Institut Pasteur de Montevideo

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Scientific Report 2017

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PREFACE

The Institut Pasteur de Montevideo aims to be 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 are also committed to find new diagnostic tools, treatments and cures, contributing to the development of drugs, vaccines and biomarkers of disease.

By December 2017, more than 260 people worked or studied at the IP Montevideo, including almost 100 established researchers, technicians or assistants coming from several partner institutions and 40 collaborators working in administration and research support units.

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. These programs are mainly funded by institutional grants or grants from several agencies including National Agency for Innovation and Research (ANII) National Institute for Agricultural Research (INIA), National Republic University FOCEM (Mercosur), Interamerican Development Bank (IDB), 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 2017, a significant investment was made to purchase one equipment to study “circular dichroism” and one device for “in vivo imaging”.

The number and impact of the publications have reached the average of 80 publications/year. According to international databanks, publications from the IP Montevideo have a cumulated average of >19 citations per publication, which can be considered of competitive international standard. Remarkably, new patents on a new drug-class technology have been filed in the last year; some of them have been licensed to a start-up company.

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 2017, we harbor more than 140 MSc, doctoral and postdoctoral fellows. We have also organized several international courses on different topics of molecular medicine. In 2017, 71 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.

Finally, the 2017 annual budget of the IP Montevideo was close to 8,5 million dollars, mainly coming from the Uruguayan national budget and from own incomes by service sales, grants and research contracts.

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

Luis Barbeito
Executive Director
Institut Pasteur Montevideo

RESEARCH

CORE FACILITIES

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

Analytical Biochemistry and Proteomics Unit

MEMBERS

Rosario Durán, PhD (Head, Investigator IIBCE – IP Montevideo**)**

Magdalena Portela (Technical Assistant – School of Sciences/IP Montevideo)

Analía Lima, MSc. (Technical Assistant, PhD student)

Jessica Rossello, Biochemist (Technician, PhD student)

Bernardina Rivera, Biochemist (Technician, Graduate student)

Alejandro Leyva (Technician, PhD student)

Associate Members

Andrés Kamaid, PhD (Associate Investigator)

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

Students

Analía Lima, MSc. (PhD student).

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

Alejandro Leyva, Biochemist (PhD student; ANII Fellow)

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

MAIN EQUIPMENT

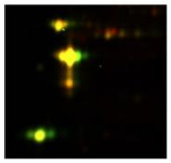
- HPLC, Agilent 1200
- Capilar HPLC, Agilent 1200;
- Nano HPLC, Easy-nLC 1000, Thermo
- Nano HPLC Ultimate 300, Thermo
- 2D Electrophoresis, EttanIPGphor + EttanDaltSix
- Typhoon FLA 9500, GE Healthcare
- 4800 MALDI TOF/TOF Mass Spectrometer, Abi Sciex
- LTQ Velos + ETD Mass Spectrometer, Thermo
- Q-exactive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

4800 MALDI-TOF/TOF, Abi Sciex

LTQ Velos, Thermo

Q-Exactive, Thermo

Ultimate 3000, Thermo



Typhoon FLA 9500, GE

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 2017we analyzed 1394 samples, both routine and non-routine analysis. We would like to highlight that those analysis include around **700 samples for quantitative proteome analysis**.

RESEARCH

The main objectives of the Analytical Biochemistry and Proteomics Unit are: to perform and support biomedical research projects based on mass spectrometry and proteomics, to provide researchers with training, scientific assistance and access to MS-based proteomic technologies, and to actively contribute to local programs and regional education in this area.

A major contribution made by the Analytical Biochemistry and Proteomics Unit was the incorporation of mass spectrometry based proteomics to our local academy, bringing totally new analytical capabilities to perform comprehensive protein studies, including high throughput protein identification and quantitation. During the last years, the Unit has gradually incorporated mass spectrometers and know-how in order to continuously expand the quality and type of analytical procedures available. Currently our analytical portfolio includes shotgun and gel-based comparative and quantitative proteomics strategies, *in vivo* and *in vitro* interactomics, and protein posttranslational modification analysis.

Our own research interest is centered on the characterization of signaling pathways in mycobacteria using proteomic approaches. The ability of *Mycobacterium tuberculosis* to survive in the intracellular environment of host is central to tuberculosis pathogenesis. The signalling cascades mediated by Ser/Thr kinases are key players in this process. In particular one of these enzymes, PknG, became of special interest as it was found to play dual roles in mycobacterial metabolism and bacterial survival inside the host through mechanisms still not completely

understood. In order to unveil PknG partners in mycobacteria we developed a tailored interactomic approach to indentify new substrates and interactors of PknG, which were further validated using *in vitro* and *in vivo* approaches. Interestingly, some of the identified substrates contained a Forkhead-associated domain (FHA) that recognizes phospho-Thr residues and participates in the assembly of multi-component signalling complexes in transduction pathways. Currently, we are characterizing at the molecular level the protein complexes formed *in vivo* by the FHA domain-containing substrates of PknG, and its dynamics in response to the biochemical stimuli found in the host. The experimental strategy we are using combines *in vivo* crosslinking with mass spectrometry, to provide a snapshot of protein interactions in the mycobacteria Altogether, our results point to nitrogen metabolism, cell envelope biosynthesis and response to hypoxia as the main processes regulated by PknG. Through the execution of this project we aim to contribute to unravel the processes controlled by PknG signalling cascades mediated by its FHA domain containing substrates and its possible role in the adaptation to the host environment.

STAFF TRAINING

Internships

- Alejandro Leyva. Laboratory of Toxinology, Fiocruz, Rio de Janeiro, 22- 25 mayo de 2017
- Jessica Rossello, Structural Microbiology Laboratory, Institut Pasteur, Paris. Setiembre-Noviembre 2017. Fellowship: PEDECIBA, Uruguay.
- Magdalena Portela, Structural Microbiology Laboratory, Institut Pasteur, Paris. Setiembre 2017. Fellowship: Institut Pasteur International Network (RIIP):

Courses

- Alejandro Leyva -Sao Paulo School of Advanced Science on Mass Spectrometry-based Proteomics. SPSAS-MS. August 28 to September 6, 2017.
- Analía Lima -Sao Paulo School of Advanced Science on Mass Spectrometry-based Proteomics. SPSAS-MS. August 28 to September 6, 2017.
- "Bernardina Rivera. Espectometría de Masas en Química Clínica. Ïn: Congreso Latinoamericano de Bioquímica Clínica, XI Congreso Uruguayo de Bioquímica Clínica. Punta del Este, Maldonado, Uruguay. September 2017

MEETINGS

Presentations at National Meetings

J. Rossello; A. Lima; M. Gil; J Rodriguez Duarte; A. Correa; P.Carvalho; A. Kierbel; R. Durán. La fosfodiesterasa FcsR de *P. aeruginosa* regula la síntesis de proteínas de flagelo, sistema de secreción tipo III y quimiotaxis en forma independiente de su actividad enzimática. National Congress of Biosciences. May 12 to-14, 2017. Montevideo, Uruguay. Best Poster Award.

B. Rivera; M. Gil; E. Urdániz; M. Portela; M. Piuri; C.Batthyány; R. Durán. Identificación de interactores in vivo de la proteína FhaA en micobacterias. National Congress of Biosciences May 12 to-14, 2017. Montevideo, Uruguay.

A. Lima; M. Gil; A. Cascioferro; J. Rossello; B. Rivera; N. Lisa; M. Bellinzoni; M. Álvarez; C. Batthyány; A. Wehenkel; R. Brosch; P:M. Alzari; R. Durán. Rol de la Ser/Thr-quinasa PknG en la fisiología y patología de *Mycobacterium tuberculosis.* National Congress of Biosciences May 12 to-14, 2017. Montevideo, Uruguay.

A. Lima; M. Gil; A. Cascioferro; J. Rossello; B. Rivera; N. Lisa; M. Bellinzoni; M. Álvarez; C. Batthyány; P. Carvalho; A. Wehenkel; R. Brosch; P:M. Alzari; R. Durán. Rol de la Ser/Thr-quinasa PknG en la fisiología y patología de *Mycobacterium tuberculosis*. I Jornadas Científicas "Prof. Clemente Estable". September 26-27, 2017. Montevideo, Uruguay.

B. Rivera; M. Gil; E. Urdániz; M. Portela; C. Batthyány; M. Piuri; R. Durán. Identificación de interactores *in vivo* de la proteína FhaA en micobacterias. 5to. Encuentro Nacional de Química, ENAQUI, October 2017, Montevideo, Uruguay.

B. Rivera; M. Gil; E. Urdániz; M. Portela; A. Wkenhel; C. Batthyány; M. Piuri; P. Alzari; R. Durán. Identificación *in vivo* de interactores de la proteína FhaA de *M. tuberculosis.* Primeras Jornadas Científicas "Profesor Clemente Estable", September 26-27, 2017. Montevideo, Uruguay.

Presentations at International Meetings

B. Rivera; M. Gil; E. Urdániz; M. Portela; A. Wekenhel; C. Batthyány; M. Piuri; P. Alzari; R. Durán. Identificación in vivo de interactores de la proteína FhaA de M. tuberculosis. XXIII Congreso Latinoamericano de Bioquímica Clínica, XI Congreso Uruguayo de Bioquímica Clínica, Setiembre 2017, Maldonado, Uruguay. Fundación Wiener lab prize.

A. Lima; M. Gil; A. Cascioferro; J. Rossello; B. Rivera; M. Portela; N. Lisa; M. Bellinzoni; M. Alvarez; C. Batthyány; P. Carvalho; R. Brosch; P. Alzari; R. Durán. New substrates and processes regulated by the Mycobacterium tuberculosis Ser/Thr protein kinase PknG revealed by proteomics and interactomic analyses. Sao Paulo School of Advanced Science on Mass Spectrometry-based Proteomics. SPSAS-MS. 28 de agosto de 2017 a 6 de setiembre de 2017. Best Poster Award , 2nd place.

D. Prieto; N. Seija; N. Sotelo; C. Abreu; C. Ortega; R. Durán; M. Gil; V. Irigoin; C. Oliver; A.I. Landoni; R. Gabús; P. Oppezzo. Proteomic characterization of CLL plasma exosomes during disease evolution identify S100-A9 protein as a key molecule in the activation of the canonical NF-kB pathway.1. 6th Young Investigators' Meeting on chronic lymphocytic leukemia, New York, Mayo 2017.

R. Durán "Proteómica y sus aportes a la bioquímica clínica" en XXIII Congreso Latinoamericano de Bioquímica Clínica, 17 - 20 de septiembre de 2017, Punta del Este, Uruguay. Oral presentation.

COURSES

Organization/Collaboration

Course: "Integrating IP Montevideo technologies" Module: Protein characterization by Mass Spectrometry. A. Lima and M. Portela Organizers

GRANTS

As Principal Investigator

Fondo Clemente Estable, Modalidad I. Redes de señalización mediadas por dominios FHA en micobacterias y su rol en la adaptación al ambiente del hospedero (2015-2018) PI. Rosario Durán. FCE_1_2014_1_104045.

As Advisor/Researcher

PICT- 201- 0287. Evaluación de las diferencias genómicas y fenotípicas asociadas con la virulencia Clemente Estable - 2017 Análisis de la proteostasis de un beta rizobio durante el establecimiento de la simbiosis con su hospedero mediante ribosomeprofiling y proteómica en cepas locales de Mycobacterium avium subsp. PI. M Paz Santangelo, INTA Castellar, Bs As.
Fondo Clemente Estable - 2017 Análisis de la proteostasis de un beta rizobio durante el establecimiento de la simbiosis con su hospedero mediante ribosome profiling y proteómica de alto rendimiento. FCE_1_2017_1_136082. PI: Raul Platero (IIBCE).

Fondo Clemente Estable – 2017. Abordaje genómico y proteómico de los mecanismos moleculares de la resistencia al colistin y de la evolución de clones epidémicos de Klebsiella pneumoniae multiresistentes. FCE_1_2017_1_136412. PI. C. Márquez, Fac. Química

PUBLICATIONS

Fló M., Margenat M., Pellizza L., Graña M., Durán R., Báez A., Salceda E., Soto E., Alvarez B., Fernández C. 2017. Functional diversity of secreted cestode Kunitz proteins: Inhibition of serine peptidases and blockade of cation channels. PLoS Pathogens. 13(2):e1006169

Prieto D., Sotelo N., Seija N., Sernbo S., Abreu C., Durán R., Gil M., Sicco E., Irigoin V., Oliver C., Landoni A.I., Gabus R., Dighiero G., Oppezzo P. 2017. S100-A9 protein in exosomes from chronic lymphocytic leukemia cells promotes NF-κB activity during disease progression. Blood. 130(6):777-788

Silva A.R.F., Lima D.B., Leyva A., Duran R., Batthyany C., Aquino P.F., Leal J.C., Rodriguez J.E., Domont G.B., Santos M.D.M., Chamot-Rooke J., Barbosa V.C., Carvalho P.C. 2017DiagnoProt: a tool for discovery of new molecules by mass spectrometry. Bioinformatics. 33(12):1883-1885. doi: 10.1093/bioinformatics/btx093

Cabrera G., Lundberg U., Rodríguez-Ulloa A., Herrera M., Machado W., Portela M., Palomares S., Espinosa L.A., Ramos Y., Durán R., Besada V., Vonasek E., González L.J. 2017 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. 150:183-200

Folle A.M., Kitano E.S., Lima A., Gil M., Cucher M.; Mourglia-Ettlin G., Iwai L.K., Rosenzvit M., Battyány C., Ferreira A.M. Characterisation of Antigen B protein species present in the hydatid cyst fluid of *Echinococcus canadensis* G7 genotype. PLoS Neglected Tropical Diseases, 2017.

Rossello J., Lima A., Gil M., Duarte J.R., Correa A., Carvalho P.C., Kierbel A., Durán R. 2017. The EAL-domain protein FcsR regulates flagella, chemotaxis and type III secretion system in Pseudomonas aeruginosa by a phosphodiesterase independent mechanism. ScientificReports. 7(1): 10281.

INTERNATIONAL PATENT: under the Patent Cooperation Treaty (PCT)

1. "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

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

Recombinant Proteins Unit

MEMBERS

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

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 (Cº 12 to Cº 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*).

COURSES

"Integrating IP Montevideo technologies" Organizer Agustín Correa. A module-based course for IP Montevideo's staff.

PUBLICATIONS

Selected articles

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

Potent and specific inhibition of glycosidases by small artificial binding proteins (affitins). Correa A, Pacheco S, Mechaly AE, Obal G, Béhar G, Mouratou B, Oppezzo P, Alzari PM, Pecorari F. PLoS One. 2014 May 13;9(5):e97438. doi: 10.1371/journal.pone.0097438. eCollection 2014.

Generation of a vector suite for protein solubility screening. Correa A, Ortega C, Obal G, Alzari P, Vincentelli R, Oppezzo P. Front Microbiol. 2014 Feb 25;5:67. doi: 10.3389/fmicb.2014.00067. eCollection 2014.

Tuning different expression parameters to achieve soluble recombinant proteins in E. coli: advantages of high-throughput screening. Correa A, Oppezzo P. Biotechnol J. 2011 Jun;6(6):715-30. doi: 10.1002/biot.201100025. Epub 2011 May 12. Review.

Protein Crystallography Facility (PXF)

MEMBERS

Alejandro Buschiazzo, PhD (Head of the Lab)
Joaquin Dalla Rizza (Technician)
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

The purpose of the Protein Crystallography Facility (PXF) is to provide equipment, training, assistance, and technological innovations for determining three-dimensional structures of protein and other macromolecules and macromolecular assemblies. X-ray crystallography is one of the most powerful techniques to study the three-dimensional structures of macromolecules and it has transformed our understanding of biological processes. This facility allows users to crystallize macromolecules and solve their 3D structures by X ray diffraction.

MAIN EQUIPMENT

Crystallization robot – Honeybee963®

The Honeybee963® (Digilab) robot is a bench-top system for the automation and miniaturization of vapor diffusion in sitting-drop protein crystallization experiments. Proprietary Cartesian synQUAD® dispensers couple high-speed micro-solenoid valves with high resolution syringe pumps, dispensing volumes down to 100 nL. We currently use 200-300 nL nanodrops to maximize precision and crystallizability.
The 96-needle arm allows for very fast dispensing of reservoir solutions on a 96-well setup. Three independent protein synQUAD® needles then proceed to dispense up to three different proteins, variable volumes are defined using the robot's software. Automation enables the assay of typically hundreds of different potential crystallogenesis conditions in a matter of minutes, allowing to greatly increase the search space, a known requisite to raising the probability of finding hits.

Screen Production and Optimization robot – Alchemist DT®

The Alchemist DT ® (Rigaku) is a bench-top liquid handling robot for the screen production and optimization of crystallization conditions. It provides consistent, precise and accurate liquid dispensing in a volume range of 1 µl to 10 ml into SBS, Linbro ® and Nextal® footprint plates Due to its technology, elimination of tubing means no waste and removes the possibility of cross-contamination.
CrystalTrak™, the integrated software package, is designed specifically for protein crystallography. Once the screen is designed, CrystalTrak™ automatically calculates the recipe and defines the necessary stock solutions for use with the Alchemist. 26 different stock solutions can be stored on the deck at one time. Stock management tools and barcode tracking ensure that the correct stock solutions and necessary volumes of solutions are available on the deck before any plate generation begins.

X ray generator – Rigaku MicroMax-007HF®

Micromax007-HF® (Rigaku) is an X-ray generator with a 0.07 mm diameter effective focal size at the source. Equipped with a Cu rotating anode, it provides an output of 1200 W and a brightness of 31 kW/mm2. In combination with the installed optics (Varimax-HF®, Rigaku) which consists of confocal multilayer mirrors, the resulting X rays focused on the crystalline sample are ultra-bright, and can be used effectively for various measurement purposes. We can solve structures with atoms that scatter anomalously at 1.5418Å wavelength (S, I, Cs, lanthanides, among the most used). Molecular replacement problems can be tackled, as well as data collection for high-resolution refinement (including ligands, inhibitors, drugs, point-mutation protein variants, etc).

Image plate area detector – MAR345®

The MAR345® (Mar Research) detector installed on a MAR345dtb®table is an image plate detector that enables us to collect data up to 1.2Å resolution on our geometric setup (taking full advantage of the 2θ angle). It is a single Φ-angle oscillation setup, equipped with a convenient χ-motor that facilitates crystal mounting under cryogenic conditions. Read-out cycles range from 108 to 34 seconds, depending on pixel size and effectively scanned plate diameter.

The read-out system of the Mar345 is unique in its use of a single high performance 85mW laser which delivers more than 0.8 μJ/pixel at the plate. This ensures that an extremely high percentage (>95%) of trapped F-centers are transformed into photostimulated luminiscence.

X ray cryosystem – 700 series Cryostream®

The Cryostream® (Oxford Cryosystems) allows a continuous laminar flow of gas nitrogen at cryogenic temperatures during single crystal data collection. Fast cool-down to 100 Kelvin is achievable in 20 minutes. It has a fairly low liquid nitrogen consumption with a variable flow from 5 to 10 litre/minute.

We have an accessory auto-fill system that uses a level probe in the cryosystem's Dewar, that automates the topping-up of the Dewar basically during data collections that last for several hours/days.

Liquid nitrogen generator – LN40®

The LN40® (Rigaku) is a helium compressor-based machine able to produce up to 40lts/day of highly pure (>98%). liquid nitrogen. The gas input comes from the air pumped by the included PSA (Pressure Swing Adsorption) system.

The operation of the cryogenic refrigeration system is based on a closed-loop Gifford-McMahon (GM) helium expansion cycle. The PSA system consists of two basic components: a vessel(s) containing "carbon molecular sieve"(CMS) and an air compressor(s) or source of clean dry air.

SERVICES

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

COURSES

Sixth edition of our regular workshop “Macromolecular Crystallography School “Structural Biology to enhance high impact research in health and disease”. Organizers: Alejandro Buschiazzo (Inst Pasteur Montevideo) and Ronan Keegan (CCP4, United Kingdom). November 13-23rd, 2017. Full-time course, with advanced theory and hands-on practice with real structure solution problems. 23 students (21 international), 17 lecturers (14 international). Supported by: CCP4, IUCr and CeBEM.

“Macromolecular Crystallography”: 2-days module within the course “Integrating Technologies at IP Montevideo”. 10 undergraduate and graduate students.

PUBLICATIONS

San Martin F, Mechaly AE, Larrieux N, Wunder EA Jr, Ko AI, Picardeau M, Trajtenberg F, Buschiazzo A. Crystallization of FcpA from *Leptospira*, a novel flagellar protein that is essential for pathogenesis. Acta Crystallogr F Struct Biol Commun. 2017 Mar 1;73(Pt 3):123-129. doi: 10.1107/S2053230X17002096.

Mechaly AE, Soto Diaz S, Sassoon N, Buschiazzo A, Betton JM, Alzari PM. Structural coupling between autokinase and phosphotransferase reactions in a bacterial histidine kinase. Structure. 2017 Jun 6;25(6):939-944.e3. doi: 10.1016/j.str.2017.04.011.

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 (Research assistant)
Gregorio Iraola, PhD (PosDoc)
Pablo Fresia, PhD (Research Assistant)
Ignacio Ferrés (Bioinformatics MSc student)
Verónica Antelo (Biology PhD student)
Daniela Costa (Biology PhD student)
Raúl Feijo (Msc 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.

COURSES

"Herramientas prácticas para el análisis de GWAS en cultivos" May 22nd –June 2nd, 2017. Organizer PhD Luisa Berná. 13 lecturers (2 international). 17 students (9 international). Supported by INIA, CABIO, FOCEM. Collaborating lecturers

"Hands on Metagenomics data analysis: tools for bioprospection in environmental and clinical microbiology"September 25th – October 6th, 2017. 11 lecturers (7 international), 22 students (14 international). Supported by: UNU BIOLAC, French Cooperation, CAMPUS FRANCE and FOCEM. Organizers

"Deciphering regulator RNA functions by high-throughput sequencing". December 4-8th, 2017. 15 lecturers (7 international), 18 students (9 international). International course funded by: UNU BIOLAC, FOCEM, and private sponsors. Collaborating lecturers

GRANTS

1. "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)
2. "Strengthening technical and human capacities for genomic services exports". Funded by IDB 2014/2017. H Naya
3. "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.
4. "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)
5. Proyecto Metagenómica – ANII (Gregorio Iraola)

PUBLICATIONS

- Cáceres A., Muñoz I., Iraola G., Díaz-Viraqué F., Collado L. 2017. Campylobacter ornithocola sp. nov., a novel member of the Campylobacter lari group isolated from wild bird faecal samples. International Journal of Systematic and Evolutionary Microbiology. 67(6) 001822: 1643-1649
- Calleros L., Betancor L., Iraola G., Méndez A., Morsella C., Paolicchi F., Silveyra S., Velilla A., Pérez R. 2017. Assessing the intra-species genetic variability in the clonal pathogen

Campylobacter fetus: CRISPRs are highly polymorphic DNA markers. Journal of Microbiological Methods. 132: 86-94.

- Dallagiovanna B., Pereira I.T., Origa-Alves A.C., Shigunov P., Naya H., Spangenberg L. 2017. lncRNAs are associated with polysomes during adipose-derived stem cell differentiation. Gene. 610: 103-111
- Fresia P., Jara R., Sierra R., Ferrés I., Greif G., Iraola G., Collado L. 2017. Genomic and clinical evidence uncovers the enterohepatic species Helicobacter valdiviensis as a potential human intestinal pathogen. Helicobacter. 22(5): e12425.
- Iraola G., Forster S.C., Kumar N., Lehours P., Bekal S., García-Peña F.J., Paolicchi F., Morsella C., Hotzel H., Hsueh P.-R., Vidal A., Lévesque S., Yamazaki W., Balzan C., Vargas A., Piccirillo A., Chaban B., Hill J.E., Betancor L., Collado L., Truyers I., Midwinter A.C., Dagi H.T., Mégraud F., Calleros L., Pérez R., Naya H. & Lawley T.D. 2017. Distinct Campylobacter fetus lineages adapted as livestock pathogens and human pathobionts in the intestinal microbiota. Nature Communications, 8:1367.
- Iraola G., Pérez R., Betancor L., Marandino A., Morsella C., Méndez A., Paolicchi F., Piccirillo A., Tomás G., Velilla A., Calleros L. 2016. A novel real-time PCR assay for quantitative detection of Campylobacter fetus based on ribosomal sequences. BMC Veterinary Research. 12(1): 286
- Luna F., Naya H., Naya D.E. 2017. Understanding evolutionary variation in basal metabolic rate: An analysis in subterranean rodents. Comparative Biochemistry and Physiology -Part A: Molecular and Integrative Physiology. 206:87-94.
- Naya D.E., Naya H., Cook J. 2017. Climate change and body size trends in aquatic and terrestrial endotherms: Does habitat matter? PLoS ONE. 12(8): e0183051.
- Naya H., Peñagaricano F., Urioste J.I. 2017. Modelling female fertility traits in beef cattle using linear and non-linear models. Journal of Animal Breeding and Genetics. 134(3):202-212.
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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. Festari M.F., Trajtenberg F., Berois N., Pantano S., Revoredo L., Kong Y., Solari-Saquieres P., Narimatsu Y., Freire T., Bay S., Robello C., Bénard J., Gerken T.A., Clausen H., Osinaga E. 2017. Revisiting the human polypeptide GalNAc-T1 and T13 paralogs. Glycobiology. 27(1):140-153

Cell Biology Unit

MEMBERS:

Mariela Bollati-Fogolín, PhD (Head)
Karen Perelmuter, MSc (Staff TA)
Sabina Victoria, MSc (Staff TA)
Romina Pagotto, PhD (Research Associate)
Cecilia Abreu, PhD (Research Associate)
Vanesa Piattoni, PhD (Postdoctoral fellow)
Hellen Daghero (Master student)
Karin Grunberg, (Technician, MSc student)
Constanza Silvera, (Intern, Undergraduate student)

Micaela Sureda (Research internship) – past member
Inés Tiscornia, MSc (Staff TA) – past member
Giuliana Mastropietro (BSc student) – finished August 2015
Soledad Astrada, (PhD student, finished December 2016)

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)

References:

- Astrada S *et al*. 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.
- Bürgi M *et al*. WISH cell line: From the antiviral system to a novel reporter gene assay to test the potency of human IFN-α and IFN-β. J Immunol Methods. 2012, 381(1-2):70-4.
- Bürgi M, *et al*. Screening and characterization of molecules that modulate the biological activity of IFNs-I. Journal of Biotechnology, 10.1016/j.jbiotec.2016.06.021 (accepted).
- Fernández Massó JR *et al*. The Antitumor Peptide CIGB-552 Increases COMMD1 and Inhibits Growth of Human Lung Cancer Cells. J Amino Acids. 2013; 251398.
- Núñez de Villavicencio-Díaz T *et al*. Comparativeproteomicsanalysis of theantitumoreffect of CIGB-552 peptide in HT-29 colon adenocarcinoma cells. J Proteomics. 2015-a May 24;126:163-171. doi: 10.1016/j.jprot.2015.05.024.
- 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

- Tiscornia I *et al*. Human monocyte-derived dendritic cells from leukoreduction system chambers after plateletpheresis are functional in an in vitro co-culture assay with intestinal epithelial cells. J ImmunolMethods. 2012, 384(1-2):164-70.
- Vallespí MG *et al*. Antitumorefficacy, pharmacokinetic and biodistributionstudies of theanticancerpeptide CIGB-552 in mouse models. J Pept Sc (2014), 20(11):850-9.

COURSES

"Cell Animal Models for Drug Discovery" October 16-27th 2017, 7 national lecturers, 8 international lecturers, 26 students (20 international). Funded by ICGEB, RIIP, UNU BIOLAC and FOCEM.

GRANTS

1. 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.
2. 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.
3. 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.

Other fundings:

4. Postdoctoral fellowship IP de Montevideo, V. Piattoni, 2015-2017.
5. Master fellowship ANII-POS_NAC_2015_1_109487, H. Daghero, 2016- 2018.ICGEB, RIIP and UNUBiolac Grants to organize the course CELL AND ANIMAL MODELS FOR DRUG DISCOVERY, October 2017.

PUBLICATIONS

Eugenia Schroeder M., Russo S., Costa C., Hori J., Tiscornia I., Bollati-Fogolín M., Zamboni D.S., Ferreira G., Cairoli E., Hill M. 2017. Pro-inflammatory Ca++-activated K+ channels are inhibited by hydroxychloroquine. Scientific Reports. 7(1):01836-8.

Mulet AP, Perelmuter K, Bollati-Fogolin M, Crispo M, Grompone G. 2017. Forkhead Box Protein O1 is Linked to Anti-Inflammatory Probiotic Bacteria Acting through Nuclear Factor-κB Pathway. J Microb Biochem Technol Volume 9(3):074-081

Schroeder M.E., Russo S., Costa C., Hori J., Tiscornia I., Bollati-Fogolín M., Zamboni D.S., Ferreira G., Cairoli E., Hill M. 2017. Pro-inflammatory Ca++ -activated K+ channels are inhibited by hydroxychloroquine. Scientific Reports. 7, Article number: 1892.

Transgenic and Experimental Animal Unit

MEMBERS

Martina Crispo, DVM, PhD (Head)
Ana Paula Mulet, PhD (Technician)
Tatiana Basika, PhD (Technician)
Geraldine Schlapp, MSc (Full time technician)
María Noel Meikle, MSc (Technician)
Ana Paula Arévalo, **TMN** (Technician, MSc student)
Gabriel Fernández, BSc (Technician)
Sergio Ancheta (Animal caretaker)
Andrés Pereyra (Animal caretaker)
Nicolás Fiore (Animal caretaker)

Postgraduate students in collaboration with IRAUy (National Institute for Animal Reproduction)
Pedro Dos Santos, DVM, MSc (PhD student)
Natalibeth Barrera, BSc (MSc Student)
Federico Cuadro, DVM (MSc Student)

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 transgenic mice by CRISPR/Cas9 technology
- Generation of transgenic mice by pronuclear microinjection of DNA fragments
- Generation of transgenic mice by gene-targeting in embryonic stem cells
- Generation of transgenic mice by Sleeping Beauty Transposons technology
- Embryo and sperm cryopreservation
- Rederivation of mouse lines
- In vitro fertilization
- Breeding and housing of SPF and conventional mice (C57BL/6J, BALB/cJ, DBA/2J, Nude, several hybrids and aprox. 30 different transgenic lines). Actual production: aprox. 2000/month.
- Four trials of acute safety of probiotic bacteria for Biopolis Company
- Estudio de la actividad hipolipemiante, capacidad antioxidante y actividad anti-inflamatoria de los componentes del extracto de pericarpio derivado de girasol “violeta” (EPGv)”.
- 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).

RESEARCH

Our scientific proposal is to provide high-level regional support in the field of animal genome modification including mice, rats 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.

Since 2007 we work with national and international biotechnological companies, offering *in vivo* biological trials in mice and rats, under GLP standards.

Undergraduated and posgraduated students undergo their thesis in our Laboratory, using the above technologies to obtain different animal models.

We organize national and international courses in the fields of Transgenesis, Genome Edition, Embryo & Sperm Cryopreservation and Lab Animal Science, training hundreds of technicians and scientist from the region and the world. We also provide personal internships in the above fields for technicians or veterinarians who run their own services.
UATE is part of the Programa de Tecnología Molecular, Celular y Animal (ProTeMCA) of the IPMon, providing know how related to animal models.

Our staff are members of National and International Councils related to our field, as the Comité de Articulación Institucional (CAI), Comisión Nacional de Experimentación Animal (CNEA), Asociación Uruguaya de Ciencia y Tecnología de Animales de Laboratorio (AUCyTAL), Federación Sudamericana de CIencia de Animales de Laboratorio (FESSACAL), International Society for Transgenic Technologies (ISTT). In addition, we actively participate in the institutional life in several Committees.

In summary, we have managed to position the Unit at a high regional level in science and technology, which has also resulted in the formation of several human resources and peer review publications.

PROJECTS

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.

TRAINING

Anatomical Bases of Mouse Multimodal Imaging Course. CCP. Prague, Chzeck Republic. January 2017.

II Curso de Diseño y ejecución de ensayos biológicos aplicados a la biomedicina. Centro de Medicina Comparada, Esperanza, Santa Fe, Argentina. December 2017.

MEETINGS

- Transgenic Technologies – May 2017, Harvard, Boston, US - Speaker
- TT2017 – October 2017, St Lake City, Utah, US - Speaker
- Simposio Microorganismos para la Agricultura – Canelones, Uy – October 2017 - Participant

COURSES

"Curso Teórico- Practico de Animales de Laboratorio" March 13-21st 2017, 19 national lecturers, 1 international lecturer, 21 national students. Supported by FOCEM and PEDECIBA.

"Cell Animal Models for Drug Discovery" October 16-27th 2017, 7 national lecturers, 8 international lecturers, 26 students (20 international). Funded by ICGEB, RIIP, UNU BIOLAC and FOCEM.

"Integrando las tecnologías del IP Montevideo" IP Montevideo internal course – October 2017.

"TRANSGENIC TECHNOLOGIES in MODELING HUMAN DISEASES: Principles, Associated technologies, Animal Management and Ethics" – June 2017, Athens, Greece. Co-organizer.

GRANTS

- Akouos Gene Editing Sheep Model (IRAUy-Harvard U)
- ProTeMCA

PUBLICATIONS

1. dos Santos-Neto P.C., Cuadro F., Barrera N., Crispo M., Menchaca A. 2017. Embryo survival and birth rate after minimum volume vitrification or slow freezing of in vivo and in vitro produced ovine embryos. Cryobiology. 78(8): 14.
2. Menchaca A., Schlapp G., Meikle M.N., Crispo M. 2017. Transgenesis and Gene Edition in Mammals. Reference Module in Life Sciences. Elsevier. V.1: 1-9 pp (Book chapter).

3. Menchaga A., AP Mulet, P. Dos Santos Neto, M. Crispo 2017. Transgenesis and Gene Edition in Small Ruminants. Revista Brasileira de Reprodução Animal, v.: 41 1, 2017

4. Mulet AP, Perelmuter K, Bollati-Fogolin M, Crispo M, Grompone G. 2017. Forkhead Box Protein O1 is Linked to Anti-Inflammatory Probiotic Bacteria Acting through Nuclear Factor-κB Pathway. J Microb Biochem Technol Volume 9(3):074-081

Biopharmaceutical Quality Control & Development Laboratory

MEMBERS

Alejandro Ricciardi, PharmD. (Head)
Larissa Armas, Technical Assistant
Virginia Bengochea, Technical Assistant (MSc student)
Julia Sanguinetti, MSc., Technical Assistant
Bernardina Rivera, Technical Assistant

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

Estudios Conformacionales de proteínas y Biofarmarcos Mediante Dicroismo Circular (PEC_1_2016_1_131933). ANII

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.

RESEARCH

PROGRAMS and their respective fundational LABORATORIES and Core Facilities

Animal Health Program

- ImmunoVirology
- Molegcular & 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

- Vascular Biology and Drug Development
- 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

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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

- 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

Fló M., Margenat M., Pellizza L., Graña M., Durán R., Báez A., Salceda E., Soto E., Alvarez B., Fernández C. 2017. Functional diversity of secreted cestode Kunitz proteins: Inhibition of serine peptidases and blockade of cation channels. PLoS Pathogens. 13(2):e1006169

Molecular and Structural Microbiology

MEMBERS

Alejandro Buschiazzo, PhD (Head of the Lab)
Joaquin Dalla Rizza (Technician)
Juan Andrés Imelio (MSc student)
Natalia Lisa, 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)

Past members
Horacio Botti
Mathias Ferrari
Sofía Horjales
Frank Lehmann (Technician) - past member, currently Lab Manager at University of Basel (Switzerland)
Ariel Mechaly (currently Honorary Research Associate)
Natalia Morero
Natalia Ruétalo

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 currently comprises at least 13 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 the pathogen *L. interrogans*, as well as the saprophytic model *L. biflex*a.

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.

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.

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; Adhikarla et al. *Front Cell Infect Microbiol.* 2018 8:45). 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.

In collaboration with Dr Hugo Gramajo (Instituto de Biologia Molecular y Celular IBR, Rosario, Argentina), we aim at elucidating the crystal structures and molecular mechanisms of transcription factors that regulate the biosynthesis of long-chain fatty acids in *Mycobacterium tuberculosis*. Dr Julia Lara from the Gramajo lab, spent time in our laboratory (2017), and a first manuscript is now being prepared.

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. *Acta Crystallogr F* 2017 73:123; Wunder et al. *Front Cell Infect Microbiol.* 2018 8:130).

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 University Medical School (Instituto de Higiene, UdelaR), the Ministry of Livestock, Agriculture and Fishery (DILAVE, MGAP) and the National Agronomic Research Institute (INIA), is essential for this initiative's great success. *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 >50 strains (Zarantonelli et al., 2018, under revision), along a 3-years project that will finish at the end of 2018. 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.

The recently created Joint International Unit "Integrative Microbiology of Zoonotic Agents" (IMiZA), associates our laboratory with the Picardeau group at Institut Pasteur Paris (Biology of Spirochetes Unit, Dept of Microbiology). Activities started in 2017, with a kick-off meeting in Montevideo. IMiZA is being instrumental for the Cecilia Nieves's MSc Thesis (expected to defend in July 2018), as well as for Juan Imelio's PhD Thesis work just starting: Imelio spent 3 months at the Picardeau lab nov 2017-feb 2018, as part of his PhD project.

COLLABORATIVE WORK

1. Coordination of a multicentric consortium in Uruguay, working with teams at the Ministry of Livestock, Agriculture and Fishery (Alejandra Suanes, Rodolfo Rivero, DILAVE, MGAP), the National Institute of Agronomic Research (Franklin Riet, INIA), the Bacteriology Dept at the University Medical School (Felipe Schelotto, Instituto de Higiene, UdelaR). Tackling issues of veterinarian *Leptospira* strains isolation, typing, diagnostics and vaccine development.

2. Structural biology of bacterial and plant malic enzymes, work led by the Drincovich lab (CEFOBI, Rosario, Argentina), led to two joint papers (Alvarez et al. *Acta Crystallogr D* 2018 74:332; Bovdilova et al. 2018 submitted)

3. Structural biology of metallo-β-lactamases, work led by the Vila lab (IBR, Rosario, Argentina), leading to a sustained collaboration (Morán-Barrio et al. *Antimicrob Agents Chemother.* 2016 60:6013).

MEETINGS

- April 2017 - 2nd meeting "Protein Biophysics at the End of the World", Buenos Aires (Argentina). Invited speaker (A Buschiazzo), delivered the talk "Bacterial signaling: from structure to movement, and back again".
- June 2017 – XLV Jornadas Uruguayas de Buiatria, Paysandu (Uruguay). Invited speaker (A Buschiazzo) delivered the lecture "Avances en la epidemiología y diagnóstico de la Leptospirosis bovina en Uruguay".
- Aug 2017 – 24th Congress and General Assembly of the International Union of Crystallography, Hyderabad (India). Poster presentation (Imelio et al.) "Two-component systems in bacteria: how is the signal unidirectionally transmitted?"

- Nov 2017 - 10th International Leptospirosis Society Meeting, Palmerston North (New Zealand). Poster presentation (Zarantonelli at al.) "Isolation and Typing of *Leptospira* spp. from Urine and Kidney of Naturally Infected Cattle in Uruguay"
- Invited speaker (A Buschiazzo) to the Gordon Research Conference "Sensory Transduction in Microorganisms", Ventura CA (USA) to be held in Jan 2018.

GRANTS

- 2014-2017 "Tipificación y diagnóstico de *Leptospira* spp. usando aproximaciones moleculares: hacia el diseño de vacunas recombinantes" - ANII, Fondo Sectorial Innovagro #FSA_1_2013_1_12557 (Uruguay). Role: Principal Investigator (A Buschiazzo). Colaborators: Vet A Suanes (DILAVE-MGAP) and Dr M Picardeau (Biology of Spirochetes Unit, Institut Pasteur, France).
- 2015-2018 "Creación y caracterización de un banco de cepas de *Leptospira* spp. aisladas de casos de leptospirosis bovina en Uruguay" - Agencia Nacional de Investigación e Innovación ANII, Programa Alianzas # ALI_1_2014_1_4982 (Uruguay). Role: co-Principal Investigator (A Buschiazzo). Together with: Prof F Schelotto (Medical School, Univ de la Republica, Uruguay), Vet A Suanes, R Rivero (DILAVE,MGAP) and Vet F Riet (Instituto Nacional de Investigaciones Agronómicas INIA).
- 2017-2018 "Estudios estructurales y funcionales del endoflagelo de *Leptospira*: un componente esencial en la patogenicidad de las espiroquetas" - ANII, Fondo Clemente Estable #FCE_3_2016_1_126797 (Uruguay). Role: Principal Investigator (F Trajtenberg).
- 2017-2018 "Banco de Cepas nativas de enfermedades zoonóticas que afectan al ganado Uruguayo para desarrollos de I+D de la industria Uruguaya" - Ministerio de Industria, Energía y Minería MIEM, programa Fondo Industrial de la Dirección Nacional de Industrias, # 2016-8-2-002671 (Uruguay) . Role: collaborator group.
- 2016-2021 "Integrative Microbiology of Zoonotic Agents" - Institut Pasteur (Francia) / Institut Pasteur de Montevideo (Uruguay) - International Joint Units program. Role: co-Principal Investigator (A Buschiazzo), together with Dr P Picardeau (Biology of Spirochetes Unit, Institut Pasteur, France)

PUBLICATIONS

Mechaly A.E., Soto Diaz S., Sassoon N., Buschiazzo A., Betton J.-M., Alzari P.M. 2017. Structural coupling between autokinase and phosphotransferase reactions in a bacterial histidine kinase. *Structure*. 25:939-44.

San Martin F, Mechaly AE, Larrieux N, Wunder EA Jr, Ko AI, Picardeau M, Trajtenberg F, Buschiazzo A. 2017 Crystallization of FcpA from *Leptospira*, a novel flagellar protein that is essential for pathogenesis. *Acta Crystallogr F Struct Biol Commun*. 73:123-9.

Host-Pathogen Interactions

MEMBERS

Carlos Robello, PhD (Head)
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Dolores Piñeyro, PhD (Associated Researcher, Facultad de Medicina)
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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

Berná L., Chiribao M.L., Greif G., Rodriguez M., Alvarez-Valin F., Robello C. 2017. Transcriptomic analysis reveals metabolic switches and surface remodeling as key processes for stage transition in trypanosoma cruzi. PeerJ 5:e3017.

de Oliveira T.C., Rodrigues P.T., Menezes M.J., Gonçalves-Lopes R.M., Bastos M.S., Lima N.F., Barbosa S., Gerber A.L., Loss de Morais G., Berná L., Phelan J., Robello C., de Vasconcelos A.T.R., Alves J.M.P., Ferreira M.U. 2017 Genome-wide diversity and differentiation in New World populations of the human malaria parasite Plasmodium vivax. PLoS Neglected Tropical Diseases. 11(7):e0005824.

Carasi P., Rodríguez E., da Costa V., Frigerio S., Brossard N., Noya V., Robello C., Anegón I., Freire T. 2017 Heme-oxygenase-1 expression contributes to the immunoregulation induced by Fasciola hepatica and promotes infection. Frontiers in Immunology. 8, JUL, 883.

Satragno D., Faral-Tello P., Canneva B., Verger L., Lozano A., Vitale E., Greif G., Soto C., Robello C., Basmadjián Y. 2017. Autochthonous outbreak and expansion of canine visceral Leishmaniasis, Uruguay. Emerging Infectious Diseases. 23(3):536-538. Letter.

Valentin-Kahan A., García-Tejedor G.B., Robello C., Trujillo-Cenóz O., Russo R.E., Alvarez-Valin F. 2017. Gene expression profiling in the injured spinal cord of Trachemys scripta elegans: An amniote with self-repair capabilities. Frontiers in Molecular Neuroscience. 10(17).

Functional Genomics

MEMBERS

Alfonso Cayota, MD, PhD (Head)
Juan Pablo Tosar PhD (Post-doc)
Fabiana Gambaro (MSc Student)

Currently abroad

Ma. Rosa García
Braulio Bonilla MSc

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.

COURSES

"Deciphering regulator RNA functions by high-throughput sequencing". December 4-8th, 2017. 15 lecturers (7 international), 18 students (9 international). Organizer: Dr. Cayota. International course funded by: UNU BIOLAC, FOCEM, and private sponsors.

GRANTS

1. "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) Period 2016-2018
2. "tRNA-derived small RNAs as mediators of survival and growth signals. Granted to Alfonso Cayota by CSIC, Universidad de la República. Period 2017-2019

PUBLICATIONS

1. Tosar J.P., Cayota A., Eitan E., Halushka M.K., Witwer K.W. 2017. Ribonucleic artefacts: Are some extracellular RNA discoveries driven by cell culture medium components? Journal of Extracellular Vesicles. 6(1): 1272832.
2. Mateescu B., Kowal E.J.K., van Balkom B.W.M., Bartel S., Bhattacharyya S.N., Buzás E.I., Buck A.H., de Candia P., Chow F.W.N., Das S., Driedonks T.A.P., Fernández-Messina L., Haderk F., Hill A.F., Jones J.C., Van Keuren-Jensen K.R., Lai C.P., Lässer C., di Liegro I., Lunavat T.R., Lorenowicz M.J., Maas S.L.N., Mäger I., Mittelbrunn M., Momma S., Mukherjee K., Nawaz M., Pegtel D.M., Pfaffl M.W., Schiffelers R.M., Tahara H., Théry C., Tosar J.P., Wauben M.H.M., Witwer K.W., Nolte-'t Hoen E.N.M. 2017. Obstacles and opportunities in the functional analysis of extracellular vesicle RNA - An ISEV position paper. Journal of Extracellular Vesicles. 6(1):1286095

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. Barrera E.E., Frigini E.N., Porasso R.D., Pantano S. 2017. Modeling DMPC lipid membranes with SIRAH force-field. Journal of Molecular Modeling. 23(9): 259.
2. Brandner A., Schüller A., Melo F., Pantano S. 2017. Exploring DNA dynamics within oligonucleosomes with coarse-grained simulations: SIRAH force field extension for protein-DNA complexes. Biochemical and Biophysical Research Communications. Sept 2017 doi: 10.1016/j.bbrc.2017.09.086
3. Calì T., Frizzarin M., Luoni L., Zonta F., Pantano S., Cruz C., Bonza M.C., Bertipaglia I., Ruzzene M., De Michelis M.I., Damiano N., Marin O., Zanni G., Zanotti G., Brini M., Lopreiato R., Carafoli E. 2017. The ataxia related G1107D mutation of the plasma membrane Ca2 + ATPase isoform 3 affects its interplay with calmodulin and the autoinhibition process. Biochimica et Biophysica Acta - Molecular Basis of Disease. 1863(1): -165-173
4. Festari M.F., Trajtenberg F., Berois N., Pantano S., Revoredo L., Kong Y., Solari-Saquieres P., Narimatsu Y., Freire T., Bay S., Robello C., Bénard J., Gerken T.A., Clausen H., Osinaga E. 2017. Revisiting the human polypeptide GalNAc-T1 and T13 paralogs. Glycobiology. 27(1):140-153
5. MacHado M.R., González H.C., Pantano S. 2017. MD Simulations of Viruslike Particles with Supra CG Solvation Affordable to Desktop Computers. Journal of Chemical Theory and Computation. 13(10): 5106-5116
6. Marcello A., Pantano S. 2017. Interdisciplinary approaches to the study of flavivirus. Biochemical and Biophysical Research Communications. 492(4):531-532
7. Surdo N.C., Berrera M., Koschinski A., Brescia M., Machado M.R., Carr C., Wright P., Gorelik J., Morotti S., Grandi E., Bers D.M., Pantano S., Zaccolo M. 2017. FRET biosensor uncovers cAMP nano-domains at b-adrenergic targets that dictate precise tuning of cardiac contractility. Nature Communications. 8, Article number: 15031.

COURSES AND CONFERENCES

“Performing Molecular Simulations with SIRAH force field”. December 11-15th 2018. Organizer: PhD. Sergio Pantano. 5 lecturers, 15 international students. Supported by FOCEM.

“Integrando las tecnologías del IP Montevideo” IP Montevideo internal training course based on the technologies available at the institute. Collaborator: Matías Machado

GRANTS

Redox Biology of Trypanosomes

MEMBERS

Marcelo Comini, PhD (Principal Investigator)
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Cecilia Ortíz (PhD student)
Diego Benitez, PhD
Florencia Sardi (PhD Student)
Jaime Franco (MSc Student)
Matías Deambrosi (MSc student)

RESEARCH

By means of a multidisciplinary approach we study the biochemical, structural and biological features that distinguish several key components of the redox system from pathogenic trypanosomatids, parasites that are causative agents of severe diseases in animals and humans. Several essential cellular processes are regulated and/or depend on redox reactions that have cysteine residues as targets or mediators. Important from a therapeutic point of view, the components of the redox system from trypanosomatids significantly differ from those present in the mammalian hosts, which opens the possibility for a selective inhibition of parasite proliferation. Our research aims at deepening the understanding on parasite redox biology to tackle it selectively with compounds having pharmacological potential.

FUNDAMENTAL ASPECTS OF TRYPANOTHIONE METABOLISM: SYNTHESIS, REDUCTION AND UTILIZATION

Our research focuses on key components of the trypanothione system, its biochemical and structural features. 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

Our laboratory exploit and develop novel fluorescent protein-based redox biosensors to monitor intracellular redox changes on real-time and by non-invasive methods. We have generated redox reporter cell lines of different trypanosomatids that 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. Drug repositioning is also an active area of research in our group. Compounds' 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.

COURSES

1. "Curso Teórico- Práctico de Animales de Laboratorio" March 13-21st 2017, 20 lecturers (1 international), 21 national students. Supported by FOCEM and PEDECIBA.
2. "Cell and Animal Models for Drug Discovery" October 16-27th 2017, 15 lecturers (8 international), 26 students (20 international). Funded by ICGEB, RIIP, UNU BIOLAC and FOCEM.
3. "Integrando las tecnologías del IP Montevideo" IP Montevideo internal training course based on the technologies available at the institute

GRANTS

1. The thioredoxin-fold diversity in trypanosomatids and tapeworms. Co-PI: M. Comini. Euros 45000 ICGEB 2015-2017 . Project CRP/URU 14-01–, 2015-2017
2. Target-based drug discovery of compounds interfering with trypanothione biosynthesis in trypanosomatids. PI: M. Comini. Euros 70000. ACIP Grant - Project A-17-2015, 2015-2017.
3. Diseño y producción de nuevas variantes de la hormona folículo estimulante (FSH) para su empleo en especies de interés productivo. PI: M. Bollati. Alianza para la Innovación, Modalidad Desarrollo Tecnológico, ANII. ALI_1_2015_1_5084 (2016-2019).
4. Diseño de biosensores para monitoreo simultáneo de señalización redox y cAMP: desde la computadora la célula y vuelta a la computadora. PI: S. Pantano. Fondo María Viña, ANII, FMV_1_2014_1_104000 (2015-2018).

PUBLICATIONS

1. Fonseca M.S., Comini M.A., Resende B.V., Santi A.M.M., Zoboli A.P., Moreira D.S., Murta S.M.F 2017. Ornithine decarboxylase or gamma-glutamylcysteine synthetase overexpression protects Leishmania (Vianna) guyanensis against antimony. Experimental Parasitology 175: 36-43

2. Franco J., Medeiros A., Benítez D., Perelmuter K., Serra G., Comini M.A., Scarone L. 2017. In vitro activity and mode of action of distamycin analogues against African trypanosomes. European Journal of Medicinal Chemistry 126: 776-788.

3. Franco J., Sardi F., Szilágyi L., Kövér K.E., Fehér K., Comini M.A. 2017. Diglycosyl diselenides alter redox homeostasis and glucose consumption of infective African trypanosomes. International Journal for Parasitology: Drugs and Drug Resistance 7(3): 303-313

4. Manta B, Bonilla M, Fiestas L, Sturlese M, Salinas G, Bellanda M, Comini MA. 2017. Polyamine-based thiols in Trypanosomatids: evolution, protein structural adaptations and biological functions. Antioxid Redox Signal. 2017 Oct 19. doi: 10.1089/ars.2017.7133. [Epub ahead of print] PubMed PMID: 29048199.

Chronic Lymphocytic Leukemia

MEMBERS

Pablo Oppezzo, PhD (Head)
Pablo Morande, PhD (Post-doctoral position)
Sandra Sernbo, PhD (Post-doctoral position)
Agustín Correa, PhD (Assistant Researcher)
Claudia Ortega, PhD (Technical Assistant)
Daniel Prieto, MSc (PhD student)
Noé Seija, BSc (MSc. student)

RESEARCH

The major focus of the Research Laboratory on Chronic Lymphocytic Leukemia ***(rCLL)*** has been the study of the mechanisms involved in the origins and progression of Chronic Lymphocytic Leukemia (CLL). The B lymphocyte is one of the most specialized cells able to re-edit its DNA to diversify the repertoire of immunoglobulin. This action is dependent of the enzyme activation-induced cytidine deaminase (AID) which is responsible for Class Switch Recombination (CSR) and Somatic Hypermutation process. Since these events are essential for the immune response, the B lymphocyte is continuously exposed to a dangerous mutagenic mechanism that should be tightly regulated. In absence of these safeguard mechanisms AID appears to be responsible of tumor development. **Our advances are related to the characterization of AID expression in patients with CLL.** We are the first to report an anomalous expression of AID in the peripheral blood of progressive CLL patients (**Oppezzo et al., Blood, 2003)** and that its expression is tightly regulated by a spliced form of the transcription factor Pax-5a **(Oppezzo et al., Blood, 2005).** Next, we reported that AID expression in leukemic cells is mainly confined to a small and proliferative subset of tumor cells ongoing CSR **(Palacios et al., Blood, 2010)** and we characterized the molecular mechanism that underlines the proliferative behavior of this subset, describing that the PI3K/AKT pathway is activated after up regulation of miR-22 **(Palacios et al, Leukemia 2015 and Palacios et al. Leukemia & Lymphoma, 2015)**. Furthermore, we recently developed a double transgenic mice model (DT-TCL1/AID) emulating unmutated progressive CLL patients that over-express AID. Our results show that **constitutive AID expression in this model leads to disease progression, tumor proliferation and diminished survival (Morande P., Leukemia & Lymphoma, iwCLL-2015, Sydney, Australia).** Altogether, these results led us to propose that: ***i)*** tumor proliferative subset is a key issue during CLL progression and ***ii)*** AID over-expression is a consequence of a continuous antigenic stimulation of the tumor clone in an inflammatory microenvironment. In this line, recent works of our laboratory have been focused on the study of the plasma-derived exosomes at the proteomic level of these progressive CLL patients. We found that equally to AID protein, S100A9 protein appears to be another important piece of the puzzle of disease progression in CLL. Our data demonstrate that **in an inflammatory context the leukemic clone is able to express high amounts of S100A9 protein which in turn is able to activate NF-κB signaling, one of the main pathways responsible for AID expression (Prieto, et al. Blood, 2017).**

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.

MEETINGS

International workshop on Chronic Lymphocytic Leukemia. iwCLL-2017, New York, USA. Participants, Noe Seija, Pablo Morande and Agust[in Correa.

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**).

COURSES

Academia de Hematología. Training course organized by AbbVie, Institut Pasteur de Montevideo and Hospital Maciel for AbbVie staff. Three in the year

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

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3. Navarrete M.A., **Oppezzo P**. 2017. The pathogenesis of follicular lymphoma, beyond apoptosis resistance. 2017. **Translational Cancer Research**. 6: S529-S532.

4. Prieto D., Sotelo N., Seija N., Sernbo S., Abreu C., Durán R., Gil M., Sicco E., Irigoin V., Oliver C., Landoni A.I., Gabus R., Dighiero G., **Oppezzo P.** 2017. S100-A9 protein in exosomes from chronic lymphocytic leukemia cells promotes NF-κB activity during disease progression. **Blood.** 130(6):777-788.

5. Prieto D, **Oppezzo P**., Lipoprotein Lipase Expression in Chronic Lymphocytic Leukemia: New Insights into Leukemic Progression. **Molecules.** 2017 Dec 5;22(12). pii: E2083. doi: 10.3390/molecules22122083. Review. PMID: 29206143

Tumor Immunology and Glycobiology

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

PUBLICATIONS

Berois N., Touya D., Ubillos L., Bertoni B., Osinaga E., Varangot M. 2017. Prevalence of EGFR Mutations in Lung Cancer in Uruguayan Population. Journal of Cancer Epidemiology. J Cancer Epidemiol. 2017: 6170290

Festari M.F., Trajtenberg F., Berois N., Pantano S., Revoredo L., Kong Y., Solari-Saquieres P., Narimatsu Y., Freire T., Bay S., Robello C., Bénard J., Gerken T.A., Clausen H., Osinaga E. 2017. Revisiting the human polypeptide GalNAc-T1 and T13 paralogs. Glycobiology. 27(1):140-153

Nozar F., Greif D., Franciulli A., Barrios E., Osinaga E., Berois N. 2017. Prevalence and Distribution of High-Risk Human Papillomavirus Genotypes in Invasive Carcinoma of the Uterine Cervix in Uruguay. An update on clinical outcome. Medical Research Archives. Volume 5, issue 5. May 2017

Immunoregulation and Inflammation

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Past members

Maite Duhalde (Post-doc)
María Eugenia Schroeder (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

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GRANTS

1. CABBIO 2015-2017
2. IP de Montevideo PTR
3. FMV. ANII. 2016-2017. Immunointervention in cancer: new therapeutic opportunities
4. Agence des Universités Francophones/FAPESP. 2017-2018. Characterization of novel molecular players in the control of obesity and obesity-induced inflammation.
5. FCE. ANII. 2017-2018. Characterization of a novel regulator of inflammation.

PUBLICATIONS

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Molecular Human Genetics

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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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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).

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

GRANTS

1. Doctoral Fellowship – Belén Torrado – 2016-2019 – ANII
2. CAP Fellowship – Paola Lepanto – 2016-2017 - UDELAR
3. CAP Fellowship – Matías Fabregat – 2016-2017 - UDELAR
4. 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

PUBLICATIONS

Prieto-Echagüe V., Lodh S., Colman L., Bobba N., Santos L., Katsanis N., Escande C., Zaghloul N.A., Badano J.L. 2017. BBS4 regulates the expression and secretion of FSTL1, a protein that participates in ciliogenesis and the differentiation of 3T3-L1. Scientific Reports. 7(1): 9765

Metabolic Diseases and Aging

MEMBERS

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Maria Caggiani, MD (MSc Student)
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Laura Colman, MSc (PhD Student)
Pía Garat, Engineer in Biotechnology (Entrepreneur BIOESPINN). Currently in Argentina as CEO of EOLO Pharma
Alejandro Rodríguez, **BSc** (MSc Student)
Karina Cal, MSc (Lab technitian) Working for EOLO Pharma

Former members
Natalia Bobba, MSc student 2014-2016. Currently at Columbia University as graduate student. Fullbright Scholar

RESEARCH

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 AngIIinduced 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-/-ApoB100/100only)
- Hypertension (AngII infusion)
- Chronic Kidney Disease (Nephrectomy + AngII – under development)
- Genetically modified mice: DBC1-/-, BBS4-/-, CD38-/-, FSTL1 loxp/loxp, SIRT6loxp/loxp Currently developing DBC1loxp/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. 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

Matalonga J., Glaria E., Bresque M., Escande C., Carbó J.M., Kiefer K., Vicente R., León T.E., Beceiro S., Pascual-García M., Serret J., Sanjurjo L., Morón-Ros S., Riera A., Paytubi S., Juarez A., Sotillo F., Lindbom L., Caelles C., Sarrias M.-R., Sancho J., Castrillo A., Chini E.N., Valledor A.F. 2017. The Nuclear Receptor LXR Limits Bacterial Infection of Host Macrophages through a Mechanism that Impacts Cellular NAD Metabolism. Cell Reports. 18(5):1241-1255

Prieto-Echagüe V., Lodh S., Colman L., Bobba N., Santos L., Katsanis N., Escande C., Zaghloul N.A., Badano J.L. 2017. BBS4 regulates the expression and secretion of FSTL1, a protein that participates in ciliogenesis and the differentiation of 3T3-L1. Scientific Reports. 7(1): 9765

Vascular Biology and Drug Development

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Lucía Collela, MSc student

Mariana Ingold, MSc student

Federico Ortiz, MSc Student

RESEARCH

The Laboratory of Vascular Biology and Drug Development has been focused on understanding the molecular and cellular basis of atherosclerosis and other inflammation related diseases in collaboration with other groups of our institute. We developed novel strategies for the prevention and treatment of these diseases. In the short term, the goal of the laboratory is to perform early-stage clinical trials with our leading compound.

Initially, we envisioned a new pharmacological strategy for the treatment and prevention of atherosclerosis. ***We designed a hybrid compound analog of α-tocopherol to which we added the electrophilic nitroalkene group.*** The rationale of our idea was that the nitroalkene-tocopherol analog would be selectively incorporated into the lipoprotein particles during their normal metabolism due to the presence of the chromanol structure of the α-tocopherol molecule. In order to test our hypothesis and to test our compound **in vivo**, we developed different mice models of cardiovascular diseases. Recently we have demonstrated, that once incorporated into the lipoproteins, the LDL transport the compound through the body, including the atherosclerotic lesions, were it exerts the anti-inflammatory and anti-atherogenic properties the nitroalkene functional group, inhibiting the development of atherosclerosis (J. Rodriguez-Duarte et al., 2018; submitted to Scientific Report).

After the development of our first generation of synthetic nitroalkene, we focused our research activities in the design & development of other novel non-conventional anti-inflammatory drugs that may tackle cell signaling cascades involved in the generation of the inflammatary response that underlies the pathogenesis of different chronic diseases (e.g. atherosclerosis, obesity-induced Insulin Resistance, hypertension). **We *designed and developed three novel families of non-conventional anti-inflammatory compounds that were protected in the USA*. We licensed our IP portfolio and we are moving our leading compound into early stage clinical trials (phase I/II).**

PATENTS

Batthyány, C., Lopez, G. V., Dapueto, R., Escande, C. & Rodriguez-Duarte, J. Nitroalkene Trolox derivatives and methods of use thereof in the treatment and prevention of inflammation related conditions; WO/2018/037279 A1. USA, PCT patent (2018).

Batthyány, C., Lopez, G. V., Escande, C., Porcal, W., Rodriguez-Duarte, J., Dapueto, R., Galliussi, G., Garat, M. P., Invernizzi, P., Ingold, M. & Collela, L. Nitroalkene Non Steroidal Anti-Inflammatory Drugs (NA-NSAIDs) and Methods of Treating Inflammation Related Conditions; PCT/IB2017/058443. USA, PCT patent (2017).

Batthyány, C., Lopez, G. V., Escande, C., Porcal, W., Dapueto, R., Rodriguez, J., Galliussi, G., Garat, M. P., Hill, M. & Segovia, M. Methods of treatment of inflammation related conditions using pluripotent anti-inflammatory and metabolic modulators; PCT/IB2017/056417. USA, PCT patent (2017).

Batthyány, C. & López, G. V. Nitroalkene Tocopherol and Analogs for Use in the Treatment and Prevention of Inflammation Related Conditions; WO/2015/073527 A1. USA, PCT patent (2015).

Batthyány, C. & Duran, R. Composition and Method for Inhibition of PknG from Mycobacterium Tuberculosis; WO/2014/204872. USA, PCT patent (2014).

GRANTS

2017-2018: "Developmenent of Eolo Pharma S.A: perform a clinical trial with a non conventional anti-inflammatory & immunomodulator compound"; CITES-GSS, Argentine (USD 629.000/2 years; together with Dr. C. Escande and V. López)

PUBLICATIONS

Rodriguez-Duarte, J., Dapueto, R., Galliusi, G., Turell, L., Kamaid, A., Khoo, N. K., Schopfer, F. J., Freeman, B. A., Escande, C., Batthyány, C.#, Ferrer-Sueta G.# & Lopez, G. V.# Electrophilic nitroalkene-tocopherol derivatives: synthesis, physicochemical characterization and evaluation of anti-inflammatory signaling responses. Submitted to Scientific Reports SREP-18-17694. # co-corresponding authors

Rodriguez-Duarte, J., Dapueto, R., Galliusi, G., Invernizzi, P., Rossello, J., Leyva, A., Bresque, M., Colman, L., Malacrida, L., Kamaid, A., Schopfer, F. J., Contreras, P., Segovia, M., Botti, H., Lopez, G. V.#, Escande, C.# & Batthyány, C.# Novel pharmacological strategies for atherosclerosis and insulin resistance treatment: design & development of vitamin E derivatives with anti-inflammatory properties. Submitted to Scientific Reports SREP-18-12801.

Ingold, M., Dapueto, R., Victoria, S., Galliusi, G., Batthyány, C., Bollati-Fogolin, M., Tejedor, D., Garcia-Tellado, F., Padron, J. M., Porcal, W. & Lopez, G. V. A green multicomponent synthesis of tocopherol analogues with antiproliferative activities. European journal of medicinal chemistry 143, 1888-1902, doi:10.1016/j.ejmech.2017.11.003.

Batthyány C., Bartesaghi S., Mastrogiovanni M., Lima A., Demicheli V., Radi R. 2017. Tyrosine-nitrated proteins: Proteomic and bioanalytical aspects. Antioxidants and Redox Signaling. 26 (7): 313-328

Folle A.M., Kitano E.S., Lima A., Gil M., Cucher M., Mourglia-Ettlin G., Iwai L.K., Rosenzvit M., Batthyány C., Ferreira A.M. 2017. Characterisation of Antigen B Protein Species Present in the Hydatid Cyst Fluid of Echinococcus canadensis G7 Genotype. PLoS Neglected Tropical Diseases. 11(1): e0005250

Silva A.R.F., Lima D.B., Leyva A., Duran R., Batthyany C., Aquino P.F., Leal J.C., Rodriguez J.E., Domont G.B., Santos M.D.M., Chamot-Rooke J., Barbosa V.C., Carvalho P.C. 2017. DiagnoProt: A tool for discovery of new molecules by mass spectrometry. Bioinformatics. 33(12):1883-1885

Cell Biology of Neural Development

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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.

COURSES

"Curso Teórico- Práctico de Animales de Laboratorio" March 13-21st 2017, 20 lecturers (1 international), 21 national students. Supported by FOCEM and PEDECIBA.

"Cell and Animal Models for Drug Discovery" October 16-27th 2017, 15 lecturers (8 international), 26 students (20 international). Funded by ICGEB, RIIP, UNU BIOLAC and FOCEM.

"Integrando las tecnologías del IP Montevideo" IP Montevideo internal training course based on the technologies available at the institute

SCIENCE DIFFUSION

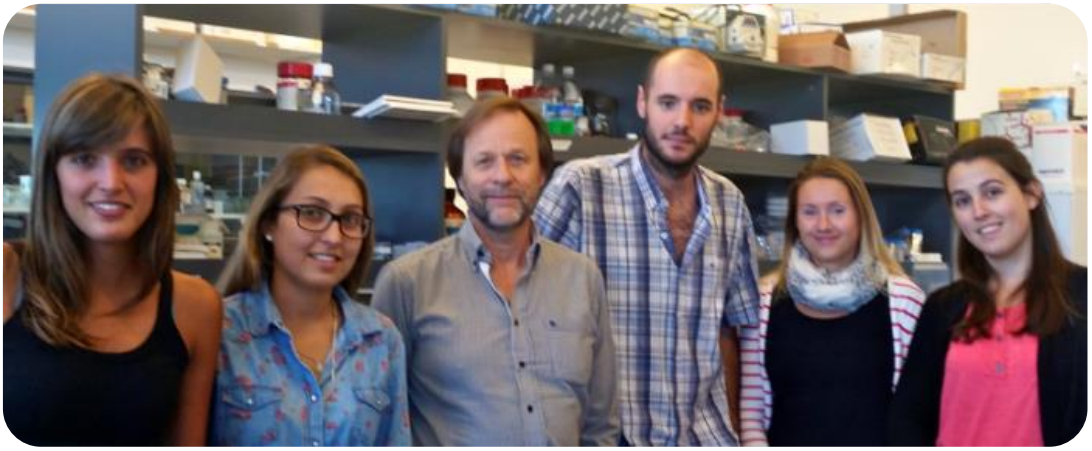
1. Brain Awareness Week, Montevideo, Uruguay.
2. Semanacyt (Science and Technology Week). Uruguay.
3. Facultad de Ciencias (UdelaR) Open Doors Day.
4. Institut Pasteur de Montevideo Open Doors Day.

GRANTS

1. Orientación neuronal en el ambiente polarizado de la retina neural en desarrollo: influencia de las proteínas Slit. PI: Zolessi. FCE_1_2014_1_104160 – ANII, Uruguay. F. Zolessi.

PUBLICATIONS

1. Álvarez G., Perdomo C., Coronel C., Aguilera E., Varela J., **Aparicio** G., **Zolessi** F.R., Cabrera N., Vega C., Rolón M., De Arias A.R., Pérez-Montfort R., Cerecetto H., González M. 2017. Multi-anti-parasitic activity of arylidene ketones and thiazolidene hydrazines against Trypanosoma cruzi and Leishmania spp. Molecules. 22(5): 709
2. Prieto D., Zolessi F.R. 2017. Functional Diversification of the Four MARCKS Family Members in Zebrafish Neural Development. Journal of Experimental Zoology Part B: Molecular and Developmental Evolution. 328(1-2):119-138.



Neurodegeneration

MEMBERS

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Romina Barreto (Msc student)
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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. Research contracts from Megapharma, Uruguay (2015-2017) and AbScience, France (2016-2017) for drug discovery in ALS rat models.

2. 2017 – 2018 – Ecos-Sud Institut IMAGINE, France– Characterization of mast cell infiltration in the peripheral motor pathway in ALS.

PUBLICATIONS

1. Trias E., Ibarburu S., Barreto-Nuñez R., Varela V., Moura I.C., Dubreuil P., Hermine O., Beckman S.J., Barbeito L. 2017. Evidence for mast cells contributing to neuromuscular pathology in an inherited model of ALS. Journal of Clinical Investigation-Insight. 2(20):e95934
2. Ibarburu S, Trias E., Lago N, Barreto-Nuñez R, Varela V, Beckman SJ, Barbeito L.. 2017. Focal Transplantation of Aberrant Glial Cells Carrying the SOD1G93A Mutation into Rat Spinal Cord Induces Extensive Gliosis. Neuroimmunomodulation. 24(3):143-153
3. Trias E., Ibarburu S., Barreto-Núñez R., Barbeito L. 2017. Significance of aberrant glial cell phenotypes in pathophysiology of amyotrophic lateral sclerosis. Neuroscience Letters. 636:27-31. Review.
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Neuroinflammation and Gene Therapy

MEMBERS

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Members at the IPMontevideo

Natalia Lago, PhD (Assistant Researcher)
Luciana Negro (PhD Student and Lecturer at the School of Medicine, UdelaR)
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)

Members at the Faculty of Medicine

Nathalia Vitureira, PhD (Guest Researcher at IP Montevideo)

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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COURSES

"Cell Animal Models for Drug Discovery". October 16-27th 2017. 7 national lecturers (8 international), 26 students (20 international). Supported by ICGEB, RIIP, UNU BIOLAC and FOCEM. Participation as lecturer.

GRANTS

Proyecto Grupos I+D "Neuroinflamación y glia". PI: Patricia Cassina/Luis Barbeito. Universidad de la República, CSIC (Grupos I+D 2014-143), Uruguay. 2015-2019.

"Inmunoreceptores como diana terapéutica para el tratamiento de la lesión medular: papel del par CD200-CD200R". PI: Natalia Lago/Hugo Peluffo. Universidad de la República, CSIC (CSIC I+D 2016), Uruguay. 2017-2019.

"Medicina de precisión aplicada a la lesión cerebral traumática: una alianza estratégica BSE-IPMon". Hugo Peluffo/Natalia Lago. Banco de Seguros del Estado, Uruguay. 2017-2020.

PUBLICATIONS

1. Lima T.Z., Sardinha L.R., Sayós J., Mello L.E., Peluffo H. 2017. Astrocytic expression of the immunoreceptor CD300f protects hippocampal neurons from amyloid-β oligomer Toxicity in vitro. Current Alzheimer Research. 14(7):778-783.

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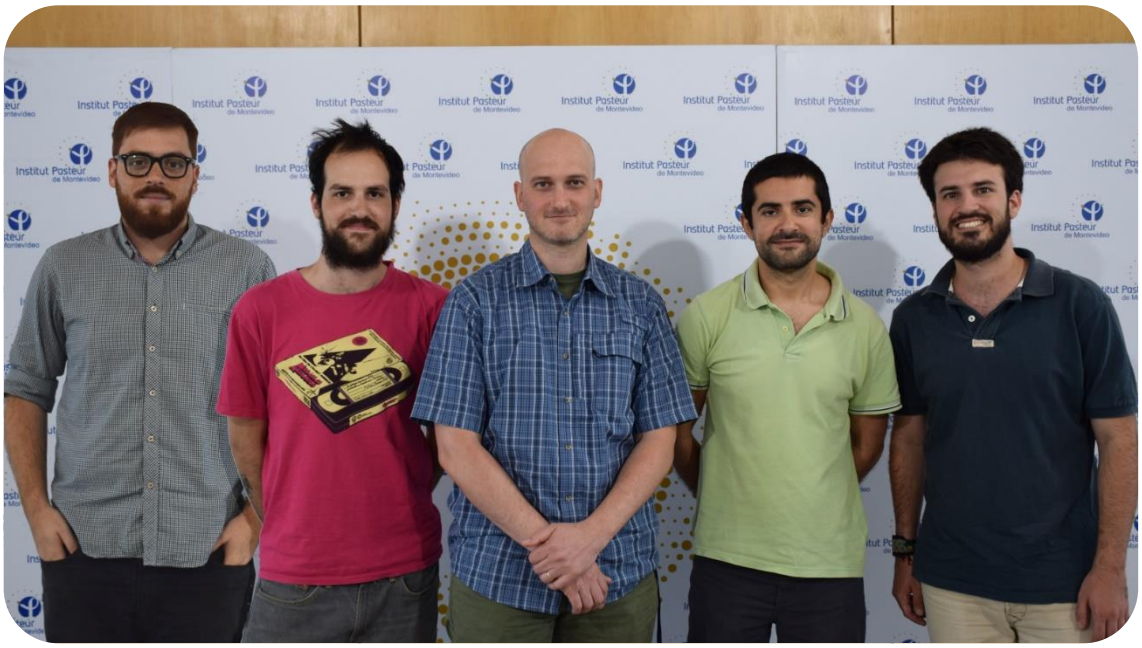

Signal Processing

MEMBERS

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

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.

COURSES

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 November 2017 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.

GRANTS

1. INNOVA II

PUBLICATIONS

1. Megrian D., Aguilar P.S., Lecumberry F. 2017. Similarity measure for cell membrane fusion proteins identification. Lecture Notes in Computer Science (including subseries Lecture Notes in Artificial Intelligence and Lecture Notes in Bioinformatics). 10125 LNCS. 257-265 Conference Paper

Worm Biology

MEMBERS

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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

"Expanding C. elegans research: First Latin American Worm Meeting". February 22-24th 2017. Organizer PhD Gustavo Salinas and PhD Inés Carrera. 30 lecturers (28 international), 52 students (42 international). Supported by: ICGEB, FOCEM, PEDECIBA, CSIC, USA Embassy, BNAI Brit, Phylumtech and other sponsors.

"Cell and Animal Models for Drug Discovery" October 16-27th 2017, 15 lecturers (8 international), 26 students (20 international). Funded by ICGEB, RIIP, UNU BIOLAC and FOCEM.

"Integrando las tecnologías del IP Montevideo" IP Montevideo internal training course based on the technologies available at the institute

YEARLY POSTGRADUATE COURSES

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

YEARLY UNDERGRADUATE COURSES

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

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. 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)
- The thioredoxin-fold in trypanosomatids and tapeworms. ICGEB (Italia) 2014-2017. Shared Project with Marcelo Comini).
- Redox Chemistry and Biology of Thiols, International postgraduate course and Symposium, supported by ICGEB, RIIP and PEDECIBA. The course was organized together with Marcelo Comini, Beatriz Alvarez and Madia Trujillo.
- Reinsertion funds for Inés Carrera. PEDECIBA.
- CSIC, Universidad de la República. Research Inititation Grants to Laura Romanelli.

PUBLICATIONS

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COURSES

Title	Organizers	Date	Foreign Speakers	Foreign Students	Finantial Agencies
"Expanding C. elegans research: First Latin American Worm Meeting"	G. Salinas I. Carrera	Feb 22nd – 24th	28	42	ICGEB PEDECIBA CSIC FOCEM BNAI Brit US Embassy Phylumtech
"Curso Teórico- Practico de Animales de Laboratorio"	M. Crispo M. Comini F. Zolessi Among others	Mar 13th – 21st	1	0	FOCEM PEDECIBA
"Herramientas de Manipulación Genética en Parásitos Unicelulares"	M.E. Francia C. Robello A. Cabrera	Mar 20th - 24th	1	0	ANII PEDECIBA SPONSORS
"Herramientas prácticas para el análisis de GWAS en cultivos"	L. Berná H. Naya	May 22nd –Jun 2nd	2	9	INIA CABBIO FOCEM
"Hands on Metagenomics data analysis: tools for bioprospection in environmental and clinical microbiology"	G. Iraola R. Ehrlich H. Botti A. Buschiazzo	Sep 25th – Oct 6th	7	14	FOCEM UNU BIOLAC COOP FRANCE CAMPUS FRANCE
"Cell Animal Models for Drug Discovery"	M. Bollati M. Comini M. Crispo H. Peluffo G. Salinas F. Zolessi E. Trías	Oct 16th - 27th	8	20	FOCEM ICGEB RIIP UNU BIOLAC
"Macromolecular Crystallography School "Structural Biology to enhance high impact research in health and disease"	F. Trajtenberg A. Buschiazzo N. Larrieux	Nov 13th – 23rd	14	21	FOCEM CCP4 IUCR CEBEM
"Deciphering regulator RNA functions by high-throughput sequencing"	A. Cayota R. Ehrlich C. Robello	Dec 4th - 8th	7	9	UNU BIOLAC FOCEM SPONSORS
"Performing Molecular Simulations with SIRAH force field"	S. Pantano M. Machado F. Klein E. Barrera M. Soñora	Dec 11th - 15th	0	15	FOCEM
"Integrando las tecnologías del IP Montevideo"	A. Correa M. Machado G. Greif	Sep 1st – Nov 17th	0	0	IP MONTEVIDEO

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IP Montevideo

at a glance

Staff

Human Resources	Dec 2010	Dec 2015	Dec 2016 Dec	Dec 2017
Scientific & Technical Staff	70	189	203	217
Administration & Support Staff	30	37	40	41
Total	150	226	243	258

Publications and Citations (Scopus)

Year	2007-2010	2011	2012	2013	2014	2015	2016	2017	Total
Publications	128	39	48	49	82	90	77	79	592

Aggregate Record	IPM 2007-2017
Number of Publications	561
Accumulated citations	11.253
Citations per publication	20

Human Resources Training

	2014	**2015**	**2016**	**2017**
Post-docs	24	22	26	**14**
PhD students	32	33	44	**40**
Master students	44	47	51	**37**
Undergraduate students	25	35	19	**5**
TOTAL	**125**	**137**	140	**96**

Budget Overview