas-Rodriguez, M. and J.L. Badano, *Ciliary Biology: Understanding the Cellular and Genetic Basis of Human Ciliopathies.* Am J Med Genet Part C Semin Med Genet, 2009. **151C**: p. 263-280.

3. Irigoin, F. and J.L. Badano, *Keeping the balance between proliferation and differentiation: the primary cilium.* Curr Genomics, 2011. **12**(4): p. 285-297.
4. Kim, S.K., et al., *Planar Cell Polarity Acts Through Septins to Control Collective Cell Movement and Ciliogenesis.* Science, 2010. **329**: p. 1337-1340.
5. Leitch, C.C., et al., *Hypomorphic mutations in syndromic encephalocele genes are associated with Bardet-Biedl syndrome.* Nat Genet, 2008. **40**: p. 443-448.
6. Marion, V., et al., *Exome sequencing identifies mutations in LZTFL1, a BBSome and smoothened trafficking regulator, in a family with Bardet--Biedl syndrome with situs inversus and insertional polydactyly.* J Med Genet, 2012. **49**: p. 317-321.
7. Otto, E.A., et al., *Candidate exome capture identifies mutation of SDCCAG8 as the cause of a retinal-renal ciliopathy.* Nat Genet, 2010. **42**: p. 840-850.
8. Scheidecker, S., et al., *Exome sequencing of Bardet-Biedl syndrome patient identifies a null mutation in the BBSome subunit BBIP1 (BBS18).* J Med Genet, 2014. **51**(2): p. 132-6.
9. Aldahmesh, M.A., et al., *IFT27, encoding a small GTPase component of IFT particles, is mutated in a consanguineous family with Bardet-Biedl syndrome.* Hum Mol Genet, 2014. **23**(12): p. 3307-15.
10. Ansley, S.J., et al., *Basal body dysfunction is a likely cause of pleiotropic Bardet-Biedl syndrome.* Nature, 2003. **425**: p. 628-633.
11. Blacque, O.E., et al., *Loss of C. elegans BBS-7 and BBS-8 protein function results in cilia defects and compromised intraflagellar transport.* Genes Dev, 2004. **18**: p. 1630-1642.
12. Fan, Y., et al., *Mutations in a member of the Ras superfamily of small GTP-binding proteins causes Bardet-Biedl syndrome.* Nat Genet, 2004. **36**: p. 989-993.
13. Gerdes, J.M., et al., *Disruption of the basal body compromises proteasomal function and perturbs intracellular Wnt response.* Nat Genet, 2007. **39**: p. 1350-1360.
14. Jin, H., et al., *The conserved Bardet-Biedl syndrome proteins assemble a coat that traffics membrane proteins to cilia.* Cell, 2010. **141**: p. 1208-1219.
15. Kim, J.C., et al., *The Bardet-Biedl protein BBS4 targets cargo to the pericentriolar region and is required for microtubule anchoring and cell cycle progression.* Nat Genet, 2004. **36**: p. 462-470.
16. Kim, J.C., et al., *MKKS/BBS6, a divergent chaperonin-like protein linked to the obesity disorder Bardet-Biedl syndrome, is a novel centrosomal component required for cytokinesis.* J Cell Sci, 2005. **118**: p. 1007-1020.
17. Li, J.B., et al., *Comparative genomic identification of conserved flagellar and basal body proteins that includes a novel gene for Bardet-Biedl syndrome.* Cell, 2004. **117**: p. 541-552.
18. Loktev, A.V., et al., *A BBSome subunit links ciliogenesis, microtubule stability, and acetylation.* Dev Cell, 2008. **15**: p. 854-865.
19. Marion, V., et al., *Transient ciliogenesis involving Bardet-Biedl syndrome proteins is a fundamental characteristic of adipogenic differentiation.* Proc Natl Acad Sci U S A, 2009. **10**: p. 1820-1825.
20. Nachury, M.V., et al., *A core complex of BBS proteins cooperates with the GTPase Rab8 to promote ciliary membrane biogenesis.* Cell, 2007. **129**(6): p. 1201-1213.
21. Ross, A.J., et al., *Disruption of Bardet-Biedl syndrome ciliary proteins perturbs planar cell polarity in vertebrates.* Nat Genet, 2005. **37**: p. 1135-1140.
22. Wiens, C.J., et al., *Bardet-Biedl syndrome-associated small GTPase ARL6 (BBS3) functions at or near the ciliary gate and modulates Wnt signaling.* J Biol Chem, 2010. **285**: p. 16218-16230.
23. Zhang, Q., et al., *BBS proteins interact genetically with the IFT pathway to influence SHH-related phenotypes.* Hum Mol Genet, 2012. **21**: p. 1945-1953.
24. Gascue, C., et al., *Direct role of Bardet-Biedl syndrome proteins in transcriptional regulation.* J Cell Sci, 2012. **125**: p. 362-375.
25. Leitch, C.C., et al., *Basal body proteins regulate Notch signaling through endosomal trafficking.* J Cell Sci, 2014. **127**(Pt 11): p. 2407-19.
26. Badano, J.L., et al., *Dissection of epistasis in oligogenic Bardet-Biedl syndrome.* Nature, 2006. **439**: p. 326-330.
27. Cardenas-Rodriguez, M., et al., *Characterization of CCDC28B reveals its role in ciliogenesis and provides insight to understand its modifier effect on Bardet-Biedl syndrome.* Hum Genet, 2013. **132**(1): p. 91-105.

28. Cardenas-Rodriguez, M., et al., *The Bardet-Biedl syndrome-related protein CCDC28B modulates mTORC2 function and interacts with SIN1 to control cilia length independently of the mTOR complex.* Hum Mol Genet, 2013. **22**(20): p. 4031-42.
29. Dishinger, J.F., et al., *Ciliary entry of the kinesin-2 motor KIF17 is regulated by importin-beta2 and RanGTP.* Nat Cell Biol, 2010. **12**(7): p. 703-10.

Research Lines

- CCDC28B and the BBS proteins in theregulation of ciliogenesis and cilia length.
- Cilia targeting: similaritieswiththe nuclear transportprocess
- BBS proteins in intracellulartrafficking: implicationsfor human disease
- Cilia in thedevelopment of the retina

Publications 2014

1- ShigunovShigunov P, Sotelo-Silveira J, Stimamiglio MA, Kuligovski C, **Irigoín F**, **BadanoJL**, Munroe D, CorreaA, Dallagiovanna B. Ribonomicanalysis of human DZIP1 revealsitsinvolvement in ribonucleoproteincomplexes and stress granules (**2014**) BMC Molecular Biology, 15, art. no. 12. – *IF: 2.057*

2- Leitch CC, Lodh S, **Prieto-Echagüe V**, **Badano JL**, Zaghloul NA. Basal body proteins regulate notch signaling through endosomal trafficking (**2014**) Journal of Cell Science, 127 (11), pp. 2391-2400. – *IF: 5.325*

Grants

1. Master Fellowship – Belén Torrado – 2013-2015 – ANII
2. Doctoral Fellowship – Paola Lepanto – 2013-2016 – ANII
3. Master Fellowship – Matías Fabregat – 2014-2016 – ANII
4. Fondo Clemente Estable – Dr. Flavio Zolessi – "Rol de las cilias y proceso de ciliogénesis durante la generación y diferenciación de neuronas en el sistema nervioso central de vertebrados."- 2013-2015 – ANII
5. CSIC Project – Florencia Irigoín – "Estudios funcionales y estructurales de CCDC28B, un modificador del Síndrome de Bardet-Biedl." – 2013-2015 – I+D Program, CSIC, UDELAR
6. Proyecto Transversal – Florencia Irigoín – "Protein sorting and transport to the ciliar and nuclear compartments: common and distinctive mechanisms" – 2013-2014 – IP Montevideo

Other activities

TRAINING COURSES

1. Molecular Biology of the Cell International Course, January 2014, Institut Pasteur Paris, Paris, France (RossinaNovas)

TRAINING OF STUDENTS

1. Internship in the Laboratory of Lucia Poggi, Dept. of Animal Physiology & Developmental Biology, COS, Heidelberg, Germany (Paola Lepanto)

CONGRESS

1. III LAZEN Meeting, April 11-13, Valparaíso, Chile (José Badano)
2. 64thLindau Nobel Laureate Meeting, Lindau, Germany (Magdalena Cárdenas)
3. Cilia 2014, Novembre 18th-21st, Institut Pasteur Paris, Paris, France (Paola Lepanto, FlorenciaIrigoín, José Badano)

Cellular Membranes Laboratory

Head: Pablo S. Aguilar, PhD

Members: ***Agustina Olivera-Couto*** (Ph.D. Student, *Plasma membrane organization*)
Marcos Nieves (M. Sc. Student, *Plasma membrane signaling*)
Natalia Carbó (Postdoc, *Cell-cell fusion)*
Daniela Megrian (M. Sc. Student, *Cell-cell fusion)*
Andrés Cabrera (Technician)

GOALS

Our scientific proposal is to understand basic cellular processes that take place at the plasma membrane

Research

Focusing on the plasma membrane, we seek to understand how the cell organizes at its boundaries.

Research Lines

A. EISOSOMES AND PLASMA MEMBRANE ORGANIZATION

Eisosomes define sites of endocytosis in Saccharmoycescerevisiae (Nature, 439: 998-1003, 2006). These structures, lay underneath the plasma membrane and are mainly composed by thousands of copies of two paralog proteins Pil1 and Lsp1 (Mol Bio Cell, 20, 809-818, 2009). Eisosomes are plasma membrane domain organizers that concentrate lipids and proteins around them. In doing so, they are thought to help coordinate spatial and temporal execution of diverse plasma membrane functions such as small molecules transport, signaling and bulk endocytosis (EMBO J., 26, 4946-4955, 2007; J Cell Biol. 185:1227-1242, 2009; Nat Struct Mol Biol, 17: 901-908, 2010).

We have found that Pil1 and Lsp1 belong to a superfamiliy of proteins that work as membrane shape modifiers. Overall, our results support a model where eisosome core proteins-mediated bending of the plasma membrane is followed by local recruitment of proteins and lipids resulting in stable plasma membrane domains (Mol Bio Cell, 22: 2360-2372, 2011, Mol Genet Genomics 287: 607-620, 2012).

We are currently challenging this model applying different live fluorescence microscopy approaches. By studying the dynamics of eisosome biogenesis we expect to build a quantitative model of plasma membrane domain organization.

B. CELL-CELL FUSION

Eukaryotic cells use intracellular membrane fusion events to move cargo through the various compartments while maintaining compartmental identity. Membrane fusion also when gametes fuse their cell membranes to form zygotes and when enveloped viruses deliver their genome into host cells during infection. Membrane fusion reactions are catalyzed by proteins collectively referred to as “fusases”. Remarkably, to this day, only intracellular and viral fusases have been identified and characterized. Cell-cell fusion stands as the field where the fusases still remain elusive.

We use yeast mating as a model of cell-cell fusion. We identified new proteins involved in this process and studied the role that extracellular calcium plays in cell fusion and cellular lysis (*Mol Biol Cell.* 18, 547-556, 2007). We also determined the structural role that sterols

play during cell polarization and cell fusion (*Proc NatlAcadSci USA.*, 107: 4170-4175, 2010). Overall, our results support a model in which the still unidentified cell fusion machinery promotes both: fusion and lysis. In collaboration with the Bioinformatics Unit of the IP Montevideo we are currently executing a battery of genetic and bioinformatic screens to identify cellular fusases. Envisioning a large set of data to be experimentally analyzed we are currently devolping a high-throughput cell-cell fusion assay in collaboration with the Cell Biology Unit of our institute. We are also analyzing the role of different genes involved in pheromone-induced calcium uptake as lysis enhancers.

C. ***CELL BIOLOGY OF BACTERIAL SIGNAL TRANSDUCTION***

In collaboration with Alejandro Buschiazzo's lab (IP Mont) and Mathieu Picardeau (IP Paris) we are addressing the subcelular localization of the two-component fluidity sensor DesK-DesR. In Gram-positive bacteria this system is crucial to adjust plasma membrane fluidity according to need (*EMBO J.* 20, 1681-1691, 2001; *Proc NatlAcadSci USA*, 106: 16185-16190, 2009). Subcelular compartmentalization of signal transduction systems is a common eukayotic feature that is currently poorly studied in prokaryotic organisms. We hope to expand the knowledge we gain throughout this model to study similar systems in the pathogenic bacteria of the genus *Leptospira*.

Publications 2014

1. **Pizzo L,** Fariello MI, **Lepanto P, Aguilar PS, Kierbel A**. An image analysis method to quantify CFTR subcellular localization. Molecular and CellularProbes, Volume 28, Issue 4, August **2014**, Pages 175-180, ISSN 0890-8508. – *IF: 1.859*
2. Zhang S, Zheng H, Long N, **Carbó N,** Chen P, **Aguilar PS**, Lu L. FigA, a putative homolog of low-affinity calcium system member Fig1 in Saccharomyces cerevisiae, is involved in growth and asexual and sexual development in Aspergillusnidulans (**2014**) Eukaryotic Cell, 13 (2), pp. 295-303. – *IF: 3.179*
3. **Lepanto P,** Lecumberry F, **Rossello J, Kierbel A**. A confocal microscopy image analysis method to measure adhesion and internalization of Pseudomonas aeruginosa multicellular structures into epithelial cells (**2014**) Molecular and Cellular Probes, 28 (1), pp. 1-5. – *IF: 1.859*
4. Giorello, F.; Berná, L.; Greif, G.; Camesasca, L.; **Salzman, V**.; Medina, K.; Robello, C.; Gaggero, C.; **Aguilar, P.S.** and Carrau, F. (**2014**) Genomesequence of the native apiculate wine yeast Hansenia sporavineae T02/19AF. ***GenomeAnnouncements***,2(3). pii: e00530-14. doi: 10.1128/genomeA.00530-14.
5. Trajtenberg, F.; Albanesi, D.; Ruétalo, N.; Botti, H.; Mechaly, A.E.; **Nieves, M**.; **Aguilar, P.S.**; Cybulski, L.; Larrieux, N.; de Mendoza, D. and Buschiazzo, A. (**2014**) Allostericactivation of bacterial response regulators: the role of the cognate histidine kinase beyond phosphorylation. ***MBio.*** 18;5(6):e02105. doi: 10.1128/mBio.02105-14.

Grants

1. "Yeast Mating as a model of Cell-Cell Fusion". International Centre for Genetic Engineering and Biotechnology Collaborative Research Programme (ICGEB-CRP). Funding period 2012-2015. € 57.000.

2. “Cell signaling in bacterial pathogenesis: iron metabolism regulation in Leptospira as a working model”. Réseau International des Institut Pasteur (RIIP). Funding period: 2012-2013. € 21.900.
3. “Apareamiento de levaduras como modelo de fusión célula-célula”. ANII-FCE-6682. Fundingperiod: 2013-2014. USD 28.000.
4. “Análisis cuantitativo de ensamble de dominios de membrana mediante microscopía de fluoresencia in vivo.” ANII-FCE-5942. Fundingperiod: 2013-2014. USD 13.000.

Other Funding:

- ✓ Agustina Olivera-Couto, ANII Doctoral fellowship, 2013-2015. Journal of Cell Science and American Society and international Union for Biochemistry and Molecula Biology, (ASBMB/IIUBMB) Traveling Awards, 2013.
- ✓ Natalia Carbó, ANII- Caldeyro Barcia postdoctoral fellowship, 2013-2015.
- ✓ Marcos Nieves,ANII Doctoral fellowship, 2014-2015.

Redox Biology of Trypanosomes Laboratory

Head: Marcelo Comini, PhD

Members: ***Andrea Medeiros*** (Postdoc)
Mariana Bonilla (Postdoc)
Bruno Manta (Postdoc)
Cecilia Ortíz (PhD student)
Diego Benitez (PhD student)
Lucía Fiestas (MSc Student, finished in Oct. 2014)
Florencia Sardi (MSc Student)
Jaime Franco (MSc Student)
Sofía Zardo (BSc Student)
Cecilia Maciel (BSc Student)
Matías Deambrosi (Technical assistant)
Oliver Orban (traineeship PhD student)

GOALS

Our scientific proposal is to contribute to the understanding of the redox biology of pathogenic trypanosomatids, as well as to the discovery and characterization of anti-trypanosomatid drug candidates.

Research

Several trypanosomatid species cause highly disabling and often fatal diseases of human and live-stock (e.g. African sleeping sickness, Chagas' disease, black-fever and Nagana cattle-disease, and Sura disease), for which safe and efficacious treatments are lacking. Trypanosomatids have evolved a specific thiol-redox metabolism that relies on the use of bis-glutathionylspermidine (trypanothione) as redox cofactor and thiol ligand. Trypanothione is involved in a variety of essential cellular processes and, important from a therapeutic point of view, is absent in mammals. By means of a multidisciplinary approach, research in our laboratory aims at:

1- gain further understanding into trypanothione-dependent metabolism by studying its synthesis, recycling and role in several cell functions,
2- develop and exploit the use of novel redox biosensors to unravel fundamental questions on parasite biology, host/parasite interaction and phenotype-based compound screening,
3- identify and characterize novel drug target candidates.

Research Lines

A. FUNDAMENTAL ASPECTS OF TRYPANOTHIONE METABOLISM: SYNTHESIS, REDUCTION AND UTILIZATION

By a multidisciplinary approach we study the biochemical, structural and biological features that distinguish several key components of the trypanothione system. Using animal infection models we further investigate the role these (macro)molecules play in parasite biology and pathogenesis. The data from these studies is used to validate drug target candidates and guide novel drug development strategies.

B. ***MONITORING INTRACELLULAR REDOX CHANGES WITH NOVEL REDOX BIOSENSORS***

Short lived reactive oxygen species act as second messengers producing changes in the redox poise of relevant redox couples within biological systems. A steadily increasing number of evidences support a key role for redox signaling in the regulation of a wide diversity of cellular and (patho) physiological processes. The measurement of physiological oxidants and intracellular redox changes on real-time and by non-invasive methods has recently been possible due to the development of fluorescent redox biosensors [Meyer and Dick 2010 Antiox Redox Signal]. Transgenic cell lines of *T. brucei* and *T. cruzi* expressing a redox biosensor have been generated in our laboratory and are currently used to address the role of redox signaling and oxidative stress in events such as parasite-host interaction, cell differentiation, cell cycle, apoptosis and metabolic dysfunction. The reporter cell lines are also employed in phenotypic drug-screening campaigns.

C. ***EARLY PHASE DRUG DISCOVERY PROJECTS***

We apply target- and phenotypic-based approaches to screen synthetic and natural compounds against the enzyme responsible of trypanothione biosynthesis in different trypanosomatid species and infective forms of the pathogens. Compound mode of action at cellular and enzyme level is studied to drive drug optimization. To conduct these studies our laboratory relies on an important network of local and international groups working on (medicinal) chemistry. In 2014, our group joined the European Consortium COST "Targeted chemotherapy towards diseases caused by endoparasites" (Action CM1307) and became member of "The Research Network Natural Products against Neglected Diseases" (ResNetNPND).

Publications 2014

1- Randall LM, **Manta B**, Hugo M, Gil M, Batthyàny C, Trujillo M, Poole LB, Denicola A. Nitration transforms a sensitive peroxiredoxin 2 into a more active and robust peroxidase (**2014**) Journal of Biological Chemistry, 289 (22), pp. 15536-15543. – *IF: 4.6*

2- Maiwald F, **Benítez D, Charquero D**, Dar MA, Erdmann H, Preu L, Koch O, Hölscher C, Loaëc N, Meijer L, **Comini MA**, Kunick C. 9- and 11-substituted 4-azapaullones are potent and selective inhibitors of African trypanosome (**2014**) European Journal of Medicinal Chemistry, 83, pp. 274-283. – *IF: 3.432*

3- Sturlese M, Lelli M, **Manta B**, Mammi S, **Comini MA,** Bellanda M. 1H, 13C and 15N resonance assignment of the mature form of monothiol glutaredoxin 1 from the pathogen Trypanosoma brucei (**2014**) Biomolecular NMR Assignments. – *IF: 0.82*

4- Peña S, Fagundez C, **Medeiros A**, **Comini M**, Scarone L, Sellanes D, Manta E, Tulla-Puche J, Albericio F, Stewart L, Yardley V, Serra G. Synthesis of cyclohexapeptides as antimalarial and anti-trypanosomal agents (**2014**) MedChemComm, 5 (9), pp. 1309-1316. – *IF: 2.62*

5- Sousa AF, Gomes-Alves AG, **Benítez D, Comini MA**, Flohé L, Jaeger T, Passos J, Stuhlmann F, Tomás AM, Castro H. Genetic and chemical analyses reveal that trypanothione synthetase but not glutathionylspermidine synthetase is essential for Leishmania infantum (**2014**) Free Radical Biology and Medicine, 73, pp. 229-238. – *IF: 5.71*

6- Hiller C, Nissen A, **Benítez D, Comini MA**, Krauth-Siegel RL. Cytosolic Peroxidases Protect the Lysosome of Bloodstream African Trypanosomes from Iron-Mediated Membrane Damage (**2014**) PLoS Pathogens, 10 (4), art. no. e1004075. – *IF: 8.057*

Grants

1. Fiocruz-Pasteur Grant - “Trypanosoma´s prostaglandin metabolism: role in infection, pathogenesis and drug resistance”, 2014-2016. M. Comini (Principal Investigator).
2. National Institute of Health USA, NIH (IRIDA) Program (R01) - “Trypanosoma cruzi Antioxidant System”, 2011-2014. M. Comini (Associate Researcher).

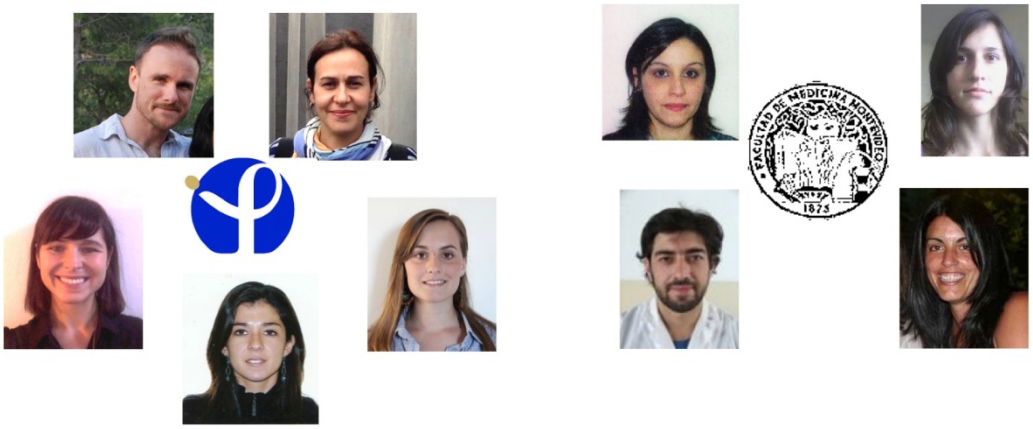
Other activities

EDUCATION AND TRAINING

1. Members of the lab. participated as lecturer and/or instructor in the international courses “Flow Cytometry and Cell Sorting in Biotechnology and Biomedicine Research” (March 17-28, 2014) and “Modern Approaches in Drug Discovery for Neglected Infectious Diseases” (Nov 3-8, 2014), both held in Montevideo, Uruguay.
2. A research traineeship in protein NMR techniques was performed by B. Manta at the Department of Chemical Sciences, University of Padova, Italy (Oct-Dec, 2014).
3. A research traineeship in leishmanial infection models (*in vitro* and *in vivo*) was performed by A. Medeiros at the Laboratory of Immunoparasitology, Instituto Fiocruz Bahia, Brazil (Oct.-Nov. 2014).
4. The PhD student Oliver Orban from the Teschnische Universitaet Braunschweig performed an 8-weeks research traineeship in our laboratory (Sep-Oct, 2014).

Neuroinflammation and Gene Therapy Laboratory

Head: Hugo Peluffo, PhD



Members: **Natalia Lago** (PhD, Assitant Researcher)
Luciana Negro (PhD Student)
Emilia Villamil (Student)
Alejandra Silva Santisteban (MD Student)
Nathalia Vitureira (PhD, Associate Researcher)

Members at the Faculty of Medicine:
Daniela Alí (MSc Student)
Eliseo Taranto (MD)
Daniela Blanco (MSc Student)

GOALS

The group is devoted to the understanding of the role of neuroinflammatory checkpoints in traumatic nervous system injuries. We belive the targeting of neuroinflammation by advanced gene therapy strategies will produce effective therapeutics for traumatic brain injury and spinal cord injury.

Research

Nervous System Traumatic Injuries. Traumatic injuries to the Nervous System, including traumatic brain injury (TBI) and spinal cord injury (SCI), remain one of the leading causes of mortality and morbidity in both industrialized and developing countries, being of increased importance in the latter. TBI is frequently referred to as the "silent epidemic", as beyond symptoms like paralysis, additional complications such as changes affecting intellectual abilities, sensation, language, or emotions, may not be readily apparent. In fact, studies including several European countries showed that TBI resulted between the highest injury burden pathologies due to permanent disability, and among the highest costs for the health system. Extensive efforts have been made to reach neuroprotective therapies for these devastating disorders, but despite interesting preclinical results, no successful outcomes have been observed in human clinical trials to date. Following the initial mechanical insult, focal TBI and SCI results in a complex delayed secondary progressing injury due to anatomical, neurochemical, metabolic, inflammatory and cellular changes that account for many of the neurological deficits observed. Inflammatory and immune reactions are present in all acute and chronic neurological pathologies. Interestingly, these processes are not only a consequence of neurodegeneration but also a critical mediator of the neurotoxic or neuroprotective mechanisms. Thus their modulation has emerged as an important therapeutic opportunity.

Neuroinflammation and CNS Damage. Although the brain has long been considered an "immune-privileged" organ, this status is far from absolute. CNS cells have innate immune functions and express a range of receptors capable of detecting and clearing apoptotic cells and regulating inflammatory responses. Among them, bone marrow-derived MICROGLIAL CELLS, are the main nervous component of the innate immune system. Resident microglia survey the CNS and act as the first line of defense against pathogen invasion by recognizing, sequestering and processing antigens, but also participate in processes regarding neuronal communication and homeostasis. Acute lesions induce tissue damage and neurodegeneration

which, in turn, incite an inflammatory response characterized by the activation of microglia, astrocytes, endothelial cells, blood leukocytes, in a process highly dependent on the type of injury and the degree of tissue damage. Several evidences have shown that the production of inflammatory molecules and oxidative stress by inflammatory cells determine the final extent of tissue damage and the death or survival of neuronal cells in surrounding or distally connected areas. Moreover, the inflammatory processes also contribute to the barriers for regeneration and plasticity.

Control of Inflammation. Inflammation is a set of complex interactions between soluble factors, extracellular matrix and cells, which is induced in any tissue in response to injury or infection. Inflammation in peripheral organs normally leads to tissue recovery, but if destruction of pathogens and resolution of damaged cells and matrix are not adequately controlled, inflammation can lead to persistent damage. For this reason, checkpoints for the control of inflammatory mechanisms, which are induced as a result of the activation of the inflammatory cascade, have gained a high degree of importance and interest in the field of immunology. Importantly, recent findings suggest that the anti-inflammatory state is not only a passive state resulting from an absence of inflammatory stimuli, but an active condition that requires participation of several molecules responsible for the supression of potentially inflammatory stimuli. **This is one of the central hypothesis of our research group.**In this sense, regulation of immune cell function by inhibitory/regulatory receptors has been characterized in the immune system, and just recently few studies have attempted their participation in the regulation of microglial cell activation after acute CNS injury. Balance between the destructive/protective events of the innate response must be precisely regulated in order to limit initial toxicity and promote CNS repair and a return to homeostatic conditions. In the last few years, promising activating/inhibitory immune receptors have been highlighted as new targets for the control and modulation of microglia/macrophage responses include the CD200/CD200R system, TREM-2 receptor and the recently described family of CD300 receptors [1-5].

CD300 Family of Receptors.

The human IREM/CD300 family of activating/inhibitory receptors is composed by six members, CD300a/IRP60, CD300b/IREM3, CD300c/CMRF35, CD300d, CD300e/IREM2 and CD300f/IREM1[6]. The importance of this family of receptors is highlighted by the fact that CD300a is the second gene with strongest evidence for positive selection between human and chimpanzee[7]. Moreover, CD300a and CD300f are among the 10 highest genes upregulated after rat SCI[8]. All of the members share an extracellular region comprising a single Ig-like domain and, with the exception of CD300a, a myeloid linage restricted pattern of expression. Two of the activating members, CD300b and CD300e, fit to the classical scheme for activating receptors with a positive charge within their membrane domain. They recruite the transmembrane adaptor molecule DAP-12 through the positive charge in its transmembrane domain andhave a functional tyrosine residue in its cytoplasmic tail able to recruit Grb2,thus signalling through two different pathways. The CD300 family contains two inhibitory receptors, CD300a and CD300f. Both display a long cytoplasmic tail with a variety of different tyrosine-based motifs and both are able to recruit SHP-1 phosphatase and therefore deliver inhibitory signals. The most interesting difference between these molecules, besides their different pattern of expression, is the existence of two binding motifs for the p85 subunit of PI3Kinase in

the cytoplasmic tail of CD300f. In fact, it has been shown that CD300f delivers in vitro both inhibitory and activating signals, thus revealing a remarkable functional duality of this receptor, similar to what has been shown for TREM2, another dual activating receptor. However, in vivo CD300f has shown to be mainly an inhibitory receptor, as shown in CD300f knockout animals in the EAE model of Multiple Sclerosis[4], and very recently in several models of Allergy[9]and in a model of Lupus Eritematosus[10]. It has been shown that the CD300 receptors are able to form complexes on the cell surface through the interaction among their extracellular immunoglobulin domain,and their combination in a complex differentially modulated the signalling outcome, suggesting how CD300 complexes could regulate the activation of myeloid cells upon interaction with their natural ligands. It was published that the T cell immunoglobulin mucin (TIM) proteins TIM-1 and TIM-4, which regulate T cell activation and tolerance, are ligands for the mouse CD300b receptor [49]. However, very recent reports suggest the existence of othermain ligands for the CD300 receptors as the phospholipids phosphatidylserine, phosphatidylcholine or sphingomyelin and ceramide.

Innovative Gene Therapy Strategies For Traumatic Injury of The Nervous System. The introduction of functional genes into an organism, as well as the regulation of gene expression has emerged in the past few decades as a powerful tool for treating or correcting multiple pathologies. To date, over 2076 gene therapy clinical trials have been completed, are ongoing or have been approved worldwide. The first commercial gene therapy drug has been approved and there are several advanced clinical trials (75 in phase III and 2 in phase IV), showing the important development of this area. One of the main focuses in gene therapy has been the development of sophisticated delivery systems, which can constitute the bottleneck for the achievement of clinical effects. As virus are evolutionary optimized for this purpose, viral vectors tend to be the most effective carriers of nucleic acids into foreign cells. In particular, several vectors show promising features for the use in the nervous system including non-integrating lentiviral vectors and some serotypes of Adenoassociated viral vectors with blood-brain barrier crossing potential. One of the most popular types of viral vectors for treating CNS disorders are HIV 1-derived lentiviral vectors. They have been tailored in the past years to reduce their biological risks and to display several features that make them excellent candidates for treating CNS disorders including low immunogenicity and transduction of post-mitotic cells. Non-viral vectors have also gained attention, and in particular, vehicles based on multifunctional proteins in DNA complexes constitute a very versatile type of carriers for therapeutic nucleic acids. They are constructed by the combination of appropriate functional domains fused in a single polypeptide chain[11]. This approach has generated the first prototypes of modular recombinant protein nano-vectors where the integrated domains enable the whole construct to mimic the infective viral cycle, which is necessary to the targeted delivery of nucleic acids. Thus, this type of nanoparticles have been also termed artificial virus. The modular nature of such constructs allows the selection of different features using well-characterized peptides and a functional redesign in iterative improvement processes. Several of these nano-vectors have shown successful transfection *in vitro* and therapeutic effects *in vivo*, suggesting their potential in the clinical context. One of these, the modular nano-vector termed NLSCt, is based on the tetramer carrier protein β-Galactosidase engineered with a polylysine K10 tail which bind and condense DNA, a NLS motif for nuclear localization and an prototypic integrin-interacting RGD domain which binds to membrane integrins and promotes cell internalization. We showed for the first time that these types of

vectors induce biologically relevant concentrations of transgenic protein after acute excitotoxic brain injuries[5, 12-15]. Interestingly, the RGD interacting motif of the NLSCt protein was able to induce neuroprotection *per se*, enabling the possibility of directing rapid actions of the vectors through the selection of their functional motifs, constituting the proof-of-principle for a "trophic vector"[16]. The modular principles underlying the NLSCt vector were further improved by generating two smaller nano-vectors termed HKRN and HNRK, based on alternative direct combination of the three functional domains RGD, NLS and K10 in a single small polypeptide, with the addition of a poly-histidine domain H6 that provides endosomal escape and purification properties. These nano-vectors achieved significant transgene expression levels in culture cells, and in vivo after a TBI [17].

Importantly, the original hypothesis that a very efficient vector could be used for most gene therapy application has evolved to the notion that each particular pathological condition may need a particular vector. For instance, for the treatment of acute traumatic CNS injuries, a gene therapy vector should induce a rapid but not permanent induction of transgene expression, it should not be proinflammatory as inflammation is a key mediator of the neuropathology, and a specific cell type may or may not needed to be targeted depending on the mechanism of action of the transgene. However other variables are less evident: i) which are the desired levels of transgene expression? ii) Which is the ideal time frame of expression? iii) Should the vector induce widespread or a localized transduction? The lack of detailed comparisons of different types of vectors in the same model under identical conditions hamper the selection of the best vector under these particular pathological conditions. This has an important impact on useful translational medicine approaches, were detailed comparative estudies are essential. **In fact, this constitutes one of the main focuses of our research group**.

1. Hoek RM, Ruuls SR, Murphy CA, Wright GJ, Goddard R, Zurawski SM, Blom B, Homola ME, Streit WJ, Brown MH, et al: **Down-regulation of the macrophage lineage through interaction with OX2 (CD200).***Science* 2000, **290:**1768-1771.
2. Kleinberger G, Yamanishi Y, Suarez-Calvet M, Czirr E, Lohmann E, Cuyvers E, Struyfs H, Pettkus N, Wenninger-Weinzierl A, Mazaheri F, et al: **TREM2 mutations implicated in neurodegeneration impair cell surface transport and phagocytosis.***Sci Transl Med* 2014, **6:**243ra286.
3. Neumann H, Takahashi K: **Essential role of the microglial triggering receptor expressed on myeloid cells-2 (TREM2) for central nervous tissue immune homeostasis.***J Neuroimmunol* 2007, **184:**92-99.
4. Xi H, Katschke KJ, Jr., Helmy KY, Wark PA, Kljavin N, Clark H, Eastham-Anderson J, Shek T, Roose-Girma M, Ghilardi N, van Lookeren Campagne M: **Negative regulation of autoimmune demyelination by the inhibitory receptor CLM-1.***J Exp Med* 2010, **207:**7-16.
5. Peluffo H, Ali-Ruiz D, Ejarque-Ortiz A, Heras-Alvarez V, Comas-Casellas E, Martinez-Barriocanal A, Kamaid A, Alvarez-Errico D, Negro ML, Lago N, et al: **Overexpression of the immunoreceptor CD300f has a neuroprotective role in a model of acute brain injury.***Brain Pathol* 2011, **22:**318-328.
6. Borrego F: **The CD300 molecules: an emerging family of regulators of the immune system.***Blood*.
7. Nielsen R, Bustamante C, Clark AG, Glanowski S, Sackton TB, Hubisz MJ, Fledel-Alon A, Tanenbaum DM, Civello D, White TJ, et al: **A scan for positively selected genes in the genomes of humans and chimpanzees.***PLoS Biol* 2005, **3:**e170.
8. Torres-Espin A, Hernandez J, Navarro X: **Gene expression changes in the injured spinal cord following transplantation of mesenchymal stem cells or olfactory ensheathing cells.***PLoS One* 2013, **8:**e76141.
9. Izawa K, Yamanishi Y, Maehara A, Takahashi M, Isobe M, Ito S, Kaitani A, Matsukawa T, Matsuoka T, Nakahara F, et al: **The receptor LMIR3 negatively regulates mast cell activation and allergic responses by binding to extracellular ceramide.***Immunity* 2012, **37:**827-839.
10. Tian L, Choi SC, Murakami Y, Allen J, Morse HC, 3rd, Qi CF, Krzewski K, Coligan JE: **p85alpha recruitment by the CD300f phosphatidylserine receptor mediates apoptotic cell clearance required for autoimmunity suppression.***Nat Commun* 2014, **5:**3146.
11. Peluffo H: **Modular Multifunctional Protein Vectors for Gene Therapy.** In *Non-Viral Gene Therapy.* Edited by Yuan X: InTech; 2011: 597-614

12. Peluffo H, Acarin L, Aris A, Gonzalez P, Villaverde A, Castellano B, Gonzalez B: **Neuroprotection from NMDA excitotoxic lesion by Cu/Zn superoxide dismutase gene delivery to the postnatal rat brain by a modular protein vector.***BMC Neurosci* 2006, **7:**35.
13. Peluffo H, Aris A, Acarin L, Gonzalez B, Villaverde A, Castellano B: **Nonviral gene delivery to the central nervous system based on a novel integrin-targeting multifunctional protein.***Hum Gene Ther* 2003, **14:**1215-1223.
14. Gonzalez P, Peluffo H, Acarin L, Villaverde A, Gonzalez B, Castellano B: **Interleukin-10 overexpression does not synergize with the neuroprotective action of RGD-containing vectors after postnatal brain excitotoxicity but modulates the main inflammatory cell responses.***J Neurosci Res*, **90:**143-159.
15. Peluffo H, Gonzalez P, Acarin L, Aris A, Beyaert R, Villaverde A, Gonzalez B: **Overexpression of the nuclear factor kappaB inhibitor A20 is neurotoxic after an excitotoxic injury to the immature rat brain.***Neurol Res* 2013, **35:**308-319.
16. Peluffo H, Gonzalez P, Aris A, Acarin L, Saura J, Villaverde A, Castellano B, Gonzalez B: **RGD domains neuroprotect the immature brain by a glial-dependent mechanism.***Ann Neurol* 2007, **62:**251-261.
17. Negro-Demontel ML, Saccardo P, Giacomini C, Yáñez-Muñoz RJ, Ferrer-Miralles N, Vazquez E, Villaverde A, Peluffo H: **Comparative analysis of lentiviral vectors and modular protein nanovectors for traumatic brain injury gene therapy.***Molecular Therapy — Methods & Clinical Development* 2014, **1:**14047.

Publications 2014

1. **Lago N**, Quintana A, Carrasco J, Giralt M, Hidalgo J, Molinero A. Absence of metallothionein-3 produces changes on MT-1/2 regulation in basal conditions and alters hypothalamic-pituitary-adrenal (HPA) axis. *Neurochemistry International* Volume 74, July **2014**, Pages 65-73. – *IF: 2.65*
2. Negro-Demontel ML**,**Saccardo P, Giacomini C, Yáñez-Muñoz RG, Ferrer-Miralles N, Vazquez E, Villaverde A & **Peluffo H**. Comparative analysis of lentiviral vectors and modular protein nanovectors for traumatic brain injury gene therapy. Molecular Therapy — Methods &Clinical Development 1, Article number: 14047 (**2014**) Published online 15 October 2014. – *IF: Molecular Therapy 6,42, Sister journal Mol. Ther. Meth. CLin. Dev. Waiting for IF.*

Grants

1. Project FundacióMarató TV3, Catalunya, España. “Modulation of immune receptors function as a novel therapeutic strategy for acute CNS damage”. (2012-2014) Amount Granted € 80.000.
2. Project CSIC-UDELAR: “Gene therapy applied to brain trauma: comparative preclinical studies using modular recombinant vectors and lentiviral vectors”. (2013-2014) Amount granted USD 40.000.

Other activities

TRAINING COURSES

Regional Course&Symposium: "Neuron Glia Interactions in health and disease: from basic Biology to translationalNeuroscience - 2nd edition" 29 september-2 october, 2014.

Cell Biology of Neural Development Laboratory

Head: Flavio Zolessi, PhD.

Members: ***Gonzalo Aparicio*** (Master Student)
Camila Davison (Master Student)
Paola Lepanto (Doctoral Student, co-directed with J. Badano)
Ileana Sosa (Master Student, co-directed with G. Bedó, UdelaR)

GOALS

The CBND lab is a joint Unit between Institut Pasteur de Montevideo and Facultad de Ciencias, Universidad de la República (UdelaR). Its main mission is the establishment of a research group on the basic aspects of neural development in vertebrates, with a focus in neuronal differentiation and survival in relation to cell polarity. In addition, the group has a strong involvement in teaching, as most members are part of the Cell Biology Section at Facultad de Ciencias, and actively participate in cell biology and developmental biology courses for undergraduates.

Research

In vertebrates, the central nervous system neurons arise from an extremely ordered tissue, the neuroepithelium. Our group is interested in understanding the mechanisms that underlie neuroepithelial differentiation (during neurulation) and neuronal differentiation, focusing in the roles and transitions of cell polarity during these processes. For this, we use both zebrafishans chick embryos. The zebrafish has great advantages for these studies, such as the accessibility to genetic manipulation and the unique optical transparency of embryos.

Publications 2014

1. Prieto, D., **Aparicio, G.**, Morande, P.E.**, Zolessi, F.R.** A fast, low cost, and highly efficient fluorescent DNA labeling method using methyl green (**2014**) Histochemistry and Cell Biology, 142 (3), pp. 335-345. – *IF: 2.*927
2. Tinoco L.W., Fraga J.L., AnoBom C.D., **Zolessi F.R.**, Obal G., Toledo A., Pritsch O., Arruti C. (**2014**) Structural characterization of a neuroblast-specific phosphorylated region of MARCKS. BiochimBiophysActa 1844(4): 837-849.

Grants

1. FCE 5888 – ANII, Uruguay. F. Zolessi.
2. Initiation to Research Grant – CSIC, UdelaR, Uruguay. G. Aparicio.

Other activities

TRAINING COURSES

1. IV Course and Symposium on Development and Plasticity of the Nervous System. Montevideo, Uruguay. Organizers: F. Zolessi, M. Brauer (IIBCE), F. Rossi (UdelaR). 06/10-08/11.

2. ICY Training Course –Program (Internal IP Montevideo training course). Instructor: A. Dufour (IP Paris). Organizers: F. Zolessi, P. Aguilar, F. Lecumberry.15-17/12.

TRAINING OF STUDENTS

María Eugenia Cruces. Undergraduate Thesis in Biology.
Director: Mercedes González; Co-director: F. Zolessi. Training on Fish Embryo Toxicity (FET) test for potential therapeutical compounds using zebrafish.

CONGRESSES

XV Jornadas de la Sociedad Uruguaya de Biociencias (SUB). Piriápolis, Uruguay. 5-7/09. President: F. Zolessi.

SCIENCE DIFFUSION

1- Brain Awareness Week, Montevideo, Uruguay.

2- Semanacyt (Science and Technology Week). Uruguay.

3- Facultad de Ciencias (UdelaR) Open Doors Day.

4- Institut Pasteur de Montevideo Open Doors Day.

Laboratory of Immunoregulation and Inflammation

Head: Marcelo Hill MD, PhD.

Members: ***Mercedes Segovia*** (Post-doc)
Maite Duhalde (Post-doc)
Sofía Russo (Master)
Mª Eugenia Schroeder (Master)
Florencia Rammauro (Master)
Matías Jeldres (stage)
Mariana Seija (stage)

Research

Dysregulation of immune responses leads to chronic inflammatory disorders collectively called immune-mediated inflammatory diseases (IMIDs). More than 80 clinically distinct diseases have been identified within this category, including classical autoimmune diseases, graft rejection and graft-versus host disease, asthma and atopy, psoriasis, immunodeficiencies, and chronic inflammatory diseases such as inflammatory nephropathies and atherosclerosis (Baeten 2009). IMIDs affect approximately 10 percent of the population and, for reasons that are not yet clear, the prevalence of these diseases appears to be rising. Cancer is another pathophysiological scenario where inflammation is part of the natural history of the disease. Furthermore, the biological basis of the effect obtained with pharmacological agents such as hydroxychloroquine (HCQ), currently used to impair immune-mediated damage, are not fully understood. Basic research is therefore needed to better characterize immunoregulatory mechanisms and new targets to control inflammation.

We have recently described new physiologic mechanisms which can control immune-mediated damage (Guillonneau et al. 2007; Hill et al. 2007a; Hill et al. 2007b; Hill et al. 2011). This knowledge can help to understand the natural history of IMIDS at the cellular and molecular level. Moreover, characterization of novel immunoregulatory mechanisms is an important issue to rationalize immunointerventional strategies (Hill et al. 2011) as well as to understand the pharmacodynamics of currently used anti-inflammatory drugs.

The laboratory of immunoregulation and inflammation has been established at the IP Montevideo in October 2013. Our laboratory studies cellular and molecular mechanisms which can control inflammation and adaptive immune responses. We are focused on the biology of dendritic cells (DCs). This is a particular subset of leukocytes which can trigger effector but also regulatory immune responses.

We are interested in the study of inflammasomes activation. Inflammasomes are cytoplasmic multi-protein complexes composed of a sensor protein (NOD-like) and an adaptor (ASC) which, once assembled, are able to activate pro-caspase-1 into caspase-1. Some of the targets of activated caspase-1 are pro-IL-1beta and pro-IL-18. Proteolytical process of these precursors by caspase-1 transforms them into active cytokines with a powerful pro-inflammatory action. In the last years, the cytoplasmic ionic composition has emerged as a one of the most studied mechanisms by which inflammasome activation is regulated (Latz et al. 2013). Indeed, characterizing new ion channel will certainly shed light on original mechanisms of inflamasomme activation. This context gives the main rational bases of our lines of investigation.

Research Lines

A. Study of the impact of new ion channels on the activation of inflammasomes.

B. Analysis of novel ion channels in cancer patients and in tumoral experimental models.

C. Characterization of novel anti-inflammatory mechanisms triggered by hydroxychloroquine through the inhibition of ion channels.

D. Study of the expression of new ion channels in autoimmune patients.

- Baeten, D. (2009). "Memorandum of understanding for the implementation of a European Concerted Research Action designates as COST Action BM0907: European Network for Translational Immunology Research and Education (ENTIRE): From Immunomonitoring to personalized immunotherapy."
- Guillonneau, C., M. Hill, F. X. Hubert, E. Chiffoleau, C. Hervé, X.-L. Li, M. Heslan, C. Usal, L. Tesson, S. Ménoret, A. Saoudi, B. Le Mauff, R. Josien, M. C. Cuturi and I. Anegon (2007). "CD40Ig treatment results in allograft acceptance mediated by CD8+CD45RClow T cells, IFN-gamma and indoleamine 2,3-dioxygenase." J Clin Invest **117**(4): 1096-106.
- Hill, M., S. Tanguy-Royer, P. J. Royer, C. Chauveau, K. Asghar, L. Tesson, F. Lavainne, S. Rémy, R. Brion, F. X. Hubert, M. Heslan, M. Rimbert, L. Berthelot, J. Moffett, R. Josien, M. Gregoire and I. Anegon (2007a). "IDO expands human CD4+CD25high regulatory T cells by promoting maturation of LPS-treated dendritic cells." Eur J Immunol. **37**(11): 3054-62.
- Hill, M., R. Zagani, C. Voisine, C. Usal and I. Anegon (2007b). "Nitric oxide and indoleamine 2,3-dioxygenase mediate CTLA4Ig-induced survival of heart allografts in rats." Transplantation **84**(8): 1060-3.
- Hill, M., P. Thebault, M. Segovia, C. Louvet, G. Beriou, G. Tilly, E. Merieau, I. Anegon, E. Chiffoleau and M. C. Cuturi (2011). "Cell therapy with autologous tolerogenic dendritic cells induces allograft tolerance through interferon-gamma and epstein-barr virus-induced gene 3." Am J Transplant **11**(10): 2036-45.
- Latz, E., TS. Xiao and A. Stutz. (2013). "Activation and regulation of the inflammasomes". Nat Rev Immunol **13**: 397-411

Publications 2014

1- **Segovia M**, Louvet C, Charnet P, Savina A, Tilly G, Gautreau L, Carretero-Iglesia L, Beriou G, Cebrian I, Cens T, Hepburn L, Chiffoleau E, Floto RA, Anegon I, Amigorena S, **Hill M**, Cuturi MC. Autologous dendritic cells prolong allograft survival through Tmem176b-dependent antigen cross-presentation (**2014**) American Journal of Transplantation, 14 (5), pp. 1021-1031. – *IF: 6.1*

2- Drujont L, Carretero-Iglesia L, Bouchet-Delbos L, Beriou G, Merieau E, **Hill M**, Delneste Y, Cuturi MC, Louvet C. Evaluation of the therapeutic potential of bone Marrow-Derived Myeloid Suppressor Cell (MDSC) adoptive transfer in mouse models of autoimmunity and allograft rejection (**2014**) PLoS ONE, 9 (6), art. N° e100013. – *IF: 3.534*

3- Moreau A, Vandamme C, **Segovia M,** Devaux M, Guilbaud M, Tilly G, Jaulin N, Le Duff J, Cherel Y, Deschamps JY, Anegon I, Moullier P, Cuturi MC and Adjali O. Generation and in vivo evaluation of IL10-treated dendritic cells in a nonhuman primate model of AAV-based gene transfer Citation: Molecular Therapy — Methods & Clinical Development (**2014**) 1, 14028; doi:10.1038/mtm.2014.28 © 2014 The American Society of Gene & Cell Therapy All rights reserved 2329-0501/14. – *IF:* --

4- Baas MC, Kuhn C, Valette F, Mangez C, Duarte MS, **Hill M**, Besançon A, Chatenoud L, Cuturi MC, You S. Combining autologous dendritic cell therapy with CD3 antibodies promotes regulatory T cells and permanent Islet allograft acceptance. *Journal of Immunology* Volume 193, Issue 9, 1 November **2014**, Pages 4696-4703. – *IF: 5.362*

Grants

1. CABBIO 2015-2017

2. FMV 2014-2016

Worm Biology Laboratory

Head: Gustavo Salinas, PhD

Members: ***Lucía Otero Larre Borges*** (PhD)
Inés Carrera (PhD)
Hugo Bisio (Post-graduatestudent)
Laura Romanelli (Post-graduatestudent)
Gastón Risi (Undergraduate student)

GOALS

Our scientific proposal is to understand redox pathways and redox regulated processes in parasitic flatworms and nematodes. This knowledge will provide a better understanding of the metabolism of worms and will lead to new therapeutic and disease-preventive agents, particularly targeting essential parasite metabolic pathways. We also seek to foster research in the nematode *C. elegans*, a formidable animal model for studying fundamental biological problems and human diseases, largely ignored in Uruguay. In education, our goal is to position IP as a Latin America reference for thiol-redox biology.

Research

The Worm Biology Laboratory research is focused on: i) thiol- and selenol-dependent pathways from flatworm parasites and nematodes, and ii) energy-harvesting pathways of flatworms and nematodes. We are characterizing,biochemically and structurally, the unique linked thioredoxin-glutathione pathways present in parasitic flatworms; in particular, we are addressing the function of redox and iron-sulfur thioredoxins and glutaredoxins. We are also investigating the fermentative metabolism of worms, with special emphasis on the malate dismutation, an essential pathway for energy harvesting in helminthsabsent in vertebrates.Our lab also focuses on drug discovery, particularly targeting the selenoenzymethioredoxin glutathione reductase and the malate dismutation pathway of parasitic helminths.

Publications 2014

1) **Otero L**, **Romanelli-Cedrez L**, Turanov AA, Gladyshev VN, Miranda-Vizuete A,**Salinas G.** (**2014**) Adjustments, extinction and remains of selenocysteine incorporation machinery in the nematode lineage.RNA20(7):1023-34.– *IF: 5.377*
2) Saiz C, Castillo V, Fontán P, Bonilla M, **Salinas G,** Rodríguez-Haralambides A, Mahler SG. Discovering Echinococcusgranulosusthioredoxin glutathione reductase inhibitors through site-specific dynamic combinatorial chemistry (**2014**) Molecular Diversity, 18 (1), pp. 1-12. – *IF: 2.544*

Grants

1. The thioredoxin-fold in trypanosomatids and tapeworms. ICGEB (Italia) 2014-2017. 15.000 €/year, (shared Project with Marcelo Comini).
2. Redox Chemistry and Biology of Thiols, International postgraduate course and Symposium, supported by ICGEB, RIIP and PEDECIBA. (USD 28.000 USD). The course was organized together with Marcelo Comini, Beatriz Alvarez and Madia Trujillo.
3. Reinsertion funds for Inés Carrera. PEDECIBA 5.000 USD
4. CSIC, Universidad de la República. Research Inititation Grants to Laura Romanelli and Hugo Bisio.5.000 USD.

Otheractivities

POSTGRADUATE COURSES:

Participated in several postgraduate courses: Enzymology (PEDECIBA Biología, Uruguay), Mitochondria (PEDECIBA Biología, Uruguay), Molecular Evolution(PEDECIBA Biología, Uruguay) and Molecular Biology of Platyhelminths(Universidad de Buenos Aires).

UNDERGRADUATE COURSES:

Introductory Biology, Faculty of Chemistry, Universidad de la República. (26 lectures per year)

TRAINING OF STUDENTS

Postgraduate students: Lucía Otero (defendedher PhD in 2014).
Hugo Bisio (started the postgraduate studies in 2013).
Laura Romanelli (started the postgraduate studies in 2013).
GastónRisi (undergraduate student, final degree thesis).

CONGRESSES

Oral presentation at Evolutionary Biology of Caenorhabditis and other Nematodes.Wellcome Trust Genome Campus. Hinxton, Cambridge, UK.Gustavo Salinas.
Invited Conference X Congreso Argentino de Protozoología y Enfermedades Parasitarias. Mar del Plata. Gustavo Salinas.

STAGES/INTERNSHIPS ABROAD

Laura Romanelli: 3 months internship at Mark Alkema´s Lab, Univerity of Massachusetts

OTHER SICENTIFIC ACTIVITIES of the PI

Ad hoc reviewer for The Journal of Biological Chemistry and Antioxidant Redox Signaling among other Journals.

Invited Seminar at The Wellcome Trust Sanger Centre, Pathogen Group, Cambridge, UK. Talk: “Unusual aspects of metabolism in flatworm parasites: implications for rational drug design”.

Co-organizer, together with Dr. Martina Crispo, of the Session “Model Organisms”.XV Meeting of the Biosciences Uruguayan Society. September 2014.

Signal Processing Laboratory

Head: Federico Lecumberry, Dr. Ing.

Members: ***Martín Etchart***, Ing.

GOALS

Our scientific proposal is to create and consolidate an interdisciplinary research group on Signal Processing and Biomedical Imaging within the Institut Pasteur de Montevideo working in joint research projects. We promote the interaction of different actors (such as engineers, physician, physicist, biologists, technicians) in research projects, seminars and workshops, and undergraduate and graduate courses in signal processing and its related application areas.

The Signal and Processing Laboratory (LPS) is part of the Signal Processing Department (DPS) at the School of Engineering (Universidad de la República), sharing some members and lines of research.

Research

The Signal Processing Laboratory (LPS) research interests are related to Signal and Image Processing and its applications to Biology and Biomedicine, in particular toStructuralBiology.

The first goal as a line of research is the formation and consolidation of a joint interdisciplinary research group in biomedical signal and image processing, with the participation of members of the DPS and the Institut Pasteur de Montevideo. In this sense, signal processing provides an objective approach to automatize and systematize the analysis of data generated by the wide range of techniques and equipments used in the IP Montevideo. Thus, an interdisciplinary approach to problems allows to develop methodologies and algorithms that incorporate from the beginning the knowledge of the different actors (such as biologists, physicians, engineers, physicists), the DPS have a large experience in this kind of collaborations. One way to achieve this goal is to identify common tasks or procedures to different research groups, usually associated with a technology platform such as epifluorescence microscopy, and develop them a set of tools adapted to these tasks.

A second line of research is related to signal processing with applications to structural biology. Solving the molecular structure of complex macromolecules usually requires the integration of different techniques, the combination of X -ray Crystallography and Cryo-Electron Microscopy (CryoEM) allows to integrate molecular and cellular approaches in order to determine high resolution density maps. Thus, it aims to create a group of researchers with expertise in signal processing with applications to crystallography and CryoEM. For the success of this line of research is essential the collaboration with other research groups within IP Montevideo and the identification of specific joint projects of interest.

Publications 2014

1- MatíasTailanián, **Federico Lecumberry**, Alicia Fernández, Giovanni Gnemmi, Ana Meikle, Isabel Pereira, and Gregory Randall. Dairy cattle sub-clinical uterine disease diagnosis using pattern recognition and image processing techniques. In Progress in Pattern Recognition, Image Analysis, Computer Vision, and Applications, volume 8827 of Lecture Notes in Computer Science, pages 690–697. Springer International Publishing, **2014**.

Grants

1- INNOVA II

Other activities

TRAINING COURSES

We are currently organizing the theoretical and practical course "Processing and Analysis of Fluorescence Microscopy Images" to be held at the Institut Pasteur de Montevideo in March 2016. The global aim of this course is to equip students to address fluorescence microscopy imaging questions from a comprehensive and quantitative perspective, and to foster two types of students: those with biological background and those trained in quantitative sciences such as mathematics and physics. Theoretical and practical sessions will be organized in a way that the skills of one group of students will help the other group.

As part of the regular courses offered by the Signal Processing Department (Universidad de la República) the LPS connect the student with introductory and advanced courses in signal and image processing, pattern recognition, programming, information theory, among others.

TRAINING OF STUDENTS

Research Assistants at the LPS are performing posgraduate thesis or are at the last stages of their undergraduate degree in electrical engineering.The LPSalso promotes short internships for undergraduate student from the School of Engineering (Universidad de la República) for working in interdisciplinary projects.

CONGRESSES

Currently, membersodthe LPS are partoftheOrganizingCommitteeofthe 20th Iberoamerican Congresson Pattern Recognition(CIARP 2015) to be held in Montevideo in November 2015.CIARP 2015 isorganized by theUruguayan IAPR Chapter, includingmembersfrom Universidad de la República andUniversidad Católica. Heldeveryyear, CIARP is the most important Iberoamerican conference in patternrecognition, computervisionand multimedia. Amongtheextensivelistofapplicationareascovered by thecongress,bioinformatics and human and animal healthareoneofthemostrelevanttopics.

- **Epigenetics of Cancer and Aging Laboratory**
- **Metabolic Diseases and Aging Laboratory**

Epigenetics of Cancer and Aging Laboratory

Head: Ruben Agrelo, MD

Members: ***Miguel Arocena*** (Postdoctoral Fellow)
Santiago González (MSc Student)
Fabián Aldunate (MSc Student)
Valeria Da Costa (MSc Student)

GOALS

We are commited to investigate how epigenetics impacts on aging and cancer.Because of the increased life expentacy in modern societies; understanding these phenomena is crucial

Research

Our group is interested in the influence of epigenetics on cell proliferation, cell senescence and cell differentiation. We want to understand how epigenetics impacts cell and tissue aging, cancer progression and response to cancer therapies.

Research Lines

A. ***EPIGENETICS AT THE AGING-CANCER INTERFACE***

Abnormal epigenetic signaling plays a critical role in tumorigenesis. Epigenetic changes can also be important determinants of cellular senescence and organism aging. The best-defined epigenetic modifications are DNA methylation and histone posttranscriptional modifications. The best known examples are global loss of DNA methylation in aging and cancer and the promoter hypermethylation of genes with a dual role in tumor suppression and progeria, such as *WRN (involved in Werner Syndrome)* and *LMNA.*

In particular we are interested in exploring how epigenetic alterations are accumulated during aging and how these events contribute to the cell transformation process**.**

We are also interested in research towards premature aging therapeutic approaches based on epigenetic drugs

B. ***EPIGENETICS AND NUCLEAR MECHANICS***

A-type lamins are essential components of the nuclear lamina. Together with B-type lamins, they are the most prominent intermediate filaments forming this network of 10-nM diameter filaments located on the inner side of the nuclear membrane. LMNA has been found to be hypermethylated in hematological tumors. Moreover LMNA is mutated in aging related diseases such as Hutchinson Guilford progeria (HGPS). By the activation of a cryptic donor splice site progerin is expressed in HGPS cells affecting the epigenetic control of facultative and constitutive heterochromatin.

We want to elucidate how these epigenetic modifications impact in nuclear mechanics.

C. ***EPIGENETIC CONTEXT OF CANCER PROGENITORS***

Using tools derived from the mammalian dosage compensation system are useful for defining the epigenetic context for Xist mediated silencing. This has led to the identification of an epigenetic context that is linked to the potential of Xist to induce chromosome-wide gene silencing in cancer progenitors. The aim of this project is to better characterize this cellular context and its components. As a consequence we expect to gain insight into normal cell biology and identify novel therapeutic targets in cancer.

Grants and Fellowships

3. URUGUAY INNOVA (URU-EU)-ANII
4. PEDECIBA
5. Postdoctoral Fellowship-Miguel Arocena- Sutz -ANII

Other activities

CONGRESS

Invited Participant and Chairman: Epigenetics, Pluripotency and Cell Reprogramming Session, The Department of Develomental & Stem Cell Biology-Pasteur Paris Retreat- Morzine –France-. October 2014

Participation in the Theoretial Biophysics Department Seminars .Humboldt University Berlin October 2014

Metabolic Diseases and Aging Laboratory

Head: Carlos Escande, Ph. D

Members: ***Paola Contreras***, PhD - (Research Associate)
Mariana Bresque, MSc - (Reasearch Assistant) – PhD Student
Natalia Bobba, BSc - (Research Assitant) – MSc Student
Adriana Carlomagno, MD – MSc Student
Rosina DapuetoMSc - PhD Student
Pía Garat – Undergraduate Student

GOALS

Our scientific proposal is to study the molecular mechanisms of metabolic diseases, including obesity, type II diabetes and cardiovascular disease, with the ultimate goal of helping to develop new therapeutical approaches to treat them.

Research

The Metabolic Diseases and Aging Laboratory research is focused on studying the role of a family of protein called Sirtuins in the control of metabolism and metabolic diseases. Sirtuins are NAD+-dependent protein deacylases with a critical role in metabolism, genome stability and cancer. We try to understand how sirtuins are regulated, and how changes in metabolism affect their function.

Specifically, we are focusing in the following projetcts:

- Role of DBC1, a negative SIRT1 regulator, in the development of insulin resistance and type II diabetes
- Potencial involvement of SIRT6 in obesity-driven inflammation and celular senescence in fat tissue.
- Development of new CD38 inhibitors for the treatment of metabolic diseases.
  DBC1 as a novel regulator of cardiovascular function.

Publications 2014

1: Clasen BF, Poulsen MM, **Escande C**, Pedersen SB, Møller N, Chini EN, Jessen N, Jørgensen JO. Growth hormone signaling in muscle and adipose tissue of obese human subjects: associations with measures of body composition and interaction with resveratrol treatment. J Clin Endocrinol Metab. **2014** Dec;99(12):E2565-73. doi: 10.1210/jc.2014-2215. PubMed PMID: 25050904.
2: **Escande C**, Nin V, Pirtskhalava T, Chini CC, Thereza Barbosa M, Mathison A, Urrutia R, Tchkonia T, Kirkland JL, Chini EN. Deleted in Breast Cancer 1 regulates cellular senescence during obesity. Aging Cell. **2014** Oct;13(5):951-3. doi: 10.1111/acel.12235. Epub 2014 Jul 3. PubMed PMID: 24992635; PubMed Central PMCID: PMC4172532.
3: Nin V, Chini CC, **Escande C**, Capellini V, Chini EN. Deleted in breast cancer 1 (DBC1) protein regulates hepatic gluconeogenesis. J Biol Chem. **2014** Feb 28;289(9):5518-27. doi: 10.1074/jbc.M113.512913.
4: Calliari A, **Bobba N, Escande C**, Chini EN. Resveratrol delays Wallerian degeneration in a NAD(+) and DBC1 dependent manner. Exp Neurol. **2014** Jan;251:91-100. doi: 10.1016/j.expneurol.2013.11.013. Epub 2013 Nov 16. PubMed PMID: 24252177.
5: Chini CC, Guerrico AM, Nin V, Camacho-Pereira J, **Escande C**, Barbosa MT, Chini EN. Targeting of NAD metabolism in pancreatic cancer cells: potential novel therapy for pancreatic

tumors. Clin Cancer Res. **2014** Jan 1;20(1):120-30. doi: 10.1158/1078-0432.CCR-13-0150. PubMed Central PMCID: PMC3947324.

Grants

1- INNOVA - ANII– Young leaders grant.

Other activities

Participation in Conferences as Invited Speaker

- **"International Symposium on Mitochondria and Cell Metabolism"** July, 2014. Montevideo, Uruguay
- **Regional symposium "Diabetes, from basics to the clinic"** June, 2014, Montevideo, Uruguay
- **SUB** (Uruguayan Society of Biosciences) Anual meeting**, October, 2014**
- **SAIC** (Argentine Society of Clnical Investigation) anual meeting, November, 2014

COURSES

Title:	Organizers:	Date	Foreign Speakers	Foreign Students	Finantial Agencies
"Flow Cytometry and cell sorting in Biotechnology and Biomedicine Research"	M. Bollati	March 17-28th	8	21	ICGEB UNU BIOLAC RIIP TWAS
"Introduction to functional and structural analysis of proteins"	A. Correa F. Trajtenberg M. Machado H. Botti	August 4-October 13th	1	2	IP MONTEVIDEO
"Proteome Analysis by Mass Spectrometry"	R. Duran C. Batthyany	September 1-12th	6	12	UNU BIOLAC RIIP IP MONTEVIDEO
"Neuron glia interactions in health and disease: from basic Biology to translational Neuroscience-3rd edition"	H. Peluffo L. Barbeito P. Cassina S. Olivera N. Lago	September 29 - October 4th	10	10	CAEN isn PEDECIBA BIOLOGIA ANII IBRO LARC MULTIPLE SCLEROSIS CSIC
"Modern Approaches in Drug Discovery for Neglected Infectious diseases"	A. Buschiazzo	November 3-8th	3	14	CEBEM UNU BIOLAC IP MONTEVIDEO
"Functional Genomics and its applications in Biomedicine: Host Pathogen - Interaction"	C. Robello A. Cayota N. Rego G. Greif G. Libisch G. Iraola	November 10-19th	4	12	UNU BIOLAC RIIP
"Hands-on course on High-Throughput Sequencing data analysis"	H. Naya M. Fontes	December 1-12th	2	16	RIIP
Total			34	87	USD 210.000

1. "Flow Cytometry and Cell Sorting In Biotechnology and Biomedicine Research"; March 17- 28th.

Theoretical and Practical Course:

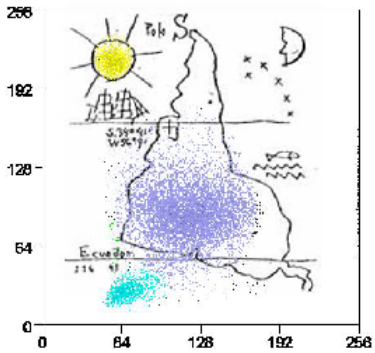
FLOW CYTOMETRY AND CELL SORTING IN BIOTECHNOLOGY AND BIOMEDICINE RESEARCH

17th-28th March, 2014 - Institut Pasteur de Montevideo - Montevideo, Uruguay

The aim of this course is to build regional capacity in flow cytometry, covering all aspects of this powerful technique, starting from experimental designs to data acquisition & analysis. The course is intended mainly for graduate students, postdoctoral researchers and young scientists in the area of biomedicine and biotechnology. Applicants from all around the globe will be welcomed.

ORGANISER:
Mariela BOLLATI -FOGOLÍN, IP Montevideo, Montevideo - Uruguay

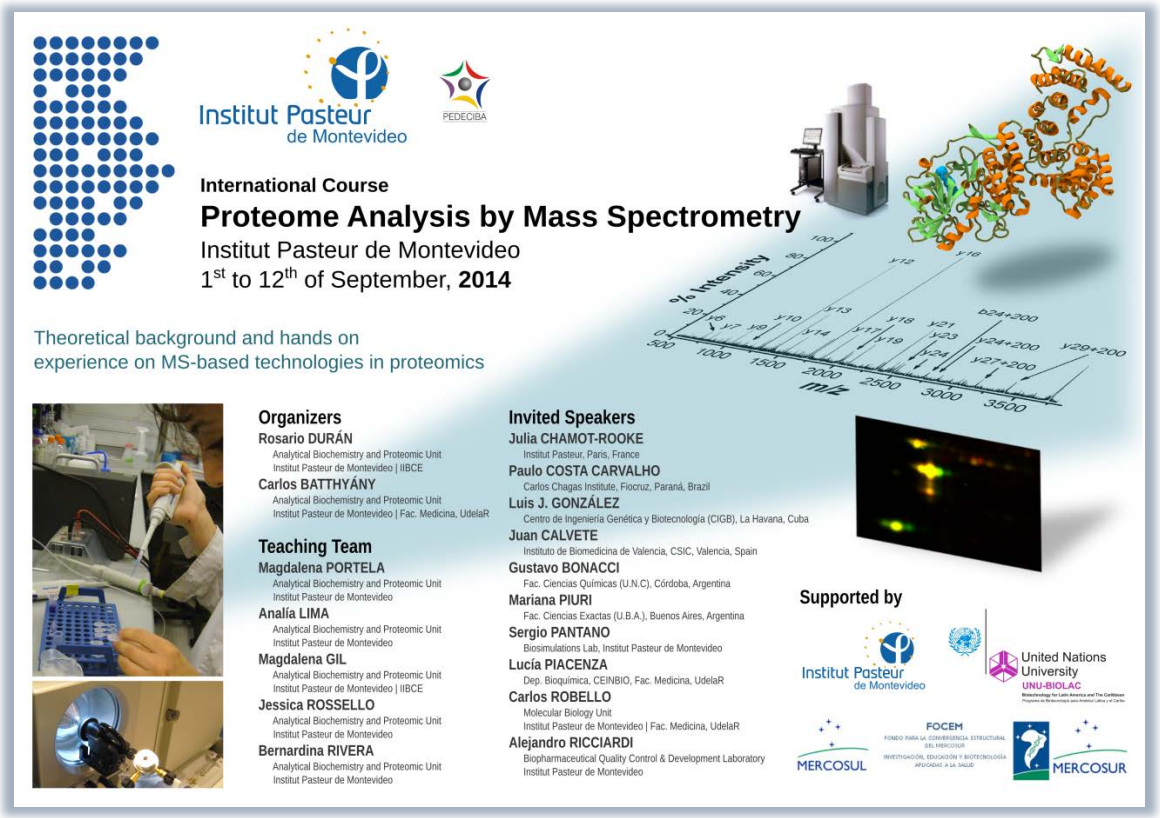
CO-ORGANISERS:
Lothar GRÖBE. Helmholtz Centre for Infection Research - Germany
Jean-Michel GARCÍA. Institut Pasteur Korea - Korea



CONFIRMED SPEAKERS
Cecilia ALONSO. CURE, Rocha - Uruguay
Nicole BORTH. BOKU, Wien - Austria
Marcelo COMINI. IP Montevideo, Montevideo - Uruguay
Andrew FILBY. London Research Institute, London - UK
Gustavo FOLLE. IIBCE, Montevideo - Uruguay
Jean-Michel GARCIA, Institut Pasteur Korea - Korea
María Inés GOLDARACENA, BD Biosciences, Buenos Aires - Argentina
Lothar GRÖBE. Helmholtz Centre for Infection Research, Braunschweig - Germany
Daniel SCOTT ALGARA. Institut Pasteur Paris, Paris - France

2. “Introduction to functional and estructural protein analysis”; August 4th – October 13th.

3. “Proteome Analysis by Mass Spectrometry - International Course”; September, 1-12th.



4. “Neuron glia interactions in health and disease: from basic Biology to translational; Neuroscience-3rd edition”; September 29th – October 4th.

September 29th to October 4th, 2014
Institut Pasteur de Montevideo
Uruguay

LOCAL ORGANIZERS:
Luis Barbeito – Institut Pasteur de Montevideo, Uruguay
Natalia Lago – Institut Pasteur de Montevideo, Uruguay
Patricia Cassina – Facultad de Medicina, Montevideo, Uruguay
Hugo Peluffo – Facultad de Medicina, Montevideo, Uruguay
Silvia Olivera – Instituto Clemente Estable, Montevideo, Uruguay

CO-ORGANIZER:
Juana Pasquini - Universidad de Buenos Aires, Argentina

CONFIRMED SPEAKERS FOR SYMPOSIUM:
Etty Benveniste - University of Alabama,United States
Alain Chedotal - Institut de la Vision, INSERM, UPMC, Paris, France
Felipe A Court - Pontificia Universidad Católica de Chile, Chile
Babette Fuss - Virginia Commonwealth University Medical Center, United States
Javier Ramos - Facultad de Medicina, UBA, Argentina
Brigitte van Zundert - Universidad Andres Bello, Chile

AIMS
The growing importance that glial cells are having in the function and pathology of the nervous system justifies a periodic update and deepening into this research field. The course is conceived to introduce the students to the basic biology of glial cells up to their clinical relevance. It will be focused on the role of different glial during during normal nervous system biology as well as in disorders and diseases. Strategies for glia targeted therapeutics will be specially introduced and discussed. Special attention will be also delivered to metabolic and inflammatory features of glial cells, including mitochondria respiration and other redox signals.

5. “Modern Approaches in Drug Discovery for Neglected Infectious diseases”; November, 3 – 8th.

6. "Functional Genomics in Biomedicine: Host–Pathogen interaction"; November 10-19th.

7. “Hands-on course on High-Throughput Sequencing data analysis”; December, 1 – 12th.

Montevideo NGS Course Dec 2014

December 1st to 12th, 2014
Institut Pasteur de Montevideo
Montevideo, Uruguay

Organizers:
Hugo Naya
Magnus Fontes

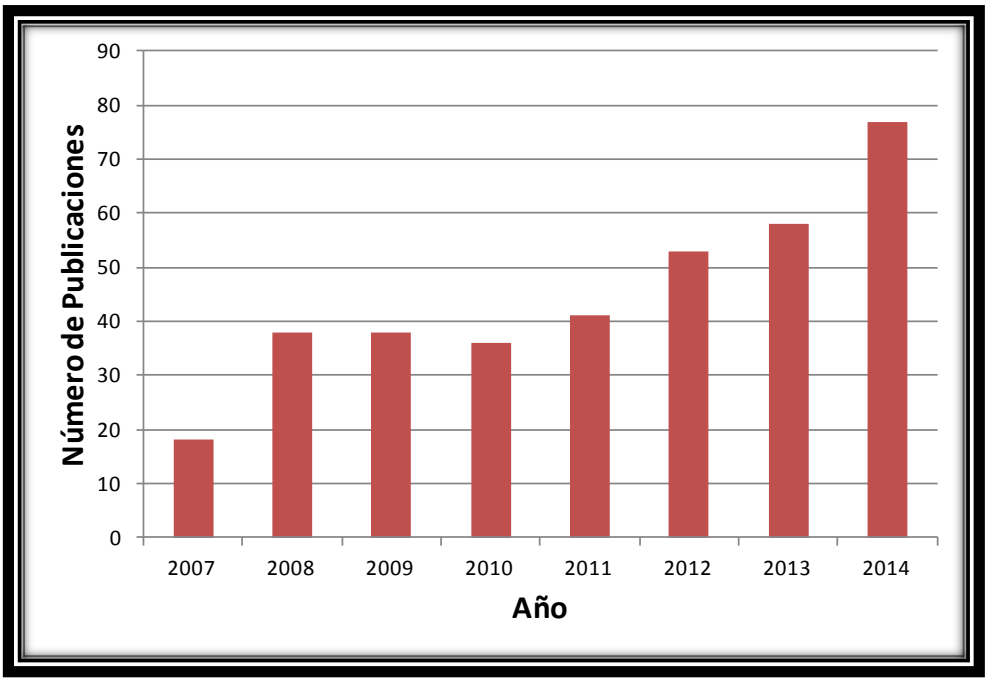
Invited Speaker
Rasmus Henningsson

Teaching Team
Magnus Fontes – Institut Pasteur, France
Hugo Naya – Institut Pasteur de Montevideo, Uruguay
Rasmus Henningsson – Institut Pasteur, France
Héctor Romero - Facultad de Ciencias – UdelaR, Uruguay
Lucía Spangenberg – Institut Pasteur de Montevideo, Uruguay
María Inés Fariello
Luisa Berná
Natalia Rego
Tamara Fernandez-Calero
Gregorio Iraola
Sebastián Valenzuela

Topics
- Introduction to HTS technologies
- Basics statistics and probability concepts applied to HTS. Particularities of HTS sampling, underlying distributions, etc. Statistics for Differential Expression. Multivariate Data Reduction techniques. Introduction to experimental design
- Mapping and assembling algorithms
- Quality analysis of HTS data
- Re-sequencing and variant analysis, de-novo sequencing
- Transcriptomics andChIP-Seq (Differential Expression, ontologies, regulatory networks)
- Metagenomics
- Introduction to Linux, R and general bioinformatics tools

PUBLICATIONS

A. Historical Evolution of IPMont Publicatons



B. 2014 Publications

1. **Trochine A**, Alvarez G, Corre S, **Faral-Tello P**, **Duran R**, **Batthyany C**, Cerecetto H, Gonzalez M, **Robello C** (2014) Trypanosoma cruzi chemical proteomics using immobilized benznidazole. Exp Parasitol 140: 33-38.
2. Turell L, **Botti H**, Bonilla L, Torres MJ, Schopfer F, Freeman BA, Armas L, Ricciardi A, Alvarez B, Radi R (2014) HPLC separation of human serum albumin isoforms based on their isoelectric points. J Chromatogr B Analyt Technol Biomed Life Sci 944: 144-151.
3. **Trajtenberg F**, Albanesi D, Ruetalo N, **Botti H**, **Mechaly AE, Nieves M, Aguilar PS**, Cybulski L, **Larrieux N**, de Mendoza D, **Buschiazzo A** (2014) Allosteric activation of bacterial response regulators: the role of the cognate histidine kinase beyond phosphorylation. MBio 5: e02105.
4. Randall LM, **Manta B**, Hugo M, **Gil M**, **Batthyany C**, Trujillo M, Poole LB, Denicola A (2014) Nitration transforms a sensitive peroxiredoxin 2 into a more active and robust peroxidase. J Biol Chem 289: 15536-15543.
5. **Morero NR**, **Botti H**, Nitta KR, **Carrion F, Obal G**, Picardeau M, **Buschiazzo A** (2014) HemR is an OmpR/PhoB-like response regulator from Leptospira, which simultaneously effects transcriptional activation and repression of key haem metabolism genes. Mol Microbiol 94: 340-352.

6. Mon ML, Moyano RD, Viale MN, Colombatti Olivieri MA, Gamietea IJ, Montenegro VN, Alonso B, Santangelo Mde L, Singh M, **Duran R**, Romano MI (2014) Evaluation of cocktails with recombinant proteins of Mycobacterium bovis for a specific diagnosis of bovine tuberculosis. Biomed Res Int 2014: 140829.
7. Martinez A, Peluffo G, Petruk AA, Hugo M, Pineyro D, Demicheli V, Moreno DM, Lima A, **Batthyany C**, **Duran R**, Robello C, Marti MA, **Larrieux N, Buschiazzo A**, Trujillo M, Radi R, Piacenza L (2014) Structural and Molecular Basis of the Peroxynitrite-mediated Nitration and Inactivation of Trypanosoma cruzi Iron-Superoxide Dismutases (Fe-SODs) A and B: Disparate Susceptibilities Due To The Repair Of Tyr35 Radical By Cys83 In Fe-SodB Through Intramolecular Electron Transfer. J Biol Chem 289: 12760-12778.
8. **Malacrida L**, Reta G, Piriz H, Rocchiccioli F, **Botti H**, Denicola A, Briva A (2014) Sevoflurane anesthesia deteriorates pulmonary surfactant promoting alveolar collapse in male Sprague-Dawley rats. Pulm Pharmacol Ther 28: 122-129.
9. Dieterle ME, Bowman C, **Batthyany C**, Lanzarotti E, Turjanski A, Hatfull G, Piuri M (2014) Exposing the secrets of two well-known Lactobacillus casei phages, J-1 and PL-1, by genomic and structural analysis. Appl Environ Microbiol 80: 7107-7121.
10. **Palacios F, Abreu C**, **Prieto D**, **Morande P**, Ruiz S, Fernández-Calero T, Naya H, Libisch G, **Robello C**, Landoni AI, Gabus R, **Dighiero G**, **Oppezzo P**. Activation of the PI3K/AKT pathway by microRNA-22 results in CLL B-cell proliferation ***Leukemia. 2014*** Jan;29(1):115.
11. Fischer S, Echeverría N, Moratorio G, **Landoni AI, Dighiero G**, Cristina J, **Oppezzo P**, **Moreno P**. Human endogenous retrovirus np9 gene is over expressed in chronic lymphocytic leukemia patients. ***Leuk Res Rep. 2014*** Jul 25;3(2):70-2. doi: 10.1016/j.
12. **Correa A, Ortega C, Obal G**, Alzari P, Vincentelli R, **Oppezzo P.** Generation of a vector suite for protein solubility screening. ***Front Microbiol. 2014*** Feb 25. eCollection 2014.
13. **Correa A**, Pacheco S, **Mechaly AE, Obal G**, Béhar G, Mouratou B, **Oppezzo P**, Alzari PM, Pecorari F. Potent and specific inhibition of glycosidases by small artificial binding proteins (affitins). ***PLoS One. 2014*** May 13;9(5) eCollection 2014.
14. Borge M, Lenicov FR, Nannini PR, De Los Ríos Alicandú MM, Podaza E, Ceballos A, Grecco HF, Cabrejo M, Bezares RF, **Morande PE, Oppezzo P,** Giordano M, Gamberale R. The expression of sphingosine-1 phosphate receptor-1 in chronic lymphocytic leukemia cells is impaired by tumor microenvironmental signals and enhanced by piceatannol and R406 ***J Immunol. 2014*** Sep 15;193(6):316.
15. Alem D, Díaz-Dellavalle P, Leoni C, De-Simone SG, **Correa A, Oppezzo P**, Rizza MD. In search of topical agricultural biofungicides: Properties of the recombinant antimicrobial peptide TrxAq-AMP Obtained from Amaranthus quitensis (2014) ***Journal of Microbial and Biochemical Technology***, 6 (5), pp. 268-273.
16. **Libisch MG**, Casás M, Chiribao M, Moreno P, **Cayota A, Osinaga E**, **Oppezzo P, Robello C.** GALNT11 as a new molecular marker in chronic lymphocytic leukemia. ***Gene. 2014*** Jan 1;533(1):270.
17. **Prieto D**, **Aparicio G**, **Morande PE, Zolessi FR**. A fast, low cost, and highly efficient fluorescent DNA labeling method using methyl green (2014) Histochemistry and Cell Biology, 142 (3), pp. 335-345.
18. **Trajtenberg F**, Altabe S, **Larrieux N**, Ficarra F, de Mendoza D, **Buschiazzo A,** Schujman GE. Structural insights into bacterial resistance to cerulenin. (2014) *FEBS J.* **281**:2324-2338.
19. **Garcia-Silva MR**, das Neves RF, Cabrera-Cabrera F, **Sanguinetti J**, Medeiros LC, **Robello C**., **Naya H, Fernandez-Calero T**, Souto-Padron T, de Souza W, **Cayota A.** Extracellular vesicles shed by Trypanosoma cruzi are linked to small RNA pathways, life cycle regulation, and susceptibility to infection of mammalian cells. Parasitol Res. 2014;113:285-304.

20. **Greif G**, **Iraola G, Berná L**, Coitinho C, Rivas C, **Naya H**, **Robello C.** 2014. Complete genome sequence of Mycobacterium tuberculosis strain MtURU-001, isolated from a rapidly progressing outbreak in Uruguay. Genome Announcements. – *IF:* --
21. **Chiribao ML, Libisch G, Parodi-Talice A, Robello C.** Early trypanosoma cruzi infection reprograms human epithelial cells (2014) BioMed Research International, 2014, art. no. 439501. – *IF:* --
22. **Faral-Tello P,** Liang M, Mahler G, Wipf P, **Robello C**. Imidazolium compounds are active against all stages of Trypanosoma cruzi (2014) International Journal of Antimicrobial Agents, 43 (3), pp. 262-268. – *IF: 4.259*
23. **Trochine A**, Creek DJ, **Faral-Tello P**, Barrett MP, **Robello C.** Benznidazole Biotransformation and Multiple Targets in Trypanosoma cruzi Revealed by Metabolomics (2014) PLoS Neglected Tropical Diseases, 8 (5), art. no. e2844. – *IF: 4.489*
24. **Berná L, Iraola G, Greif G**, Coitinho C, Rivas C, **Naya H, Robello C**. 2014. Whole-Genome Sequencing of an Isoniazid-Resistant Clinical Isolate of Mycobacterium tuberculosis Strain MtURU-002 from Uruguay. Genome Announcements. – *IF:* --
25. **Chiribao ML, Libisch G, Parodi-Talice A, Robello C.** Early trypanosoma cruzi infection reprograms human epithelial cells (2014) BioMed Research International, 2014, art. no. 439501. – *IF:* --
26. Márquez VE, Arias DG, **Chiribao ML, Faral-Tello P, Robello C**, Iglesias AA, Guerrero SA. Redox metabolism in Trypanosoma cruzi. Biochemical characterization of dithiol glutaredoxin dependent cellular pathways (2014) Biochimie. – *IF: 3.123*
27. Coitinho C, **Greif G, Robello C,** Laserra P, Willery E, Supply P. Rapidly progressing tuberculosis outbreak in a very low risk group. Eur Respir J. 2014 Mar;43(3):903-6. doi: 10.1183/09031936.00150413. PubMed PMID: 24114961. – *IF: 7.125*
28. León IE, **Porro V**, **Astrada S**, Egusquiza MG, Cabello CI, **Bollati-Fogolin M,** Etcheverry SB. Polyoxometalates as antitumor agents: Bioactivity of a new polyoxometalate with copper on a human osteosarcoma model. Chem Biol Interact. (2014), 222C:87-96. – *IF: 2.982*
29. **Pagotto RM**, Pereyra EN, Monzón C, Mondillo C, Pignataro OP. Histamine inhibits adrenocortical cell proliferation but does not affect steroidogenesis. J Endocrinol (2014); 221(1):15-28. – *IF: 3.586.*
30. Vallespí MG, Pimentel G, Cabrales-Rico A, Garza J, Oliva B, Mendoza O, Gomez Y, Basaco T, Sánchez I, Calderón C, Rodriguez JC, Markelova MR, Fichtner I, **Astrada S, Bollati-Fogolín M**, Garay HE, Reyes O. Antitumor efficacy, pharmacokinetic and biodistribution studies of the anticancer peptide CIGB-552 in mouse models. J Pept Sc (2014), 20(11):850-9. – *IF: 1.862*
31. Domínguez MF, Koziol U, **Porro V**, Costábile A, Estrade S, Tort J, **Bollati-Fogolin M**, Castillo E. A new approach for the characterization of proliferative cells in cestodes (2014) Experimental Parasitology, 138 (1), pp. 25-29. – *IF: 1.859*
32. Leon IE, **Porro V**, Di Virgilio AL, Naso LG, Williams PAM**, Bollati-Fogolín M**, Etcheverry SB. Antiproliferative and apoptosis-inducing activity of an oxidovanadium(IV) complex with the flavonoid silibinin against osteosarcoma cells (2014) Journal of Biological Inorganic Chemistry, 19 (1), pp. 59-74. – *IF: 3.164*
33. Fernández-Calero T, **Astrada S**, Alberti Á, Horjales S, Arnal JF, Rovira C, **Bollati-Fogolín M**, Flouriot G, Marin M. The transcriptional activities and cellular localization of the human estrogen receptor alpha are affected by the synonymous Ala87 mutation (2014) Journal of Steroid Biochemistry and Molecular Biology, 143, pp. 99-104. – *IF: 4.049*
34. Beedessee G, Ramanjooloo A, **Tiscornia I**, Cresteil T, Raghothama S, Arya D, Rao S, Gowd KH, **Bollati-Fogolin M**, Marie DEP. Evaluation of hexane and ethyl acetate extracts of the sponge Jaspis diastra collected from Mauritius Waters on HeLa cells (2014) Journal of Pharmacy and Pharmacology, 66 (9), pp. 1317-1327. – *IF: 2.161*
35. Ceaglio N, **Bollati-Fogolín M**, Oggero M, Etcheverrigaray M, Kratje R (2014). Chapter 6.2: High cell density cultivation processes. In Animal Cell Biotechnology, Eds Wagner R and

Hauser H. De Gruyter. Berlín, Alemania (ISBN 3110278863, 9783110278866), pp. 427 – 454.

36. **Crispo M, Vilariño M**, dos Santos-Neto PC, Núñez-Olivera R, Cuadro F, **Barrera N, Mulet AP**, Nguyen TH, Anegón I, Menchaca A. Embryo development, fetal growth and postnatal phenotype of eGFP lambs generated by lentiviral transgénesis. Transgenic Res. 2015 Feb;24(1):31-41. doi: 10.1007/s11248-014-9816-x. Epub 2014 Jul 22.
37. **Schlapp G**, Goyeneche L, Fernández G, Menchaca A, **Crispo M**. Administration of the nonsteroidal antiinflammatory drug tolfenamic acid at embryo transfer improves maintenance of pregnancy and embryo survival in recipient mice, Journal of Assisted Reproduction and Genetics, accepted.
38. **Meikle MN**; **Arévalo AP**; **Schlapp G**; Fernández G., **Graña M**; **Crispo M.** Environmental enrichment effects on reproductive performance of foster females used in transgenesis and rederivation techniques. Transgenic research 2014, 23; 897 – 897.
39. **Schlapp G**; **Meikle MN**; **Crispo M.** Slow freezing versus vitrification of transgenic mouse embryos obtained by in vitro fertilization (IVF) intended for rederivation use. Transgenic research 2014, 23; 891 – 891.
40. Tinoco LW, Fraga JL, Anobom CD, Zolessi FR, **Obal G,** Toledo A, **Pritsch O**, Arruti C. Structural characterization of a neuroblast-specific phosphorylated region of MARCKS (2014) Biochimica et Biophysica Acta - Proteins and Proteomics, 1844 (4), pp. 837-849. – *IF: 1.094*
41. **Tosar JP**, Rovira C, **Naya H, Cayota A**. Mining of public sequencing databases supports a non-dietary origin for putative foreign miRNAs: Underestimated effects of contamination in NGS (2014) RNA, 20 (6), pp. 754-757. – IF: 5.377
42. **Garcia-Silva MR, Sanguinetti J**, Cabrera-Cabrera F, Franzén O, **Cayota A**. A particular set of small non-coding RNAs is bound to the distinctive Argonaute protein of Trypanosoma cruzi: Insights from RNA-interference deficient organisms (2014) Gene, 538 (2), pp. 379-384. – IF: 2.082
43. **Garcia-Silva MR**, Cabrera-Cabrera F, Cura Das Neves RF, Souto-Padrón T, De Souza W, **Cayota A**. Gene expression changes induced by Trypanosoma cruzi shed Microvesicles in mammalian host cells: Relevance of tRNA-derived halves (2014) BioMed Research International, 2014, art. no. 305239.
44. **Berois N**, Heard I, Fort Z, Alonso R, Sica A, **Moerzinger P**, Rodriguez G, Sancho-Garnier H, **Osinaga E**, Favre M. Prevalence of type-specific HPV infection in Uruguay (2014) Journal of Medical Virology, 86 (4), pp. 647-652. – *IF: 2.217*
45. **Berois N, Osinaga E.** Glycobiology of neuroblastoma: Impact on tumor behavior, prognosis, and therapeutic strategies (2014) Frontiers in Oncology, 4 MAY, art. no. 114. – *IF: --*
46. Ikemori R, Longo Machado C, Furuzawa K, Nonogaki S, **Osinaga E**, Umezawa K, Carvalho MA, Verinaud L and Chammas R. Galectin-3 up-regulation in hypoxic and nutrient deprived microenvironments promotes cell survival. Plos One (2014). – *IF: 3.534*
47. Isasi E, **Barbeito L**, Olivera-Bravo S. Increased blood-brain barrier permeability and alterations in perivascular astrocytes and pericytes induced by intracisternal glutaric acid. Fluids Barriers CNS. 2014 Jul 24;11:15.
48. Miquel E, Cassina A, Martínez-Palma L, Souza JM, Bolatto C, Rodríguez-Bottero S, Logan A, Smith RA, Murphy MP, **Barbeito L**, Radi R, Cassina P. Neuroprotective effects of the mitochondria-targeted antioxidant MitoQ in a model of inherited amyotrophic lateral sclerosis. Free Radic Biol Med. 2014 70:204-13.

49. Sanguinetti M, Amillis S, **Pantano S**, Scazzocchio C and Ramón A. Modeling and mutational analysis of AspergillusnidulansUreA, a member of the subfamily of urea/H+ transporters in fungi and plants. Open Biology, 2014, 4:140070
50. Zecchin A, Pattarini L, Gutierrez MI, Mano M, Mai A, Valente S, Myers MP, **Pantano S**, and Giacca M. Reversible acetylation regulates vascular endothelial growth factor receptor-2 activity. Journal of Molecular Cell Biology, 2014, 6:116.
51. Shigunov Shigunov P, Sotelo-Silveira J, Stimamiglio MA, Kuligovski C, **Irigoín F**, **Badano JL**, Munroe D, Correa A, Dallagiovanna B. Ribonomicanalysis of human DZIP1 revealsitsinvolvement in ribonucleoproteincomplexes and stress granules (2014) BMC Molecular Biology, 15, art. no. 12. – *IF: 2.057*
52. Leitch CC, Lodh S, **Prieto-Echagüe V**, **Badano JL**, Zaghloul NA. Basal body proteins regulate notch signaling through endosomal trafficking (2014) Journal of Cell Science, 127 (11), pp. 2391-2400. – *IF: 5.325*
53. **Pizzo L,** Fariello MI, **Lepanto P, Aguilar PS, Kierbel A**. An image analysis method to quantify CFTR subcellular localization, Molecular and CellularProbes, Volume 28, Issue 4, August 2014, Pages 175-180, ISSN 0890-8508. – *IF: 1.859*
54. Zhang S, Zheng H, Long N, **Carbó N,** Chen P, **Aguilar PS**, Lu L. FigA, a putative homolog of low-affinity calcium system member Fig1 in Saccharomyces cerevisiae, is involved in growth and asexual and sexual development in Aspergillusnidulans (2014) Eukaryotic Cell, 13 (2), pp. 295-303. – *IF: 3.179*
55. **Lepanto P, Lecumberry F**, **Rossello J, Kierbel A**. A confocal microscopy image analysis method to measure adhesion and internalization of Pseudomonas aeruginosa multicellular structures into epithelial cells (2014) Molecular and Cellular Probes, 28 (1), pp. 1-5. – *IF: 1.859*
56. Giorello, F.; **Berná, L.; Greif, G.**; Camesasca, L.; **Salzman, V**.; Medina, K.; **Robello, C.**; Gaggero, C.; **Aguilar, P.S.** and Carrau, F. (2014) Genomesequence of the native apiculate wine yeast Hansenia sporavineae T02/19AF. ***GenomeAnnouncements***,2(3). pii: e00530-14. doi: 10.1128/genomeA.00530-14.
57. Maiwald F, **Benítez D, Charquero D**, Dar MA, Erdmann H, Preu L, Koch O, Hölscher C, Loaëc N, Meijer L, **Comini MA**, Kunick C. 9- and 11-substituted 4-azapaullones are potent and selective inhibitors of African trypanosome (2014) European Journal of Medicinal Chemistry, 83, pp. 274-283. – *IF: 3.432*
58. Sturlese M, Lelli M, **Manta B**, Mammi S, **Comini MA,** Bellanda M. 1H, 13C and 15N resonance assignment of the mature form of monothiol glutaredoxin 1 from the pathogen Trypanosoma brucei (2014) Biomolecular NMR Assignments. – *IF: 0.82*
59. Peña S, Fagundez C, **Medeiros A**, **Comini M**, Scarone L, Sellanes D, Manta E, Tulla-Puche J, Albericio F, Stewart L, Yardley V, Serra G. Synthesis of cyclohexapeptides as antimalarial and anti-trypanosomal agents (2014) MedChemComm, 5 (9), pp. 1309-1316. – *IF: 2.62*
60. Sousa AF, Gomes-Alves AG, **Benítez D, Comini MA**, Flohé L, Jaeger T, Passos J, Stuhlmann F, Tomás AM, Castro H. Genetic and chemical analyses reveal that trypanothione synthetase but not glutathionylspermidine synthetase is essential for Leishmania infantum (2014) Free Radical Biology and Medicine, 73, pp. 229-238. – *IF: 5.71*
61. Hiller C, Nissen A, **Benítez D, Comini MA**, Krauth-Siegel RL. Cytosolic Peroxidases Protect the Lysosome of Bloodstream African Trypanosomes from Iron-Mediated Membrane Damage (2014) PLoS Pathogens, 10 (4), art. no. e1004075. – *IF: 8.057*
62. **Lago N**, Quintana A, Carrasco J, Giralt M, Hidalgo J, Molinero A. Absence of metallothionein-3 produces changes on MT-1/2 regulation in basal conditions and alters hypothalamic-pituitary-adrenal (HPA) axis. Neurochemistry International Volume 74, July 2014, Pages 65-73. – IF: 2.65
63. **Negro-Demontel ML**,Saccardo P, Giacomini C, Yáñez-Muñoz RG, Ferrer-Miralles N, Vazquez E, Villaverde A & **Peluffo H**. Comparative analysis of lentiviral vectors and

modular protein nanovectors for traumatic brain injury gene therapy. Molecular Therapy — Methods &Clinical Development 1, Article number: 14047 (2014) Published online 15 October 2014. – IF: Molecular Therapy 6,42, Sister journal Mol. Ther. Meth. CLin. Dev.

64. Tinoco L.W., Fraga J.L., AnoBom C.D., **Zolessi F.R.**, Obal G., Toledo A., Pritsch O., Arruti C. (2014) Structural characterization of a neuroblast-specific phosphorylated region of MARCKS. BiochimBiophysActa 1844(4): 837-849.
65. **Segovia M**, Louvet C, Charnet P, Savina A, Tilly G, Gautreau L, Carretero-Iglesia L, Beriou G, Cebrian I, Cens T, Hepburn L, Chiffoleau E, Floto RA, Anegon I, Amigorena S, **Hill M**, Cuturi MC. Autologous dendritic cells prolong allograft survival through Tmem176b-dependent antigen cross-presentation (2014) American Journal of Transplantation, 14 (5), pp. 1021-1031. – *IF: 6.1*
66. Drujont L, Carretero-Iglesia L, Bouchet-Delbos L, Beriou G, Merieau E, **Hill M**, Delneste Y, Cuturi MC, Louvet C. Evaluation of the therapeutic potential of bone Marrow-Derived Myeloid Suppressor Cell (MDSC) adoptive transfer in mouse models of autoimmunity and allograft rejection (2014) PLoS ONE, 9 (6), art. N° e100013. – *IF: 3.534*
67. Moreau A, Vandamme C, **Segovia M,** Devaux M, Guilbaud M, Tilly G, Jaulin N, Le Duff J, Cherel Y, Deschamps JY, Anegon I, Moullier P, Cuturi MC and Adjali O. Generation and in vivo evaluation of IL10-treated dendritic cells in a nonhuman primate model of AAV-based gene transfer Citation: Molecular Therapy — Methods & Clinical Development (2014) 1, 14028; doi:10.1038/mtm.2014.28
68. Baas MC, Kuhn C, Valette F, Mangez C, Duarte MS, **Hill M**, Besançon A, Chatenoud L, Cuturi MC, You S. Combining autologous dendritic cell therapy with CD3 antibodies promotes regulatory T cells and permanent Islet allograft acceptance. *Journal of Immunology* Volume 193, Issue 9, 1 November 2014, Pages 4696-4703. – *IF: 5.362*
69. **Otero L**, **Romanelli-Cedrez L**, Turanov AA, Gladyshev VN, Miranda-Vizuete A, **Salinas G.** (2014) Adjustments, extinction and remains of selenocysteine incorporation machinery in the nematode lineage.RNA20(7):1023-34.– *IF: 5.377*
70. Saiz C, Castillo V, Fontán P, Bonilla M, **Salinas G,** Rodríguez-Haralambides A, Mahler SG. Discovering Echinococcusgranulosusthioredoxin glutathione reductase inhibitors through site-specific dynamic combinatorial chemistry (2014) Molecular Diversity, 18 (1), pp. 1-12. – *IF: 2.544*
71. MatíasTailanián, **Federico Lecumberry**, Alicia Fernández, Giovanni Gnemmi, Ana Meikle, Isabel Pereira, and Gregory Randall. Dairy cattle sub-clinical uterine disease diagnosis using pattern recognition and image processing techniques. In Progress in Pattern Recognition, Image Analysis, Computer Vision, and Applications, volume 8827 of Lecture Notes in Computer Science, pages 690–697. Springer International Publishing, 2014.
72. Clasen BF, Poulsen MM, **Escande C**, Pedersen SB, Møller N, Chini EN, Jessen N, Jørgensen JO. Growth hormone signaling in muscle and adipose tissue of obese human subjects: associations with measures of body composition and interaction with resveratrol treatment. J Clin Endocrinol Metab. 2014 Dec;99(12):E2565-73. doi: 10.1210/jc.2014-2215. PubMed PMID: 25050904.
73. **Escande C**, Nin V, Pirtskhalava T, Chini CC, Thereza Barbosa M, Mathison A, Urrutia R, Tchkonia T, Kirkland JL, Chini EN. Deleted in Breast Cancer 1 regulates cellular senescence during obesity. Aging Cell. 2014 Oct;13(5):951-3. doi: 10.1111/acel.12235. Epub 2014 Jul 3. PubMed PMID: 24992635; PubMed Central PMCID: PMC4172532.
74. Nin V, Chini CC, **Escande C**, Capellini V, Chini EN. Deleted in breast cancer 1 (DBC1) protein regulates hepatic gluconeogenesis. J Biol Chem. 2014 Feb 28;289(9):5518-27. doi: 10.1074/jbc.M113.512913. Epub 2014 Jan 10. PubMed PMID: 24415752; PubMed Central PMCID: PMC3937628.

75. Calliari A, **Bobba N, Escande C**, Chini EN. Resveratrol delays Wallerian degeneration in a NAD(+) and DBC1 dependent manner. Exp Neurol. 2014 Jan;251:91-100. doi: 10.1016/j.expneurol.2013.11.013. Epub 2013 Nov 16. PubMed PMID: 24252177.
76. Chini CC, Guerrico AM, Nin V, Camacho-Pereira J, **Escande C**, Barbosa MT, Chini EN. Targeting of NAD metabolism in pancreatic cancer cells: potential novel therapy for pancreatic tumors. Clin Cancer Res. 2014 Jan 1;20(1):120-30. doi: 10.1158/1078-0432.CCR-13-0150. Epub 2013 Sep 11. PubMed PMID: 24025713; PubMed Central PMCID: PMC3947324.

Staff

Human Resources	Dic 2010	Dic 2014
Scientific & Technical Staff	70	102
Administration & Support Staff	30	34
Associated Researchers & Students	50	80
Total	150	216

Publications 2014* (Scopus)

Año	2007	2008	2009	2010	2011	2012	2013	2014*	Total
Publications	18	37	38	35	39	49	49	82	347

* Jan - Oct 2014

Aggregate Record	IP Mont 2007- 2014
Number of Publications	347
Accumulated Citations	5625
Citations per Publication	16,2

2014 Budget Overview

INCOMES - 2014 In thousands dollars		
FINANCIAL SOURCES	INCOME	%
IP MONTEVIDEO	4.261	60%
GRANTS	1.075	15%
SERVICES	836	12%
INNOVA	817	12%
FOCEM	102	1%
TOTAL	7.091	100%