Institut Pasteur de Montevideo

Scientific Report 2019

DIRECTOR'S PROLOGUE

The Institut Pasteur de Montevideo aims to be a "state-of-the-art" curiosity-driven research center with international projection in the field of biomedicine with a focus on molecular mechanisms of human and animal diseases following the "one health concept". We are also committed to finding new diagnostic tools, treatments, and cures, contributing to the development of drugs, vaccines, and biomarkers of disease closing the circle between curiosity-driven science and knowledge valorization.

By December 2018, when my team of Direction assumed, ***we focused our initial efforts on trying to improve our institutional values*** and environment trying to avoid authoritarianism, gender misconduct, and other non-desirable behaviors from our staff. ***We believe that working with empathy, joy, commitment, and respect for everybody does not ensure results, but it does guarantee dedication and a team attitude that are the basis for achieving them in the long run, our aim.*** We tried to develop an institutional culture of respect, teamwork, and to eradicate any gender inequity ***following the first law of leadership: be human first, scientist second*** (Nature, Nov. 2018). For doing this, we hire a new Chief of Human Resources that will be focusing on providing and helping our scientists with leadership skills, soft skills, improving their emotional intelligence in order to deal with human resources with empathy, creating a professional but healthy, friendly work environment.

During 2019 and following exciting teamwork with the academic council and almost all the principal investigators of our institute. We discussed, plan, and wrote the institutional strategic plan for the period 2020-2025 (see the attached version). In an attempt to highlight the most notable and innovative points of the same, I would mention 1. the four prioritized scientific objectives, 2. the plan to restructure the technological platforms and create new technological nuclei, and 3. the innovation program. This one not only contemplates open innovation but more importantly ***we are developing a "Venture and Company Builder" innovation program, based on intellectual property that seeks, together with private capital, to promote the creation of 20 scientific-technological-based startups in the next ten years in life sciences that will achieve the global market from day zero***. With this program, the institute intends to close the virtuous circle between science guided by curiosity and the valorization of knowledge, in an attempt to show that in Uruguay it is possible to dream with a knowledge society that helps the country begin to walk the path of sustainable development by relying on in science, technology, and innovation.

Research groups were organized into institutional programs focused on the One-Health concept, with an emphasis on multidisciplinary molecular approaches in human and veterinary medicine including environmental interactions. These programs are mainly funded by institutional grants or grants obtained 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).
With the aim of promoting more collaborations between our groups to achieve an improvement in the science we are doing, we are also promoting the concept of multi-

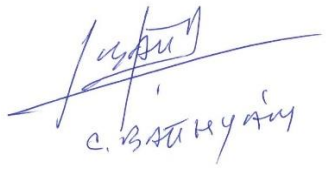
principal investigator groups and developing an institutional program of adjunct full-time researchers that may elect the Principal Investigator to work for a 4 year period, after which they will be evaluated and, if they are doing well, they will be able to continue in the same lab or may have the option to move to another lab.

The number and impact of the publications have reached an average of 90 publications/year. According to international databanks, publications from the IP Montevideo have a cumulated average of >20 citations per publication, which can be considered of competitive international standard. Remarkably, new international patents on two different areas have been filed in the last year; that have been licensed to two startup companies.

Our research laboratories provide an environment for the training of advanced graduate students. The IP Montevideo also contributes to the training of human resources in collaboration with the national and international postgraduate programs. In 2019, we harbor 120 undergraduate (16), MSc (32), PhD (51) and postdoctoral (21) fellows. We have also organized several international courses on different topics of molecular medicine that were attended by 235 students from the region and abroad. One hundred 5 distinguished invited professors 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.

Finally, the 2019 annual budget of the IP Montevideo was close to 4,5 million dollars, mainly coming from the Uruguayan national budget (@ 60%) and from own incomes by service sales, grants, and research contracts (@ 40%)

I wish to thank my team, all our researchers, and each of the members of our Institute for their dedication, support, teamwork spirit, and commitment. In addition, I wish to acknowledge the great contribution and trust from our partner institutions in Uruguay and France.



Carlos Batthyany, M.D. Ph.D.
Executive Director
Institut Pasteur de Montevideo

PREFACE

RESEARCH LABS

BIOMOLECULAR SIMULATIONS LAB

Biomolecular simulations make use of computer programs to recreate and visualize the behavior and phenomena that rule biological processes at the molecular level. These tools make it possible to simulate experiments in more controlled conditions than in cells or living organisms, and to perform "theoretical experiments" that would be technically impossible.

These possibilities have resulted in important advances in biomedicine, facilitating the understanding of mechanisms of diseases and the development of drugs, for example.

In the Biomolecular Simulations Laboratory, we apply different molecular modelling techniques and simulations to several problems of biomedical interest, such as the stability of viral particles of Zika and Dengue or interactions between proteins that participate in the contraction of the cardiac muscle. These activities are carried out in collaboration with experimental groups in Uruguay and abroad.

Finally, an important part of our work is dedicated to the development of coarse-grained methods to perform advanced simulations at low computational cost. These methods offer the possibility of improving the comparability of theoretical studies with biochemical / biophysical or molecular biology experiments.

RESEARCH LINES

Development of the SIRAH coarse-grained force field (Southamerican Initiative for a Rapid and Accurate Hamiltonian).
Our group develops and maintains one of the broadest coarse-grained force fields for existing biomolecular simulations. SIRAH (www.sirahff.com) uses a Top Down approach and a classic Hamiltonian, common to atomic force fields. SIRAH is freely distributed with easy-to-use analysis tools, parameters and topologies to simulate DNA, proteins, explicit solvent and phospholipids. Currently, representations are being developed for metal ions, glycans and RNA. This line is entirely developed by our group.

Development of FRET sensors for cyclic nucleotide and redox signalling pathways.
Using bioinformatics and structural modelling, together with coarse-grained simulations, we have developed a new generation of FRET sensors for signaling cAMP, cGMP and redox conditions. These biosensors allow us to reach an unprecedented spatial resolution since they can be genetically fused to the C-terminal of virtually any protein, directing them to any cell compartment. This research line continues with the design of new generations of biosensors in the framework of the ProTeMCA program and in collaboration with foreign experimental groups.

Flavivirus stability studies.
Using multiscale simulations, we study the different factors that affect the stability of viral particles (Virus-like Particles). The reduced computational cost of our simulation scheme allows us to perform comparative simulations of different flaviviruses varying the temperature and pH conditions. The availability of experimental structures of viral particles of Zika, Dengue, Japanese encephalitis (JEV) and tick-borne Encephalitis virus (TBEV) allows us to study the accessibility of different epitopes, helping to understand the mechanisms of viral neutralization by antibodies. Additionally, the computational approach enables the identification of amino acids involved in the acid firing mechanism of flaviviruses, which could contribute significantly to the

development of vaccines through the creation of attenuated viruses. These studies are part of collaborations with national and foreign experimental groups.

PUBLICATIONS

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[7] **Machado**, M.R., Zeida, A., **Darré**, L., **Pantano**, S. From quantum to subcellular scales: Multi-scale simulation approaches and the SIRAH force field (2019) Interface Focus, 9 (3), art. no. 20180085. DOI: 10.1098/rsfs.2018.0085 IF 3.1

[8] **Otero**, L., **Martínez-Rosales**, C., **Barrera**, E., **Pantano**, S., **Salinas**, G. Complex I and II Subunit Gene Duplications Provide Increased Fitness to Worms (2019) Frontiers in Genetics, 10, art. no. 1043. DOI: 10.3389/fgene.2019.01043 IF 3.5

[9] **Piattoni**, C.V., **Sardi**, F., **Klein**, F., **Pantano**, S., **Bollati-Fogolin**, M., **Comini**, M. New red-shifted fluorescent biosensor for monitoring intracellular redox changes (2019) Free Radical Biology and Medicine, 134, pp. 545-554. DOI: 10.1016/j.freeradbiomed.2019.01.035 IF 5.6

[10] Riccardi, E., **Pantano**, S., Potestio, R. Envisioning data sharing for the biocomputing community (2019) Interface Focus, 9 (3), art. no. 20190005. DOI: 10.1098/rsfs.2019.0005 IF 3.1

CHRONIC LYMPHOID LEUKEMIA LAB

Our laboratory focuses on the study of the mechanisms involved in the origins and progression of Chronic Lymphoid Leukemia (CLL), the most common type of leukemia in people over 50 years, whose incidence in Uruguay is 5 cases per 100,000 people. CLL originates in cells of the immune system known as B lymphocytes, which lose their ability to die and begin to accumulate in the blood. Although many patients with CLL respond to current treatments, some don't.

The B lymphocyte is one of the most specialized cells of the immune system and is able to re-edit its DNA, thanks to the action of the cytidine deaminase (AID), an enzyme necessary to respond in the presence of different infections. However, the mutagenic action of the AID enzyme also has its negative aspects since B lymphocytes are continually exposed to damage of their DNA. Although the control mechanisms on AID are many and redundant, they sometimes fail, and in their absence this enzyme can be overexpressed in tumour cells, causing cancer progression and / or refractoriness in its treatment.

Our advances are related to the characterization of the expression of the AID enzyme in patients with CLL, and the development of animal models to study the causes of the progression of the disease and the refractoriness in the treatment.

RESEARCH LINES

Characterization of the origins of the immunological microenvironment in tumour progression and its correlation with the constitutive expression of AID in progressive patients with CLL
The objective is to corroborate if the constitutive expression of the enzyme cytidine deaminase (AID) during the evolution of the disease is a key event in the progression of CLL.

Deciphering of the effect of new inhibitors of kinases and proapoptotic molecules on the proliferative subpopulations of CLL.
It aims to characterize the molecular and phenotypic effects of new kinase inhibitors and the action of proapoptotic molecules in proliferating subpopulations of the tumour clone during treatment.

The S100A9 protein as a new therapeutic target in CLL. Linkage of inflammation, microenvironment and clinical evolution.
We aim to deepen the knowledge of the role of inflammation in the clinical evolution of patients with CLL.

Development of an artificial binding protein (ABP) platform to generate new prognostic and treatment tools in the LLC.
The objective is to create a therapeutic tool (ABP) capable of simultaneously recognizing the tumor cell and recruiting natural killer cells (NK) to destroy the tumor clone.

PUBLICATIONS

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[2] **Ortega**, C., **Abreu**, C., **Oppezzo**, P., **Correa**, A. Overview of high-throughput cloning methods for the post-genomic era (2019) Methods in Molecular Biology, 2025, pp. 3-32. DOI: 10.1007/978-1-4939-9624-7_1 IF N/A

[3] Stanganelli, C., Torres, D.C., **Ortega**, C., Sotelo, N., Márquez, M.E., Segges, P., Muniz, M.T.C., Bigni, R.S., Campregher, P.V., Sabino, A., Gomes, C.P., Lopes Ferrari deChauffaille, M.L., Arrais Rodrigues, C., Yamamoto, M., Abdelhay, E., Cabrera, J., Deglesne, P.-A., López, J.L., Galvano, C., Krzywinski, A., Bezares, R., Lang, C., Zanella, L., Agriello, E., Cabrejo, M., Gabús, R., Dighiero, G., **Oppezzo**, P., Hassan, R., Slavutsky, I., On behalf of the Latin American Group of Chronic Lymphocytic Leukemia (LAG-CLL) Distinctive IGHV gene usage and stereotyped receptors in South American patients with chronic lymphocytic leukemia (2019) Hematological Oncology, **Article in Press**. DOI: 10.1002/hon.2661 IF 3.4

FUNCTIONAL GENOMICS LABORATORY

Our scientific goal is to clarify the biological role of small regulatory RNAs in the realm of human cancer. Moreover, we work in close collaboration with the University Hospital and the National Cancer Program, to which we provide technological and experimental support for Clinical Oncology research, as well as developing new diagnostic biomarkers for cancer and disease.

In recent years, our main research goals focused on the study of a new class of molecules called small non-coding RNAs. Our work shows that fragmentation of these RNAs generates molecules capable of regulating cell survival pathways related to cell stress and proliferation. We primarily focus on fragments derived from transfer RNAs and Y-RNAs and their secretion from normal tumor cells. We study how other cells are able to receive and sense these RNAs that were previously released onto the extracellular environment. We evaluate these pathways as a new communication mechanism between cells. This research and other lines of work at Functional Genomics aim at identifying new molecular pathways in cancer initiation and progression with special emphasis on new therapeutic targets and diagnostic biomarkers.

RESEARCH LINES

Small RNAs derived from tRNA and Y-RNAs as new molecular pathways in cancer and their potential as a source of new diagnostic biomarkers in cancer.

Our main line of research is oriented to the role of new classes of small regulatory RNAs derived from tRNA and Y-RNA as central actors in the proliferation and survival responses to stress, and its potential as new molecular mechanisms in the initiation and progression of cancer. The focus of our current research activities is on the extracellular biology of these regulatory RNAs, and their ability to work as signaling molecules between cells.

Part of our work has shown that these small RNAs are actively secreted by cells through extracellular vesicles or extra-vesicular fractions, being transferred to other cells, constituting a new mechanism of intercellular communication and transfer of genetic information. Additionally, its unique stability in the extracellular environment allows its detection in biological fluids, positioning itself as potential molecular biomarkers in human cancer.

Currently, in collaboration with the Clinical Oncology Service of the Hospital de Clínicas and the National Cancer Institute, we are studying the diagnostic value of small circulating RNAs derived from tRNAGlu, tRNAGly and Y4-RNA in patients with lung cancer.

Research and Development activities with health centers.
Our laboratory is currently participating in a series of initiatives to incorporate new diagnostic biomarkers in oncology, as well as genomic and molecular tools.

Genetic susceptibility to breast cancer. Together with the Hospital de Clínicas of the Faculty of Medicine (Udelar), we have incorporated in the oncology routine the study of mutations of a panel of 11 hereditary predisposition genes to breast and ovarian cancer. This procedure uses next-generation deep sequencing for analysis including the BRCA1 and BRCA2 genes among others.

Biomarkers of therapeutic prediction in lung cancer. Our laboratory performs fluorescent in situ hybridization (FISH) assays to detect translocations of the ALK gene, as a way to predict sensitivity to treatment with inhibitors (Crizotinib) in lung cancer.

PUBLICATIONS

[1] Fromm, B., Kang, W., Rovira, C., **Cayota**, A., Witwer, K., Friedländer, M.R., **Tosar**, J.P. Plant microRNAs in human sera are likely contaminants (2019) Journal of Nutritional Biochemistry, 65, pp. 139-140. DOI: 10.1016/j.jnutbio.2018.07.019 IF 4.5

[2] Fromm, B., **Tosar**, J.P., Aguilera, F., Friedländer, M.R., Bachmann, L., Hejnol, A. Evolutionary implications of the microRNA- and piRNA complement of Lepidodermella squamata (Gastrotricha) (2019) Non-coding RNA, 5 (1), art. no. 19. DOI: 10.3390/ncrna5010019 IF N/A

HOST-PATHOGEN INTERACTIONS LAB

The Host-Pathogen Interaction Laboratory is focused on the study of human and animal pathogens, in particular the protozoan parasites Trypanosoma cruzi —which causes Chagas disease—, T. vivax and T. evansi; the causative agent of Leishmania, and the Mycobacterium prokaryote —associated with tuberculosis—, with emphasis on its functional genomics and its interactions with the host.

On Chagas Disease, our main focus of study, we have described a set of proteins related to the redox metabolism of trypanosomatids. We seek to deepen its inhibition, as well as its use in the development of possible therapeutic strategies and preventive Likewise, we work on the characterization of gene expression changes produced by T. cruzi in human cells. We have shown that there is a cellular reprogramming by this parasite, which allows the establishment and persistence of the infection.

We are focus on the identification of virulence factors, the development of new prophylactic strategies, and the improvement of diagnostic techniques, as well as in understanding aspects of the basic biology of T. cruzi and other related pathogens that are important for human and animal health.

RESEARCH LINES

Study of the intracellular host-pathogen interaction with a systemic approach

At the lab we have studied virulence factors of intracellular pathogens, as well as the host genes and routes necessary for the establishment of the infection, and the interface between them. We set out as main objectives the design of new strategies for the treatment or prevention of various infectious pathologies, such as Chagas disease, African trypanosomiasis, leishmaniasis, and tuberculosis

Determination of virulence factors necessary for infection with T.cruzi

Regarding the establishment and persistence of T. cruzi infection, we have described a set of proteins related to the redox metabolism of trypanosomatids (triparedoxin cytosolic and mitochondrial peroxidase, triparredoxins, glutarredoxins, pteridin reductase), and we have characterized their structure and function. We are developing strategies for its inhibition, as well as its application in the development of therapeutic and preventive strategies.

Characterization of the changes in gene expression produced by intracellular parasites in human cells

We have shown that there is a cellular reprogramming led by T. cruzi that allows the establishment and persistence of the infection in human cells. One of the main objectives we are currently pursuing is to identify host proteins, which, when inhibited, prevent T. cruzi infection. We are also extending this strategy for genomic and molecular biology studies in leishmaniasis, African trypanosomiasis, neosporosis and tuberculosis.

PUBLICATIONS

[1] Aguilera, E., Perdomo, C., Espindola, A., Corvo, I., Faral-Tello, P., **Robello**, C., Serna, E., Benítez, F., Riveros, R., Torres, S., Vera de Bilbao, N.I., Yaluff, G., Alvarez, G. A nature-inspired design yields a new class of steroids against trypanosomatids (2019) Molecules, 24 (20), art. no. 3800, DOI: 10.3390/molecules24203800 IF 3.1

[2] **Cabrera**, A., **Fresia**, P., **Berná**, L., Silveira, C., Macías-Rioseco, M., **Arevalo**, A.P., **Crispo**, M., **Pritsch**, O., Riet-Correa, F., Giannitti, F., **Francia**, M.E., **Robello**, C. Isolation and molecular characterization of four novel Neospora caninum strains (2019) Parasitology Research. DOI: 10.1007/s00436-019-06474-9 IF 2.1

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[4] **Díaz-Viraqué**, F., **Pita**, S., **Greif**, G., **de Souza**, R.C.M., **Iraola**, G., **Robello**, C. Nanopore Sequencing Significantly Improves Genome Assembly of the Protozoan Parasite Trypanosoma cruzi (2019) Genome biology and evolution, 11 (7), pp. 1952-1957. DOI: 10.1093/gbe/evz129 IF 3.7

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[7] Mesías, A.C., Sasoni, N., Arias, D.G., Pérez Brandán, C., Orban, O.C.F., Kunick, C., **Robello**, C., **Comini**, M.A., Garg, N.J., Zago, M.P. Trypanothione synthetase confers

growth, survival advantage and resistance to anti-protozoal drugs in Trypanosoma cruzi (2019) Free Radical Biology and Medicine, 130, pp. 23-34. DOI: 10.1016/j.freeradbiomed.2018.10.436 IF 5.6

[8] **Piñeyro**, M.D., **Arias**, D., **Ricciardi**, A., **Robello**, C., **Parodi-Talice**, A. Oligomerization dynamics and functionality of Trypanosoma cruzi cytosolic tryparedoxin peroxidase as peroxidase and molecular chaperone (2019) Biochimica et Biophysica Acta - General Subjects, 1863 (10), pp. 1583-1594. DOI: 10.1016/j.bbagen.2019.06.013 IF 3.7

[9] **Pita**, S., **Dıaz-Viraque**, F., **Iraola**, G., **Robello**, C. The tritryps comparative repeatome: Insights on repetitive element evolution in trypanosomatid pathogens (2019) Genome Biology and Evolution, 11 (2), pp. 546-551. DOI: 10.1093/gbe/evz017 IF 3.7

[10] **Robello**, C., Maldonado, D.P., Hevia, A., Hoashi, M., Frattaroli, P., Montacutti, V., Heguy, A., Dolgalev, I., Mojica, M., **Iraola**, G., Dominguez-Bello, M.G. The fecal, oral, and skin microbiota of children with Chagas disease treated with benznidazole (2019) PLoS ONE, 14 (2), art. no. e0212593. DOI: 10.1371/journal.pone.0212593 IF 2.8

[11] **Spangenberg**, L., **Graña**, M., Mansilla, S., Martínez, J., Tapié, A., **Greif**, G., Montano, N., Vaglio, A., Gueçaimburú, R., **Robello**, C., Castro, L., Quijano, C., Raggio, V., **Naya**, H. Deep sequencing discovery of causal mtDNA mutations in a patient with unspecific neurological disease (2019) Mitochondrion, 46, pp. 337-344. DOI: 10.1016/j.mito.2018.09.004 IF 3.4

IMMUNOREGULATION AND INFLAMATION LABORATORY

The deregulation of the immune system can lead to chronic conditions that are known as immune-mediated inflammatory diseases (IMIDs). IMIDs include more than 80 clinical entities such as autoimmune and autoinflammatory diseases that affect up to 10% of the population in the western world. Basic research has allowed to characterize physiological mechanisms responsible for controlling the development of inflammatory and adaptive responses mediating pathological effects, and this knowledge is critical to innovate at the level of strategies directed at the immune system and to understand the mechanism of action of drugs currently being used in the clinic.

Our group is interested in the study of cellular and molecular mechanisms that control the inflammatory process and the adaptive immune response. We focus on the biology of dendritic cells (DCs), since they constitute a sub-population of leukocytes able to orchestrate effector adaptive immune responses, while having powerful strategies capable of regulating the development of the inflammatory process and the adaptive response. Our work tries to cover relevant and original aspects at the level of molecular mechanisms while looking for pertinence in human health.

In this framework, the laboratory has characterized the emerging ion transporters TORID-1 (Tmem176b) and TORID-2 (Tmem176a), which are critical regulators of NLRP3 inflammasome activation. Inflammasome regulation by TORID-1 has been shown to be relevant in the anti-tumor immune response. We have characterized pharmacological inhibitors of TORID-1 and TORID-2 that have been characterized at a pre-clinical level as promising anti-tumor drugs.

RESEARCH LINES

Role of TORID-1 as an innate checkpoint in tumor immunity.

Role of TORID-1 and TORID-2 in the biology of chronic lymphocytic leukemia. (Collaboration with Pablo Oppezzo)

Characterization of small molecules capable of inhibiting or activating the conductance mediated by TORID-1 and 2.

Role of the intracellular protein TORID-1 in obesity and inflammation induced by obesity. (Collaboration with Carlos Escande)

Study of non-conventional anti-inflammatory drugs as immunomodulators in organ transplantation. (Collaboration with Carlos Batthyany)

PUBLICATIONS

[1] Gualdoni, G.S., Jacobo, P.V., Sobarzo, C.M., Pérez, C.V., Matzkin, M.E., Höcht, C., Frungieri, M.B., **Hill**, M., Anegon, I., Lustig, L., Guazzone, V.A. Role of indoleamine 2,3-dioxygenase in testicular immune-privilege (2019) Scientific Reports, 9 (1), art. no. 15919. DOI: 10.1038/s41598-019-52192-8 IF 4.0

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IMMUNOVIROLOGY LAB

At the Immunovirology Laboratory, we study the cellular and molecular processes involved in leukemic transformation by oncogenic viruses. In particular, we work with the Enzootic Bovine Leukemia (LBE) caused by the Bovine Leukemia Virus (BLV), which has a high prevalence in dairy cattle, generating important economic losses for our country.

With the aim of elucidating the mechanisms involved in viral infection, we have analyzed the genetic variability of BLV in Uruguay comparing their circulating genotypes with those described in other countries. We have characterized at the molecular and structural level the main BLV proteins: the glycoprotein of envelope (ENV), capsid (CA) and protease (PR). Also, we studied the interaction between these proteins with different components of the infected cell.

Also, using transcriptomic analysis, we studied the differences of gene expression between infected and non-infected animals with BLV, to know possible mechanisms involved in the control of the infection.

The knowledge we have obtained of this pathology and its causal agent has also allowed us to develop new technologies for the diagnosis both serologically and molecularly, as well as the generation of immunogenic preparations from the viral proteins that are being tested in animal models.

The results of our work will allow us to better understand the mechanisms that cause the leukemic transformation, generating new tools to optimize its diagnosis, and new procedures to improve the control and prevention of viral transmission

RESEARCH LINES

Biophysical and structural characterization of the BLV capsid protein (BLV-CA).
The mechanism of BLV capsid formation through the self-assembly of thousands of copies of BLV-CA represents a key event in the retrovirus cycle. To understand this mechanism we have characterized the biochemical and biophysical properties that affect this process, and in collaboration with the Laboratory of Molecular and Structural Microbiology of the IPMontevideo we elucidate the three-dimensional structure of BLV-CA, showing that it is organized in a pseudohexagonal with an important conformational plasticity. On the other hand, we generated nano-antibodies directed against the viral capsid and we studied the effect of the interaction with capsid on the modulation of self-assembly.

Characterization of the interactions between BLV-CA and intracellular components of the host cell
The transit of the viral capsid between the plasma membrane and the cell nucleus depends on the interaction with various cellular proteins. Other retroviral models have described restriction factors that disturb the conformation of the capsid generating anti-viral conditions. For the case of delta-retroviruses such as BLV we have no confirmed evidence on these mechanisms. In the HIV model it has been demonstrated that the interaction between capsid and nucleoporins would participate in this transit and in the entrance to the nucleus through the nuclear pore. Based on the ability of BLV-CA to self-assemble in vitro in tubular or supramacromolecular structures, and using affinity and mass spectrometry techniques, our objective is to identify and characterize the interactions between BLV-CA and the factors of the cellular guest involved in this traffic. We will also look for partners of BLV-CA that can act as innate immune sensors when analyzing cell lysates of permissive and non-permissive cells to

BLV infection. The engineering 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 as microscopy approaches. The results obtained in BLV will then be transferred to the HTLV-1 research to define common and diverse mechanisms adopted by these delta retroviruses when establishing the viral infection.

Caracterización bioquímica, estructural e inmunológica de la proteína de la envoltura BLV
The env of BLV has a crucial role in the determination of viral infectivity, and is responsible for inducing the fusion of viral and cellular membranes after recognition of specific cell surface receptors.

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

Env is one of the main targets of antiviral immune responses, which generate both humoral neutralizing antibodies and T-cell specific adaptive immunity. Other retroviruses have been

reported to be the presence of an immunosuppressive peptide (isu) in the structure of the glycoprotein Env. It could be important in its ability to immunomodulate immune responses. We are interested in studying the effect of amino acid modifications in the isu domain on humoral and cellular adaptation responses to the 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 hope to identify the modifications that reduce the immunosuppressive activity of this domain and, therefore, increase its immunogenicity. This result could be useful for the rational design of effective vaccines against this retrovirus.

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

Genetic characteristics identification associated with the Enzootic Bovine Leukemia (LBE) natural control

Given the high prevalence of Enzootic Bovine Leukemia (LBE) in Uruguay, the strategy to eradicate the disease implemented in Europe and Oceania is impracticable in our country. An alternative control strategy using vaccines is promising, but there are still no effective products on the market. Considering that recent results show that the LBE has a hereditary component that reaches 8%, a third strategy to control the disease would involve the herding of flocks by increasing the frequency of genotypes associated with resistance to infection.

In an experimental herd with high prevalence of BLV infection, we have analyzed a the groups of animals: one control group characterized by a low proviral load and low titers of anti-BLV antibodies; one “non-control” group with high proviral load and high specific antibody titers, and a “negative” group, without detectable presence of BLV.

Using peripheral blood mononuclear cells (PBMC) from these animals, we are characterizing, in collaboration with Natalia Rego and Hugo Naya of the Bioinformatics Unit (IP Montevideo), the transcriptomic representatives of these groups by mass sequencing of mRNA (ARNseq). We hope to identify genes and isoforms differentially expressed in control animals and interpret these differences in the context of biological processes, ontologies of sub or overrepresented metabolic pathways.

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[2] **Gil**, M., **Lima**, A., Rivera, B., Rossello, J., Urdániz, E., Cascioferro, A., Carrión, F., Wehenkel, A., Bellinzoni, M., Batthyány, C., Pritsch, O., Denicola, A., Alvarez, M.N., Carvalho, P.C., Lisa, M.-N., Brosch, R., Piuri, M., Alzari, P.M., Durán, R. New substrates and interactors of the mycobacterial Serine/Threonine protein kinase PknG identified by a tailored interactomic approach (2019) Journal of Proteomics, 192, pp. 321-333. DOI: 10.1016/j.jprot.2018.09.013 IF 3.5

METABOLIC DISEASES AND AGING

Obesity is a serious medical problem that involved a high percentage of the world population. Traditionally conceived as a disease of developed countries, it is now recognized as a pandemic by WHO. In Uruguay, nearly 60% of the adult population are considered overweight or obese. Moreover, about 10% of the child population is overweight or obese, and they suffer hypertension and diabetes, pathologies associated with obesity. Understanding the molecular mechanisms involved in pathophysiology of obesity, diabetes and other associated diseases is our main goal. Research aims on Metabolic Diseases and Aging Lab are:

a) to do basic science focused on the molecular mechanisms of metabolic diseases, with a strong emphasis on Sirtuins;

b) To develop novel pharmacological strategies to treat obesity and metabolic diseases.

RESEARCH LINES

DBC1 as modulator of metabolic function. The main focus of our research has been to continue to understand the role of the protein Deleted in Breast Cancer-1 (DBC1), a SIRT1 regulator, in the control of metabolism and metabolic diseases. In order to achieve that, we took four different scientific approaches. A) We continued using the genetic deletion of DBC1 mouse model as an experimental paradigm in metabolism regulation; B) we engaged in studying how DBC1 function is regulated in vivo. C) We decided to generate a loxp/loxp DBC1 mouse model as a tool for tissue-specific knockout of DBC1. D) Based on our previous data that DBC1 regulates the “healthy obesity” phenotype, we began to search for secreted targets of DBC1 that may account for its effects.

Novel regulators of metabolism and metabolic diseases: focus on Inflammation. Chronic inflammation has emerged in the past few years as a major player in the development of metabolic diseases, with accumulating evidences showing that both innate and adaptive immune cells are involved in the onset and progression of obesity, type II diabetes and atherosclerosis. During this period, we begun to work with two different proteins in context of chronic inflammation and metabolic diseases: SIRT6 and “TMEM176B.

Research and development in anti-obesity drugs. The development of novel compounds for pharmacological treatment of metabolic diseases was a seminal part of our G5 proposal. We associated in close collaboration with Dr. Carlos Batthyany, who together with Dr. Virginia Lopez had already designed several compounds aimed to treat atherosclerosis. Together, we showed that these compounds are effective in vivo for atherosclerosis. In the course of this scientific collaboration, we designed together a novel family of compounds, one of which is showing striking results on prevention of obesity, insulin resistance and non-alcoholic liver steatohepatitis (NASH).

PUBLICATIONS

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[2] **Rodriguez-Duarte**, J., **Galliussi**, G., **Dapueto**, R., **Rossello**, J., **Malacrida**, L., **Kamaid**, A., Schopfer, F.J., **Escande**, C., **López**, G.V., **Batthyány**, C. A novel nitroalkene-α-tocopherol analogue inhibits inflammation and ameliorates atherosclerosis in Apo E knockout mice (2019) British Journal of Pharmacology. DOI: 10.1111/bph.14561 IF 6.6

[3] **Santos**, L., **Colman**, L., **Contreras**, P., Chini, C.C., **Carlomagno**, A., **Leyva**, A., **Bresque**, M., Marmisolle, I., Quijano, C., **Durán**, R., **Irigoín**, F., **Prieto-Echagüe**, V., Vendelbo, M.H., Sotelo-Silveira, J.R., Chini, E.N., **Badano**, J.L., **Calliari**, A.J., **Escande**, C. A novel form of Deleted in breast cancer 1 (DBC1) lacking the N-terminal domain does not bind SIRT1 and is dynamically regulated in vivo (2019) Scientific Reports, 9 (1), art. no. 14381. DOI: 10.1038/s41598-019-50789-7 IF 4.0

MOLECULAR AND HUMAN GENETICS LAB

In the lab we are interested in understanding different aspects of the biology of primary cilia, organelles that are present in the vast majority of cell types in the human body and that play a role as signaling hubs, acting as cellular antennae that are critical for the interaction between cells and the environment.

It has been shown that ciliary dysfunction underlies a number of human conditions collectively known as ciliopathies. Among them, we focus on Bardet-Biedl Syndrome (BBS), a ciliopathy characterized primarily by obesity, polydactyly, mental retardation, retinal degeneration, renal and gonadal malformations and that can include additional features such as asthma, diabetes, anosmia and congenital heart disease.

We are focused on BBS and other cilia-associated proteins and perform both in vitro and in vivo assays to determine their function. The goal is to gain insight into basic aspects of ciliary biology as well as to understand the cellular and molecular basis of different phenotypes that characterize the ciliopathies. To this end, a significant effort of the lab is centered on the institutional program InDICyO (Investigación en Diabetes, Inflamación, Enfermedades Cardiovasculares y Obesidad), where we aim to understand the role of cilia and proteins of interest in the development of obesity and atherosclerosis.

RESEARCH LINES

CCDC28B and BBS proteins in the regulation of ciliogenesis and cilia length.
In the lab we have been dissecting the biological role of a number of BBS proteins and the BBS-associated protein CCDC28B (coiled-coil domain containing protein 28B). In patients it was first reported that a reduction in CCDC28B protein levels, in the presence of mutations in BBS genes, results in a more severe presentation of the syndrome. CCDC28B interacts with a number of BBS proteins and we have demonstrated that it is a novel regulator of ciliary length, both in cells and in vivo in zebrafish. We know that the function of CCDC28B in the cilium relies, at least in part, on its interaction with SIN1 and the molecular motor kinesin 1. Currently, we continue characterizing CCDC28B with the goal of understanding the mechanim by which it regulates cilia.

Cilia targeting: similarities with nuclear transport.
Cilia are highly conserved organelles that protrude from the cellular plasma membrane while their interior is connected to the cytosol. However, the composition of cilia appears to be highly regulated and different from that of the membrane and cellular interior. Importantly, this

particular composition is important for the function of the organelle. For example, receptors and mediators of different signaling cascades are concentrated inside the cilium and ciliary entry of molecules has been shown to be regulated. However, the mechanisms involved in directing molecules into the cilium as well as in mediating their entry are not completely understood. Different lines of evidence show a similarity with nuclear import. In this context, we have been studying the role of the nuclear import machinery in transporting proteins into the cilium, looking at proteins that are able to localize to both compartments. In particular, we have focused on Gli2, a transcription factor for Hedgehog signaling, that changes its localization in response to pathway activation. We were able to show that Gli2 used importins to enter both the nucleus and the cilium but importantly, it uses different importins in each case. We continue studying this process with the aim of gaining a better understanding of the mechanism.

Bardet-Biedl associated proteins in intracellular trafficking

The functional characterization of BBS proteins has led us to uncover different extra-ciliary roles. For example, we showed that BBS7 is able to enter the nucleus where it modulates the activity of RNF2, a chromatin remodeling factor. Thus, defects in BBS7 result in changes in gene expression. More recently, we also documented a role for CCDC28B in the nucleus that is linked to cilia length regulation in a mechanism that we still do not completely understand. In collaboration with Dr. Norann Zaghloul at the University of Maryland, USA, we have shown that BBS proteins not only transport molecules to the base of cilia but have a broader role in intracellular transport and secretion of at least a subset of proteins. We have shown that BBS4 is required for the correct secretion of FSTL1, a function that we believe relevant to understand the etiology of BBS.

The role of BBS proteins and cilia in the development of ciliopathy associated phenotypes.

Dissecting the biological role of the BBS proteins and CCDC28B is critical to understand the mechanism underlying the development of BBS associated phenotypes. Therefore, in addition of studying the function of these proteins at a cellular and molecular level, we also evaluate their function in models that are relevant to the pathology. For example, we have studied the role of cilia in the development of the retina in zebrafish. Currently, we are studying the role of BBS proteins and cilia in adipogenesis and the development of obesity. Our results show that defects in BBS4 lead to a reduction in the secretion of FSTL1 (as previously mentioned). Importantly, we uncovered a novel role for FSTL1 in ciliogenesis and adipogenesis. This line of research is being followed in a collaboration with the laboratory of Dr. Escande in the context of the INDICyO Program.

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[3] Alciaturi, J., Anesetti, G., **Irigoin**, F., Skowronek, F., Sapiro, R. Distribution of sperm antigen 6 (SPAG6) and 16 (SPAG16) in mouse ciliated and non-ciliated tissues (2019) Journal of Molecular Histology, **ARTICLE IN PRESS** DOI: 10.1007/s10735-019-09817-z IF 2.9

MOLECULAR AND STRUCTURAL MICROBIOLOGY LAB

The Laboratory of Molecular and Structural Microbiology (LMSM) seeks to understand how bacteria sense signals from their environment and internal milieu, to thereafter respond and adapt. Pathogenic bacteria are a particular interest, focused on the Spirochete genus Leptospira.

Signaling is mediated by proteins, which change their 3D structures in a signal-dependent way, structures that are often stabilized by phosphorylation. Phosphoryl-transfer along such signal transduction pathways is thus a key enzymatic reaction, the mechanistic features of which our lab wants to uncover at the molecular level.

Our lab uses X-ray crystallography, to image the 3D structures of target proteins, such as sensory histidine kinases and response regulators, alone and in complex. By combining these high-resolution images with other sources of information —especially issued from biochemistry, genetics and microbiology experiments— we wish to understand the function and then contribute to the development of applications, such as vaccines against microbial diseases.

The Leptospira genus comprises many species, at least 10 of them cause a serious disease: leptospirosis. This zoonosis (that is, a disease that transmits from animals to humans), affects the reproductive capacity of cattle in Uruguay. It also causes an acute disease in humans, sometimes deadly, for which there are still no effective vaccines. Signaling systems in Leptospira shall uncover virulence and pathogenesis mechanisms, still poorly understood in these spirochetes. Key virulence proteins will be attractive targets to develop intervention strategies, and effectively control the zoonosis, with anticipated veterinarian and public health applications.

RESEARCH LINES

Signaling and regulation in microorganisms

We are particularly interested in two component systems (TCS) in bacteria. TCSs are central in mediating signaling and regulation, almost ubiquitous in prokaryotes and archaea, they are also present in fungi and plants. Other regulatory proteins, such as one component systems, are also being studied. The general question behind this line of research is: how do cells use these sensory and regulatory proteins to detect extra- and intra-cellular signals, and then regulate specific functions? To answer this question, saprophytic bacteria (such as Bacillus subtilis and Leptospira biflexa) are studied, as well as pathogenic ones (Leptospira interrogans, L. borpetersenii, L. noguchii, Enterococcus faecium, Mycobacterium tuberculosis). In these models, several key biological processes are studied, such as the regulation of lipid synthesis (Albanesi et al., Proc Natl Acad Sci USA 2009, 106: 16185, Trajtenberg et al., J Biol Chem 2010, 285: 24892, Trajtenberg et al. al., mBio 2014, 5: e02105; Trajtenberg et al., eLife 2016, 5: e21422; Imelio et al., Bio-protocol 2017, 7: e2510; Lara et al., 2018 submitted), heme metabolism (Morero et al., Mol Microbiol 2014 , 94: 340), or virulence in pathogenicity (Adhikarla et al., Front Cell Infect Microbiol 2018, 8:45).

Molecular and structural biology of Leptospira

Different species of Leptospira cause leptospirosis. This zoonosis is the most widespread one around the world, reemerging as an important problem in human and animal health. In Uruguay, it is a significant issue, provoking cattle abortions and reproductive failure, and transmitting to humans causing acute disease. Our lab wants to elucidate the molecular mechanisms that determine and/or regulate virulence and pathogenicity in Leptospira. There are currently two main projects to do this: the study of the motility apparatus and systematic efforts to isolate and type local Leptospira strains in Uruguay.

i- The motility apparatus of Leptospira. Active translational motility of leptospira (swimming) is central for the virulence of the pathogenic species that cause leptospirosis. The essential organelle used for swimming is the flagellum. Our lab is currently studying the detailed molecular architecture of the flagellar appendage. The filament of these spirochetes' flagella is confined within the periplasm, a unique feature shared by all Spirochetes, including bacteria like Treponema pallidum (the agent of syphilis) or Borrelia burgdorferi (Lyme disease). In collaboration with the Ko and Sindelar labs in Yale University, as well as the Picardeau lab at the Institut Pasteur (Paris), we have shown that the flagellar filament from Leptospira is much more complex compared to better-known bacterial models used so far as a paradigm of swimming motility in bacteria. As an example, filaments from Salmonella and other Enterobacteria are built as homopolymers of a single protein species (flagellin), whereas Leptospira comprise at least eight different proteins polymerized into the periplasmic filament assembly (Wunder et al., Mol Microbiol 2016, 101: 457; San Martin et al., Acta Crystallogr F 2017, 73:123; Wunder et al., Front Cell Infect Microbiol 2018, 8:130). We have recently crystallized two new proteins from both pathogenic and saprophytic Leptospira spp. (San Martín et al., Acta Crystallogr F 2017, 73:123),and solved their 3D structures (unpublished results), revealing novel protein folds. We are now moving forward towards their physiological interactions and interaction partners, enabling for the flagellar filament role in spirochete motility.

ii- isolation and typing of autochthonous Leptospira spp. strains. This line is being developed in the context of a multicentric collaborative project, aims to isolating native strains from Leptospira spp. from biological samples obtained from infected cattle and other animal reservoirs. These isolates are typed by complementary techniques: classic serologic methods and novel molecular approaches, ultimately achieving greater sensitivity and specificity (Zarantonelli et al., PLoS Negl Trop Dis 2018, 12:e0006694). This effort has led to the creation of a biobank of strains of Leptospira, which up to now was not available in Uruguay, reporting the identity of the serovars that circulate in natural infections. This biobank will be useful in the formulation of more efficacious vaccines, in the improvement of diagnostic methods, as well as in further investigations of leptospirosis in Uruguay.

Collaborations

Our lab works in collaboration with Dr. Hugo Gramajo (Institute of Molecular and Cellular Biology, IBR, Rosario, Argentina) and his team, to elucidate crystallographic structures and mechanisms of action of Mycobacterium tuberculosis transcription factors (one component regulator systems), playing essential roles in regulating fatty acid metabolism in this pathogen.

We also collaborate with Dr. Mathieu Picardeau (Institut Pasteur, Paris, France) and Dr. Albert Ko (Yale University, New Haven, USA) on motility of Leptospira and pathogenesis mechanisms of these bacteria at the molecular level.

In addition, our lab integrates a multicentric consortium in Uruguay, working together with teams from the Veterinary Laboratories Division ("Miguel C Rubino" DILAVE) of the Ministry of Livestock, Agriculture and Fishery (Alejandra Suanes, Rodolfo Rivero), National Institute of Agricultural Research (Franklin Riet), Hygiene Institute at the Medical School (Felipe Schelotto), addressing problems of isolation, typing, diagnostics and genomics of Leptospira.

PUBLICATIONS

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[4] **Ortíz**, C., **Botti**, H., **Buschiazzo**, A., **Comini**, M.A. Glucose-6-Phosphate Dehydrogenase from the Human Pathogen Trypanosoma cruzi Evolved Unique Structural Features to Support Efficient Product Formation (2019) Journal of Molecular Biology, 431 (11), pp. 2143-2162. DOI: 10.1016/j.jmb.2019.03.023 IF 5.1

NEURODEGENERATION

In most neurodegenerative diseases such as Alzheimer, Parkinson and Amyotrophic Lateral Sclerosis (ALS), neuronal pathology begins as a focal process that extends to other regions of the nervous system.

Our research aims to understand the biological mechanisms underlying the neurodegenerative process in ALS. Likewise, our research points to the development of new drugs that could stop or slow disease progression, which would allow a significant improvement in patient's quality of life.

The experimental approach is based on characterizing the neurodegenerative "cellular microenvironment", unravelling the role of neurons and glial cells as well as immune and vascular cells. We have recently identified new cell types with aberrant phenotype as well as new protein mediators and receptors that promote the degenerative process and that can be targeted by specific drugs.

The results of these studies will impact on a better understanding, diagnosis and treatment of neurodegenerative diseases.

RESEARCH LINES

Characterization of aberrant phenotypes of glial cells during ALS.

Influence of mast cells and neutrophils on the degeneration of motor neurons and loss of neuromuscular synapses.

Development of new neuroinflammatory modulators for the treatment of ALS

Nitro-NGF as a new glial factor that mediates the apoptosis of the motor neuron.

PUBLICATIONS

[1] **Kovacs**, M., **Trias**, E., **Varela**, V., **Ibarburu**, S., Beckman, J.S., Moura, I.C., Hermine, O., King, P.H., Si, Y., Kwon, Y., **Barbeito**, L. CD34 identifies a subset of proliferating microglial cells associated with degenerating motor neurons in ALS (2019) International Journal of Molecular Sciences, 20 (16), art. no. 3880. DOI: 10.3390/ijms20163880 IF 4.2

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NEUROINFLAMMATION AND GENE THERAPY LAB

Traumatic injuries affecting the brain or spinal cord due to traffic or work accidents, sports activities or violence, constitute in Uruguay the main cause of death and disability in people under 40 years. These injuries are in many cases progressive, and could trigger psychiatric pathologies, in particular depression and dementias like Alzheimer disease.

The objective of our group is to understand the consequences that these traumas could have for the nervous system, and in this way decipher how to reduce them. The main responsible for the progression of the traumatic damage is the inflammatory process that is triggered after the trauma. Our hypothesis is that by stimulating the positive part of this process (tissue cleansing and healing) and restraining its negative components (oedema-swelling, toxic mediators) by means of modulating immune receptors, we may promote functional recovery.

We focus on the study of molecules capable of acting as checkpoints of inflammation, called immune inhibitory receptors such as CD300f or CD200R1. Also, working with experts from the State Insurance Bank of Uruguay (Banco de Seguros del Estado-BSE), we search for possible blood molecules that may represent biomarkers of the progression of these injuries in traumatic brain injury patients.

Through the administration of recombinant proteins (proteins produced outside our body) or the use of gene therapy (introduction or manipulation of genes in cells of the brain or spinal cord), we aim to stimulate cells to produces a specific therapeutic protein that modulates the response of the tissue towards the alleviation of inflammation.

RESEARCH LINES

Modulation of the microglial and macrophage phenotype by activation of the CD200R1 immune receptor after traumatic injuries in the brain, spinal cord and peripheral nerve.

Modulation of the microglial and macrophage phenotype by the CD300f immune receptor and its role after traumatic injuries of the brain, spinal cord and peripheral nerve.

Role of the CD300f immune receptor in psychiatric conditions such as major depressive disorder or autistic spectrum disorder.

Preclinical and clinical research in traumatic brain injuries in association with the State Insurance Bank (BSE). Determination of blood biomarkers that could contribute to improve patient stratification and precision medicine approaches.

PUBLICATIONS

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REDOX BIOLOGY OF TRYPANOSOMES LAB

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 (Chagas disease, Leishmaniasis and African sleeping sickness).

These studies allow us to identify and understand the role these components play in parasite biology (e.g. growth, infection, and pathogenesis).

Our research aims to gain understanding into the redox biology of trypanosomatids to guide novel strategies and the development of the safer and more efficacious drugs against this disease.

RESEARCH LINES

Fundamental aspects of trypanothione metabolism: synthesis, reduction and utilization

We study the biochemical, structural and biological features that distinguish several key components of the trypanothione system from pathogenic trypanosomatids. Using animal

infection models, we investigate the role these molecules play in parasite biology and pathogenesis. The data from these studies allows to validate new drug target candidates, their inhibitors as well as to guide novel drug development strategies.

Development of biosensors for non-invasive and high-content studies

Our laboratory is interested in the development of different types of biosensors (fluorescence- and luminescence-based) that allow the real time and non-invasive monitoring of parasite proliferation, redox state and major signaling pathways. The transgenic cell lines expressing the biosensors allow us to study the role played by the oxidative stress and redox signaling in a variety of cellular processes (e.g. host-pathogen interaction, cell differentiation, cell cycle, apoptosis and metabolic dysfunction). The reporter cell lines are also used in phenotypic drug-screening campaigns and to investigate drug mode of action.

Early phase drug discovery projects

We apply target- and phenotypic-based approaches to screen synthetic and natural compounds that affect, in a selective manner, the growth of the infective form of different trypanosomatid species. Drug repurposing is also an active area of research in our lab.

Drug mode of action at cellular and enzymatic level is addressed to foster and guide drug optimization. Our laboratory relies on an important network of local and international groups working on (medicinal) chemistry to fulfill this goal.

PUBLICATIONS

[3] Currier, R.B., Ulrich, K., Leroux, A.E., Dirdjaja, N., **Deambrosi**, M., **Bonilla**, M., Ahmed, Y.L., Adrian, L., Antelmann, H., Jakob, U., **Comini**, M.A., Krauth-Siegel, R.L. An essential thioredoxin-type protein of Trypanosoma brucei acts as redox-regulated mitochondrial chaperone (2019) PLoS Pathogens, 15 (9), art. no. e1008065. DOI: 10.1371/journal.ppat.1008065 IF 6.2

[4] Ferrer, M.J., Wehrendt, D.P., **Bonilla**, M., **Comini**, M.A., Tellez-Iñón, M.T., Potenza, M. Production of recombinant *Trypanosoma cruzi* antigens in Leishmania tarentolae (2019) Methods in Molecular Biology, 1955, pp. 105-118. DOI: 10.1007/978-1-4939-9148-8_8 IF N/A

[5] **Manta**, B., Möller, M.N., **Bonilla**, M., **Deambrosi**, M., Grunberg, K., Bellanda, M., **Comini**, M.A., Ferrer-Sueta, G. Kinetic studies reveal a key role of a redox-active glutaredoxin in the evolution of the thiol-redox metabolism of trypanosomatid parasites (2019) Journal of Biological Chemistry, 294 (9), pp. 3235-3248. DOI: 10.1074/jbc.RA118.006366 IF 4.1

[6] Mesías, A.C., Sasoni, N., Arias, D.G., Pérez Brandán, C., Orban, O.C.F., Kunick, C., **Robello**, C., **Comini**, M.A., Garg, N.J., Zago, M.P. Trypanothione synthetase confers growth, survival advantage and resistance to anti-protozoal drugs in Trypanosoma cruzi (2019) Free Radical Biology and Medicine, 130, pp. 23-34. DOI: 10.1016/j.freeradbiomed.2018.10.436 IF 5.6

[7] **Ortíz**, C., **Botti**, H., **Buschiazzo**, A., **Comini**, M.A. Glucose-6-Phosphate Dehydrogenase from the Human Pathogen Trypanosoma cruzi Evolved Unique Structural Features to Support Efficient Product Formation (2019) Journal of Molecular Biology, 431 (11), pp. 2143-2162. DOI: 10.1016/j.jmb.2019.03.023 IF 5.1

[8] **Piattoni**, C.V., **Sardi**, F., **Klein**, F., **Pantano**, S., **Bollati-Fogolin**, M., **Comini**, M. New red-shifted fluorescent biosensor for monitoring intracellular redox changes

(2019) Free Radical Biology and Medicine, 134, pp. 545-554. DOI: 10.1016/j.freeradbiomed.2019.01.035 IF 5.6

[9] Rivas, F., **Medeiros**, A., **Comini**, M., Suescun, L., Rodríguez Arce, E., Martins, M., Pinheiro, T., Marques, F., Gambino, D. Pt-Fe ferrocenyl compounds with hydroxyquinoline ligands show selective cytotoxicity on highly proliferative cells (2019) Journal of Inorganic Biochemistry, 199, art. no. 110779. DOI: 10.1016/j.jinorgbio.2019.110779 IF 3.2

[10] Rodríguez Arce, E., Putzu, E., Lapier, M., Maya, J.D., Olea Azar, C., Echeverría, G.A., Piro, O.E., **Medeiros**, A., **Sardi**, F., **Comini**, M., **Risi**, G., **Salinas**, G., Correia, I., Pessoa, J.C., **Otero**, L., Gambino, D. New heterobimetallic ferrocenyl derivatives are promising antitrypanosomal agents (2019) Dalton Transactions, 48 (22), pp. 7644-7658. DOI: 10.1039/c9dt01317b IF 4.0

[11] Talevi A, Carrillo C, **Comini** MA. The thiol-polyamine metabolism of Trypanosoma cruzi: molecular targets and drug repurposing strategies Current Medical Chemistry, 2018 Sep 26. doi: 10.2174/0929867325666180926151059. [**Epub ahead of print**] IF 3.9

[12] Vairoletti, F., **Medeiros**, A., Fontán, P., Meléndrez, J., Tabárez, C., **Salinas**, G., Franco, J., **Comini**, M.A., Saldaña, J., Jancik, V., Mahler, G., Saiz, C. Synthesis of bicyclic 1,4-thiazepines as novel anti-: Trypanosoma brucei brucei agents (2019) MedChemComm, 10 (8), pp. 1481-1487. DOI: 10.1039/c9md00064j IF 2.4

TUMOR IMMUNOLOGY AND GLYCOBIOLOGY LAB

Proteins and carbohydrates (sugars) interaction plays an essential role in several cellular processes, such as cell proliferation and differentiation, as well as in different diseases, including viruses, bacteria and parasites infections. To perform these specialized functions, some types of carbohydrates bind to proteins creating complex structures called glycoproteins, which are found on the surface of cells and which influence cellular communication. Glycobiology is a discipline that studies the structure and function of carbohydrates as entities linked to cell function.

Our laboratory is focused on the identification and study of some alterations of glycosylation in cancer. Altered glycosylation produces some structures that are tumour-specific. Against them, we generate different types of biotechnological developments in view of their biomedical application. Our lines of research are oriented to understand characteristics of tumour biology and, especially, to develop new molecular procedures in view of their application to the diagnosis and treatment of the disease.

RESEARCH LINES

On biopharmaceuticals, the focus of study of the laboratory, we have developed specific kits and analytical methodologies base on requests from productive sector. Some of the projects that we are working on are:

Anti-TN antibody engineering in molecular imaging and cancer immunotherapy.
Using different recombinant antibodies and fragments of them, we found that the Tn antigen is a molecular target of interest for the diagnosis and treatment of human tumours. We develop fragments of antibodies (scfv, minibodies and nanobodies) for their application in molecular

imaging. We also investigated the development of immuno-nanoparticles to direct drugs towards tumours.

O-glycosylation enzymes as predictive and predictive biomarkers in oncology.
We evaluated whether the expression of GalNAc-Ts enzymes, which catalyse the first stage of O-glycosylation, can influence the biological properties of cancer cells (invasiveness, chemo-resistance, etc.), to subsequently develop useful diagnostic procedures in clinical oncology.

Anti-tumour immunotherapy using parasite molecules.
We found that some cancer-specific glyco-antigens are also expressed in certain parasites. We observed that immunization with extracts of Trypanosoma cruzi and Echinococcus granulosus induces anti-tumour immune responses in animal models. We seek to elucidate the molecular and cellular bases of this type of immune responses in order to generate a new type of anti-tumour therapeutic strategy.

VASCULAR BIOLOGY AND DRUG DEVELOPMENT LAB

The Laboratory of Vascular Biology and Drug Development has focused on the understanding of the molecular and cellular bases of atherosclerosis and other diseases in which chronic inflammation and low noise play a central pathogenic role (obesity, type II diabetes, neurodegenerative diseases , etc.).

In collaboration with other groups of our institute, we develop new strategies for the prevention and treatment of these diseases. The initial objective of the laboratory was to develop a new pharmacological strategy for the prevention and treatment of atherosclerosis, based on the design of a hybrid compound analogous to Vitamin E that possesses non-conventional anti-inflammatory properties.

Subsequently, we focus our research activities on the design and development of other non-conventional anti-inflammatory drugs that can be used for the treatment of other diseases in which chronic inflammation plays a central role (insulin resistance induced by obesity, hypertension). In this way, we have currently designed and developed four new families of non-conventional anti-inflammatory compounds that are patented in the US and internationally. We have recently licensed our portfolio of intellectual property and are committed to conducting clinical trials in humans (phase I / II) with our leading compound.

RESEARCH LINES

Development of a new pharmacological strategy for the treatment and prevention of atherosclerosis.
We designed a hybrid compound analogous to □-tocopherol to which we added a nitroalkenyl group. The rationale of our idea was that the nitroalkene analogous to tocopherol should be selectively incorporated into lipoprotein particles (particularly LDL) during its normal metabolism, due to the presence of chromanol in its structure and as does □-tocopherol. Once incorporated, LDL would act as carriers of our compound to the whole organism but including atherosclerotic damage, where the compound could exert the anti-inflammatory and anti-atherogenic properties of nitroalkenes. In order to test our hypothesis and test our compound in vivo, we developed different models of cardiovascular diseases in mice and demonstrated the

efficacy of our compound for the prevention of atherosclerosis (J Rodríguez-Duarte et al., 2018; British Journal of Pharmacology).

Development of new pharmacological strategies for the prevention and treatment of diseases related to chronic inflammation.

After the development of our first generation of synthetic nitroalkenes, we focus our research activities on the design and development of non-conventional anti-inflammatory drugs for the prevention and treatment of different diseases in which chronic inflammation plays a central pathogenic role. We design and develop three new families of unconventional anti-inflammatory compounds that are protected in the USA. UU We licensed our portfolio of intellectual property and created a "Startup" (EOLO Pharma S.A.) to test our leading compound in clinical trials (phase I / II).

PUBLICATIONS

[1] Ingold, M., Colella, L., Hernández, P., Batthyány, C., Tejedor, D., Puerta, A., García-Tellado, F., Padrón, J.M., Porcal, W., López, G.V. A Focused Library of NO-Donor Compounds with Potent Antiproliferative Activity Based on Green Multicomponent Reactions (2019) ChemMedChem, 14 (18), pp. 1669-1683. DOI: 10.1002/cmdc.201900385 IF 3.0

[2] Jobbagy, S., Vitturi, D.A., Salvatore, S.R., Turell, L., Pires, M.F., Kansanen, E., Batthyany, C., Lancaster, J.R., Jr., Freeman, B.A., Schopfer, F.J. Electrophiles modulate glutathione reductase activity via alkylation and upregulation of glutathione biosynthesis (2019) Redox Biology, 21, art. no. 101050, DOI: 10.1016/j.redox.2018.11.008 IF 7.8

[3] Ramos-Artuso, F., Galatro, A., Lima, A., Batthyány, C., Simontacchi, M. Early events following phosphorus restriction involve changes in proteome and affects nitric oxide metabolism in soybean leaves (2019) Environmental and Experimental Botany, 161, pp. 203-217. DOI: 10.1016/j.envexpbot.2019.01.002 IF 3.7

[4] Rodriguez-Duarte, J., Galliussi, G., Dapueto, R., Rossello, J., Malacrida, L., Kamaid, A., Schopfer, F.J., Escande, C., López, G.V., Batthyány, C. A novel nitroalkene-α-tocopherol analogue inhibits inflammation and ameliorates atherosclerosis in Apo E knockout mice (2019) British Journal of Pharmacology. DOI: 10.1111/bph.14561 IF 6.6

WORM BIOLOGY LAB

Our laboratory studies how worms harvest energy. Parasitic worms —also known as helminths— infect a quarter of the world's population. Eight of the twenty diseases catalogue by WHO as unattended are caused by helminths, while infections by these organisms in livestock and crops constitute a major economic problem for developing countries.

For these parasites there are no commercial vaccines available and there is an urgent need for new antihelmintics. We seek to identify "Achilles heels" of helminth metabolism. In particular, we study helminth metabolic pathway that harvest energy under hypoxic conditions, such as those found in the gastrointestinal tract of their hosts. We also established whole organism motility bioassays in order to discover new antihelmintics that paralyze worms.

Our laboratory also investigates in selenium biology, an essential micronutrient for most organisms, including mammals. For most of our research we use the C. elegans worm as a model.

RESEARCH LINES

Malate dismutation in helminths.
We aim to elucidate aspects of this metabolic pathway that allows parasitic worms to harvest energy under hypoxic conditions, such as those found in the gastrointestinal tract of their hosts.

Thioredoxin and glutathione systems of parasitic flatworms.
These organisms have linked pathways of thioredoxin and glutathione, with thioredoxin glutathione reductase as the sole enzyme serving both pathways. We are currently investigating structural aspects of this enzyme and determinants of redox function and iron-sulfur binding of thioredoxins and glutaredoxins.

Search for new anthelmintics.
Based on a motility test of C. elegans we aim to identify, from libraries of natural and synthetic products, new anthelmintics.

Selenium metabolism.
We seek to understand pathways of metabolization and response to the trace element selenium in animals, by direct and reverse genetic approaches using C.elegans as a model organism.

PUBLICATIONS

[1] Mariotti, M., Salinas, G., Gabaldón, T., Gladyshev, V.N. Utilization of selenocysteine in early-branching fungal phyla (2019) Nature Microbiology, 4 (5), pp. 759-765. DOI: 10.1038/s41564-018-0354-9 IF 14.3

[2] Otero, L., Martínez-Rosales, C., Barrera, E., Pantano, S., Salinas, G. Complex I and II Subunit Gene Duplications Provide Increased Fitness to Worms (2019) Frontiers in Genetics, 10, art. no. 1043. DOI: 10.3389/fgene.2019.01043 IF 3.5

[3] Roberts Buceta, P.M., Romanelli-Cedrez, L., Babcock, S.J., Xun, H., VonPaige, M.L., Higley, T.W., Schlatter, T.D., Davis, D.C., Drexelius, J.A., Culver, J.C., Carrera, I., Shepherd, J.N., Salinas, G. The kynurenine pathway is essential for rhodoquinone biosynthesis in Caenorhabditis elegans (2019) Journal of Biological Chemistry, 294 (28), pp. 11047-11053. DOI: 10.1074/jbc.AC119.009475 IF 4.1

[4] Rodríguez Arce, E., Putzu, E., Lapier, M., Maya, J.D., Olea Azar, C., Echeverría, G.A., Piro, O.E., Medeiros, A., Sardi, F., Comini, M., Risi, G., Salinas, G., Correia, I., Pessoa, J.C., Otero, L., Gambino, D. New heterobimetallic ferrocenyl derivatives are promising antitrypanosomal agents (2019) Dalton Transactions, 48 (22), pp. 7644-7658. DOI: 10.1039/c9dt01317b IF 4.0

[5] Vairoletti, F., Medeiros, A., Fontán, P., Meléndrez, J., Tabárez, C., Salinas, G., Franco, J., Comini, M.A., Saldaña, J., Jancik, V., Mahler, G., Saiz, C. Synthesis of bicyclic 1,4-thiazepines as novel anti-: Trypanosoma brucei brucei agents (2019) MedChemComm, 10 (8), pp. 1481-1487. DOI: 10.1039/c9md00064j IF 2.4

PROMOTION OF YOUNG RESEARCHERS: 4-YEAR RESEARCH GROUPS (G4)

OBJECTIVES

1. The promotion of young researchers affiliated / linked to IP Montevideo (current or previous), and who have already obtained their PhD

2. That the applicant carry out a project that proves to be independent, and not an extension of a line of research of the group of origin.

3. Once the 4 years of the Program have concluded, it is intended that the person responsible for the project can apply for the creation of a new Research Unit or Laboratory at IP Montevideo or at other academic institutions.

The program was inaugurated by 2018 and by 2019 had the following groups.

APICOMPLEXAN BIOLOGY LAB

We focus on the study of a group of parasites, causative agents of both human and veterinary disease, collectively known as Apicomplexa. We aim to unravel the molecular mechanisms underlying these parasites' ability to multiply and disseminate. With this, we aim to identify novel drug targets and drugs, to prevent these processes and more rapidly and effectively alleviate the diseases they cause.

Many, but not all, apicomplexans are able to cross the placental barrier causing abortion or congenital disease. We are interested in understanding the molecular basis of this capacity to cross such barrier, and their interactions with the host. Moreover, we are interested in understanding which specific strains of apicomplexans are present in our country, and to which extent, in order to optimize treatments and diagnostics to what is locally relevant.

Finally, we work in collaboration with the Parasitology and Micology department of the School of Medicine to provide molecular unequivocal diagnosis of parasitic diseases. This allows us to carry out epidemiological surveys, and to better and more effectively treat individual patients.

RESEARCH LINES

Systematic identification of proteins with a role in regulating T. gondii's cell division

Identification of novel centrosome components in T. gondii.

Identification of the molecular basis of differential pathogenesis among N. caninum strains.

Typing of prevalent Cryptosporidium species in Uruguay – in collaboration with the Parasitology and Mycology Department, School of Medicine.

Incidence of T. gondii in sheep abortion rates, and T. gondii presence in wildlife with a focus in the autochthonous members of the felidae family (Leopardus geoffroyi and Leopardus wiedii) Collaborative project with the Uruguayan Wool Secretiarat and the

Biodiversity and Genetics Department from the Clemente Estable Research Institute.

PUBLICATIONS

[1] **Cabrera**, A., **Fresia**, P., **Berná**, L., Silveira, C., Macías-Rioseco, M., **Arevalo**, A.P., **Crispo**, M., **Pritsch**, O., Riet-Correa, F., Giannitti, F., **Francia**, M.E., **Robello**, C. Isolation and molecular characterization of four novel Neospora caninum strains (2019) Parasitology Research. DOI: 10.1007/s00436-019-06474-9 IF 2.1

MICROBIAL GENOMICS LAB

The Microbial Genomics Laboratory is focused on the study of the microbial world (mainly bacteria), given the relevance of microorganisms for both human and livestock health. To achieve this we use genomics, metagenomics, bioinformatics and bacteriology as fundamental tools. The information we generate help us to understand the underlying evolutionary mechanisms that shape the acquisition of antibiotic resistance, host adaptation and transmission between hosts and the environment. Accordingly, our work contributes to generate new diagnostic approaches for infectious disease, to understand epidemiological patterns of bacterial infections and to identify biomarkers in microbial communities associated to several diseases.

RESEARCH LINES

– Characterization of evolutionary forces, inference of epidemiological and biogeographical patterns and characterization of virulence and antibiotic resistance determinants in environmental, livestock and human bacteria. The comparative analysis of bacterial genomes allows to make inferences about the biology of bacteria, for example, how they adapt, transmit, cause disease in different hosts, how they disseminate in time and space or how they acquire virulence or antibiotic resistance. The Microbial Genomics Laboratory leads local and international initiatives for studying diverse pathogenic bacteria important for human and livestock health like Campylobacter, Leptospira, Myocbacterium or Clostridium.

– Characterization of human, livestock and environmental microbiomes, with emphasis on the transmission dynamics of antibiotic resistance determinants between commensal and pathogenic bacteria. Today the role of the microbiome in human (and animal) health is well-known, but microbiome variability patterns in different populations and the mechanisms by which these bacteria can transmit between hosts still require to be explored in depth. The Microbial Genomics Laboratory is focused on determining the variability patterns of human microbiome in Latin American populations under a one-health perspective, given we consider the role of the environment and other animals (mainly livestock) as reservoirs of pathogens and antibiotic resistance mechanisms.

– Development of new analytical approaches for computational microbiology. Bioinformatic tools that we apply for the analysis of microbial genomes and metagenomes need to be optimized and constantly updated. Accordingly, the development and standardization of new bioinformatic approaches constitutes an important research line within the Microbial Genomics Laboratory. Specifically, we aim to develop and automatize pipelines for the comparative

analysis of bacterial gennomes, i.e. genotyping or pangenome reconstruction approaches. Also, we aim to consolidate the application of third-generation sequencing technologies like Oxford Nanopore in microbial genomics.

PUBLICATIONS

[1] **Costa**, D., **Iraola**, G. Pathogenomics of emerging Campylobacter species (2019) Clinical Microbiology Reviews, 32 (4), art. no. e00072-18. DOI: 10.1128/CMR.00072-18 IF 17.7

[2] **Costa** D, Betancor L, Gadea P, Cabezas L, Caiata L, Palacio R, Seija V, Galiana A, Vieytes M, Cristophersen I, Calleros L, **Iraola** G. Polyclonal Campylobacter fetus infections among unrelated patients, Montevideo, Uruguay, 2013-2018. Clin Infect Dis. 2019 Jul 15. pii: ciz657. doi: 10.1093/cid/ciz657. IF 9.1

[3] **Díaz-Viraqué**, F., **Pita**, S., **Greif**, G., **de Souza**, R.C.M., **Iraola**, G., **Robello**, C. Nanopore Sequencing Significantly Improves Genome Assembly of the Protozoan Parasite Trypanosoma cruzi (2019) Genome biology and evolution, 11 (7), pp. 1952-1957. DOI: 10.1093/gbe/evz129 IF 3.7

[4] **Ferrés** I, **Fresia** P, **Iraola** G, simurg: simulate bacterial pangenomes in R. Bioinformatics, 2019, **Epub ahead of print** DOI: 10.1093/bioinformatics/btz735 IF 4.5

[5] **Fresia**, P., **Antelo**, V., **Salazar**, C., Giménez, M., D'Alessandro, B., Afshinnekoo, E., Mason, C., Gonnet, G.H., **Iraola**, G. Urban metagenomics uncover antibiotic resistance reservoirs in coastal beach and sewage waters (2019) Microbiome, 7 (1). DOI: 10.1186/s40168-019-0648-z IF 10.5

[6] Muñoz, M., Restrepo-Montoya, D., Kumar, N., **Iraola**, G., Camargo, M., Díaz-Arévalo, D., Roa-Molina, N.S., Tellez, M.A., Herrera, G., Ríos-Chaparro, D.I., Birchenall, C., Pinilla, D., Pardo-Oviedo, J.M., Rodríguez-Leguizamón, G., Josa, D.F., Lawley, T.D., Patarroyo, M.A., Ramírez, J.D. Integrated genomic epidemiology and phenotypic profiling of Clostridium difficile across intra-hospital and community populations in Colombia (2019) Scientific Reports, 9 (1), art. no. 11293. DOI: 10.1038/s41598-019-47688-2 IF 4.0

[7] Muñoz, M., Restrepo-Montoya, D., Kumar, N., **Iraola**, G., Herrera, G., Ríos-Chaparro, D.I., Díaz-Arévalo, D., Patarroyo, M.A., Lawley, T.D., Ramírez, J.D. Comparative genomics identifies potential virulence factors in Clostridium tertium and C. paraputrificum (2019) Virulence, 10 (1), pp. 657-676. DOI: 10.1080/21505594.2019.1637699 IF 4.7

[8] **Nieves**, C., **Ferrés**, I., **Díaz-Viraqué**, F., **Buschiazzo**, A., **Zarantonelli**, L., & **Iraola**, G. (2019). Draft Genome Sequences of 40 Pathogenic Leptospira Strains Isolated from Cattle in Uruguay. Microbiology Resource Announcements, 8(47),e00893-19 IF N/A

[9] **Pita**, S., **Dıaz-Viraque**, F., **Iraola**, G., **Robello**, C. The tritryps comparative repeatome: Insights on repetitive element evolution in trypanosomatid pathogens (2019) Genome Biology and Evolution, 11 (2), pp. 546-551. DOI: 10.1093/gbe/evz017 IF 3.7

[10] **Robello**, C., Maldonado, D.P., Hevia, A., Hoashi, M., Frattaroli, P., Montacutti, V., Heguy, A., Dolgalev, I., Mojica, M., **Iraola**, G., Dominguez-Bello, M.G. The fecal, oral, and skin microbiota of children with Chagas disease treated with benznidazole (2019) PLoS ONE, 14 (2), art. no. e0212593. DOI: 10.1371/journal.pone.0212593 IF 2.8

EXPERIMENTAL EVOLUTION OF VIRUS LAB

Virus epidemics cause millions of infections each year, putting thousands of lives at risk. Treatment development frequently fails due to the great ability of these pathogens to evade the immune response, partly as a consequence of their high mutation rates. Our laboratory studies viral evolution through experimental approaches, in order to understand how viruses change in action.

The main objective is to understand how the genetic information of a virus determines its capacity for replication, transmission and virulence. Likewise, we will test evolutionary hypotheses in order to understand how viruses jump from species and adapt to new hosts. These experiments will be carried out through the use of synthetic biology in order to specifically alter, through genetic modifications, the replicative and / or transmission properties of the viruses to be studied.

Our focus of study will be viruses whose genomes are made up of RNA. We will mainly focus on viruses transmitted by arthropods (called arboviruses) and within these, those that use mosquito vectors such as dengue or Zika. We will also investigate enteroviruses and viruses that can potentially emerge and cause a similar problem, such as the Mayaro virus, so that we are better prepared for future epidemics.

RESEARCH LINES

Restriction of the evolutionary potential of arboviruses by synthetic biology.

Study of the mutation buffering capacity (genetic robustness) of RNA viruses.

Use of genetically modified enteroviruses as potential oncolytic agents.

PUBLICATIONS

[1] Carrau, L., Rezelj, V.V., Noval, M.G., Levi, L.I., Megrian, D., Blanc, H., Weger-Lucarelli, J., **Moratorio**, G., Stapleford, K.A., Vignuzzi, M. Chikungunya virus vaccine candidates with decreased mutational robustness are attenuated in vivo and have compromised transmissibility (2019) Journal of Virology, 93 (18), art. no. e00775-19. DOI: 10.1128/JVI.00775-19 IF 4.3

[2] Cifuente, J.O., **Moratorio**, G. Evolutionary and Structural Overview of Human Picornavirus Capsid Antibody Evasion (2019) Frontiers in Cellular and Infection Microbiology, 9, art. no. 283. DOI: 10.3389/fcimb.2019.00283 IF 4.3

[3] Danet, L., Beauclair, G., Berthet, M., **Moratorio**, G., Gracias, S., Tangy, F., Choumet, V., Jouvenet, N. Midgut barriers prevent the replication and dissemination of the yellow fever vaccine in Aedes aegypti (2019) PLoS Neglected Tropical Diseases, 13 (8), art. no. e0007299. DOI: 10.1371/journal.pntd.0007299 IF 4.5

[4] Roesch, F., Fajardo, A., **Moratorio**, G., Vignuzzi, M. Usutu virus: An arbovirus on the rise (2019) Viruses, 11 (7), art. no. 640. DOI: 10.3390/v11070640 IF 3.8

TECNOLOGICAL UNITS and LABS

The institute has a set of technological equipment organized on platforms —also called units— with the capacity to collaborate with the research work of scientists from within and outside the institute. These units provide services that are available to the local scientific community and to the national and regional productive sector.

ANALYTICAL BIOCHEMISTRY AND PROTEOMICS UNIT

The Analytical Biochemistry and Proteomics Unit (Ubypa) —an IP Montevideo and Instituto de Investigaciones Biológicas Clemente Estable (IIBCE) Mixed Unit— has the objective of carrying out and supporting biomedical research projects based on mass spectrometry (MS) and proteomics. It also offers training, scientific assistance and access to EM-based proteomic technologies to the local scientific community; and contributes to local and regional education programs in this area.

During the last years, the Unit incorporated mass spectrometers and developed the know-how to expand the quality and type of analytical procedures available. Currently, our analytical portfolio includes “shotgun” proteomic strategies, as well as gel-based strategies; in vivo and in vitro interactome studies; and analysis of post-translational protein modifications.

The research projects of our group focus on the study of signaling mechanisms in mycobacteria using proteomic approaches, with emphasis on the analysis of protein phosphorylation. In particular, we are interested in understanding some key processes for pathogenic mycobacteria, such as Mycobacterium tuberculosis, which are related to their ability to survive inside the host.

RESEARCH LINES

The role of protein phosphorylation in the regulation of biological processes in Mycobacterium tuberculosis.

Our work focuses on the characterization of signaling pathways mediated by phosphorylation in mycobacteria. In the past, we described the regulatory mechanisms of Ser/Thr kinases and identified some of their substrates and targets “downstream” in the signaling pathways. At present, we are using in vivo cross-linking in combination with mass spectrometry to obtain a “snapshot” of the protein-protein interactions in the living bacteria, with emphasis in the study of the interactome of the previously identified kinase-substrates. This has allowed us to begin to elucidate phosphorylation-dependent interactions that participate in the regulation of nitrogen uptake and cell division.

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

SERVICES

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.

To perform a routine analysis, please contact the technical team to confirm availability to the email ubypa@pasteur.edu.uy. When your application has been accepted, we will contact you to schedule the analysis.

Non-Routine Service

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

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

For scientific inquiries, please write to ubypa@pasteur.edu.uy. We will try to be flexibile to find the best way to use our facilities.

PUBLICATIONS

[1] Bellinzoni, M., Wehenkel, A.M., **Durán**, R., Alzari, P.M. Novel mechanistic insights into physiological signaling pathways mediated by mycobacterial Ser/Thr protein

kinases (2019) Genes and Immunity, 20 (5), pp. 383-393. DOI: 10.1038/s41435-019-0069-9 IF 2.6

[2] Chavarría, C., Trostchansky, A., **Durán**, R., Rubbo, H., Souza, J.M. Nitroalkylation of α-synuclein by nitro-oleic acid: Implications for parkinson's disease (2019) Advances in Experimental Medicine and Biology, 1127, pp. 169-179. DOI: 10.1007/978-3-030-11488-6_11 IF 2.1

[3] **Gil**, M., **Lima**, A., Rivera, B., Rossello, J., Urdániz, E., Cascioferro, A., Carrión, F., Wehenkel, A., Bellinzoni, M., Batthyány, C., Pritsch, O., Denicola, A., Alvarez, M.N., Carvalho, P.C., Lisa, M.-N., Brosch, R., Piuri, M., Alzari, P.M., Durán, R. New substrates and interactors of the mycobacterial Serine/Threonine protein kinase PknG identified by a tailored interactomic approach (2019) Journal of Proteomics, 192, pp. 321-333. DOI: 10.1016/j.jprot.2018.09.013 IF 3.5

[4] Miles, S., **Portela**, M., Cyrklaff, M., Ancarola, M.E., Frischknecht, F., **Durán**, R., Dematteis, S., Mourglia-Ettlin, G. Combining proteomics and bioinformatics to explore novel tegumental antigens as vaccine candidates against Echinococcus granulosus infection (2019) Journal of Cellular Biochemistry, 120 (9), pp. 15320-15336. DOI: 10.1002/jcb.28799 IF 3.4

[5] **Santos**, L., **Colman**, L., **Contreras**, P., Chini, C.C., **Carlomagno**, A., **Leyva**, A., **Bresque**, M., Marmisolle, I., Quijano, C., **Durán**, R., **Irigoín**, F., **Prieto-Echagüe**, V., Vendelbo, M.H., Sotelo-Silveira, J.R., Chini, E.N., **Badano**, J.L., **Calliari**, A.J., **Escande**, C. A novel form of Deleted in breast cancer 1 (DBC1) lacking the N-terminal domain does not bind SIRT1 and is dynamically regulated in vivo (2019) Scientific Reports, 9 (1), art. no. 14381. DOI: 10.1038/s41598-019-50789-7 IF 4.0

BIOINFORMATICS UNIT

Bioinformatics is the application of computational and statistical techniques for the analysis of data of biological origin, which also includes its organization and storage as well as the development of tools to manage them.

The volume of data currently generated by sequencing techniques promotes a constant development of methods to deal efficiently with numerous genomes and proteomes, without losing sight of the biologically relevant data.

At the Bioinformatic Unit we apply various tools to the comparative study of genomes on a global scale, as well as specific families of genes and proteins. Historically we have focused on bacteria, human pathogens and the human genome. We have also developed specific tools to deal with the new methodological challenges posed by the information explosion. These applications and developments have a place in genetics and biomedicine, and most recently in metagenomics, the genomic characterization of environmental samples.

RESEARCH LINES

Human genomics:

The URUGENOMES project seeks to create local capacities in the area of human genomics and especially medical genomics. In this context, 50 genomes were sequenced, which are being analyzed from the point of view of population genomics (ancestry), and 30 other genomes of patients with rare diseases, which are analyzed in the context of medical genomics.

Human transcriptomics:
One of the areas in which the unit specializes is human transcriptomics. Several projects are currently being developed within this line. One of them is dedicated to the study of differentiation processes of stem cells to different cell types (adipogenesis, osteogenesis and cardiomyogenesis). Of special interest is the post transcriptional regulation that takes place during these processes. Another project within this research line aims to analyze the response of human cells to the infection of pathogens of different origins.

Structural bioinformatics:
We seek to exploit data on protein structures, generated by X-ray crystallography and other techniques (NMR, cryo-electromicroscopy), in the functional understanding of protein families. Using three-dimensional structures and genomic sequences often provides clues about the function of a protein and, sometimes, generates functional hypotheses about whole families of 'hypothetical' genes (genes without known function).

Genomics and microbial evolution:
It seeks to understand the evolution of pathogens that affect livestock and domestic animals using phylodynamics; and fundamentally the discovery of the evolutionary forces that shape the genomes of zoonotic bacteria such as Campylobacter, Leptospira and Mycobacterium. Among the ongoing projects within this line, there is currently a Latin American joint effort to study the population dynamics of Clostridium difficile using genomic epidemiology approaches. Recently, one of the projects of this line seeks to analyze the dynamics of resistance to antibiotics in enterobacteria of urban environments through the application of metagenomics.

Statistical genetics and its application to evolution

SERVICES

- **NGS and microarrays data analysis:**
    1. Genome Assembly
    2. Variant analysis
    3. Transcriptomic and ribosomal profile analyses
    4. Small RNAs data analysis
- Sequence alignment and phylogenetic inference
- Biostatistics and advice on specific software usage
- Metagenomic data analyses and experimental design.

PUBLICATIONS

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[2] Marcon, B.H., Shigunov, P., **Spangenberg**, L., Pereira, I.T., de Aguiar, A.M., Amorín, R., Rebelatto, C.K., **Correa**, A., Dallagiovanna, B. Cell cycle genes are downregulated after adipogenic triggering in human adipose tissue-derived stem

cells by regulation of mRNA abundance (2019) Scientific Reports, 9 (1), art. no. 5611. DOI: 10.1038/s41598-019-42005-3 IF 4.0

[3] Pereira, I.T., **Spangenberg**, L., Robert, A.W., Amorín, R., Stimamiglio, M.A., **Naya**, H., Dallagiovanna, B. Cardiomyogenic differentiation is fine-tuned by differential mRNA association with polysomes (2019) BMC Genomics, 20 (1), art. no. 219. DOI: 10.1186/s12864-019-5550-3 IF 3.5

[4] **Spangenberg**, L., **Graña**, M., Mansilla, S., Martínez, J., Tapié, A., **Greif**, G., Montano, N., Vaglio, A., Gueçaimburú, R., **Robello**, C., Castro, L., Quijano, C., Raggio, V., **Naya**, H. Deep sequencing discovery of causal mtDNA mutations in a patient with unspecific neurological disease (2019) Mitochondrion, 46, pp. 337-344. DOI: 10.1016/j.mito.2018.09.004 IF 3.4

[5] Zarate YA, Bosanko KA, Caffrey AR, Bernstein JA, Martin DM, Williams MS, Berry-Kravis EM, Mark PR, Manning MA, Bhambhani V, Vargas M, Seeley AH, Estrada-Veras JI, van Dooren MF, Schwab M, Vanderver A, Melis D, Alsadah A, Sadler L, Van Esch H, Callewaert B, Oostra A, Maclean J, Dentici ML, Orlando V, Lipson M, Sparagana SP, Maarup TJ, Alsters SI, Brautbar A, Kovitch E, Naidu S, Lees M, Smith DM, Turner L, Raggio V, **Spangenberg** L, Garcia-Miñaúr S, Roeder ER, Littlejohn RO, Grange D, Pfotenhauer J, Jones MC, Balasubramanian M, Martinez-Monseny A, Blok LS, Gavrilova R, Fish JL. Mutation update for the SATB2 gene. Human Mutation, Variation, Informatics, and Disease 2019 Aug; 40(8):1013-1029. doi: 10.1002/humu.23771. IF 5.3

BIOPHARMACEUTICAL DEVELOPMENT LAB

The Biopharmaceutical Development Lab offers vast experience in bioassays and protein chemistry, as well as a wide range of analytic techniques and lab equipment.

It provides solutions in the field of analytical control of biopharmaceuticals, either using pre-established methodologies based on international guides and pharmacopeia, or developing new analytic tools in order to meet and follow bioanalytical strategies. Our assays follow ICH guidelines and the FDA and EMA regulations.

Created in June 2009 under the direction of Alejandro Ricciardi, the lab was established by the Ministry of Public Health as a national reference laboratory for the control of biopharmaceuticals sold in the Uruguayan market.

To fulfill this task, the Biopharmaceutical Development Laboratory has highly qualified human resources, equipment and the support of different technological platforms of the Institut Pasteur de Montevideo. Our services are developed under GLP conditions (Certified by LSQA, Enabled by MSP), and in accordance with the directives established by the ICH guidelines, as well as the FDA and EMA agencies.

RESEARCH LINES

The unit has developed biopharmaceuticals kits and analytical methodologies for the productive sector. An example of this have been the following projects:

Development of Methodologies to Quantify Proteins and DNA Contaminant Derivatives of the Host Cell in Recombinant Bio-Pharmaceuticals. Funded by the National Research and

Innovation Agency. (Project ALIANZA – Laboratorio Celsius S.A. and IP Montevideo) (2011 – 2012)

Methodological development for quantification of immunogenicity generated by administration of Interferon beta1a in patients, by means of a Bioassay based on cell culture and Real Time PCR. Funded by Laboratorios Clausen S.A. (2010).

Participation in a Multicenter Study for the Determination of the Biological Potency of the First Filgrastim Standard of the United States Pharmacopeia (USP). (2012)

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

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.

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.

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.

CELL BIOLOGY UNIT

The Cell Biology Unit (UBC) is a technology-based platform whose mission is to offer advice and support to several research groups or industries in different projects that use cell cultures and flow cytometry as a main technology. Since 2007, our unit carries out collaborative actions with the Servicio de Clasificación Celular y Citometría de Flujo (SECIF) from Clemente Estable Institute.

Using these technologies (cell culture and flow cytometry) we develop research projects in the area of biotechnology.

The UBC is also member of the Molecular, Cellular and Animal Technology Program (ProTeMCA), one of the institutionally supported programs.

RESEARCH LINES

Cell technology:
Our group has been dedicated to the generation and characterization of recombinant cell lines of biomedicine and biotechnological interest. Among them, we use a great variety of reporter cell lines (IFN type I, NF-kB, redox biosensors, among others) to search for and characterize substances that interfere with the signaling pathways of type I IFNs (Burgi et al, 2012 and Burgi et al, 2016). Also we use them in in-vitro models of inflammation (NF-kB, Tiscornia et al, 2012 Mastropietro et al, 2015; Rolny et al, 2016) or to improve the metabolism/productivity of cells of biotechnological interest (redox biosensors within the framework of ProTeMCA).

Environmental toxicology:
Endocrine disruptors (ED) are anthropogenic substances present in the environment, capable of altering the homeostasis of the endocrine system of organisms, contributing to the development of pathologies. Our working hypothesis is that the increase of certain reproductive pathologies is caused, in part, by the increasing exposure to EDs present in the environment. In this context,

we have validated an in vivo model —the transgenic mouse Oct4-GFP— for toxicological monitoring of environmental estrogens (Porro et al, 2015). We have also developed an in vitro assay that uses a dual reporter cell line and allows us to evaluate in a single test the estrogenic or androgenic activity of a putative ED. In this line of research, we are collaborating with Dr. Rodríguez (ISAL, CONICET-UNL, Argentina).

Antitumoral peptides:
Since 2011, we have collaborated with Dr. Vallespi, from the Center for Genetic Engineering and Biotechnology (CIGB), in Cuba, on the project "CIGB-552: novel peptide with antitumor and anti-inflammatory properties useful for cancer treatment".

We demonstrate that CIGB-552 is effective in reducing the size of tumours present in mice and we identify the COMMD1 protein as a key mediator for its antitumor activity (Fernández Massó et al, 2013, Vallespí et al., 2014, Núñez de Villavicencio et al., 2015).

Recently, we described the minimum functional unit of CIGB-552 necessary to exercise its biological activity (ability to penetrate tumor cells, interact with COMMD1 and induce apoptosis, Astrada et al, 2016 and 2018).

Tissue engineering: intestinal organoids and 3d crops:
Traditional cell cultures, in two dimensions, offer a simple, rapid, economic and reproducible evaluation system. However, they lack the ability to recreate the cellular interactions that take place in a tissue in vivo, limiting its predictive power. Our goal is to generate and characterize cellular models in three dimensions (3D) that resemble living systems, improving the correlation of results. In particular, we propose to establish a murine intestinal organoid culture, either from primary tissue or by differentiation of induced pluripotent stem cells (from the English iPSC) and apply them in the study of inflammation, cancer and probiotic screening.

EQUIPMENT

Laboratory of cellular crops

- We have several laboratories equipped to work in an aseptic environment, such as biological safety cabinets, CO2incubators, cytological centrifuges and inverted microscopes.
  We also have an Automatic Analyzer for Glucose and Lactate Bioprofile Basic 2 Analyzer (Nova Biomedical)

Flow cytometry laboratory

Cellular analyzers:

- CyAn™ ADP (Beckman Coulter)
- BD Accuri™ C6 (BD Biosciences)
- Attunne Nxt (Thermo Fisher Scientific)
- BD FACSAria™ Fusion (BD Biosciences) High speed cellular classifier

Software for data analysis:

– Summit V4.3

– BD Accuri C6 Software V1.0.264.21

– FACSDiva

– FlowJo

We organize an annual training course for new users of the installed equipment. We are open to establish collaborations and to participate in research projects providing technical and scientific assistance.

For more information and making reservations, please contact us at: citometria@pasteur.edu.uy

SERVICES

- Culture, amplification and storage of cell lines. We have a cellular bank with more than 20 certified cell lines (ATCC, DSMZ, Riken).
- Detection of contamination by Mycoplasma sp in cell cultures using PCR.
- Quantification of glucose and lactate in cell culture supernatants.
- Cell-based assays: cytotoxicity, proliferation, transfection, generation of stable cell lines.
- Experimental design, sample processing, acquisition and analysis by flow cytometry.
- Training and advice for cytometry users.
- Isolation of different cell populations from a mixture by high-speed cell separation (up to 4 pathways).
- Cellular cloning by depositing a single cell per well using a cell sorter.
- Analysis by Flow Cytometry: Procedure and Costs
- Request form for analysis using Analytical Cytometers-CyAn ADP / BD Accuri C6 / Attune NXT
- Analysis request in BD FACSAria Fusion

PUBLICATIONS

[1] Bürgi M, Hernández P, Cabrera M, Cerecetto H, González M, Kratje R, Raimondi A, Oggero M, **Bollati-Fogolín** M. Identification and characterization of human interferon alpha inhibitors through a WISH cell line-based reporter gene assay. Bioorg Chem. 2019 Oct 28:103372. doi: 10.1016/j.bioorg.2019.103372. **[Epub ahead of print]** IF 3.9

[2] **Daghero**, H., **Pagotto**, R., Vallespí, M.G., **Bollati-Fogolín**, M. Generation of stable reporter breast and lung cancer cell lines for NF-κB activation studies (2019) Journal of Biotechnology, 301, pp. 79-87. DOI: 10.1016/j.jbiotec.2019.05.014 IF 3.2

[3] Martínez, J., Tarallo, D., Martínez-Palma, L., **Victoria**, S., **Bresque**, M., Rodríguez-Bottero, S., Marmisolle, I., **Escande**, C., Cassina, P., Casanova, G., **Bollati-Fogolín**, M., Agorio, C., Moreno, M., Quijano, C. Mitofusins modulate the increase in mitochondrial length, bioenergetics and secretory phenotype in therapy-induced senescent melanoma cells (2019) Biochemical Journal, 476 (17), pp. 2463-2486. DOI: 10.1042/BCJ20190405 IF 4.3

[4] **Piattoni**, C.V., **Sardi**, F., **Klein**, F., **Pantano**, S., **Bollati-Fogolin**, M., **Comini**, M. New red-shifted fluorescent biosensor for monitoring intracellular redox changes (2019) Free Radical Biology and Medicine, 134, pp. 545-554. DOI: 10.1016/j.freeradbiomed.2019.01.035 IF 5.6

MOLECULAR BIOLOGY UNIT

The Molecular Biology Unit (UBM) offers standardized nucleic acid sequencing services and fragment analysis by capillary electrophoresis. These services are aimed to provide technical support and scientific advice to researchers of the institute and other public or private research organizations, companies and centers across the region.

It also offers advice for the experimental design of genomic and transcriptomic studies through DNA microarrays or massive sequencing. It has a microarray scanner (Agilent) and a high capacity sequencer (Illumina) that allows the realization of a wide spectrum of experiments.

RESEARCH LINES

Study of the intracellular host-pathogen interaction with a systemic approach

At the UBM we studied virulence factors of intracellular pathogens, as well as the host genes and routes necessary for the establishment of the infection, and the interface between them. Based on this, one of our objectives is the design of new strategies for the treatment or prevention of various infectious pathologies, like Chagas disease, African trypanosomiasis, leishmaniasis, and tuberculosis.

Determination of virulence factors necessary for T.cruzi infection.

For the establishment and persistence of T. cruzi infection, we have described a set of proteins related to the redox metabolism of trypanosomatids (triparedoxin cytosolic and mitochondrial peroxidase, triparredoxins, glutarredoxins, pteridin reductase), characterizing them from the structural and functional point of view. We are developing strategies to inhibit them, and applying them in the development of therapeutic and preventive strategies.

Characterization of changes in gene expression produced by intracellular parasites in human cells

We have shown that T.cruzi triggers a cellular reprogramming that allows the establishment and persistence of the infection in human cells. One of our objectives is to identify host proteins, which, when inhibited, prevent T. cruzi infection. We are also extending this strategy for genomic and molecular biology studies in leishmaniasis, African trypanosomiasis, neosporosis and tuberculosis.

EQUIPMENT

- DNA sequencer / analyzer
- Real-time PCR
- Microarray reader
- Microarray hybridizer
- BioAnalyzer
- General molecular biology instrumentation
- MiSeq Illumina

SERVICES

- **DNA sequencing (Sanger method)**

The UBM has the only facility in Uruguay for DNA sequencing service that performs sequencing using the Sanger Method and fragment analysis. We are working with a Genetic Analyzer 3130 (Applied BioSystems), and recently we acquired a 3500 Genetic Analyzer (Applied BioSystems), which will improve performance. The unit receives samples from private and public institutions throughout the country.
Bellow we will find the cost of the services provided by the unit:

Service	**Cost (US$)***	**Coupon book x100 (US$)***	**Private**
Secuence	7	600	20
Fragments	2,5	200	5
Purification	2,5	200	5

** Prices for academic sector (national and international)*

The coupons expires 12 months after purchase.

– For the purchase of services, please write to: tecnologia@pasteur.edu.uy

– For consultation techniques or other services, please write to: ubm@pasteur.edu.uy

– To submit samples it is necessary to complete the following forms:

Sequence Request Form

Fragment Analysis Form

- **Microarrays**

The Agilent platform is still being used for transcriptomic studies of complex organisms, mainly human and bovine samples. There is also sporadic cooperation with private clinics that use Comparative Genomic Hybridization in diagnostics (for example, genetic diagnoses prior to implantation).

- **Deep sequencing**

The Illumina device has led to the beginning of the Genomics Program, and is now widely used in different applications, mainly genomics and transcriptomics of pathogens (bacteria and unicellular protozoa). We also provide practical training on 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 in their research.

PUBLICATIONS

See HOST-PATHOGENS INTERACTION LAB.

PROTEIN CRYSTALLOGRAPHY UNIT

The purpose of the Unit is to set up and maintain a Protein Crystallography Facility PXF, to provide equipment, training, assistance, and technological innovations for determining three-dimensional structures of proteins and other macromolecules and macromolecular assemblies.

X-ray crystallography is one of the most powerful techniques to study the 3D structures of macromolecules and it has transformed our understanding of biological processes. Our setup allows users to crystallize macromolecules and solve their three-dimensional structures using X-ray diffraction.

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, and thus the probability of finding hits.

- **Automatic setup and optimization of screen assays – 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: vessels containing "carbon molecular sieve" (CMS) and an air compressor as a source of clean dry air.

SERVICES

To access the facility users should write to pxf@pasteur.edu.uy making a short request. A form will be provided, which includes information about the project and specifications on the macromolecule and other details. This will be evaluated by the Facility staff in terms of technical feasibility and scientific relevance. All requests will have a due response.

Experimental approaches currently available for users

• Protein crystallization screenings (manual and robotic [Honeybee963® 96-well robot)

• Follow-up and optimization of initial crystallization hits (manually and robot-assisted with an Alchemist® instrument)

• X ray Diffraction – Testing & Crystal Characterization

• X ray Diffraction – single crystal data collection

• Crystal Structure Determination & Refinement

PUBLICATIONS

See MOLECULAR AND STRUCTURAL MICROBIOLOGY LAB

RECOMBINANT PROTEINS UNIT

Recombinant proteins —those produced in the laboratory by genetic engineering using cells different from nature— have shown a high impact in basic research and in the biomedical field for drug manufacture. However, in many cases it is not possible to obtain a stable, soluble and homogeneous product, and this limits its applications. Several strategies were developed in the last decades to overcome these limitations. In this sense, our group has generated a set of vectors that facilitates cloning and allows the evaluation of several parameters that can improve the soluble expression of a target protein.

In the context of cancer therapeutic tools, our group recently focused on the generation of artificial binding proteins known as Affitins. This class of proteins has a wide range of advantages compared to classical therapeutic antibodies that could be taken into account in the development of therapeutic approaches.

RESEARCH LINES

Vector Suite and Artificial Binding Proteins (ABP)

Our laboratory has developed a set of vectors that allow the evaluation of different promoters and proteins that enhance solubility, through an efficient cloning strategy. This vector suite was extended in order to generate a set of vectors to include the evaluation of expression of recombinant proteins in different cellular compartments and cellular hosts. This helps to overcome the limitations encountered when working with a single subcellular location and a single type of host. In addition, these vectors also allow the evaluation of alternative purification strategies for the improvement of the yields of the target protein.

In the context of cancer therapeutic tools, our group recently focused on the generation of artificial binding proteins known as Affitins. This class of proteins has a wide range of advantages compared to classical therapeutic antibodies that could be taken into account in the development of therapeutic approaches.

Compared to classical therapeutic antibodies, Affitins are able to maintain constant high affinity even when their molecular weight remains small. This could be very useful in lymphoid neoplasms, in order to gain access to solid tissues as secondary lymphoid organs, where leukemic cells receive survival signals that acquire favourable conditions of proliferation.

In this line, a new generation of combinatorial protein engineering technologies has recently been created in our laboratory. The results have made it possible to propose the use of these

artificial binding proteins as versatile selective glycosidase inhibitors and, potentially, as enzymatic inhibitors in general, which could be foreseen for future tumour therapy strategies (Correa et al., PlosOne, 2014).

EQUIPMENT

- ÄKTAxpress / ÄKTA Pure and ÄKTA Purifier
- Benchtop Bioreactor BIOSTAT® B plus (Prokaryotic culture)
- CelliGen 310 Bioreactor (Eukaryotic culture)
- BelloCell 3000 Bioreactor (Eukaryotic culture)
- EmulsiFlex-C5 Homogenizer
- Multitron 2 Incubated Shaker

SERVICES

- Expression of recombinant proteins (PR) in prokaryotic and eukaryotic systems:
    - PR expression in E. coli
    - PR expression in mammalian cells
    - PR expression in insect cells
- Optimization of the expression of soluble recombinant proteins

PUBLICATIONS

See CHRONIC LYMPHOID LEUKEMIA LAB.

TRANSGENIC AND EXPERIMENTAL ANIMAL UNIT

The unit provides high-level support in the field of animal genome modification (mice, rats and ruminants). We offer several techniques, such as pronuclear microinjection, homologous recombination in embryonic stem cells, lentiviral injection, transposons and the revolutionary CRISPR / Cas9 system. We also provide cryopreservation, in vitro fertilization and embryo rederivation in mice. Besides, the unit supplies mice and rats with high genetic and health status for researchers within the institute and the region.

Since 2007 we have worked with national and international biotechnology companies offering in vivo biological assays in mice and rats, under GLP standards. We receive undergraduate and graduate students who do thesis using different technologies to obtain animal models. We also organize courses for technicians and scientists, and offer internships for technicians or veterinarians who administer their own services.

Our staff is member of organizations, such as the International Society for Transgenic Technologies (ISTT), the Institutional Articulation Committee (CAI), the National Commission of Animal Experimentation (CNEA), the Uruguayan Association of Animal Science and Technology Laboratories (AUCyTAL), and the Federation of South American Associations for Laboratory Animal Science (FESSACAL)

RESEARCH LINES

Gene editing in rodents

Gene editing in ruminants

Cryopreservation of embryos and mammals sperm

In vitro fertilization of rodents and ruminants

EQUIPMENT

Facilities

- Animal Facility: SPF (Specific Pathogen Free) and Conventional area
- Microinjection Laboratory
- Assisted Reproduction Techniques Laboratory (ART)
- Molecular Biology Laboratory

SERVICES

- Generation of transgenic animals using CRISPR / Cas9 technology, pronuclear microinjection of DNA fragments, gene-targeting in embryonic stem cells, Sleeping Beauty Transposons technology.
- Cryopreservation of embryos and sperm.
- Rederivation of murine lines
- In vitro fertilization of murine lines
- Crosses and maintenance under SPF and conventional conditions of various strains mice (C57BL / 6J, BALB / cJ, DBA / 2J, Nude, several hybrids and about 30 different transgenic lines). Approximate production (currently): 2.000 mice per month.
- Immunomodulatory capacity assays of probiotic bacteria
- Biological activity assays of recombinant erythropoietin. Our Bioterio is certified by the Ministry of Public Health
- Toxicity tests for biotechnological products (EPO, Filgen, Interferon)
- Production of polyclonal antibodies in mice

PASTEUR+INIA JOINT UNIT

The Institut Pasteur of Montevideo (IP Montevideo) and the National Institute of Agricultural Research (INIA) Joint Unit was created in October 2014 with the aim of promoting and adding value to knowledge and combining research areas of both institutions. It also seeks to provide solutions to the agricultural sector based on the use of technologies developed from multidisciplinary approaches in animal health, microbiology, bioinformatics, molecular genetics and generation of bio-inputs.

This Unit intends to carry out joint research projects, train human resources and create associative networks in areas of mutual interest, meeting the needs of the agricultural sector.

As an open platform, the unit is available to researchers from IP Montevideo, INIA and other research institutions, promoting the linkage of the productive sector, academy and industry.

RESEARCH LINES

Bioinputs

We study new bio-inputs formulated with microorganisms or microbial bioactive compounds to improve the productivity, quality and health of the plants or the characteristics of the soils. We seek the development of products and technologies that allow improving the sustainability of food production systems, complying with safety standards and reducing the use of agrochemicals.

Infectious diseases affecting livestock

We study Enzootic Bovine Leukosis, Tuberculosis, Campylobacteriosis, Leptospirosis and Neosporosis. These diseases affect animal health and have a negative impact on livestock production. Some of these infectious diseases can also affect humans and put at risk the health of those who work in contact with infected animals. The objective is to develop technologies for the diagnosis, treatment and control of these diseases, seeking to improve the global health status of Uruguayan cattle.

EQUIPMENT AND SERVICES

The UMPI has a laboratory with Biosafety level II to work with pathogenic microorganisms. This makes it possible to strengthen the work capacities of the researchers that work under the Animal Health program at the institute.

The laboratory has areas equipped and dedicated for:

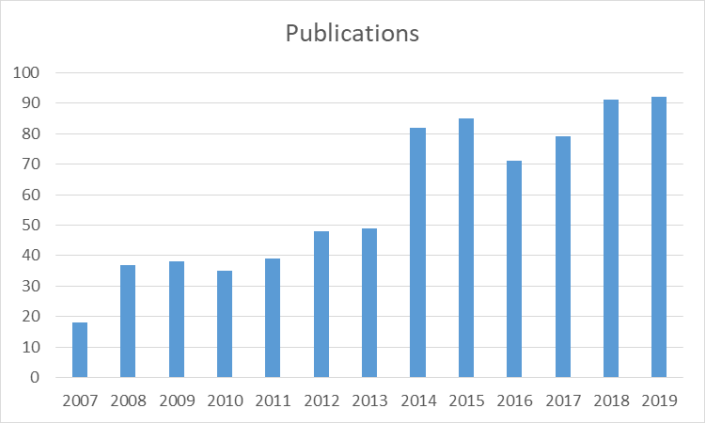
- Cultivation of aerobic microorganisms
- Cultivation of eukaryotic cells
- Extraction of nucleic acids and proteins
- Preparation of reaction mixtures for the amplification of nucleic acids (Pre-PCR area)
- Amplification by conventional or real time PCR (PCR zone)
- Quantification and analysis of nucleic acid amplification products (Post-PCR area)
- Protein analysis (ELISA / Western Blot)

PUBLICATIONS

[1] **Hamond**, C., Silveira, C.S., Buroni, F., Suanes, A., **Nieves**, C., Salaberry, X., Aráoz, V., Costa, R.A., Rivero, R., Giannitti, F., **Zarantonelli**, L. Leptospira interrogans serogroup Pomona serovar Kennewicki infection in two sheep flocks with acute leptospirosis in Uruguay (2019) Transboundary and Emerging Diseases, 66 (3), pp. 1186-1194. DOI: 10.1111/tbed.13133 IF 3.5

INSTITUT PASTEUR DE MONTEVIDEO AT A GLANCE – 2019

PUBLICATIONS



INTERNATIONAL COURSES

	2013	2014	2015	2016	2017	2018	2019
Courses	7	8	6	9	9	11	13
Students	85	174	49	260	217	163	235
Regional Students	40	51	68	91	139	121	230
Invited Professors	44	36	65	57	71	79	105
Funding (k USD)	140	211	190	201	250	148	183

HUMAN RESOURCES TRAINING

	2014	2015	2016	2017	2018	2019
Post-docs	24	22	26	14	14	21
PhD students	32	33	44	40	47	51
MSc students	44	47	51	37	40	32
Undergraduate students	25	35	19	5	17	16
TOTAL	**125**	**137**	**140**	**96**	**118**	**120**