Institut Pasteur de Montevideo

Institut Pasteur de Montevideo

Scientific Report 2015

INDEX

PREFACE

The IP Montevideo's mission is to become a "state-of the-art" research center with international projection in the field of biomedicine with focus on molecular mechanisms of human and animal diseases. We also seek to pave the way for new treatments and cures, contributing to the development of vaccines, diagnostic devices and new drugs. 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 2015, more than 240 people worked or studied at the IP Montevideo, including almost 100 researchers, technicians or assistants and 38 collaborators in administration and research support units.

One key event in 2015 was the attainment of first ranks publications, including the breakthrough discovery of the structural conformation of the retroviral capsid from bovine leukosis virus published in the journal Science.

The IP Montevideo has established central core facilities with "state-of-the-art" equipments to study genomic, proteomic, structural & cell biology and animal research. In 2015, an ambitious investment was performed to purchase a modern confocal microscope, a capilar electrophoresis and two new autoclaves.

Seventeen research groups with personnel from different institutions are organized into institutional Programs focused on Genomics, Cell and Animal Technology, Metabolic, Cardiovascular and Inflammatory Related Diseases and Zoonotic diseases. These programs are funded in part by institutional grants or agreements with FOCEM (Mercosur), Interamerican Development Bank (IDB), National Institute for Agricultural Research (INIA).

The IP Montevideo also contributes to the training of human capacities in collaboration with the national and international postgraduate programs. In 2015, we received more than 70 graduate, doctoral and postdoctoral fellows.

Our research laboratories provide an environment for the training of advanced graduate students. We have experienced great success in the organization of 6 international courses on different topics of molecular medicine. In 2015, more than 60 distinguished professors and dozens of advanced students from abroad attended the courses. In addition, the last Friday of each month, we received school and high school students and present different activities of science pop. All over the year this activity allows us to spread science among hundreds of young students.

The number and impact of the publications further increased in 2015, reaching more than 90 publications in the period, reaching a cumulated average of 16,5 citations per

Transfer technology to public or private companies is also a major activity of the IP Montevideo, contributing to the development of biotechnology and supporting the creation of start up companies. In 2015, our start up incubator Bioespinn supported the consolidation of 7 start-up companies. Biospinn is partially funded by the Uruguayan Agency for Innovation and Research (ANII).

Finally, the 2015 annual budget of the IP Montevideo was close to 6 million dollars, 2/3 coming from our government and 1/3 obtained by our self services, grants and research contracts, including those allocated by European Union (Uruguay-Innova) and FOCEM (MERCOSUR)]

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

Luis Barbeito
Executive Director
Institut Pasteur Montevideo

RESEARCH

CORE FACILITIES

- Analytical Biochemistry and Proteomics Unit
- Recombinant Proteins Unit
- Protein Crystallography
- Bioinformatics Unit
- Molecular Biology Unit
- Cell Biology Unit
- Transgenic and Experimental Animal Unit
- Bioparmaceutical Quality Control and Development Laboratory
- Microscopy Unit

Analytical Biochemistry and Proteomics Unit

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 Rossello, Biochemist** (Technician, PhD student; ANII Fellow)
- **Bernardina Rivera, Biochemist** (Technician, Graduate 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)

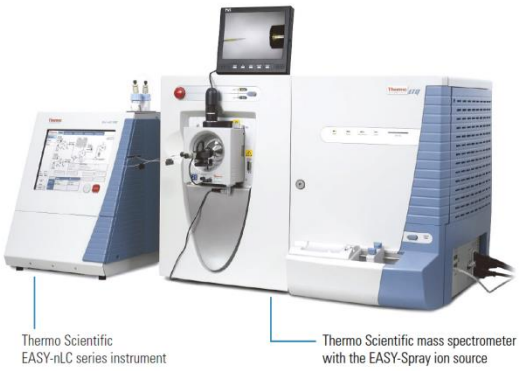
Students

- **Jorge Rodriguez, Biochemist** (PhD student; ANII Fellow)
- **Rosina Dapueto,** M.Sc. (PhD Student, ANII Fellow)
- **Alejandro Leyva, Biochemist** (PhD student; ANII Fellow)
- **Gonzalo Spera, M.D.** (MSc student)
- **Adriana Carlomagno, M.D.** (MSc student)
- **Germán Galliusi** (MSc student, ANII Fellow**)**
- **Rosina Toledo** (MSc student, CSIC)
- **Josefina Peña** (Undergraduate student, ANII Fellow)

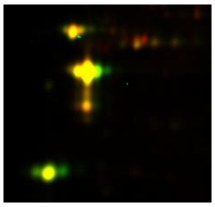
MAIN EQUIPMENT

- HPLC, Agilent 1200
- Capilar HPLC, Agilent 1200;
- Nano HPLC, Easy-nLC 1000, Thermo-Proxeon
- 2D Electrophoresis, Ettan IPGphor + Ettan DaltSix
- Typhoon FLA 9500, GE Healthcare
- 4800 MALDI TOF/TOF Mass Spectrometer, Abi Sciex
- LTQ Velos + ETD Mass Spectrometer, Thermo

4800 MALDI-TOF/TOF Analyzer, Abi Sciex



LTQ Velos, Thermo



Typhoon FLA 9500, GE

SERVICES

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.

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 simple preparation.
- Post-translational modification analysis.
- 2-D gel electrophoresis based proteomics.
- “Shotgun” proteomics.
- Quantitative proteomics.
- De novo peptide sequencing.

RESEARCH

In the past years, members of our group have been involved in different areas of biological/biomedical & 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:

CELL SIGNALING PATHWAYS IN PATHOGENIC BACTERIA: A PROTEOMIC APPROACH

- Characterization of *Mycobacterium tuberculosis* Ser/Thr kinase PknG signaling pathways. PI: Rosario Durán; Graduate Thesis Project of M. Gil, PEDECIBA, UdelaR, 2011-to date.
- 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.
- 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).

BIOLOGICAL EFFECTS OF NEW GENERATION OF 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.

1. 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).
2. Electrophilic mediated protein modifications.

1. "Composition and Method for Inhibition of PknG from *Mycobacterium tuberculosis*", PIs C. Batthyány & R. Durán (U.S. PCT Application No. 61/835,416; 2014).
2. Molecular Mechanisms of Nitroalkene mediated anti-inflammatory cell signaling events; PIs C. Batthyány & H. Botti.

EDUCATION

TRAINING OF STUDENTS

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.

Magister in Science students:

- **Jessica Rossello**. PEDECIBA Biología. "Estudio de la adhesión y agregación de *Pseudomonasaeruginosa* en células epiteliales mediante aproximaciones proteómicas" Director: R. Durán Co-Director: A. Kierbel. **Finished Dec 2015**
- **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. **Finished Dec. 2015**
- **Bernadina Rivera**. PEDECIBA Química. "Vías de señalización mediadas por PknG y su regulación en micobacterias". Director: Dra R. Durán, Co-Director: Dr. C. Batthyány.

Under gradúate students:

- **Josefina Peña**. Lic. Bioquímica. "Modulación del proteoma del fagosoma por una quinasa de *Mycobacteriumtuberuculosis*". Initiation into Research Fellowship ANII. Director: R. Durán; Co-Director: A. Lima. **Finished Sep 2015**

- **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. **Finished June 2015**
- **Rosina Toledo Gallo.** Lic. Bioquímica. "Purificación Analítica y Preparativa de las Proteínas hidrofóbicas del Surfactante Pulmonar". Initiation into Research Fellowship ANII. **Finished Apr 2015**

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. **"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**.
3. **"Identification of tumor associated antigens"**; (2014 – 2015); Private Company: Contrato de Servicio; PIs R. Durán & C. Batthyány; **Amount Granted: USD 35.500**.
4. "**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**.
5. "**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.**
6. "**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.**
7. "**Surfactante Pulmonar durante la Lesión Pulmonar Aguda: Abordaje estructural, dinámico y funcional**" (2013 – 2015) CSIC I+D 2012, **PI: L. Malacrida, AmountGranted: USD 38.000**.
8. **"Redes de señalización mediadas por dominios FHA en micobacterias y su rol en la adaptación al ambiente del hospedero"** (2015-2018); R. Durán (FCE_1_2014_1_104045); **Amount Granted: USD 50.000**
9. **Desarrollo y validación de procesos para el estudio y valorización de nutracéuticos: creación de la primer empresa uruguaya del tipo "Contract of Research Organization".** (2015-2017); C. Batthyany (ALI_2-2014-1-5055); **Amount Granted: USD 302.000**

PUBLICATIONS

1. Wagner, T., Alexandre, M., Durán, R., Barilone, N., Wehenkel, A., Alzari, P.M.& Bellinzoni, M. **2015**. The crystal structure of the catalytic domain of the Ser/Thr kinase PknA from *M. tuberculosis* shows an Src-like autoinhibited conformation. **Proteins 83:982-988**.

2. Lisa, M.N., Gil, M., André-Leroux, G., Barilone, N., Durán, R., Biondi, R.M. & Alzari, P.M. **2015**. Molecular Basis of the Activity and the Regulation of the Eukaryotic-like S/T Protein Kinase PknG from *Mycobacterium tuberculosis*. **Structure 23:1039-1048**.

3. Margenat ,M., Labandera, A.M., Gil, M., Carrion, F., Purificação, M., Razzera, G., Portela, M.M., Obal, G., Terenzi, H., Pritsch, O., Durán, R., Ferreira, A.M. & Villarino, A. **2015**. New potential eukaryotic substrates of the mycobacterial protein tyrosine phosphatase PtpA: hints of a bacterial modulation of macrophage bioenergetics state. **Sci. Rep.5: 8819**.

4. Yunes Quartino, P.J., Portela, M., Lima, A., Durán, R., Lomonte, B. & Fidelio, G.D. **2015**. A constant area monolayer method to assess optimal lipid packing for lipolysis tested with several secreted phospholipase A2. **Biochim Biophys Acta. 1848: 216-224.**

5. Cabrera, .G, Salazar, V., Montesino, R., Támbara, Y., Struwe, W.B., Lugo, E.L., Harvey, D.J., Antoine, L., Rincón, M., Domon, B., Méndez Martínez, M.D., Portela, M., González-Hernández, A., Triguero, A., Durán, R., Lundberg, U., Vonasek, E. & González, L.J. **2015** Structural characterization and biological implications of sulfated N-glycans in a serine protease from the neotropical moth *Hylesia metabus* (Cramer [1775]) (Lepidoptera: Saturniidae). **Glycobiology**. 2015 Nov 3. pii: cwv096. [Epub ahead of print]

INTERNATIONAL PATENT: under the Patent Cooperation Treaty (PCT)

1. Nitroalkene tocopherols and analogs thereof for use in the treatment and prevention of inflammation related conditions. Batthyány, C. & G.V. López Inventors; WO 2015/073527 A1; WIPO PCT

2. Composition and method for inhibition of PknG from Mycobacterium Tuberculosis; Batthyány, C. & R. Durán Inventors; US 2015/0051283 A1

Recombinant Proteins Unit

MEMBERS

- **Pablo Oppezzo, PhD** (Head)
- **Agustín Correa, PhD** (Principal technical assistant)
- **Claudia Ortega, PhD** (Technical Assistant)
- **Cecilia Abreu, PhD** (Technical Assistant)

MAIN EQUIPMENT

ÄKTAxpress

ÄKTAxpress for protein purification gives you the highest possible purity needed for structural and functional studies. Optimized protocols with a choice of up to four purification steps minimize the need for chromatography expertise. Tag removal and maintenance procedures can be integrated into the purification protocols, eliminating manual interference during a run. Purification schemes for double affinity-tagged proteins are also supported. A four-step protocol may consist of affinity chromatography (AC), desalting (DS), ion exchange chromatography (IEX) and gel filtration (GF).

ÄKTA Pure

ÄKTA pure is a flexible and intuitive chromatography system for fast purification of proteins, peptides, and nucleic acids from microgram levels to tens of grams of target product. Is a reliable system where hardware and UNICORN™ system control software are designed to work together with columns and media to meet any purification challenge.

ÄKTA Purifier

ÄKTA™purifier systems are designed for fast, high-resolution separation and characterization of proteins at laboratory scale. The systems perform all chromatographic techniques and scout for optimal binding and elution conditions, pH, gradient shapes, and flow rates. This system can produce 25 MPa and flow rates up to 10 ml/min, become ideal for laboratory purification and high-resolution analysis.

Robot Tecan Genesis 200

The Tecan Genesis 200 is a versatile robot for automating pipetting tasks. It is equipped with two arms, the LiHa (liquid handler) and the RoMa (robot manipulator). The LiHa is a conductivity-sensing 8-channel pipetting arm capable of dispensing volumes of 5-1000ul. The RoMa is a manipulating arm capable of picking up and moving objects on the workstation.

Benchtop Bioreactor BIOSTAT® B plus (Prokaryotic culture)

The BIOSTAT® B fermenter bioreactor has been specially designed to cover the wide variety of requirements in biotechnological and biopharmaceutical research and development. The wide range of configurations is available to choose from for animal, plant and insect cell cultivation as well as for microbial fermentation. Volumes range from 1L to 2L to 5L. Typical areas of application include the following:

- Process development for the manufacture of vaccines, recombinant proteins and monoclonal antibodies
- Process strategy development using a batch, fed-batch, continuous or perfusion mode

- Scale-up and scale-down tests for commercial-scale manufacture
- Small-scale (pre-) production
- High cell-density fermentation
- Adherent cell cultures on microcarriers
- Low-shear-stress cell cultivation of sensitive organisms
- Cultivation of filamentous organisms
- Dual usage both for cell cultures and microbial applications, such as in academic research.

CelliGen 310 Bioreactor (Eukaryotic culture)

CelliGen® 310 is a benchtop, autoclavable bioreactor with advanced controller and touchscreen interface capable of operating up to four reactors simultaneously. The CelliGen 310 is an advanced benchtop cell culture bioreactor ideal for research through production. This powerful system can regulate up to 32 parameters each, in one to four vessels. Over 120 parameters total. So, you can integrate and control your own analyzers, pumps, sensors and other ancillary devices directly from the CelliGen 310 bioreactor. Some bioreactor´s characteristics are:

- Available 5.0 L culture,
- Batch, fed-batch & continuous modes for growing high-density cultures of mammalian, insect & plant cells
- For secreted products, a patented packed-bed basket option is available to maximize cell productivity regardless of cell type
- Fully-integrated system is ready for out-of-the box startup.
- Mass flow controller with 4-gas control. Vessel, pH/DO/and level/foam probes, hoses are present.

BelloCell 3000 Bioreactor (Eukaryotic culture)

The eucaryote cell culture system BelloCell 3000 provides a protected, controlled, and contained environment for the growth of cell cultures. Maximal capacity is equivalent to extremely high yields — averaging 2.4 x 1010cells for a system with four bottles. The BelloCell 3000 consists of three major components; a control box, the BelloStage unit, and ready-to-use disposable 500 ml bottles. The BelloStage unit, which holds up to four disposable cell culture bottles, moves the bottles' contents up and down according to your program, using a platform to compress and expand the bellows built into each bottle, to optimize oxygenation. As the platform lifts, it compresses the bellows, sending the media into the chamber that contains the BioNOC II® disks; as the platform descends, the media returns to the expanding bellows, exposing the carrier disks to the atmospheric

environment. The growth cells in these carriers grow along the fibers, and then pile up to fill the space in the net.

EmulsiFlex-C5 Homogenizer

The EmulsiFlex-C5 has an air/gas driven, single-acting, high-pressure pump. Quiet operation is due to a specially designed pump motor pilot valve.
The EmulsiFlex-C5 has a capacity of 1-5L/hr. The flow rate depends upon the selected homogenizing pressure. Samples, as small as 7mL, can be processed with a hold back volume of less than 1mL. AVESTIN provides stainless steel heat exchangers to control inlet and outlet temperatures. The entire EmulsiFlex-C5 can be immersed in a water bath for temperature control. The pressure is adjustable between 500-30,000psi (30-2,000bar), which is high enough for virtually every homogenization application.
The equipment is Steam-In-Place (SIP) sterilizable. It is suitable for clean room and GMP manufacturing. All wetted parts are autoclavable. For inspection, all wetted parts can be disassembled and reassembled in a short time.

Multitron 2 Incubated Shaker

The Multitron II is a large capacity shaking incubator which combines flexibility and operational safety with the optimum utilization of space by its modular construction. UPR laboratory have three units with culture capacity of 5 liters each one for differents procariotyc cultures. In addition integral microprocessor offers a wide variety of control possibilities, including high temperature range (C° 12 to C° 65), oxygenation control and light intensity.

SERVICES

Services that are currently being provided

1. Protein expression in prokaryotic and eukaryotic systems:

- E. coli expression
- Baculovirus system
- Mammalian cells expression
- Drosophila expression system

2. Optimizing conditions for the expression of Recombinant Proteins
3. Refolding and soluble production of Recombinant Proteins (RP)
4. Maintaining collections of expression vectors and bacterial strains
5. Developing and testing new vectors and protocols

RESEARCH

Recombinant proteins, have demonstrated a high impact in basic research as well as in the biomedical field. However, in many cases obtaining a soluble and homogenous product is not possible, limiting their applications. Several strategies were developed over the last decades to overpass these limitations. In this regard, our group had generated a vector suite that facilitates the cloning steps and allows the evaluation of several parameters that can improve the soluble expression of a target protein. At the moment the vector suite is used not only in our group, but by several groups from the IPMONT and from laboratories from Argentina, France, USA, Sweden and India among others (*Correa et al., Front Microbiol., 2014; Correa et al., Biotechnol J 2011; Correa et al., Methods Mol Biol 2015*).

In the context of therapeutics tools related with cancer, our group is recently focused on the generation artificial binding proteins know as Affitins. This class of proteins, present a broad range of advantages when compared with classical therapeutics antibodies that could be taken into account in the development of therapeutic approaches. Compared with classical therapeutics antibodies Affitins 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 acquiring favorable 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 these artificial binding proteins 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*).

EDUCATION-COURSES

1. “Introduction to structural and functional analysis of proteins,” Coordinators: Agustin Correa,Horacio Botti,Matias Machado,Felipe Trajtenberg,Lucia Turell, Bruno Manta . Institut Pasteur de Montevideo. Setiembre-Noviembre 2014.
2. Curso de postgrado: “Expression, Purification and Crystallization of Recombinant Proteins by High-throughput Methodologies”. Coordinadores: Pablo Oppezzo y Renaud Vincentelli. Febrero 2013.
3. Curso de postgrado: “Expresión de Proteínas Recombinantes”. PEDECIBA- Biología-Maestría en Biotecnología, Facultad de Ciencias – Institut Pasteur de Montevideo. Coordinadores: Oppezzo P. (IP Montevideo) y Marín M. (FC, UdelaR) – 5 de noviembre – 10 de diciembre de 2008.

GRANTS

- Fondo María Viña – Dr. Pablo Oppezzo – "Development of Artificial Binding Proteins (Affitins) to evaluate new prognosis and treatment strategies in Chronic Lymphocytic Leukemia"– 2015-2017 – ANII, Uruguay
- CSIC, I+D2014 – Dr. Pablo Oppezzo – "Implicancias de la expresión anómala de la enzima mutagénica AID en la progresión de la Leucemia Linfoide Crónica" – 2014-2017 –Comisión sectorial de investigación científica de la Universidad de la República, Uruguay.
- 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, Uruguay.
- 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, Uruguay
- 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, Uruguay
- Fondo CYTED – Dr. Pablo Oppezzo – "Red-iberoamericana de Leucemia Linfoide Crónica: hacia el desarrollo de nuevos marcadores pronósticos" – 2011-2014 – CYTED.
- Proyectos Transversales IPMont – 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, Uruguay.
- Fondo Lady Tata – Dr. Pablo Oppezzo – "Characterisation of the proliferating pool in CLL. Is AID expression a marker of this subpopulation?" – 2008-2011 – Lady Tata Foundation, United Kingdom.

PUBLICATIONS

1. **Correa A, Oppezzo P.** Overcoming the solubility problem in E. coli: available approaches for recombinant protein production. **Methods Mol Biol. 2015**;1258:27-44. doi: 10.1007/978-1-4939-2205-5_2.

Protein Crystallography

MEMBERS

Alejandro Buschiazzo, PhD (Head of the Lab)
Nicole Larrieux (Technician)
Frank Lehmann (Technician) - past member
Felipe Trajtenberg, PhD (Research Scientist)

TECHNOLOGICAL FACILITY

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 2015

1. Four new structures have been released in the PDB: 4PH0, 4PH1, 4PH2 and 4PH3, corresponding to BLV capsid structures. And six new structures have been solved (on hold until publication: 5IUJ, 5IUK, 5IUL, 5IUM, 5IUN and 5CEE), adding up to ten (10) crystal structures deposited in the Protein Data Bank.
2. A new collaboration has started with Dr Luis Javier Gonzalez (Centro de Ingeniería Genética y Biotecnología, Havana, Cuba). We wish to determine the crystal structure of human epidermal growth factor (hEGF) and selected variants, in complex with the extracellular portion of its receptor. hEGF variants under study have been developed by the Cuban team as an effective biological medicinal therapeutic.
3. Two interns (junior tenured researchers) were hosted in 2015, both coming from the Institut Pasteur (Paris): Francesca Gubellini (Dept of Structural Biology) from June to Aug; and Francesca Di Nunzio (Dept of Virology) from Oct to Dec. Dr Di Nunzio undertook a joint internship in the Pritsch lab and in ours here at IPMontevideo.

OTHER ACTIVITIES

WORKSHOPS, COURSES, TRAINING

1. Organization of the joint CeBEM-CCP4-RIIP Macromolecular Crystallography School - "From data processing to structure refinement and beyond". Venue: Institut Pasteur de Montevideo, Uruguay. Hands-on international workshop.

April 6-16, 2015.

Funded by CeBEM, CCP4, IUCr, Inst Pasteur de Montevideo and the RIIP (Inst Pasteur International Network).

This fourth edition is in continuity with the previous Workshops organized at IPMontevideo in 2010 and 2013, and in Sao Carlos (Inst de Fisica de Sao Carlos, Univ de Sao Paulo, Brazil) in 2014. Twenty students, fourteen invited speakers and tutors (http://www.ifsc.usp.br/mx2014/).

Attendance of 24 students from 10 different countries; staff of 15 invited teachers and tutors coming from several of the best centers involved in Protein Crystallography around the world. Satellite scientific sympoisum “Structural Biology in Infection and Disease”, sponsored by the Institut Pasteur International Network, with seven invited speakers from different Pasteur Institutes.

2. Incorporation of two new members to the laboratory:
•Marcos Nieves (PhD student)
•Natalia Lisa (postdoc fellow, to start beginning 2016)

Frank Lehmann left our lab, finding a new position at the Membrane Protein Laboratory (Imperial College London, at Diamond synchrotron UK), still working in collaboration with our group. A new technician shall be incorporated in 2016 in our protein crystallography facility.

3. Creation of the Joint International Unit “Integrative Microbiology of Zoonotic Agents” - IMiZA. IMiZA links our lab with the Picardeau team at the Institut Pasteur, Paris. The Joint Unit will start operating in 2016, for a first five-years period.

Bioinformatics Unit

MEMBERS

Hugo Naya, PhD (Head)
Martín Graña, PhD (Associated Researcher)
Natalia Rego (Technical Assistant, MSc student in Zoology)
Lucía Spangenberg, PhD
María Inés Fariello, PhD (Research Assistant, funded by Facultad de Ingrniería)
Tamara Fernandez, PhD (Biology student, Research assistant)
Gregorio Iraola, PhD (PosDoc)
Pablo Fresia, PhD
Sebastián Valenzuela (Bioinformatics MSc student)
Daniela Megrian (Bioinformatics MSc student)
Ignacio Ferrés (Bioinformatics MSc student)
Daniela Costa (Biology PhD student)
Gonzalo Collazo (Undergraduated student)

SERVICES

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

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.

EDUCATION-COURSES

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).

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.

PUBLICATIONS

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Molecular Biology Unit

MEMBERS

Carlos Robello, PhD (Head)
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)
Lucía López (Estudiante Maestría)

MAIN EQUIPMENT

- DNA Sequencer/analyzer
- Real Time PCR
- Microarray reader
- Microarray hybridizer
- BioAnalyzer
- General Molecular Biology Equipment

SERVICES

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

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

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.

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*.

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.

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.

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CELL BIOLOGY UNIT

MEMBERS:

Mariela Bollati-Fogolín, PhD (Head)
Karen Perelmuter, MSc (Staff TA)
Sabina Victoria, MSc (Staff TA)
Romina Pagotto, PhD (Postdoctoral fellow)
Vanesa Piattoni, PhD (Postdoctoral fellow)
Soledad Astrada, MSc (PhD student)
Hellen Daghero (Master student)
Micaela Sureda (Research internship)

Inés Tiscornia, MSc (Staff TA) – past member
Giuliana Mastropietro (Undergraduate student – finished August 2015)

MISSION

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

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

CORE FACILITIES - SERVICES

The CBU has cell culture and flow cytometry facilities. The flow cytometry labs are equipped with a FACSAria Fusion (BD) cell sorter and two analytical cytometer, CyAn ADP (Beckman Coulter) and Accuri C6 (BD). In particular the cell sorter was installed in February 2015 and can operate under a Class II Type A2 biosafety cabinet.

The routine services that we offer are:

1. Culture, amplification and storage of different cell lines.
2. Detection of Mycoplasma contamination in cell culture by PCR.
3. Quantification of glucose and lactate in cell culture supernatants.
4. Cell-based assays: cytotoxicity, proliferation, biological activity.
5. Generation of recombinant or reporter stable cell lines.
6. Flow cytometry analysis: DNA content and cell cycle analysis, fluorescent proteins detection, apoptosis, multicolor analysis, cytokine quantification by Multiplexing.
7. Sorting of heterogeneous cell populations into homogeneous populations: sterile sorting, cloning by single cell deposition, up to 4 way sorting.
8. Training and advice for flow cytometry users

RESEARCH

In addition to the core facility activities and services the CBU has interest in different research projects:

CELL CULTURE TECHNOLOGY:

During the last years, our group has generated a variety of reporter cell lines with broader applications (type I IFN, redox biosensors, NF- kB, 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 Burgi *et al*, 2016), 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 (Porro *et al*, 2015 and one manuscript is in preparation). To accomplish the Project we had the financial support from ANII (2013-2015), one post-doctoral fellow ANII (R. Pagotto). In the frame of this project we are actively collaborating with Dr. Rodríguez (ISAL, CONICET-UNL, Santa Fe, Argentina).

COLLABORATIVE PROJECT:

Since 2011 we are collaborating with Dr. Vallespi, 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, four articles were published (Fernández Massó *et al*, 2013, Vallespí *et al*., 2014, Núñez de Villavicencio-Díaz *et al*, 2015a and 2015b), and one manuscript is submitted. Two students (S. Astrada, PhD student and H. Daghero, Master student) are performing their thesis under the supervision of Bollati M. and Vallespi M. Both students have fellowships support from ANII.

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- Porro V, Pagotto R, Harreguy MB, Ramírez S, Crispo M, Santamaría C, Luque EH, Rodríguez HA, Bollati-Fogolín M. Characterization of Oct4-GFP transgenic mice as a model to study the effect of environmental estrogens on the maturation of male germ cells by using flow cytometry. J Steroid Biochem Mol Biol. 2015 Jul 4. pii: S0960-0760(15)30006-6. doi: 10.1016/j.jsbmb.
- 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

PUBLICATIONS

2015

1- Leon IE, Cadavid Vargas JF, Tiscornia I, Porro V, Castelli S, Desideri A, Bollati-Fogolín M, Etcheverry SB. Oxidovanadium (IV) Complexes with chrysin and silibinin. Anticancer activity and mechanisms of action in a human colon adenocarcinoma model. J Biol Inorg Chem. 2015 Oct; 20(7):1175-91. doi: 10.1007/s00775-015-1298-7.

2- Núñez de Villavicencio-Díaz T, Ramos Gómez Y, Oliva Argüelles B, Fernández Masso JR, Rodríguez-Ulloa A, Cruz García Y, Guirola-Cruz O, Perez-Riverol Y, Javier González L, Tiscornia I, Victoria S, Bollati-Fogolín M, Besada Pérez V, Guerra Vallespi M. Data for comparative proteomics analysis of the antitumor effect of CIGB-552 peptide in HT-29 colon adenocarcinoma cells. Data Brief. 2015 Jul 8; 4:468-73. doi: 10.1016/j.dib.2015.06.024.

3- Negrotto S, Mena HA, Ure AE, Jaquenod De Giusti C, Bollati-Fogolín M, Vermeulen EM, Schattner M, Gómez RM. Human Plasmacytoid Dendritic Cells Elicited Different Responses after Infection with Pathogenic and Nonpathogenic Junin Virus Strains. J Virol. 2015 Jul 15; 89(14):7409-13. doi: 10.1128/JVI.01014-15.

4- Porro V, Pagotto R, Harreguy MB, Ramírez S, Crispo M, Santamaría C, Luque EH, Rodríguez HA, Bollati-Fogolín M. Characterization of Oct4-GFP transgenic mice as a model to study the effect of environmental estrogens on the maturation of male germ cells by using flow cytometry. J Steroid Biochem Mol Biol. 2015 Jul 4. pii: S0960-0760(15)30006-6. doi: 10.1016/j.jsbmb.

5- Salzman V, Porro V, Bollati-Fogolín M, Aguilar PS. Quantitation of yeast cell-cell fusion using multicolor flow cytometry. Cytometry A. 2015 May 28. doi: 10.1002/cyto.a.22701.

6- Núñez de Villavicencio-Díaz T, Ramos Gómez Y, Oliva Argüelles B, Fernández Masso JR, Rodríguez-Ulloa A, Cruz García Y, Guirola-Cruz O, Perez-Riverol Y, Javier González L, Tiscornia I, Victoria S, Bollati-Fogolín M, Besada Pérez V, Guerra Vallespi M. Comparative proteomics analysis of the antitumor effect of CIGB-552 peptide in HT-29 colon

adenocarcinoma cells. J Proteomics. 2015 May 24;126:163-171. doi: 10.1016/j.jprot.2015.05.024.
7- 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 Inflamm.; 2015: 860534. doi: 10.1155/2015/860534.

GRANTS

1. Separador celular de alta velocidad, multiparamétrico y bioseguro para su utilización en biomedicina y biotecnología. PI: M Bollati, ANII (EQC_2013_X_1_2, Uruguay), 2014-2015. Amount 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_2_2011_1_6046), 2013 – 2015. Amount granted: $U 525,000.
3. Diseño de biosensores para monitoreo simultáneo de señalización redox y cAMP: Desde la computadora a la célula y vuelta a la computadora. PI: S. Pantano. M. Bollati participated as associate research. ANII (FMV_1_2014_1_104000), 2015-2018. Amount granted: $U 1,300,000.
4. Diseño y producción de nuevas variantes de la hormona folículo estimulante (FSH) para su empleo en especies de interés productivo. PI: M Bollati, ANII (ALI_1_2015_1_5084), 2016-2019. Amount granted: U$S 290,000 (Joint project with industry).

Other fundings:

5. Doctoral fellowship ANII- POS_NAC_2012_1_8523, S. Astrada, 2013-2015 (3 years).
6. Postdoctoral fellowship ANII- PD_NAC_2013_1_10903, R. Pagotto, 2013-2015 (2 years).
7. Postdoctoral fellowship Instituto Pasteur de Montevideo, V. Piattoni, 2015-2017 (2 years).
8. Master fellowship ANII-POS_NAC_2015_1_109487, H. Daghero, 2016- 2018 (2 years).

OTHER ACTIVITIES

We cooperated as lecturers and instructors in:

- ✓ May 2015: Basic Cell Culture course, IIBCE, PEDECIBA. Montevideo, Uruguay. K. Perelmuter, S. Victoria and M. Bollati participated in the theoretical and practical activities.
- ✓ June, 2015: M. Bollati was invited speaker in the Symposium of Cronic Inflammation: Advances and therapeutic prospects, Montevideo, Uruguay.
- ✓ July, 2015: M. Bollati was invited speaker in the Sixth International School on Production of Biopharmaceuticals in Animal Cell Cultures, UFRJ, Rio de Janeiro, Brazil.
- ✓ August 2015: M. Bollati was invited speaker in Production of recombinant protein, Facultad de Ciencias, PEDECIBA, Montevideo, Uruguay.

TRAINING OF RRHH

Students performing their thesis work at the CBU:

- Soledad Astrada. PhD student. Supervisors: M. Bollati and M. Guerra Vallespi.
- Hellen Daghero, Master student. Supervisors: M. Bollati and M. Guerra Vallespi.

- Giuliana Mastropietro, diploma thesis in Biotechnology Engineering, Universidad ORT, Uruguay. Supervisors: I. Tiscornia, K. Perelmuter & M. Bollati. Finished in August 2015.

In addition, the CBU has hosted traineeships for postdoctoral fellows and visiting scientist:

- PhD. Ana Belén Heredia Gutiérrez: from Instituto Universitario de Ingeniería de Alimentos para el Desarrollo, Universidad Politécnica de Valencia (Spain). March - May (3 months). Iberoamerica fellowship from Santander Bank.
- PhD. David Romanin: from Instituto de Estudios Inmunológicos y Fisiopatológicos, Universidad Nacional de La Plata (Argentina). February - March (1 month).
- Biochem. Julián Abud: PhD student from Facultad de Bioquímica y Cs. Biológicas, Universidad Nacional del Litoral Santa Fe (Argentina). February (1 month).

M. Bollati has also contributed as:

- Reviewer of peer-reviewed journals, including PLoS ONE, J Biotechnol, Beneficial Microbes, among others.
- Reviewer of scientific projects for CONICYT (Chile), ANII (Uruguay agency).
- Member of the jury for the poster award in the 24th ESACT meeting (Barcelona, Spain).

Members of the CBU presented their work in several international scientific meetings:

- Use of Genetically Encoded Fluorescent biosensor to monitor intracellular redox changes in CHO-K1 recombinant protein-producing cells. Perelmuter K, Tiscornia I, Comini M, Bollati-Fogolín M. Poster at the 24th ESACT Meeting, Barcelona (Spain), May-June 2015.
- HT-29 and Caco-2 Reporter cell lines for functional studies of nuclear factor kappa-B activation. Mastropietro G, Perelmuter K, Tiscornia I, Bollati-Fogolín M. Poster and selected for oral presentation at the 24th ESACT Meeting, Barcelona (Spain), May-June 2015.
- Methyl green: old dye with new applications. Victoria S, Bollati-Fogolín M. Poster at 30th Congress of the International Society for Advancement of Cytometry, Glasgow (Scotland), June 2015. Victoria S. received the SRL Junior Staff Travel Award.
- Characterization of a novel antitumoral peptide: CIGB-552. Astrada S, Vallespí MG, Bollati-Fogolín M. Oral presentation at the BCI Departmental Retreat, IP Paris, November 25-27, 2015 Morzine, France. Astrada S received the RIIP fellowship.
- Characterization of Oct4-GFP transgenic mice as a model to study the effect of environmental estrogens on the maturation of male germ cells by using flow cytometry. Pagotto R, Porro V, Harreguy MB, Ramírez S, Crispo M, Rodríguez HA, Bollati-Fogolín M. Oral presentation at IP Montevideo Retreat Meeting. October 2015.
- CIGB-552 derived-metabolites: characterization and analysis of their antitumoral properties Astrada S, Gomez Y, Obal G, Vallespí MG, Bollati-Fogolín M. Poster at IP Montevideo Retreat Meeting. October 2015.

Transgenic and Experimental Animal Unit

MEMBERS

Martina Crispo, DVM, PhD (Head)
Geraldine Schlapp, MSc (Full time technician)
María Noel Meikle, MSc (Technician)
Ana Paula Arévalo, TMN (Technician, MSc student)
Gabriel Fernández, **BSc** (Technician)
Sergio Anchetta (Animal caretaker)
Martín Mereles (Animal caretaker)
Mario Borges (Animal caretaker)
Casandra Carrillo (Animal caretaker)
Ana Paula Mulet, MSc (PhD student)
Pedro Dos Santos, DVM, MSc (PhD student)
Natalibeth Barrera, BSc (MSc Student)
Federico Cuadro, DVM (MSc Student)
Micaela Sureda, BSc (Internship)

MISSION

Our scientific proposal is to provide high-level regional support in the field of animal gene edition including mice, rats, zebrafish and ruminants. For that, several techniques are offered nowadays, as pronuclear microinjection, homologous recombination in embryonic stem cells, lentiviral injection, transposons and the revolutionary CRISPR/Cas9 system. Associated to these techniques, we offer cryopreservation, in vitro fertilization and embryo rederivation. We also provide mice and rats with high genetic and sanitary status.

RESEARCH

During 2015, our Unit obtained important achievements, generating genetically modified models in mice and large animals using the latest technology known as CRISPR/Cas9. This positioned us as one of the only technological platform offering this system to regional researchers.

We have managed the extension of the conventional area of the animal house, increasing five times the existing capacity, including also pathogens models and rats.

We have strengthened established collaborations and our own research, reflected in six full papers in international journals and several abstracts.

We continue working with national and international biotechnological companies, with grants close to 50.000 USD and the services offered resulting in a new project granted by ANII Alliances to the Metabolic Diseases and Aging Lab, with an estimated amount of 300.000 USD shared with others research groups from IPMon and a Company created (NutraScan).

Students were formed under my leadership in activities developed in Uruguay, Hungary, Argentina, Brazil and Spain. Several posgraduated students follow their projects in our Unit.

We organized the Symposium and International Course "ADVANCES IN THE GENERATION OF GENETICALLY MODIFIED ANIMAL MODELS" with 60 registered participants from the region and 2 the world. This was the first time a course with these characteristics was dictated in our region, triggering lot of interest.

The fourth 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, we have managed to position the Unit at a high level in science and technology, which has also resulted in the formation of human resources.

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.

2015-2018 Nuevas estrategias para la criopreservación de ovocitos y embriones ovinos producidos in vitro. PhD thesis, Co-tutor.

2015-2018 Desarrollo de inoculantes para la movilización del fósforo como insumo en la producción agrícola. Tipo de participación: Integrante del Equipo.

2015-2018 Desarrollo y validación de procesos para el estudio y valorización de nutracéuticos: creación de la primera empresa uruguaya del tipo. Tipo de participación: Integrante del Equipo.

CORE FACILITIES - SERVICES

Generation of gene edited mice by CRISPR/Cas9 system (1 project – Spats1)

Generation of chimeric mice by homologous recombination in ES cells (1 project – Ccar2)

Generation of transgenic mice by pronuclear microinjection (1 project - RO GFP).

Embryo and sperm cryopreservation (three projects running).

In vitro fertilization: several murine lines successfully cryopreserved and rederived using CARD protocol

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. 18.000/year.

Three trials of acute safety of probiotic bacteria for Biopolis Company

Trials of biological activity for recombinant eritropoyetin (Lab. Clausen & LCB) (aprox. 12 per month). The animal facility is certified by the Ministry of Health.

Trials of toxicity for biotechnological products (EPO, Filgen, Interferon) for Lab. Clausen & LCB (10 per month).

PUBLICATIONS

1. Crispo M, Schlapp G, Meikle MN, Mulet AP, Barrera N, Cuadro F, Dos Santos-Neto PC, Menchaca A. Advances in the Generation of Genetically Modified (GM) Animal Models: Meeting report. Transgenic Res. 2015 Dec;24(6):1087-90. doi: 10.1007/s11248-015-9913-5.
2. Dos Santos Neto PC, Vilariño M, Barrera N, Cuadro F, Crispo M, Menchaca A. Cryotolerance of Day 2 or Day 6 in vitro produced ovine embryos after vitrification by Cryotop or Spatula methods. Cryobiology. 2015 Feb;70(1):17-22. doi: 10.1016/j.cryobiol.2014.11.001
3. Crispo M, Mulet AP, Tesson L, Barrera N, Cuadro F, dos Santos-Neto PC, Nguyen TH, Crénéguy A, Brusselle L, Anegón I, Menchaca A. Efficient Generation of Myostatin Knock-Out Sheep Using CRISPR/Cas9 Technology and Microinjection into Zygotes. PLoS One. 2015 Aug 25;10(8):e0136690. doi: 10.1371/journal.pone.0136690.
4. 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.
5. 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, 2015 Feb;32(2):271-5. doi:10.1007/s10815-014-0378-x.
6. Porro, V.; R. Pagotto; M.B. Harreguy; S. Ramírez; M. Crispo; C. Santamaría; E.H. Luque; H.A. Rodríguez; M. Bollati-Fogolín. Characterization of Oct4-GFP transgenic mice as a model to study the effect of environmental estrogens on the maturation of male germ cells by using flow cytometry. Journal of Steroid Biochemistry and Molecular Biology, v.: 154, p.: 53 - 61, 2015.
7. Menchaca, A; Dos Santos Neto, PC; Cuadro, F; Barrera, N; Crispo, M. Biotecnologías de la reproducción en ovejas y cabras: lo último y lo mejor (Review y Conferencia), 2015. Congreso de la Sociedad Latinoamericano de Reproducción Animal, Buenos Aires, Argentina, 2015
8. Morande, P.; Y. Yoy; N. Sotelo; D. Prieto; N. Seija; M. Crispo; N. Chiorazzi; P.Oppezzo. Constitutive AID expression in CCL mouse model leads to disease progression, tumor proliferation and diminished survival, 2015. XVI International 4 Workshop on Chronic Lymphocytic Leukaemia and the Young Investigators Meeting 2015, Sydney.
9. Cuadro, F.; P. Dos Santos-Neto; D. Bosolasco; V. Brum; M. Crispo; A. Menchaca. Effect of the treatment with progesterone and FSH on follicular aspiration of the first follicular wave in ewes. XXIX Reunión Anual de la Sociedad Brasilera de Tecnología de Embriones, Gramado, 2015.

GRANTS

“Ensayo de ingesta aguda de 3 cepas probióticas en ratones BALB/cJ". Biópolis.
Responsables Mariela Bollati, Martina Crispo (2015) USD 42.000.

RIIP and UNU-Biolac International Course “ADVANCES IN THE GENERATION OF GENETICALLY MODIFIED (GM) ANIMAL MODELS - International Mini Symposium: TRANSGENIC TECHNOLOGIES, THE LATEST TRENDS”. Main organizer. EUR 57.500.

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 Dos Santos Neto, MSc - Nuevas estrategias para la criopreservación de ovocitos y embriones ovinos producidos in vitro. PhD Thesis (2015-2019). UNIVERSIDADE FEDERAL DE SANTA CATARINA, Brasil, CNPq (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, PEDECIBA Biología (Co-tutor).
- Federico Cuadro, DVM - Influencia de la progesterona en el desarrollo folicular sobre la maduración y fertilidad ovocitaria en ovinos. MSc thesis, Facultad de Veterinaria, UdelaR (Co-tutor).
- Ana Paula Arevalo, TMN - Evaluación por imagenología molecular del diabody anti-Tn en modelo murino de cáncer de pulmón. MSc Thesis (2014-2016). UdelaR PRO.IN.BIO (Tutor).
- Micaela Sureda, Biol. Cultivo primario de organoides intestinales murinos en 3D in vitro. Internship (2015-2016). IPMon

MEETINGS

- Sociedad Latinoamericana de Reproducción Animal (SOLARA). Marzo 2015, Buenos Aires, Argentina.
- XXIX Reunión Anual de la Sociedad Brasilera de Tecnología de Embriones, Gramado, 2015.
- 66th AALAS (American Association for Laboratory Animal Science) National Meeting, Phoenix, USA; November. ICLAS-ARC Laboratory Animal Specialist Award (Geraldine Schlapp).
- ORAL PRESENTATIONS IN NATIONAL AND INTERNATIONAL MEETINGS
- Martina Crispo: Estado de la Transgénesis Animal en Uruguay. Sociedad Latinoamericana de Reproducción Animal (SOLARA). Marzo 2015, Buenos Aires, Argentina.

INTERNSHIPS & COURSES

- March – Ana Paula Mulet: Internship in Dr. Martín Rumbo’s Lab, Instituto de Estudios Inmunológicos y Fisiopatológicos, Universidad Nacional de La Plata, Argentina
- April - Ana Paula Arévalo & Gabriel Fernández: IV Curso de técnicas avanzadas en experimentación animal. Critical Care Training Center - Getafe University Hospital. Modalidad on – Line.
- May – Ana Paula Mulet: SALAAM Training School: Transposons and CRISPRs for large animals. Godollo, Hungary.
- June – Gabriel Fernández: Capacitación para Miembros de Comités de Bioética Animal, beca ICLAS. Online.

- October - Ana Paula Arévalo: Curso de Postgrado Estrategias en Bioseguridad y Biocontención, Universitat Autònoma de Barcelona, Spain.

TEACHING

- Organization of the International Course “ADVANCES IN THE GENERATION OF GENETICALLY MODIFIED (GM) ANIMAL MODELS – International Mini Symposium: TRANSGENIC TECHNOLOGIES, THE LATEST TRENDS. September 2015. More than 60 participants from the region and the world. One meeting report published in Transgenic Research (Crispo et al, 2015)
- Organization of the IP Montevideo internal course 2015: Manejo, técnicas de administración de sustancias y obtención de muestras en ratones, for 20 researchers that uses mice and zebrafish 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
Researcher Level 3 PEDECIBA
Member of the International Society for Transgenic Technologies (ISTT) Council (2014-2017)
Member of Scientific Comittee of Centro Multidisciplinario para Investigação Biológica (CEMIB) Universidad de Campinas (2010 - to date)
Members of Comisión Nacional de Experimentación Animal (CNEA) (2014 - 2018)
Members of Comisión de Evaluación del Riesgo en Bioseguridad, MGAP (2009 - to date)
Members of Comité de Ética en el Uso de Animales (CEUA). Institut Pasteur de Montevideo (2009 - to date)
Members of Comité de Ética en el Uso de Animales (CEUA). Facultad de Ciencias, UdelaR (2011 to date)
Member of Comisión de Bioseguridad. Institut Pasteur de Montevideo.
Members of Board of Asociación Uruguaya de Ciencia y Tecnología de Animales de Laboraotrio (AUCyTAL).
IPMon Posdoc proyect evaluation committee (2015)
Member of Organizing Committee for TT2016
Evaluation of scientific abstracts for TT2016 meeting
Evaluation of scientific abstracts for 42nd International Embryo Transfer Society

Biopharmaceutical Quality Control & Development Laboratory

MEMBERS

Alejandro Ricciardi, PharmD. (Head)
Larissa Armas, Technical Assistant
Diego Charquero, BSc
Sofía Horjales, PhD

MAIN EQUIPMENT

PA 800 Plus Capillary Electrophoresis (Beckman Coulter)
HPLC Prominence with DAD, RID and Fluorescence detectors (Shimadzu)
Multiskan Spectrum Spectrophotometer and Plate Reader (Thermo Scientific)
Class II, Type A2 Biological Safety Cabinet (Thermo Scientific)
CO2Incubator (Thermo Scientific)
InvertedMicroscope (Nikon)
Freezer -20 °C and Fridge (Angelantonni)
PLA® 2.0 , Stegmann Systems.
Combistats® , EDQM.

SERVICES

A) Routine

We carry out **Biological Activity** assays such as: cell-based bioassays, kinetic assays, and *in vivo* assays in different species.

Purity assays are performed by HPLC, SDS-PAGE, zone and Capillary Electrophoresis, isoelectric focusing or 2D electrophoresis and either ELISA to quantify protein contaminants or hybridization for DNA contaminants.

Identification assays are done through immunochemistry techniques, peptide mapping, N-glycan profiling and**Quantification** assays through colorimetric and HPLC techniques.

B) Institutional Technological Platform for Biopharmaceutical Comparability Studies

The current regulations and international guidelines establish new and rigorous quality requirements to demonstrate biosimilarity among the innovative products already existent in the market and its possible copies.

These requirements are important in a potential biosimilar development stage, to generate scientific evidence supporting the quality, efficacy and safety of the biosimilar to be as close as possible to the reference product.

The comparability study from which biosimilarity should be inferred consists overall in three steps:

1) Physicochemical and biological quality comparability "*in vitro*"
2) Non-clinical comparability
3) Clinical comparability

The physicochemical and biological characterization is the analytical founding for the development and comparison of the possible biosimilars, and the amount of possible reduction for non-clinical and clinical comparison studies depends of the success in this first stage.

We have experience in biosimilars head to head physicochemical comparability studies in our Lab, together with other platforms of the Institute, following WHO and EMA international guidelines.

Besides the previously described assays, the analytical set for comparability studies include: binding assays, folding assays, characterization and quantification of molecular aggregates, thermal stability, and tertiary structure determination among others.

C) ANALYTICAL TARGETS

The current analytical bioportfolio includes the determination of the quality specifications for the following biopharmaceuticals: Interferon-α, Interferon-β, Filgrastim (G-CSF), PEGylated derivates of Interferon and G-CSF, Molgramostim (GM-CSF), Interleukin-2, Erythropoietin, Insulin, Heparin and low molecular weight Heparin, Albumin, Immunoglobulin, Somatropin, Coagulation Factor VIII and certain monoclonal antibodies (Adalimumab, Rituximab and Abciximab).

This is an open list that continues to increase as new technical and technological possibilities are available.

RESEARCH

The Quality Control and Development Laboratory of Biopharmaceuticals offers vast experience in methodologies development, bioassays and protein chemistry, as well as a wide range of analytic techniques and lab equipment.

This Lab was conceived to provide solutions in the field of analytical control of Biopharmaceuticals, either using pre-established methodologies by the international guides and pharmacopeias or developing new analytic tools in order to meet and follow the current bioanalytical strategy.

The Lab offers a variety of support services to Biopharmaceuticals products for human use in GLP conditions. Our assays follow the directions established by the ICH guidelines and the FDA and EMA agencies.

From its beginning as analysis laboratory in June 2009, it was named by the Public Health Ministry authorities as the reference lab qualified to perform in-country release testing for Biopharmaceuticals sold in the Uruguayan Market.

The compliance of GLP conditions was not only certified by the government health authorities, but also by several quality audits performed by our clients, both national and international (Current MSP Certification).

The purpose of this Lab belonging to the Institute's core facilities is to allow the collaboration with other units, thus amplifying the set of analysis to obtain a complete physicochemical characterization of biopharmaceuticals quality properties.

Additionally, it is an advantage to face new regulatory challenges regarding the appearance of biosimilars and to be able to offer a broad analytical platform to perform *head to head* physicochemical comparability studies against the original therapeutic molecules.

With the recent introduction of new national and regional regulations for Biosimilar pharmaceuticals, the capability to apply analytical tools in order to perform comparability studies plays a substancial role.

Therefore, it is essential to know the *state of the art* regarding these analytical technologies to evaluate in depth biopharmaceuticals or potential biosimilars. The regulatory health agencies have established three characteristics as priority to identify and analyse in detail biopharmaceuticals, grouped in the following analytical specifications i) possible post-translational modifications; ii) tertiary structure and iii) possible protein aggregates.

Within the Lab's study object framework – biopharmaceuticals – specific *kits* and methodologies have been developed on demand. Some examples of these projects are:

i) "Methodology Development to Quantify Host Cell Protein and DNA contaminants in recombinant biopharmaceuticals". Financed by the National Agency for Research and Innovation: ANII (ALIANZA project between Laboratorio Celsius S.A. and IP Montevideo) (2011-2012)

ii) "Methodological development to quantify generated immunogenicity by Interferon beta1a administration in patients, through cell based and RT PCR bioassays". Financed by Laboratorio Clausen S.A. (2010)

Moreover, we participated in a multicenter study for the determination of the biological activity of the first filgrastim USP reference standard (2012).

Development of the analytical methodology for the characterization of the N-glycosilated chains of therapeutic proteins using hydrophilic interaction HPLC with fluorescence detection (HILIC-FLD), as well as MALDI-TOF mass spectrometry (Tech transfer from CIGB-CUBA).

GRANTS

Tech transfer for Laboratorio Celsius S.A. (Uruguay) for the filgrastim biological activity bioassay (2009).

Methodological development to quantify generated immunogenicity by the Interferon beta1a administration in patients, through cell based and RT PCR bioassays.Laboratorio Clausen S.A. (Uruguay), (2010).

Biopharmaceutical analytical tech transfer for ConsorcioBiocertifica (Chile), (2010).

Methodology development to quantify Host Cell Protein and DNA contaminants in recombinant biopharmaceuticals.ALIANZA project financed by ANII (National Agency for Research and Innovation) and Laboratorio Celsius S.A. (2011-2012).

Bioassay validation study for fligrastim biological activity for Eurofarma Laboratory (Brasil), (2012).

Physicochemical comparability study between two commercial Abciximab biopharmaceuticals.Laboratorio Libra S.A (Uruguay), 2013.

Tech transfer from Biogen Idec.(USA) for Interferon beta1a biological activity bioassay (2013).

Validation study for peg-fligrastim biological activity bioassay for Eurofarma Laboratory (Brasil), (2014).

Participation in physicochemical comparability studies for the development of a filgrastim-based biosimilar (Fiprima) of Eurofarma Laboratory (Brasil). First biosimilar of original production in Latin America approved by ANVISA (2011 – 2015).

Tech transfer to Eurofarma Laboratory (Brasil) of the analytical methodology to perform the filgrastim and peg-filgrastim biological activity cell-based bioassay, (2016).

Microscopy Unit

MEMBERS

Flavio Zolessi, PhD (Head)
Federico Lecumberry, PhD (Head)
Marcela Díaz, MSc (Technician)
Tabaré de los Campos (Technician)

DESCRIPTION

The Microscopy service of the Institut Pasteur from Montevideo (IP Montevideo) has equipment for performing fluorescence and confocal microscopy. These microscopes are available to all researchers in the public or private sector who wish to view and take pictures of fluorescent or confocal microscopy.

Our service is dedicated to making assistance and image processing as well as providing technical advice. We have high quality equipment that allow to obtain high resolution images of materials of biological and non-biological origins.

MAIN EQUIPMENT

Inverted fluorescence microscope

Brand: Olympus

Model: IX81

Camera: ORCA, Hamamatsu

The fluorescence microscope is equipped with dichroic filters that allow observation of a wide range of fluorochromes:

Available fluorescence filters:

- 1 – U-MNUA2: Excitation 360-370nm / Emission 420-460nm (DAPI)
- 2 – U-MNIBA3: Excitation 470-495nm / Emission 510-550nm (FITC, GFP)
- 3 – U-MWIG3: Excitation 530-550nm / Emission 570nm (Rhodamine, TRITC, Cy3, Texas Red)
- 4- GOLD: Excitation 300-400nm / Emission 515nm (sybr GOLD)

It also has polarizing filters for observations of differential interference contrast (Nomarski technique).

Acquisition Software:

Image pro-plus (SCOPE-PRO)

Available Objectives:

- 10X NA 0.30
- 40X LUCP LWD NA 0.6
- 60X NA 1.25 OIL IMMERSION
- 100X NA 1.40 OIL IMMERSION

Confocal Microscopy

Brand: Leica

Model: DMI6000, TCS-SP5

Available filters:

- 1 – A: Excitation 340-380nm / Emission 425 (DAPI)
- 2 – I3: Excitation 450-490nm / Emission 515 (FITC, GFP)
- 3 – N2.1: Excitation 515-560nm / Emission 590nm (TRITC, Cy3, Texas Red)

Available Objectives:

- 20X NA 0.70 WATER / GLYCEROL /OIL IMMERSION
- 63X NA 1.42 OIL IMMERSION

Available Lasers:

- Argon 488nm: 458/476/488/496/514
- HeNe 543nm
- HeNe 594nm
- HeNe 633nm
- Diode 405 nm

Software acquisition:

The confocal laser microscope is an essential tool for the study of intracellular localization and colocalization of fluorescently labeled signals. The software LASAF (Leica Application Suite Advanced Fluorescence) allows acquisition and image processing.

Confocal Microscopy II

Brand: Zeiss

Model: Axio Observer Z1, LSM 800

- Transmitted Light Basis Set with DIC for LSM 800

Available fluorescence filters:

- 1 – DAPI: Ex 365 nm, Em 445/50
- 2 – FITC, GFP: Ex 450 – 490, Em 515 – 565
- 3 –TRITC, RODAMINE: Ex 546/12, Em 575 – 640

Available Objectives:

- 25X LD LCI Plan-Apochromat 0,8 AN/ WD:0,57/ cover glass 0-0,17.

Imm DIC. cod: 420852-9871-000

- 63X LCI-plan-Neofluar/1,3 AN/DIC cod: 420882-9970-000
- Objective i Plan-Apochromat 63x/1.4 Oil DIC M27 with insulation ring for optimized temperature at the specimen, (WD=0.18mm) incl. Immersol 518 F, oiler 20ml and Cover glasses, high performance, D=0.17mm

Available Lasers:

- Diode laser 405nm, 5mW, laser class 3B
- Diode laser 488nm, 10mW, laser class 3B
- Diode laser (SHG) 561nm, 10mW, laser class 3B
- Diode laser 640nm, 5mW, laser class 3B

Acquisition Software:

ZEN 2.1 system Hardware License Key
Image acquisition and processing under Win 7 x64. User interface configurable, control of the Carl
Zeiss microscope systems and components, extensive
acquisition and analysis. CZI image format.

The following modules are included:
– ZEN Module Measurement
– ZEN Module Multi Channel
– ZEN Module Image Analysis
– ZEN Module Time Lapse
– ZEN Module Z Stack
– ZEN Module Extended Focus
– ZEN Module Autofocus
– ZEN Module Colocalisation
– ZEN Module Spectral Unmixing
– ZEN Module Tiles & Positions

SERVICES

- Capture images using confocal microscopy
- Capture images using epifluorescence microscopy

LABORATORIES

- Functional Genomics
- Neurodegeneration
- Tumor Immunology & Glycobiology
- BioMolecular Simulation
- Molecular & Human Genetics
- Metabolic Diseases & Aging
- Redox Biology of Tryponosomes
- Neuroinflammation & Gene Therapy
- Worm Biology
- Cell Biology of Neural Development
- Immunoregulation & Inflammation
- Signal Processing
- Molegcular & Structura Microbiology
- Chronic Lymphocytic Leukemia
- Structural and Molecular Microbiology
- Host – Pathogen Interactions

Functional Genomics

MEMBERS

Alfonso Cayota, MD, PhD (Head)
Julia Sanguinetti (Msc Student)
Juan Pablo Tosar (Doctoral Student)
Braulio Bonilla (MSc Student)
Fabiana Gambaro (Undergraduate Student)

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.

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.

PUBLICATIONS

Linhares-Lacerda, L., Palu, C.C., Ribeiro-Alves, M., Paredes, B.D., Morrot, A., Garcia-Silva, M.R., Cayota, A., Savino, W. Differential expression of microRNAs in thymic epithelial cells from Trypanosoma cruzi acutely infected mice: Putative role in thymic atrophy (2015) Frontiers in Immunology, 6 (AUG), art. no. 00428,

Tosar, J.P., Gámbaro, F., Sanguinetti, J., Bonilla, B., Witwer, K.W., Cayota, A. Assessment of small RNA sorting into different extracellular fractions revealed by high-throughput sequencing of breast cell lines (2015) Nucleic Acids Research, 43 (11), pp. 5601-5616.

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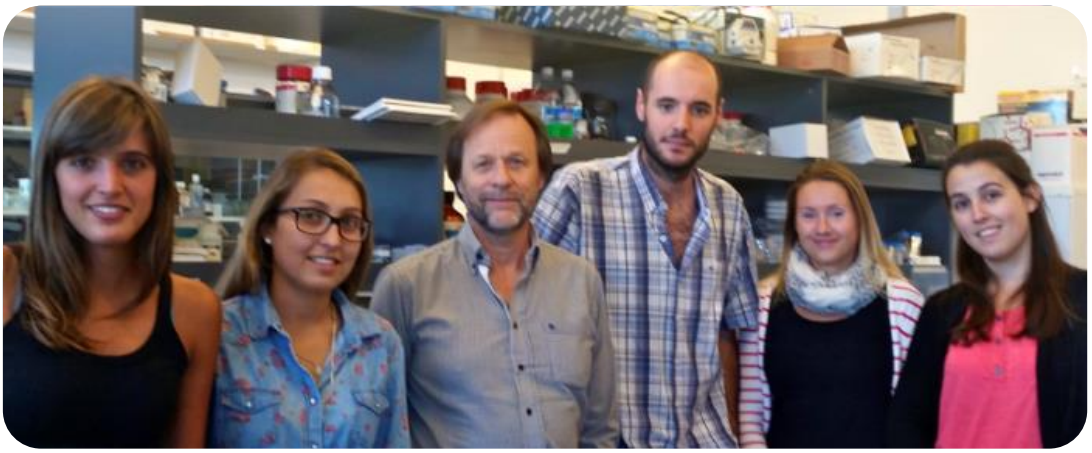

Neurodegeneration

MEMBERS

Luis Barbeito, MD (Head)
Emiliano Trías (PhD student)
Valentina Varela (Msc student)
Romina Barreto (Msc student)
Sofía Ibarbouru (Msc student)

RESEARCH

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 SOD1G93A rats, their appearance being closely associated with the progression of paralysis in SOD1G93A 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.

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.

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 p75NTR receptors, they re-express p75NTR following nerve injury or in ALS, thus becoming sensitive to NGF-induced apoptosis. We found that spinal cord extracts from ALS-affected SODG93A 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.

- Richter et al. (2015). *Post-translational modification of nerve growth factor by peroxynitrite: pathogenic significance in neurodegenerative diseases*. European Journal of neurodegenerative diseases.

GRANTS

1. Movilidad Bilateral Uruguay-Brasil – DICYT (MEC) (2013-2015) Amount Granted USD 6.000.
2. Proyecto ECOS U014S02 “Mastocitos y neuroinflamación en enfermedades neurodegenerativas: caracteización de los mecanismos impllicados y nuevos blancos terapéuticos” 2014-2016.

PUBLICATIONS

1. Ghisoni K, Martins Rde P, Barbeito L, Latini A. Neopterin as a potentialcytoprotective brain molecule. J Psychiatr Res. 2015 Dec; 71:134-9.
2. Olivera-Bravo, S., Barbeito, L. A role of astrocytes in mediating postnatal neurodegeneration in Glutaric acidemia-type 1 (2015) FEBS Letters, 589 (22), pp. 3492-3497.
3. Olivera-Bravo, S., Ribeiro, C.A.J., Isasi, E., Trías, E., Leipnitz, G., Díaz-amarilla, P., Woontner, M., Beck, C., Goodman, S.I., Souza, D., Wajner, M., Barbeito, L. Striatal neuronal death mediated by astrocytes from the Gcdh-/- mouse model of glutaric acidemia type I (2015) Human Molecular Genetics, 24 (16), art. no. ddv175, pp. 4504-4515

Tumor Immunology and Glycobiology

MEMBERS

Eduardo Osinaga MD, PhD (Head)
Nora Berois, MD, PhD (Associate Investigator)
Edgardo Berriel MD, MSc (PhD student)
María Florencia Festari, MSc (PhD student)
Diego Touyá, MD (MSc student)
Claudia Schvartzman (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:

1. 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.
2. 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).

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.

BioMolecular Simulation

MEMBERS

Sergio Pantano, PhD (Head)
Matías Machado, PhD (Staff Member)
Astrid Brandner, MSc (Staff Member)
Gaston Hugo (Staff Member)
Steffano Silva (Undergraduate Student)

RESEARCH

The Group of BioMolecular Simulations develops and applies cutting-edge modeling and simulation methods to study problems of biomedical relevance.

We develop and maintain 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 at Coarse Grained and multiresolution level. 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).

Latest developments include:

- Parameters for phospholipids (In collaboration with E. Frigini and R. Porasso, Universidad Nacional de San Luis, Argentina)
- Supra molecular water models for multiresolution simulations. These are applied to the study of entire viral capsids (In collaboration with D. Guerin and M. Costabel).
- Parameters for divalent Cations (Ca^{2+}, in collaboration with Carlos Cruz, Aggeu Magalhães Research Center –Fiocruz)
- Refining of Protein-DNA interacting parameters (In collaboration with Prof. Francisco Melo, Catholic University, Santiago de Chile)

These tools have been applied to the development and optimization of a FRET sensor for cAMP and cGMP in living cells. The sensor for cAMP has been used to characterize signaling processes with unprecedented spatial resolution, uncovering the presence of sub micrometer scale compartments. A research paper was submitted, which is currently under revision in Nature Communications. This project is carried out in collaboration with the group of Prof. Manuela Zaccolo at the University of Oxford.

The availability of parameters for lipids and Calcium allowed us to undertake the study of Calcium mediated gating mechanism of Connexin channels. This leaded to the description of an open state of the channel, which allows explaining experimental results of permeation of dyes and signaling molecules larger than the pore size described by the X-ray structures available. This project is in collaboration with Drs. Francesco Zonta (ShanghaiTech University, China) and Fabio Mammano (University of Padua, Italy).

PUBLICATIONS

1. Machado MR and Pantano S. Exploring LacI–DNA Dynamics by Multiscale Simulations Using the SIRAH force field. JCTC, 2015, 11:5012.
2. Jäger AV, De Gaudenzi JG, Mild JG, Cormack BM, Pantano S, Altschuler DL, Edreira MM. Identification of novel cyclic nucleotide binding proteins in Trypanosoma cruzi. Mol Biochem Parasitol. 2015, 198:104.

3. Darré L, Machado MR, Brandner AF, Ferreira S, Gonzalez HC, Pantano S. SIRAH: a structurally unbiased coarse-grained force field for proteins with aqueous solvation and long-range electrostatics. JCTC, 2015, 11:723.
4. Morande PE, Borge M, Abreu C, Galletti J, Zanetti SR, Nannini P, Bezares RF, Pantano S, Dighiero G, Oppezzo P, Gamberale R, Giordano M. Surface localization of high-mobility group nucleosome-binding protein 2 (HMGN2) on leukemic B cells from chronic lymphocytic leukemia patients is related to secondary autoimmune hemolytic anemia. Leuk Lymphoma. 2015. Jan 21:1-8.

ACADEMIC TRAINING

In June 2015 Astrid Brandner, staff member of the group, defended her Master Thesis in Bioinformatics.

COURSES AND CONFERENCES

During 2015 our group organized:

- OpenLab: Performing Molecular Simulations with the Sirah Force Field.

This course was held at the IPM form May 4th - 8th, 2015 and received 17 students from Argentina, Brazil, Chile, Peru and Uruguay.

This OpenLab experience was performed within the context of the FOCEM project INVESTIGACIÓN, EDUCACIÓN Y BIOTECNOLOGÍA APLICADAS A LA SALUD and entirely organized by the members of our group. The initiative received support from ANII and the auspice of UNESCO.

We received very favorable comments from the students who delivered oral presentations exposing in which way the contents of the course were of practical help in their current research projects.

- Workshop of South American PIs in Molecular Simulations.

This meeting was held at the IPM from May 8th to 9th and gathered 24 scientists from Argentina, Chile, Brazil and Uruguay.

This meeting was the first opportunity for South American Principal Investigators to gather in a relaxed environment to exchange ideas and information about their resources, expertise, strengths and weaknesses in the regional context. Each participant had a slot for an oral presentation followed by an entire afternoon of free discussions about future actions and regional collaborative projects.

- VIII PosLatAm course (Postgrado Latinoamericano en Biofísica)

This meeting was held in November, in the city of Salto, Uruguay. About 40 students from the region and 15 invited professors.

- Binational meeting of the Argentinean and Uruguayan biophysical societies.

After the PosLatAm, we organized an Argentinean-Uruguayan meeting of both Biophysics Societies with more than 150 participants and speakers, from Argentina, Brazil, Chile, Denmark, Spain, Portugal, Uruguay, USA and Venezuela. From the number of participants, the scientific and personal exchange and incipient collaborations it is possible to conclude that both events were very successful conferring a large visibility to the the

Both events received with support from ANII, IUPAB, Biophysical Society, UdelaR, FOCEM, PEDECIBA, and Comision Tecnica Mixta de Salto Grande.

Detailed information about scientific program, participants, book of abstracts and pictures are available at:

http://masbiofisica.fcien.edu.uy/latin-american-crosstalk-in-biophysics-sbf-uy-sab

http://masbiofisica.fcien.edu.uy/poslatamviii

https://www.facebook.com/SBF.uy/

Molecular and Human Genetics

MEMBERS

José Badano, PhD (Head)
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)

RESEARCH

In our laboratory we study different aspects related to the biology of a particular cellular organelle: the primary cilium. This organelle is present in the vast majority of cells in the human body and plays a critical role in the interaction of cells with their environment, participating in signal sensing and transduction [1]. Consequently, their dysfunction has been shown to result in a number of human conditions collectively known as ciliopathies [2]. Among these, we study genes and proteins that when mutated caused Bardet-Biedl syndrome (BBS), one of the most pleiotropic ciliopathies [3].

One particular aspect of cilia biology that we are interested in is cilia formation and maintenance, a process where the BBS associated proteins have been shown to play a role. 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 [4]. 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 symmetry and hydrocephaly [5].

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 identified 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 interaction 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 [6]. We have continued the characterization of CCDC28B, finding and characterizing novel interactions. That approach is allowing us to both understand the mechanism by which CCDC28B regulates ciliogenesis and uncover novel proteins important in the process.

The BBS proteins have also been implicated in cilia formation. However, we have uncovered novel extra-ciliary roles for at least some of these proteins (see also below for BBS7). In collaboration with Dr. Norann Zaghloul at University of Maryland, Baltimore, USA, we have described a role for BBS4 in the regulation of intracellular traffic. In this work it was shown that depletion of BBS4 and other BBS proteins results in impaired endosomal sorting of the Notch receptor with the consequent impairment of recycling to the cilium but also to the plasma membrane [7].

In another line of research we study the process of ciliary import. Albeit the interior and membrane of cilia are continuous with the cytosol and plasma membrane respectively, the cilium presents a defined composition, which is critical for the function of the organelle. To achieve this, the process of ciliary import is highly regulated. Interestingly, recent reports have shown striking similarities between ciliary and nuclear import. In this context, we have been studying the process of ciliary import for proteins that present the capacity of localizing to both the cilium and the nucleus. This research line was initially fueled by our previous studies on BBS7 that led to the demonstration that at least some BBS proteins play extraciliary roles in the nucleus modulating gene transcription [8]. In this project, which is being guided by Dr. Irigoín, we are working 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 collaborating 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).

Another important interest of the laboratory is to understand the role of the cilium and ciliary proteins (BBS and others) during development and in tissue maintenance. In other words, gain insight to understand why cilia dysfunction results in the different phenotypes that characterize the ciliopathies. One project has been centered on understanding the role of cilia in the early development of the zebrafish retina. Neurons are highly specialized cells in which cilia are present. However, the role of this organelle in these cells is not completely known yet. Focusing on retinal ganglion cells (in collaboration with Dr. Flavio Zolessi) we were able to characterize the dynamics of cilia assembly in the early retina. We also showed that cilia are important both for proliferation and differentiation of these cells [9].

Finally, as mentioned above one important goal is to understand why cilia impairment results in different phenotypes in humans. One hallmark phenotype of the ciliopathies is obesity and in the Institut we are part of a multi-group interdisciplinary program focused on the study of obesity, inflammation and other metabolic related disorders: INDICyO. One main contribution to this program has been centered on studying the role of BBS proteins and the cilium in one critical aspect, the differentiation of adipocytes. Here, we are using cell-based studies to address the relationship between the cilium and the function of some of the BBS proteins in the process, which in turn are regulating the production and secretion of likely relevant proteins. Therefore, we are studying aspects related to the cellular basis of obesity in BBS and at the same time going further into understanding the role of BBS proteins in intracellular trafficking.

PUBLICATIONS

1. Novas, R., Cardenas-Rodriguez, M., Irigoín, F., Badano, J.L. Bardet-Biedl syndrome: Is it only cilia dysfunction? FEBS Lett, 2015. 589(22): p. 3479-91

RESEARCH LINES

- CCDC28B and the BBS proteins in the regulation of ciliogenesis and cilia length.
- Cilia targeting: similarities with the nuclear transport process.

- BBS proteins in intracellular trafficking: implications for human disease.
- Cilia in the development of the retina.

EDUCATION-COURSES

TRAINING COURSES

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

TRAINING OF STUDENTS

1. Sheila Castro Sánchez. 3 month Internship (from Universidad de Vigo, Spain).

CONGRESS

1. Latin American Society for Developmental Biology, October 20-23, 2015, Santos, Brasil. Invited speaker (José Badano).
2. Latin American Society for Developmental Biology, October 20-23, 2015, Santos, Brasil. Poster presentation (Paola Lepanto).

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

CSIC Project – Florencia Irigoín – "Estudios funcionales y estructurales de CCDC28B, un modificador del Síndrome de Bardet-Biedl." – 2013-2015 – I+D Program, CSIC, UDELAR

Metabolic Diseases and Aging

MEMBERS

Carlos Escande, PhD (Head)
Paola Contreras, PhD (Research Associate)
Mariana Bresque, MSc (Research Assistant, PhD Student)
Natalia Bobba, BSc (Research Assistant, MSc Student)
Adriana Carlomagno, MD (MSc Student)
Laura Colman, MSc (PhD Student)
Rosina Dapueto, MSc (PhD Student)
Leonardo Santos, MSc (PhD Student)
Pía Garat, Engineer in Biotechnology (Entrepreneur BIOESPINN)
Alejandro Rodríguez, BSc (MSc Student)

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.
- DBC1 as a novel regulator of cardiovascular function.

EDUCATION-COURSES

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

GRANTS

1. INNOVA – ANII – Young leaders grant. 2014-2019
2. Fondo Clemente Estable – ANII – 2015-2017
3. Alianza Pasteur-Granuy – ANII – 2015-2017

PUBLICATIONS

1. Mathison A*, **Escande C***, Calvo E, Seo S, White T, Salmonson A, Faubion WA Jr, Buttar N, Iovanna J, Lomberk G, Chini EN, Urrutia R. Phenotypic Characterization of Mice Carrying Homozygous Deletion of KLF11, a Gene in Which Mutations Cause Human Neonatal and MODY VII Diabetes. Endocrinology. 2015 Oct;156(10):3581-95. doi: 10.1210/en.2015-1145. Epub 2015 Aug 6. PubMed PMID: 26248217; PubMed Central PMCID: PMC4588811. **Shared First Authorship**

2. **Escande C**, Nin V, Pirtskhalava T, Chini CC, Tchkonia T, Kirkland JL, Chini EN. Deleted in breast cancer 1 limits adipose tissue fat accumulation and plays a key role in the development of metabolic syndrome phenotype. Diabetes. 2015 Jan;64(1):12-22. doi: 10.2337/db14-0192. Epub 2014 Jul 22. PubMed PMID: 25053585; PubMed Central PMCID: PMC4274806.

Redox Biology of Trypanosomes

MEMBERS

Marcelo Comini, PhD (Principal Investigator)
Andrea Medeiros, PhD (Postdoc)
Mariana Bonilla, PhD (Postdoc)
Bruno Manta, PhD (Postdoc, till October 2015)
Cecilia Ortíz (PhD student)
Diego Benitez (PhD student)
Florencia Sardi (MSc Student)
Jaime Franco (MSc Student)
Sofía Zardo (BSc Student)
Karin Grunberg (BSc Student)

RESEARCH

By means of a multidisciplinary approach we study the biochemical, structural and biological features that distinguish several key components of the redox system from pathogenic trypanosomatids, parasites that are causative agents of severe diseases in animals and humans. Several essential cellular processes are regulated and/or depend on redox reactions that have cysteine residues as targets or mediators. Important from a therapeutic point of view, the components of the redox system from trypanosomatids significantly differ from those present in the mammalian hosts, which opens the possibility for a selective inhibition of parasite proliferation. Research in our laboratory aims at:

1. gaining further understanding into the thiol-based redox metabolism of trypanosomatids by studying its synthesis, recycling and role in several cell functions,
2. developing and exploiting the use of novel redox biosensors to unravel fundamental questions on parasite biology, host/parasite interaction and phenotype-based compound screening,
3. Identifying and characterizing novel drug target candidates.

EDUCATION-COURSES

Co-organization of the 4th Course "Redox Chemistry and Biology of Thiols" and 2nd International Symposium "Thiol Metabolism and Redox Regulation of Cellular Functions", February 23rd-March 7th 2015, Montevideo, Uruguay.

Members of the lab. participated as lecturer or presented works in several international meetings, congresses and courses: "Symposium on Structural biology in infection and disease", Montevideo-Uruguay (10/4/2015); "VI Kinetoplastid Molecular Cell Biology Meeting", Woods Hole-USA (25-29/4/2015); "Sao Paulo School of Advance Science on Neglected Diseases Drug Discovery- focus on Kinetoplastids", Sao Paulo-Brazil (14-24/6/2015); Conference on "Antiparasitic chemotherapy-CM1307", Belgrade-Serbia (26-28/10/2015); Course "Molecular aspects of Chemotherapy, Drug resistance and immunoprophylaxis in diseases caused by trypanosomatids", Fiocruz Belo Horizonte, Brazil (3-6/11/2015); "IV Encuentro Nacional de Química", Montevideo-Uruguay (4-6/11/2015); "XX Simposio Nacional de Química Orgánica", Mar del Plata-Argentina (11-14/11/2015); "16° Brazilian Meeting on Organic Synthesis", Buzios-Brazil (15-18/11/2015).

The PhD student Oliver Orban from the Teschnische Universitaet Braunschweig, Germany and Dr. Ma. Laura Sbaraglini Universidad Nacional de la Plata, Argenitna performed an 8-weeks research traineeship in our laboratory (Sep-Oct, 2015).

GRANTS

1. Fiocruz-Pasteur Grant –"Trypanosoma´s prostaglandin metabolism: role in infection, pathogenesis and drug resistance"–, 2014-2016. M. Comini (Principal Investigator).
2. ACIP Grant –"Target-based drug discovery of compounds interfering with trypanothione biosynthesis in trypanosomatids"–, Project A-17-2015, 2015-2017. M. Comini (Principal Investigator).
3. ICGEB Grant –"The thioredoxin-fold diversity in trypanosomatids and tapeworms" Project CRP/URU 14-01–, 2015-2017. M. Comini (co-Principal Investigator).
4. FMV Grant –"Diseño de biosensors para monitoreo simultáneo de señalización redox y cAMP: desde la computadora a la célula y vuelta a la computadora"–, Project FMV_1_2014_1_104000, 2015-2018. M. Comini (Associate Researcher).

PUBLICATIONS

Musunda B, Benítez D, Dirdjaja N, Comini MA, Krauth-Siegel RL. (2015) Glutaredoxin-deficiency confers bloodstream Trypanosoma brucei with improved thermotolerance. Mol Biochem Parasitol. 204(2): 93-105.

Bisio H, Bonilla M, Manta B, Graña M, Salzman V, Aguilar PS, Gladyshev VN, Comini MA, Salinas G. (2015) A New Class of Thioredoxin-Related Protein Able to Bind Iron-Sulfur Clusters. Antioxid Redox Signal. Oct 27. [Epub ahead of print] PubMed PMID: 26381228.

Rodríguez Arce E, Sarniguet C, Moraes TS, Vieites M, Tomaz AI, Medeiros A, Comini MA, Varela J, Cerecetto H, González M, Marques F, García MH, Otero L, Gambino D (2015) A new ruthenium cyclopentadienyl azole compound with activity on tumor cell lines and trypanosomatid parasites. J Coord. Chem. 1-15.

Sturlese M, Lelli M, Manta B, Mammi S, Comini MA, Bellanda M (2015) (1)H, (13)C and (15)N resonance assignment of the mature form of monothiol glutaredoxin 1 from the pathogen Trypanosoma brucei. Biomol NMR Assign 9: 143-146.

Fernández M, Arce ER, Sarniguet C, Morais TS, Tomaz AI, Azar CO, Figueroa R, Diego Maya J, Medeiros A, Comini M, Helena Garcia M, Otero L, Gambino D. (2015) Novel ruthenium(II) cyclopentadienyl thiosemicarbazone compounds with antiproliferative activity on pathogenic trypanosomatid parasites. J Inorg Biochem. 153: 306-314.

Miserachs HG, Cipriani M, Grau J, Vilaseca M, Lorenzo J, Medeiros A, Comini MA, Gambino D, Otero L, Moreno V. (2015) Antitumor and antiparasitic activity of novel ruthenium compounds with polycyclic aromatic ligands. J Inorg Biochem. 150: 38-47.

Comini MA. Measurement and meaning of cellular thiol: disulphide redox status. Free Radic. Res. 2015.

Olivera-Couto, A., Salzman, V., Mailhos, M., Digman, M.A., Gratton, E., Aguilar, P.S. Eisosomes are dynamic plasma membrane domains showing Pil1-Lsp1 heteroligomer binding equilibrium (2015) Biophysical Journal, 108 (7), pp. 1633-1644.

Stefani, M., Sturlese, M., Manta, B., Löhr, F., Mammi, S., Comini, M., Bellanda, M. 1H, 13C and 15N resonance assignment of the cytosolic dithiol glutaredoxin 1 from the pathogen Trypanosoma brucei (2015) Biomolecular NMR Assignments, 4 p.

Neuroinflammation and Gene Therapy

MEMBERS

Hugo Peluffo, PhD (Joint Position, Facultad de Medicina, Universidad de la República)

Members at the IPMontevideo

Natalia Lago, PhD (Assistant Researcher, Part Time)
Luciana Negro (PhD Student)
Bruno Pannunzio (Student)
Daniela Alí (MSc Student)
Andrés Cawen (Student)

Members at the Faculty of Medicine

Daniela Blanco (MSc Student)
Nathalia Vitureira, PhD (Investigador Asociado IPMon)

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**.

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GRANTS

1. Project FundacióMarató TV3, Catalunya, España. "Modulation of immune receptors function as a novel therapeutic strategy for acute CNS damage". (2012-2015) Amount Granted € 80.000.
2. Project CSIC-UDELAR: "Gene therapy applied to brain trauma: comparative preclinical studies using modular recombinant vectors and lentiviral vectors". (2013-2015) Amount granted USD 40.000.
3. Project CSIC-UDELAR Grupos I+D: Neuroinflammation and glia". (2015-2018) Coordinated project.

PUBLICATIONS

1. Aroa Ejarque-Ortiz, Carme Solà, Águeda Martínez-Barriocanal, Simó Schwartz Jr., Margarita Martín, **Hugo Peluffo**, Joan Sayós. *The receptor cmrf35-like molecule-1 (clm-1) enhances the production of LPS-induced pro-inflammatory mediators during microglial activation*. **PLoS ONE** DOI: 10.1371/journal.pone.0123928, 2015.
2. **Peluffo H**, **Solari-Saquieres P, Negro-Demontel ML**, Isaac Francos-Quijorna, Navarro X, Ruben López-Vales, Sayós J, **Lago N**. *CD300f immunoreceptor contributes to peripheral nerve regeneration by the modulation of macrophage inflammatory phenotype*. **J. Neuroinflammation**, 12:145 (12 August) 2015.
3. **Hugo Peluffo**, Ugutz Unzueta, **María Luciana Negro**, Zhikun Xu, Esther Vazquez, Neus Ferrer-Miralles and Antonio Villaverde. *BBB-targeting, protein-based nanomedicines for drug and nucleic acid delivery to the CNS*.**Biotechnology Advances**, 33(2):277-287, 2015.
4. Santos-Nogueira, López-Serrano, Hernández, **Lago N**, Astudillo AM, Balsinde J, Estivill-Torrús G, de Fonseca FR, Chun J, López-Vales R. *Activation of Lysophosphatidic Acid Receptor Type 1 Contributes to Pathophysiology of Spinal Cord Injury*. **Journal of Neuroscience,** 4703-14, 2015.

5. Ejarque-Ortiz, A., Solà, C., Martínez-Barriocanal, Á., Schwartz, S., Martín, M., Peluffo, H., Sayós, J. The receptor CMRF35-like molecule-1 (CLM-1) enhances the production of LPS-induced pro-inflammatory mediators during microglial activation (2015) PLoS ONE, 10 (4), art. no. e0123928 Cited 1 time.

Worm Biology

MEMBERS

Gustavo Salina, PhD (Head)
Inés Carrera, PhD
Lucía Otero Larre Borges, PhD
Jorge Pórfido, PhD
Laura Romanelli (PhD student)
Cecilia Martínez (PhD student)
Gastón Risi (MSc student)

MAIN EQUIPMENT

We have specific equipment needed to work with *C. elegans,* including a WMicroTracker that has the capability to measure in an easy, fast and reproducible way the locomotive activity of small animals cultured in microtiter plates.

SERVICES

High-Throughput Screening assay to evaluate toxicity and pharmacological effects in the eukaryotic animal *C. elegans*.

RESEARCH

METABOLIC PATHWAYS OF PARASITIC WORMS

Our research focuses on helminth metabolic pathways essential for parasite survival. We study thiol- and selenol-dependent pathways and energy-harvesting pathways of flatworms and nematodes. We are characterizing the unique linked thioredoxin-glutathione pathways present in parasitic flatworms and addressing the function of redox and iron-sulfur thioredoxins and glutaredoxins. We are also studying flatworm and nematode metabolic pathways that allow parasites to harvest energy under low oxygen tension. For some of our studies we use the nematode *C. elegans* as a model organism.

ANTHELMINTIC DRUG DISCOVERY

Our lab focuses on worm drug discovery, particularly targeting the selenoenzyme thioredoxin glutathione reductase. We have also a set up a reproducible and automatized whole animal bioassay for anthelmintics drug screening and discovery.

EDUCATION-COURSES

POSTGRADUATE COURSES

- Redox Chemistry and Biology of Thiols (co-organizer)
- Recombinant Proteins (co-organizer)

UNDERGRADUATE COURSES

1. Introductory Biology, Faculty of Chemistry, Universidad de la República (28 lectures per year)

TRAINING OF STUDENTS

- Lucía Otero (defended PhD in 2014).
- Hugo Bisio (defended MSc in 2015).
- Laura Romanelli (started PhD in 2013).
- GastónRisi (started MSc in 2016).

- Cecilia Martínez (Started PhD in 2016)
- Enrique Ladós (Veterenary graduate student, 2015)

CONGRESSES AND INVITED SEMINARS

- Invited Seminar at The Wellcome Trust Sanger Centre, Pathogen Group, Cambridge, UK. "Unusual aspects of metabolism in flatworm parasites: implications for rational drug design". July 2014.
- Invited seminar at the National Jewish Health & University of Colorado School of Medicine. Denver, USA. "Redox networks in flatworm parasites: implications for rational drug design and treatment of neglected diseases" July 2015.
- Oral presentation at Evolutionary Biology of Caenorhabditis and other Nematodes. Wellcome Trust Genome Campus. Hinxton, Cambridge, UK. Gustavo Salinas. 2014.
- Invited Conference X Congreso Argentino de Protozoología y Enfermedades Parasitarias. Mar del Plata. Gustavo Salinas. 2014.

STAGES/INTERNSHIPS ABROAD

- Hugo Bisio: 3 months internship at Massimo Bellanda´s Lab, Università di Padova (2015)
- Laura Romanelli: 3 months internship at Mark Alkema´s Lab, Univerity of Massachusetts (2014)

OTHER SICENTIFIC ACTIVITIES of the PI

- Member of the Editorial Board of *The Journal of Biological Chemistry* (2016-2021)
- Editor of the Forum Issue "Parasite Redox Control" for *Antioxidant Redox Signaling*
- Ad hoc reviewer for several journals including PLoS Pathogens, Antioxidant Redox Signaling, BBA General Subjects, PLoS ONE, BMC Genomics, Molecular and Biochemical Parasitology, International Journal for Parasitology.
- Member of scientific boards of Universidad de la República and National Agency for Innovation and Research (ANII)

GRANTS

- Studies on helminth mitochondrial metabolism: molecular basis of the malate dismutation. ANII (Uruguay) 2015-2017) 15.000 USD/year
- The thioredoxin-fold in trypanosomatids and tapeworms. ICGEB (Italia) 2014-2017. 15.000 €/year, (shared Project with Marcelo Comini).
- Redox Chemistry and Biology of Thiols, International postgraduate course and Symposium, supported by ICGEB, RIIP and PEDECIBA. (28.000 USD). The course was organized together with Marcelo Comini, Beatriz Alvarez and Madia Trujillo.

- Reinsertion funds for Inés Carrera. PEDECIBA 5.000 USD.
- CSIC, Universidad de la República. Research Inititation Grants to Laura Romanelli and Hugo Bisio. 5.000 USD each.

PUBLICATIONS

1. **Pasquet V**, **Bisio H**, López GV, **Romanelli-Cedrez L**, **Bonilla M**, Saldaña J and **Salinas G** (**2015**) Inhibition of tapeworm thioredoxin and glutathione pathways by an oxadiazole N-oxide leads to reduced Mesocestoides vogae infection burden in mice. Molecules 20(7), 11793-807. – *IF: 2.416.*
2. Silva V, Folle M, Ramos AL, Zamarreño F, Costabel M, García-Zepeda E, **Salinas G**, Córsico B, Ferreira AM (**2015**) Echinococcus granulosus antigen B: a hydrophobic ligand binding lipoprotein at the host-parasite interface. Prostaglandins, Leukot Essent Fatty Acids 93: 17–23. – *IF: 2.346*
3. Stefanakis, N., Carrera, I., Hobert, O. Regulatory Logic of Pan-Neuronal Gene Expression in C. elegans (2015) Neuron, 87 (4), pp. 733-750

Cell Biology of Neural Development

MEMBERS

Flavio Zolessi, PhD (Head)

Gonzalo Aparicio, MSc (Doctoral Student)

Camila Davison, BSc (Graduate Student)

ASSOCIATED MEMBERS

Paola Lepanto, MSc (Doctoral Student, MHGL)

Ileana Sosa, BSc (Master Student, Sección Genética, Facultad de Ciencias)

Marcela Díaz, MSc (Microscopy Unit)

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.

EDUCATION-COURSES

TRAINING COURSES

1. Course on Processing and Analysis of Fluorescence Microscopy Images (PAFMI), Uruguay. Organizers: F. Zolessi, F. Lecumberry, P. Aguilar. 29/02-11/03/2016.
2. IV Course and Symposium on Development and Plasticity of the Nervous System. Montevideo, Uruguay. Organizers: F. Zolessi, M. Brauer (IIBCE), F. Rossi (UdelaR). 06/10-08/11/2014.
3. ICY Training Course –Program (Internal IP Montevideo training course). Instructor: A. Dufour (IP Paris). Organizers: F. Zolessi, P. Aguilar, F. Lecumberry.15-17/12/2014.

TRAINING OF STUDENTS

1. Antonella Alba, Antonella Arrieta, Lucía Veloz. PAIE-CSIC 2016. Director: F. Zolessi; Co-director: G. Aparicio.
2. 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 (2014-2015).

CONGRESSES

1. Symposium on Genome Editing, XVI Congreso Latinoamericano de Genética (ALAG 2016). Co-organized by F. Zolessi and G. Bedó. Montevideo, Uruguay. 9-12/10/2016.
2. 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.

GRANTS

1. FCE_1_2014_1_104160 – ANII, Uruguay. F. Zolessi.

PUBLICATIONS

1. Prieto D., **Aparicio G.**, Machado, M., **Zolessi F.R.** (2015) Application of the DNA-Specific Stain Methyl Green in the Fluorescent Labeling of Embryos. J. Vis. Exp. (99), e52769, doi: 10.3791/52769.
2. Paolini A, Duchemin AL, Albadri S, Patzel E, Bornhorst D, González Avalos P, Lemke S, Machate A, Brand M, Sel S, Di Donato V, Del Bene F, **Zolessi FR**, Ramialison M, Poggi L. (2015) Asymmetric inheritance of the apical domain and self-renewal of retinal ganglion cell progenitors depend on Anillin function. Development 142(5): 832-9. doi: 10.1242/dev.118612
3. Prieto D., **Aparicio G.**, Morande P.E., **Zolessi F.R.** (2014) A fast, low cost, and highly efficient fluorescent DNA labeling method using methyl green. Histochem Cell Biol 142(3):335-45. doi: 10.1007/s00418-014-1215-0
4. 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. Biochim Biophys Acta 1844(4): 837-849.

Immunoregulation and Inflammation

MEMBERS

Marcelo Hill MD, PhD (Head)
Mercedes Segovia (Post-doc)
Maite Duhalde (Post-doc)
Sofía Russo (PhD student)
Florencia Rammauro (Master student)
Matías Jeldres (Master student)

RESEARCH

Regulation of immune responses is a critical issue to achieve physiological homeostasis. 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. 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 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. Recently, we characterized the intracellular protein TORID as an emergent regulator of immune responses (Segovia et al. 2014). TORID is highly expressed by dendritic cells, where it is localized within the phagosomal membrane. We demonstrated that TORID is a non-specific cation channel which promotes V-ATPase activity. Through that mechanism, TORID control antigen processing, particularly through the cross-presentation pathway.

The characterization of the immunoregulatory properties of TORID is the main focus of our laboratory.

Research lines

- Role of TORID in anti-tumoral immune responses (Cancer Immunology Program)
- Role of TORID in chronic lymphocytic leukemia biology (Cancer Immunology Program)
- Characterization of small molecules able to inhibit or activate TORID-mediated conductance.
- Characterization of the role played by TORID in anti-viral immune responses.
- Role of TORID in obesity and obesity induced inflammation (INDICyO Program).
- Role of TORID in cellular immunity against Leptospira spp

1. 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."
2. 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.
3. 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.
4. 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.
5. 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.

GRANTS

1. FMV 2014-2015
2. CABBIO 2015-2017
3. IP de Montevideo PTR

PUBLICATIONS

1. Gaudin A, Renard E, Hill M, Bouchet-Delbos L, Bienvenu-Louvet G, Farges JC, Cuturi MC, Alliot-Licht B. Phenotypic analysis of immunocompetent cells in healthy human dental pulp. J Endod. 2015 May;41(5):621-7. doi: 10.1016/j.joen.2015.01.005. Epub 2015 Feb 18.

Signal Processing

MEMBERS

Federico Lecumberry, Eng, PhD (Head)
Martín Etchart, Eng
Mauricio Ramos

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.

EDUCATION-COURSES

We co-organized the theoretical and practical course “Processing and Analysis of Fluorescence Microscopy Images“ at the Institut Pasteur de Montevideo in March 2016. The global aim of this course was 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 wereorganized in a way that the skills of one group of students 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.

Among this course, an introductory course on image processing for biology and medicine will be organized on July 2016 at Facultad de Ingeniería. See “Procesamiento de Imágenes para Biología y Medicina” (PIMBIO) at http://www.imagina.ei.udelar.edu.uy/pimbio/ for more information.

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

Members of the LPS were part of the Organizing Committee of the 20th Iberoamerican Congresson Pattern Recognition (CIARP 2015) held in Montevideo in November 2015. CIARP 2015 was organized by the Uruguayan IAPR Chapter, including members from Universidad de la República and Universidad Católica. Held every year, CIARP is the most important Iberoamerican conference in pattern recognition, computer vision and multimedia. Among the extensive list of application areas covered by thecongress, bioinformatics and human and animal health are one of the most relevant topics.

GRANTS

1. INNOVA II

Research Laboratory on Chronic Lymphocytic Leukemia

MEMBERS

Pablo Oppezzo, PhD (Head)
Pablo Morande, PhD (Post-doctoral position)
Sandra Sernbo, PhD (Post-doctoral position)
Cecilia Abreu, PhD (Technical Assistant)
Agustín Correa, PhD (Principal technical assistant)
Claudia Ortega, PhD (Technical Assistant)
Florencia Palacios, PhD (Technical Assistant)
Daniel Prieto, MSc (PhD student)
Noé Seija, BSc (MSc. student)

RESEARCH

Our work is focus in the haematology area, tumoral immunology and the recombinant antibodies production. 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 (Recombinant Protein Platform).

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.

Our work concentrates in the study of Chronic Lymphocytic Leukemia (CLL) 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.

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.

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. We first 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 described and characterized one of the proliferative tumor subsets in this leukemia. This subpopulation express the mutagenic enzyme AID by Activation-Induced Cytidine Deaminase and is associated with expression of tumor anti-apoptotic and cell proliferation markers (*Palacios and Moreno et al, Blood, 2010*). We also 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*).

Development of new prognostic and therapeutic tools in CLL

This research line is outlined by our double profile as a research/facility group. Concerning the development of prognostic markers in CLL, we previously 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, our group is recently focused on the generation of new therapeutics molecules named Artificial Binding Proteins (Affitins). Compared with classical therapeutics antibodies Affitins 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 acquiring favorable 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*).

Networking

Production of recombinant antibodies as well as new protein scaffolds targeting tumor antigens constitutes a very useful tool to evaluate different prognostic and/or therapeutic molecules in cancer. To develop new therapeutic and prognostic methods in CLL it is mandatory to constitute a CLL network, that engages a continuous and coordinate work between our group (focused in the CLL biology) and different medical groups (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 VH genes (IgVH) in CLL. The establishment of this standard procedure as a routine laboratory practice allowed us to start a strong collaboration with clinical hematologic groups of Hospital Maciel and Hospital de Clínicas in Montevideo and with the clinical hematologic group of Academy of Medicine in Buenos Aires , Argentine. These collaborations resulted in the foundation of the first LatinAmerican CLL group (LAG-CLL) with the participation of different laboratories of Argentine, Brasil and Uruguay. The consolidation of this network was recently achieved after obtaining the funds supported by CYTED. Oppezzo's lab is the principal coordinator of this program (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)

EDUCATION-COURSES-CONGRESS

1. First Iberoamerican meeting on Chronic Lymphocytic Leukemia. Date and place: 15-17 November **2013**, Montevideo, Uruguay. Participants: 285
2. 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
3. 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
4. Third LatinAmerican Workshop on prognosis markers in CLL: *"Analysis of mutational profile of immunoglobulin VHgenes in CLL"*. Date and place: 20-22 May **2015**, Montevideo, Uruguya. Participants: 42

GRANTS

- Fondo María Viña – Dr. Pablo Oppezzo – "Development of Artificial Binding Proteins (Affitins) to evaluate new prognosis and treatment strategies in Chronic Lymphocytic Leukemia"– 2015-2017 – ANII, Uruguay
- CSIC, I+D2014 – Dr. Pablo Oppezzo – "Implicancias de la expresión anómala de la enzima mutagénica AID en la progresión de la Leucemia Linfoide Crónica" – 2014-2017 –Comisión sectorial de investigación científica de la Universidad de la República, Uruguay.
- 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, Uruguay.
- 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, Uruguay
- 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, Uruguay
- Fondo CYTED – Dr. Pablo Oppezzo – "Red-iberoamericana de Leucemia Linfoide Crónica: hacia el desarrollo de nuevos marcadores pronósticos" – 2011-2014 – CYTED.
- Proyectos Transversales IPMont – 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, Uruguay.
- Fondo Lady Tata – Dr. Pablo Oppezzo – "Characterisation of the proliferating pool in CLL. Is AID expression a marker of this subpopulation?" – 2008-2011 – Lady Tata Foundation, United Kingdom.

PUBLICATIONS

1. Montamat-Sicotte D, Litzler LC, **Abreu C**, Safavi S, Zahn A, Orthwein A, Müschen M, **Oppezzo P**, Muñoz DP, Di Noia JM. HSP90 inhibitors decrease AID levels and activity in mice and in human cells. ***Eur J Immunol. 2015***Aug;45(8):2365-76. doi: 10.1002/eji.201545462.
2. **Morande PE**, Borge M, **Abreu C**, Galletti J, Zanetti SR, Nannini P, Bezares RF, Pantano S, **Dighiero G, Oppezzo P**, Gamberale R, Giordano M. Surface localization of high-

mobility group nucleosome-binding protein 2 on leukemic B cells from patients with chronic lymphocytic leukemia is related to secondary autoimmune hemolytic anemia. **Leuk Lymphoma. 2015** Apr;56(4):1115-22. doi: 10.3109/10428194.2014.957205.

3. **Palacios F**, **Prieto D**, **Abreu C**, Ruiz S, Morande P, Fernández-Calero T, Libisch G, Landoni AI, **Oppezzo P**. Dissecting chronic lymphocytic leukemia microenvironment signals in patients with unmutated disease: microRNA-22 regulates phosphatase and tensin homolog/AKT/FOXO1 pathway in proliferative leukemic cells. **Leuk Lymphoma. 2015** May;56(5):1560-5. doi: 10.3109/10428194.2014.990900.
4. Correa, A., **Oppezzo, P**. Overcoming the solubility problem in E. coli: Available approaches for recombinant protein production (2015) Methods in Molecular Biology, 1258, pp. 27-44.
5. Fischer, S., Echeverría, N., Moratorio, G., Landoni, A.I., Dighiero, G., Cristina, J., **Oppezzo**, P., Moreno, P. Human endogenous retrovirus np9 gene is over expressed in chronic lymphocytic leukemia patients (2015) Leukemia Research Reports, 3 (2), pp. 70-72.

Structural and Molecular Microbiology

MEMBERS

Alejandro Buschiazzo, PhD (Head of the Lab)
Juan Andrés Imelio (MSc student)
Nicole Larrieux (Technician)
Frank Lehmann (Technician) - past member
Natalia Lisa, PhD (Postdoctoral fellow) - recruited
Ariel Mechaly, PhD (Postdoctoral fellow)
Cecilia Nieves (MSc student)
Marcos Nieves (PhD student)
Fabiana San Martin (MSc student)
Felipe Trajtenberg, PhD (Research Scientist)
Leticia Zarantonelli, PhD (Associated Research Scientist)

Scientific interests

We wish to understand how cells sense specific signals and subsequently respond through cell regulation at the molecular level. Particular emphasis is given to signaling in 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. Macromolecular crystallization and single crystal X-ray diffraction experiments, all the way to 3D structure determination, are available at our facility. 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 retroviral capsid self-assembly and uncoating. The Pritsch lab has a long standing expertise in studying the delta-retrovirus Bovine Leukemia Virus (BLV), which infects cattle and produces a malignant transformation of B lymphocytes. 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.

Apart from our own main lines of research, we carry on several projects as collaborators, both within and beyond the Institut Pasteur de Montevideo, contributing with our expertise in protein science and structural biology. In particular, recent progress has been obtained in collaboration with the Hugo Gramajo team (at IBR institute, Rosario, Argentina), focused on fatty acid synthesis regulation in 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 most recent and ongoing challenge is the successful incorporation of Microbiology approaches into an 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.

Our main contributions concern the molecular and structural studies of TCS in *B. subtilis* (Albanesi et al., *Proc Natl Acad Sci USA* 2009, 106:16185-90; Trajtenberg et al., *J Biol Chem* 2010, 285:24892-903; Trajtenberg et al., *mBio* 2014, 5:e02105-14) and *Leptospira* (Morero et al., *Mol Microbiol* 2014, 94:340-52). We have thus contributed to uncovering the mechanistic workings of histidine kinase-mediated signal transduction, including the details of response regulators' activation switch. In collaboration with Prof Roland Wedlich (Munster) we are also attempting to integrate the molecular details of single protein components (histidine kinase, response regulators), with measurements of time and spatial organization of such species in the living cell.

2. MOLECULAR AND STRUCTURAL BIOLOGY OF LEPTOSPIRA

Leptospira spp. are spirochete 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 also significant. A collaborative partnership has been established with Albert Ko's (Yale University) and Mathieu Picardeau's (Institut Pasteur) laboratories.

We are actively engaged in understanding motility in *Leptospira*, from a structural point of view, focused on the flagellar architecture, recently achieving important progress. The discovery of several proteins, up to now considered hypothetical (with no orthologs in other species), as constituents of the flagellar filament, has resulted in solving two new crystal structures revealing novel 3D folds.

In the context of a collaborative multicentric project, we are also pursuing the aim of isolating autochthonous strains of *Leptospira* bacteria from biologic samples obtained from infected bovine cattle. Interaction with the Medical School (Instituto de Higiene), the Ministry of Livestock, Agriculture and Fishery (DILAVE) and the National Agronomic Research Institute (INIA), is central to this initiative. *Leptospira* isolates are being typed with complementary techniques (serologic already available in the country, as well as introducing molecular techniques). A biobank of native *Leptospira* strains is thus being built, which shall be instrumental in the preparation of efficacious bacterin-based

vaccines. Our team is also using this information to characterize vaccine candidates, to eventually be optimized through protein engineering.

In 2015 we submitted a proposal through an open call to create a Joint International Unit "Integrative Microbiology of Zoonotic Agents". The project strongly links our lab with the Picardeau group at Institut Pasteur - Paris (Biology of Spirochetes Unit, Dept of Microbiology). The 5-years project was selected after scientific evaluation, focused in leptospirosis, to be launched in 2016.

STRUCTURAL VIROLOGY

In collaboration with Dr Otto Pritsch (Inst Pasteur de Montevideo), this 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. 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.

The structure-based hypotheses of assembly/disassembly could be useful in the design and optimization of antiretroviral compounds. Our lab is interested in pursuing this line of research, focusing on capsid interactions with host cell proteins, important in the physiologic regulation of the assembly process during the retrovirus life cycle.

3. COLLABORATIVE WORK

i. 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*, key regulators of the lipid metabolism in this pathogen.

ii. Dr Roland Wedlich-Söldner (Institute of Cell Dynamics and Imaging, University of Munster, Germany). We wish to understand the integrated cellular and molecular mechanisms governing bacterial signal transduction mediated by histidine kinases.

iii. Dr Rosario Duran (Inst Pasteur de Montevideo), starting a collaboration to elucidate the protein composition and protein:protein interacting architecture within the *Leptospira* flagellar filament assembly.

iv. Drs Marcelo Hill and Otto Pritsch (Inst Pasteur de Montevideo), the former focusing on immunological aspects of the host adaptive response to *Leptospira*, and the latter on viral related protein targets (BLV and Ebola).

v. Drs Mathieu Picardeau (Institut Pasteur, Paris, France) and Albert Ko (Yale University, New Haven, USA), working in in Leptospira motility and molecular mechanisms of pathogenesis.

PUBLICATIONS

1. Saita E, Abriata LA, Tsai YT, **Trajtenberg F,** Lemmin T, **Buschiazzo A,** Dal Peraro M, de Mendoza D, Albanesi D. A coiled coil switch mediates cold sensing by the thermosensory protein DesK. (2015) *Mol Microbiol.* **98:**258-71.
2. Obal G, **Trajtenberg F,** Carrión F, Tomé L, **Larrieux N,** Zhang X, Pritsch O, **Buschiazzo A.** Conformational plasticity of a native retroviral capsid revealed by x-ray crystallography. (2015) *Science* **349:**95-8.
3. Methot SP, Litzler LC, **Trajtenberg F,** Zahn A, Robert F, Pelletier J, **Buschiazzo A**, Magor BG, Di Noia JM. Consecutive interactions with HSP90 and eEF1A underlie a functional maturation and storage pathway of AID in the cytoplasm. (2015) *J Exp Med* **212**:581-96.
4. Saita, E., Abriata, L.A., Tsai, Y.T., Trajtenberg, F., Lemmin, T., Buschiazzo, A., Dal Peraro, M., de Mendoza, D., Albanesi, D A coiled coil switch mediates cold sensing by the thermosensory protein DesK (2015) Molecular Microbiology, 98 (2), pp. 258-271.

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)

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. ***"Determination of molecular and immunologic characteristics of the secreted glycoprotein from Ebola virus".***

Institut Pasteur (France), program Ebola Task Force.

2015-2018

Role: collaborator scientist. (PI Felix Rey, IP, France).

Partners: Dr Otto Pritsch (Inst Pasteur de Montevideo), and over 10 teams at Institut Pasteur-Paris.

4. "Integrative Microbiology of Zoonotic Agents".
Institut Pasteur (France), Joint International Units program, International Affairs Direction.

2016-2021

Role: co-PI (together with Mathieu Picardeau, IP, France).

OTHER ACTIVITIES

NETWORKING, SCIENTIFIC MEETINGS, PRIZES AND HONORS

2. Sustained contribution of our group to the Center for Structural Biology of the Mercosur (Centro de Biologia Estructural del Mercosur, CeBEM) *www.cebem-lat.org* 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.
3. A Buschiazzo (Head of the Unit) earned a promotion to the position of Associate Professor (Directeur de Recherche), Institut Pasteur, Paris 2015.
4. Attendance to scientific meetings and conferences :

- May 2015 - "Hands-on training course: Bioinformatics Applied to Vaccinology" - Univ. de Sao Paulo (Sao Paulo, Brazil), F San Martin.
- June 2015 - 11 Encuentro Nacional de Microbiologos, Sociedad Uruguaya de Microbiologia - SUM (Montevideo, Uruguay), L Zarantonelli, C Nieves, F Trajtenberg poster / A Buschiazzo, oral presentation.
- August 2015 - 23rd Congress of the International Union of Biochemistry and Molecular Biology (IUBMB) and 44th Annual Meeting of the Brazilian Society for Biochemistry and Molecular Biology – SBBq (Foz do Iguaçu, Brazil). A Buschiazzo - invited speaker.
- Sep 2015 - 22nd meeting Associação Brasileira de Cristalografia - ABCr and 1st Latin American Crystallographic Association congress (Sao Paulo, Brazil). A Buschiazzo - invited speaker.
- Oct 2015 - Scientific Meeting of the Institut Pasteur International Network, Institut Pasteur (Paris, France). A Buschiazzo - invited speaker
- Oct 2015 - Meeting of the "Sociedad de Bioquímica y Biología Molecular" SBBM - (Montevideo, Uruguay), J Imelio, oral presentation.

- Nov 2015 - Meeting of the Seccional Biofísica de la Sociedad Uruguaya de Biociencias (SBF.uy-SUB) “Latin American Crosstalk in Biophysics and Physiology”, and VIII PosLatAm course: “Membrane Lipids, Transporters, Channels...and all that crosstalk” (Salto, Uruguay). A Buschiazzo - invited speaker to the meeting and course teacher.
- Nov 2015 - 11 Annual Meeting of the Asociacion Argentina de Cristalografia - AACr (La Plata, Argentina). A Buschiazzo, plenary lecture.

Host-Pathogen Interactions

MEMBERS

Carlos Robello, PhD (Head)
Adriana Parodi-Talice, PhD (Associated Researcher, Facultad de Ciencias)
Dolores Piñeyro, PhD (Associated Researcher, Facultad de Medicina)
Gonzalo Greif, PhD
Luisa Berná (Postdoctoral Researcher- INNOVA II)
Ma. Laura Chiribao (PhD Student, Facultad de Medicina)
Paula Faral (PhD Student)
Gabriela Libisch (PhD Student)
Andrés Cabrera (PhD Student)
Cecilia Portela (Tecnician, Facultad de Ciencias)
Florencia Díaz (MSc student)
Fernanda Matto (MSc student)
Moira Lasserre (MSc student)
Lucía López (Master student)

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

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.

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*.

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.

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.

PUBLICATIONS

1. Coitinho C, Greif G, van Ingen J, Laserra P, Robello C, Rivas C. First case of Mycobacterium heckeshornense cavitary lung disease in the Latin America and Caribbean region. New Microbes New Infect. 2015 Dec 18;9:63-5.
2. Dusfour I, Zorrilla P, Guidez A, Issaly J, Girod R, Guillaumot L, Robello C, Strode C. Deltamethrin Resistance Mechanisms in Aedes aegypti Populations fromThree French Overseas Territories Worldwide. PLoS Negl Trop Dis. 2015 Nov20;9(11).
3. Lasserre M, Berná L, Greif G, Díaz-Viraqué F, Iraola G, Naya H, Castro-Ramos M, Juambeltz A, Robello C. Whole-Genome Sequences of Mycobacterium bovis StrainMbURU-001, Isolated from Fresh Bovine Infected Samples.Genome Announc. 2015 Nov 5;3(6).
4. Greif G, Rodriguez M, Reyna-Bello A, Robello C, Alvarez-Valin F. Kinetoplast adaptations in American strains from Trypanosoma vivax. Mutat Res. 2015 Mar;773:69-82.
5. Fernandez-Calero T, Garcia-Silva R, Pena A, Robello C, Persson H, Rovira C, Naya H, Cayota A. Profiling of small RNA cargo of extracellular vesicles shed by Trypanosoma

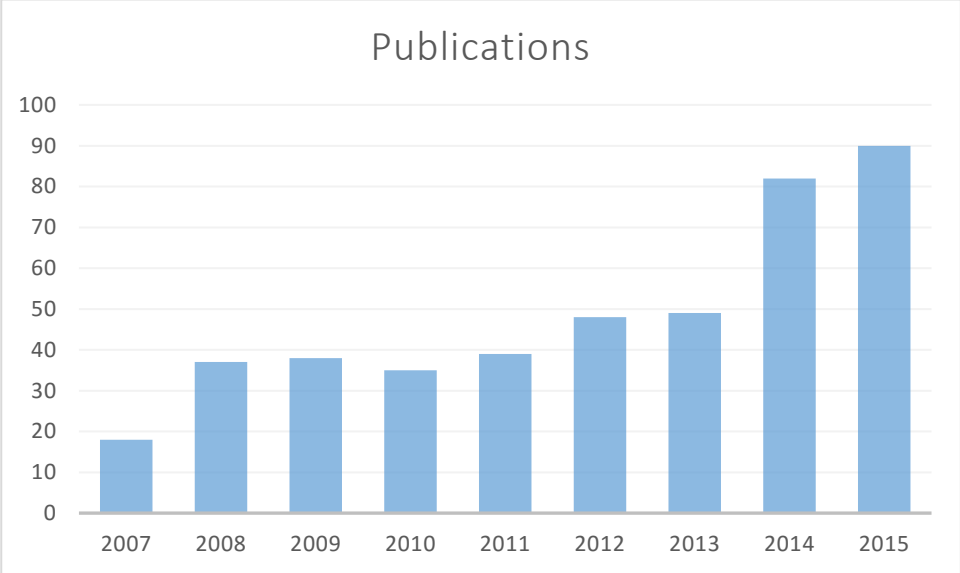
cruzi reveals a specific extracellular signature. Mol Biochem Parasitol. 2015 Jan-Feb;199(1-2):19-28.

6. Arias DG, Piñeyro MD, Iglesias AA, Guerrero SA, Robello C. Molecular characterization and interactome analysis of Trypanosoma cruzi tryparedoxin II. JProteomics. 2015 Apr 29;120:95-104.
7. Trochine A, Creek DJ, Faral-Tello P, Barrett MP, Robello C. Bestatin induces specific changes in Trypanosoma cruzi dipeptide pool. Antimicrob Agents Chemother. 2015 May;59(5):2921-5.
8. Querido JF, Echeverría MG, Marti GA, Costa RM, Susevich ML, Rabinovich JE, Copa A, Montaño NA, Garcia L, Cordova M, Torrico F, Sánchez-Eugenia R, Sánchez-Magraner L, Muñiz-Trabudua X, López-Marijuan I, Rozas-Dennis GS, Diosque P, de Castro AM, Robello C, Rodríguez JS, Altcheh J, Salazar-Schettino PM, Bucio MI, Espinoza B, Guérin DM, Silva MS. Seroprevalence of Triatoma virus (Dicistroviridae: Cripaviridae) antibodies in Chagas disease patients. Parasit Vectors. 2015 Jan 17;8:29.
9. Capdevila, D.A., Marmisollé, W.A., Tomasina, F., Demicheli, V., Portela, M., Radi, R., Murgida, D.H. Specific methionine oxidation of cytochrome c in complexes with zwitterionic lipids by hydrogen peroxide: Potential implications for apoptosis (2015) Chemical Science, 6 (1), pp. 705-713. Cited 2 times.
10. Iraola, G., Betancor, L., Calleros, L., Gadea, P., Algorta, G., Galeano, S., Muxi, P., Greif, G., Pérez, R. A rural worker infected with a bovine-prevalent genotype of Campylobacter fetus subsp. fetus supports zoonotic transmission and inconsistency of MLST and whole-genome typing (2015) European Journal of Clinical Microbiology and Infectious Diseases, 34 (8), pp. 1593-1596..

COURSES

Title	Organizers	Date	Foreign Speakers	Foreign Students	Finantial Agencies
“Redox Chemestry and Biology of Thiols”	M. Comini G. Salinas B. Álvarez M. Trujillo	Feb 23rd - Mar 7th	7	27	ICGEB PEDECIBA RIIP FOCEM SFRBM
“Macromolecular Crystallography School "From data processing to structure refinement and beyond"	A. Buschiazzo R. Keegan	Apr 06th - 16th	15	19	CeBEM CCP4 IUCr RIIP FOCEM
“Analysis & Prediction of Complex Traits Using Whole-Genome Regression Methods”	H. Naya M.I. Fariello D. Gianola	Apr 20th - 24th	1	0	ANII IP MONTEVIDEO
“Performing Molecular Simulations with SIRAH force field”	S. Pantano M. Machado	May 04th - 08th	1	13	UNESCO FOCEM
“Advances in the generation of Genetically Modified (GM) Animal Models”	M. Crispo A. Menchaca	Sep 07th - 18th	13	11	ANII FOCEM ISTT RIIP TWASS UNU BIOLAC OTHER INCOMES - SPONSORS
“International Workshop on Human and Bovine Tuberculosis”	C. Robello O. Pritsch	Sep 28th - 29th	6	5	FOCEM
“Untangling Genomes through bioinformatics using R/Bioconductor and tools for pathway analysis”	N. Rego L. Berná F. Álvarez	Oct 05th - 16th	7	8	ANII FOCEM CABBIO PEDECIBA
“VII PosLatAm Course: Membrane Lipids, Transporters, Channels ... and all that crosstalk”	S. Pantano L. Malacrida M. San Román L. Coitiño D. Peluffo	Nov 23rd - 25th	8	36	ANII FOCEM IUPAB PEDECIBA BIOPHYSICAL SOCIETY CSIC
“Modeling and data analysis for the Healthy Human Global project-Research camp”	H. Naya M. Fontes M.I. Fariello A. Bordería G. Moratorio	Dec 14th - 17th	19	11	RIIP

A. Historical Evolution of IPM Publications



B. Publications 2015

1) Agrelo, R., Sutz, M.A., Setien, F., Aldunate, F., Esteller, M., Da Costa, V., Achenbach, R. A novel werner syndrome mutation: Pharmacological treatment by read-through of nonsense mutations and epigenetic therapies (2015) Epigenetics, 10 (4), pp. 329-341. Cited 1 time.

2) Arias, D.G., Piñeyro, M.D., Iglesias, A.A., Guerrero, S.A., Robello, C. Molecular characterization and interactome analysis of Trypanosoma cruzi tryparedoxin II (2015) Journal of Proteomics, 120, pp. 95-104.

3) Aroa Ejarque-Ortiz, Carme Solà, Águeda Martínez-Barriocanal, Simó Schwartz Jr., Margarita Martín, Hugo Peluffo, Joan Sayós. The receptor cmrf35-like molecule-1 (clm-1) enhances the production of LPS-induced pro-inflammatory mediators during microglial activation. PLoS ONE DOI: 10.1371/journal.pone.0123928, 2015.

4) Báez, A., Salceda, E., Fló, M., Graña, M., Fernández, C., Vega, R., Soto, E.α-Dendrotoxin inhibits the ASIC current in dorsal root ganglion neurons from rat (2015) Neuroscience Letters, 606, art. no. 31507, pp. 42-47.

5) Berná, L., Alvarez-Valin, F. Evolutionary volatile Cysteines and protein disorder in the fast evolving tunicate Oikopleura dioica (2015) Marine Genomics.

6) Bisio H, Bonilla M, Manta B, Graña M, Salzman V, Aguilar PS, Gladyshev VN, Comini MA, Salinas G. A New Class of Thioredoxin-Related Protein Able to Bind Iron-Sulfur Clusters. Antioxid Redox Signal. 2015 Oct 27. [Epub ahead of print] PubMed PMID: 26381228.-

7) Cabrera G, Salazar V, Montesino R, Támbara Y, Struwe WB, Lugo EL, Harvey DJ, Antoine L, Rincón M, Domon B, Méndez Martínez MD, Portela M, González-Hernández A, Triguero A, Durán R, Lundberg U, Vonasek E, González LJ. Structural characterization and biological implications of sulfated N-glycans in a serine protease from the neotropical moth Hylesia metabus (Cramer [1775]) (Lepidoptera: Saturniidae). Glycobiology. 2015 Nov 3. pii: cwv096. [Epub ahead of print]

8) Capdevila, D.A., Marmisollé, W.A., Tomasina, F., Demicheli, V., Portela, M., Radi, R., Murgida, D.H. Specific methionine oxidation of cytochrome c in complexes with zwitterionic lipids by hydrogen peroxide: Potential implications for apoptosis (2015) Chemical Science, 6 (1), pp. 705-713. Cited 2 times.

9) Coitinho C, Greif G, van Ingen J, Laserra P, Robello C, Rivas C. First case of Mycobacterium heckeshornense cavitary lung disease in the Latin America and Caribbean region. New Microbes New Infect. 2015 Dec 18;9:63-5.

10) Correa A, Oppezzo P. Overcoming the solubility problem in E. coli: available approaches for recombinant protein production. Methods Mol Biol. 2015;1258:27-44. doi: 10.1007/978-1-4939-2205-5_2.

11) Carrau, F., Gaggero, C., Aguilar, P.S. Yeast diversity and native vigor for flavor phenotypes (2015) Trends in Biotechnology, 33 (3), pp. 148-154. Cited 2 times.

12) Comini MA. Measurement and meaning of cellular thiol: disulphide redox status. Free Radic. Res. 2015.

13) Correa, A., Oppezzo, P. Overcoming the solubility problem in E. coli: Available approaches for recombinant protein production (2015) Methods in Molecular Biology, 1258, pp. 27-44.

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IP Montevideo

at a glance

Staff

Human Resources	Dec 2010	Dec 2015
Scientific & Technical Staff	70	105
Administration & Support Staff	30	37
Associated Researchers & Students	50	84
Total	150	226

2015 Publications (Scopus)

Year	2007	2008	2009	2010	2011	2012	2013	2014	2015	Total
Publications	18	37	38	35	39	48	49	82	86	432

Aggregate Record	IPM 2007-2015
Number of Publications	432
Accumulated citations	6752
Citations per publication	16,5

2015 Budget Overview

Incomes – 2015 in thousand dollars		
FINANTIAL SOURCES	INCOME	%
IP MONTEVIDEO	3.777	64%
GRANTS	1.385	23%
SERVICES	765	13%
TOTAL	5.927	100%