Institut Pasteur de Montevideo

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Scientific Report 2015-2016

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PREFACE

The IP Montevideo celebrated its 10th anniversary in December 2016. Still, the original mission remains the same: 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, biological markers of disease and new drugs.

By December 2016, more than 240 people worked or studied at the IP Montevideo, including almost 100 established researchers, technicians or assistants coming from several partner institutions and 40 collaborators in administration and research support units.

Seventeen research groups are organized into institutional programs focused on the One-Health concept, with emphasis in multidisciplinary molecular approaches in human and veterinary medicine including environmental interactions. The federative programs are focused on i) Genomics, ii) Molecular, Cell and Animal Technology, iii) Obesity, Diabetes, Inflammatory and Cardiovascular Diseases, iv) Cancer and, v) Animal Health. These programs are mainly funded by institutional grants or agreements with FOCEM (Mercosur), Interamerican Development Bank (IDB), the National Institute for Agricultural Research (INIA) and the National Agency for Innovation and Research (ANII) and have close interactions with the national Republic University and the Institut Pasteur International Network (IPIN).

The IP Montevideo has established central core facilities with "state-of-the-art" equipment to study genomic, proteomic, structural & cell biology and animal research. In the 2015-2016, an ambitious investment was performed to purchase a modern confocal microscope, capillary electrophoresis and a new mass spectrometer equipment for proteomic analysis.

The number and impact of the publications further increased in 2015-2016 period, reaching an average of 80 publications/year. According to international data banks, publications from the IP Montevideo have a cumulated average of >15 citations per publication, which can be considered of competitive international standard. Remarkably, 3 patents on a new drug-class technology have been filed in the last year, which will be likely licensed to a regional start-up company for the treatment of inflammatory diseases.

Our research laboratories provide an environment for the training of advanced graduate students. The IP Montevideo also contributes to the training of human capacities in collaboration with the national and international postgraduate programs. In 2016, we harbor more than 140 MSc, doctoral and postdoctoral

fellows. We have also organized several international courses on different topics of molecular medicine. In 2016, 60 distinguished professors and dozens of advanced students from abroad attended the courses. In addition, we have received hundreds of elementary and high school students for different science pop activities. All over the year this activity allows us to spread science among hundreds of young students.

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. At present, our start-up incubator Bioespinn supports the consolidation of 10 start-up companies in different developmental stages. Bioespinn is partially funded by the National Agency for Innovation and Research (ANII).

Finally, the 2016 annual budget of the IP Montevideo was close to 7,5 million dollars, mainly coming from the Uruguayan national budget and from own incomes by service sales, grants and research contracts, including those allocated by European Union (Uruguay-Innova) and FOCEM (MERCOSUR).

I wish to thank all our researchers and members for their dedication, continued support and commitment. Also, I wish to acknowledge the great contribution and trust from our partner institutions in Uruguay and France.

Luis Barbeito
Executive Director
Institut Pasteur Montevideo

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
- Biopharmaceutical 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; Academic Coordinator-IP Montevideo)
Horacio Botti, MD, PhD (Adjunct Investigator, IP Montevideo), until 2015
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), until 2015
Jessica Rossello, Biochemist (Technician, PhD student)
Bernardina Rivera, Biochemist (Technician, Graduate student)
Alejandro Leyva (Technician, PhD student)

Associate Members

Virginia López, PhD (Adjunct Professor of Organic Chemistry, School of Chemistry and Science, UdelaR)
María Noel Álvarez, PhD (Associate Investigator, Adjunct Professor of Biochemistry, School of Medicine, UdelaR, Uruguay)
Andrés Kamaid, PhD (Associate Investigator)

Leonel Malacrida, PhD (Associate Investigator, Assistant Profesor, Pathophysiology Department, School of Medicine, UdelaR, Uruguay)

Students

Magdalena Gil, Biochemist (PhD student, ANII Fellow). **Dissertation May 2016**.

Analía Lima, MSc. (PhD student).

Jorge Rodriguez, Biochemist (PhD student; ANII Fellow).

Rosina Dapueto, M.Sc. (PhD Student, ANII Fellow).

Jessica Rossello, Biochemist (PhD student; ANII Fellow).

Alejandro Leyva, Biochemist (PhD student; ANII Fellow)

Gonzalo Spera, M.D. (M.Sc. student, Pro.In.Bio. Fellow). **Dissertation Dec 2015**

Adriana Carlomagno, M.D. (M.Sc. student, Pro.In.Bio. Fellow)**. Dissertation Jul 2016.**

Bernardina Rivera, Biochemist (Graduate student, ANII Fellow).

Germán Galliusi (MSc student; Pro.In.Bio. Fellow).

Rosina Toledo (MSc student, ANII Fellow).

Josefina Peña (Undergraduate student, ANII Fellow), until 2015

MAIN EQUIPMENT

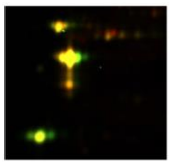
- HPLC, Agilent 1200
- Capilar HPLC, Agilent 1200;
- Nano HPLC, Easy-nLC 1000, Thermo
- Nano HPLC Ultimate 300, Thermo
- 2D Electrophoresis, EttanIPGphor + EttanDaltSix
- Typhoon FLA 9500, GE Healthcare
- 4800 MALDI TOF/TOF Mass Spectrometer, Abi Sciex
- LTQ Velos + ETD Mass Spectrometer, Thermo
- Q-exactive (Q-Orbitrap), Thermo

4800 MALDI-TOF/TOF, Abi Sciex

LTQ Velos, Thermo

Q-Exactive, Thermo

Ultimate 3000, Thermo



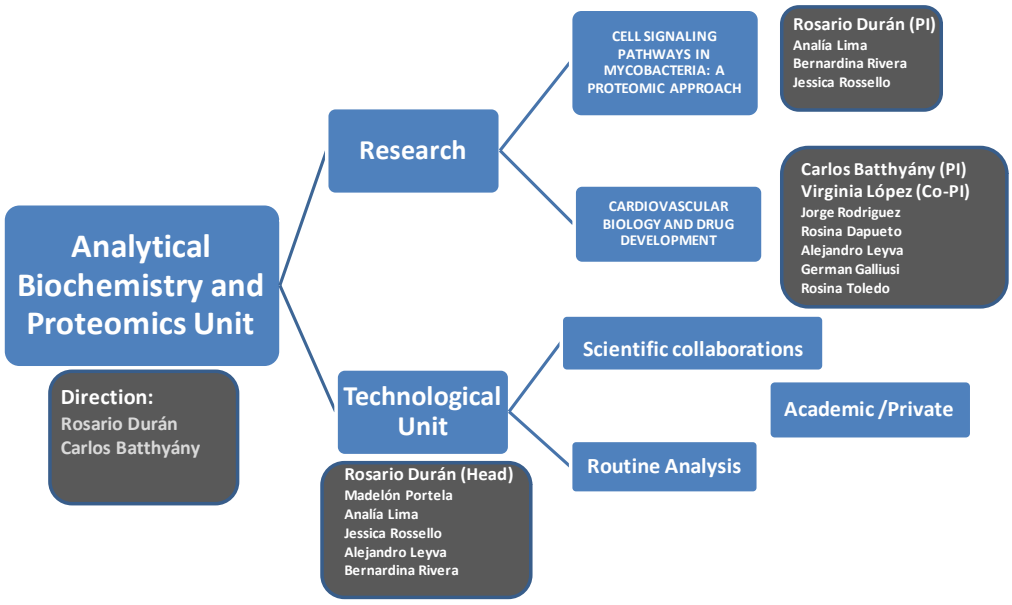
Typhoon FLA 9500, GE

GOALS

The goals of the Analytical Biochemistry and Proteomics Unit (UByPA) is to:
1. perform and support mass spectrometry (MS)/analytical biochemistry based research;
2. provide researchers with training, scientific assistance and access to MS-based proteomic related technologies;
3. improve available MS & 2D-DIGE proteomic techniques for biomedical research;
4. contribute to local and regional training and education programs in MS-based proteomics.

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

UNIT ORGANIZATION



SERVICES

Routine Analysis

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-translationalmodificationanalysis.
- 2-D gel electrophoresis based proteomics.
- "Shotgun" proteomics.
- Quantitative proteomics.
- De novo peptide sequencing.

- ✓ In 2016 we analyzed 1227 samples, both routine and non-routine analysis. We would like to highlight that those analysis include around **400 samples for quantitative proteome analysis**.
- ✓ **Due to this high demand, we acquired and installed a new state of the art nanoLC-mass spectrometer (Ultimate nLC/Q-Orbitrap, Q Exactive Plus, Thermo). This nLC-mass spectrometer is now fully operative and the staff has already been trained.**

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 mass spectrometry (MS) based proteomics 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

The ability of *M. tuberculosis* to inhibit phagosome maturation and to survive in the intracellular environment of host is central to tuberculosis pathogenicity. The signaling cascades mediated by Ser/Thr kinases are key players in these processes. In particular one of these enzymes, PknG, became of special interest as it was found to play dual roles in mycobacterial metabolism and pathogenesis through mechanisms still not completely understood. In order to unveil PknG partners in mycobacteria we developed an affinity purification-sequential elution-mass spectrometry strategy to identify substrates and interactors of the kinase. This strategy allowed us to identify new PknG substrates that were further validated by *in vitro* and aslo *in vivo* by proteomic analysis of a *pknG* null mutant strain. We provide evidence that PknG regulates nitrogen assimilation and cell wall synthesis in mycobacteria. These are essential processes not only for bacterial physiology but also for the pathogen survival in the nutrient deficient environment within the host. Interestingly, some of the identified substrates contain a Forkhead-associated domain (FHA). The FHA domain is a protein module that specifically recognizes phospho-Thr residues and that participates in the assembly of multicomponent signaling complexes in transduction pathways regulated by phosphorylation in prokaryotes and eukaryotes. The genome of *M. tuberculosis* codifies five proteins with FHA domain, which have been reported as in vitro substrates of several Ser/Thr kinases. The presence of signaling networks between Ser/Thr kinases and FHA domain-containing proteins in mycobacteria has been postulated, however nowadays our knowledge about its architecture, the protein-protein interactions underlying this network and the biological process under control is very scarce. Our research aims to contribute to the elucidation of FHA domain mediated signaling networks in mycobacteria by characterizing at the molecular level the signaling complex formed *in vivo* by FHA domain-containing proteins and its dynamics in response to the biochemical stimuli found in the host. We are focusing in two FHA domain-containing proteins, GarA and FhaA, which we identify as substrates of PknG. The experimental strategy proposed combines the specific purification of tagged proteins with *in vivo* crosslinking to provide a snapshot of protein interactions in the mycobacteria. This approach will contribute to unravel the processes controlled by PknG signaling cascades mediated by its FHA domain containing substrates and its possible role in the adaptation to the host environment.

VASCULAR BIOLOGY AND DRUG DEVELOPMENT:

Metabolic diseases (obesity, metabolic syndrome and type II diabetes) and cardiovascular diseases (Hypertension, atherosclerosis and its complications - AMI, stroke) are the main cause of morbidity and mortality worldwide. In the pathogenesis of this set of diseases one can highlight the generation of a chronic inflammatory response at low noise where cell signaling through the proinflammatory transcription factor NFkB and activation of the inflammasome by sterile signals play major roles.

My main line of research since 2011 has been the development of a new pharmacological strategy for the prevention and treatment of this set of diseases, being the idea to tackle the chronic inflammatory response that promotes the development of them. To do this, we initially take into account the metabolism of alpha-tocopherol and the role LDL and the chronic/oxidative inflammatory process play in the development of atheroma plaques. We design and synthesize a series of hybrid compounds formed by a mimetic structure of alpha-tocopherol (Vitamin E) adding a nitroalkene group which exerts potent anti-inflammatory and anti-atherogenic effects. The rational of this new pharmacological strategy is that the hybrid molecule is going to be selectively incorporated into the LDL due to the presence of the chromanol structure, characteristic of alpha-tocopherol, and to the specific action of alpha-tocopherol transfer proteins which recognize this structure. The LDL particle is then goong to be used as a carrier of the hybrid compound to the lesions where it will exert, *in situ*, its anti-inflammatory and antiatherogenic properties (see invention patents). With this new pharmacological strategy we were able to demonstrate that the hybrid compound not only inhibits the development of atherosclerosis in apo $E^{-/-}$ mice but also inhibits the development of hypertension in mice treated with a continuous infusion of angiotensin II (see Patents of Invention).

We subsequently expanded our portfolio of molecules and developed and protected them (see Patents of Invention) two new families of non-conventional anti-inflammatory compounds that block the cellular signaling pathways involved in the generation of the chronic inflammatory response, the critical pathogenic step in the development of metabolic and cardiovascular diseases. Preclinical results in animal models obtained so far are very encouraging and have allowed us to license our intellectual property portfolio to CITES (http://cites-gss.com/), the first technology incubator in Latin America. Our project was selected and funded with the objective of completing the development and preclinical studies of our compounds and perform the first clinical trial (Phase I) with the leader compound that we select.

EDUCATION

TRAINING OF STUDENTS

PhD 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.
- **Jessica Rossello**. Project: "Fosforilación de sustratos de PknG involucrados en el metabolismo de nitrógeno en micobacterias: Rol en la adaptación al ambiente del hospedero" Director: Rosario Durán. Co-Director: Pedro Alzari.
- **Alejandro Leyva.** Project: "Nuevos mecanismos moleculares y farmacológicos involucrados en la patogenia de la Enfermedad Renal Crónica". Directors: C. Escande & C. Batthyany

MSc 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.
- **Adriana Carlomagno, M.D.** Project: "Nuevas estrategias farmacológicas para el tratamiento de la hipertensión arterial sistémica. Ensayos pre-clínicos en un modelo animal". Directors: C. Escande, C. Batthyany & P. Contreras. **Finished Jul 2016**
- **Germán Galliusi.** Project: "Desarrollo de nuevos moduladores metabolicos e inflamatorios" Director: C. Batthyany

Under graduate 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 primera empresa uruguaya del tipo "Contract of Research Organization".** (2015-2017); C. Batthyany (ALI_2-2014-1-5055); **Amount Granted: USD 302.000**
10. "**Development, synthesis and characterization of novel anti-inflammatory compounds".** (2016-2018); C. Batthyany, V. López, C. Escande (CITES, http://cites-gss.com/); **Amount Granted: USD 630.000.**

STUDENTS FELLOWSHIP

- PhD fellowship - Rosina Dapueto; 2014 (2 years); ANII
- PhD fellowship - Jorge Rodríguez; 2013 (3 years); ANII
- PhD Fellowship – Jessica Rossello; 2016 (3 years); ANII
- PhD Fellowship-Alejandro Leyva ; 2016 (3 years); ANII
- MSc. Fellowship –Bernardina Rivera; 2015 (2 years); ANII

PUBLICATIONS

2015

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.

2016

5. **C. Batthyány**, S. Bartesaghi, M., Mastrogiovanni, **A. Lima**, V. Demicheli, R. Radi. Tyrosine-Nitrated Proteins: Proteomic and Bioanalytical Aspects. Antioxid Redox Signal. (2016) Jul 22. [Epub ahead of print]

6. V. Demicheli, D.M. Moreno, G.E. Jara, **A. Lima**, S. Carballal, N. Ríos, **C. Batthyany**, G Ferrer-Sueta, C. Quijano, D.A. Estrín, M.A. Martí, R. Radi. Mechanism of the Reaction of Human Manganese Superoxide Dismutase with Peroxynitrite: Nitration of Critical Tyrosine 34. Biochemistry. 55 (2016) 3403-3417.

7. M.E. Dieterle, J. Fina Martin, **R. Durán**, S.I. Nemirovsky, C. Sanchez Rivas, C. Bowman, D. Russell, G.F. Hatfull, C. Cambillau, M. Piuri. Characterization of prophages containing “evolved” Dit/Tal modules in the genome of Lactobacillus casei BL23, Applied Microbiology and Biotechnology 100 (2016) 9201-9215.

8. G. Cabrera, U. Lundberg, A. Rodriguez-Ulloa, M. Herrera, W. Machado, **M. Portela**, S. Palomares, L.A. Espinosa, Y. Ramos, **R. Duran**, V. Besada, E. Vonasek, L.J. Gonzalez. , Protein content of the Hylesiametabus egg nest setae (Cramer [1775]) (Lepidoptera: Saturniidae) and its association with the parental investment for the reproductive success and lepidopterism, J Proteomics 150 (2016) 183-200.

9. G. Cabrera, V. Salazar, R. Montesino, Y. Tambara, W.B. Struwe, E. Leon, D.J. Harvey, A. Lesur, M. Rincon, B. Domon, M. Mendez, **M. Portela**, A. Gonzalez-Hernandez, A. Triguero, **R. Duran**, U. Lundberg, E. Vonasek, L.J. Gonzalez. Structural characterization and biological implications of sulfated N-glycans in a serine protease from the neotropical moth Hylesiametabus (Cramer [1775]) (Lepidoptera: Saturniidae), Glycobiology 26 (2016) 230-250.

10. Müller Virginia, Gustavo Bonacci, Carlos **Batthyany**, María Valeria Amé, Fernando Carrari, Jorge Omar Gieco, and Dr. Ramon Asis Peanut seed cultivars with contrasting resistance to Aspergillus parasiticus colonization display differential temporal response of protease inhibitors. Phytopathology Accepted for publication. Posted online on 14 Nov 2016

INTERNATIONAL PATENT:
under the Patent Cooperation Treaty (PCT)

1. "Methods of treatment of inflammation related conditions using pluripotent anti-inflammatory and metabolic modulators"; inventors Batthyany, C., Lopez, G.V., Escande, C., Porcal, W., Dapueto, R., Rodriguez, R., Galliussi, G., and Garat, M.P. 2016. USA patent provisional application; to be assigned.

2. "Trolox derivatives and methods of use thereof in the treatment and prevention of inflammation related conditions"; inventors Batthyany, C., Lopez, G.V., Dapueto, R., Escande, C., and Rodriguez, R. 2016. USA patent non-provisional application; to be assigned.

3. "System, Method and Device for Identifying Discriminant Biological Factors and for Classifying proteomic profiles"; inventors Carvalho PC, Batthyany C, Silva A; Lima D; Leyva A; Barbaosa V; Durán, R. 2016, USA provisional patent application; to be assigned

4. 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. It 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 different 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*).

PUBLICATIONS

1. **Ortega C, Abreu C, Oppezzo P and Correa A.** Cloning methods for the post-genomic era. **Methods Mol Biol.** Submitted
2. **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 Facility (PXF)

MEMBERS

Alejandro Buschiazzo, PhD (Head of the Lab)
Joaquin Dalla Rizza (Technician) - recruited 2016
Nicole Larrieux (Technician)
Felipe Trajtenberg, PhD (Research Scientist)

Frank Lehmann (Technician) - past member, moved to Biozentrum, University of Basel (Switzerland) as Lab Manager (2016)

TECHNOLOGICAL FACILITY

Experimental approaches currently available for users

1. Protein crystallization screenings: manual and robot-assisted (Honeybee963®, Isogen Life Science)
2. Follow-up and optimization of initial crystallization hits: manual and robot-assisted (Alchemist®, Rigaku)
3. X ray Diffraction – Testing and initial characterization of crystals
4. X ray Diffraction – single crystal data collection
5. Crystal structure determination & refinement

Progress 2015-2016

1. Eleven new structures have been released in the PDB: 4PH0, 4PH1, 4PH2, 4PH3 (corresponding to BLV capsid structures, Obal et al. *Science* 2015); 5IUJ, 5IUK, 5IUL, 5IUM, 5IUN (structures of the complex between a His-kinase and its cognate response regulator, from *B. subtilis*, Trajtenberg et al. *eLife* 2016); 5CEE (malic enzyme from *Candidatus Phytoplasma* AYWB in complex with NAD and Mg^{2+}, ms in preparation); and, 5K0W (the crystal structure of the metallo-beta-lactamase GOB-18 from *Elizabethkingia meningoseptica*, Moran-Barrio, et al. *Antimicrob Agents Chemother* 2016).

2. A research contract service was launched with Dr Luis Javier Gonzalez (Centro de Ingeniería Genética y Biotecnología, Havana, Cuba), set up 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, and this crystallographic service is embedded within several biosimilarity studies.

3. The core facility was used to undertake macromolecular crystallography studies led by several teams: a vaccine candidate protein from *Fasciola hepatica* (Carlos Carmona, Inst de Higiene, Uruguay); a pathogenic factor from *Leptospira interrogans* (Elsio Wunder, Yale University, USA); malic enzymes from bacteria and plants (Fabiana Drincovich/Clarisa Alvarez, CEFOBI, Argentina); bacterial cellulase fusions with biotechnological aims (Agustin Correa, IPMont, Uruguay); redox enzymes from trypanosomatid parasites Glc-6-P-dehydrogenase and trypanothione synthase (Marcelo Comini, IPMontevideo, Uruguay); among others.

4. 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. April 6-16, 2015.

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.
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. A subsequent edition has been held in Sao Carlos in 2016, and funding has been secured to run our sixth edition in Oct 2017.
In 2015 we hosted 24 students from 10 different countries; a staff of 15 invited teachers and tutors coming from several of the best centers in the world involved in Protein Crystallography methods development. A satellite scientific symposium was hel during the workshop, titled “Structural Biology in Infection and Disease”, and sponsored by the Institut Pasteur International Network, with seven invited speakers from different Pasteur Institutes.

2. February 16-19, 2016

Organization of the international workshop “Integrative methods in Structural Biology to enhance high impact research in health and disease”, co-organized by Prof Dave Stuart (University of Oxford, UK) and Alejandro Buschiazzo, at the Institut Pasteur de Montevideo. It hosted 40 students, 20 coming from UK and 20 from Uruguay, and top scientists and teachers from Europe and the Mercosur region. The workshop concluded with the signature of a Memorandum of Understanding between IPMontevideo and the European Structural Biology Network INSTRUCT, to foster future collaborations and greater exchange.

3. Incorporation of Joaquin Dalla Rizza (staff technician, started Nov 2016)

Frank Lehmann (staff technician) left our lab, finding a first job at the Membrane Protein Laboratory (Imperial College London, at Diamond synchrotron UK), still working in collaboration with our group. He then obtained a staff position as Lab Manager at Biozentrum, University of Basel (Switzerland). A new technician replacing Frank, was recently incorporated to our protein crystallography facility (Joaquin Dalla Rizza), succeeding a competitive open call.

4. 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. Two successive grants from the Ministerio de Educacion y Cultura (Uruguay) were obtained (40K u$s), 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.

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 Ingeniería)
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)
Melanie Nuesch (Undergraduated 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, automation 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 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.

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. We have performed transcriptome analysis that 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.

Finally, we are in charge of the Uruguayan Human Genome Project (URUGENOMES), funded by the InterAmerican Development Bank, in cooperation with the Seoul National University. In the framework of this project we are analyzing samples from natives and descendants to reconstruct our past. In addition, we are entering in the last phase of this project, related to rare-diseases, and we have performed several preparatory actions involving the use of NGS for diagnostic, particularly in mitochondrial diseases and Sudden Infant Death Syndrome.

EDUCATION-COURSES

We are currently involved in several teaching activities, mainly on bioinformatics-related topics. The 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.

This year, we have created the intensive international course Human Genome Tour 2016 with 120h of direct teaching in three weeks. In this course participated 16 students from abroad and 16 students from Uruguay. Several professors came from abroad (USA, Sweden, Korea) and several from Uruguay. Additionally, we participated in international courses of the IPIN.

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 (more than ten students 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$S 20000.
3. “Identification of microRNAs regulating infection of human cells by Listeria monocytogenes”. PROGRAMMES TRANSVERSAUX DE RECHERCHE – PTR Institut Pasteur 2016/2018 Javier Pizarro-Cerdá (H Naya) €250.000.
4. “Strengthening technical and human capacities for genomic services exports”. Funded by IDB 2014/2017. H Naya U$S600.000.
5. "Modulación del sistema ubiquitina-proteasoma por electrófilos y oxidantes: estudio de redes moleculares" (FCE_3_2016_1_126877). Fondo Clemente Estable - 2016- Agencia Nacional de Investigación e Innovación. Responsable: Horacio Botti, Colaboradores: Gregorio Iraola, Álvaro Cabana, Martín Hugo, Marcelo Hill. $U 1.000.000.
6. "Evolución genómica de Mycobacterium avium paratuberculosis en animales silvestres y de producción" (FCE_3_2016_1_126791). Fondo Clemente Estable - 2016- Agencia Nacional de Investigación e Innovación. Pablo Fresia (Responsable), Gregorio Iraola, Ignacio Ferres, Hugo Naya, Fernando Paolicci (INTA). $U 1.000.000.

PUBLICATIONS

2015

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- Fernandez-Calero T, Garcia-Silva R, Pena A, Robello C, Persson H, Rovira C, **Naya H**, Cayota A (2015). "Profiling of small RNA cargo of extracellular vesicles shed by Trypanosoma cruzi reveals a specific extracellular signature". Mol Biochem Parasitol. pii: S0166-6851(15)00013-4. doi:10.1016/j.molbiopara.2015.03.003.

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2016

- Gianola D, **Fariello MI**, **Naya H**, Schön CC (2016). "Genome-wide association studies with a genomic relationship matrix: a case study with wheat and Arabidopsis". G3 (Bethesda) 6(10):3241-3256. doi: 10.1534/g3.116.034256.

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- **Iraola G, Spangenberg L**, Lopes-Bastos B, **Graña M**, Vasconcelos L, Almeida A, Greif G, Robello C, Ristow P, **Naya H** (2016). "Transcriptome sequencing reveals wide expression reprogramming of basal and unknown genes in Leptospira biflexa biofilms". mSphere 10.1128/mSphere.00042-16.

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Molecular Biology Unit

MEMBERS

Carlos Robello, PhD (Head)
Luisa Berná (Postdoctoral Researcher- INNOVA II)
Gonzalo Greif, PhD
Gabriela Libisch (PhD Student)
Cecilia Portela (Technician, Facultad de Ciencias)

MAIN EQUIPMENT

- DNA Sequencer/analyzer
- Real Time PCR
- Microarray platform
- Microarray hybridizer
- BioAnalyzer
- General Molecular Biology Equipment
- MiSeq Illumina

SERVICES

1. **DNA sequencing (Sanger method)**

   The DNA sequencing service is the unique facility in Uruguay making sequencing by the Sanger method, and fragment analysis. Working until now with a 3130 Genetic Analyzer (Applied BioSystems), we have recently acquired a 3500 Genetic Analyzer (Applied BioSystems) which will allow improving the performance of the platform. The platform receives samples from private and public institutions from all the country.

2. **Microarrays**

   This Agilent platform is still being used for transcriptomic studies of complex organisms, mainly human and bovine samples. There are also sporadic cooperation with private clinics that use Comparative Genomic Hybridization in diagnostics (e.g. pre-implantation genetic diagnostics).

3. **Deep sequencing**

   The Illumina equipment has allowed to initiate the Genomics Program, and it is now widely used in different applications, mainly genomics and transcriptomics of pathogens (bacteria and unicellular protozoa). We also provide practical advice of how to use the equipment and analyze the data, in order to stimulate the autonomy of other laboratories in the application of next generation sequencing on their research.

PUBLICATIONS

1. Iraola G, Spangenberg L, Lopes Bastos B, Graña M, Vasconcelos L, Almeida Á, Greif G, Robello C, Ristow P, Naya H. Transcriptome Sequencing Reveals Wide Expression Reprogramming of Basal and Unknown Genes in Leptospira biflexa Biofilms. mSphere. 2016 Apr 6;1(2). pii: e00042-16.

2. Spangenberg L, Graña M, Greif G, Suarez-Rivero JM, Krysztal K, Tapié A, Boidi M, Fraga V, Lemes A, Gueçaimburú R, Cerisola A, Sánchez-Alcázar JA, Robello C, Raggio V, Naya H. 3697G>A in MT-ND1 is a causative mutation in mitochondrial disease. Mitochondrion. 2016 May;28:54-9.

3. 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.

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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 (Research Associate)
Vanesa Piattoni, PhD (Postdoctoral fellow)
Soledad Astrada, (PhD student, dissertation December 2016)
Hellen Daghero (Master student)

Micaela Sureda (Research internship) – past member
Inés Tiscornia, MSc (Staff TA) – past member
Giuliana Mastropietro (BSc 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 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; Rolny *et al*, 2016).

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). In the frame of this project we are actively collaborating with Dr. Rodríguez (ISAL, CONICET-UNL, Santa Fe, Argentina) and we had the financial support from ANII-CONICET (MOV_CO_2015_1_110054).

COLLABORATIVE PROJECT:

Since 2011 we are collaborating with Dr. M. 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". In this research line, we have demonstrated that CIGB-552 is effective in reducing tumors size in mice and that COMMD1 protein is a key mediator for its antitumor activity (Fernández Massó *et al*, 2013, Vallespí *et al*., 2014, Núñez de Villavicencio-Díaz *et al*, 2015a and 2015b). Recently, we described the minimal functional unit of the CIGB -552 (the minimum amino acid sequence) necessary to exert its biological activity (ability to penetrate into tumor cells, interact with COMMD1 and induce of apoptosis, Astrada *et al*, 2016)

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- Núñez de Villavicencio-Díaz T et al. Data forcomparativeproteomicsanalysis of theantitumoreffect of CIGB-552 peptide in HT-29 colon adenocarcinoma cells. Data Brief. 2015-b Jul 8;4:468-73. doi: 10.1016/j.dib.2015.06.024
- Mastropietro G *et al*. HT-29 and Caco-2 Reporter Cell Lines for Functional Studies of Nuclear Factor Kappa B Activation.MediatorsInflamm. 2015; 2015:860534. doi: 10.1155/2015/860534.
- Porro V *et al*. 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.
- Rolny I *et al*. Lactobacillus delbrueckii subsp lactis CIDCA 133 modulates the response of human epithelial and dendritic cells infected with Bacillus cereus. Benef Microbes. 2016 Nov 30;7(5):749-760

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- Vallespí MG *et al*. Antitumorefficacy, pharmacokinetic and biodistributionstudies of theanticancerpeptide CIGB-552 in mouse models. J Pept Sc (2014), 20(11):850-9.

PUBLICATIONS

2015

1- Cabrera M, de Ovalle S, **Bollati-Fogolín M**, Nascimento F, Corbelini P, Janarelli F, Kawano D, Eifler-Lima VL, González M, Cerecetto H. New hits as phase II enzymes inducers from a focused library with heteroatom-heteroatom and Michael-acceptor motives. Future Sci OA. 2015 Nov 1;1(3):FSO20. doi: 10.4155/fso.15.18. eCollection 2015.

2- 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.

3- 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.

4- 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.

5- 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.

6- 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.

7- 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.

8- **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.

2016

9- **Astrada S**, Gomez Y, Barrera E, Obal G, Pritsch O, Pantano S, Vallespí MG, **Bollati-Fogolín M***. Comparative analysis reveals amino acids critical for anticancer activity of peptide CIGB-552. J Pept Sci. 2016 Nov;22(11-12):711-722. doi: 10.1002/psc.2934.

10- Malacrida L, **Astrada S**, Briva A, **Bollati-Fogolín M**, Gratton E, Bagatolli LA. Spectral phasor analysis of LAURDAN fluorescence in live A549 lung cells to study the hydration and time evolution of intracellular lamellar body-like structures. Biochim Biophys Acta. 2016 Nov;1858(11):2625-2635. doi: 10.1016/j.bbamem.2016.07.017.

11- Rolny I, **Tiscornia I,** Racedo SM, Pérez PF, **Bollati-Fogolín M***. *Lactobacillus delbrueckii subsp lactis* CIDCA 133 modulates the response of human epithelial and dendritic cells infected with Bacillus cereus. Benef Microbes. 2016 Nov 30;7(5):749-760.

12- Bürgi M, Zapol'skii VA, Hinkelmann B, Köster M, Kaufmann DE, Sasse F, Hauser H, Etcheverrigaray M, Kratje R, **Bollati-Fogolín M***, Oggero M. Screening and characterization of molecules that modulate the biological activity of IFNs-I. J Biotechnol. 2016 Sep 10;233:6-16. doi: 10.1016/j.jbiotec.2016.06.021.

13- García EP, **Tiscornia I**, Libisch G, Trajtenberg F, **Bollati-Fogolín M,** Rodríguez E, Noya V, Chiale C, Brossard N, Robello C, Santiñaque F, Folle G, Osinaga E, Freire T. MUC5B silencing reduces chemo-resistance of MCF-7 breast tumor cells and impairs maturation of dendritic cells. Int J Oncol. 2016 May;48(5):2113-23. doi: 10.3892/ijo.2016.3434.

14- Ubillos L, Freire T, Berriel E, Chiribao ML, Chiale C, Festari MF, Medeiros A, Mazal D, Rondán M, **Bollati-Fogolín M**, Rabinovich GA, Robello C, Osinaga E. Trypanosoma cruzi extracts elicit protective immune response against chemically induced colon and mammary cancers. Int J Cancer. 2016 Apr 1;138(7):1719-31. doi: 10.1002/ijc.29910.

*: last /corresponding author.

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. Granted: U$S 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. 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. Granted: $U 1,300,000.
4. Diseño y producción de nuevas variantes de la hormona folículo estimulante (FSH) para su empleo en especies de interés productivo. PI: M Bollati, ANII (ALI_1_2015_1_5084), 2016-2019. Granted: U$S 290,000.
5. Generación y caracterización de modelos in vitro para el estudio de perturbadores endócrinos. ANII-CONICET (MOV_CO_2015_1_110054), 2016-2018, PI: M Bollati. Granted: U$S 6,540.

Other fundings:

6. Postdoctoral fellowship ANII-PD_NAC_2013_1_10903, R. Pagotto, 2013-2015.
7. Doctoral fellowship ANII-POS_NAC_2012_1_8523, S. Astrada, 2013-2016.

8. Postdoctoral fellowship IP de Montevideo, V. Piattoni, 2015-2017.
9. Master fellowship ANII-POS_NAC_2015_1_109487, H. Daghero, 2016- 2018.ICGEB (15,000 Euros), RIIP (22,000 Euros) and UNUBiolac (15,000 USD) Grants to organize the course CELL AND ANIMAL MODELS FOR DRUG DISCOVERY, October 2017.

OTHER ACTIVITIES

In February 2016 we have organized the "Flow cytometry basic course and its applications in research" at the Institut Pasteur de Montevideo, with the support of PEDECIBA.

During 2015 and 2016 we have collaborated 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.
- ✓ May 2016: Basic Cell Culture course, IIBCE, PEDECIBA. Montevideo, Uruguay. K. Perelmuter, S. Victoria and M. Bollati participated in the theoretical and practical activities.
- ✓ June 2016: Interacción microorganismo-hospedador: mecanismos moleculares y celulares. CERELA, Tucumán, Argentina. M. Bollati was invited speaker.
- ✓ September 2016: CABBIO course Glicoproteínas terapéuticas: diseño, expresión y análisis de sus glicanos, Santa Fe, Argentina.M. Bollati was invited speaker.
- ✓ October 2016: Proinbio Course Técnicas de procesamiento, cultivo y caracterización de células, Hospital de Clínicas, Montevideo, Uruguay. S. Victoria and M. Bollati participated in the theoretical and practical activities
- ✓ November 2016: VII SLATCC and Curso Avanzado en Cultivo de Células; IBT, UNAM, México. M. Bollati was invited speaker at both events.

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), PICT (Argentina).
- Member of the jury for the poster award in the 24th ESACT meeting (Barcelona, Spain).

Students performing their thesis work at the CBU:

- Soledad Astrada has accomplished her PhD thesis in December 2016. Supervisor: M. Bollati.
- Giuliana Mastropietro, has accomplished her diploma thesis in Biotechnology Engineering in August 2015. Supervisors: M. Bollati.
- Hellen Daghero, Master student. Supervisor: M. Bollati. On going.

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)
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)
Sergio Anchetta (Animal caretaker)
Martín Mereles (Animal caretaker)
Mario Borges (Animal caretaker)
Casandra Carrillo (Animal caretaker)
Gisell González (Animal caretaker)

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 in mice. We also provide mice and rats with high genetic and sanitary status for researchers of the institution and the region.

CORE FACILITIES - SERVICES

Generation of gene edited mice by CRISPR/Cas9 system (3 projects)

Generation of gene edited sheep by CRISPR/Cas9 system (2 projects)

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

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

Embryo and sperm cryopreservation (3 projects).

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 and growing.

Production of polyclonal antibodies (10 proteins).

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).

Capacitation in Transgenesis technologies.

Capacitation in embryo transfer and cryopreservation.

Capacitation in BSL2 facility management.

RESEARCH

During 2015 and 2016, our Unit obtained important achievements, generating genetically modified models in mice and large animals using the latest technology for gene edition

known as CRISPR/Cas9. This positioned us as one of the only technological platform offering this system to national and regional researchers.

We have managed the extension of the conventional area of the animal house, increasing five times the existing capacity, including also BSL2 pathogen models and rats. We have increased also the SPF area capacity by incorporating four new ventilated racks for mice. Actual capacity is near 1100 cages (about 6500 mice). We improved several process in order to manage the facility more efficiently.

We have strengthened established collaborations and our own research, reflected in 10 full papers (7 as first or corresponding author) in international journals and several abstracts.

We continue working with national and international biotechnological companies, with grants close to USD 100.000. We received five technicians from Mexico, Chile and Argentina for capacitation in transgenesis, cryopreservation and BSL2 management technologies.

Students were formed under my leadership in activities developed in Uruguay, Hungary, Czech Republic, France, 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 the world. This was the first time a course with these characteristics was dictated in our region, triggering lot of interest. The same Course will be organized in Hellenic Pasteur Institute during 2017 under my co-organization.

The fourth and fifth edition of the internal course for researchers of Institut Pasteur de Montevideo: "**Manejo, técnicas de administración de sustancias y obtención de muestras en ratones"** was organized, being mandatory for working with mice at our facility. Several lectures for undergraduated and posgraduated students were given by members of the UATE. During 2016, this course was approved by the **Comisión Nacional de Experimentación Animal** (CNEA) as one of the references courses to obtain national accreditation (Category A and B) for working with Lab animals.

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 and several publications.

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. Participation: Research member.

2015-2018 Desarrollo y validación de procesos para el estudio y valorización de nutracéuticos: creación de la primera empresa uruguaya del tipo. Participation: Research member.

2016-2019 Diseño y producción de nuevas variantes de la hormona folículo estimulante (FSH) para su empleo en especies de interés productivo. Participation: Research member.

PUBLICATIONS (full papers)

1. **Crispo M**, Dos Santos-Neto PC, Vilariño M, Mulet AP, de León A, Barbeito L, Menchaca A. RAPID COMMUNICATION: Nerve growth factor influences cleavage rate and embryo development in sheep. J Anim Sci. 2016 Oct;94(10):4447-4451. doi: 10.2527/jas.2016-0736.

2. Menchaca A, Anegon I, Whitelaw CB, Baldassarre H, **Crispo M**. New insights and current tools for genetically engineered (GE) sheep and goats. Theriogenology. 2016 Jul 1;86(1):160-9. doi: 10.1016/j.theriogenology.2016.04.028. Review.

3. Menchaca A, Barrera N, dos Santos Neto PC, Cuadro F, **Crispo M**. Advances and limitations of in vitro embryo production in sheep and goats. Anim. Reprod.2016 v.13, n.3, p.273-278.

4. Tamowski S, Luo J, Kanzler B, Whitelaw B, **Crispo M**, Doglio L, Jerchow B, Parker-Thornburg J. TT2016 meeting report on the 13th Transgenic Technology meeting in Prague, Czech Republic. Transgenic Res. 2016 Aug;25(4):553-9. doi: 10.1007/s11248-016-9966-0.

5. **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.

6. 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

7. **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.

8. **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.

9. 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.

10. 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.

GRANTS

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

- 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** (RIIP and UNU-Biolac).

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). March 2015, Buenos Aires, Argentina.
- XXIX Reunión Anual de la Sociedad Brasilera de Tecnología de Embriones, Gramado, August 2015.
- 66th AALAS (American Association for Laboratory Animal Science) National Meeting, Phoenix, USA; November. ICLAS-ARC Laboratory Animal Specialist Award (**Geraldine Schlapp**).
- TT2016 Meeting of the International Society for Transgenic Technologies (ISTT). Prague, Czech Republic, March 2016.
- 14 Congreso de la Sociedad Brasilera de Ciencia de Animales de Laboratorio, Porto Alegre, Brazil. May 2016.

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.
- Martina Crispo: Curso CABBIO de Tecnologías Transgénicas. September 2016, Porto Alegre, Brazil.
- Martina Crispo: INSERM/Wellcome Trust workshop “CRISPR/Cas9”. November 2016, Buenos Aires, Argentina.

INTERNSHIPS & COURSES

2015

- March 2015 – Ana Paula Mulet: Internship in Dr. Martín Rumbo’s Lab, Instituto de Estudios Inmunológicos y Fisiopatológicos, Universidad Nacional de La Plata, Argentina
- April 2015- Ana Paula Arévalo & Gabriel Fernández: IV Curso de técnicas avanzadas en experimentación animal. Critical Care Training Center - Getafe University Hospital. Modalidad on – Line.

- May 2015– Ana Paula Mulet: SALAAM Training School: Transposons and CRISPRs for large animals. Godollo, Hungary.
- June 2015– Gabriel Fernández: Capacitación para Miembros de Comités de Bioética Animal, beca ICLAS. Online.
- October 2015- Ana Paula Arévalo: Curso de Postgrado Estrategias en Bioseguridad y Biocontención, Universitat Autònoma de Barcelona, Spain.

2016

- September 2016- Ana Paula Mulet. Curso CABBIO en Tecnologías Transgénicas. Porto Alegre, Brazil.
- October 2016 – Maria Noel Meikle. CARD course in embryo in vitro fertilization and cryopreservation. Institut Pasteur, Paris, France.
- November 2016 – Ana Paula Arévalo. Internship in mouse imagenology. Instituto del corazón, Sao Paulo, Brazil.

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 2015-2016: **"MANEJO, TÉCNICAS DE ADMINISTRACIÓN DE SUSTANCIAS Y OBTENCIÓN DE MUESTRAS EN RATONES"**, for 40 researchers that uses mice, rats 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 Laboratorio (AUCyTAL).
- IPMon Posdoc project evaluation committee (2015)
- Member of Organizing Committee for TT2016
- Member of Organizing Committee for TT2017
- Evaluation of scientific abstracts for TT2016 meeting
- Evaluation of scientific abstracts for 42nd International Embryo Transfer Society
- Evaluation of scientific projects CONACyT (Paraguay)
- Reviewer for different scientific journals

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 de 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:

- U-MNUA2: Excitation 360-370nm / Emission 420-460nm (DAPI)
- U-MNIBA3: Excitation 470-495nm / Emission 510-550nm (FITC, GFP)
- U-MWIG3: Excitation 530-550nm / Emission 570nm (Rhodamine, TRITC, Cy3, Texas Red)
- 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:

- A: Excitation 340-380nm / Emission 425 (DAPI)
- I3: Excitation 450-490nm / Emission 515 (FITC, GFP)
- 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:

- DAPI: Ex 365 nm, Em 445/50
- FITC, GFP: Ex 450 – 490, Em 515 – 565
- 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

Two kind of services are offered by the technicians at the Microscopy Unit. First, in the microscopes' manipulation and image acquisition. Second, basic image processing and analysis, including image enhancing (denoising, deconvolution), image segmentation, colocalization, among others.

STATISTICS OF THE PLATFORM

Users of the microscopy platform

The microscopes were used in 2016 by 88 users from 18 different units fo the Institut Pasteur de Montevideo. Among them, 87 use the inverted fluorescence microscope and 57 the confocal microscopes. The following table shows the distribution of users among the units

		2016		
UNIDADES	18		Epi	Confocal
GMH	Genética Molecular Humana	7	7	7
NEURO	Neurodegeneración	5	5	5
UBC	Unidad de Biología Celular	5	5	2
UByPA	unidad de Bioquímica y Proteómica Analítica	8	8	5
iLLC	Unidad de Proteínas Recombinantes	3	3	3
UBM	Unidad de Biología Molecular	8	8	6
UATE	Unidad de Animales Transgénicos Experimentales	6	6	2
BRT	Biología Redox de Tripanosomas	6	6	2
LIRI	Laboratorio de Inmunoregulación e Inflamación	5	5	4
GF	Genómica Funcional	3	3	1
LGIT	Laboratorio de Glicobiología e Inmunología Tumoral	7	7	3
BCDN	Biología Celular del Desarrollo Neural	4	4	4
PME	Patologías del Metabolismo y Envejecimiento	9	9	4
NFTG	Neuneuroinflamación y Terapia Génica	7	6	7
BG	Biología de Gusanos	2	2	2
CB	Control de Biofármacos	1	1	0
UBP	Unidad de biofísica de proteínas	1	1	0
CP	Cristalografía de proteínas	1	1	
	TOTAL	88	87	57
	PROMEDIO DE USUARIOS POR UNIDAD	5		

There were also 18 users from external institutions: Facultad de Ciencias, Instituto Clemente Estable, Facultad de Agronomía, Facultad de Química, Hospital de Clínicas and Laboratorios Santa Elena.

Use of the microscopes

In 2016 the Leica Confocal Microscope was used for 1533 hours, the Zeiss Confocal Microscope was used for 482 hours and the Olympus Microscope 584.5 hours; Figure 1 shows this distribution.

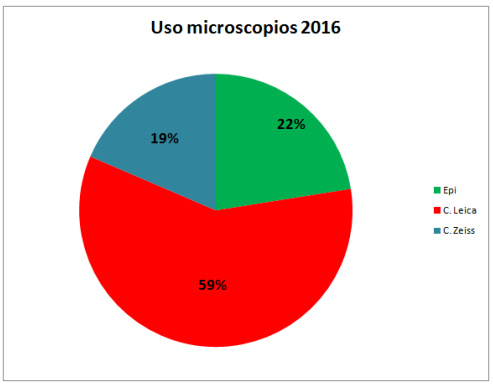

Figure 1: Relative utilization of the microscopes.

The following table shows the distribution (in hours) of the microscopes among the users and is presented in Figure 2.

Microscopes	Internal users (hs)	External users (hs)	Courses (hs)
Leica Confocal	1445	44.5	13.5
Zeiss Confocal	427.5	54.5	0
Olympus	482.5	98.5	3.5

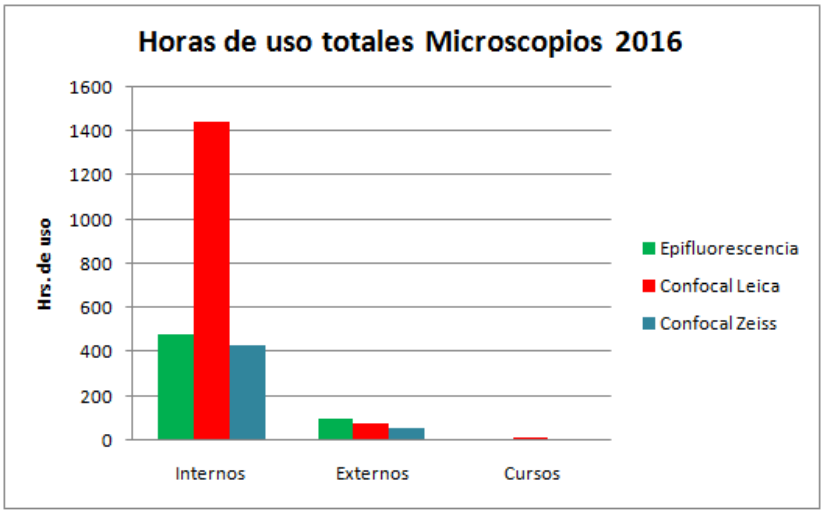

Figure 2: Use of the microscopes by users.

Courses in 2016

- November 17: Laboratorio de Ingeniería Genómica, licenciatura en biotecnología, ORT. Responsable: Alexandra Castro. Confocal.
- August 22: "Biología Molecular Vegetal", FCIEN. Responsable: Sabina Vidal. Confocal.
- April 5-7: Laboratorio de control de calidad, módulo vacunas, ORT. Responsable: Patricia Acuña. Epifluorescencia.
- March 30-31: Laboratorio de biología celular, licenciatura en biotecnología, ORT. Responsable: Mariela Bollati.

Users training

Eleven new internal users were trained in 2016, with a dedication of 39.5 hours for the Olympus microscope, 67 hours for the Leica microscope and 124.5 hours for the Zeiss microscope.

Access charge

TIPO DE USUARIOS	TARIFAS EN USD (POR HORA) Horario central (10 - 18 hs)		TARIFAS EN USD (POR HORA) Horario no central (8 - 10 hs, 18 - 20 hs y fines de semana)		TARIFAS EN USD (OVERNIGHT)	
	MICROSCOPIO EPIFLUERESCENCIA	MICROSCOPIO CONFOCAL	MICROSCOPIO EPIFLUERESCENCIA	MICROSCOPIO CONFOCAL	MICROSCOPIO EPIFLUERESCENCIA	MICROSCOPIO CONFOCAL
EXTERNOS - Institucionaes académicas públicas nacionales o regionales	4	10	*NO SE BRINDARÁ SERVICIO*		*NO SE BRINDARÁ SERVICIO*	
- Instituciones académicas privadas y empresas nacionales o regionales	10	25				
INTERNOS - Unidades Científicas y Cursos organizados por Personal del Instituto	3	6	2	4	15	

PROGRAMS and their respective fundational LABORATORIES and Core Facilities

Animal Health Program

- ImmunoVirology
- Molecular & Structural Microbiology
- Host – Pathogen Interactions*
- Bioinformatics Unit*

Genomics Program

- Bioinformatics Unit*
- Host – Pathogen Interactions*
- Functional Genomics

ProTeMCA Program

- Redox Biology of Tryponosomes
- BioMolecular Simulation
- Transgenic and Experimental Animal Unit*
- Cell Biology Unit

Cancer Program

- Chronic Lymphocytic Leukemia
- Tumor Immunology & Glycobiology
- Immunoregulation & Inflammation*
- Transgenic and Experimental Animal Unit*

INDICYO Program

- Analytical Biochemistry and Proteomics Unit
- Molecular Human Genetics
- Immunoregulation & Inflammation*
- Metabolic Diseases & Aging

* Laboratory or Core Facility belonging to more than one Program
In blue: Core Facilites presented in the previous section

LABORATORIES

- Cell Biology of Neural Development
- Neuroinflammation & Gene Therapy
- Neurodegeneration
- Signal Processing
- Worm Biology

Laboratory of ImmunoVirology

MEMBERS

Otto Pritsch, PhD (Head)
Federico Carrión (Staff Technical Assistant, PhD student)
Sergio Bianchi, PhD (Postdoctoral fellow)
Natalia Olivero, MSc (PhD student)
Andrés Addiego, MD (MSc student)
Martín Fló, PhD (Postdoctoral fellow)
Mariana Margenat, PhD (Postdoctoral fellow)
Natalia Ibañez, MD (MSc student)

RESEARCH

We are interested in studying Enzootic Bovine Leukemia (EBL), an infectious disease caused by an oncogenic member of the genus Deltaretrovirus, the Bovine Leukemia Virus (BLV), affecting more than 60% of dairy cattle in Uruguay. At the moment, no vaccine against BLV is available. In order to gain insight into the degree of genetic variability of BLV in our country we have performed a phylogenetic analysis of Env sequences and revealed the presence of seven BLV genotypes in the South American region (Moratorio et al, 2010). We also performed a detailed molecular analysis of complete bovine leukemia virus genomes isolated from B-cell lymphosarcoma, and compared with other BLV full-length sequences from other clinical manifestations (Moratorio et al, 2013). In parallel we developed a rapid and sensitive real time PCR assay using SYBR green chemistry to detect and quantify BLV proviral DNA from blood obtaining an increased sensitivity over the ELISA and AGID tests (Rama, 2010).

We also initiated the characterization of the main BLV proteins at the molecular and structural levels. In particular, we analyzed the self-assembly process of the purified recombinant BLV capsid (BLV-CA) protein providing the first description of their assembly properties. On the other hand, BLV-CA full-length and separate N- and C-terminal domains were expressed and purified to homogeneity. In order to obtain insights into the detailed molecular structure and self-assembly process of a native, non-engineered retroviral CA, we solved the crystal structure of the mature BLV-CA at 2.75Å resolution, showing a 2D hexagonal lattice displaying both lateral 3-fold and 2-fold interactions between asymmetric CA hexamers. This work was done in collaboration with IPMont Protein Cristallography Unit. (Obal et al, 2015).

In the context of this project we have organized a multidisciplinary group to work on BLV, funded by the Institut Pasteur de Montevideo, the National Institute of Agronomic Research of Uruguay (INIA), the Universidad de la República de Uruguay and the Centre National de la Recherche Scientifique (CNRS, France).

RESEARCH LINES

In general, retroviruses use very similar principles in their biological cycles: assembly and budding of an immature particle, proteolytic capsid maturation, entry through membrane fusion via interactions of the envelope glycoprotein complex with a cellular receptor, reverse transcription of the viral genome, mature capsid uncoating, transport of the pre-integration complex into the nucleus and integration of the provirus.

Our principal research lines are:

Characterization of the biophysical and structural basis of BLV capsid protein

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

To study this issue we have performed a comprehensive characterization of the biophysical properties of the CABLV assembly process. By exploring a wide range of conditions we have characterized the parameters affecting the self-assembly process. Particularly, we focused on analyzing the effect of compounds in near-physiological conditions mimicking the virus intra-particle environment.

Despite the essential role of the retroviral core, its high polymorphism has hindered high-resolution structural analyses. In collaboration with Alejandro Buschiazzo´s lab, we have elucidated the x-ray structure of the native capsid (CA) protein from bovine leukemia virus. CA is organized as hexamers that deviate substantially from sixfold symmetry, yet adjust to make two-dimensional pseudohexagonal arrays that mimic mature retroviral cores. Intra- and interhexameric quasi-equivalent contacts are uncovered, with flexible trimeric lateral contacts among hexamers, yet preserving very similar dimeric interfaces making the lattice. The conformation of each capsid subunit in the hexamer is therefore dictated by long-range interactions, revealing how the hexamers can also assemble into closed core particles, a relevant feature of retrovirus biology. (Obal et al, 2015).

Characterization of the interactions between BLV capsid and host factors

The early steps of infection of delta retroviruses are not well known. Interesting, recent structural studies showed similarities between HIV and BLV CA, suggesting that molecular common partners, such as nucleoporins would participate in viral intracellular traffic. Based on the ability of BLV CA to self-assemble into tubular or planar structures, and by using affinity and mass spectrometry technologies, we aim to identify and characterize the

interactions between BLV CA and cellular host factors involved in this traffic. We will also look for BLV CA partners that could act as innate immune sensors by analyzing cell lysates from permissive and non-permissive cells to BLV infection. Engineered cells generated by Francesca Di Nunzio in IP Paris, will be used to identify new restriction factors or functional viral partners by mass spectrometry. We will also design and purify nanobodies against BLV CA that will be labeled microscopy approaches. Results obtained on BLV will be then transferred on HTLV-1 research to define common and diverse mechanisms adopted by these delta retroviruses when establishing viral infection.

Characterization of the biochemical, structural and immunological features of BLV envelope protein

The BLV env complex plays a crucial role in determining viral infectivity, being responsible for inducing fusion of viral and cellular membranes after recognition of specfic cell-surface receptors.

We have optimized the expression of the soluble env ectodomain in Drosophila S2 cells, with a natural and an altered furin cleavage site. Protein expression and secretion into supernatant was induced by divalent metals, and protein purification was performed by affinity chromatography using a StrepTactin column followed by size exclusion chromatography. Protein quality control was assessed by mass spectrometry. This system should allow the production of sufficient material for crystallization trials, electron cryo-microscopy of isolated trimers, and biophysical studies of the multimeric complex formed by the recombinant proteins.

Env is one of the main targets of the antiviral immune responses, generating both humoral neutralizing antibodies and T-cell specific adaptive immunity. It has been reported for other retrovirus that the presence of an immunosuppressive (isu) peptide in Env glycoprotein structure could be important in their ability to immunomodulate immune responses. We are interested in studying the effect of amino acid modifications in the isu domain in humoral and cellular adaptive responses against challenge with modified Env glycoproteins. This will allow us to understand one of the mechanisms involved in the generation of resistance used by BLV to escape the antiviral immune response. On the other hand, we also expect to identify the modifications that reduce the immunosuppressive activity of this domain and therefore increase their immunogenicity. This result could be useful for the rational design of effective vaccines against this retrovirus.

By using BLV Env purified protein we have also developed a new ELISA test to be used in the diagnosis of enzootic bovine leukosis. In collaboration with ATGen SA we are now generating a new EBL diagnostic kit which will be used in a field experiment with more than 50.000 dairy cows.

Identification of genetic characteristics associates with natural control of EBL

Given the high prevalence of EBL in Uruguay, the strategy to eradicate the disease implemented in Europe and Oceania, is impracticable in our country. An alternative control strategy by using vaccines is promising, but there are still no effective products on the market. Taking into account that recent results show that EBL has a heritable component that reaches 8%, a third strategy to control disease would involve breeding herds by increasing the frequency of genotypes associated with resistance to infection.

We have analyzed in a experimental herd with high prevalence of BLV infection, a group of animals defined as "controllers" of the disease and characterized by low proviral load and low titers of anti-BLV antibodies. Another groups defined as "non-controllers" with high proviral load and high titers of specific antibodies, and “negative” without detectable BLV presence.

By using peripheral blood mononuclear cells (PBMC) from these animals, we are characterizing, in collaboration with Natalia Rego and Hugo Naya from the IPMON Bioinformatic Unit, the transcriptomes representatives of these groups by massive sequencing of mRNA (RNAseq). We expect to identify genes and isoforms differentially expressed in “controller” animals, and interpret these differences in the context of biological processes, metabolic pathways ontologies sub- or overrepresented.

EDUCATION

TRAINING OF STUDENTS

Four students are performing their postgraduation thesis work at the lab

- Federico Carrión, MSc-PhD student PEDECIBA, Biophysical characterization of nanobodies against viral proteins.

- Natalia Olivero, PhD student PEDECIBA, Production and characterization of immunogens against Bovine Leukemia Virus.

- Andrés Addiego, MSc student ProInBio, Development of new immunological methods for the diagnosis of Enzootic Bovine Leukosis.

- Natalia Ibañez, MSc student ProInBio, Identification of intracellular interactors for Bovine Leukemia Virus capsid protein.

GRANTS AND FELLOWSHIPS

- Identificación de marcadores moleculares asociados con la resistencia a la infección por el Virus de la Leucosis Bovina mediante análisis transcriptómico de individuos controladores de la carga viral. Responsable científico: Otto Pritsch. Código del Proyecto: FSA_1_2013_1_12970, Fondo INNOVAGRO, Noviembre 2014 – Noviembre 2016.
- Producción y Caracterización de Inmunógenos contra el Virus de la Leucosis Bovina. Responsable Científico: Otto Pritsch. CSIC I+D 2014, Período Abril 2015 – Abril 2017.
- International Associated Laboratory on Structural Virology. Centre National de la Recherche Scientifique (CNRS) - IPMont-LIA. Period: December 2014 – December 2017. Felix Rey, CNRS URA 3015 Virology, Institut Pasteur, Paris - Otto Pritsch. Institut Pasteur de Montevideo.
- Desarrollo y validación de un kit para el diagnóstico serológico de la Leucosis Enzootica Bovina. Responsable científico: Otto Pritsch. Código del Proyecto: ALI_1_2016_2_129851. Proyecto Alianza Academia – Empresa ANII. Período: 2017 – 2019.
- Postdoctoral fellowship IPMON, Martín Fló, March 2016 - 2018
- Postdoctoral fellowship PEDECIBA (Programa Uruguay retiene), Mariana Margenat March 2017 - 2019
- Doctoral fellowship ANII- POS_NAC_2015_1_555, Natalia Olivero, 2016-2019

PUBLICATIONS

1. New potential eukaryotic substrates of the mycobacterial protein tyrosine phosphatase PtpA: hints of a bacterial modulation of macrophage bioenergetic statemitochondrial functions. Margenat M, Labandera A, Gil M, Carrión F, Purificação M, Razzera G, Portela MM, Obal G, Terenzi H, Pritsch O, Durán R, Ferreira AM, Villarino A. Scientific Reports 5: 8819: DOI: 10.1038, 2015
2. Conformational plasticity of the native retroviral capsid revealed by X ray crystallography. Obal G, Trajtenberg F, Carrión F, Tomé L, Larrieux N, Zhang X, Pritsch O, Buschiazzo A. Science 349(6243):95-98, 2015.

3. Inmunidad innata frente a retrovirus. González L, Ibañez N, Mateus M, Romero K, Pritsch O. Anales de Facultad de Medicina (Univ Repúb Urug) 2(Supl):18-32, 2015.
4. Effective anti-tumor therapy based on a novel antibody drug-conjugate targeting the Tn carbohydrate antigen. Sedlik C, Heitzmann A, Viel S, Ait Sarkouh R, Batisse C, Schimdt F, De La Rochere P, Amzallag N, Osinaga E, Oppezzo P, Pritsch O, Sastre-Garau X, Hubert P, Amigorena S, Piaggio E. Oncoimmunology Apr 22;5(7):e1171434, 2016.
5. Comparative analysis reveals amino acids critical for the anticancer activity of peptide CIGB-552. Astrada S, Gomez Y, Barrera E, Obal G, Pritsch O, Pantano S, Vallespí MG, Bollati-Fogolín M. Journal of Peptide Science 22(11-12): 711-722, 2016.

Molecular and Structural Microbiology

MEMBERS

Alejandro Buschiazzo, PhD (Head of the Lab)
Joaquin Dalla Rizza (Technician) - recruited 2016
Juan Andrés Imelio (MSc student)
Nicole Larrieux (Technician)
Natalia Lisa, PhD (Postdoctoral fellow) - recruited 2016
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)

Frank Lehmann (Technician) - past member, moved to Biozentrum, University of Basel (Switzerland) as Lab Manager (2016)

Scientific interests

We wish to understand how bacteria 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 of bacteria, both pathogenic as well as non-pathogenic, with molecular and structural approaches.

Among pathogenic bacteria, we are interested in learning about *Leptospira*, a genus that comprises 11 species that cause leptospirosis. This is the most widespread zoonosis throughout the globe. In Uruguay it represents one of the main issues for reproductive diseases in cattle, leptospirosis is a cause of abortions, with great impact given that beef/dairy exports are one of the main sources of income for the country. The risk for human infection is proven and mainly linked to rural activities. The global burden of human leptospirosis is very high and increasing (1 million cases and 60,000 deaths per year), its morbidity is higher than visceral leishmaniasis and severe dengue (Costa et al., *PLoS Negl Trop Dis.* 2015 9:e0003898). Yet the lack of efficacious vaccines and a significant knowledge-gap concerning its pathogenicity mechanisms and main virulence factors, classes leptospirosis as a neglected disease. We wish to uncover molecular mechanisms of leptospirosis pathogenicity and environmental/host-adaptation.

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

Leptospira spp., prokaryotic Spirochetes related to *Treponema* -the agent of syphilis- and *Borrelia* -Lyme disease-, are 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 causative agents of leptospirosis) and *L. biflex*a (a saprophytic model, highly related to the pathogenic relatives).

Using *Leptospira* we are also studying the structure of the motility apparatus of Spirochetes, which is quite unique in many ways: it has a periplasmic filament and not extracellular, with an unusually complex protein composition, rather than the flagellin-only type of filament as found in *Salmonella* and other extensively studied organisms. In the long-term, our aim is to understand the function and regulation of spirochetal flagella.

We also continue our work with *Bacillus subtilis* (Firmicutes), a well-established model of Gram+ bacteria, to answer questions about two-component system-mediated signaling and cell regulation.

A more recent line of research is done in collaboration with the Pritsch lab at the Inst Pasteur Montevideo. We want to unveil 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.

Apart from our own main lines of research, we carry out 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 approaches fuse 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 TCSs 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; Saita et al., *Mol Microbiol* 2015, 98:258-71; Trajtenberg et al., *eLife* 2016, 5:e21422) and *Leptospira* (Morero et al., *Mol Microbiol* 2014, 94:340-52; Fouts et al., *PLoS Negl Trop Dis.* 2016, 10:e0004403; Hadhikarla et al., 2016 submitted). 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-Söldner (Institute of Cell Dynamics and Imaging, Univ of Münster, Germany) we are also integrating the molecular details of single protein components (histidine kinases, 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 Wunder et al., *Mol Microbiol* 2016, 101:457-70), has resulted in solving two new crystal structures revealing novel 3D folds (San Martin et al., 2016 submitted).

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 methods already available in the country, as well as now molecular techniques that are being put forward by us at Inst Pasteur Montevideo). A biobank of native *Leptospira* strains is thus being built, having isolated >25 strains during the first 1.5 years of this 3-years project (Zarantonelli et al., 2016 manuscript in preparation). These strains have been unequivocally identified (three different species have so far been pinpointed), and typed to the levels of serogroup and serovar. Such data shall be instrumental in guiding formulations of more efficacious bacterin-based vaccines, the ones available in the market have been questioned, and so far do not contain several of the serovars that we have isolated in the field. Our team is also using these strains and the bank of sera, to characterize vaccine candidates, which could eventually be optimized through protein engineering.

In 2015 we submitted a proposal through an open call at Inst Pasteur Paris, to create a Joint International Unit. 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 “Integrative Microbiology of Zoonotic Agents” (IMiZA) was selected after scientific evaluation, focused on leptospirosis, and eventually launched at the end of 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 the protein p24 from the Bovine Leukemia Virus (BLV), we have been able to solve its crystal structure, which corresponds to the mature, native form of the protein. We have thus uncovered a plastic architecture that is consistent with the assembled core particle of retroviruses as observed by cryo-electron microscopy, turning our findings into a first as far as native retroviral capsid structures are concerned (Obal et al., *Science* 2015, 349:95-8). 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.

In 2016 we started a collaboration within the Ebola Task Force coordinated in Inst Pasteur Paris, joining efforts with the Pritsch lab to recombinantly produce and characterize the secreted non-structural glycoprotein from Ebola virus (EBOV sGP). A graduate student from the Pritsch lab, Federico Carrion, is pushing forward this aim, which made nice progress in 2016 obtaining good quantities of highly pure sGP, from transformed Schneider 2 cells (*Drosophila*). Crystallographic studies are currently underway.

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. We have recently solved the 3D structure of the long-acyl chain regulator FasR, in acyl-bound and apo states (Lara et al., 2016 ms in prep).

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. We co-supervise a PhD student, Marcos Nieves, doing his graduate studies in Germany and Uruguay.

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, by protein cross-linking and mass spectrometry. Also in collaboration with Drs Paulo Carvalho (Fiocruz, Curitiba) and Fabio Gozzo (Univ de Campinas) in Brazil.

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. 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. *Both authors equally contributed to this work ; † Co-corresponding authors.
2. 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.

3. 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.

4. East A, **Mechaly AE**, Huysmans GH, Bernarde C, Tello-Manigne D, Nadeau N, Pugsley AP, **Buschiazzo A**, Alzari PM, Bond PJ, Francetic O. Structural basis of pullulanase membrane binding and secretion revealed by X-ray crystallography, molecular dynamics and biochemical analysis. (2016) *Structure* **24**:92-104.

5. Fouts DE, Matthias MA, Adhikarla H, Adler B, Amorim-Santos L, Berg DE, Bulach D, **Buschiazzo A**, Chang YF, Galloway RL, Haake DA, Haft DH, Hartskeerl R, Ko AI, Levett PN, Matsunaga J, **Mechaly AE**, Monk JM, Nascimento AL, Nelson KE, Palsson B, Peacock SJ, Picardeau M, Ricaldi JN, Thaipandungpanit J, Wunder EA Jr, Yang XF, Zhang JJ, Vinetz JM. What makes a bacterial species pathogenic?: comparative genomic analysis of the genus *Leptospira*. (2016) *PLoS Negl Trop Dis* **10**:e0004403.

6. Meyer PA, Socias S, Key J, Ransey E, Tjon EC, **Buschiazzo A**, Lei M, Botka C, Withrow J, Neau D, Rajashankar K, Anderson KS, Baxter RH, Blacklow SC, Boggon TJ, Bonvin AM, Borek D, Brett TJ, Caflisch A, Chang CI, Chazin WJ, Corbett KD, Cosgrove MS, Crosson S, Dhe-Paganon S, Di Cera E, Drennan CL, Eck MJ, Eichman BF, Fan QR, Ferré-D'Amaré AR, Fromme JC, Garcia KC, Gaudet R, Gong P, Harrison SC, Heldwein EE, Jia Z, Keenan RJ, Kruse AC, Kvansakul M, McLellan JS, Modis Y, Nam Y, Otwinowski Z, Pai EF, Pereira PJ, Petosa C, Raman CS, Rapoport TA, Roll-Mecak A, Rosen MK, Rudenko G, Schlessinger J, Schwartz TU, Shamoo Y, Sondermann H, Tao YJ, Tolia NH, Tsodikov OV, Westover KD, Wu H, Foster I, Fraser JS, Maia FR, Gonen T, Kirchhausen T, Diederichs K, Crosas M, Sliz P. Data publication with the structural biology data grid supports live analysis. (2016) *Nat Commun* **7**:10882.

7. Wunder EA Jr, Figueira CP, Benaroudj N, Hu B, Tong BA, **Trajtenberg F**, Liu J, Reis MG, Charon NW, **Buschiazzo A**, Picardeau M, Ko AI. A novel flagellar sheath protein, FcpA, determines filament coiling, translational motility and virulence for the *Leptospira* spirochete. (2016) *Mol Microbiol* **101**:457-70.

8. Morán-Barrio J*, **Lisa MN***, **Larrieux N**, Drusin SI, Viale AM, Moreno DM, **Buschiazzo A†**, Vila AJ†. Crystal Structure of the Metallo-β-Lactamase GOB in the Periplasmic Dizinc Form Reveals an Unusual Metal Site. (2016) *Antimicrob Agents Chemother* **60**:6013-22. *Both authors equally contributed to this work ; † Co-corresponding authors.

9. **Trajtenberg F, Imelio JA,** Machado MR, **Larrieux N,** Marti MA, Obal G, **Mechaly AE, Buschiazzo A**. 1. Regulation of signaling directionality revealed by 3D snapshots of a kinase:regulator complex in action. (2016) *eLife* **5**:e21422.

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 (Ministry of Livestock, Agriculture and Fishery, DILAVE) and Dr M Picardeau (Biology of Spirochetes Unit, Inst Pasteur)

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 (Ministry of Livestock, Agriculture and Fishery, 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).

5. ***"Native strains biobanking of zoonotic agents affecting Uruguayan cattle, for R&D within the Uruguayan veterinarian industry".***

Ministry of Industry, Energy and Mining (MIEM, Uruguay), Industrial Funds #2016-8-2-0002671.

2016-2017

Role: Principal Investigator.

Partners: Vet Alejandra Suanes and Rodolfo Rivero (Ministry of Livestock, Agriculture and Fishery, DILAVE).

OTHER ACTIVITIES

NETWORKING, SCIENTIFIC MEETINGS, PRIZES AND HONORS

1. A Buschiazzo earned a promotion to the position of Associate Professor (Directeur de Recherche), Institut Pasteur, Paris 2015. A position that effectively started Jan 2016.
2. The co-authors of the paper published in Science 2015, were awarded two prizes in Uruguay:
    i. Premios de la Cultura Uruguaya Morosoli 2015 Morosoli Institucional (delivered by the Fundación Lolita Rubial, Uruguay), for the contribution to the cultural Uruguay in Science and Technology, to the team of researchers (BLV) Alejandro Buschiazzo (Institut Pasteur de Montevideo - Medical School, UdelaR).
    ii. Premio Nacional Ciudadano de Oro (delivered by the Centro Latinoamericano de Desarrollo - CELADE, Uruguay), in consideration of the merits of your outstanding work, service quality and your contribution to the sustained process of national development.
3. A dedicated prize was awarded by the Institut Pasteur de Montevideo to **F Trajtenberg** and G Obal, as first authors of the paper published in Science 2015, in occasion of the scientific annual retreat, Institut Pasteur de Montevideo.
4. Attendance to scientific meetings and conferences :
5. May 2015 - “Hands-on training course: Bioinformatics Applied to Vaccinology” - Univ. de Sao Paulo (Sao Paulo, Brazil), F San Martin.
6. 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.
7. 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.
8. Sep 2015 - 22nd meeting Associação Brasileira de Cristalografia - ABCr and 1st Latin American Crystallographic Association congress (Sao Paulo, Brazil). A Buschiazzo - invited speaker.
9. Oct 2015 - Scientific Meeting of the Institut Pasteur International Network, Institut Pasteur (Paris, France). A Buschiazzo - invited speaker
10. Oct 2015 - Meeting of the “Sociedad de Bioquímica y Biología Molecular” SBBM - (Montevideo, Uruguay), J Imelio, oral presentation.

11. 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.

12. Nov 2015 - 11 Annual Meeting of the Asociacion Argentina de Cristalografia - AACr (La Plata, Argentina). A Buschiazzo, plenary lecture.

13. Jan 206 - Gordon Research Conference, Biology of Spirochetes (Ventura, CA, USA). A Buschiazzo, invited speaker (“An extra twist in *Leptospira* flagella: filament structure, flagellin, and beyond”). F Trajtenberg and AE Mechaly, presented posters.

14. Jan 2016 - Gordon Research Conference, Sensory Transduction in Microorganisms (Ventura, CA, USA). A Buschiazzo, AE MEchaly, presented posters.

15. April 2016 - Macromolecular Crystallography School 2016 "From data processing to structure refinement and beyond" http://www.ifsc.usp.br/mx2016/ Dr Felipe Trajtenberg, invited speaker.

16. April 2016 - Microbiology Dept Retreat, Institut Pasteur (Paris). A Buschiazzo, invited speaker (Delivered lecture: "Two component systems signaling: from molecular structure to physiology...”).

17. April 2016 - Program of Seminars and Journal Club at Institut Pasteur de Montevideo. A Buschiazzo, plenary lecture “Leptospira flagella are essential for pathogenesis: will structure help us understand cellular function?”

18. June 2016 - Kick-off meeting of the International Joint Unit “Integrative Microbiology of Zoonotic Agents”. Organized at the Institut Pasteur de Montevideo, we hosted Drs Mathieu Picardeau, Pascale Bourhy and Nadja Benaroudj, from our partner Biology of Spirochetes Unit - IPasteur Paris. Three days full-time discussions and presentations, with attendance of all the Lab of Mol & Structural Microbiology members.

19. June 2016 - National meeting of the INIA Platform of Animal Health “Advances in Animal Health Research”. A Buschiazzo, invited plenary lecturer: "Creation and characterization of a biobank of *Leptospira spp.* strains isolated from bovine leptospirosis cases in Uruguay”. L Zarantonelli and C Nieves attended.

20. July 2016 - Program of Seminars and Journal Club, Dept Microbiology at Instituto de Investigaciones Biologicas Clemente Estable (Montevideo, Uruguay). A Buschiazzo invited plenary lecturer.

21. Aug 2016 - Organization of the 2nd Meeting of the Uruguayan Crystallography Network, Institut Pasteur de Montevideo. Dr F Trajtenberg, member of the local organizing committee. A Buschiazzo, invited plenary lecturer: "Integrative Structural Biology: new challenges and opportunities for Macromolecular Crystallography ". Posters presented by N Lisa, AE Mechaly, F Trajtenberg, JA Imelio (best poster prize winner) and F San Martin.

22. Aug 2016 - BCA/CCP4 Protein Crystallography Summer School. Attendee: F San Martin (obtained full scholarship from CCP4). Diamond Light Source, Oxfordshire, UK.

23. Aug 2016 - 7th Brazil School for Single Particle Cryo-Electron Microscopy. Attendee: AE Mechaly. Supported with CeBEM funds. Laboratorio Nacional de Nanotecnologia, CNPEM, Campinas, Brazil.

24. Oct 2016 - First Panafrican Conference of Crystallography, IUCr. A Buschiazzo, Invited speaker (title "Macromolecular Crystallography within Integrative Landscapes: new challenges and opportunities"). Dschang, Camerun.

25. Nov 2016 - V Latin American Protein Society meeting, A Buschiazzo invited plenary lecturer ("The times they are a-changin': can protein structure and flexibility be seen, together?"). Rio de Janeiro, Brazil.

26. Nov 2016- Argentinian Society of Biochemistry meeting (SAIB). JA Imelio (selected for oral presentation); N Lisa, poster. Cordoba, Argentina.

27. Nov 2016 - Institut Pasteur International Network Scientific Symposium. Poster presentation of advances in the Ebola task force project "**Recombinant EBOV sGP: A Potential Biomarker for Fast Diagnosis of Ebola Virus Infection"** by *F. Carrion*, N. Olivero, N. Larrieux, S. Bianchi, M. Flo, A. Addiego, F. Trajtenberg, O. Pritsch & A. Buschiazzo.

28. Nov 2016 - FAPESP Week Montevideo 2016, scientific meeting co-organized by FAPESP agency (Brazil) and Universidad de la Republica (Uruguay). A Buschiazzo, invited speaker (Title "How do bacteria perceive signals in their environment?"). Montevideo, Uruguay.

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)
Luisa Berná (Postdoctoral Researcher- INNOVA II)
María Eugenia Francia (Calmette-Yersin/RIIP Postdoctoral Position)
Gonzalo Greif, PhD
Ma. Laura Chiribao, PhD
Paula Faral (PhD Student)
Gabriela Libisch (PhD Student)
Andrés Cabrera (PhD Student)
Cecilia Portela (Technician, Facultad de Ciencias)
Florencia Díaz (MSc student)
Fernanda Matto (MSc student)
Moira Lasserre (MSc student)
Lucía López (MSc student)

RESEARCH

The Host Pathogen Interactions Laboratory is the research branch of the Unit of Molecular Biology. It is focused on human and animal pathogens, in particular the protozoan parasites *T. cruzi*, *T. vivax* and *Leishmania*, and the prokaryote *Mycobacterium*, with emphasis in genomics and functional genomics of those pathogens, and their interactions with hosts.

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

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

2016

Zago MP, Hosakote YM, Koo SJ, Dhiman M, Piñeyro MD, Parodi-Talice A, Basombrio MA, Robello C, Garg NJ. TcI Isolates of Trypanosoma cruzi Exploit the Antioxidant Network for Enhanced Intracellular Survival in Macrophages and Virulence in Mice. Infect Immun. 2016 May 24;84(6):1842-56.

García EP, Tiscornia I, Libisch G, Trajtenberg F, Bollati-Fogolín M, Rodríguez E, Noya V, Chiale C, Brossard N, Robello C, Santiñaque F, Folle G, Osinaga E, Freire T. MUC5B silencing reduces chemo-resistance of MCF-7 breast tumor cells and impairs maturation of dendritic cells. Int J Oncol. 2016 May;48(5):2113-23.

Ubillos L, Freire T, Berriel E, Chiribao ML, Chiale C, Festari MF, Medeiros A, Mazal D, Rondán M, Bollati-Fogolín M, Rabinovich GA, Robello C, Osinaga E. Trypanosoma cruzi extracts elicit protective immune response against chemically induced colon and mammary cancers. Int J Cancer. 2016 Apr 1;138(7):1719-31.

Pizzo C, Faral-Tello P, Yaluff G, Serna E, Torres S, Vera N, Saiz C, Robello C, Mahler G. New approach towards the synthesis of selenosemicarbazones, useful compounds for Chagas' disease. Eur J Med Chem. 2016 Feb 15;109:107-13.

2015

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Arias DG, Piñeyro MD, Iglesias AA, Guerrero SA, Robello C. Molecular characterization and interactome analysis of Trypanosoma cruzi tryparedoxin II. J Proteomics. 2015 Apr 29;120:95-104.

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Palacios F, Abreu C, Prieto D, Morande P, Ruiz S, Fernández-Calero T, Naya H, Libisch G, Robello C, Landoni AI, Gabus R, Dighiero G, Oppezzo P. Activation of the PI3K/AKT pathway by microRNA-22 results in CLL B-cell proliferation.Leukemia. 2015 Jan;29(1):115-25.

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.

Functional Genomics

MEMBERS

Alfonso Cayota, MD, PhD (Head)
Juan Pablo Tosar PhD (Post-doc)
Julia Sanguinetti MSc (Technical Assistant)
Ma. Rosa García (Researcher)
Braulio Bonilla MSc (PhD. Student)
Fabiana Gambaro (MSc Student)

RESEARCH

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

In the last years, our main focus of research has been centered on the biology of tRNA-derived small RNAs in the regulation of gene expression with special 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.
3. “Implementation of genetic tests for breast cancer risk by deep sequencing of BRCA1 and BRCA2 genes in Uruguayan women” National Agency for Research and Innovation (ANII) Amount Granted USD 40.000 – Period 2016-2018
4. “tRNA-derived small RNAs as mediators of survival and growth signals. Granted to Alfonso Cayota by CSIC, Universidad de la República. Amount Granted USD 45.000 – Period 2017-2019

PUBLICATIONS

1- 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,

2- Doldan, X., Fagundez, P., Cayota, A., Laiz, J. and Tosar, J.P. (2016) Electrochemical Sandwich Immunosensor for Determination of Exosomes Based on Surface Marker-Mediated Signal Amplification. Analytical chemistry 88: 10466-10473.

3- Tosar, J.P., Gambaro, F., Sanguinetti, J., Bonilla, B., Witwer, K.W. and Cayota, A. (2015) Assessment of small RNA sorting into different extracellular fractions revealed by high-throughput sequencing of breast cell lines. Nucleic acids research 43: 5601-5616.

Other activities

PARTICIPATION IN MULTICENTRIC CANCER PROGRAMS

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

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BioMolecular Simulation

MEMBERS

Sergio Pantano, PhD (Head)
Matías Machado, PhD (Staff Member)
Exequiel Barrera, PhD (PosDoc CONICET-IP)
Florencia Klein, Lic. (MSc. student)
Steffano Silva (Undergraduate Student)
Astrid Brandner, MSc (Staff Member) until 2015
Gaston Hugo (Staff Member) until 2015

RESEARCH

The Group of BioMolecular Simulations develops and applies cutting-edge modeling and simulation methods to study problems of biomedical relevance. Within the line of methodological work, we undertook the development of a set of computational tools to analyze and visualize the results of Coarse-Grained (CG) simulations. This led to the creation of a software package called SirahTool, which was published in Bioinformatics (see paper by Machado & Pantano in PUBLICATIONS). Additionally, we finished the parameterization calcium and phospholipids started in 2015. Application of these parameters to concrete biomedical problems resulted in the publication of two papers in collaboration with external and intramural colleagues. In the first case, we applied CG simulations to provide a molecular level rational to the reduced binding of an Ataxia related mutation in the PMCA3 Ca^{2+} pump (see paper by Cali et al in PUBLICATIONS). In the second case, and in collaboration with the Cell Biology Unit, we took profit of the availability of interaction parameters for zwitterionic phospholipid to describe the mechanism of permeation of cell penetrating peptide (see paper by Astrada et al in PUBLICATIONS). These parameters, along with all the software developed in the group are freely available from our web page (www.sirahff.com), which received nearly 1.500 visitors/month in 2016.

We continued appling these computational tools to the development and optimization of a FRET sensor for cyclic nucleotides. A paper has been recently accepted in Nature Communications describing a functioning of a novel architecture of a cAMP sensor targetable to virtually any subcellular compartment. This line of research is carried out in collaboration M. Zaccolo (Oxford), but also in the framework of a local project funded by ANII. The project of development of fluorescent sensors is also integrated within ProTeMCA (Programa de Tecnologia Molecular, Celular y Animal). As a result of this intramural cooperation we have computationally developed a shifted RedOx sensor, which is currently being characterized in vitro and in cells in collaboration with the groups of Drs. Comini and Bollati.

We also made progresses in the study of the gating mechanism of Connexin hemichannels. Using a series of simulations techniques we were able to describe what we believe corresponds to the voltage gating of Connexins. In particular, using CG simulations we predicted and experimentally confirmed a point mutation on a transmembrane helix that inhibits the opening of the channel upon a decrease in the extracellular calcium levels. Furthermore, the speed up granted by the CG approach allowed us to reproduce computationally current traces alike those coming from electrophysiology experiments in a completely unbiased manner. A manuscript is in preparation in collaboration with Drs. Francesco Zonta (ShanghaiTech University, China) and Fabio Mammano (University of Padua, Italy).

Finally, we started collaborations with the company LAGASH to develop software for 3D visualization using mixed reality technology called Hololens, recently launched by Microsoft. A prototype for visualization of proteins is already available. Currently, we are jointly exploring alternatives to find financial support to complete this project and extend it to 3D visualization of biomedical images (confocal microscopy and medical imaging).

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.
5. Revisiting the human polypeptide GalNAc-T1 and T13 paralogs MF Festari, F Trajtenberg, N Berois, S Pantano, L Revoredo, Y Kong, et al Glycobiology; 2016.
6. Comparative analysis reveals amino acids critical for anticancer activity of peptide CIGB-552. S Astrada, Y Gomez, E Barrera, G Obal, O Pritsch, S Pantano, et al. Journal of Peptide Science 22 (11-12), 711-722; 2016.
7. SIRAH Tools: mapping, backmapping and visualization of coarse-grained models. M Machado, S Pantano. Bioinformatics 32(10):1568-70; 2016.
8. F. Trajtenberg; J. A. Imelio; M. Machado; N. Larrieux; M. A. Marti; G. Obal; et al. Regulation of signaling directionality revealed by 3D snapshots of a kinase: regulator complex in action. eLife, 2016,5:e21422.

ACADEMIC TRAINING

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

On March 2016 Florencia Klein joined the group as MSc student in Chemistry in our group, directed by Sergio Pantano and Matias Machado.

On June 2016 Florencia Sardi started her PhD in Biotechnology within the framework of an ANII project and ProTeMCA. She is currently being supervised by Drs. M. Comini and S. Pantano.

Exequiel Frigini continues his PhD started on 2015 at the Department of Physics of the University of San Luis, Argentina. He is currently under the direction of Dr. R. Porasso (San Luis, Arg.) and Dr. Pantano.

Internships: During 2016 we received the following interns for periods between 3 and 6 months:

Benoiˆt Malye (Nice, France); Carlos Cruz (Fiocruz, Pernambuco, Br); Fabian Gonzalez (Univ. Talca, Chile); Gabriel J. Olguín-Orellana (Univ. Talca, Chile).

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/

During 2016 our group organized:

The 42nd edition of the QUITEL (Congreso de Quimica Teorica de Expresion Latina). This conference was held at the Radisson Hotel from Nov. 19 to 25 2016 and received nearly 200 participants from 21 countries. Further information is available at http://quitel2016.org.uy

Practical course: "Enhanced sampling techniques in classical MD simulations". This course was organized as a complementary activity to the QUITEL at the IP Montevideo from Nov. 20 to 25 2016. Theoretical/practical lectures were delivered by V. Leone and F. Marinelli (NIH, USA) and IP. It received 20 students from Argentina, Brazil, Chile, Colombia, Spain and Uruguay. This activity was sponsored by FOCEM.

"Molecular dynamics simulations with SIRAH force field". M. Machado and S. Pantano, at the III CCES Workshop & SAIMS, Universidade Estadual de Campinas

"Introduction to multiscale molecular dynamics simulations". M. Machado, at the School on Bioinformatics Engineering, University of Talca, Chile.

OTHER:

a) Members of the group delivered the following seminars as invited speakers:

-*Coarse Grained Approaches and the Sirah force field*. S. Pantano, Summer Course "GPU Molecular Dynamics Simulation to Accelerate The Drugs Design" at Universidad de Concepcion, Chile.

-*Completando el campo de fuerzas SIRAH: Fosfolípidos e iones divalentes para electrofisiología computacional en canales de Conexinas*. S. Pantano, Workshop Latinoamericano de Modelado Molecular & Simulacion Computacional, Bs. As. Argentina.

-*Design of a universal FRET-tag reveals cAMP nano-domains at β-adrenergic cascades*. S. Pantano, XXXIX SBBM, Puerto Varas, Chile.

-Affordable viral particle's simulations on desktop computers using SIRAH force field. M. Machado, XLV Annual Meeting of SAB, Tucuman, Arg.

-Parameterization of phospholipids at a coarse grain level, modeling lipid membranes with SIRAH force field. E. Barrera, 42nd Quitel Conference. Montevideo, Uy.

-Holographic visualization of macromolecular structures. S. Pantano. 42nd Quitel Conference. Montevideo, Uy.

-A universal FRET-tag reveals cAMP nano-domains at the β-adrenergic cascade. S. Pantano, BBRC Symposium, Toronto, Canada.

GRANTS

"Design of biosensors for simultaneous detection of cAMP and redox signalling: from in silico to in vivo and back". Fondo Maria Viñas ANII.

"Diseño Y Producción De Nuevas Variantes De La Hormona Foliculo Estimulante (Fsh) Para Su Empleo En Especies De Interés Productivo". Project "Alianza ANII".

"Use and improvement of the SIRAH force field for Coarse-Grained Simulations applied to intermolecular interactions of proteins". Programa De Cooperación Internacional Proyectos De Apoyo A La Formación De Redes Internacionales Entre Centros De Investigación Convocatoria 2015. Conicyt-Chile. Project 224841.

"Caracterização in silico de alvos de medicamentos para Zika e Dengue". Supercomputer time grant (8.600.000 hours/node) at Santos Dumont (http://sdumont.lncc.br).

Florencia Klein received a MSc fellowship by ANII.

Florencia Sardi received a PhD fellowship by ANII.

Other activities of the group leader:

S. Pantano has been appointed as Executive Editor of the journal Biochemical and Biophysical Research Communications (BBRC).

S. Pantano has participated as external examiner in the following postdoctoral theses:

Raúl Estaban Ithuralde, Facultad de Ciencias Exactas, UBA, Arg.;

Fabiana San Martin, evaluation of MSc. thesis, PEDECIBA, Biologia. ;

Carlos Navarro Retamal, PhD thesis, Univ. Talca, Chile.

Diego Carvalho MSc. thesis PEDECIBA Bioinformatics.

Redox Biology of Trypanosomes

MEMBERS

Marcelo Comini, PhD (Principal Investigator)
Andrea Medeiros, PhD (Postdoc)
Mariana Bonilla, PhD (Postdoc)
Bruno Manta, PhD (Postdoc, until October 2015)
Cecilia Ortíz (PhD student)
Diego Benitez, PhD
Florencia Sardi (PhD Student)
Jaime Franco (MSc Student)
Sofía Zardo (BSc Student, until May 2016)
Karin Grunberg (BSc Student, until December 2016)

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.

FUNDAMENTAL ASPECTS OF TRYPANOTHIONE METABOLISM: SYNTHESIS, REDUCTION AND UTILIZATION

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

MONITORING INTRACELLULAR REDOX CHANGES WITH NOVEL REDOX BIOSENSORS

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

differentiation, cell cycle, apoptosis and metabolic dysfunction. The reporter cell lines are also employed in phenotypic drug-screening campaigns.

EARLY PHASE DRUG DISCOVERY PROJECTS

We apply target- and phenotypic-based approaches to screen synthetic and natural compounds against the enzyme responsible of trypanothione biosynthesis in different trypanosomatid species and infective forms of the pathogens. Compound mode of action at cellular and enzyme level is studied to drive drug optimization. To conduct these studies our laboratory relies on an important network of local and international groups working on (medicinal) chemistry. Since 2011, our group was invited to join two European Consortium COST devoted to drug discovery against neglected diseases (Action CM0801: "New Drugs for Neglected Diseases" and Action CM1307: "Targeted chemotherapy towards diseases caused by endoparasites") and became member of "The Research Network Natural Products against Neglected Diseases" (ResNetNPND).

EDUCATION-COURSES

2015

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).

2016

- Lecture at "Curso Teórico-Práctico de Animales de Laboratorio", April 4-12, 2016, Montevideo, Uruguay.
- Lectures at "Curso de Educación Permamente "Leishmania en Uruguay: Aspectos Moleculares y Epidemiológicos", August 4, 2016, Montevideo, Uruguay.
- Lecture at "Simposio Internacional sobre Temas de Actualidad en Interacción Huésped-Patógeno", August 10-12, 2016, Asunción, Paraguay
- Lectures at "International Workshop on Drug Design and Neglected Tropical Diseases", November 1-8, 2016, La Plata, Argentina.
- Members of the lab. participated as main authors or co-authors of different studies presented in several international meetings, congresses and courses: "VI Simposio Internacional de Química", Santa María, Cuba (June 7-10, 2016); "Brazilian Society for Biochemistry and Molecular Biology", Natal, Brazil (June 18-22, 2016); "Gordon Research Conference: Thiol-Redox Regulation and Signaling", Vermont, USA (Aug 6-7, 2016); "Conference on Antiparasitic Chemotherapy for Human and Veterinary Use", Madrid-Spain (Oct 24-26, 2016); "IX IberoAmerican Congress of Biophysics", Tucumán, Argentina (Nov 23-25, 2016); "Brazilian Symposium on Medicinal Chemistry", Buzios-Brazil (Nov 27-30, 2016); "IV Encuentro Nacional de Química", Montevideo-Uruguay (4-6/11/2015); "Scientific Symposium of Institut Pasteur International Network" Paris, France (Nov 29 – Dec 2, 2016).
- The PhD student Karla Pérez Treviño from the Universidad Autónoma de Nuevo León, México, and Dr. Ma. Laura Sbaraglini Universidad Nacional de la Plata, Argentina performed research traineeship in our laboratory for 3- (Aug 15th – Sep 3rd, 2016) and 8-weeks (May 2nd – June 17th, 2016), respectively.

GRANTS/AWARDS

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).
5. Best ACIP-project poster at the "Scientific Symposium of Institut Pasteur International Network" Paris, France (Nov 29 – Dec 2, 2016).

PUBLICATIONS

1. 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.
2. 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.
3. 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.
4. 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.
5. 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.
6. 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.
7. Comini MA. Measurement and meaning of cellular thiol: disulphide redox status. Free Radic. Res. 2015.
8. 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.

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10. Orban OCF, Korn RS, Benítez D, Medeiros A, Preu L, Loaëc N, Meijer L, Koch O, Kunick C and Comini MA (2016) 5-Substituted 3-chlorokenpaullone derivatives are potent inhibitors of *Trypanosoma brucei* bloodstream forms. Bioorg Med Chem. 24(16):3790-800.

11. Benítez D, Medeiros A, Fiestas L, Panozzo-Zenere EA, Maiwald F, Prousis KC, Roussaki M, Calogeropoulou T, Detsi A, Jaeger T, Šarlauskas J, Peterlin Mašič L, Kunick C, Labadie GR, Flohé L, Comini MA. (2016) Identification of Novel Chemical Scaffolds Inhibiting Trypanothione Synthetase from Pathogenic Trypanosomatids. PLoS Negl Trop Dis. 10(4): e0004617.

12. Ortiz C, Moraca F, Medeiros A, Botta M, Hamilton N, Comini M. (2016) Binding Mode and Selectivity of Steroids towards Glucose-6-phosphate Dehydrogenase from the Pathogen Trypanosoma cruzi. Molecules 21(3), 368-382.

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14. Alberca LN, Sbaraglini ML, Balcazar D, Fraccaroli L, Carrillo C, Medeiros A, Benitez D, Comini M, Talevi A. (2016) Discovery of novel polyamine analogs with anti-protozoal activity by computer guided drug repositioning. J Comput Aided Mol Des. 30(4): 305-321.

15. Comini MA (2016) Measurement and meaning of cellular thiol:disufhide redox status. Free Radic Res. 50(2): 246-71.

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 Laboratory on Chronic Lymphocytic Leukemia).

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&Lymphoma 2015*). Microenvironment signals are not only provided by cell to cell interactions but also by different molecules as soluble factors or exosomes which play a key role in tumor-host crosstalk. Our last work in this research line investigates the proteomic profiling of plasma-derived exosomes during CLL evolution, and has resulted in a manuscript entitled "S100-A9 protein in exosomes from chronic lymphocytic leukemia cells promotes NF- B activity during disease progression", which has been recently submitted to Blood.

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*). Recently, the first affitins "made in Uruguay" recognizing the specific CLL tumor antigen ROR-1 have been developed in our laboratory. Functional and biological tests on primary CLL cells are being carried out.

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 IberoAmerican CLL meeting (1st IBAM-CLL) was carried out in November 15th, 2013 in Punta del Este, receiving 285 participants. (http://www.clliberoamericangroup.com), and the second IBAM-CLL was held on the 23rd of September, 2016 in San Pablo, Brasil.

Finally, the most important advance at the regional level was the approval to develop the first clinical trial in CLL involving Argentine, Brazil and Uruguay. This clinical trial was presented by our group in Uruguay in November 2014 and was approved in December 2016. This is the first project started by the LAG-CLL and allows us to treat, for the first time, those

CLL patients that have been become refractory to the standard treatment. This trial aims to evaluate the new drug Ibrutinib (Janssen) plus high doses of Methylprednisolone. At this time Ibrutinib emerges as the new drug to treat refractory CLL patients. However, in our region the economical reason is the main problem that should be solved to extend this treatment to most of refractory patients. The clinical trial starts with patient enrollment in Abril 2017 and it will be open until Abril 2019.

Combined with this trial the global scientific committee of Janssen also approves us a basic research project that aims to understand the molecular basis of the Ibrutinib cure in CLL, characterizing the effects of this drug in the proliferative and quiescent CLL subsets described by our group.

EDUCATION-COURSES-CONGRESS

HUMAN RESOURCES

- On March 2014 Daniel Prieto started his PhD program.
- On March 2015 Noé Seija oined our group as MSc student.

COURSES AND CONFERENCES

As organizer:

1. Second IberoAmerican meeting Chronic Lymphocytic Leukemia. 23-26 September 2016, San Pablo, Brazil. Participants: 382
2. 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

As invited speakers:

1. XVI INTERNATIONAL WORKSHOP ON CHRONIC LYMPHOCYTIC LEUKEMIA. 2015.Proteomic characterization of CLL exosomes during disease evolution, Australia;

2. JORNADA LATINOAMERICANA DE LA SOCIEDAD ARGENTINA DE HEMATOLOGÍA. 2016. Leucemia Linfática Crónica: Desde el conocimiento molecular a la vida real; Sociedad Argentina de Hematología. Argentina

3. XIV CONGRESO URUGUAYO DE HEMATOLOGIA 2016.El microambiente tumoral y la progresión leucémica. Preguntas claves. Sociedad Uruguaya de Hematología. Colonia del Sacramento. Uruguay

4. VII SIMPÓSIO IBEROAMERICANO DE PLANTAS MEDICINAIS III SIMPÓSIO IBEROAMERICANO DE INVESTIGAÇÃO EM CÂNCER UNIVALI. 2016. Preguntas claves en la biologia de la Leucemia Linfoide Crónica ITAJAÍ. Brazil

As chairman:

1. Neoplasias Linfoides: Chronic Lymphocytic Leukemia , 2016 Progresos en Oncología Molecular y su impacto a nivel clínico. Uruguay. Organizado por ROCHE S.A.

2. JORNADA LATINOAMERICANA DE LA SOCIEDAD ARGENTINA DE HEMATOLOGÍA. 2016. Mesa de Leucemia Linfática Crónica: Desde el conocimiento molecular a la vida real; Sociedad Argentina de Hematología. Argentina

3. XIV CONGRESO URUGUAYO DE HEMATOLOGIA 2016. Mesa de Leucemia Linfoide Crónica. Sociedad Uruguaya de Hematología. Colonia del Sacramento. Uruguay

Other activities of the group leader:

From January 2017 P. Oppezzo has been appointed as part of the International Scientific Committee of Young Investigator Meeting (YIM) associated to the iwCLL committee (**international workshop on Chronic Lymphocytic Leukemia**).

P. Oppezzo has participated as external examiner in the following post grade theses:

Paula I. Seoane, MSc thesis Facultad de Ciencias - UDeLaR - Uruguay

Carolina Ottati, MSc thesis Facultad de Ciencias - UDeLaR - Uruguay

GRANTS

- Fondo Clemente Estable – Dr. Agustin Correa- “Diseño y desarrollo de proteínas de unión artificiales con potencial uso en la biomedicine” 2016-2018 – ANII, Uruguay

- 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.

PUBLICATIONS

1. Almejún MB, Campos BC, Patiño V, Galicchio M, Zelazko M, Oleastro M, **Oppezzo P**, Danielian S. Noninfectious complications in patients with pediatric-onset common variable immunodeficiency correlated with defects in somatic hypermutation but not in class-switch recombination. ***J Allergy Clin Immunol. 2016*** Oct 3. pii: S0091-6749(16)31054-5. doi: 10.1016/j.jaci.2016.08.030.
2. Sedlik C, Heitzmann A, Viel S, Ait Sarkouh R, Batisse C, Schmidt F, De La Rochere P, Amzallag N, Osinaga E, **Oppezzo P**, Pritsch O, Sastre-Garau X, Hubert P, Amigorena S, Piaggio E. Effective antitumor therapy based on a novel antibody-drug conjugate targeting the Tn carbohydrate antigen. ***Oncoimmunology***. ***2016*** Apr 22;5(7):e1171434. doi: 10.1080/2162402X.2016.1171434.
3. 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.
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 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.
5. **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.
6. 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.

7. 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.

Tumor Immunology and Glycobiology

MEMBERS

Eduardo Osinaga MD, PhD (Head)
Nora Berois, MD, PhD (Associate Investigator)
Alvaro Pittini, PhD (Assistant Investigator)
María Florencia, PhD
Edgardo Berriel MD, MSc, (PhD student)
Sabrina Fischer, MSc, (PhD student)
Mariel Flores, MSc, (PhD student)
Patricia Solari, (MSc student)
Guillermo Tramontín (MSc 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).

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).

3. Production of recombinant antibodies and antibody fragments specific for tumor-associated glyco-antigens. Evaluation in molecular imaging of cancer (immuno-PET) and cancer therapy.

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.

3. **"Anticuerpos por ingeniería genética para diagnóstico y tratamiento del cáncer".** ANII – I+I. U$S 140.000, 2015-2017
4. **"Inmunología Tumoral"**. Proyecto Grupo I+D CSIC-UdelaR. U$S 80.000, 2015-2019.
5. **"Inmuno-nanopartículas en tratamiento del cáncer".** MIEM - Vinculación con la Diáspora Calificada. U$S 25.000, 2017

PUBLICATIONS

Ubillos L, Freire T, Berriel E, Chiribao ML, Chiale C, Festari MF, Medeiros A, Mazal D, Rondán M, Bollati-Fogolín M, Rabinovich GA, Robello C and Osinaga E. *Trypanosoma cruzi* extracts elicits protective immune response against chemically-induced colon and mammary cancers. *Int J Cancer 138:1719-31 (2016)*

Osinaga E, Freire T and Ubillos L. Author's reply to: Could cross-immunological reactivity to *Trypanosoma cruzi* antigens be considered a rational strategy for designing vaccines against cancer? *Int J Cancer. 139:2144 (2016)*

García E, Tiscornia I, Libisch G, Trajtenberg F, Bollati-Fogolín M, Rodríguez E, Noya V, Chiale C, Brossard N, Robello C, Santiñaque F, Folle G, Osinaga E and Freire T. MUC5B silencing reduces chemo-resistance of MCF-7 breast tumor cells and impairs maturation of dendritic cells. *Int. J. Oncol. 48:2113-23 (2016)*

Sedlik C, Heitzmann A, Viel S, Ait Sarkouh R, Batisse C, Schmidt F, De La Rochere P, Amzallag N, Osinaga E, Oppezzo P, Pritsch O, Sastre-Garau X, Hubert P, Amigorena S, Piaggio E. Effective antitumor therapy based on a novel antibody-drug conjugate targeting the Tn carbohydrate antigen. *Oncoimmunology. 5:e1171434 (2016)*

Festari MF, Trajtenberg F, Berois N, Pantano S, Revoredo L, Kong Y, Solari-Saquieres P, Narimatsu Y, Freire T, Bay S, Robello C, Bénard J, Gerken TA, Clausen H and Osinaga E. Revisiting the human polypeptide GalNAc-T1 and T13 paralogs. *Glycobiology (2016, in press)*

Immunoregulation and Inflammation

MEMBERS

Marcelo Hill MD, PhD (Head)
Mercedes Segovia (Researcher)
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
4. CABBIO 2015-2016. A novel ion channel as a therapeutic target to modulate inflammation.
5. FMV. ANII. 2016-2017. Immunointervention in cancer: new therapeutic opportunities
6. Agence des Universités Francophones/FAPESP. 2017-2018. Characterization of novel molecular players in the control of obesity and obesity-induced inflammation.
7. FCE. ANII. 2017-2018. Characterization of a novel regulator of inflammation.

CONTRACTS

RosasT. 2016. Characterization of the in vivo anti-tumoral effect of polyclonal sera.

CONGRESS ORAL PRESENTATIONS

2015. XL Congress of the Brazilian Society of Immunology. Innate Immunity. "Novel regulators of inflammasome activation". M Hill.

2015. Satellite course of the LXIII Annual Meeting of the Argentinean Society of Immunology, IV Meeting of LASID (Latin American Society for Immunodeficiencies) and the Second French-Argentinean Immunology Congress. "Novel regulators of inflammasome activation". M. Hill.

2016. 3rd **Institut Pasteur International Network Symposium.** "Tmem176b is a checkpoint in IL-1β-dependent tumor immunity" S. Russo.

INTERNATIONAL COURSES ORGANIZATION

2016. Update on Immunology: from mechanisms to immunotherapy and viceversa.

INVENTION DISCLOSURES

2016. Pharmacologic inhibition of novel immune checkpoints in cancer.

PUBLICATIONS

1. Comparative Study of the Immunoregulatory Capacity of In Vitro Generated Tolerogenic Dendritic Cells, Suppressor Macrophages, and Myeloid-Derived Suppressor Cells.

Carretero-Iglesia L, Bouchet-Delbos L, Louvet C, Drujont L, Segovia M, Merieau E, Chiffoleau E, Josien R, Hill M, Cuturi MC, Moreau A.
Transplantation. 2016 Oct;100(10):2079-2089.

2. Generation and Characterization of Mouse Regulatory Macrophages.

Carretero-Iglesia L, Hill M, Cuturi MC.
Methods Mol Biol. 2016;1371:89-100. doi: 10.1007/978-1-4939-3139-2_6.

3. Phenotypic analysis of immunocompetent cells in healthy human dental pulp.

Gaudin A, Renard E, Hill M, Bouchet-Delbos L, Bienvenu-Louvet G, Farges JC, Cuturi MC, Alliot-Licht B.
J Endod. 2015 May;41(5):621-7. doi: 10.1016/j.joen.2015.01.005

Molecular Human Genetics

MEMBERS

José Badano, PhD (Head)
Florencia Irigoín, PhD (Research associate)
Victoria Prieto, PhD (Postdoctoral Fellow)
Magdalena Cárdenas, MSc (Postdoctoral FellowPhD student) until 2015
Paola Lepanto (PhD student)
Rossina Novas, Bach (PhD student)
Belén Torrado (MSc student)
Matías Fabregat (MSc student)

RESEARCH

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. We have focused on dissecting the role of 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 interact 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. Using yeast two-hybrid screens and co-immunoprecipitation as tools we have uncovered a few interacting proteins that are shedding light into the function of the BBS modifier. First we characterized an interaction with the mTORC2 component SIN1. Importantly, 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 (*6*). More recently we have been working on characterizing the interaction between CCDC28B and cytoskeletal components that is informing us about the mechanism by which this protein regulates SIN1 function and ciliogenesis (manuscript submitted and currently being revised).

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 studies on BBS7 that led to the demonstration that at least some BBS proteins play extraciliary roles in the nucleus modulating gene transcription (*7*). In this project, led by Dr. Irigoín, we worked with the transcription factor Gli2, a protein that shuttles between the cilium and the nucleus, to understand whether similar mechanisms are used. Our results demonstrated that Gli2 uses two classical nuclear localization signals (NLS) to transport into the nucleus and that

these motifs are not required to enter the cilium. However, ciliary import of Gli2 does utilize a RAN GTP/GDP gradient and a different importin, Imp-β2 (*8*). Intriguingly, Imp-β2 also collaborates in GLi2 nuclear translocation and therefore we are now focused on understanding how is the final destination decided.

Another important interest of the laboratory is to understand the role of the cilium and ciliary proteins (BBS and others) in development and disease pathogenesis. 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, 10*).

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. Our main contribution to this program has been centered on studying the role of BBS proteins and cilia in the differentiation of adipocytes. In addition to their role in ciliogenesis, we have uncovered novel extra-ciliary roles for at least some of the BBS proteins that are relevant in this context. Previously, 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 (*11*). We have now expanded these studies to characterize the functional interaction between BBS4 and a secreted protein implicated in adipogenesis. Therefore, we have been using cell-based studies to address the role of the cilium and the BBS proteins in the production and secretion of relevant proteins for adipogenesis (manuscript under preparation). Thus, this line of research will likely provide important insight to understand the cellular basis of phenotypes associated with BBS and the ciliopathies.

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1. M. Cardenas-Rodriguez, J. L. Badano, Ciliary Biology: Understanding the Cellular and Genetic Basis of Human Ciliopathies. Am J Med Genet Part C Semin Med Genet 151C, 263-280 (2009).
2. J. L. Badano, N. Mitsuma, P. L. Beales, N. Katsanis, The Ciliopathies: An Emerging Class of Human Genetic Disorders. Annu Rev Genomics Hum Genet 22, 125-148 (2006).
3. R. Novas, M. Cardenas-Rodriguez, F. Irigoin, J. L. Badano, Bardet-Biedl syndrome: Is it only cilia dysfunction? FEBS Lett 589, 3479-3491 (2015).
4. J. L. Badano et al., Dissection of epistasis in oligogenic Bardet-Biedl syndrome. Nature 439, 326-330 (2006).
5. M. Cardenas-Rodriguez et al., Characterization of CCDC28B reveals its role in ciliogenesis and provides insight to understand its modifier effect on Bardet-Biedl syndrome. Hum Genet 132, 91-105 (2013).
6. M. Cardenas-Rodriguez et al., The Bardet-Biedl syndrome-related protein CCDC28B modulates mTORC2 function and interacts with SIN1 to control cilia length independently of the mTOR complex. Hum Mol Genet 22, 4031-4042 (2013).
7. C. Gascue et al., Direct role of Bardet-Biedl syndrome proteins in transcriptional regulation. J Cell Sci 125, 362-375 (2012).
8. B. Torrado, M. Grana, J. L. Badano, F. Irigoin, Ciliary Entry of the Hedgehog Transcriptional Activator Gli2 Is Mediated by the Nuclear Import Machinery but Differs from Nuclear Transport in Being Imp-alpha/beta1-Independent. PLoS One 11, e0162033 (2016).
9. P. Lepanto, J. L. Badano, F. R. Zolessi, Neuron's little helper: The role of the primary cilium in neurogenesis. Neurogenesis 3, e1253363 (2016).
10. P. Lepanto, C. Davison, G. Casanova, J. L. Badano, F. R. Zolessi, Characterization of primary cilia during the differentiation of retinal ganglion cells in the zebrafish. Neural Dev 11, 10 (2016).

11. C. C. Leitch, S. Lodh, V. Prieto-Echague, J. L. Badano, N. A. Zaghloul, Basal body proteins regulate Notch signaling through endosomal trafficking. J Cell Sci 127, 2407-2419 (2014).

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
2. B. Torrado, M. Grana, J. L. Badano, F. Irigoin, Ciliary Entry of the Hedgehog Transcriptional Activator Gli2 Is Mediated by the Nuclear Import Machinery but Differs from Nuclear Transport in Being Imp-alpha/beta1-Independent. PLoS One 11, e0162033 (2016).
3. P. Lepanto, C. Davison, G. Casanova, J. L. Badano, F. R. Zolessi, Characterization of primary cilia during the differentiation of retinal ganglion cells in the zebrafish. Neural Dev 11, 10 (2016)
4. P. Lepanto, J. L. Badano, F. R. Zolessi, Neuron's little helper: The role of the primary cilium in neurogenesis. Neurogenesis 3, e1253363 (2016).

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.
- INDICyO: understanding the cellular and molecular basis of obesity in BBS

EDUCATION-COURSES

TRAINING COURSES 2016

1. 4th South American Workshop on new trends in Advanced Fluorescence Microscopy Techniques, January 25-29, School of Chemical Sciences, Universidad de Concepción, Chile (Belén Torrado)
2. Course “Image Processing in Biology and Medicine” (PIMBIO), July 6-22, School of Engineering, Montevideo, Uruguay (Belén Torrado)
3. IV Latin American Zebrafish Network Course and Symposium, December 2016, Porto Alegre, Brazil (Rossina Novas)

TRAINING OF STUDENTS 2015

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).
3. Invited visit and Genomics Seminar Speaker, November 8-11, 2016, Pennsylvania State University, State College, Pennsylvania, USA (José Badano).
4. Frontiers in Bioscience 2, Max-Planck Symposium, November 17-19, Buenos Aires, Argentina (Belén Torrado and 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
5. CSIC Project – Florencia Irigoín – "Estudios funcionales y estructurales de CCDC28B, un modificador del Síndrome de Bardet-Biedl." – 2013-2015 – I+D Program, CSIC, UDELAR
6. Doctoral Fellowship – Belén Torrado – 2016-2019 – ANII
7. Doctoral Fellowship – Paola Lepanto – 2013-2016 – ANII
8. CAP Fellowship – Paola Lepanto – 2016-2017 - UDELAR
9. CAP Fellowship – Matías Fabregat – 2016-2017 - UDELAR
10. CSIC Project – Florencia Irigoín & José Badano – "Estudio funcional de la interacción CCDC28B-BBS4 y su impacto en la patogénesis del síndrome de Bardet-Biedl". Awarded on 2016 to be executed 2017-2018 – I+D Program, CSIC, UDELAR

Metabolic Diseases and Aging

MEMBERS

Carlos Escande, **PhD** (Head)
Paola Contreras, **PhD** (Research Associate)
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Leonardo Santos, MSc (Research Assistant, PhD Student)
Natalia Bobba, BSc (Research Assistant, MSc Student)
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Rosina Dapueto, 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 trying to understand the molecular mechanisms that are involved in the control of metabolism and metabolic diseases, such as obesity, type II diabetes, and cardiovascular diseases. In particular, we are interested in the regulation of Sirtuins, a conserved family of proteins with key roles in metabolism and aging. Sirtuins are NAD^+-dependent protein deacylases that have been implicated in the control of several cellular functions, from cell cycle control, genomic stability and DNA repair, mitochondrial biogenesis and function, gene expression and metabolic control. One common feature of all Sirtuins is that they need oxidized NAD in order to be active and, in fact, their enzymatic activity is dramatically affected by changes in intracellular NAD levels. Indeed, it has been extensively shown that Sirtuins can act as metabolic sensors, re-shaping the metabolic program of cells and tissues in response to different stressors, including changes in caloric input. All these findings make Sirtuins extremely interesting candidates for interventions aimed to prevent and treat metabolic diseases.

Deleted in Breast Cancer 1 (DBC1), a SIRT1 protein regulator and beyond

Sirt1, one of the seven Sirtuins expressed by mammals and founder of the family, has received special attention due to its massive impact on metabolism and metabolic diseases. Several independent investigators have shown that activation of Sirt1 (genetically or pharmacologically) prevents obesity induced insulin resistance, protects against atherosclerosis, improves cardiac function, and prevents neurodegenerative disorders, among other pathologies.

SIRT1 is not only regulated by changes in NAD^+ levels, but also by post-translational modifications and by protein-protein interactions. In particular, we have extensively shown that a SIRT1 binding protein, named DBC1 (Deleted in Breast Cancer 1), binds and inhibits SIRT1 *in vivo* and that this binding is tightly regulated by the metabolic state of the organism. Interestingly, we found that knock-out of DBC1 leads to a “healthy obesity” phenotype, a condition where mice become morbidly obese but are protected against insulin resistance, liver steatosis and atherosclerosis. Currently, one of the main focuses in the laboratory is to continue to understand the role of DBC1 in the regulation of metabolism and other cellular processes. Specifically, we are very interested understanding how DBC1 function is regulated in normal and pathological conditions in order to understand its cellular functions. In addition, we and others have recently shown that many DBC1 functions are SIRT1-independent, showing that DBC1 role in the control of metabolism likely goes beyond what we were originally thinking.

- **A novel isoform of DBC1 regulated by cell cycle status**. During the search for new pathways that regulate DBC1 function, we found a novel form of DBC1 that lacks the N-terminal domain (which has been shown to bind most of interactors, including SIRT1). This form of DBC1, which we believe is a result of partial proteolytic processing, is only present when cells enter the G0 phase of cell cycle and it disappears as soon as the cell re-enters the cell cycle. Importantly, when cells are forced to stop the cell cycle in different checkpoints (G1/S, G2/M), this short form of DBC1 is not present. This makes us believe that it is exclusive of quiescent cells. Interestingly, our preliminary data suggest that cells lacking DBC1 are delayed when forced to re enter the cell cycle form G0 both *in vitro* and *in vivo*. We are now working on mapping the N-terminal deletion, and unwinding the molecular mechanisms that control the production of this short form of DBC1. Our hypothesis is that this novel form of DBC1 regulates G0/G1 transitions.
- **Follistatin like 1 (FSTL1)**: **a secreted protein regulated by DBC1 with potential role in metabolic diseases.** On the search for secreted factors regulated by DBC1 that regulate metabolism and could explain the "healthy obesity" phenotype observed in the DBC1 KO mice, and in close collaboration with Dr. José Badano, we found that FSTL-1 expression and secretion is regulated by DBC1 and SIRT1 both in vitro and in vivo. FSTL1 is a glycoprotein that has been recently linked to cardiac regeneration during infarction, renal protection, and inflammation. Our findings show that FSTL1 is regulated by DBC1 *in vitro* and *in vivo*. Also, we found that FSTL1 expression is regulated in fat tissue during obesity and that FSTL1 plays a role during adipocyte differentiation. Importantly, we found that this regulation also occurs in obese patients. We are currently working on understanding the relevance of FSTL1 in fat tissue function in vivo. We are generating tissue-specific FSTL1 KO mice and also expanding our preliminary findings in patients.
- **DBC1 as a novel regulator of cardiovascular function**. In previous work we showed that DBC1 KO mice are protected against atherosclerosis. However, our findings pointed to fat tissue playing a key role in this vascular phenotype. To gain insight into the role of DBC1 in cardiovascular function, we started to work on AngII-induced hypertension. Our preliminary data show that DBC1 KO mice present less thickness of arterial wall in response to AngII, although they develop hypertension to a similar extent of WT mice. Moreover, DBC1 KO mice show fewer incidences of aortic aneurysms and reduced kidney damage in response to AngII. We are currently trying to understand the molecular mechanisms underlying these effects.

- **SIRT6: a Sirtuin with novel unexpected functions in the control of the inflammatory response.** The role of this Sirtuin in metabolic diseases has been much less studied than SIRT1. SIRT6 is a protein that is tightly bound to chromatin that has been shown to play a key role in genomic stability and DNA repair. Interestingly, SIRT6 controls glycolysis in such a tight way that mice that are KO for SIRT6 die early in life from hypoglycemia, suggesting that SIRT6 also plays a central role in metabolic control. Very recently is has been proposed that, in contrast to other sirtuins, SIRT6 positively regulates inflammation by promoting the secretion of TNFalpha. Also, it has been recently shown that free-fatty acids, which are elevated during obesity and insulin resistance, stimulate SIRT6 activity. Based on these novel findings, we decided to explore the role of SIRT6 on chronic inflammation during these pathologies. Our preliminary data shows that both chronic and acute inflammation drive a very rapid increase in SIRT6 expression, which is inhibited by anti-inflammatory compounds. Although preliminary, our data strongly suggest that SIRT6 expression and activation is an intrinsic part of the inflammatory response.

Animal models for biomedical research in metabolic diseases

We have put a strong effort in setting a battery of animal models suitable for biomedical research in metabolic diseases. Many of these models, together with our expertise, made possible not only to develop our research projects but also to establish new and fruitful intramural and external collaborations. It also helped to get private companies to get interested in our capabilities and led to novel funding opportunities. Below there is a list of animal models already available in our laboratory:

- Diet induced obesity and insulin resistance
- Atherosclerosis (ApoE$^{-/-}$ and LDLR$^{-/-}$ApoB$^{100/100only}$)
- Hypertension (AngII infusion)
- Chronic Kidney Disease (Nephrectomy + AngII – under development)
- Genetically modified mice: DBC1$^{-/-}$, BBS4$^{-/-}$, CD38$^{-/-}$, FSTL1 $^{loxp/loxp}$, SIRT6$^{loxp/loxp}$
  Currently developing DBC1$^{loxp/loxp}$ at IPMON in collaboration with José Badano

INTRAMURAL NETWORKING

As part of an Institutional effort to promote synergistic interactions between different research groups at IPMON, in 2016 we launched **INDICyO** (a Spanish acronym for Research in Diabetes, Inflammation, Cardiovascular Diseases and Obesity), a multidisciplinary program that brought together four different labs to collaborate in order

to improve the quality of our science. This program is based in two basic principles: multidisciplinarity and solidarity. The founding laboratories of this program are the ones led by Drs. Carlos Batthyany (UBYPA), Marcelo Hill (LIRI), José Badano (GMH) and Carlos Escande (PME). Since it was launched in 2016 the program has achieved the following milestones:

1. Four PhD Students and two MSc students share advisors between INDICYO members and develop research projects that are collaborative
2. Two research projects with international funding and three with intramural funding
3. Weekly seminars with PIs and students for papers and results discussions
4. Two patents and one paper submitted

Our laboratory is deeply involved in the development of this program. The following research projects are under development with active participation of our laboratory.

- TMEM176b: A novel regulator of inflammation and insulin resistance during obesity. Co-PI with Marcelo Hill (LIRI). Funded intramural and international grants
- Role of FSTL1 in the regulation of adipogenesis and adipose tissue function during obesity. Co-PI with José Badano (GMH). Funded by intramural grant. International grant submitted (ADA, USA)
- Evaluation of new anti-inflammatory compounds for the treatment of metabolic diseases. Co-PI with Carlos Batthyany (UBYPA). Funded by private international company.

PATENTS

1. “Methods of treatment of inflammation related conditions using pluripotent anti-inflammatory and metabolic modulators”; inventors Batthyany, C., Lopez, G.V., **Escande, C**., Porcal, W., Dapueto, R., Rodriguez, R., Galliussi, G., and Garat, M.P. 2016. USA patent provisional application; to be assigned.
2. “Trolox derivatives and methods of use thereof in the treatment and prevention of inflammation related conditions”; inventors Batthyany, C., Lopez, G.V., Dapueto, R., **Escande, C**., and Rodriguez, R. 2016. USA patent non-provisional application; to be assigned.

GRANTS

1. INNOVA – ANII – Young leaders grant. 2014-2019
2. Fondo Clemente Estable – Role of the protein DBC1 in fat tissue physiology during obesity - ANII – 2015-2017
3. Alianza Pasteur-Granuy – Creation and development of NutraScan - ANII – 2015-2017 (Co-PI with Carlos Batthyany)
4. Agence universitaire de la Francophonie (AUF) – 2016-2018 (Co-PI with Marcelo Hill, LIRI)
5. I+D Grants – CSIC – Novel role of CD38 in the regulation of the acute inflammatory response. 2017-2019 (Co-PI with Paola Contreras)
6. Intramural collaborative grant - IPMON. Development of a model of atherosclerosis in Zebrafish 2015-2016. (Coordinator)
7. CITES-SANCOR – Eolo Pharma: A pharmaceutical company for the development of novel compounds for the treatment of metabolic and cardiovascular diseases. (Co-PI with Carlos Batthyany and Virginia Lopez; Scientific Director of the company) 2017-2018

PUBLICATIONS

1. Camacho-Pereira J, Tarragó MG, Chini CC, Nin V, **Escande C**, Warner GM, Puranik AS, Schoon RA, Reid JM, Galina A, Chini EN. CD38 Dictates Age-Related NAD Decline and Mitochondrial Dysfunction through an SIRT3-Dependent Mechanism. **Cell Metab. 2016** Jun 14;23(6):1127-39. doi: 10.1016/j.cmet.2016.05.006. PubMed PMID: 27304511; PubMed Central PMCID: PMC4911708.
2. Chini CC, Espindola-Netto JM, Mondal G, Guerrico AM, Nin V, **Escande C**, Sola-Penna M, Zhang JS, Billadeau DD, Chini EN. SIRT1-Activating Compounds (STAC) Negatively Regulate Pancreatic Cancer Cell Growth and Viability Through a SIRT1 Lysosomal-Dependent Pathway. **Clin Cancer Res. 2016** May 15;22(10):2496-507. doi: 10.1158/1078-0432.CCR-15-1760. PubMed PMID: 26655844; PubMed Central PMCID: PMC4867252.
3. **Santos L, Escande C**, Denicola A. Potential Modulation of Sirtuins by Oxidative Stress. **Oxid Med Cell Longev. 2016**; 2016:9831825. doi: 10.1155/2016/9831825. Review. PubMed PMID: 26788256; PubMed Central PMCID: PMC4691645.
4. 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**

5. **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.

Cell Biology of Neural Development

MEMBERS

Flavio Zolessi, PhD (Head)

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Magela Rodao, BSc (Graduate Student)

ASSOCIATED MEMBERS

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

Marcela Díaz, MSc (Microscopy Unit)

RESEARCH

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 zebrafish and chick embryos. The zebrafish has great advantages for these studies, such as the accessibility to genetic manipulation and the unique optical transparency of embryos.

In vertebrates, the central nervous system is generated during the process of neurulation from the ectoderm, by a series of morphogenetic movements that generate a tube-like structure, the neural tube. The cell-shape change that is needed for these movements depends on the modulation of the cytoskeleton. In particular, the contraction of actin filaments accumulated at the apical side of these cells is thought to be one of the major motors for apical constriction and thus bending of the neural plate. Actin-modulating proteins, such as those of the MARCKS family, are essential for this process in mice and are apically accumulated in the chick neural plate only during this process (Stumpo et al., 1995; Zolessi and Arruti, 2001).

Cells in the neural tube are organized as a pseudostratified epithelium, where nuclei constantly move between the apical and basal sides following the cell cycle stages ("interkinetic nuclear migration"). These cells are in close contact to one another, being mostly attached at the apical side through adherens junctions and at the basal side to a basal lamina. Neurons arise from these neuroepithelial cells once they have a last cell division, and differentiate in this epithelial environment through a series of stages that imply a gradual transition from epithelial to neuronal morphology as we have previously shown in the retina of the zebrafish (Zolessi et al., 2006). Here, retinal ganglion cells, which are the first neurons to differentiate, always form their axon in a basal direction and dendrites in an apical direction. Signals responsible for this stereotyped behavior have been largely elusive, and could be different for different neuronal types. In the case of the retinal ganglion cells, Laminin-1 in the basal lamina of the retinal neuroepithelium is necessary for axon outgrowth towards the basal side (Randlett et al., 2011), but all experimental evidence suggests that there must be other, still unidentified, molecules collaborating in the final and correct orientation of these neurons.

Research line 1: The role of MARCKS family proteins in neurulation and early neural differentiation in vertebrates. MARCKS proteins are unique in many aspects. They are naturally unfolded phosphoproteins highly enriched in the central nervous system. In most vertebrates this family is only composed by two relatively small proteins, MARCKS and

MARCKS Like-1, each encoded by a different gene, with no splice isoforms. We have further analyzed the roles of MARCKS in chick neurulation, by a combination of genetic manipulation approaches including gene knockdown and the expression of mutant forms of the proteins, with pharmacological treatments on cultured embryos to affect the phosphorylation and localization of MARCKS, as well as the actin cytoskeleton. We found that MARCKS phosphorylation by PKC prevents neural plate folding by deeply affecting neuroepithelial polarity and integrity, but not acto-myosin contractility (Aparicio et al., in preparation).

In the teleosts, including the zebrafish, however, there are four genes encoding four proteins, two MARCKS and two MARCKS Like-1. This finding added to the knowledge that in teleosts primary neurulation has important differences with that of amniotes, made us wonder what could be the functions of these four proteins. By knocking-down each of these genes in zebrafish embryos, we found that even if all of them appear to be essential for a correct neural development, some differences are evident in the phenotypes (Prieto and Zolessi, 2016). In particular, two of them caused particular defects in neurulation. In MARCKSB knocked-down embryos, the fourth ventricle was extremely enlarged and the hindbrain walls formed a wider angle than in controls, while neuroepithelial polarity appeared unaffected. MARCKS Like-1A knock-down, on the other hand, caused a general disorganization of the neuroepithelium, characterized by an apparent loss in apico-basal polarity. The most interesting phenotype was found in the double knock-down, where a new phenotype appeared: the duplication of the neural tube from the hindbrain to the spinal cord. This phenotype is extremely similar to that of the Vangl-2 mutant, in which planar cell polarity and convergent extension movements are affected (Tawk et al., 2007).

Research line 2: Neuronal polarization and orientation in the zebrafish neural retina. On the one hand, we have continued with the characterization of the putative signals involved in neuronal oriented differentiation *in vivo*. Our previous work suggested the existence of negative signals for axon outgrowth inside the retina, while other authors had shown in the in different species that axon guidance repulsive molecules such as Semaphorins and Slits are present in the retinal parenchyma and that they functional inactivation caused retinal ganglion cells extension inside the retina. We wondered if Slits signaling through their Robo receptors could be involved, in collaboration with Laminin-1, on defining the orientation of these cells in the early stages of differentiation. Some preliminary results suggested a particular role of Slit2 in this process. We are currently approaching this question by combining genome editing (using CRISPR) and the expression of dominant-negative forms of the receptors Robo-2 and -3 in the zebrafish (Davison, Doctoral Thesis). On the other hand, in association with Dr. Badano's lab in the Institute, we also started a characterization

of the possible roles that primary cilia could have on early retinal ganglion cells differentiation (Lepanto et al., 2016a and 2016b). Neuronal progenitors in the neuroepithelium have an apically-localized primary cilium of so-far unknown function. We have followed the behavior of these cilia as cells become post-mitotic and become differentiating neurons, with surprising observations on the extreme dynamics of the organelle. It disappears from progenitors relatively late in G2, usually re-appearing a short time after the last mitosis and remaining at the tip of the retracting apical process of these neurons until after the axon is formed. Along this time, the cilium may transitorily disappear. Once the apical process is completely retracted, apparently random movements of the cilium occur around the cell, until just before the dendritic tree starts to grow, when it stabilizes at its base, remaining there at least for a long period until eventually being reabsorbed. The knock-down of genes essential for ciliary growth and maintenance (Elipsa and IFT88) caused different effects on retinal ganglion cells generation, reducing the neurogenesis of these cells in particular, and in their morphological differentiation, particularly in the positioning of the cell body in the inner layer of the retina and in the formation of the inner plexiform layer (where their dendrites contact the afferent interneurons).

Finally, two current graduate students are beginning with the characterization of the intracellular and extracellular signals involved in the differentiation of the most intriguing cell in the retina: the photoreceptors. These cells are characterized by an apparent "double identity", with an epithelial-like morphology in general, but also behaving as neurons, being the first pre-synaptic neurons in the visual pathway. It is of particular interest that the mutation in genes involved in epithelial polarity, such as Crumbs, cause severe retinal degeneration in humans, such as that seen in retinitis pigmentosa or congenital amaurosis. On one hand, and again in collaboration with Badano's lab, we are characterizing the role of the primary cilium in the differentiation of these cells, bearing in mind that the outer segment of these cells (responsible fo photo-transduction) is actually a modified cilium (Rodao's thesis). On the other hand, we also work on the hypothesis that external signals must influence the orientation of these cells in the neuroepithelium for these cells to proeperly differentiate. In particular, we will analyze the function of some subapical adhesion molecules (such as N-Cadherin and Pals-1/nok) and eventually of axon guidance molecules, such as Slits (Aparicio's thesis). Preliminary results following the in vivo differentiation of photoreceptors through the transgenic expression of specific markers indicate, again, complex behaviors of the cells and the studied structures in the initial stages of cell differentiation after post-mitosis and before the onset of outer segment formation.

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.

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.

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.
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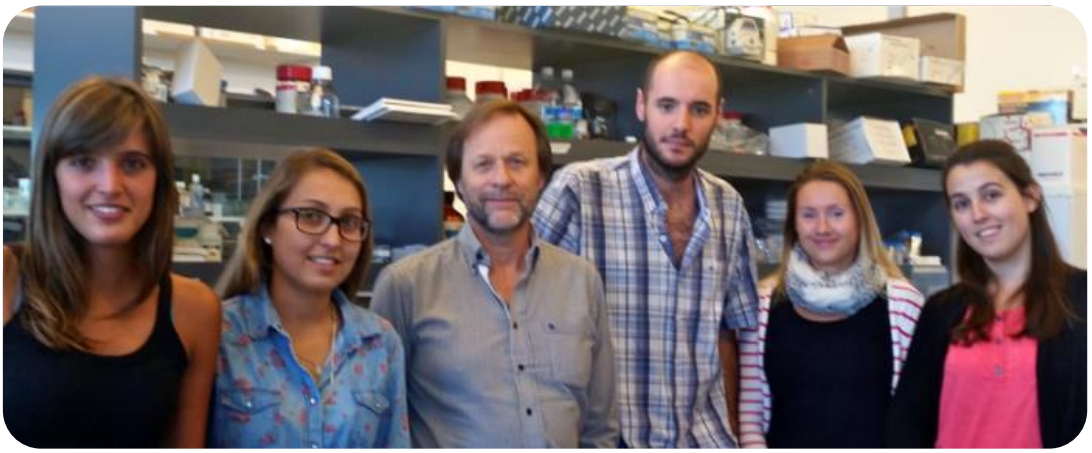
3. Lepanto P., Badano J.L., Zolessi F.R. (2016) Neuron's little helper: the role of primary cilia in neurogenesis. Neurogenesis. 3:1, e1253363. DOI: 10.1080/23262133.2016.1253363 .
4. Lepanto P., Davison C., Casanova G., Badano J.L., Zolessi F.R. (2016) Characterization of primary cilia during the differentiation of retinal ganglion cells in the zebrafish. Neural Dev. 11(1):10. doi: 10.1186/s13064-016-0064-z.



Neurodegeneration

MEMBERS

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Romina Barreto (Msc student)
Sofía Ibarbouru (Msc student)

RESEARCH

Modulation of paralysis 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 disability 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 cells are the most toxic cells yet identified to motor neurons. AbA cells actively proliferate after the onset of progressive paralysis and make intimate contact with degenerating motor neurons. By analyzing the population of proliferating glial cells in the ventral horn of symptomatic SOD1 rats, we found that microglia are the most likely cellular origin of AbA cells.

Our central hypothesis is that the spread of motor neuron disease is dependent on the formation of a neurodegenerative microenvironment surrounding damaged neurons, 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 nitrated NGF species. 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.

Another 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.

The specific research projects detailed below are being executed in collaboration through numerous intramural and external collaborations:

Targeting AbA cells and immune cells with tyrosine-kinase inhibitor drugs.
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. The main results obtained during this period have showed that masitinib exerts the following neuroprotective effects:

- Extension of post-paralysis survival in $SOD1^{G93A}$ rats when treatment started after paralysis onset.
- Prevention of microglia proliferation and proinflammatory phenotype by inhibiting CSF-1R kinase activity at nanomolar concentrations.
- Prevention of microglia transformation into AbAs.
- Reduction of the number of AbAs in the degenerating spinal cord.
- Improved motor neuron pathology after paralysis onset.
- Drastic decrease in neuromuscular junction denervation and decrease immune cell infiltration in skeletal muscle.

The protective effects of masitinib were unprecedented when compared to other drugs assayed in ALS rodent models because it the first compound to significantly delayed survival when administered up to 7 days after disease onset. Taken together, the results

support the medical plausibility of treating ALS with masitinib. Our data has supported the rational for an ongoing phase 2/3 clinical study with masitinib involving almost 400 ALS patients. Part of these studies have been published in 2016 (Trias et al, 2016 J. Neuroinflamm). Two other publications are being submitted in the first semester 2017, showing that macrophages and mast cells infiltrating the skeletal muscle and sciatic nerves are also target for tyrosine kinase inhibitors.

AbA cells transplantation into the spinal cord

AbA cells appear in the symptomatic phase of the disease surrounding motor neurons (MTNs) and increase in number during paralysis progression, suggesting they can exert local neurotoxicity or inflammation. Thus, we aim to further analyze the intrinsic activity of AbA cells by transplanting the cells into the spinal cord of wild-type rats. We hypothesize that AbA cells transplantion will induce neuronal pathology or glial cell activation, reproducing in wild-type rats some features of the ALS pathology observed in $SOD1^{G93A}$ rats. Preliminary observations indicate that AbA cell transplantation results in a large inflammatory response through the CNS, an effect that could be modulated by neuroprotective drugs such such as masitinib.

Role of nitrated-NGF species in AbA cells

We have previously reported that activated astrocytes in ALS express increased levels of NGF, which triggers p75-dependent motor neuron apoptosis. Although adult motor neurons lack TrkA and $p75^{NTR}$ receptors, they re-express $p75^{NTR}$ following nerve injury or in ALS, thus becoming sensitive to NGF-induced apoptosis. We found that spinal cord extracts from ALS-affected $SOD1^{G93A}$ mice contained a hundred-fold more active form of NGF than the mature factor. Because reactive 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 this context, our group has also developed two specific antibodies against nitrated species of NGF that allow the specific identification of AbA cells. Preliminary observations show that anti-nitrated NGF antibodies are specific for AbAs in vitro and in the spinal cord of symptomatic $SOD1^{G93A}$ rats, discriminating them from microglia or astrocytes. We are currently submitting a manuscript on the characterization of these antibodies and their potential use in neuropathological studies. There is an interest in

probing the antibodies in necropsy samples from ALS patients, to determine whether AbA-like cells can be found in ALS.

In addition, we are studying whether the anti-nitrated NGF antibodies play a neuroprotective activity. These antibodies have been developed to recognize only the nitrated tyrosine residue located that position 52, so they do not interfere with NGF signaling itself. However, the antibodies could potentially prevent an apoptotic activity of nitrated NGF in pathological conditions.

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.
3. Proyecto ECOS U014S02 "Mast cells and neuroinflamation in ALS"" 2014-2016.
4. Research contracts from Megapharma, Uruguay (2015-2017) and AbScience, France (2016-2017) for drug discovery in ALS rat models.

PUBLICATIONS

2015

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.
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Neuroinflammation and Gene Therapy

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Members at the IPMontevideo

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Bruno Pannunzio (MSc Student and hands-on teacher at the School of Medicine, UdelaR)
Daniela Alí (MSc Student with stable salary from the Ministry of Work and Social Security, MTSS)
Andrés Cawen (Student)

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Daniela Blanco (MD, MSc Student)
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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.

Neuroinflammation and CNS Damage. 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*. 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. Thus the modulation of neuroinflammation has emerged as an important therapeutic opportunity.

For these reasons, checkpoints for the control of inflammatory mechanisms have gained a high degree of importance and interest in the field of neuroimmunology. 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 suppression of potentially inflammatory stimuli. **This is one of the central hypotheses 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 to unveil their participation in the regulation of microglial cell activation in the Nervous System.

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 [1]. 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. Moreover, CD300a and CD300f are among the 10 highest spinal cord genes upregulated after a SCI, and CD300f is among the top brain upregulated genes after intraperitoneal LPS injection. Three of the activating

members, CD300b, CD300c and CD300e, fit to the classical scheme for activating receptors with a positive charge within their membrane domain. They recruit the transmembrane adaptor molecule DAP-12/DAP10 through the positive charge in its transmembrane domain and have a functional tyrosine residue in its cytoplasmic tail able to recruit Grb2. 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 and unique functional duality of this receptor. *In vivo* CD300f has shown to be mainly an inhibitory receptor, as shown in CD300f knockout animals in the EAE model of Multiple Sclerosis [2], and very recently in several models of Allergy [3] and in a model of Lupus Erythematous [4], **but this is still a critical open question, specially for the nervous system**. Recent reports suggest that the phospholipids phosphatidylserine, phosphatidylcholine or sphingomyelin are the main ligands for the CD300 and TREM receptors, but it is still another area under intense study.

Innovative Gene Therapy Strategies For Traumatic Injury Of The Nervous System. One of the main focuses in the gene therapy field 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 [5, 6]. Non-viral vectors have also gained attention, and in particular, vehicles based on multifunctional proteins in DNA complexes constitute a very versatile type of nano-carriers for therapeutic nucleic acids. They are constructed by the combination of appropriate functional domains fused in a single polypeptide chain. We showed for the first time that these types of vectors induce biologically relevant concentrations of transgenic protein after acute excitotoxic brain injuries [7]. 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” [8]. The modular principles underlying the NLSCt vector were further improved by generating two smaller nano-vectors termed HKRN and HNRK, that achieved significant transgene expression levels in culture cells, and in vivo after a TBI [9-11].

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, and **this concept constitute one of the main focuses of our research group**.

Current Situation, Milestones Achieved And Expected Outcomes

The role of the CD300 molecules in the nervous system is largely unknown, and most studies in this field have been published by our group and our collaborator Joan Sayós [12-15].

Our initial results suggested that the human CD300f overexpression could induce a significant neuroprotective effect after excitotoxic acute rat brain damage [15]. In order to test the neuroprotective effect in one of the trauma preclinical models of choice, we introduced to our country the Controlled Cortical Injury focal traumatic brain injury (CCI-TBI) model and the spinal cord contusion model of spinal cord injury (SCI).

The ligands of the CD300 family of immune receptors were suggested to be phospholipids, and in particular their presence in the CNS was unknown. We also reported, using both the human and rat CD300f-IgG2a fusion proteins, that the unknown ligands of CD300f were present in oligodendrocytes and fibrous astrocytes *in vitro* and mainly in white matter of the CNS, and also in Schwann cells in peripheral nerves [12, 15]. In collaboration with the groups of Joan Sayós and John Coligan, we have produced several unpublished results showing that some phospholipids interact with several members of the family, being phosphatidylserine and sphingomyelin the main ligands for CD300f. Interestingly, treatment of cells with trypsin abolished an important fraction of the binding of CD300f to its ligands, suggesting that there is a proteinaceous co-receptor. CD300f-Fc pull-down coupled to mass spectrometry and CRISPR knockdown experiments suggest that Annexin VII may be this co-receptor. Moreover, human brain histological samples from control or TBI patients also show the presence of the ligands.

Our most recent and exciting results involve the characterization of the behavioural phenotype of the CD300f KO animals. There are growing evidences indicating that microglia could be a central player in mayor depressive disorders (MDD)[16] and obsessive compulsive disorders (OCD)[17], in part but not exclusively by an neuroinflammatory mechanism. This prompted us to seek for behavioural phenotypes in the CD300f KO animals that could be induced by chronic low-grade inflammation. We found that these animals display a decreased spontaneous locomotor activity and slight alteration in the short-term inescapable stress behaviour (compatible with depressive symptoms). More importantly, deficits in the motivational behaviour evaluated by the latency to start grooming behaviour in the sucrose splash test were observed. The same test also showed and anhedonic-like

behaviour suggesting alterations in the self-care and the pleasure seeking behaviour. Taken together, these behavioural alterations are compatible with MDD. In addition, these animals showed alterations in the Marble burying test, suggesting an increased OCD. In accordance with the MDD, preliminary experiments showed decreased serotonin and noradrenalin levels in the hippocampus. Moreover, in vitro in mixed hippocampal glial-neuronal cultures, the inhibition of CD300f induced an increase in the number of vGlut1/Homer positive synapses as well as in the vGlut1 intensity of these synapses, suggesting that CD300f may directly or indirectly regulate synaptic plasticity. Finally, aging is a known chronic inflammatory process, and thus we used aged WT or CD300f KO animals for behavioural evaluation. Interestingly, aged KO animals displayed enhanced MDD symptoms and increased hair greying.

Part of the research strategy designed includes collaboration with Dr. John Coligan (Receptor Cell Biology Section Laboratory of Immunogenetics, NIAID/NIH) and Dr. Dorian McGavern (Viral Immunology and Intravital Imaging Section, NINDS-NIH). The CD300f KO mice have been crossed to the CX3CR1–GFP mice that display GFP labelled microglia for two photon microscopy studies in the naïve and TBI brain. One of our students has performed a research stage at his laboratory and will perform another one this year.

We expect to continue with the close on-going collaborations (Marcelo Hill, Carlos Escande, Luis Barbeito) and strengthen collaborations with other groups working at the IPMon, as for instance with the Genome Program to contribute in general terms to the consolidation of the biomedical hub of our institute. Moreover, the lentiviral technology can be applied by different groups of our Institut, including for instance the generation of transgenic animals. We will continue to strongly support the development of the *in vivo* animal experimentation area (including surgical procedures, neurological tests, histopathology, and participation in the animal and human ethical committees). We expect to be more effective in obtaining national and international funding for the research, which has been a critical problem during the last two years.

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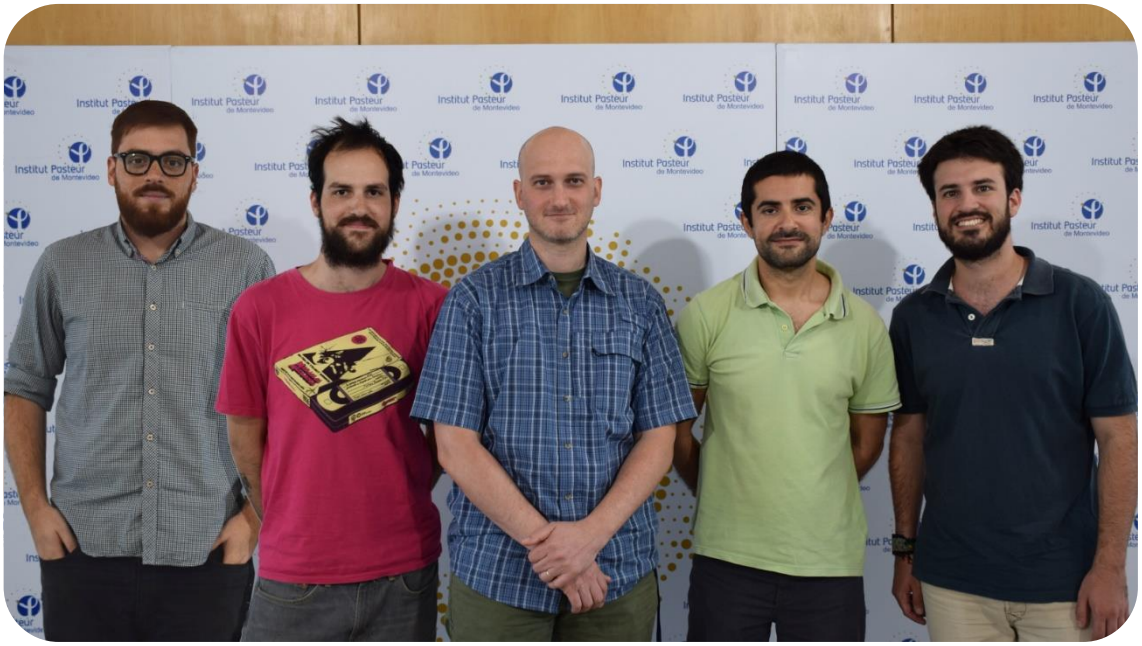
GRANTS

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 with several other groups.
4. Project approved by the Banco de Seguros del Estado (National Public Insurance Company) (2017-2020). “Presicion Medicine applied to traumatic brain injuries”. Amount of the proposal USD 200.000.

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. **Hugo Peluffo**, Patricia **Solari-Saquieres, Maria Luciana Negro-Demontel**, Isaac Francos-Quijorna, Xavier Navarro, Ruben López-Vales, Joan Sayós, Natalia **Lago**. *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 E, López-Serrano C, Hernández J, **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.



Signal Processing

MEMBERS

Federico Lecumberry, Eng, PhD (Head)
Martín Etchart, Eng
Mauricio Ramos
Bernardo Marenco, Eng
Andréi Guchin, Eng

RESEARCH

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

The main 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 Signal Processing Department (DPS) at the School of Engineering (Universidad de la República) and the Institut Pasteur de Montevideo (IP 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 (Cryo-EM) 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 Cryo-EM. 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. Currently we are working in the estimation and correction of the Contrast Transfer Function in Cryo-EM “movies” in collaboration with researchers at the NIH (Bethesda, MD, USA).

Another incipient line of research looks for develop new tools for the analysis of evolutive data of viruses, in particular to measure the mutation capacity of the virus and their ability to adapt to new environments. This relates high-dimensional data analysis with the study of RNA virus populations and NGS technologies, a challenging task as each virus population evolution over time is composed of a mutant cloud rather than genomes with the same nucleotide sequence. This line of research intends to develop a tool to analyze and visualize the data obtained from evolutive experiments of different arbovirus species in order to understand the mechanisms that underlie the adaptation of these viruses to different environments.

EDUCATION-COURSES

We co-organized the theoretical and practical course “Processing and Analysis of Fluorescence Microscopy Images” (PAFMI) 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 were organized 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 connects students with introductory and advanced courses in signal and image processing, pattern recognition, programming, information theory, among others.

Among these courses, an introductory course on image processing for biology and medicine was organized in July 2016 at the School of Engineering. See "Procesamiento de Imágenes para Biología y Medicina" (PIMBIO) at http://www.imagina.ei.udelar.edu.uy/pimbio/ for more information. More than thirty-five participants attend this three weeks course, including Master and PhD students, researchers and technicians from the microscopy units of several research centers in Montevideo.

We also have participated in other courses at the IP Montevideo, for example, lectures in Human Genome Tour (HGT 2016) in Automatic Learning Techniques applied to Whole Genome Prediction of human diseases.

TRAINING OF STUDENTS

Research Assistants at the LPS are performing postgraduate thesis or are at the last stages of their undergraduate degree in electrical engineering. The LPS also 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 Congress on 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 del Uruguay. 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 the congress, bioinformatics and human and animal health are one of the most relevant topics.

GRANTS

1. INNOVA II

PUBLICATIONS

1. Similarity measure for cell membrane fusion proteins identification. Daniela Megrian, Pablo S. Aguilar, Federico Lecumberry. Progress in Pattern Recognition, Image Analysis, Computer Vision, and Applications: 21st Iberoamerican Congress, CIARP 2016, Lima, Perú, November 8-11, 2016, Lecture Notes in Computer Science, Springer, , page 257–265 – 2016.

2. Medida del largo de cilias primarias: Un plugin para ImageJ. Mauricio Ramos, Paola Lepanto, Florencia Irigoin, Federico Lecumberry. Acta Microscópica, Volume 25 Supp A – 2016.

3. A confocal microscopy image analysis method to measure adhesion and internalization of Pseudomonas aeruginosa multicellular structures into epithelial cells. Paola Lepanto, Federico Lecumberry, Jéssica Rossello, Arlinet Kierbel. Molecular and Cellular Probes, Volume 28, Number 1, page 1-5. Feb. 2013.

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)

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: i) the unique linked thioredoxin-glutathione pathways present in parasitic flatworms and addressing the function of redox and iron-sulfur thioredoxins and glutaredoxins, ii) the malate dismutation pathway, absent in vertebrates, which allow parasites to harvest energy under low oxygen tension. For the malate dismutation pathway we use the nematode *C. elegans* as a model. In addition to understand rational pharmacological targets for worm infections our lab also focuses on worm drug discovery. We have set up a reproducible and automatized whole animal motility assay for anthelmintics drug screening and discovery. This assay is currently being used to screen synthetic and natural product chemical libraries.

COURSES & MEETINGS

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 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.
- Redox networks in flatworm parasites: implications for rational drug design and treatment of neglected diseases. Invited seminar at the National Jewish Health & University of Colorado School of Medicine. Denver, USA. Gustavo Salinas, July 2015.

- *Caenorhabditis elegans* selenoprotein T is involved in odorant aversion and is essential in the avoidance of the pathogenic bacteria *Serratia marcescens*. Romanelli et al. Poster presentation. Thiol-Based Redox Regulation & Signaling" GRC, Stoweflake USA. August 2016.
- A novel thioredoxin-related protein from flatworms binds Fe_2S_2. BIsio et al. Thiol Metabolism and Redox Regulation of Cellular Functions Symposium, Jacksonville, Uruguay. March 2015.
- Selenoproteins T affect the octanol avoidance pathway in *Caenorhabditis elegans*. Romanelli L et al. Thiol Metabolism and Redox Regulation of Cellular Functions Symposium, Jacksonville, Uruguay. March 2015.

STAGES/INTERNSHIPS ABROAD

- Laura Romanelli: 2 months internship at Mark Alkema´s Lab, Univerity of Massachusetts (2016)
- Jorge Pórfido: three weeks internship at Nétor Carrillo Lab, Institute of Molecular and Cellular Biology of Rosario (IBR), Argentina (2016)
- Hugo Bisio: 3 months internship at Massimo Bellanda´s Lab, Università di Padova (2015)

OTHER SICENTIFIC ACTIVITIES of the PI

- Member of the Editorial Board of *The Journal of Biological Chemistry* (2016-2021)
- Guest Editor, together with Marcelo Comini, of the Forum Issue “Alternative thiol-based redox systems” (2016-2017) 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, Experimental Parasitology.
- Member of scientific boards of Universidad de la República and National Agency for Innovation and Research (ANII)

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.

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SERVICES

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

GRANTS

- Expanding C. elegans Research: First Latin American Worm Meeting. Montevideo. Supported by ICGEB, USA Embassy, B´nai B´rith, IPMON, PEDECIBA, UdelaR and Company of Biologists (33.000 USD). Grant received in 2016, Symposium held in 2017. The symposium was organized together with Inés Carrera (Worm Biology Lab) and Andrea Callixto (Universidad Mayor, Chile)
- 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
4. **Romanelli-Cedrez** L, **Carrera** I, Otero L, Miranda-Vizuete A, Mariotti M, Alkema MJ and **Salinas** G. (**2016**) Selenoprotein T is required for pathogenic bacteria avoidance in Caenorhabditis elegans. Under review Free Radicals in Biol and Med. (FRBM-S-16-01292) IF: 5.894
5. **Salinas** G, Gao W, Wang Y, **Bonilla** M, Novikov A, Virginio VG, Ferreira HB, Vieites M , Gladyshev VN, Gambino D, Dai S. (**2016**) The enzymatic and structural basis for inhibition of

Echinococcus granulosus thioredoxin glutathione reductase by gold(I). Antiox Redox Signal. Under review Antiox Redox Signal (ARS-2016-6816.R1) Gustavo Salinas & Sahodong Dai corresponding authors. IF: 7.093

6. Gladyshev VN, Arnér ES, Berry MJ, Brigelius-Flohé R, Bruford EA, Burk RF, Carlson BA, Castellano S, Chavatte L, Conrad M, Copeland PR, Diamond AM, Driscoll DM, Ferreiro A, Flohé L, Green FR, Guigó R, Handy DE, Hatfield DL, Hesketh J, Hoffmann PR, Holmgren A, Hondal RJ, Howard MT, Huang K, Kim HY, Kim IY, Köhrle J, Krol A, Kryukov GV, Lee BJ, Lee BC, Lei XG, Liu Q, Lescure A, Lobanov AV, Loscalzo J, Maiorino M, Mariotti M, Prabhu KS, Rayman MP, Rozovsky S, **Salinas** G, Schomburg L, Schweizer U, Simonović M, Sunde RA, Tsuji PA, Tweedie S, Ursini F, Zhang Y. Selenoprotein Gene Nomenclature. J Biol Chem **2016** 291(46):24036-24040. IF: 4.258

7. Maggioli G, Bottini G, Basika T, Alonzo P, **Salinas** G, Carmona C. (**2016**) Immunization with Fasciola hepatica thioredoxin glutathione reductase failed to confer protection against fasciolosis in cattle. Veterinary Parasitol 224:13-9.

COURSES

2015

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

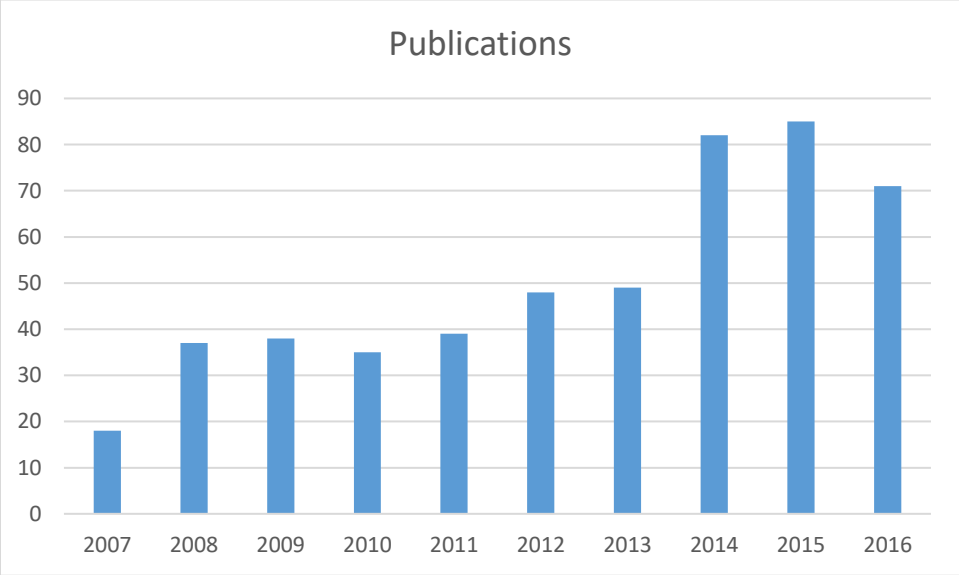
COURSES

2016

Title	Organizers	Date	Foreign Speakers	Foreign Students	Finantial Agencies
"Interactive Methods in Structural Biology to Enhance High Impact Research in Health and Disease"	D. Stuart A. Buschiazzo	Feb 16th - 19th	12	17	BRITISH COUNCIL CEBEM
"Basic Course: Cytometry and Research Applications"	M. Bollati S. Victoria K. Perelmuter	Feb 22th – 26th	0	0	IIBCE PEDECIBA
"Processing and Analysis of Fluorescence Microscopy Images"	F. Lecumberry F. Zolessi	Feb 29th – Mar 21th	6	16	ICGEB PEDECIBA RIIP FOCEM
"Human Genome Tour 2016"	H. Naya	Mar 14th – Apr 1st	5	13	BID
"Theoretical and Practical Course on Laboratory Animals"	M. Crispo A. P. Arevalo M. N. Meikle	Apr 04th - 12th	1	0	OWN FOUNDS
"Progress in Molecular Oncology and Its Impact at Clinical Level"	E. Osinaga M. Varangot	May 02nd - 06th	11	19	ROCHE ASTRAZENECA ABBVIE FOCEM NOVARTIS BIKO
"2nd Meeting of Uruguayan Network of Crystalography"	F. Trajtenberg H. Botti	Aug 29th– 30th	2	0	PEDECIBA
"Riesgo Genético en Cancer: Una Mirada Multidisciplinaria	L. Delgado A. Cayota	Oct 06th-08th	4	0	EMBASSY OF FRANCE EMBASSY OF ISRAEL
"Update on Inmunology from Mechanisms to Immunotherapy and Viceversa"	M. Hill M. Segovia	Oct 19th - 21th	7	12	ANII CLAUSEN CSIC PEDECIBA FOCEM
"Bioethical Aspects of Human Genomics Research"	R. Ehrlich L. Cuñetti	Nov 03th-04th	4	11	FOCEM EMBASSY OF FRANCE UNESCO
"Quitel"	O. Ventura L. Coitiño M. Paulino S. Pantano R. Faccio	Nov 20th - 25th	16	25	ANII PEDECIBA CSIC FOCEM
"Proteome Analysis by Mass Spectometry"	R. Duran P. Carvalho C. Batthyany	Nov 28th - Dec 02nd	5	15	PEDECIBA UNU BIOLAC TECHNOFROM

PUBLICATIONS

A. Historical Evolution of IPMontevideo 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) 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.

10) 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.

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

12) 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.

13) Crispo, M., Mulet, A.P., Tesson, L., Barrera, N., Cuadro, F., Dos Santos-Neto, P.C., Nguyen, T.H., Crénéguy, A., Brusselle, L., Anegón, I., Menchaca, A. Efficient generation of myostatin knock-out sheep using CRISPR/Cas9 technology and microinjection into zygotes (2015) PLoS ONE, 10 (8), art. no. e0136690.

14) Crispo, M., Schlapp, G., Meikle, M.N., Mulet, A.P., Barrera, N., Cuadro, F., Dos Santos-Neto, P.C., Menchaca, A. Advances in the Generation of Genetically Modified (GM) Animal Models: Meeting report (2015) Transgenic Research, 4 p.

15) Crispo, M., Vilariño, M., dos Santos-Neto, P.C., Núñez-Olivera, R., Cuadro, F., Barrera, N., Mulet, A.P., Nguyen, T.H., Anegón, I., Menchaca, A. Embryo development, fetal growth and postnatal phenotype of eGFP lambs generated by lentiviral transgénesis (2015) Transgenic Research, 24 (1), pp. 31-41. Cited 1 time.

16) 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.

17) Darré, L., Machado, M.R., Brandner, A.F., González, H.C., Ferreira, S., Pantano, S. SIRAH: A structurally unbiased coarse-grained force field for proteins with aqueous solvation and long-range electrostatics (2015) Journal of Chemical Theory and Computation, 11 (2), pp. 723-739. Cited 1 time.

18) dos Santos Neto, P.C., 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 (2015) Cryobiology, 70 (1), pp. 17-22.

19) 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).

20) East A, Mechaly AE, Huysmans GHM, Bernarde C, Tello-Manigne D, Nadeau N, Pugsley AP, Buschiazzo A, Alzari PM, Bond PJ, Francetic O. Structural Basis of Pullulanase Membrane Binding and Secretion Revealed by X-Ray Crystallography, Molecular Dynamics and Biochemical Analysis. Structure. Octubre 2015, in the press.

21) Echeverría, N., Moratorio, G., Cristina, J., Moreno, P. Hepatitis C virus genetic variability and evolution (2015) World Journal of Hepatology, 7 (6), pp. 831-845. Cited 2 times.

22) 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.

23) Escande, C., Nin, V., Pirtskhalava, T., Chini, C.C.S., Tchkonia, T., Kirkland, J.L., Chini, E.N. Deleted in breast cancer 1 limits adipose tissue fat accumulation and plays a key role in the development of metabolic syndrome phenotype (2015) Diabetes, 64 (1), pp. 12-22. Cited 2 times.

24) Fernández, M., Arce, E.R., Sarniguet, C., Morais, T.S., Tomaz, A.I., Azar, C.O., Figueroa, R., Diego Maya, J., Medeiros, A., Comini, M., Helena Garcia, M., Otero, L., Gambino, D. Novel ruthenium(II) cyclopentadienyl thiosemicarbazone compounds with antiproliferative activity on pathogenic trypanosomatid parasites (2015) Journal of Inorganic Biochemistry, .

25) 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. doi: 10.1016/j.molbiopara.2015.03.003. Epub 2015 Mar 17. PubMed PMID: 25795082.

26) 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. Cited 1 time.

27) Gaudin A1, Renard E2, Hill M2, Bouchet-Delbos L2, Bienvenu-Louvet G3, Farges JC4, Cuturi MC2, Alliot-Licht B5. 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..

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IP Montevideo

at a glance

Staff

Human Resources	Dec 2010	Dec 2015	Dec 2016
Scientific & Technical Staff	70	189	203
Administration & Support Staff	30	37	40
Total	150	226	243

Publications and Citations (Scopus)

Year	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	Total
Publications	18	37	38	35	39	48	49	82	85	71	502

Aggregate Record	IPM 2007-2016
Number of Publications	502
Accumulated citations	9464
Citations per publication	18,8

Human Resources Training

	2014	2015	2016
Post-docs	24	22	**26**
PhD students	32	33	**44**
Master students	44	47	**51**
Undergraduate students	25	35	**19**
TOTAL	**125**	**137**	**140**

2015-2016 Budget Overview