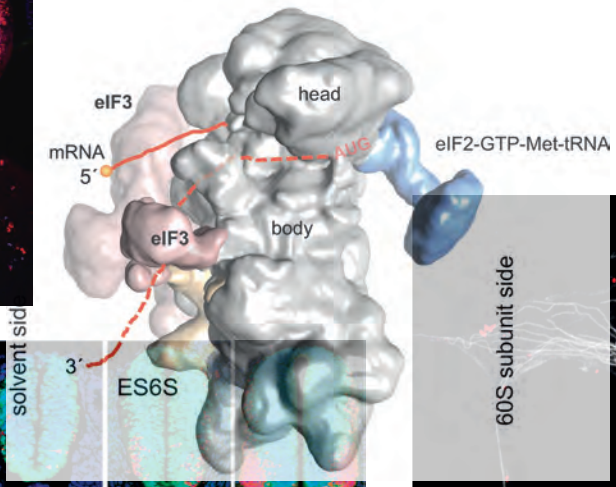
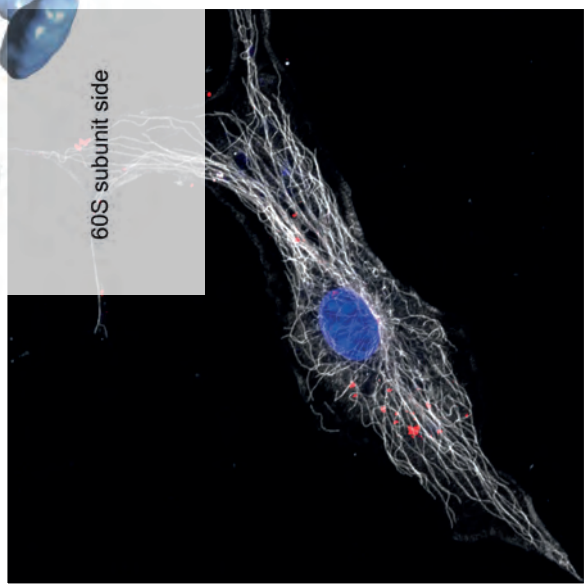
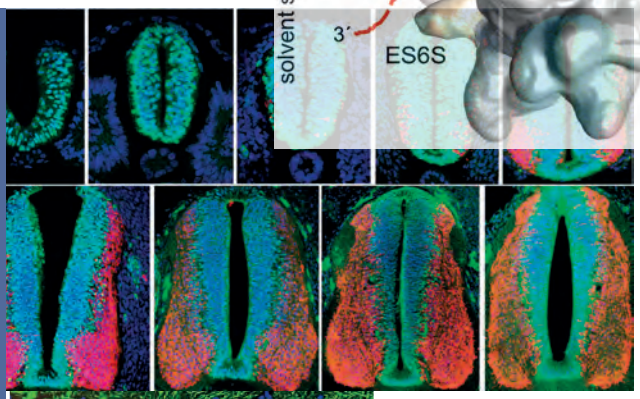
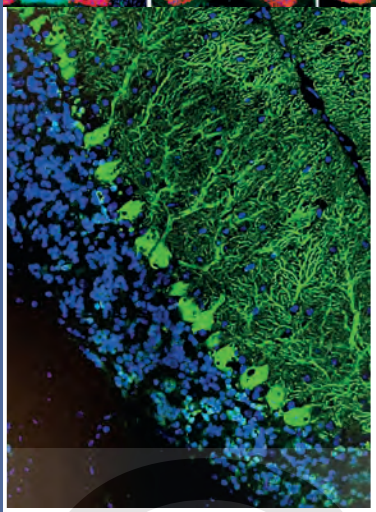
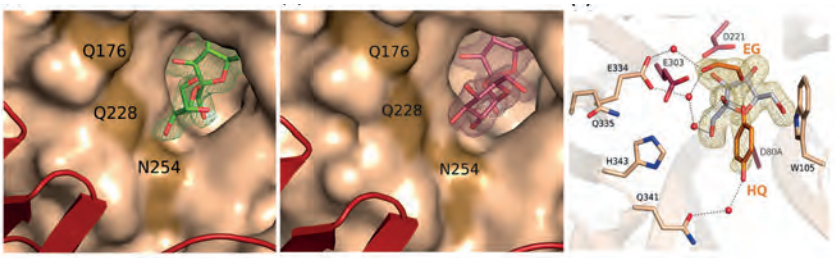
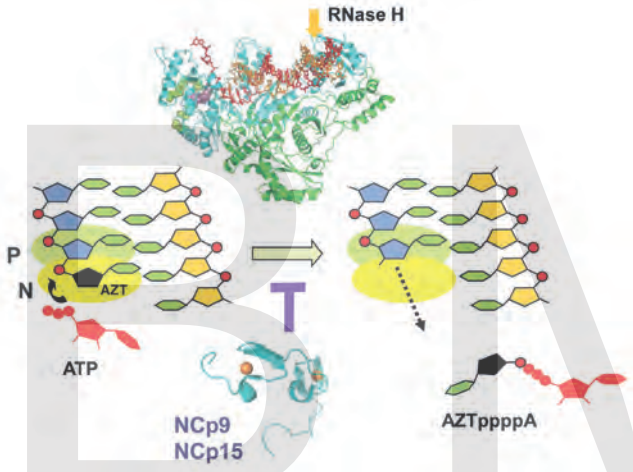


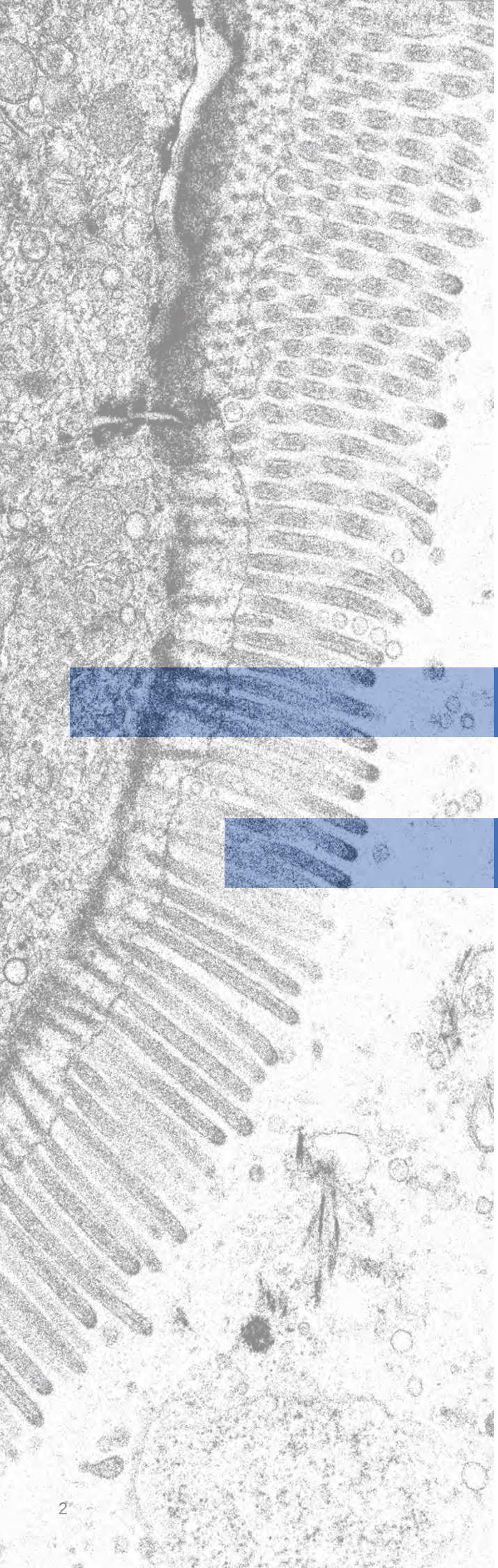





# Scientific Report 2019-2020

CENTRO DE BIOLOGÍA MOLECULAR SEVERO OCHOA




**CENTRO DE BIOLOGÍA MOLECULAR SEVERO OCHOA**

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Campus de la Universidad Autónoma de Madrid
28049 Madrid
Tel.: (+34) 91 196 4401
www.cbm.uam.es

# INDEX

**Lourdes Ruiz Desviat**

*CBM DIRECTOR*

Dear colleagues and friends,

I am pleased to present the scientific report 2019-20 of the Centro de Biología Molecular Severo Ochoa (CBM), giving an overview of the events and scientific and strategic activities during this period.

Looking back at 2019-2020, the overwhelming situation of COVID-19 pandemic, in which we are still immersed, immediately comes to everybody's mind. However, I would like to highlight 2019-2020 as the years in which our center completed a substantial remodelling process, following the advice of the SAB that visited us in December 2018 and the internal analysis and strategic plan approved by the CBM General Board (Claustro Cientifico). In these two years, we have implemented profound changes in the scientific organization and we have recruited young and extremely promising scientists, as well as some senior consolidated groups, adding their expertise to the strategic lines of the center. In its new organization, the CBM strives to foster collaborative work between senior and junior principal investigators (PIs) in 12 dynamic Units, favouring shared laboratory space and resources, according to common research interests. Units are in turn integrated into four research Programs. I would like to thank the elected Unit Coordinators and Program Directors for their work managing and organizing the new scientific structure during this initial stage. The CBM has also worked in the renewal of its infrastructures and in the incorporation of new technologies. Of note, during this period CBM researchers have obtained ERC grants (1 StG, 1 CoG, 1 AdG), as well as other highly competitive and well-funded EU grants, demonstrating their scientific excellence and potential. In addition, thanks to our rich academic life, CBM attracts the best students, as exemplified by the more than 60 predoctoral students who defended their PhD Thesis with the highest marks in 2019-20, supervised by CBM researchers.

Sadly, in November 2019 our respected and beloved colleague Prof. Margarita Salas passed away, after working enthusiastically in her lab at CBM for 42 years. On December 2020 the president of CSIC and the Rector of UAM inaugurated a bust in her honor in the Hall of CBM, to remember her as key reference and role model for present and next generations of Spanish scientists. I would also like to remember here with affection our colleague Prof. Carlos Alonso, who died victim of COVID19 in 2020. We are indebted to both of them, for their work and all their contributions to science and to our center.

When COVID-19 pandemic emerged in March 2020 we were obliged to a complete shutdown, maintaining only the essential housekeeping activities of the center. I would like to acknowledge the responsible attitude of all the personnel during this period, those coming to perform essential services and those working at home, as well as the generosity of those with experience in PCR techniques, who immediately volunteered to participate in SARS-COV2 diagnostic tests, following an urgent request from our authorities (although this was in the end never put to practice). I would also like to thank researchers and scientific/technical services for quickly setting up the conditions needed to perform these diagnostic tests in our center. Thanks to CSIC funding, the existing BSL-3 laboratory was upgraded to allow working with SARS-CoV-2 and other airborne transmitted pathogens, placing the CBM in a unique position to carry out research with this type of pathogens, both in tissue culture and in animal models. And very soon during the lockdown, several researchers redirected their work to the study of COVID-19, developing new strategies for SARS-COV2 detection, testing therapeutic approaches and studying disease biomarkers and different aspects of the immune response. In addition, one of our researchers coordinates the CSIC Interdisciplinary Thematic Platform known as Global Health Platform against COVID-19 pandemic and others lead strategic areas within this platform, such as Disease Diagnostics & Containment or Immune Response. And last but not least, CBM researchers have played an essential role in social media (television, radio, press etc), regularly explaining to the lay public the advances in detection, treatments and vaccines for COVID-19, and providing useful recommendations. I must say that in this very exceptional and dramatic situation, I am particularly proud of the relevant role that the CBM played and is playing in the fight against SARS-COV2.

In summary, having in mind the CBM reorganization and the scientific activities in progress, I am confident that after these two years we are on the right pathway to consolidate a leading position in the Spanish research landscape, maintaining an equilibrium between our well-known multidisciplinarity, which is one of our strengths, and scientific focus and innovation. I look forward to seeing the fruits of our work in the next few years, and foresee an improvement in our research outputs and the gain in internal coherence.

Finally, I would like to thank the commitment and excellent work of coordinators and personnel in scientific and technical services. Personally, I have to acknowledge the invaluable help of the Vice-director Dr Jaime Millán, of the internal Scientific Advisory Board and of the Management Committee, who have provided me with advice and support throughout 2019-20. I also want to thank the administrative and management staff for their dedication and work and for making everyday's tasks a little bit easier. It has been a privilege for me to be Director of the CBM these two years and to share my time with you all.

# Prof. Margarita Salas *In memoriam*

Margarita Salas, 80 years old, passed away in November 7, 2019. Margarita did a PhD on metabolism working at the laboratory of Alberto Sols. There, she met her future husband Eladio Viñuela. Both made their postdoctoral training in the group of Severo Ochoa at the New York University in the USA. They returned to Spain and set up their own group, at the Centro de Investigaciones Biológicas (CIB). Later on, on 1977, they moved to a brand-new Institute, the Centro de Biologia Molecular (CBM).

Among her scientific contributions are seminal studies on transfer genetic information, from DNA to protein indicating, for example, that genetic message is read in only one direction, and that UAA represents a stop codon where protein synthesis ends. By studying bacteriophage phi29, she found the presence of a terminal protein (TP) covalently bound to one end of each DNA strand. TPs were shown to be needed to start the process of viral DNA replication, not only in some bacterial, but also in some animal virus. Moreover, a specific DNA polymerase (phi29 pol) was shown to be crucial to use the TP primer, performing phi29 DNA replication with an exceptional processivity, fidelity and strand-displacement activities. These unusual properties were the basis for the biotechnological application of phi29pol, as a paradigm for the isothermal amplification of human genomic DNA from very limiting amounts of starting material, being widely used for forensic, archeologic, oncological and metagenomic analysis. The patent of the enzyme, and its commercial licensing has provided important incomes for the Spanish Research Council (CSIC). In 2019, Margarita received the "Life Achievement" award from the European Patent Office, as a relevant example that an excellent basic research is always a requisite for developing applications.

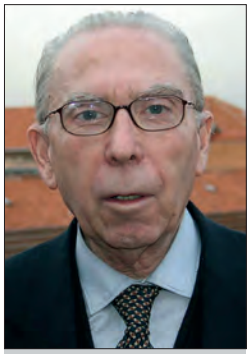
From 1992 to 1994, Margarita Salas was the Director of the CBMSO, where she was working until few weeks before her death. On October 2019, Margarita published her latest article.

Margarita Salas created a School (“the Margaritos”), by teaching molecular biologists from different origins, and telling them how to carry out a rigorous and original work. We will miss her, her mentorship and her leadership.

***Luis Blanco and Jesús Ávila***


Bust *in memoriam* of Prof. Margarita Salas, made by Victor Ochoa and inaugurated at the CBM on December 2nd, 2020.

# Prof. Carlos Alonso *In memoriam*



Carlos Alonso Bedate was born in Mota del Marqués (Valladolid) on November 29, 1935. He entered the Jesuit Order in 1953, and then pursued University studies in Philosophy (Alcalá de Henares, 1960) and Theology (Granada, 1966). At that time, in Granada, influenced by social contacts with prominent researchers, Carlos uncovered the challenges of biology and decided to become a scientist. He attained a master's degree in Genetics (University of California Davis, 1967-1969), and afterwards a Researcher position at Nijmegen University (Netherlands, 1970-1973), getting his first PhD doctorate. In parallel, he attained the Biological Sciences degree (1973) and doctorate (1974) at the University of Granada.

In 1973, as a member of the so-called *grupo granadino* headed by Prof. Federico Mayor Zaragoza, contributed to the first steps of the Molecular Biology Department at the recently inaugurated Universidad Autónoma de Madrid. From 1973 to 1978, Carlos was engaged in teaching activities as Associate Professor in the Biochemistry and Molecular Biology specialty, and was one of the research leaders of the early Centro de Biología Molecular Severo Ochoa (1976). Although he decided to follow his career path at the Spanish National Research Council (CSIC) from 1978, becoming Full Professor in 1998, he always kept his academic activities, teaching the *Molecular Interactions* subject to many generations of molecular biologists, who learned on cutting-edge topics, taught with a mixture of enthusiasm and critical view.

Carlos started his scientific activity in molecular genetics using the *Drosophila hydei* polytene chromosomes as a model, combining both molecular and microscopy approaches, and proved the real existence of the Z-DNA structure in the genome. In the early 1980s, because of his interest in applied science and neglected people and diseases, Carlos moved to the field of Parasitology, collaborating with the Colombian scientist Dr. Manuel E. Patarroyo on vaccines against malaria. After a short period working with the protozoan *Trypanosoma cruzi* (the causative agent of Chagas disease), Carlos focused on developing a vaccine against visceral leishmaniasis (VL). His key contributions in this area led in 2018 to the development of a commercially available vaccine against canine VL. After his formal retirement age, Carlos always kept very much alive his interest in science and continued working at the CBM as *ad Honorem* scientist, making contributions in the molecular evolution area.

Carlos also played a prominent role at both national and international levels in the field of Bioethics. In 1994, he participated as chair of the International Bioethics Committee, which culminated in the unanimous adoption, by the General Conference of UNESCO in 1997 and by the General Assembly of the United Nations in 1988, of the Universal Declaration on the Human Genome. Furthermore, he was a founding member of the First Spanish Bioethics Committee (2007), in which he actively participated until 2020. Carlos always stood out for the rigor, balance, and courage of his ideas, and for his willingness to generate consensus.

On 13 April 2020, the CBM community lost a very special member. Apart from his scientific contributions and mentoring of young scientists, Carlos will always be remembered at CBM as a friend and a reference, always open to help and to bring spiritual and personal support to every one of us.

***José María Requena and Federico Mayor Jr***

# Board of Directors and Manager

**LOURDES RUIZ DESVIAT**
CBM DIRECTOR

**JAIME MILLÁN MARTÍNEZ**
CBM VICE-DIRECTOR

**GERMÁN LERMA RODRIGO**
CBM MANAGER

**JOSÉ ANTONIO ESTEBAN GARCÍA**
REPRESENTANT OF CSIC AT
THE CBM SCIENTIFIC COMMITTEE

**FEDERICO MAYOR JR**
DIRECTOR OF THE INSTITUTO
UNIVERSITARIO DE BIOLOGÍA
MOLECULAR (UAM)

## *Program Directors*

**CRISANTO GUTIERREZ ARMENTA**

**GENOME DYNAMICS AND FUNCTION**

**PAOLA BOVOLENTA NICOLAO**

**TISSUE AND ORGAN HOMEOSTASIS**

**CARLOS DOTTI**

**PHYSIOLOGICAL AND PATHOLOGICAL PROCESSES**

**MARIA LUISA TORIBIO GARCÍA**

**INTERACTIONS WITH THE ENVIRONMENT**

**SONSOLES CAMPUZANO CORRALES**
CBM RERESENTANT IN GENDER
EQUALITY COMISSION

# Scientific Advisory Board

**Chairman: Dr. Matthew Freeman**
Sir Willian Dunn School of Pathology,
University of Oxford, Oxford (UK)

**Dr. Sebastian Amigorena**
INSERM U932, PSL Research University.
Institut Curie, Paris (France)

**Dr. John Diffley**
Cancer Reseach UK. London Research
Institute, London (UK)

**Dra. Anne Ephrussi**
Developmental Biology Unit. European Molecular
Biology Laboratory, Heidelberg (Germany)

**Dr. Reinhard Faessler**
Department of Molecular Medicine,
Max Plank Institute of Biochemistry,
Martinsried (Germany)

**Dr. Matthias W. Hentze**
Gene Expression Programe, European
Molecular Biology Laboratory,
Heidelberg (Germany)

**Dr. Vivek Malhotra**
Departament of Cell and Developmental Biology,
Centre de Regulació Genòmica, Barcelona (Spain)

**Dr. Ed Palmer**
Department of Biomedicine University
Hospital Basel, Basel (Switzerland)

**Dr. Felix Rey**
Molecular and Structural Microbiology,
CNRS URA 3015, Institut Pasteur,
Paris (France)

**Dr. Giampietro Schiavo**
UCL Institute of Neurology, Discoveries Centre for
Regenerative and Precision Medicine, UCL Campus
University College London, London (UK)

**Dr. Matthijs Verhage**
Department of Functional Genomics at
the VU University Medical Center, Vrije
Universiteit, Amsterdam (Netherlands)

**Dr. Waldemar Vollmer**
Institute for Cell and Molecular Biosciences,
Newcastle University, Newcastle upon Tyne (UK)

# Programs

## GENOME DYNAMICS AND FUNCTION

*13 PIs*

## TISSUE AND ORGAN HOMEOSTASIS

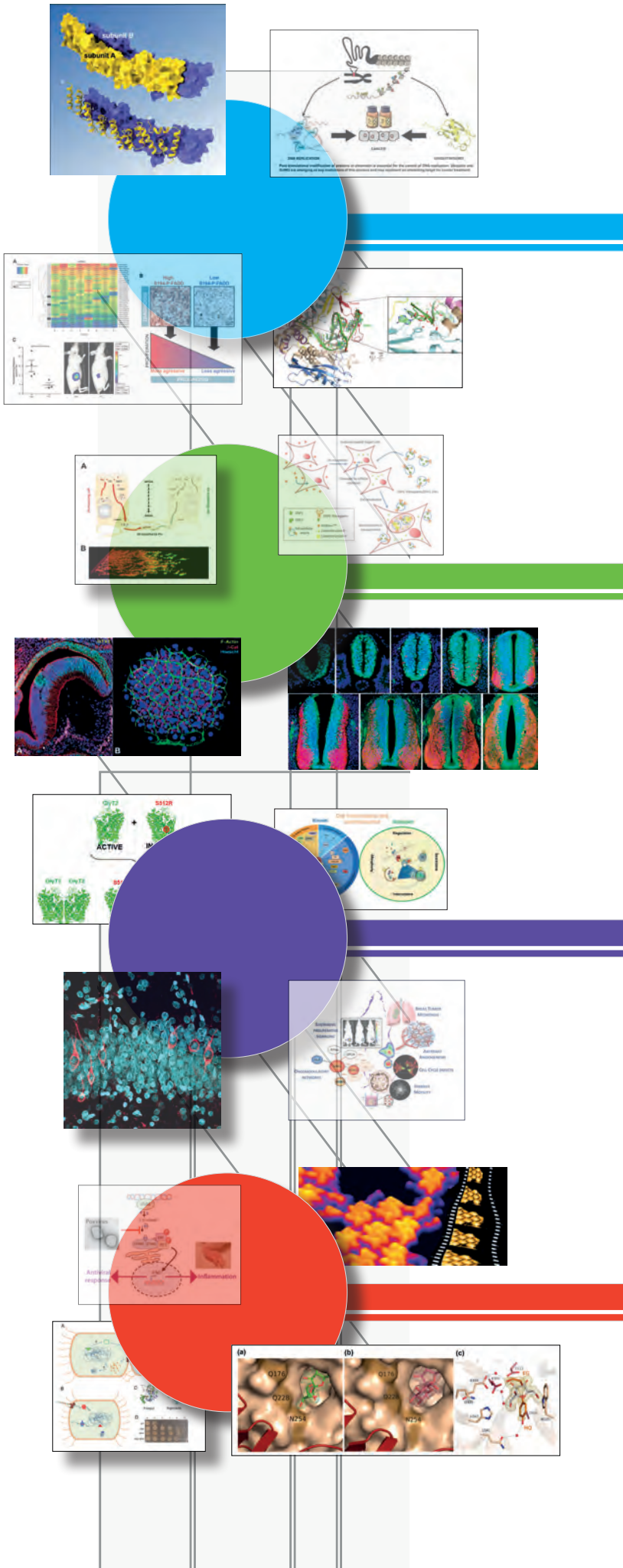
*22 PIs*

## PHYSIOLOGICAL AND PATHOLOGICAL PROCESSES

*32 PIs*

## INTERACTIONS WITH THE ENVIRONMENT

*24 PIs*



# *Units*

## GENOME DECODING

### GENOME MAINTENANCE AND INSTABILITY

## CELL ARCHITECTURE AND ORGANOGENESIS

### CELL-CELL COMMUNICATION AND INFLAMMATION

### SPECIFICATION, REPROGRAMMING AND REGENERATION

## MOLECULAR NEUROPATHOLOGY

### METABOLIC AND SIGNALING NETWORKS IN DISEASE

## IMMUNE SYSTEM DEVELOPMENT AND FUNCTION

### MICROBES IN HEALTH AND WELFARE

## Scientific Staff

**SCIENTIFIC PERSONNEL**
(CSIC-UAM)
457

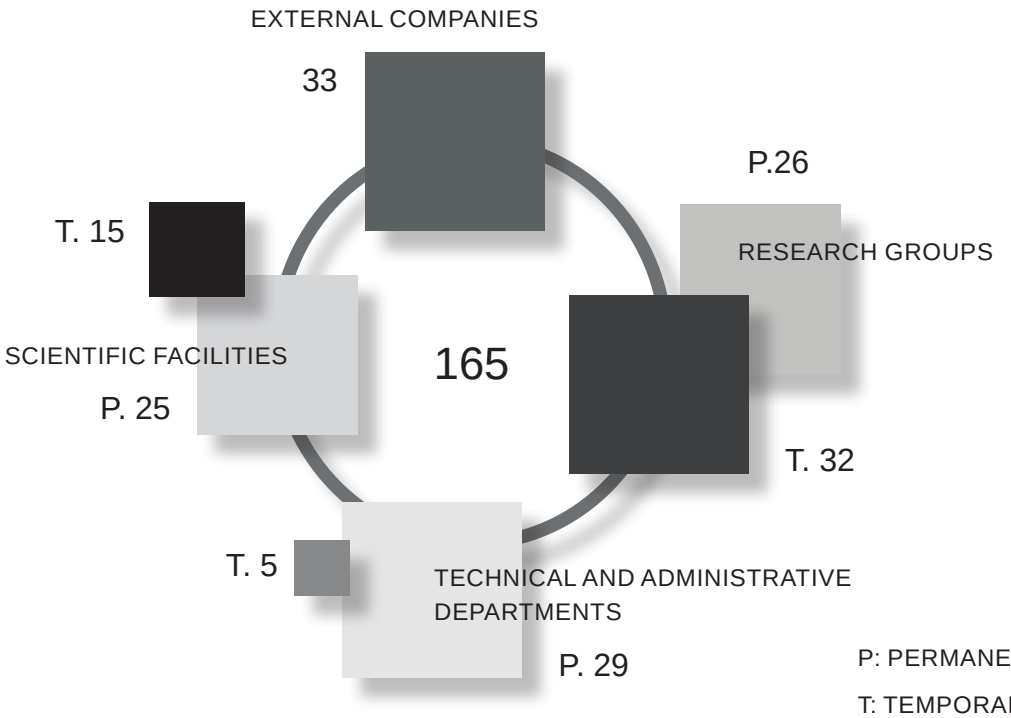
- RESEARCH PROFESSORS
- UNIVERSITY PROFESSORS — 33
- RESEARCH INVESTIGATORS
- RESEARCH SCIENTISTS
- ASSOCIATE PROFESSORS — 66
- TENURE TRACK RESEARCHERS
- TENURE TRACK PROFESSORS — 24
- "AD HONOREM" 7

- STUDENTS 105
- RESEARCHERS 222
  - PINP

CSIC

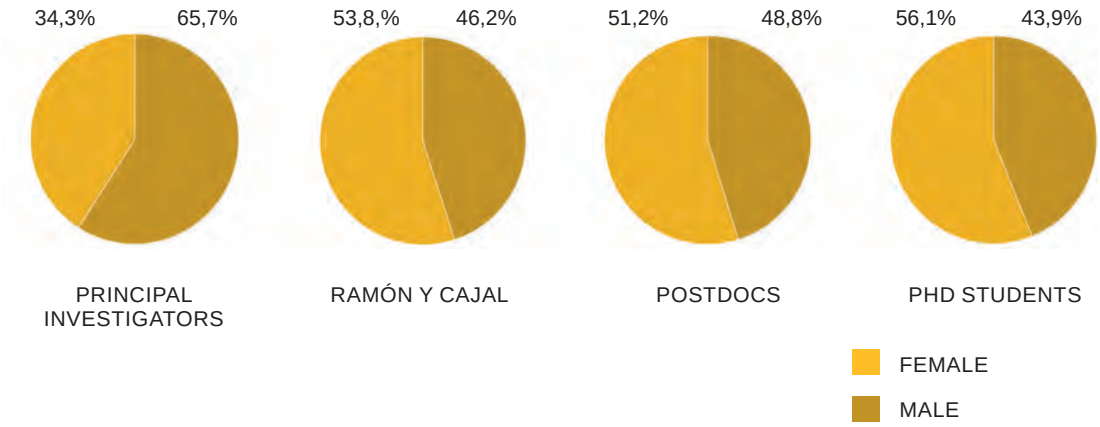
UAM Universidad Autónoma de Madrid

## Research Support Staff

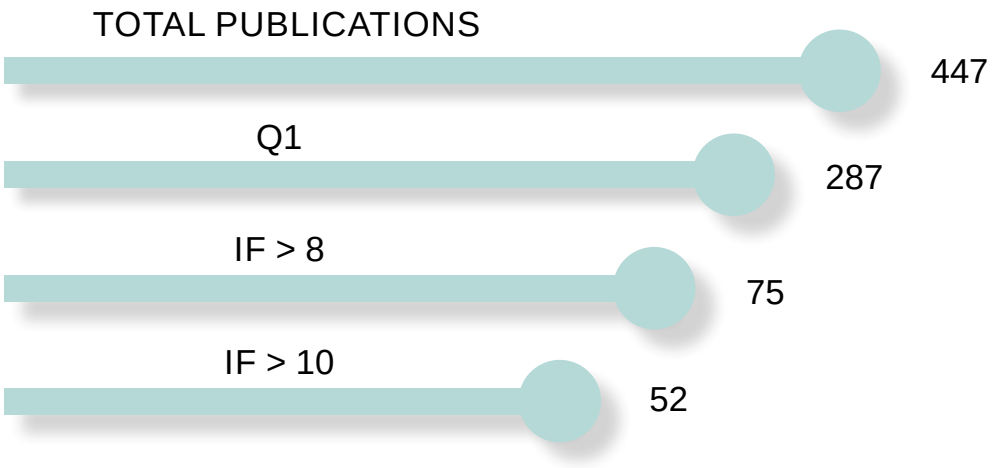



P: PERMANENT
T: TEMPORARY

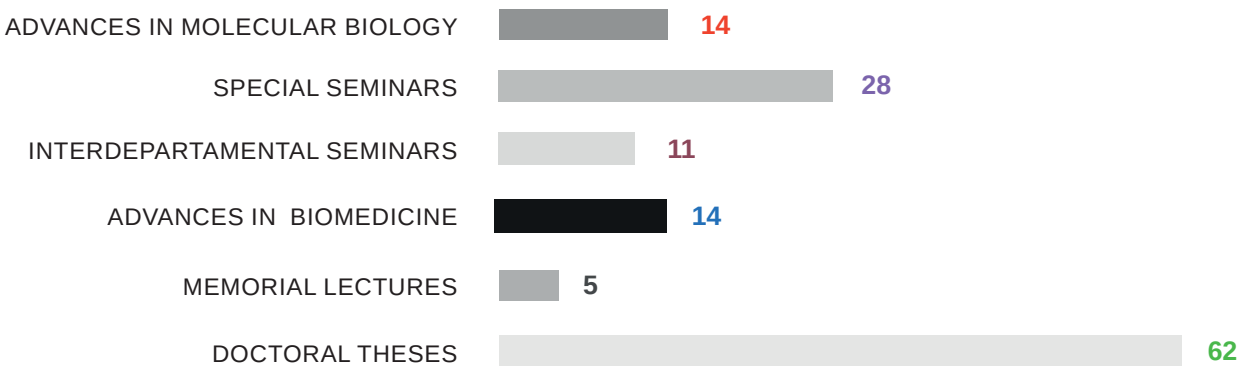
## Gender Equality



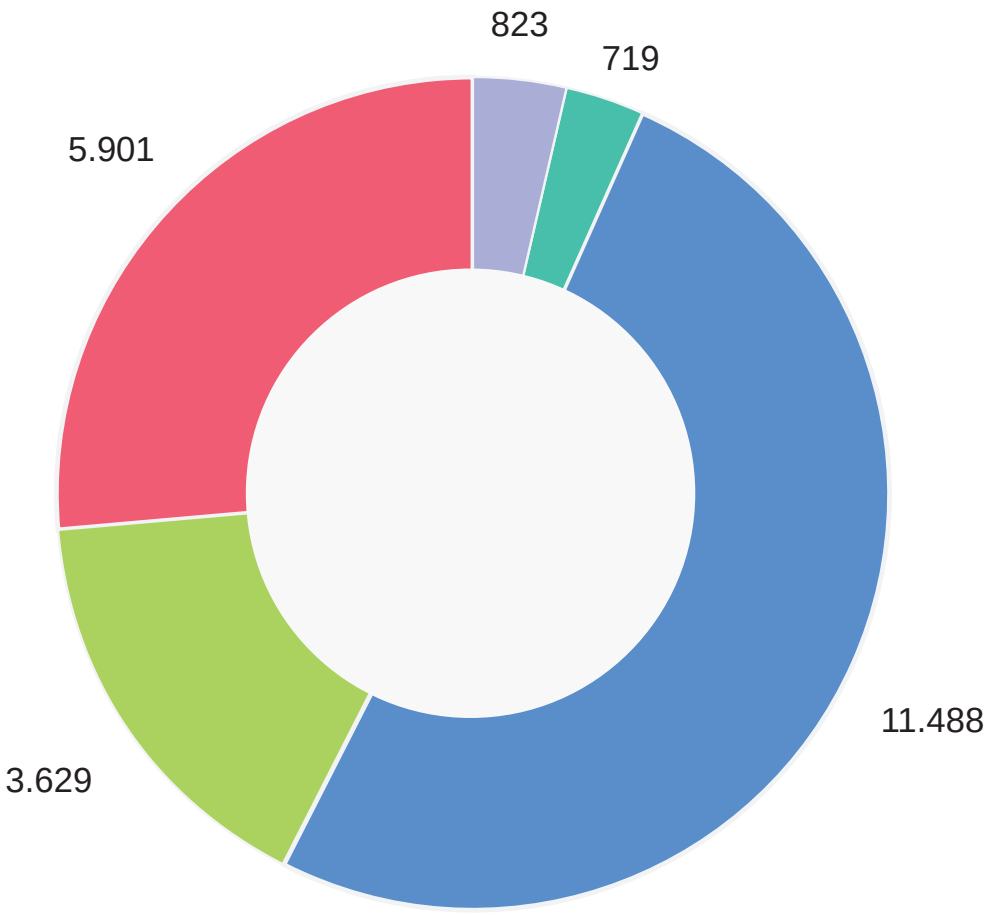

## Publications




## Seminars, Lectures and Theses



# Projects (*)




* FIGURE IN THOUSAND OF EUROS

# Services

## SCIENTIFIC FACILITIES

- ANIMAL FACILITY
- BIOINFORMATICS FACILITY
- FLOW CITOMETRY FACILITY
- ELECTRON MICROSCOPY FACILITY
- FERMENTATION FACILITY
- GENOMICS AND NEXT GENERATION SEQUENCING FACILITY
- OPTICAL AND CONFOCAL MICROSCOPY FACILITY
- PROTEOMICS AND PROTEIN CHEMISTRY CORE FACILITY
- *DROSOPHILA* TRANSGENESIS FACILITY
- CBM-CNB TRANSGENESIS FACILITY

## TECHNICAL SERVICES

- BIOLOGICAL SECURITY
- CELL CULTURE, WASHING AND STERILIZATION
- COMPUTING
- GRAPHIC DESIGN / PHOTOGRAPHY
- INSTRUMENTATION
- LIBRARY
- SAFETY AND OCCUPATIONAL RISK PREVENTION

## ADMINISTRATIVE SERVICES

- ADMINISTRATION
- HUMAN RESOURCES
- INSTITUTIONAL RELATIONS
- OTHER ADMINISTRATIVE SERVICES
- PURCHASING AND STOCKROOM

# Awards and Honors

## 2019

**MARGARITA SALAS FALGUERAS**
- Doctor Honoris Causa from the University of Burgos
- Doctor Honoris Causa from the Pontifical University of Salamanca
- Denomination "Plaza de Margarita Salas" within the IES Machado. Alcala de Henares
- Inauguration of the "Experimental and sensorial space. Margarita Salas". CP Tremañes. Gijón
- European Inventor Award 2019, obtained in the *Logro de Toda una Vida* and *Premio Popular* categories. Awarded by the European Patent Office
- Denomination "Margarita Salas" to the Hall of the INIA Acts
- Act-Tribute of the CSIC to the scientific staff most awarded for their scientific merits
- "Scientific Inspiration Award" granted by the Inspiring girls Foundation

**JOSÉ J. LUCAS LOZANO**
- Finalist in the Vanguardia de la Ciencia Award 2018 - La Vanguardia and Fundació La Pedrera
- Health Sciences Award from the Caja Rural de Granada Foundation
- Appointed Member of the Academia Europaea

**PAOLA BOVOLENTA**
- President of the Spanish Society of Neuroscience (SENC)
- Member of the SAB Institute of Biology of the Ecole Normale Supérieure (IBENS). Paris

**JOSÉ A. LÓPEZ GUERRERO**
- Plaque of honor for his scientific and informative work, Spanish Association of Scientists

**MARÍA LLORENS-MARTÍN**
- Miguel Catalán Award for Young Researchers of the Community of Madrid
- Young Female Talent Award, Biology and Geology Category of the Royal Academy of Sciences of Spain
- Finalist in the Vanguardia de la Ciencia Award 2019 - La Vanguardia and Fundació La Pedrera

## 2020

**CRISANTO GUTIERREZ ARMENTA**
- Member of the Academia Europaea Section Bochemistry and Molecular Biology
- Member of the EMBO council 2020

**ESTEBAN DOMINGO SOLANS**
- Foreign Associate Member of the US Academy of Sciences

**MARÍA LLORENS-MARTÍN**
- Miguel Catalán Award for young people under 40 years of age

# Total Honors

**2** LAUREATES OF THE PRÍNCIPE DE ASTURIAS DE INVESTIGACIÓN

**3** LAUREATES OF THE PREMIO MÉXICO DE INVESTIGACIÓN (OF 19 IN TOTAL)

**3** LAUREATES OF THE PREMIO JAIME I DE INVESTIGACIÓN

**5** LAUREATES OF THE RAMÓN Y CAJAL NATIONAL PRIZE

**19** DOCTORS “HONORIS CAUSA” OF SPANISH AND FOREIGN UNIVERSITIES

**4** MEMBERS OF THE US NATIONAL ACADEMY OF SCIENCES

**9** SPANISH ROYAL ACADEMIES MEMBERS

# ERC Projects

## BALBINO ALARCÓN

*Ref: ERC-2013-ADG*

NOVEL PROPERTIES OF ANTIGEN RECEPTORS AND INSTRUMENTS TO MODULATE LYMPHOID FUNCTION IN PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS

## MARÍA MITTELBRUNN

*Ref: 715322-EndoMitTalk-ERC-2016-STG*

ENDOLYSOSOMAL-MITOCHONDRIA CROSSTALK IN CELL AND ORGANISM HOMEOSTASIS

## CRISANTO GUTIERREZ

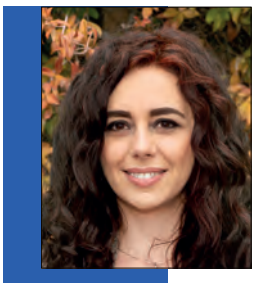
*Ref: 833617-PLANTGROWTH-ERC-2018-ADG*

EXPLOITING GENOME REPLICATION TO DESIGN IMPROVED PLANT GROWTH STRATEGIES

## EDUARDO BALSA MARTÍNEZ

*Ref: 948478 -MitoCure-2020 ERC-Stg*

MOLECULAR AND METABOLIC MECHANISMS UNDERLYING MITOCHONDRIAL DYSFUNCTION

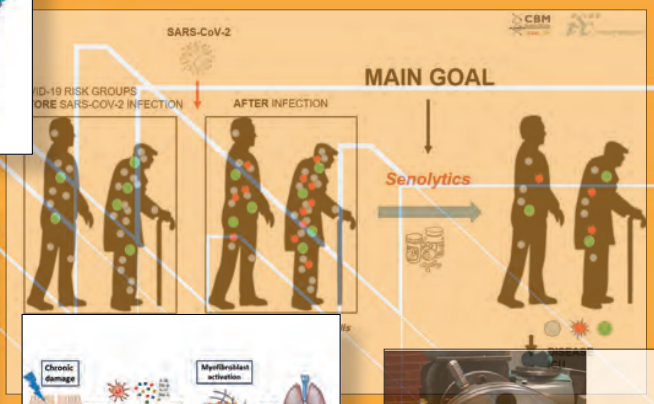


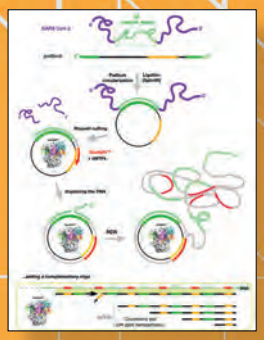
## MARÍA LLORENS-MARTÍN

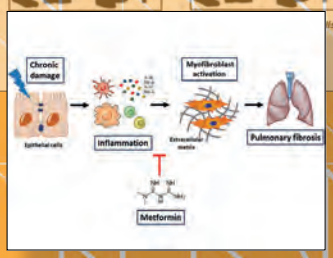
*Ref: 101001916-HumAN-ERC-2020-CoG*

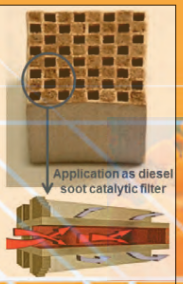
INTERROGATING HUMAN ADULT HIPPOCAMPAL NEUROGENESIS

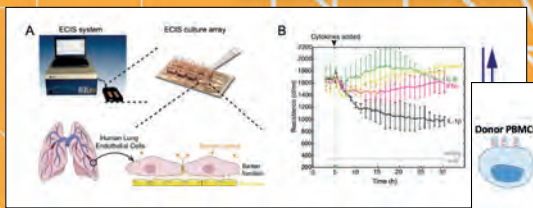
# COVID-19 Projects

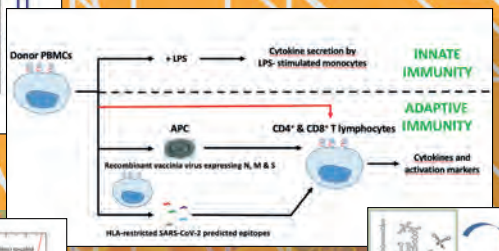


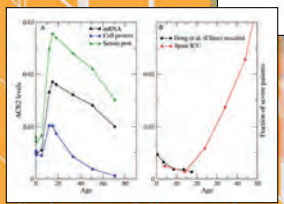





# COVID-19 PROJECTS

## BALBINO ALARCÓN

ULTRARAPID DEVELOPMENT OF HUMAN NEUTRALIZING ANTIBODIES TO SARS-COV-2 VIA *IN SILICO* AND *IN VITRO* APPROACHES

## ANTONIO ALCAMÍ

PHOTO VS SARS: REMOVAL OF SARS-COV-2 BY PHOTOCATALYSIS ASSOCIATED WITH AIR TREATMENT SYSTEMS IN HOSPITALS AND RESIDENCES

INACTIVATION OF SARS-COV-2 IN THE AIR OF CLOSED ENCLOSURES

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## MARGARITA DEL VAL

CHARACTERIZATION OF THE IMMUNE RESPONSE TO SARS-COV-2 DURING THE EPIDEMIC PHASE

## CAYETANO VON KOBBE

SENOLYTICS AS A THERAPY FOR COVID-19 TREATMENT

## ULTRARAPID DEVELOPMENT OF HUMAN NEUTRALIZING ANTIBODIES TO SARS-COV-2 VIA *IN SILICO* AND *IN VITRO* APPROACHES

PI: BALBINO ALARCÓN

Based on the published structure of a neutralizing human antibody binding to the receptor binding domain (RBD) of the SARS-CoV-1 virus, we have built a model of interaction between that antibody and the RBD of SARS-CoV-2. According to the predictions of the model, we are modifying the heavy (H) and light (L) chains of the human antibody in order to fit and neutralize the new coronavirus. The monoclonal human antibody will serve as a therapy for acute cases of COVID-19 and, in addition, will be adapted to fit the emerging variants of the virus.

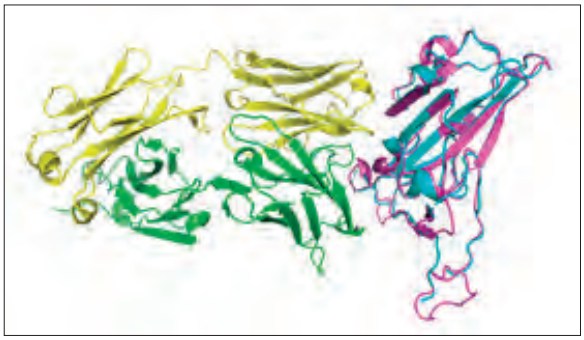


***Figure.*** *Model of interaction between human neutralizing antibody m396 (green and yellow) of SARS-CoV-1 and the S proteins of SARS-CoV-1 (magenta) and SARS-CoV2 (cyan). The Fab m396-SARSCoV RBD structure has a resolution of 2.3-Å and PDB code 2DD8. The structure of the S protein of SARS-CoV-2 was obtained by Cryo-EM and has the PDB code 6VSB.*

Publications:

Horndler L, Delgado P, Abia D, Balabanov I, Martínez-Fleta P, Cornish G, Llamas MA, Serrano-Villar S, Sánchez-Madrid F, Fresno M, van Santen HM, Alarcón B. Flow cytometry multiplexed method for the detection of neutralizing human antibodies to the native SARS-CoV-2 spike protein. *EMBO Mol Med*. 2021 Jan 20:e13549. doi: 10.15252/emmm.2020**13549**. Online ahead of print.

Patents:

Balbino Alarcón Sánchez, Lydia Horndler Gil, Pilar Delgado Cañaveras, Ivaylo Balabanov, Hisse Martien van Santen. Flow cytometry multiplexed method for the detection of SARS-CoV-2 antibodies. EP20382667.2. País de prioridad: España. Fecha de prioridad: 24-07-2020. Propietario: CSIC. Licenciatario: Vitro SA.

## PHOTO VS SARS: REMOVAL OF SARS-COV-2 BY PHOTOCATALYSIS ASSOCIATED WITH AIR TREATMENT SYSTEMS IN HOSPITALS AND RESIDENCES

PIs: FERNANDO FELDMAN (AIRE LIMPIO), BENIGNO SÁNCHEZ CABRERO (CIEMAT), JAVIER DIEGUEZ (RJB) AND ANTONIO ALCAMÍ (CBMSO)

The project, funded by the Center for Industrial Technological Development (CDTI), is a private-public collaboration between the Company AIRE LIMPIO, Center for Energy, Environmental and Technological Research (CIEMAT), Royal Botanical Garden (RJB-CSIC) and CBMSO. This project will develop a new technology based on adsorption plus photocatalysis. This technology harnesses adsorption and an oxidation-reduction reaction produced by the excitation of a semiconductor when irradiated by UVA radiation, breaking chemical and biological organic bonds. This process will damage viral capsids thus eliminating the possibility of airborne transmission of SARS-CoV-2.

***Figure.*** *Photocatalytic system developed by AIRE LIMPIO.*

## INACTIVATION OF SARS-COV-2 IN THE AIR OF CLOSED ENCLOSURES

PIs: ANTONIO ALCAMÍ (CBMSO) AND MIGUEL ANGEL BAÑARES (ICP)

The project is a collaboration with scientists at the Institute of Catalysis and Petrochemistry (ICP). New methods for the inactivation of SARS-CoV-2 in the air will be developed based on catalytic filters that can be installed in air conditioning systems. The inactivation of coronavirus present in aerosol form in the air will be carried out using ceramic or polymeric filters activated with metal and metal oxide nanoparticles. Viral inactivation will be tested in a purpose-built aerosol chamber. The project is funded by the Spanish National Research Council.

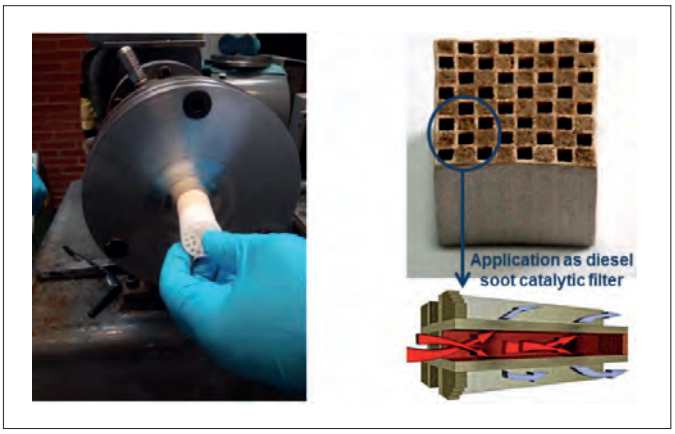



***Figure.*** *Ceramic filters developed to inactivate viruses in air samples.*

## AIRCOVID19: AIR TRANSMISSION OF SARS-COV-2, DETECTION IN HOSPITALS AND INNOVATIVE TECHNOLOGIES

PIs: ANTONIO ALCAMÍ (CBMSO), ALVARO SOMOZA (IMDEA NANOCIENCIA), XAVIER RODÓ (ISGLOBAL), CRISTINA CALVO (HOSPITAL LA PAZ) AND MARÍA LUZ GARCÍA (HOSPITAL SEVERO OCHOA)

The project, funded by the COVID-19 funds from Instituto de Salud Carlos III and coordinated by Antonio Alcamí, is a multidisciplinary approach involving virologists, molecular biologists, clinicians and nanotechnologist to characterize aerosol transmission of SARS-CoV-2. We have carried out a long-term study since the beginning of the COVID-19 pandemic to monitor SARS-CoV-2 aerosols in different areas of two major hospitals in Madrid, using technology we optimized. We are also developing point-of-care innovative technologies for rapid detection of airborne SARS-CoV-2 in hospitals and public environments, based on nanoparticles and colorimetric detection of virus or real-time detection of the virus in air particles (laser-induced fluorescence technology).

Patents:

A. Rastrojo and A. Alcamí. Device and method for capturing and analysing airborne organisms. EP20382510.4. European Patent Application 12/06/2020. Owner: CSIC.

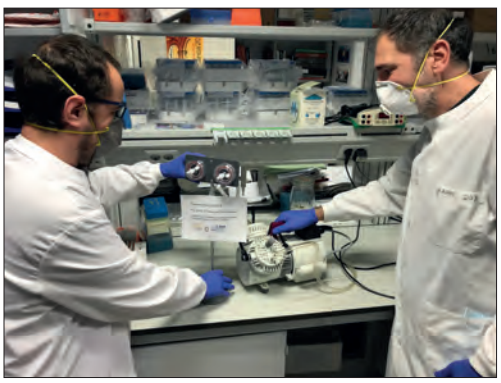


***Figure.*** *Filter technology to sample virus-containing aerosols.*

## ANGIOTENSIN CONVERTING ENZYME 2 SERUM LEVELS AS PREDICTOR OF INFECTION AND CLINICAL OUTCOME IN COVID-19

PIs: MANUEL FRESNO Y UGO BASTOLLA

Our analysis suggested that ACE2 expression correlates strongly and negatively with COVID-19 severity. We found that higher levels of ACE2 in serum indicate lower probability of contracting infection in highly exposed health workers and in persons cohabiting with seropositive relatives. ACE2 in serum correlates inversely with anti- spike (S) antibody titers, suggesting that it can act as a decoy to neutralize SARS-Cov2. Moreover, serum ACE2 may be used as a biomarker to discriminate different type of symptoms in infected people. On the other hand, highly exposed seronegative children develop a different immune response than adults.

Publications:

Horndler, L., Delgado, P., Abia, D., Balabanov, I., Martínez-Fleta, P., Cornish, G., Llamas, M.A., Serrano-Villar, S., Sánchez-Madrid, F., Fresno, M., van Santen, H.M., and Alarcón, B. (2021). Flow cytometry multiplexed method for the detection of neutralizing human antibodies to the native SARS-CoV-2 spike protein. *EMBO molecular medicine*, e13549. **10**.15252/emmm.202013549.

Bastolla U, Mathematical model of SARS-Cov-2 propagation versus ACE2 fits COVID-19 lethality across age and sex and predicts that of SARS. "https://arxiv.org/abs/2004.07224".

U Bastolla, P Chambers, D Abia, ML García-Bermejo and M Fresno, Is Covid-19 severity associated with ACE2 degradation? arxiv.

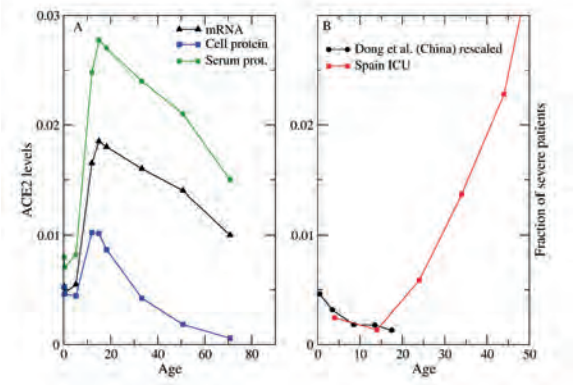



***Figure.*** *A: Proposed behavior of ACE2 with age at the three levels of mRNA, protein in the cellular membrane and protein in serum. B: The fraction of seropositive population hospitalized in Spanish ICU during the first wave of COVID-19 (red) and severe pediatric cases of COVID-19 in China (Dong et al. 2020), rescaled to take into account undetected cases, as a function of age. Comparison between the two graphs suggests an overall negative correlation between COVID severity and ACE2 expression.*

## SIMPLE SARS-COV-2 DIAGNOSIS BY PHI29 POLYMERASE AMPLIFICATION

PIs: LUIS BLANCO AND MIGUEL DE VEGA (CBM), FELIPE CORTÉS (CNIO)

We are developing a simple method to detect SARS-CoV-2 using a virus-specific linear probe that becomes circularized only in the presence of the viral RNA. Once the probe becomes circular, an engineered form of phi29 DNA polymerase (Qualiphi™; CSIC patent licensed to 4basebio) will start copying the circular template by "rolling-circle replication". Such a reaction depends on the unique and robust strand-displacement synthesis by phi29 DNA polymerase, a hallmark of the isothermal DNA amplification of full genomes used worldwide, is performed at room temperature and can be easily detected as a readout of the presence of SARS-CoV-2 genomic RNA.

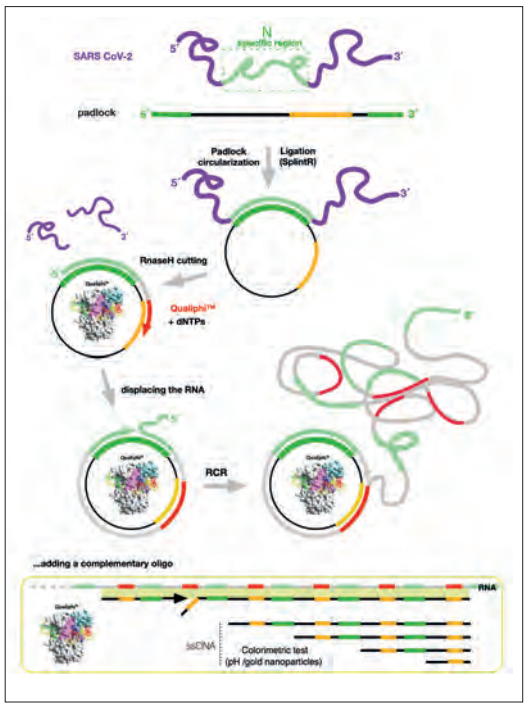



***Figure: Simple SARS-CoV-2 diagnosis by phi29 polymerase amplification.***

*Improved detection of SARS-CoV-2 using a virus-specific linear probe (padlock probe) that becomes circularized only in the presence of the viral RNA. Once the probe becomes circular, phi29 DNA polymerase (Qualiphi™) will start copying the circular template by "rolling-circle replication" (RCR) in isothermal conditions. Different methods can be used as a readout of the concatemeric product.*

## POST COVID19 PULMONARY FIBROSIS: MARKERS AND THERAPEUTIC OPTION WITH METFORMIN

PI: SANTIAGO LAMAS

In COVID19 infection, pulmonary damage is very common and is present in the 20-30% of cases. Among the complications, pulmonary fibrosis has an incidence of more than 40% and lacks prognostic and therapeutic options. In experimental models, Metformin, a widely used oral antidiabetic, prevented and slowed the development of pulmonary fibrosis. In patients with post-CoVid19 pulmonary fibrosis, the proposal aims to: a) validate one or more prognostic biomarkers based on the chronic inflammatory response associated with fibrosis b) Establish the use of Metformin as an effective drug in the treatment and prevention of post COVID19 pulmonary fibrosis.

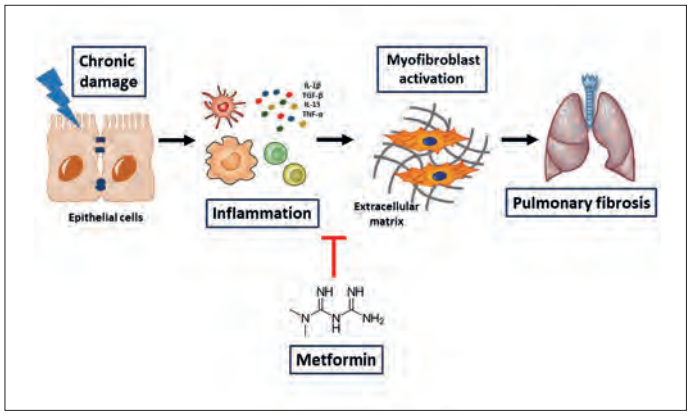



***Figure.*** *By reprogramming, metabolism Metformin may contribute to halt lung inflammation and fibrosis.*

## SEARCHING FOR NEW THERAPEUTIC TARGETS TO PREVENT LUNG MICROVASCULAR ENDOTHELIAL BARRIER DISRUPTION AND PULMONARY EDEMA CAUSED BY SARS-COV2-INDUCED CYTOKINE STORM

PI: JAIME MILLÁN

Patients severely ill with COVID19 suffer acute respiratory distress syndrome (ARDS) in which the lung microvasculature is disrupted by an inflammatory cytokine storm that induces endothelial hyperpermeability. Our group investigates the role of RhoA and Rac subfamilies of GTPases, which can be pharmacologically targeted with drugs approved for clinical use, on endothelial barrier dysfunction in response to inflammatory signaling. We propose to generate first, a platform for *in vitro* testing of drugs with potential therapeutic effects on cytokine-induced lung endothelial barrier dysfunction. Second, to investigate which combination of inflammatory mediators provokes endothelial hyperpermeability in the lung microvasculature of COVID19 patients. We will take advantage of the collection of plasma samples of COVID19 patients and healthy donors available from IdiPAZ. Third, to test whether pharmacological targeting of the RhoA and Rac protein subfamilies prevents human lung endothelial barrier dysfunction *in vitro* and *in vivo* in response to the SARS-CoV2-induced cytokine storm.

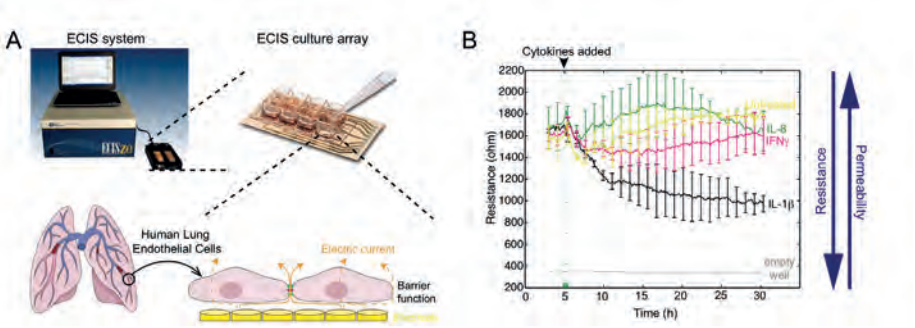



***Figure.*** *(A) The ECIS system (Applied Biophysics) measures transendothelial resistance of cells plated in gold arrays, which facilitates screening the effect of stimuli and drugs on human lung microvascular endothelial cell (HLMVEC) barrier integrity. (B) ECIS analysis of the effect of three cytokines on HLMVEC barriers. IL1b strongly reduces barrier integrity.*

## ANTIVIRAL ACTIVITY OF IMMUNOMODULATORY NON-CODING RNAS AND CELL-TARGETED COMPOUNDS AGAINST HUMAN CORONAVIRUSES

PIs: MARGARITA SÁIZ / FRANCISCO SOBRINO

In this project we aim to test the antiviral effect against SARS-CoV-2 of: i) synthetic RNA transcripts derived from the foot-and-mouth-disease virus genome (ncRNAs) known to induce a solid and broad range antiviral response in vivo and ii) valproic acid and lauryl gallate, two widely used low-cost generic drugs ready for their use in humans. The potential synergy between different molecules, including remdesivir, will be assayed. Testing the antiviral effect against human coronaviruses of a variety of molecules of known inhibitory activity against a wide range of viral pathogens could provide a basis for short/mid-term therapeutic treatments for covid-19 patients.

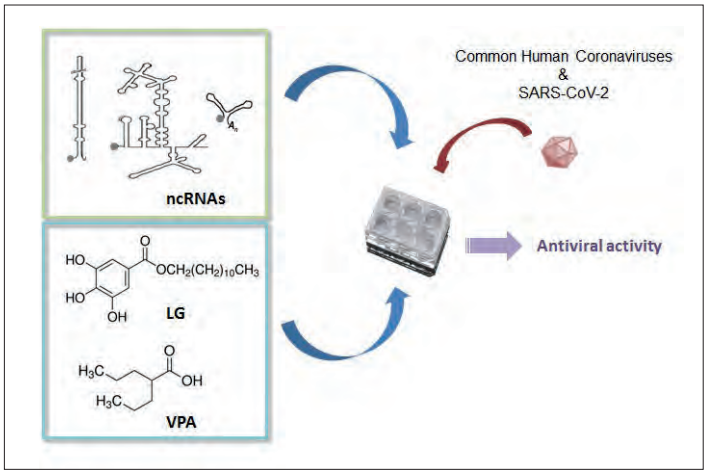



***Figure.*** *Analysis of the inhibitory effect of FMDV ncRNAs and cell-targeted compounds (LG and VPA) against infection by human coronaviruses in cultured cells.*

## CHARACTERIZATION OF THE IMMUNE RESPONSE TO SARS-COV-2 DURING THE EPIDEMIC PHASE

PI: MARGARITA DEL VAL LATORRE

Our project aims at the characterization of the adaptive cellular and trained innate immune response against SARS-CoV-2 in patients both in the acute and convalescent phase of the infection. For the analysis of adaptive immunity, we use recombinant vaccinia viruses to express SARS-CoV-2 proteins (S, M and N) in a fraction of peripheral blood mononuclear (PBMCs) from the donors, acting as antigen presenting cells for the CD4+ and CD8+ T lymphocytes in the remaining PBMCs; detection of cytokines and activation markers are the indicators for SARS-CoV-2 specific immunity. Innate immunity is detected by stimulation of monocytes by lipopolysaccharide and quantitation of the production of inflammatory cytokines.

Publications:

García-Basteiro, A.L., Legido-Quigley, H., Álvarez-Dardet, C., Arenas, A., Bengoa, R., Borrell, C., Del Val, M., Franco, M., Gea-Sánchez, M., Gestal, J., González López Valcárcel, B., Hernández-Aguado, I., March, J.C., Martin-Moreno, J.M., Menéndez, C., Minué, S., Muntaner, C., Porta, M., Prieto-Alhambra, D., and Vives Cases, C. (2020) Evaluation of the COVID-19 response in Spain: principles and requirements. *Lancet Public Health*. **5**(11), e575.

García-Basteiro, A., Alvarez-Dardet, C., Arenas, A., Bengoa, R., Borrell, C., Del Val, M., Franco, M., Gea-Sánchez, M., Otero, J.J.G., Valcárcel, B.G.L., Hernández, I., March, J.C., Martin-Moreno, J.M., Menéndez, C., Minué, S., Muntaner, C., Porta, M., Prieto-Alhambra, D., Vives-Cases, C. and Legido-Quigley, H. (2020) The need for an independent evaluation of the COVID-19 response in Spain. *Lancet*. **396**(10250), 529-530.

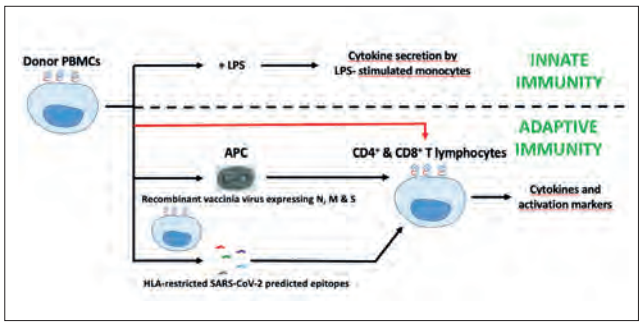



***Figure. Experimental approach to evaluate anti-SARS-CoV-2 innate and T lymphocyte adaptive immune responses in PBMCs from convalescent and healthy donors.***

## SENOLYTICS AS A THERAPY FOR COVID-19 TREATMENT

PI: CAYETANO VON KOBBE

The main objective of this project is to develop a therapy to alleviate or cure the symptoms of COVID-19 patients, by using senolytics, drugs that specifically kill senescent cells of the organism.

The starting hypothesis is that senescent cells play a central role in the development of the main symptoms of COVID-19, as the exacerbated pro-inflammatory environment ("cytokine storm"). There is evidence that indicates a greater chronic accumulation of these cells whether in diseases associated with COVID-19 risk groups (as diabetes, and cardiovascular and chronic respiratory diseases, among others), or the most affected one, the age-associated group.

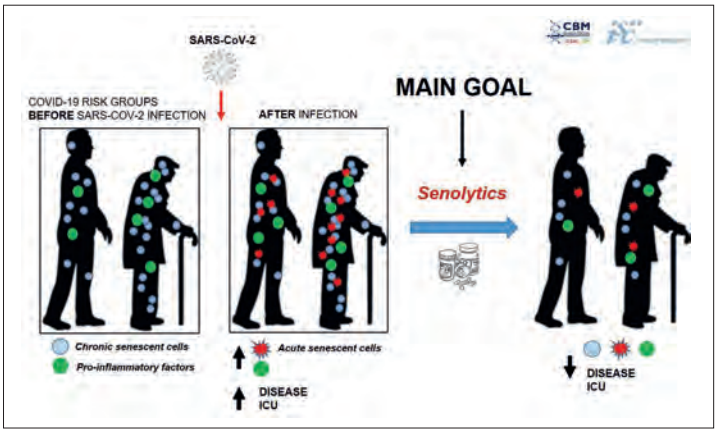
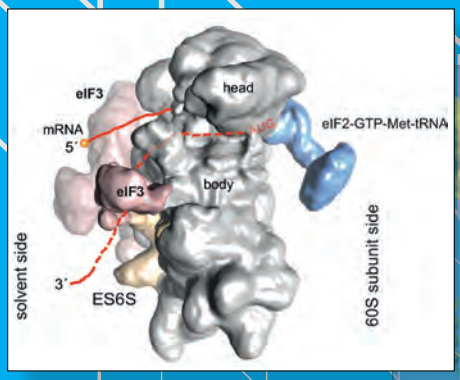
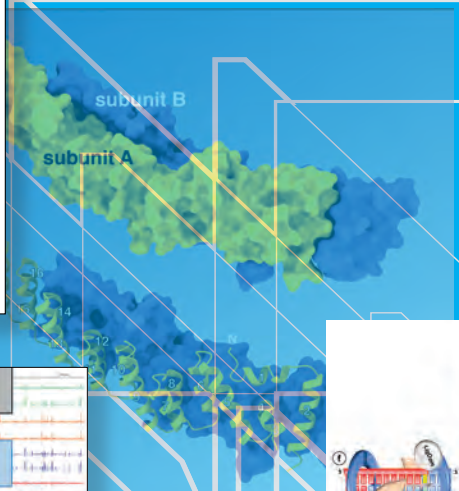


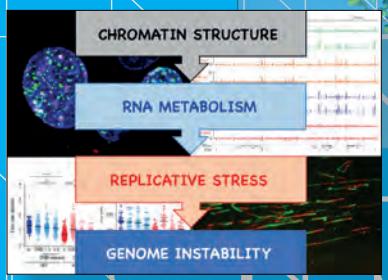

***Figure.*** *Starting Hypothesis: COVID-19´s risk groups display per se high levels of senescence, wich in turn is augmented after SARS-CoV-2 infection. Treatment with senolytics would alleviate the symptoms associated with both a chronic and acute pro-imflammatory environment.*

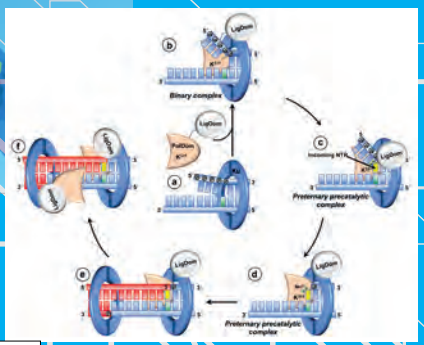
# Genome Dynamics and Function

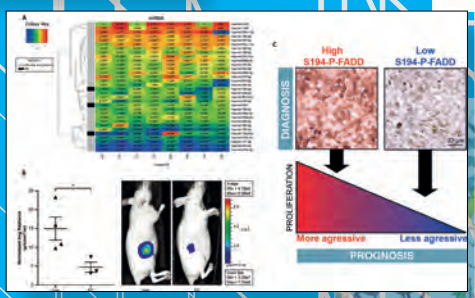
## PROGRAM






# *UNITS*

## GENOME DECODING



## GENOME MAINTENANCE AND INSTABILITY

UNIT

ENCARNACIÓN MARTÍNEZ-SALAS

# Genome Decoding

GENOME DYNAMICS AND FUNCTION

Complementary and collaborative research activities within the Genome Decoding Unit are deciphering the molecular basis of essential cellular processes to better understand how genomic information is interpreted, and to provide the basis for new diagnostic tools and therapeutic strategies. The group of Crisanto Gutiérrez (CG) is aimed at understanding key questions of cell proliferation, transcription and genome replication and how epigenetics affects their coordination in the plant Arabidopsis using a combination of cellular, molecular, genetic and genomic approaches. José María Requena (JMR) is carrying out genome-wide scale analyses and proteomics approaches for understanding gene expression in *Leishmania*. Structural research is of primary relevance to understand the function of molecular machines. Santiago Ramón-Maiques (SRM) devised a cellular assay to assess the disease-causing potential of CAD, the multienzymatic protein responsible of *de novo* pyrimidine synthesis. Similarly, the group of Ugo Bastolla (UB) characterizes protein function using dynamical couplings, conformational changes and accelerated evolutionary rates of structure divergence, and also investigates the relationship between genome replication and chromatin organization. RNA biology is a major focus of investigation at different levels in the Unit. José Fernández Piqueras/Javier Santos group (JFP/JS) integrates data from genomics and transcriptomics approaches to identify new driver-molecular mechanisms and to propose tailored treatments with the aid of bioinformatic tools. Likewise, understanding the interplay between mRNA and proteins can lead to characterize the expression of novel proteins using alternative mechanisms of translation initiation, improving the annotated proteome. Encarna Martínez-Salas (EMS) research is aimed at understanding the principles guiding alternative mechanisms of translation initiation through the characterization of multifunctional RNA-binding proteins. The group of Ivan Ventoso/Juan José Berlanga/Miguel Angel Rodríguez (IV/JJB/MAR) is focused to study regulation of mRNA translation in eukaryotes and its implications for stress response and aging.

Highlights of the unit:

CG group was awarded an Advanced ERC grant (2019)

A collaborative work between CG and UB groups have identified DNA replication origins in Arabidopsis and their chromatin landscape (*Genome Res* 2019).

The EMS group has developed a robust proteomic approach that allowed the discovery of ER-Golgi trafficking proteins Rab1b and ARF5 as regulators of IRES activity (*Life Sci Alliance* 2019).

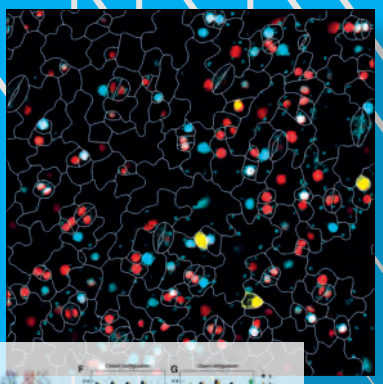
The group of IV/JJB/MAR identified the ES6S region of 40S ribosomal subunit as the gateway for mRNA entry and secondary structure unwinding during translation initiation (*Elife* 2019).

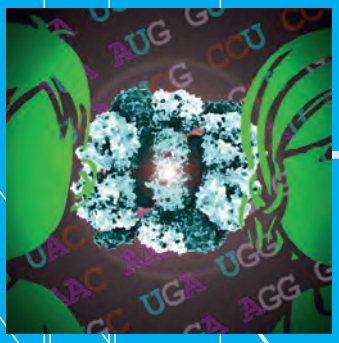
The CG group has generated a fluorescent sensor that identifies cell cycle phases in Arabidopsis in live-imaging experiments (*Nat Plants* 2020).

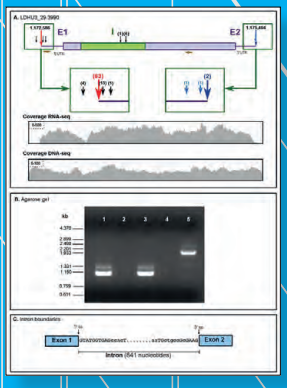
The joint research of EMS and SRM groups discovered a dimerization domain in the member of the survival of motor neurons Gemin5, which provides a hub for the assembly of complexes involved in RNA processing, stability and translation (*Nucleic Acids Res* 2020).

The SRM team has identified variants of the CAD enzyme in childrens than can benefit from uridine therapy (*Genet Med* 2020).

Research of the JFP/JS group identified the passenger ACO1-deficiency arising from 9p21 deletions to kill T-cell lymphoblastic neoplasia cells (*Carcinogenesis* 2020).







**UGO BASTOLLA**

COMPUTATIONAL BIOLOGY AND BIOINFORMATICS

**JOSÉ FERNÁNDEZ PIQUERAS / JAVIER SANTOS**

GENETICS AND CELL BIOLOGY OF CANCER: T-CELL LYMPHOBLASTIC NEOPLASMS

**CRISANTO GUTIERREZ**

CELL DIVISION, GENOME REPLICATION AND CHROMATIN

**ENCARNACIÓN MARTÍNEZ SALAS**

INTERNAL INITIATION OF TRANSLATION IN EUKARYOTIC mRNAs

**SANTIAGO RAMÓN**

STRUCTURE AND FUNCTION OF MACROMOLECULAR COMPLEXES

**JOSÉ MARÍA REQUENA**

REGULATION OF GENE EXPRESSION IN *LEISHMANIA*

**IVÁN VENTOSO / CÉSAR DE HARO /**
**JUAN JOSÉ BERLANGA / MIGUEL ÁNGEL RODRÍGUEZ**

REGULATION OF mRNA TRANSLATION IN EUKARYOTES AND ITS IMPLICATIONS FOR ORGANISMAL LIFE

## GENOME DECODING

# COMPUTATIONAL BIOLOGY AND BIOINFORMATICS

***Principal Investigator:***
Ugo Bastolla Bufalini

***Postdoctoral Fellow:***
Yves Dehouck

***Undergraduate and Master Student:***
Tadhg Cuddihy

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## Research summary

Our work focuses on three main lines: (1) Developing and applying computational methods for predicting functional protein dynamics from structural information; (2) Developing structure-aware models of protein evolution with selection on protein structure and protein folding stability, for improving phylogenetic inference and detecting selection on protein function; (3) Characterizing the relation between chromatin structure and function at the level of the initiation of genome replication and transcription regulation. Along the first line, we defined dynamical couplings that can be computed with our torsional network model based only on the protein structure and that characterize allosteric couplings between functionally distinct protein regions, we applied these calculations to neurotransmitter transport in pentameric ion channels and we studied functional conformation changes of proteins based on changes of torsion angles and their relation with torsional normal modes. Along the second line, we applied the site-dependent, stability-constrained substitution models of protein evolution that we developed for improving the inference of ancestral protein sequences and we showed that accelerated rates of protein structure divergence help identifying changes in protein function. Along the third line, in collaboration with the group of Crisanto Gutierrez, we identified subtle changes in genome replication origin activity across developmental stages of the model plant Arabidopsis thaliana and correlated these changes with the chromatin states that we recently classified; furthermore, we discovered that origin firing scores correlate with the abundance of GGN trinucleotide motifs, and we are presently investigating the relationship of GGN motifs with nucleosome occupancy and G-quadruplex DNA secondary structures. Finally, during 2020 we investigated the Covid-19 pandemics, trying to estimate its infection fatality rates in different countries by estimating undetected cases from the ratio of positive tests (https://medrxiv.org/cgi/content/short/2020.03.27.20045062v1) and we proposed an association between Covid-19 severity and the differential expression of the SARS-CoV-2 receptor protein ACE2, which plays a key role in downregulating the inflammatory peptides of the Angiotensin and Bradikinin system and whose downregulation in aging, chronic inflammatory conditions and through degradation by SARS-CoV-2 might play a key role in infection severity, https://arxiv.org/abs/2004.07224.

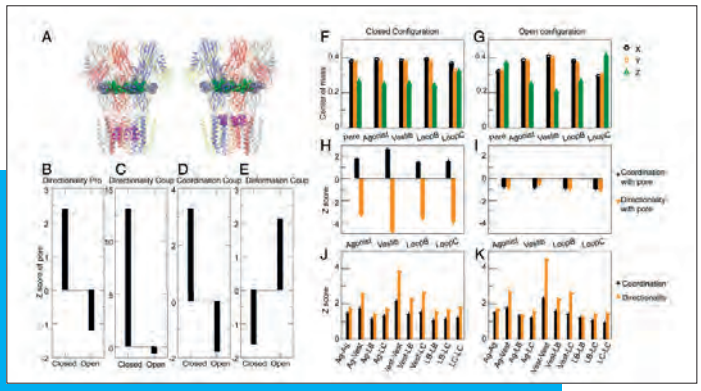

***Figure.*** *Functional dynamics in the pentameric ligand-gated ion cannel of a bacteria symbiont crystallized in the closed and open conformation (A) that show markedly different dynamical profiles (D-E). The pore where transport takes place and allosteric sites present dynamics orthogonal to the pore direction z in the closed conformation (F) where allosteric sites oppose to the pore motion (H), but this changes in the open conformation where the main dynamics is in the pore direction (G,I). Allosteric sites show coupled motions in both conformations (J,K). From Ref.[2].*

## Publications


Alfayate, A., Caceres, C.R., Gomes Dos Santos, H. and Bastolla, U (2019) Predicted dynamical couplings of protein residues characterize catalysis, transport and allostery. *Bioinformatics*. **35**:4971-4978.

Hu, H., Howard, R.J., Bastolla, U., Lindahl, E. and Delarue, M. (2020) Crystal structures of a multi-domain pentameric ion channel extend our understanding of the modulation of the gating transition in the pLGIC family. *Proc. Nat. Acad. Sci USA*, **117**:13437-13446.

Bastolla, U, and Dehouck, Y. (2019) Can conformational changes of proteins be represented in torsion angle space? A study with rescaled ridge regression. *J Chem Inf Mod*.**59**:4929-4941.

Bastolla, U. and Arenas, M. (2019) The Influence of Protein Stability on Sequence Evolution: Applications to Phylogenetic Inference. *Methods Mol Biol*.**1851**:215-231.

Arenas, M. and Bastolla, U. (2020) ProtASR2: Ancestral Reconstruction of Protein Sequences accounting for Folding Stability. *Meth. Ecol. Evol.* **11**:248–257.

Pascual-García, A., Arenas, M. and Bastolla, U. (2019) The molecular clock in the evolution of protein structures. *Systematic Biology*, **68**:987-1002.

Sequeira-Mendes, J., Vergara, Z., Peiro, R., Morata, J., Araguez, I., Costas, C., Mendez Giraldez, R., Casacuberta, J. M., Bastolla, U., and Gutierrez, C. (2019) Differences in firing efficiency, chromatin and transcription underlie the developmental plasticity of the Arabidopsis DNA replication origins. *Genome Res*. **29**:784-797.


## International projects / Research networks

- Participation in the EU-funded H2020-MSCA-RISE-2018-823922 AMR-TB project “Theoretical and computational investigation of tuberculosis antimicrobial resistance development based on extensive experimental library of mycobacterium strains”, 2019-2023.

## GENOME DECODING

# GENETICS AND CELL BIOLOGY OF CANCER: T-CELL LYMPHOBLASTIC NEOPLASMS


***Principal Investigators:***
José Fernández Piqueras
Javier Santos Hernández

***Scientific Staff:***
María del Consuelo Villa Morales
María del Pilar López Nieva
Concepción Vaquero Lorenzo
Alfonso Blázquez Castro
(entry date: 10/12/2019)
Laura González Sánchez
(leaving date: 31/12/ 2019)

***Postdoctoral Fellow:***
Iria González Vasconcellos

***Predoctoral Fellows:***
Antonio Lahera Alonso
Laura Vela Martín
(entry date: 7/11/2019)
Sara Ruiz García
(entry date: 1/9/2020)
José Luis Marín Rubio
(leaving date: 15/2/2019)

***Technicians:***
M. A. Cobos Fernández
Isabel Merlín Sastre

***Undergraduate and Master Students:***
Ana López Gómez
(21/06/2019, TFG)
Raúl Pérez Mato
(entry date: 11/ 2019, leaving date: 7/ 2020, TFM)

http://www.cbm.uam.es/jfpiqueras


## Research summary

T-cell lymphoblastic leukaemia/lymphoma (T-LBL and T-ALL) are haematological diseases with an urgent need for reliable prognostic biomarkers that allow therapeutic stratification and dose adjustment. Therefore, the major aim of our work is to decipher new molecular biomarkers and to propose more effective and less toxic treatments. To this end, we integrate data from genomics and transcriptomics approaches as a start point to identify new driver-molecular mechanisms. In the last years, we have demonstrated the overexpression of critical oncogenes in these neoplasms (such as *MYC, ABL1, BCR-ABL* and *SMO*) due to the combinatorial effect of down-regulated microRNAs, as well as the inactivation of tumour suppressor genes by allele deletion and epigenetic events (*CDKN2A, CDKN2B* and *EPHA7*). Our data revealed a considerable degree of intratumoral heterogeneity helped by the existence of RNA editing. Taking advantage of exome analyses and the versatility of RNA-Seq, we have identified new mutations and significant changes in gene expression, which served to propose new directed therapies. Our results highlight the potential of RNA-Seq to identify new cryptic fusions, which could be drivers or tumour-maintaining passenger genes. Our data also revealed that down regulation of different combinations of isoforms of the tumour suppressor *FBXW7* is a sine qua non condition to induce a proliferative pattern in a cell model system. In addition, we have demonstrated that FADD phosphorylation may serve as a predictor for T-cell lymphoblastic lymphoma aggressiveness and clinical status. Finally, we have shown that reducing the aconitase activity decreases the viability of T-cell lymphoblastic neoplasia cells in correlation to a differential ACO1 expression. These findings were confirmed in vivo on athymic nude-mice by using tumour xenografts derived from tumour T-cell lines expressing different levels of ACO1. We are currently interested in evaluating the involvement of exosome traffic and the deregulation of circular RNAs and long and short ncRNAs, in order to achieve a comprehensive view of the complex regulatory networks deregulated in these neoplasms in the context of a personalized precision medicine. Using specific algorithms we also want to prioritize anticancer drug treatments in T-ALL based on individual genomic data.

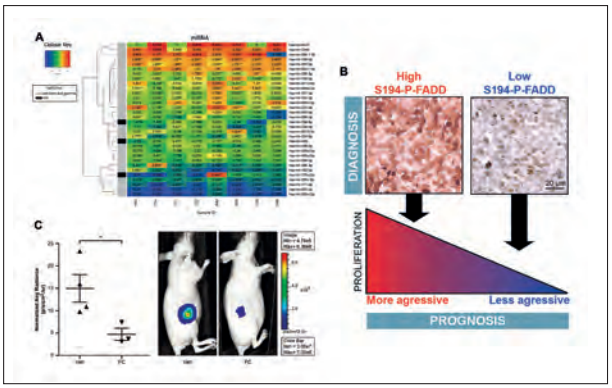


***Figure.*** *A.- Heatmap representation depicting the expression changes of selected microRNAs in tumour samples as determined by small-RNA-seq analysis. B.- Proposed model of association between FADD phosphorylation and prognosis of T-cell lymphoblastic lymphoma. C.- Effect of fluorocitrate treatment on tumour xenografts generated with a human cell line derived from T-cell lymphoblastic neoplasia.*

## Publications

Vázquez-Domínguez, I., González-Sánchez, L., López-Nieva, O., Fernández-Navarro, P., Villa-Morales, M., Cobos- Fernández, M. A., Sastre, I., Fraga, M. F., Fernández, A. F., Malumbres, M., Salazar-Roa, M., Graña-Castro, O., Santos, J., Llamas, P., López-Lorenzo, J. L., and Fernández-Piqueras, J. (2019) Downregulation of specific FBXW7 isoforms with differential effects in T-cell lymphoblastic lymphoma. *Oncogene* **38**: 4620-4636

Marín-Rubio, J. L., Pérez-Gómez, E., Fernández-Piqueras, J.*, and Villa-Morales, M.* (2019) S194-P-FADD as a marker of aggressiveness and poor prognosis in human T-cell lymphoblastic lymphoma. *Carcinogenesis*, **40**:1260-1268.

López-Nieva, P., Fernández-Navarro, P., Graña-Castro, O., Andrés-León, E., Santos, J., Villa-Morales, M., Cobos-Fernández, M. A., González-Sánchez, L., Malumbres, M., Salazar-Roa, M., and Fernández-Piqueras, J. (2019) Detection of novel fusion-transcripts by RNA-Seq in T-cell lymphoblastic lymphoma. *Scientific Reports* **9**:5179.

Marín-Rubio, J. L., Vela-Martín, L., Fernández-Piqueras, J., and Villa-Morales, M. (2019) FADD in Cancer: Mechanisms of Altered Expression and Function, and Clinical Implications. *Cancers (Basel)* **11**, 1462.

Fernandez-Navarro, P., López-Nieva, P., Piñeiro-Yañez, E., Carreño-Tarragona, G., Martinez-López, J., Sánchez, R., Aroca, A., Al-Shahrour, F., and Fernández-Piqueras, J. (2019) The use of PanDrugs to prioritize anticancer drug treatments in a case of T-ALL based on individual genomic data. *BMC Cancer* **19**(1):1005.

Salazar-Roa, M., Trakala, M., Álvarez-Fernández, M., Valdés-Mora, F., Zhong, C., Muñoz, J., Yu, Y., Peters, T. J., Graña, O., Serrano, R., Zapatero-Solana, E., Abad, M., Bueno, M. J., Gómez de Cedrón, M., Fernández-Piqueras, J., Serrano, M., Blasco, M. A., Wang, D. Z., Clark, SJ., Izpisua-Belmonte, J. C., Ortega, S., and Malumbres, M. (2019) A novel microRNA-based strategy to expand the differentiation potency of stem cells. *bioRxiv,* **1**-51. doi: http://dx.doi.org/10.1101/826446

Gonzalez-Sanchez, L., Cobos-Fernandez, M. A., Lopez-Nieva, P., Villa-Morales, M., Stamatakis, K., Cuezva, J. M., Marin Rubio, J. L., Vazquez-Dominguez, I., Gonzalez-Vasconcellos, I., Salido, E., Llamas, P., Lopez-Lorenzo, J. L., Santos, J., and Fernandez-Piqueras, J. (2020) Exploiting the passenger ACO1-deficiency arising from 9p21 deletions to kill T-cell lymphoblastic neoplasia cells. *Carcinogenesis*, **41** (8): 1113–1122.

Salazar-Roa, M., Trakala, M., Álvarez-Fernández, M., Valdés-Mora, F., Zhong, C., Muñoz, J., Yu, Y., Peters, TJ., Graña-Castro, O., Serrano, R., Zapatero-Solana, E., Abad, M., Bueno, M. J., de Cedrón, M. G., Fernández-Piqueras, J., Serrano, M., Blasco, M. A., Wang, D. Z., Clark, S. J., Izpisua-Belmonte, J. C., Ortega, S., Malumbres, M. (2020) Transient exposure to miR-203 enhances the differentiation capacity of established pluripotent stem cells. *The EMBO Journal* **39**: e104324.

Blázquez-Castro, A., Fernández-Piqueras, J. and Santos, J. (2020) Genetic Material Manipulation and Modification by Optical Trapping and Nanosurgery-A Perspective. *Frontiers in Bioengineering and Biotechnology* (Nanobiotechnology) **8**:580937.

Lopez-Nieva, P., Gonzalez-Sanchez, L., Cobos-Fernandez, M. A., Cordoba, R., Santos, J., and Fernandez-Piqueras, J. (2020) More insights on the use of γ-secretase inhibitors in cancer treatment. *The Oncologist* **26**:e298–e305.

## Awards and recognition

- President of the Animal Experimentation Ethics Committee of the Severo Ochoa Center for Molecular Biology (CEEA-CBMSO) (since February 2010 to date).

- Member of the Executive Committee of the Jiménez Díaz Commemorative Lesson (Fundación Conchita Rábago de Jiménez Díaz) (from January 16, 2015 to July 1, 2019).

- Member of the External Scientific Committee of the Research Institute Hospital 12 de Octubre (i + 12) and its Permanent Commission (since 12/12/2016 to date)

- Member of the Board of Trustees of the Severo Ochoa Foundation (since 12/17/2019 to date).

## Patents

License for use and exploitation of two cell lines (Jurkat T-cells expression FADD and Jurkat T-cells deficient in FADD), agreement signed through FUAM with the company Applied Biological Materials, Inc. Madrid, December 10, 2019. Validity 10 years, extendable.

## Doctoral Theses

**José Luis Marín Rubio** (2019) Alterations of FADD expression and phosphorylation in T-cell lymphoblastic lymphoma. Universidad Autónoma de Madrid. Directores: José Fernández Piqueras y María Villa Morales (Mención Internacional; Sobresaliente *Cum Laude;* and PINP Award to best Doctoral Thesis at CBMSO in 2019).

## International projects / Research networks

Participation in Projects financed by the European Union:

- New biomarkers in precursor T-cell lymphoblastic neoplasms: intratumoral heterogeneity, editing of mRNA and exosomes (RTI2018-093330-B-I00). (1/1/2019 to 31/12/2021). Ministry of Science, Innovation and Universities (FEDER,EU).

Membership in networks:

- Membership of CIBERER consortium (CIBER in Rare Diseases, ISCIII).

- Membership of Institute for Health Research Foundation Jiménez Diaz (IIS-FJD).

## GENOME DECODING

# CELL DIVISION, GENOME REPLICATION AND CHROMATIN

***Principal Investigator:***
Crisanto Gutierrez

***Staff Scientist:***
Bénédicte Desvoyes

***Postdoctoral Fellows:***
Julia Emiliani
(since February 2020)
Jorge Fung Uceda
(since October 2019)
María Sol Gómez
(since February 2020)
Anna González Gil
(since November 2019)

***Predoctoral Fellows:***
Nadia Casado García
Clara Echevarria Zomeño
Rocío Núñez Vázquez
Sofía Madeira
(until November 2019)

***Technicians:***
Victoria Mora-Gil Cobo
(until July 2019)
Elisa Alonso Pérez
(since August 2020)

http://www.cbm.uam.es/crisanto-gutierrez

## Research summary

The transition to multicellularity required the evolution of novel structures and mechanisms to coordinate cell division, acquisition of cell fates and the differentiation and establishment of complex regulatory networks. Our research is aimed at understanding fundamental questions on cell proliferation control, transcriptional regulation and genome replication in multicellular organisms and how epigenetics affects such coordination. To that end, we use the model plant *Arabidopsis thaliana* that offers the possibility of carrying out molecular, cellular, genetic and genomic approaches. Contrary to animals, plant development is post-embryonic and occurs during the entire life of the organism, providing excellent experimental settings to study cell proliferation, arrest and differentiation.

We have generated a whole-genome map of DNA replication origins (ORIs) in two stages of development and found that they are located in 9 different chromatin landscapes. We are expanding these studies to mutants in specific pathways to identify the molecular determinants of ORI activity in various chromatin states. We are also interested in learning the dynamics of various factors involved in the assembly of initiation complexes, as well as their regulation in response to abiotic stress.

We are also interested in understanding the relevance of chromatin dynamics on gene expression and cell proliferation control. The balance between the canonical histone H3.1 and the variant H3.3 serves to identify cell populations undergoing their last cell cycle before exit to differentiation because most of H3.1 is massively evicted in the last G2 phase, which is longer in these cells. A major advance has been the generation of Arabidopsis lines (PlaCCI) expressing three fluorescent markers (CDT1a-CFP, H3.1-mCherry and CYCB1;1-YFP) that allow the identification of each cell cycle phase by unique combinations of colors (Fig). This is analogous to the Fucci system available for animals, but in the case of plants new markers had to be identified because plants lack geminin, a key component of Fucci. The PlaCCI lines express all three markers plus an antibiotic resistance gene from a single construct, facilitating their use for genetic analysis of cell proliferation in many different organs and experimental conditions, including mutants of interest and response to stress. Our studies are ultimately aimed at designing strategies to improve plant growth and performance using pathways not explored so far.

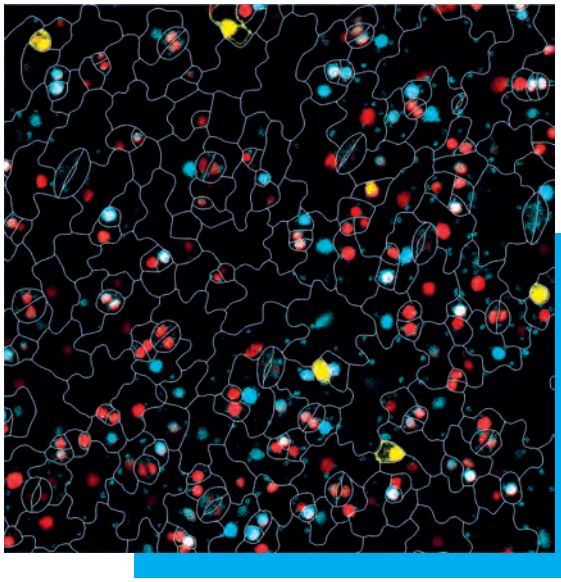

***Figure**. Epidermis of a young (7-8 day-old) leaf of Arabidopsis PlaCCI line showing cells in G1 (light blue), S and early G2 (red) and late G2 and early mitosis (yellow).*

## Publications

Sequeira-Mendes, J., Vergara, Z., Peiró, R., Morata, J., Aragüez, I., Costas, C., Mendez-Giraldez, R., Casacuberta, J. M., Bastolla, U., and Gutierrez, C. (2019) Differences in firing efficiency, chromatin and transcription underlie the developmental plasticity of Arabidopsis DNA replication origins. *Genome Res*. **29**, 784–797.

Sweetlove, L., and Gutierrez, C. (2919) The journey to the end of the chromosome: delivering active telomerase to telomeres in plants. *Plant J*. **98**, 193-194 (2019).

Cajero-Sánchez, W., Aceves-García, P., Fernández-Marcos, M., Gutiérrez, C., Rosas, U., García-Ponce, B., Alvarez-Buylla, E. R., Sánchez, M.P., and Garay-Arroyo, A. (2019) Natural root cellular variation in responses to osmotic stress in Arabidopsis thaliana accessions. *Genes* **10** (12), 983.

Ocaña-Pallarès, E., Vergara, Z., Desvoyes, B., Tejada-Jimenez, M., Romero-Jurado, A., Galvan, A., Fernandez, E., Ruiz-Trillo, I., and Gutierrez, C. (2020) Origin recognition complex (ORC) evolution is influenced by global gene duplication / loss patterns in eukaryotic genomes. *Genome Biol. Evol.* **12**(2):3878–3889.

Probst, A., Desvoyes, B., and Gutierrez, C. (2020) Similar yet critically different: The distribution, dynamics and function of histone variants. *J. Exp. Bot*. **71**, 5191-5204

Coloma, I., Cortijo, M., Fernandez-Sanchez, I., Perles, J., Priego, J. L., Gutierrez, C., Jimenez-Aparicio, R., Desvoyes, B., and Herrero, S. (2020) pH- and time-dependent release of phytohormones from diruthenium complexes. *Inorg. Chem*. **59**, 7779-7788.

Desvoyes, B., and Gutierrez, C. (2020) Roles of plant retinoblastoma protein: cell cycle and beyond. *EMBO J.* **39**: e105802.

Desvoyes, B., Arana-Echarri, A., Barea, M. D., and Gutierrez, C. (2020) A comprehensive fluorescent sensor for spatiotemporal cell cycle analysis in Arabidopsis. *Nature Plants* **6**, 1330-1334.

## Awards and recognition

- Editorial Board EMBO J., EMBO Rep.

- Editor Plant J.

- Member of Academia Europaea (2020).

## Doctoral Theses

**Ana Sofía Madeira Matos** (2019) Role of histone H3 variant, HTR6, during stress response. Universidad Autónoma de Madrid. Directores. Crisanto Gutierrez and Bénédicte Desvoyes. Jury: Aline Probst, Julio Salinas, Luis Blanco. Sobresaliente cum laude, European Mention.

## International projects / Research networks

- ERC-2018-AdG_833617-PLANTGROWTH (2019-2024).

## GENOME DECODING

# INTERNAL INITIATION OF TRANSLATION IN EUKARYOTIC mRNAs

***Principal Investigator:***
Encarnación Martínez-Salas

***Postdoctoral Fellows:***
Rosario Francisco-Velilla
Alejandra Escós Lopez

***Predoctoral Fellows:***
Azman Embark-Buh

***Technician:***
Jorge Ramajo

***Undergraduate and Master Students:***
Salvador Abellan

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## Research summary

Our aims are focused to understand the principles guiding alternative mechanisms of translation initiation in eukaryotes through the characterization of RNA-binding proteins (RBPs) interacting with mRNAs. Internal ribosome entry sites (IRES) are non-coding RNA regions that substitute the function of the 5' terminal cap of mRNAs, the anchoring point of the translation machinery. To achieve their function, IRESs assemble ribonucleoprotein complexes, including a subset of eIFs and RBPs. Our specific aims were the identification of proteins modulating IRES activity, the evaluation of synergism and/or interference with other factors, and the understanding of structural constraints which are essential for its activity. Using a novel RNA-affinity approach we have identified Rab-1b and ARF5, two components of the ER-Golgi trafficking complexes, as regulatory factors of a viral IRES. Additionally, we have shown that Gemin5 interacts with a viral IRES as well as a selective group of cellular RNAs. Gemin5 is a multitasking protein, that forms part of the survival of motor neuron (SMN) complex and performs a dowregulatory role in translation. Defects on the SMN complex levels lead to SMA (Spinal muscular atrophy), an autosomal rare disease. Similar to essential RBPs involved in translation control (eIF4G), stress response (G3BP) and antiviral response (MDA5), Gemin5 is targeted by viral proteases, resulting in a p85 fragment that enhances IRES translation. Hence, understanding the implications of Gemin5 in gene expression regulation could provide hints on the multiple roles of this protein in RNA-dependent processes. The N-terminal domain of Gemin5 is involved in the interaction with the ribosome and the snRNAs, whereas the C-terminal region harbors a non-conventional RNA-binding site (RBS1). Identification of the RNA partners of the RBS1 domain unveiled a feedback loop with its own mRNA, counteracting the negative effect of Gemin5 on translation. The RBS1 moiety coevolved with the RNA-interacting region, revealing the evolutionary selection of the RNA-protein interaction module between Gemin5 and its own mRNA. Additionaly, the midle region of the protein harbors a dimerization domain. The crystal structure of this domain consists of a tetratricopeptide (TPR)-like domain that self-assembles into a canoe-shaped dimer (Figure). The dimerization module is functional in living cells driving the interaction between the p85 fragment and the full-length Gemin5, which anchors splicing and translation members. Disruption of the dimerization capacity of Gemin5 prevents this interaction and abrogates the translation enhancement induced by p85. The characterization of this dimerization domain provides the structural basis for a role of Gemin5 as a central hub for protein-protein interaction.

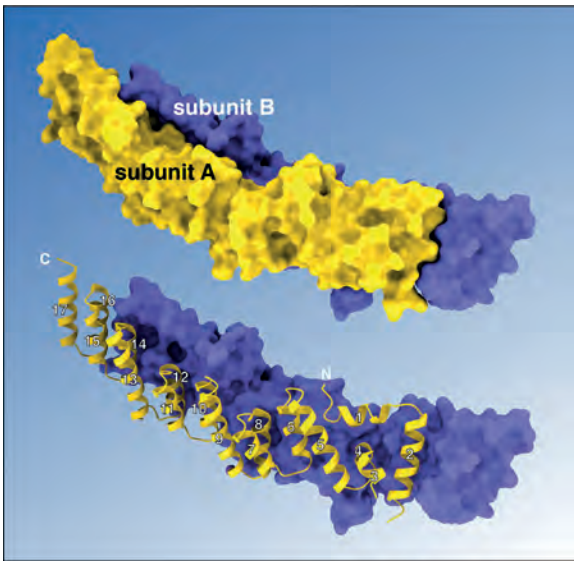


*Figure. Three-dimensional structure of the dimerization motif of Gemin5. (Top) View of the TPR-like dimer surface structure. (Bottom) Structure of G5-TPR dimer with the subunit at front depicted in yellow cartoon and the subunit at the back shown in violet surface representation.*

## Publications

Fernandez-Chamorro, J., Francisco-Velilla, R., Ramajo, J. and Martínez-Salas, E. (2019) IRES-driven RNA localization at ER-Golgi compartment is mediated by RAB1b and ARF5. *Life Sci. Alliance* **2**(1) e201800131.

Francisco-Velilla, R., Embarc-Buh, A. and Martínez-Salas, E. (2019) RNA-binding modes impacting on translation control: the versatile multidomain protein Gemin5. *BioEssays*, **41**(4):e1800241.

Lopez-Arguello, S., Rincon, V., Rodriguez-Huete, A., Martínez-Salas, E., Belsham, G. J., Valbuena, A., and Mateu, M.G. (2019) Thermostability of the Foot-and-Mouth Disease virus capsid is modulated by lethal and viability-restoring compensatory amino acids substitutions. *J. Virol*. **93**(10):e1800241.

Moreno, M., Fernandez-Algar, M., Fernandez-Chamorro, J., Ramajo, J., Martínez-Salas, E., and Briones, C. (2019). Development of a combined ELONA-qPCR method for the high-throughput screening and characterization of DNA and RNA aptamers: applicability to the selection of aptamers against PCBP-2. *Molecules*, **24**(7):1213.

Brown, P., RELISH Consortium, Zhou, Y., et al. (2019) Large expert-curated database for benchmarking document similarity detection in biomedical literature search. *Database* 2019, **1**-66.

Moreno-Morcillo, M., Francisco-Velilla, R., Embarc-Buh, A., Fernandez-Chamorro, J., Ramon-Maiques, S., and Martínez-Salas, E. (2020) Structural basis for the dimerization of Gemin5 and its role in protein recruitment and translation control. *Nucleic Acids Res*. **48**(2):788-801.

Francisco-Velilla, R., Embarc-Buh, A., Rangel-Guerrero, S., Basu S., Kundu, S., and Martínez-Salas, E. (2020) RNA-protein coevolution study of Gemin5 uncovers the role of the PXSS motif of RBS1 domain for RNA binding.. *RNA Biology*, **17**(9), 1331.1341.

Martínez-Salas, E., Embarc-Buh, A., and Francisco-Velilla, R. (2020) Emerging roles of Gemin5: from snRNPs to translation control. *Int. J. Mol. Sci.* **21**(11):3868.

Pulido, M. R, Martínez-Salas, E., Sobrino, F., and Sáiz, M. (2020) MDA5 cleavage by the Leader protease of foot-and-mouth disease virus reveals its pleiotropic effect against the host antiviral response. *Cell Death Dis*. **11**(8):718.

## Awards and recognition

Member of the Editorial Board: Virology, Virus Res, Peer J, Front in Microbiol, Cellular and Infection Microbiology, Virus and Host.

## Doctoral Theses

**Azman Embarc Buh.** (2020) Gemin5, a multifunctional RNA-binding protein involved in translation control. Universidad Autónoma de Madrid. Directora: E. Martinez-Salas, Codirectora: R. Francisco-Velilla.

## International projects / Research networks

- RNAlife-2. (2020-2022) RED2018-102467-T MINECO.

## GENOME DECODING

# STRUCTURE AND FUNCTION OF MACROMOLECULAR COMPLEXES

***Principal Investigator:***
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***Postdoctoral Fellows:***
Francisco del Caño Ochoa

***Undergraduate and Master Students:***
Ricardo García Martín (TFG)

http://www.cbm.uam.es/santiagoramon

## Research summary

Research in our group is focused on understanding the molecular mechanisms and cellular functions of proteins and how their defects can lead to disease. We are particularly interested in explaining protein malfunction and in identifying disease-causing variants, to help in the correct diagnosis of patients and to provide a rational basis for the development of new therapeutic strategies. We rely on a mixture of biochemical, biophysical and cellular methods to define the structure, function and evolution of these macromolecular targets. One of our main objectives is to determine the architecture and functioning mechanisms of CAD, a multifunctional mega-enzyme responsible for the initiation and control of de novo synthesis of pyrimidine nucleotides. In parallel to our efforts to decipher the structure, in the past two years, we studied the subcellular location of CAD as a first approach to understand how it works within the complex cellular environment. By combining CRISPR/Cas9 editing, protein engineering and fluorescence microscopy, we showed that CAD locates exclusively in the cytoplasm, contradicting other studies that reported its translocation to the nucleus. On the other hand, since the discovery in 2015 that defects in CAD cause a rare and potentially lethal congenital metabolic disorder, our group has been deeply involved in the identification of pathogenic variants of CAD, helping in the diagnosis of children affected by this illness (in collaboration with Hudson H. Freeze). Although the CAD-deficit can be treated with an oral uptake of uridine, the disease is difficult to diagnose given the large size of the protein, the >1000 missense known variants and the nonspecific clinical presentation. We aimed to set up a reliable and discerning assay to assess the pathogenicity of CAD variants. Using CRISPR/Cas9, we generated a human CAD-knockout cell line that requires uridine (a pyrimidine) for survival. Then, we tested if transient transfection of CAD bearing clinical mutations was able to restore the growth of the knockout cells in absence of uridine. The system allowed us to test 34 variants, identifying 16 as deleterious for CAD activity. Combination of these pathogenic variants confirmed that out of the 25 children with biallelic mutations in CAD included in the study, 11 were CAD-deficient and thus, could benefit from the uridine therapy. In the past two years, we also studied other disease-related proteins involved in diverse processes such as control of gene expression (in collaboration with Encarna Martínez-Salas, CBM) or glycosylation pathways (with Belén Pérez-González, CBM).

*In July 2020 the group moved to the Instituto de Biomedicina de Valencia (IBV-CSIC).*

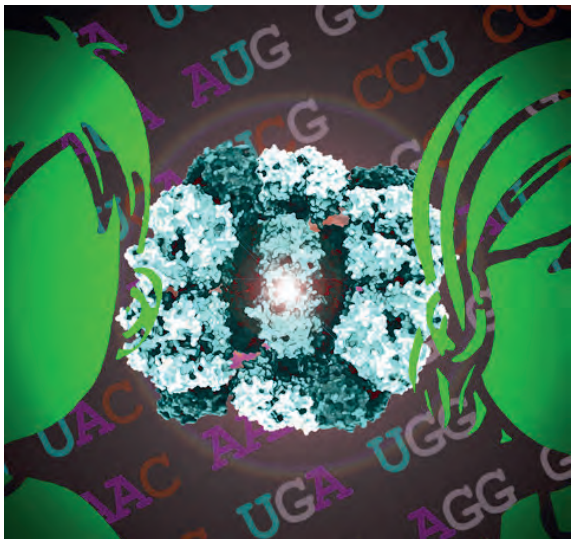


***Figure.*** *We developed a cellular assay to identify pathogenic missense variants of the protein CAD (shown in the center), helping in the diagnosis of children affected by a severe inborn metabolic disease.*

## Publications

Hruschka, N., Kalisz, M., Subijana, M., Graña-Castro, O., Del Cano-Ochoa, F., Brunet, L. P., Chernukhin, I., Sagrera, A., De Reynies, A., Kloesch, B., Chin, S. F., Burgués, O., Andreu, D., Bermejo, B., Cejalvo, J. M., Sutton, J., Caldas, C., Ramón-Maiques, S., Carroll, J. S., Prat, A., Real, F. X., and Martinelli, P. (2020) The GATA3 X308_Splice breast cancer mutation is a hormone context-dependent oncogenic driver. *Oncogene*. **39**, 5455-5467.

Del Caño-Ochoa, F., Ng, B. G., Abedalthagafi, M., Almannai, M., Cohn, R. D., Costain, G., Elpeleg, O., Houlden, H., Karimiani, E. G., Liu, P., Manzini, M. C., Maroofian, R., Muriello, M., Al-Otaibi, A., Patel, H., Shimon, E., Sutton, V. R., Toosi, M. B., Wolfe, L. A., Rosenfeld, J. A., Freeze, H. H., and Ramón-Maiques, S. (2020) Cell-based analysis of CAD variants identifies individuals likely to benefit from uridine therapy. *Genet Med*. **22**, 1598-1605.

Del Caño-Ochoa, F., and Ramón-Maiques, S. (2020) The multienzymatic protein CAD leading the de novo biosynthesis of pyrimidines localizes exclusively in the cytoplasm and does not translocate to the nucleus. *Nucleosides Nucleotides Nucleic Acids*. **39**:1320-1334.

Del Caño-Ochoa, F., Moreno-Morcillo, M., and Ramón-Maiques, S. (2019) CAD, a multienzymatic protein at the head of de novo pyrimidine biosynthesis. *Subcell Biochem*. **93**:505-538.

Moreno-Morcillo, M., Francisco-Velilla, R., Embarc-Buh, A., Fernández-Chamorro, J. Ramón-Maiques, S., and Martinez-Salas, E. (2019) Structural basis for the dimerization of Gemin5 and its role in protein recruitment and translation control. *Nucleic Acids Res*. **48**:788-801.

## International projects / Research networks

- Our group is associated with Group U739 of the CIBER de Enfermedades Raras (CIBERER), and collaborates with the Group U746 CIBERER.

## GENOME DECODING

# REGULATION OF GENE EXPRESSION IN *LEISHMANIA*


***Principal Investigator:***
Jose María Requena Rolanía

***Scientific Staff***
Manuel Soto Álvarez

***Postdoctoral Fellows:***
Laura Ramírez García
África Sanchiz Giraldo
(2019)

***Predoctoral Fellows:***
Esther Camacho Cano

***Undergraduate and Master Students:***
Alberto Cámara Ballesteros
Fernando Lominchar Villaseñor

Darío López García
Hui Miao
Daniel Mora Diego

***Visiting Scientists:***
Nahiara E. Zorgi
(State University of Campinas, Campinas, Sao Paulo, Brazil)

http://www.cbm.uam.es/jmrequena

## Research summary

Protists of the *Leishmania* genus include species causing severe diseases in humans, i.e. leishmaniasis. Moreover, *Leishmania* species are the subject of fundamental interest regarding the evolution of gene regulatory mechanisms, as these organisms have put aside transcriptional regulation. There is currently no acceptable vaccine for preventing leishmaniasis and treatment options are limited. The research activity of our group is focused on studying, on the one hand, genome organization and gene expression in this parasite, and, on the other hand, the immuno-pathological outcomes associated with the infections caused by this parasite. Our aim is contributing knowledge for the development of new methods of controlling this parasitosis.

In the last coup of years, efforts have been made to generate both complete and well-annotated genomes for the main pathogenic *Leishmania* species, bearing in mind they represent ultimate resource for genome-wide scale approaches, such as transcriptome and proteome analyses. Massive sequencing techniques have become routine tools in pursuing these objectives, which have been done in close collaboration with Dr Begoña Aguado (head of the Genomics & Massive Sequencing service at CBM). As a result, our group has generated new assemblies, improving previous ones, for the genomes of *Leishmania* donovani, causative agent of visceral leishmaniasis, and L. braziliensis, causing mucocutaneous leishmaniasis in South America. Furthermore, we are using massive RNA sequencing (RNA-seq) for the annotation of transcriptomes of several *Leishmania* species and to study changes in gene expression. For hosting this information, which is actively curated, a web page was created: http://leish-esp.cbm.uam.es. More recently, we have incorporated proteomics methodologies to analyze the proteomes of these parasites.

The alliance of proteomics, genomics, and transcriptomics has resulted in a powerful combination for improving the annotation of the *Leishmania* genomes.

Another area of active research, headed by Dr Manuel Soto, was aimed to the study of *Leishmania* – host immune response interactions. The immunoprophylactic properties of a live attenuated vaccine based on a genetically modified-*Leishmania* mutant line *(LiΔHSP70-II)* have been studied in different models of murine leishmaniasis caused by different *Leishmania* species.

Finally, as members of the Tropical Diseases network (ISCIII; http://www.ricet.es/es/), our group was engaged in collaborative research dealing with molecular diagnosis and typing of *Leishmania* strains isolated from patients.

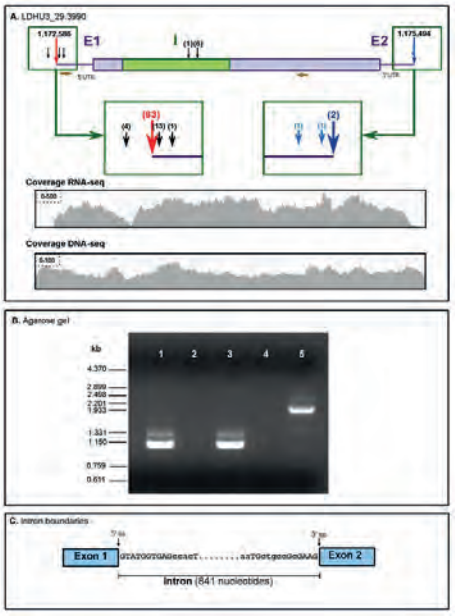


***Figure.*** *Documentation of a cis-splicing event during transcription of the Leishmania donovani gene LDHU3_29.3990. cis-splicing events are extremely rare in this parasite.*

## Publications


Camacho, E., Gonzalez-de la Fuente, S., Rastrojo, A., Peiro-Pastor, R., Solana, J. C., Tabera, L., Gamarro, F., Carrasco-Ramiro, F., Requena, J. M., and Aguado, B. (2019). Complete assembly of the *Leishmania* donovani (HU3 strain) genome and transcriptome annotation. *Sci Rep* **9**, 6127.

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Camacho, E., Rastrojo, A., Sanchiz, A., Gonzalez-de la Fuente, S., Aguado, B., and Requena, J. M. (2019). Leishmania Mitochondrial Genomes: Maxicircle Structure and Heterogeneity of Minicircles. *Genes (Basel)* **10**: 758.

Lozano-Andrés, E., Libregts, S. F., Toribio, V., Royo, F., Morales, S., López-Martín, S., Valés-Gómez, M., Reyburn, H. T. Falcón-Pérez, J. M., Wauben, M. H., Soto, M., and Yáñez-Mó, M. (2019). Tetraspanin-decorated extracellular vesicle-mimetics as a novel adaptable reference material. *J Extracell Vesicles*. **8**, 1573052.

Soto, M., Ramírez, L., Solana, J. C., Cook, E. C. L., Hernández-García, E., Charro-Zanca, S., Redondo-Urzainqui, A., Reguera, R. M., Balaña-Fouce, R., and Iborra, S. (2020). Resistance to experimental visceral leishmaniasis in mice infected with *Leishmania* infantum requires Batf3. *Front Immunol*.**11**:590934.

Solana, J. C., Ramírez, L., Cook, E. C. L., Hernández-García, E., Sacristán, S., Martín, M. E., González, V. M., Reguera, R. M., Balaña-Fouce, R., Fresno, M., Requena, J. M., Iborra, S., and Soto, M. (2020). Subcutaneous immunization of Leishmania HSP70-II null mutant line reduces the severity of the experimental visceral leishmaniasis in BALB/c mice. *Vaccines (Basel)*. **8**(1), 141.

Frezza, V., Pinto-Diez, C., Fernandez, G., Soto, M., Martin, M. E., Garcia-Sacristán, A., and Gonzalez, V. M. (2020). DNA aptamers targeting *Leishmania* infantum H3 protein as potential diagnostic tools. *Anal Chim Acta.* **1107**:155-163.

Ramírez, L., Dias de Moura, L., Lopes Fontoura Mateus, N., Hoehr de Moraes, M., do Nascimento, L., Melob, N. J., Bezerra Taketa, L., Catecati, T., Huete S. G., Penichet, K., Mattos Piranda, E., Gutierrez de Oliveira, A., Steindel, M., Barral-Netto, M., Pires e Cruz, M. S., Barral, A., and Soto, M. (2020) Improving the serodiagnosis of canine Leishmania infantum infection in geographical areas of Brazil with different disease prevalence. *Parasite Epidemiol Control*. **8**:300126.

Ruiz-Marvez, E., Ramirez, C. A., Rodriguez, E. R., Florez, M. M., Delgado, G., Guzman, F., Gomez-Puertas, P., Requena, J. M., and Puerta, C. J. (2020). Molecular Characterization of Tc964, A Novel Antigenic Protein from *Trypanosoma cruzi.* Int *J Mol Sci* **21**, 2432.

Zorgi, N. E., Arruda, L. V., Paladine, I., Roque, G. A. S., Araújo, T. F., Brocchi, M., Barral, M., Sanchiz, Á., Requena, J. M., Abánades, D. R., and Giorgio, S. (2020). Leishmania infantum transfected with toxic plasmid induces protection in mice infected with wild type L. infantum or L. amazonensis. *Mol. Immunol*. **127**, 95–106.

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## International projects / Research networks

Retics: RED DE INVESTIGACIÓN COLABORATIVA EN ENFERMEDADES TROPICALES (RICET). RD16/0027/0008, 2017-2021.

## GENOME DECODING

# REGULATION OF mRNA TRANSLATION IN EUKARYOTES AND ITS IMPLICATIONS FOR ORGANISMAL LIFE

***Principal Investigators:***
Iván Ventoso
César de Haro (until January 2020)
Juan José Berlanga
Miguel Ángel Rodríguez

***Predoctoral Fellows:***
Irene Díaz-López
(until January 2020)
Tamara Jiménez

***Undergraduate and Master Students:***
Ana Carreras-Presas

***Technicians:***
José Alcalde
Laura Barbado
(until February 2020)

https://www.cbm.uam.es/iventoso

## Research summary

We continue to investigate how eukaryotic systems (mammals, yeast and RNA viruses) regulate translation initiation at both global and message-specific manner, trying to identify new elements in ribosomes and mRNAs, and new initiation factor (eIFs) activities involved in the differential translation of mRNAs during cell proliferation and stress response. In the last two years, we have identified the ES6S region of 40S ribosomal subunit as the gateway for mRNA entry and secondary structure unwinding during the scanning process, so that blocking ES6S region differentially affected the expression of some proto-oncogenes (H-Ras, CCND3, ODC-1, etc.) and other genes involved in signal transduction with long and structured 5´UTR mRNAs. We are currently evaluating the antiviral and antitumoral activities of oligonucleotides and aptamer molecules targeting the ES6S of 40S subunit.

We are also studying how Alphaviruses have evolved to adapt translation of their mRNA to stress conditions in infected cells and tissues, a useful model that has allowed us to develop modified viruses with increased oncoselectivity for tumor virotherapy.

We continue to study how cells reprogramme translation during the stress response in yeast and mammals by modulating the activity of eIF2 and eIF2A factors, and the physiological impact of this response on cell and organismal adaptation, survival and ageing. Thus, we recently found that preventing eIF2α phosphorylation not only impaired stress response, but also accelerated ageing in yeast by a mechanism that involves proteostasis disruption.

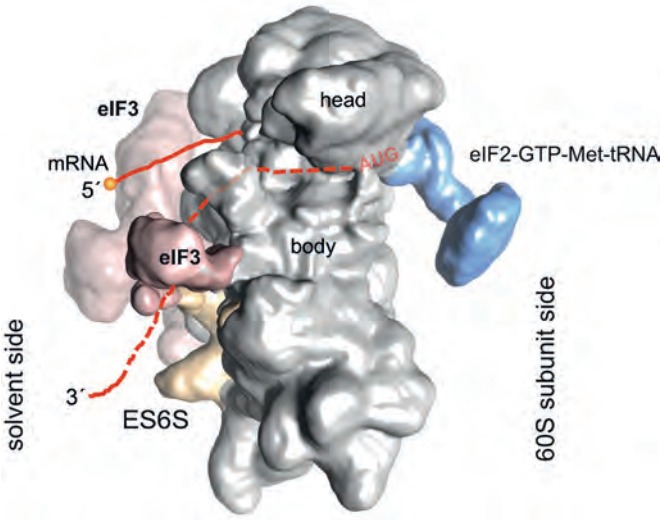


***Figure.*** *Involvement of ribosomal ES6S region and eIF2 in mRNA threading and initiation codon recognition, respectively. Model of pre-initiation 43S complex. mRNA (red) penetrates through the ES6S$^{A}$ and ES6S$^{B}$ helices of the 40S subunit (yellow). The positions of eIF3 (pink) and the ternary complex (eIF2-GTP-Met-tRNAi, blue) are also shown.*

## Publications

Díaz-López, I., Toribio, R. and Ventoso, I. (2020). Mapping mRNA-18S rRNA Contacts Within Translation Initation Complex by Means of Reverse Transcriptase Termination Sites and RNAseq. *Bio-protocol* **10**(16): e3713.

Toribio, R., Díaz-López, I., Berlanga, J. J., Molina-Jiménez, F., Majano, P., and Ventoso, I. (2020) Naturally Occurring and Engineered Alphaviruses Sensitive to Double-Stranded-RNA-Activated Protein Kinase Show Restricted Translation in Mammalian Cells, Increased Sensitivity to Interferon, and Marked Oncotropism. *J Virol*. **94**(3):e01630-19.

Losada, A., Berlanga, J. J., Molina-Guijarro, J. M., Jiménez-Ruiz, A., Gago, F., Avilés, P., de Haro, C., and Martínez-Leal, J. F. (2020) Generation of endoplasmic reticulum stress and inhibition of autophagy by plitidepsin induces proteotoxic apoptosis in cancer cells. *Biochem Pharmacol* **172**:113744.

Díaz-López, I., Toribio, R., Berlanga, J. J., and Ventoso, I. (2019) An mRNA-binding channel in the ES6S region of the translation 48S-PIC promotes RNA unwinding and scanning. *Elife*. **8**:e48246.

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## Doctoral Theses

**Irene Díaz López**, 2019. Involvement of ES6S region of 40S subunit in mRNA threading and scanning during translation initiation. Universidad Autónoma de Madrid. Directores, Iván Ventoso Bande y Francisco René Toribio López.

**Tamara Jiménez Saucedo**, 2020. Role of eIF2α-dependent translational regulation in aging. Universidad Autónoma de Madrid. Directores, Miguel Ángel Rodríguez Gabriel y Juan José Berlanga Chiquero.

UNIT

LUIS BLANCO

# Genome Maintenance and Instability

GENOME DYNAMICS AND FUNCTION

Researchers at the "Genome maintenance and instability" Unit use a variety of model systems to decipher mechanisms of genome organization, DNA and chromatin replication and repair. DNA replication itself is an important source of DNA damage, e.g., when a replication fork stalls is at risk of breaking. Luckily, specialized checkpoints and mechanisms of DNA repair, translesion synthesis, re-priming, histone reloading and telomere maintenance can prevent accumulation of DNA damage and therefore, prolong the cell and organismal fitness.

Margarita Salas group used phi29 DNA polymerase to study the coordination between DNA polymerization and proofreading activities of family B DNA polymerases. Moreover, as part of ongoing efforts to characterize bacteriophage Bam35 as a new working model, a high-quality, fully resolved genome of the host strain *Bacillus thuringiensis* HER1410 was obtained. In connection with both projects, a new variant of Bam35 DNA polymerase with enhanced capacity to amplify damaged DNA has been engineered.

Luis Blanco group characterized the mechanism of DNA primer synthesis by human PrimPol, a specific primase alleviating replicative stress both at the nucleus and mitochondria, and characterized a cancer-associated variant of human PrimPol which disables the steric gate, and is able to use ribonucleotides to build primers.

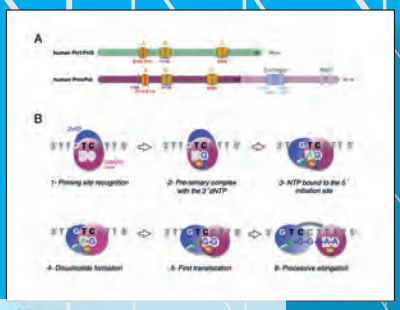
Miguel de Vega group has shown that double-stranded DNA breaks in bacteria are repaired by a specialized multifunctional enzyme, LigD, which has been shown to contain a novel AP lyase activity, thus coupling abasic site cleavage to the nonhomologous end-joining reaction. Moreover, the structural determinants responsible for the preferential ribonucleotides insertion by bacterial LigDs, and for the nucleolytic activities of bacterial/archaeal PolXs, have been identified.

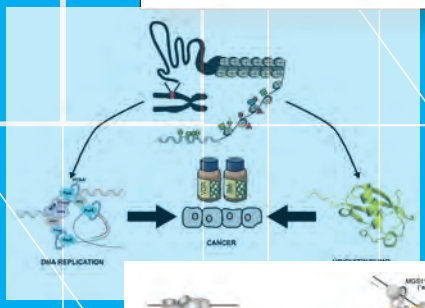
José A. Tercero group uncovered that the evolutionarily conserved Mgs1/WRNIP1 ATPase has a key role in preventing unscheduled and potentially detrimental DNA recombination at damaged replication forks. This action in turn helps to channel DNA damage bypass to error-free template switching, thus importantly contributing to the maintenance of genome stability during chromosome replication.

By integrating mammalian replication origin maps into three-dimensional chromatin interaction networks, María Gómez group has shown that origins form replication factories with correlated activation efficiencies.

Emilio Lecona group has identified a functional cooperation of the protein segregase VCP, in association with the adaptor FAF1, and the deubiquitinase USP7 during DNA replication through the control of the ubiquitin/SUMO equilibrium at the fork that determines the extraction of replication factors from chromatin.

As described in detail in this report, several labs from our Research Unit with recognized expertise in DNA amplification have engaged in collaborative projects to develop a novel diagnostic method of SARS-CoV-2 infection.






**LUIS BLANCO**

GENOME MAINTENANCE AND VARIABILITY: ENZYMOLOGY OF DNA REPLICATION AND REPAIR

**MIGUEL DE VEGA**

MAINTENANCE OF BACTERIAL GENOME STABILITY

**MARÍA GÓMEZ**

FUNCTIONAL ORGANIZATION OF THE MAMMALIAN GENOME

**EMILIO LECONA**

CHROMATIN, CANCER AND THE UBIQUITIN SYSTEM

**MARGARITA SALAS GROUP**

REPLICATION OF BACTERIOPHAGE Ø29 DNA

**JOSÉ ANTONIO TERCERO**

CHROMOSOME REPLICATION AND GENOME STABILITY

## GENOME MAINTENANCE AND INSTABILITY

# GENOME MAINTENANCE AND VARIABILITY: ENZYMOLOGY OF DNA REPLICATION AND REPAIR

***Principal Investigator:***
Luis Blanco Dávila

***Postdoctoral Fellow:***
María Isabel Martínez Jiménez

***Predoctoral Fellows:***
Alberto Díaz Talavera
Nieves Calero Muñoz
Cristina Velázquez Ruiz
Ana Martínez Carrón

***Technician:***
Susana Guerra González

***Visiting Scientist:***
Paola Libertad García Medel

http://www.cbm.uam.es/lblanco

## Research summary

Work done in the last decade has revealed that DNA replication is a major source of genomic instability, due to rearrangements that occur at sites of stalled replication forks. These problems are overall defined as “replication stress” (RS). Along the last seven years we have contributed to demostrate that the majority of DNA lesions or blocking structures occurring in the template DNA may be “tolerated” during RS, mainly through the generation of PrimPol-mediated new replicative starting points (namely primers) in the leading strand, immediately beyond the lesion. Importantly, our discovery of PrimPol leads to reconsider the molecular scenario in which the DNA lesion is subsequently repaired, implying the existence of remaining small DNA gaps containing the lesion, generated after PrimPol-mediated restart, that need to be filled, processed, and finally sealed. We are currently interested in the identification and characterization of potential PrimPol interactors, and in the basic mechanism of the subsequent damage tolerance and repair transactions needed behind, after PrimPol-mediated fork restart.

It is worth mentioning that PrimPol inactivation or removal during the replication of nuclear DNA entails a significant reduction of replication fork speed, indicating its relevant role in physiological conditions. Thus, whereas PrimPol-deficient mice and siRNA-depleted human cells are viable, they show large symptoms of genomic instability, very likely related to the augmented DNA RS in these cells. On the other hand, in the presence of RS induced by replication inhibitors or topoisomerase poisons, such as camptothecin and etoposide, PrimPol deficiency generates a strong and persistent increase in RS signaling, leading to augmented genetic instability, a hallmark of cancer cells. Altogether, our data strongly suggest that PrimPol is a novel and relevant target in cancer. Its deficiency, either due to specific inactivating mutations or to the use of specific inhibitors, in combination with other well known genotoxic agents, would lead to high replicative stress burden causing tumor cells death by replication catastrophe. Thus, another objective of our research is the analysis of human PrimPol regulation, and the characterization of human PrimPol cancer variants with altered specific activity and primase deficiency, that could have a pronostic value in cancer.

From a biotechnological perspective, we have co-developed a novel PrimPol-based DNA amplification method named TruePrime™, currently commercialized by 4BaseBio AG, for the diagnosis of cancer in liquid biopsies, and for the massive preparation of synthetic DNA to fullfill the growing demand in gene therapy and preparation of DNA vaccines.

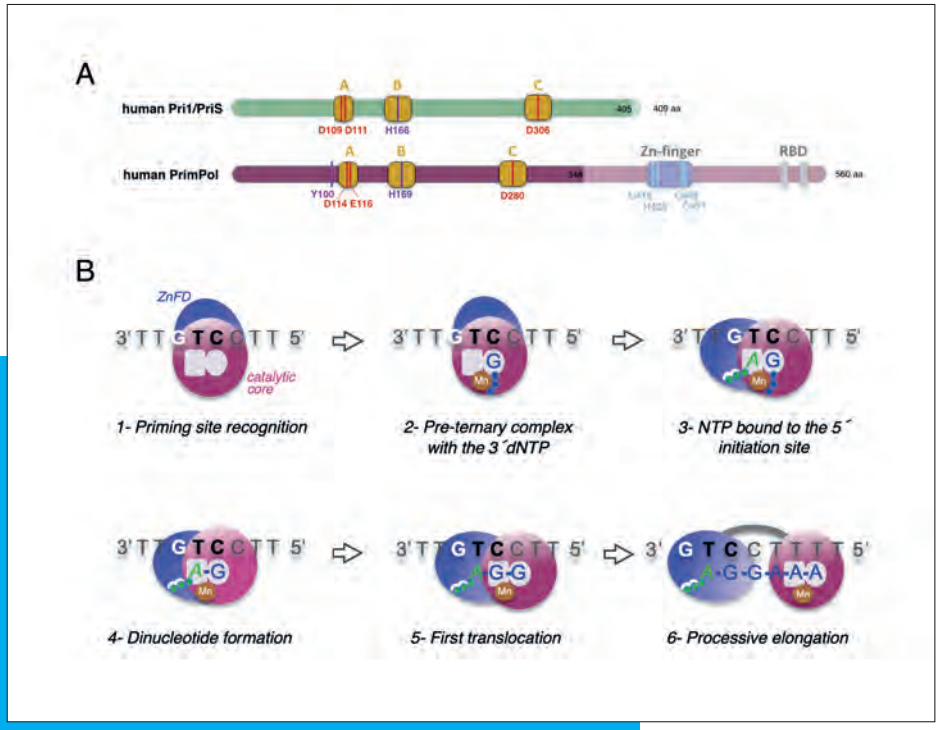



***Figure.*** *Mechanism of DNA priming by human PrimPol. A) Structural comparison of human Pri1/PriS/p49 and human PrimPol. B) Sequential steps during primer synthesis by human PrimPol.*

## Publications

Blanco, L., Calvo, P.A., Diaz-Talavera, A., Carvalho, G., Calero, N., Martínez-Carrón, A., Velázquez-Ruiz, C., Villadangos, S., Guerra, S. and Martínez-Jiménez, M.I. (2019) Mechanism of DNA primer synthesis by human PrimPol. *Enzymes* **45**, 289-310.

Calvo, P.A., Sastre-Moreno, G., Perpiñá, C., Guerra, S., Martínez-Jiménez, M.I. and Blanco, L. (2019) The invariant glutamate of human PrimPol DxE motif is critical for its Mn2+-dependent distinctive activities. *DNA Repair (Amst)* **77**, 65-75.

Díaz-Talavera, A., Calvo, P.A., González-Acosta, D., Díaz, M., Sastre-Moreno, G., Blanco-Franco, L., Guerra, S., Martínez-Jiménez, M.I., Méndez, J. and Blanco, L. (2019) A cancer-associated point mutation disables the steric gate of human PrimPol. *Sci. Rep*. Feb 4;**9**(1):1121.

Duong, V.N., Zhou, L., Martínez-Jiménez, M.I., He, L., Cosme, M., Blanco, L., Paintsil, E. and Anderson, K.S. (2020) Identifying the role of PrimPol in TDF-induced toxicity and implications of its loss of function mutation in an HIV+ patient. *Sci Rep*. **10**(1):9343.

## Doctoral Theses

**Patricia A. Calvo Fernández** (2019). "Structure-function analysis of human PrimPol". Universidad Autónoma de Madrid. Supervisors: Luis Blanco Dávila & María I. Martínez Jiménez.

**Gustavo Carvalho Dias** (2020). "Characterization of murine PrimPol as a robust DNA primase". Universidad Autónoma de Madrid. Supervisors: Luis Blanco Dávila & María I. Martínez Jiménez.

**Alberto Díaz Talavera** (2020). "Human PrimPol: a TLS DNA primase for alleviating DNA replication stress and a potential target in cancer". Universidad Autónoma de Madrid. Supervisor: Luis Blanco Dávila. International PhD mention.

## GENOME MAINTENANCE AND INSTABILITY

# MAINTENANCE OF BACTERIAL GENOME STABILITY

***Principal Investigator:***
Miguel de Vega

***Postdoctoral Fellow:***
Patricia A. Calvo

***Undergraduate and Master Students:***
Cristina San Vicente
(January-June 2020)
Amalia Buitrago
(from October 2020)

www.cbm.uam.es/de-vega-lab

## Research summary

Our main objective is the study at a molecular level of the proteins responsible for genetic stability in bacteria, using as model those from the bacterium *Bacillus subtilis*.

Double strand breaks (DSBs) are the most hazardous DNA lesions as they are lethal to dividing cells if are not repaired in a timely fashion. Many bacteria are provided with a nonhomologous end joining system (NHEJ) responsible for repairing DSBs and constituted by the ring-shaped DNA-binding Ku that recruits the multicatalytic protein Ligase D (LigD) to the ends. By using its polymerization and ligase activities, LigD fills the gaps that arise after realignment of the ends and seals the resulting nicks. Abasic (AP) sites are the most common genomic DNA lesions frequently associated with DSBs. As their presence near the break can pose a strong block to the final ligation, these lesions must be excised. In the last two years, we have found a robust AP lyase activity in the polymerization domain of LigD that cleaves AP sites preferentially when they are proximal to recessive 5'-ends. Such a reaction depends on both, metal ions and the formation of a Watson–Crick base pair between the incoming nucleotide and the templating one opposite the AP site. Therefore, the coupling of AP sites cleavage to the end-joining reaction by the bacterial LigD guarantees the repair of DSBs associated to AP sites (see Figure).

We have also been studying the biochemical features of bacterial DNA polymerases belonging to family X, involved in the base excision repair pathway and that have a C-terminal PHP domain with 3′-5′ exonuclease, AP-endonuclease, 3′-phosphodiesterase and 3′-phosphatase activities. Multiple sequence alignments allowed us to identify highly conserved residues along the PHP domain of bacterial/archaeal PolXs and whose potential role in the nucleolytic activities had not been established. Biochemical analysis of site directed mutants at the corresponding *B. subtilis* PolX residues Phe440, Arg469, Arg474, Asn498, Arg503 and Lys545, indicated a DNA binding role for those residues that were arranged in the tertiary structure in a way that form an electropositive path to the catalytic site. Those results, together with the intermolecular transference of the 3′-terminus between the PHP and polymerization active sites, and the available 3D structures of bacterial PolXs led us to propose the requirement to a great degree of a functional/structural flexibility to coordinate the synthetic and degradative activities in these enzymes.

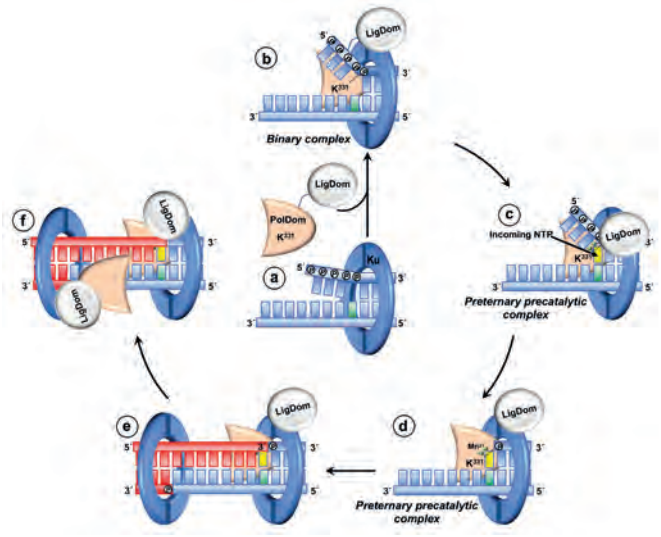



***Figure.*** *Coupling of AP sites cleavage to the end-joining reaction by the B. subtilis LigD. After the breakage, the DNA end is threaded through the open ring-like structure of the Ku dimer (a). The location of the AP site proximal to the 5'-end could promote the partial melting of the 5'-end making the AP site accessible. After its recruitment by Ku, LigD would form a complex with the DNA, most probably implying the interaction of Lys331 with one of the phosphates of the phosphodiester bond between the AP site and the next 3' nucleotide (b). The templating nucleotide opposite the AP site directs the binding of the complementary ribonucleotide, forming a Watson-Crick base pair at the polymerization site of the PolDom (c). Once the preternary-precatalytic complex is stabilized, the protein incises at the AP site, releasing the cleaved strand and giving rise to a new 5'-P end (d). PolDom mediates further synapsis between the 3' overhanging strands from opposing breaks catalyzing the in trans addition of the nucleotide to the 3'-end of the incoming primer (e). Finally, the LigDom ligates both ends (f).*

## Publications

Sánchez-Salvador, A. and de Vega, M. (2020) Structural Determinants Responsible for the Preferential Insertion of Ribonucleotides by Bacterial NHEJ PolDom. *Biomolecules* **10**(2):203.

Del Prado, A., Santos, E., Lázaro, J.M., Salas, M. and de Vega, M. (2019) The Loop of the TPR1 Subdomain of Phi29 DNA Polymerase Plays a Pivotal Role in Primer-Terminus Stabilization at the Polymerization Active Site. *Biomolecules* **24**;9(11).

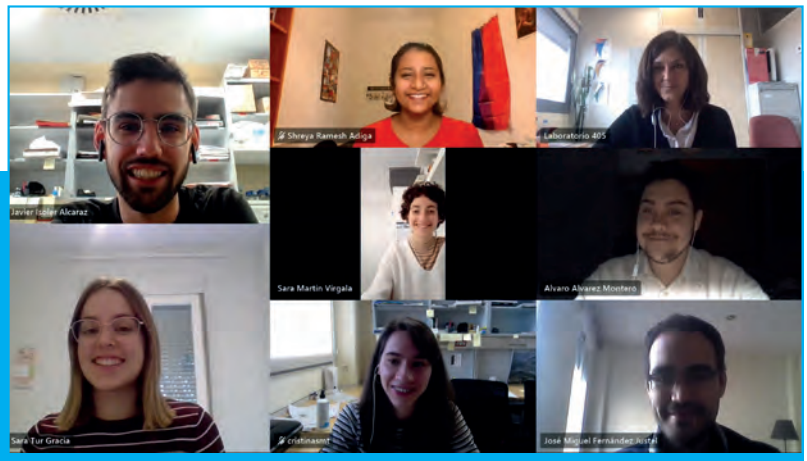
Rodríguez, G., Martín, M.T. and de Vega, M. (2019) An array of basic residues is essential for the nucleolytic activity of the PHP domain of bacterial/archaeal PolX DNA polymerases. *Sci Rep*. **9**(1):9947.

de Ory, A., Carabaña, C. and de Vega, M. (2019) Bacterial Ligase D preternary-precatalytic complex performs efficient abasic sites processing at double strand breaks during nonhomologous end joining. *Nucleic Acids Res*. **47**(10):5276-5292.

Del Prado, A., Rodríguez, I., Lázaro, J.M., Moreno-Morcillo, M., de Vega, M. and Salas, M. (2019) New insights into the coordination between the polymerization and 3'-5' exonuclease activities in ϕ29 DNA polymerase. *Sci Rep*. **9**(1):923.

## GENOME MAINTENANCE AND INSTABILITY

# FUNCTIONAL ORGANIZATION OF THE MAMMALIAN GENOME




***Principal Investigator:***
María Gómez

***Postdoctoral Fellow:***
José Miguel Fernández Justel

***Predoctoral Fellows:***
Cristina Santa María Tobías
Sara Martín Vírgala
Javier Isoler Alcaraz
(since August 2019)
(co-PI: César Cobaleda)

***Undergraduate and Master Students:***
Miriam Alvarado López (TFM)
Sofía Gutiérrez Rodríguez (TFG)
Álvaro Eugenio Álvarez Montero
TFG (from August 2020)
(co-PI: Emilio Lecona)

***Visiting Scientists:***
Martyna Dziakowicz
Erasmus Master student
(March-July 2020)
Shreya Ramesh
(from August 2020)

www.cbm.uam.es/mgomez

## Research summary

Copying and decoding the genomic information in a timely and accurate fashion is essential for life. Both tasks (DNA replication and transcription) are remarkably complex in scale and regulation, and the molecular machineries involved in them translocate on the same DNA template, often in opposite directions and at different rates. This impose cells to employ efficient mechanisms to coordinate both processes. Failure of these mechanisms can lead to catastrophic effects on genome stability and cell viability, and is the focus of much research in recent years. Very likely, dealing with this potential conflicts strongly influences many key parameters of cellular function, including genome organization, chromatin structure or mutagenesis rates. We aim to unravel the intricate relationship between the processes of replication and transcription and the impact of this crosstalk on genome homeostasis. To tackle this we employed a combination of genome-wide approaches, bioinformatics, high-resolution molecular biology and single molecule techniques.

Our expectation is that our research will help understanding a basic question in Biology: how the genome is functionally organized. Besides its relevance for fundamental biology, the knowledge generated might have important implications in disease scenarios. For example, in interpreting the mutational consequences of transcriptional changes, especially in cancer cells where upregulation of transcription is suspected to be a major contributor of oncogene-induced replication stress.

In these last two years we have focused in understanding the determinants of replication origin positioning and usage in mammalian cells using genome-wide mapping techniques, evolutionary comparisons across vertebrates, and three-dimensional chromatin network analyses. We´ve found that, while replication origins contain a short, highly conserved region at the core of the initiation site, the positional conservation is very small, indicative of rapid turnover during evolution. Origin activation efficiency, on the other hand, is strongly influenced by the three-dimensional organization of the genome, particularly by the number of contacts established between chromatin fragments containing replication origins. These results support the proposed architectural organization of origins in DNA replication factories and highlight the tight similarities between replication and transcription multimolecular assemblies in the 3D nucleus.

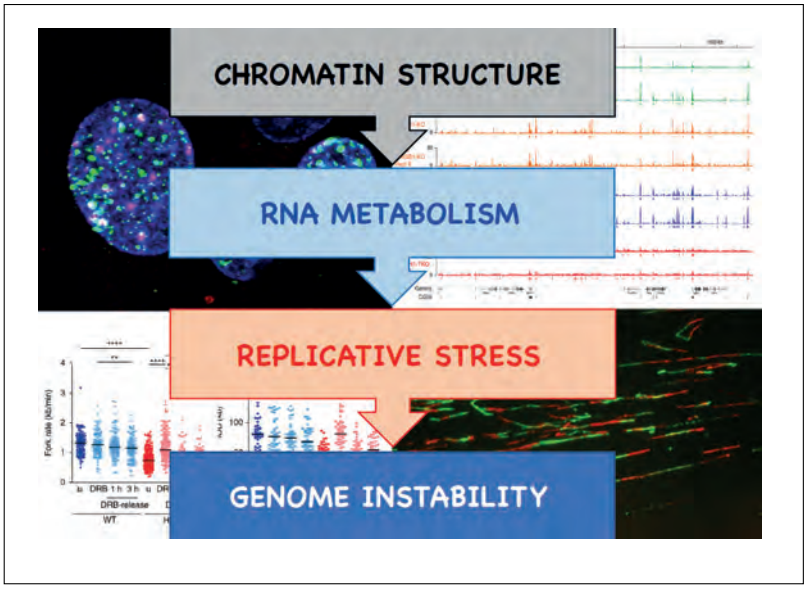



***Figure.*** *Replication-transcription crosstalk and genome homeostasis.*

## Publications

Massip, F., Laurent, M., Brossas, C., Fernández-Justel, JM., Gómez, M., Prioleau, MN., Duret, L. and Picard, F. (2019). Evolution of Replication Origins in Vertebrate Genomes: Rapid Turnover Despite Selective Constraints. *Nucl. Acids*. Res **47**: 5114-5125.

Jodkowska, K., Pancaldi, V., Almeida, R., Fernández-Justel, JM., Graña, O., Girau, M., Rubio, M., Rodríguez-Acebes, S., Carrillo, E., Pisano, D., Al-Sharour, F., Valencia, A., *Gómez, M. and *Méndez, J. *CO-CORRESPONDING AUTHORS (2019). Three-dimensional connectivity and chromatin environment mediate the stochastic activation of stress-responsive DNA replication origins. *BioRxiv*, 644971.

## International projects / Research networks

- CHROMOdyst (MINECO, 2018-2019).

# GENOME MAINTENANCE AND INSTABILITY

## CHROMATIN, CANCER AND THE UBIQUITIN SYSTEM


***Principal Investigator:***
Emilio Lecona

***Undergraduate and Master Students:***
Pilar Oroz Joven
Master Student
(November 2018-July 2019)
Guillermo de la Vega Barranco, Master Student
(from October 2020)
Victoria Menéndez García
Master Student
(September 2019-June 2020)
Antonio Pardo Melero
Undergraduate Student
(February 2019-July 2019)
Alejandro García López
Undergraduate Student
(September 2019-June 2020)
Alejandro Fernández Llorente
Undergraduate Student
(from September 2020)
Álvaro Eugenio Álvarez Montero
Undergraduate Student
(from August 2020)
(co-PI: María Gómez)

https://www.cbm.uam.es/elecona

## Research summary

DNA replication is a central process in cell biology that mediates the copy of cellular DNA to ensure the faithful transmission of genetic information through cell division. Our group is interested in unraveling the mechanisms of control of DNA replication by the protein modifiers ubiquitin and SUMO. We have described that active replication forks present a SUMO rich environment that is maintained by the action of the deubiquitinase USP7 and is essential for DNA replication (Lecona et al., NSMB 2016). We have proposed that the group modification of the replication machinery plays a role during DNA replication (Lecona and Fernández Capetillo, Bioessays 2016) and the inhibition of USP7 leads to the disassembly of the replisome and a premature activation of the mitotic program that results in cell death (Galarreta et al., *Embo J.* 2021). At the moment we are working to determine the functions of the group SUMOylation of the replisome and its interplay with the ubiquitination of replication factors.

One of the functions of SUMO and ubiquitin in chromatin is tagging proteins for their extraction by the AAA ATPase VCP through their recognition by specific partners. Our work has shown that VCP, in association with its adaptor FAF1, cooperates with the deubiquitinase USP7 to control the SUMO and ubiquitin equilibrium during DNA replication (Franz, Valledor et al, under revision). Following this work, we are now trying to understand how VCP affects the dynamics of the replication forks and to determine its targets in the replisome.

In addition, we are interested in the response to the challenges that hinder the advance of replication forks, known as replication stress. In the last years the replication stress response (RSR) has been put forward as a promising target in cancer. Initially the RSR constitutes a barrier for transformation but once cancer cells overcome this barrier their accelerated proliferation increases replication stress and makes them highly dependent on the RSR. We are analyzing the functions of the SUMO pathway and VCP in the RSR and its potential applications for cancer treatment. We are using breast cancer cell models with mutations in BRCA1, a central factor in homologous recombination, to explore the therapeutic potential of inhibitors of SUMOylation and VCP, since these tumors have been shown to be sensitive to the inhibition of the RSR.

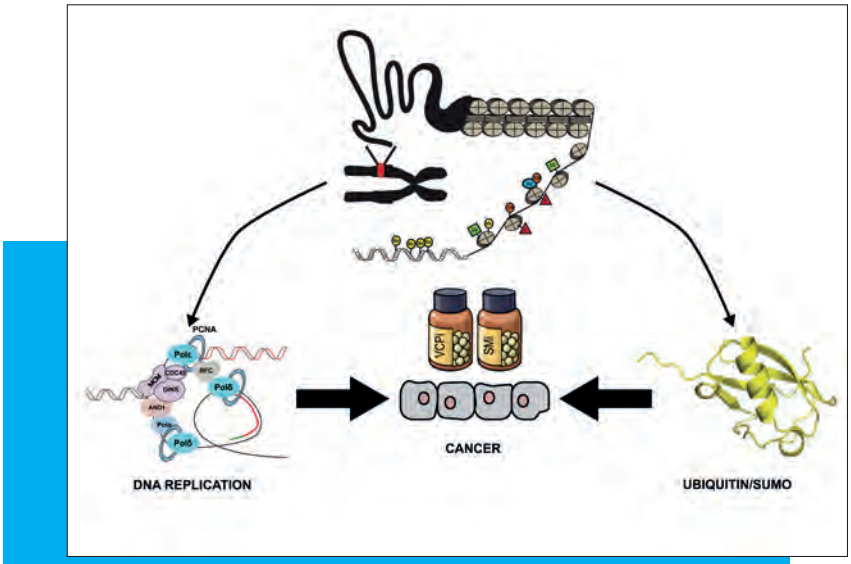



***Figure.*** *Post-translational modification of proteins in chromatin is essential for the control of DNA replication. Ubiquitin and SUMO are emerging as key modulators of this process and may represent an interesting target for cancer treatment.*

## International projects / Research networks

- *Ubiquitin like Proteins in Signaling, Proliferation and Cancer*.
Thematic Network, Spanish Ministry of Economy. Project SAF2017-90900-REDT. October 2018-September 2020.

# GENOME MAINTENANCE AND INSTABILITY

## REPLICATION OF BACTERIOPHAGE Ø29 DNA


***Principal Investigator:***
Margarita Salas
(deceased Nov. 2019)

***Postdoctoral Fellow:***
Alicia del Prado

***Predoctoral Fellows:***
Ana Lechuga Mateo
Carlos D. Ordóñez Cencerrado
Mario Rodríguez Mestre
(September-December 2020)

***Undergraduate and Master Student:***
Ane Muruzabal
(until June 2020)

***Visiting Scientist:***
Modesto Redrejo Rodríguez

http://www.cbm.uam.es/msalas

## Research summary

During these years we have been working in the study of the coordination between the polymerization and 3'-5' exonuclease activities of Φ29 DNA polymerase. We analyzed the biochemical role of the residues Tyr101 and Thr189 from the exonuclease domain that were proposed to stabilize the DNA binding. Their mutant derivatives showed a reduced 3'-5' exonuclease activity and were involved in binding the dsDNA. In addition, Tyr101 is playing a role in processivity and Thr189 seems to be an important determinant in the fidelity of the DNA polymerase. The mutants of the residue Q180 showed an enhanced 3'-5' exonuclease activity of the enzyme, not due to a polymerization defect but to a structural conformation prone to degrade the substrate.

On the other hand, the superimposition of the structures of the apo structure of the polymerase and the polymerase in the polymerase/terminal protein (TP) heterodimer shows that the structural changes are restricted almost to the TPR1 loop (residues 304–314). We disclosed the involvement of these residues in binding the DNA and the TP and our results allowed us to propose a role for the Φ29 DNA polymerase TPR1 loop in the proper positioning of the DNA and TP-priming 3'-OH termini at the preinsertion site of the polymerase to enable efficient initiation and further elongation steps during Φ29 TP-DNA replication.

We have also analyzed by site-directed mutagenesis the role of three tyrosines of the Φ29 single-stranded DNA binding protein (SSB) as ligands of ssDNA, confirming the involvement of the Tyr57 in binding the ssDNA and how the lack of this interaction was affecting the activities of the SSB derivative mutant Y57A that was unable to unwind the ssDNA or to stimulate the DNA elongation.

We have also recently made an effort in the characterization of Bam35 as a new DNA replication model. We have now generated an engineered Bam35 DNA polymerase with enhanced DNA amplification capacity as a proof-of-concept of isothermal amplification of damaged DNA.

Bam35 belong to an enigmatic group of temperate tectiviruses, recently recognized as *Betatectivirus* genus, preying members of *Bacillus cereus sensu latto* with great biotechnological and biomedical interest. As a part of ongoing efforts to disclose virus-host interactions, we have obtained a high-quality, fully resolved genome of the host strain *Bacillus thuringiensis* HER1410.

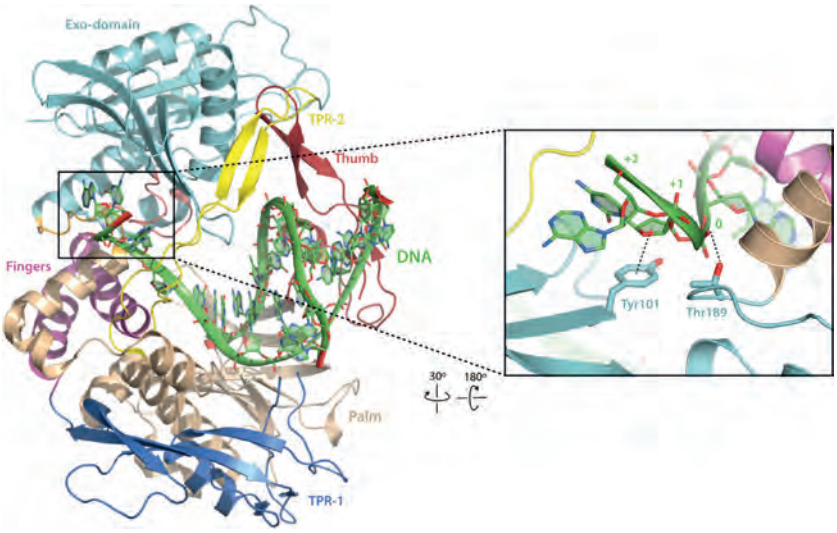



***Figure.*** *Crystallographic data corresponding to Φ29 DNA polymerase ternary complex are from Protein Data Bank (PDB) ID 2PYL. The exonuclease domain is in cyan, the thumb in red, the palm in light brown, the fingers in magenta and the subdomains TPR1 and TPR2 in dark blue and yellow, respectively. Primer terminus interacting residues Tyr101 and Thr189 residues are represented in sticks.represented in sticks.*

## Publications

del Prado A, Santos E, Lázaro JM, Salas M, de Vega M (2019). The Loop of the TPR1 Subdomain of Phi29 DNA Polymerase Plays a Pivotal Role in Primer-Terminus Stabilization at the Polymerization Active Site. *Biomolecules* **9**(11), 648.

de la Torre I, Quiñones V, Salas M, del Prado A (2019). Tyrosines involved in the activity of Φ29 single-stranded DNA binding protein. *PLoS ONE* **14**(5): e0217248.

del Prado A, Rodríguez I, Lázaro JM, Moreno-Morcillo M, de Vega M, Salas M (2019). New insights into the coordination between the polymerisation and 3´-5´exonuclease activities in Φ29 DNA polymerase. *Scientific Reports*. **9**(1):923.

Lechuga A, Lood C, Salas M, van Noort V, Lavigne R, Redrejo-Rodríguez M (2020). Completed Genomic Sequence of *Bacillus thuringiensis* HER1410 Reveals a Cry-Containing Chromosome, Two Megaplasmids, and an Integrative Plasmidial Prophage. G3: Genes, *Genomics and Genetics*. **10** (9) 2927-2939.

Flament-Simon SC, de Toro M, Chuprikova L, Blanco M, Moreno-González J, Salas M, Blanco J, Redrejo-Rodríguez M (2020). High diversity and variability of pipolins among a wide range of pathogenic *Escherichia* coli strains. *Scientific Reports*. **10**(1):12452.

Ordóñez CD, Lechuga A, Salas M, Redrejo-Rodríguez M (2020). Engineered viral DNA polymerase with enhanced DNA amplification capacity: a proof-of-concept of isothermal amplification of damaged DNA. *Scientific Reports*. **10**(1):15046.

## Awards and recognition

- Honoris Causa doctorate to Margarita Salas from the Universidad de Burgos (2019).

- Honoris Causa doctorate to Margarita Salas from the Universidad Pontificia de Salamanca (2019).

- The city of Oviedo named a public square after Margarita Salas by suggestion of the Consejería de Sanidad del Principado de Asturias (2019).

- Denomination “Plaza de Margarita Salas” to a common space in the High School “Antonio Machado”. Alcalá de Henares, Madrid (2019).

- “Espacio experimental y sensorial. Margarita Salas”. Public School Tremañes. Gijón (2019).

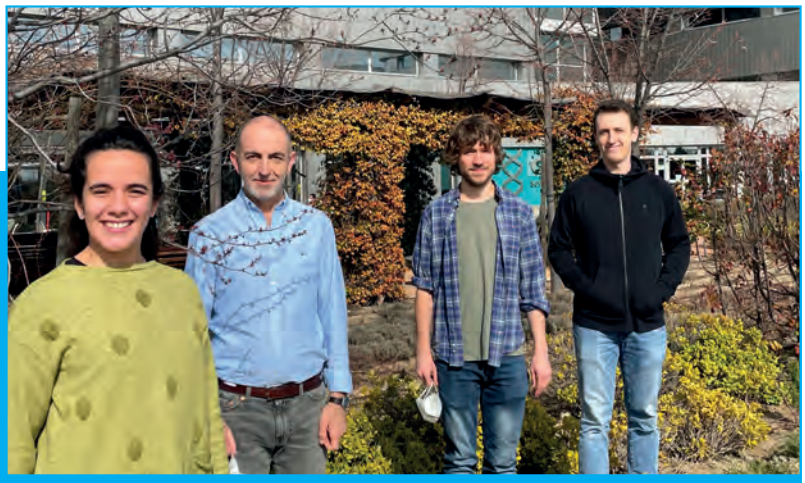
- European Inventor Award 2019 to Margarita Salas, Lifetime achievement and Popular Prize categories (European Patent Office).

## Doctoral Theses

**Ana Lechuga Mateo** (2020). Disclosing Bacillus virus Bam35 and its host. Identification and characterization of the viral SSB, host genomic characterization and phage-bacteria interactome. Universidad Autónoma de Madrid. Supervisors: Margarita Salas Falgueras y Modesto Redrejo Rodríguez. Mención Internacional.

## GENOME MAINTENANCE AND INSTABILITY

# CHROMOSOME REPLICATION AND GENOME STABILITY



***Principal Investigator:***
José Antonio Tercero

***Predoctoral Fellows:***
Paula González Fernández
Carl P. Lehmann

***Undergraduate and Master Students:***
Alejandro Aznar Casado

http://www.cbm.uam.es/jatercero

## Research summary

Genome integrity is at risk during chromosome replication, as the DNA is unpacked and unwound, which leaves it highly exposed to multiple types of insults. The maintenance of genome stability is fundamental for the correct transmission of genetic information in every cell cycle and to prevent pathological conditions. In fact, genomic instability is a hallmark of diseases such as cancer, as well as a cause of premature ageing and developmental disorders. We study how genome integrity is preserved during replication, especially under DNA damage conditions. The main mechanisms involved in genome maintenance are evolutionarily conserved, which allows us to analyse them at the molecular level using the genetically tractable yeast *Saccharomyces cerevisiae*, and then to extrapolate results from this eukaryotic model to more complex organisms. We recently investigated the relevance for genome integrity of the Mgs1 AAA+ ATPase (human WRNIP1), a protein related to DNA damage tolerance whose function was poorly understood. We also contributed to study the regulation of the structure-specific endonuclease Mus81-Mms4/Eme1.

DNA damage tolerance (DDT) is crucial for genome stability and is mainly carried out by template-switch recombination, an error-free mode of bypassing DNA lesions, or translesion DNA synthesis, which is error-prone. We investigated the role of Mgs1/WRNIP1 in modulating DDT and found that elimination of Mgs1 in cells lacking Rad5, an essential DDT protein, activates an alternative mode of DNA damage bypass, driven by recombination, which allows chromosome replication and cell viability under stress conditions that block replication forks. A combination of genetic and molecular approaches revealed that Mgs1 is required to prevent a potentially detrimental recombination salvage pathway at damaged replication forks. This in turn helps channel DNA damage bypass to template switching at the sites of perturbed DNA replication, thus significantly contributing to genome integrity maintenance.

The Mus81-Mms4/Eme1 endonuclease is important for genome stability as it allows the resolution of persistent replication-associated recombination structures. We previously showed that this complex is tightly regulated during the cell cycle, being activated by Mms4-phosphorylation only in G2/M. However, the fate of the phosphorylated-Mms4 was unknown. We contributed to uncover that Mms4 is an Esc2-STUbL-Cullin8 mitotic substrate that is modified by SUMOylation and ubiquitylation and targeted for proteasome degradation, a process linked to the cell cycle-dependent Mms4-phosphorylation. This degradation helps to prevent a phosphorylated active form of the endonuclease after mitosis and, consequently, the negative effects of Mus81-Mms4 activity on genome integrity in G1 and during chromosome replication.

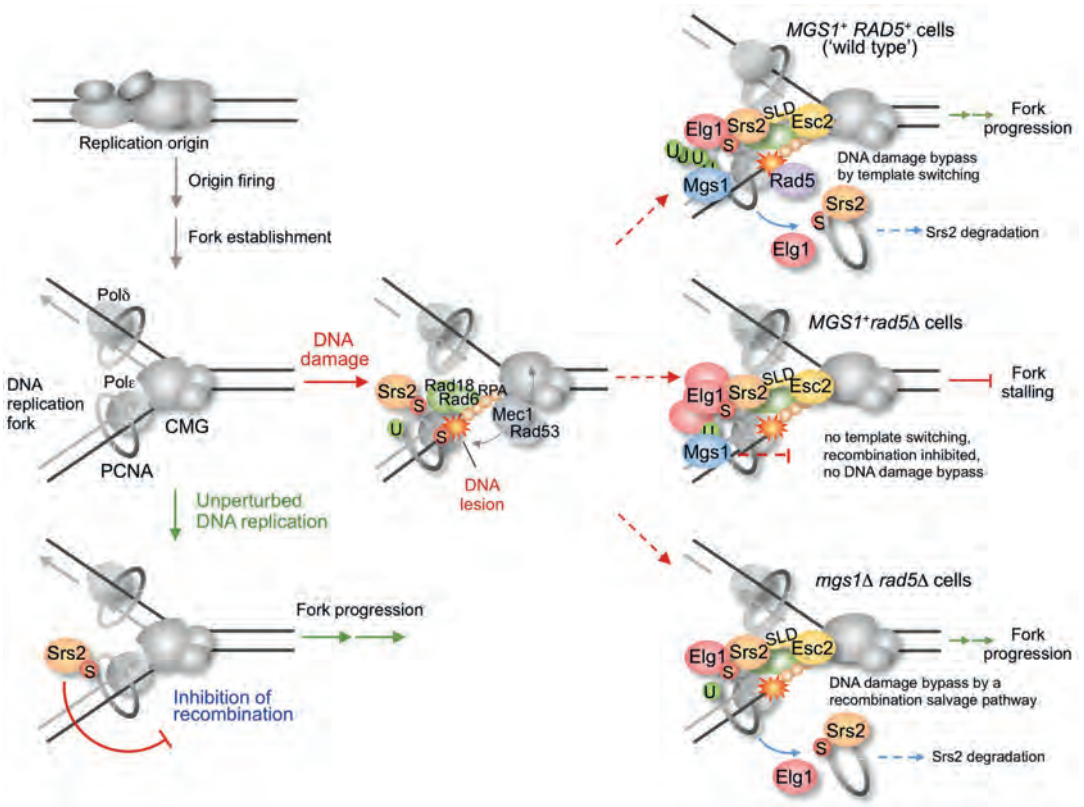



***Figure.*** *Model of DNA recombination control at replication forks, during unperturbed chromosome replication and in the presence of DNA damage (for details, see Lehmann et al. 2020).*

## Publications

Jiménez-Martín, A., Saugar, I., Joseph, C. R., Mayer, A., Lehmann, C. P., Szakal, B., Branzei, D., Tercero, J. A. (2020) The Mgs1/WRNIP1 ATPase is required to prevent a recombination salvage pathway at damaged replication forks. *Sci. Adv*. **6**: eaaz3327.

Waizenegger, A., Urulangodi, M., Lehmann, C. P., Clarisse-Reyes, T. A., Saugar, I., Tercero, J. A., Szakal, B., Branzei, D. (2020) Mus81-Mms4 endonuclease is an Esc2-STUbL-Cullin8 mitotic substrate impacting on genome integrity. *Nat. Commun*. **11**: 5746.

Lehmann, C. P., Jiménez-Martín, A., Branzei, D., Tercero, J. A. (2020) Prevention of unwanted recombination at damaged replication forks. *Curr. Genet*. **4**: 1045-1051.

## Doctoral Theses

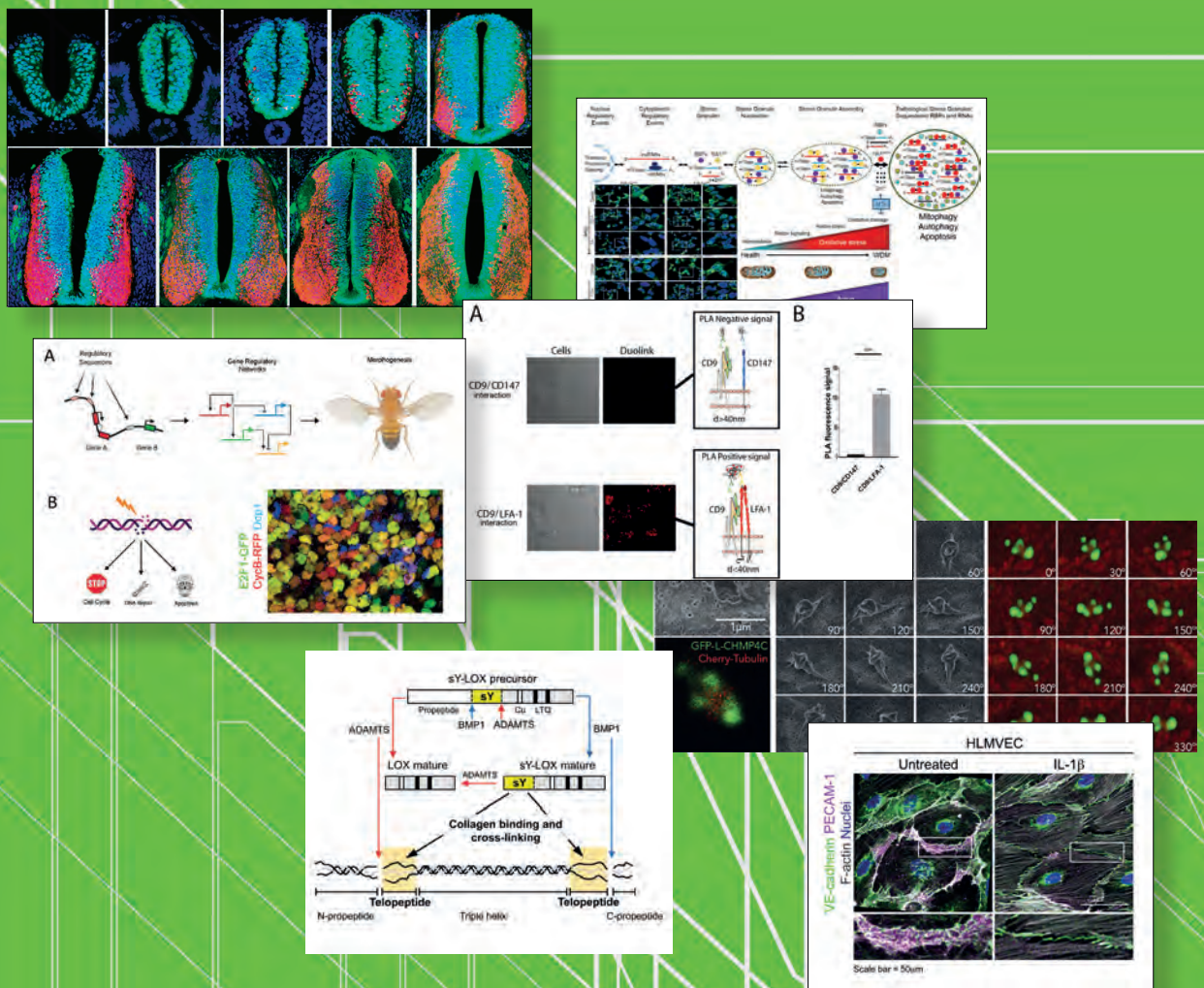
**Alberto Jiménez Martín** (2019). La AAA+ ATPasa Mgs1/WRNIP1 y su relación con la tolerancia al daño en el DNA durante la replicación cromosómica. Universidad Autónoma de Madrid. Supervisor: José Antonio Tercero.

## International projects / Research networks

- Genomic Instability (MICIU, RED2018-102372-T).

# Tissue and Organ Homeostasis

PROGRAM



# *UNITS*

## CELL ARCHITECTURE & ORGANOGENESIS



## CELL-CELL COMMUNICATION & INFLAMMATION



## SPECIFICATION, REPROGRAMMING & REGENERATION

UNIT

FERNANDO MARTÍN BELMONTE

# Cell Architecture & Organogenesis

TISSUE AND ORGAN HOMEOSTASIS

The Cell Architecture and Organogenesis (*Cellarch*) unit is part of the Tissue and Organ Homeostasis Program. It gathers together ten labs (14 PIs) interested in understanding how cells control their shape and function to organize themselves in complex tissues and organs in a cooperative manner. We are interested in addressing how this organization is achieved, maintained, and adapted during development, homeostasis, and human disease through the precise temporal and spatial orchestration of gene expression and protein function. Multiple cellular processes, from transcription and translation to membrane transport and signal transduction, must be coordinated through multilayered circuits in a cell-autonomous manner. Besides, the interaction between different cell types further coordinates these processes to ensure proper tissue structure and function, for example, by regulating asymmetric cell divisions, cellular communication, self-renewal of progenitor cells, or expansion of specific cell types. Systems-biology studies suggest that these processes are interconnected through a complex network of positive and negative feedback loops, and understanding how cells integrate all these processes requires a coordinated multidisciplinary approach. In the *Cellarch* unit, several groups with expertise in these areas and genome-wide studies will use model organisms such as flies, worms, zebrafish, and mice to study how cells integrate shape control gene expression and protein function in specific niches. These studies will address three significant aspects of these coordinated processes:

a) Obtain advanced knowledge of the cellular differentiation processes through the functional characterization of the protein machinery involved in cell polarization, signaling, and cell communication.

b) Study the regulation of gene expression during embryonic development and homeostasis, and analyze how regulatory and genome structure variations can contribute to human disease.

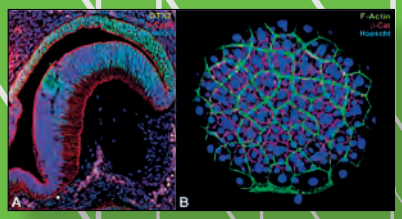
c) Integrate all this information within a specific tissue during normal physiology and pathological conditions such as inflammation using *in vivo* models.

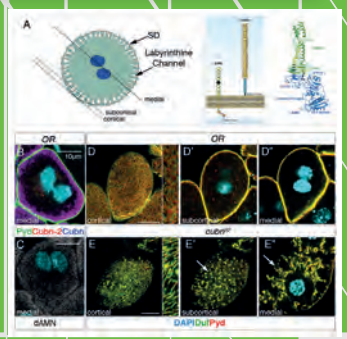
Given the number of molecular pathways involved, the Cellarch unit will specifically investigate how each of these cellular pathways modulates or interferes with the others, thus complementing the areas investigated explicitly in each of the various labs. Thus, the *Cellarch* research teams will coordinate their efforts to build realistic models that will help us to gain a deeper understanding of normal physiology and human disease.

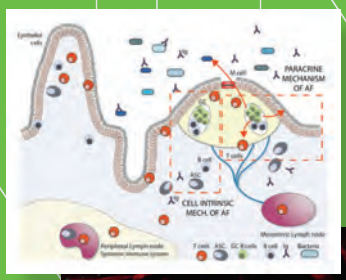
Highlights 2019-2020

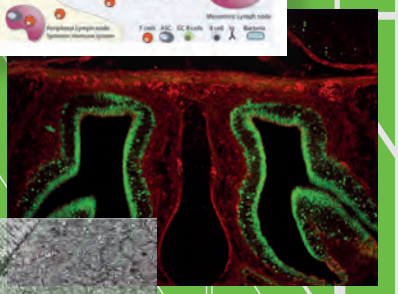
Sporadic AD patients present elevated levels of a glial-derived secreted protein (SFRP1), promoting the pathogenic cleavage of the Amyloid Precursor Protein. Neutralization of the protein activity delays disease progression in mouse models and prevents cognitive decline, pointing to a novel potential therapeutic strategy for the disease (Esteve, Bovolenta 2019 Nat Neurosci 22:1258-1268). In addition, FGF2 has a triple effect by simultaneously increasing the growth fraction, promoting symmetric divisions, and shortening the length of the cell cycle in cultures of radial glia cells (Ledesma-Terrón, Míguez 2020 Development 147(14):dev189712).

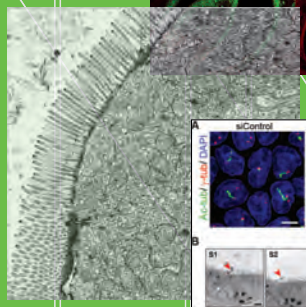
Our unit has also unveiled the mechanisms linking epithelial architecture to cancer onset, bridging developmental/cell biology with pathology (Bernascone, Martin-Belmonte 2019 Nat Comm 10: 2481). Also, analyses in *drosophila* and zebrafish suggest a conserved role for junctional protein Tjp1a/ZO-1 in promoting remodeling in podocytes that are essential for blood filtration in vertebrates and highly associated with chronic kidney diseases (Carrasco-Rando, Ruiz-Gómez, (2019) J Cell Biol 218(7):2294.

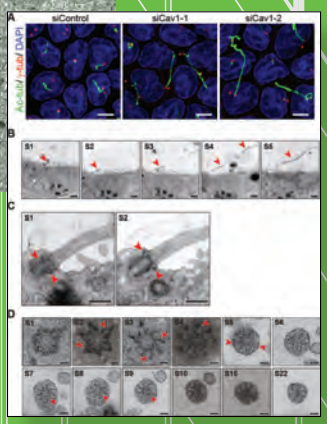







**MIGUEL ÁNGEL ALONSO**
CELL POLARITY

**PAOLA BOVOLENTA**
MORPHOGENESIS AND DIFFERENTIATION OF THE VERTEBRATE CNS

**ISABEL CORREAS**
CYTOSKELETON-PLASMA MEMBRANE INTERACTIONS

**JOSÉ FÉLIX DE CELIS**
CELL SIGNALING DURING IMAGINAL DEVELOPMENT IN *DROSOPHILA*

**CARLOS ESTELLA**
GENE EXPRESSION CONTROL, PATTERNING AND GROWTH DURING APPENDAGE DEVELOPMENT

**FERNANDO MARTÍN BELMONTE**
INTESTINAL MORPHOGENESIS AND HOMEOSTASIS

**NURIA MARTÍNEZ MARTÍN**
INTEGRATED METABOLISM IN IMMUNITY

**DAVIZ MÍGUEZ**
BIOPHYSICS AND SYSTEMS BIOLOGY

**JAIME MILLÁN**
CELL BIOLOGY OF INFLAMMATION

**MAR RUIZ GÓMEZ / JOAQUIM CULÍ**
GENETIC AND FUNCTIONAL ANALYSIS OF THE RENAL FILTRATION DIAPHRAGM IN HEALTH AND DISEASE

**ESTHER SERRANO SÁIZ**
TRANSCRIPTIONAL CONTROL OF SEXUAL DIFFERENTIATION OF THE NERVOUS SYSTEM

# CELL ARCHITECTURE & ORGANOGENESIS

## CELL POLARITY

***Principal Investigator:***
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Javier Casares Arias
Armando Rubio Ramos
Leticia Labat de Hoz

***Technician:***
Laura Fernández Martín

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## Research summary

Our aims during this period have been: 1) to study the mechanism of biogenesis of the primary cilium in polarized epithelial cells, 2) to investigate the role of the formin INF2 in disease, and 3) to characterize a novel member of the MAL family of proteins.

The primary cilium is a membrane protrusion of the cell surface that is present as a single copy in most mammalian cells, and that consists of a central microtubular scaffold surrounded by the ciliary membrane. The membrane harbors a large variety of receptors that are important for the signaling pathways involved in cell growth, migration, development and differentiation. Previous work in our laboratory established the midbody remnant's critical role in primary cilium formation in polarized epithelial cells. During this period, we have investigated the regulation of the inheritance of the midbody remnant and the mechanism by which it facilitates the formation of the cilium. We observed that the majority of midbody remnants are physically connected to the plasma membrane through a membranous stalk derived from the uncleaved arm of the cytokinetic bridge, and that the ESCRT-III subunit CHMP4C controls the integrity of this arm, ensuring continuity between the remnant membrane and the plasma membrane. We also found that a specialized patch made of condensed membranes accompanies the remnant on its journey to meet the centrosome at the middle of the apical surface. Thanks to the physical continuity between the two structures, part of the patch is delivered to the plasma membrane zone above the centrosome and is used to build the ciliary membrane. In this way, we have determined the origin of the ciliary membrane, the contribution of the midbody remnant, and the mechanism of primary ciliogenesis in polarized epithelial cells.

Formins are a widely occurring family of proteins involved in the formation of linear actin polymers. Mutations in the formin INF2 are responsible for two human hereditary diseases that affect the kidney (focal segmental glomerulosclerosis, FSGS) and the peripheral nerves (Charcot-Marie-Tooth disease, CMT) by producing podocytes and Schwann cell degeneration, respectively. We are currently investigating how the pathogenic mutations affect the activity of INF2 and the mechanism by which they damage cells and produce disease.

A third ongoing project deals with the functional characterization of human MALL, a member of the MAL family of proteins, whose pattern of expression is restricted to specific cell types and that, unlike all the other members we have previously characterized, reside within the nucleus of mammalian cells.

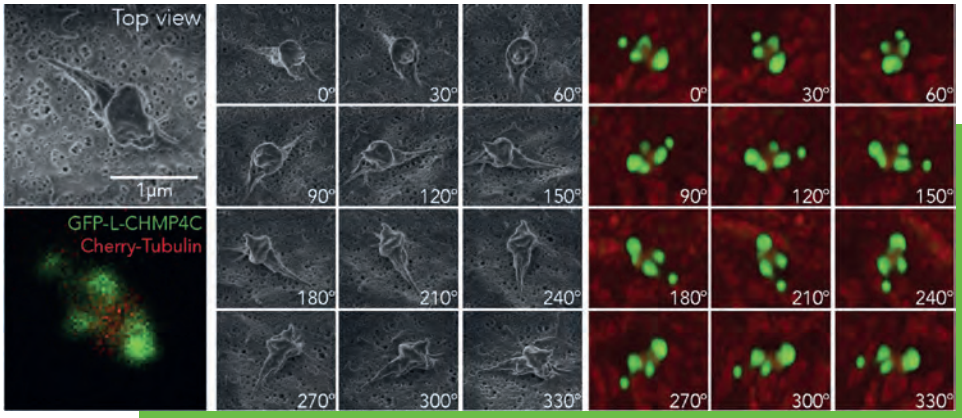


***Figure.CHMP4C is present at the membranous connection between the midbody remnant and the plasma membrane.*** *Large left panels: top-view images of connected remnants acquired by SEM (top) and confocal microscopy (bottom). Small right panels: side-view scanning electron microscopy images (left) and matching confocal microscopy images obtained by 3D reconstruction (right).*

## Publications

Rangel, L., Bernabé-Rubio, Fernández-Barrera, J, Casares-Arias, J., Millan, J., Alonso, M.A., and Correas, I. (2019) Caveolin-1α regulates primary cilium length by controlling RhoA GTPase activity. *Sci. Rep*. **9**, 1116.

Labat-de-Hoz, L., and Alonso, M. A. (2020) The formin INF2 in disease: progress from 10 years of research. *Cell. Mol. Life Sci*. **77**, 4581-4600.

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Bernabé-Rubio, M., Bosch-Fortea, M., García, E., Bernardino de la Serna, J., and Alonso, M.A. (2020) Adaptive lipid immiscibility and membrane remodeling are active functional determinants of primary ciliogenesis. *Small Methods* (in press).

## Awards and recognition

- Member of the Compartment Organization And Transport (COAT) Excellence Network.

- Member of the Scientific Review Panel of Euro-BioImaging.

## Doctoral Theses

**Javier Casares Arias** (2020). “Caracterización del remanente del cuerpo medio y la regulación de su herencia”. Univ. Autónoma de Madrid. Director: Miguel A. Alonso. Sobresaliente *cum laude* and International Mention.

## CELL ARCHITECTURE & ORGANOGENESIS

# MORPHOGENESIS AND DIFFERENTIATION OF THE VERTEBRATE CNS


***Principal Investigator:***
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***Scientific Staff:***
Pilar Esteve Pastor

***Postdoctoral Fellows:***
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Polynikis Kaimakis
Viviana Gallardo
(up to June 2019)
Javier Rueda Carrasco
(up to July 2019)

***Predoctoral Fellows:***
Carlos Camacho de la Macorra
Guadalupe Pereyra Gómez
Pablo Miaja Hernández
(November 2020)
Marcos Martínez Baños
(December 2020)
Lorena Buono
(up to March 2020)
Tania Moreno Mármol
(up to July 2019)
Inés Mateo Ruiz
(up to July 2019)

***Technicians:***
Mª Jesús Martín Bermejo
Noemí Tabanera Anguita

***Undergraduate and Master Students:***
Xabier Perosanz
(February 2019-June 2019)
Pablo Azón
(February 2019-June 2019)
Javier Cruz
(November 2019-June 2020)
Elio Escamilla
(December 2019-June 2020)
Débora Lloret
(October 2019- February 2020)
Kateryna Matveyeva
(December 2019- June 2020)
Marcos Sintes
(up to August 2019)

***Visiting Scientist:***
Sara Ester López
(September 2017-December 2020)

http://www.cbm.uam.es/pbovolenta

## Research summary

Comprehending how the brain forms and what sicken it are necessary tools for knowing how to preserve normal brain functions. Our research tackles this idea by addressing basic mechanisms of brain morphogenesis and searching for the molecular causes of its degeneration. In this frame, we have shown that the retinal pigment epithelium (RPE) actively contributes to vertebrate eye morphogenesis with a species-specific mechanism. In zebrafish, RPE cells stretch and flatten actively forcing the acquisition of the eye cup-shape, in virtual absence of cell proliferation. In amniotes instead (Fig. 1A), proliferation drives RPE expansion with a much-reduced need of cell flattening. Thus, extreme RPE flattening and accelerated differentiation are efficient solutions adopted by fast-developing species to enable timely optic cup formation. Collaborative transcriptomic studies support this concept showing that in just few hours zebrafish RPE cells acquire the molecular machinery required for their conversion from a neuroepithelial to a squamous epithelium, which further endows the stiffness and mechanosensing capability to promote eye morphogenesis. We also showed that similar mechanosensing mechanisms operate during the earliest morphogenetic rearrangements in zebrafish embryo (Fig. 1B).

We also participated in studies showing that the transcription factor Sox2 is need for the establishment of thalamic visual connections and mediates a genome-wide network of long-range interactions connecting gene promoters and SOX2-bound distal enhancers, regulating genes involved in neurodevelopmental disorders. We further contributed to show that Nr2f1 haploinsufficient mice are an excellent model for the poorly characterized Bosch-Boonstra-Schaaf Optic Atrophy syndrome that causes visual and mental disabilities.

Related to neurodegeneration, we pursued our observation that the secreted protein SFRP1 acts as a negative regulator of ADAM10, a metalloprotease involved in APP processing, synaptic plasticity and inflammation, all of which are key pathological features of Alzheimer's disease (AD). We have shown that SFRP1 levels are elevated in the brain of AD patients with degrees that correlate with disease severity, increased amyloidogenic APP processing, chronic inflammation and synaptic loss. Notably, antibody mediated neutralization of SFRP1 activity in an AD-like mouse model is sufficient to significantly reduce AD pathological traits and prevent the decrease in the LTP response that is observed in untreated AD-like mice. Besides, in mouse models of acute and chronic neuroinflammation, astrocyte-derived SFRP1 promotes and sustains microglial activation via the upregulation of components of HIF-dependent inflammatory pathway. These findings suggest that SFRPI might have a pleiotropic function in the brain that could be targeted as a novel therapeutic approach for AD.

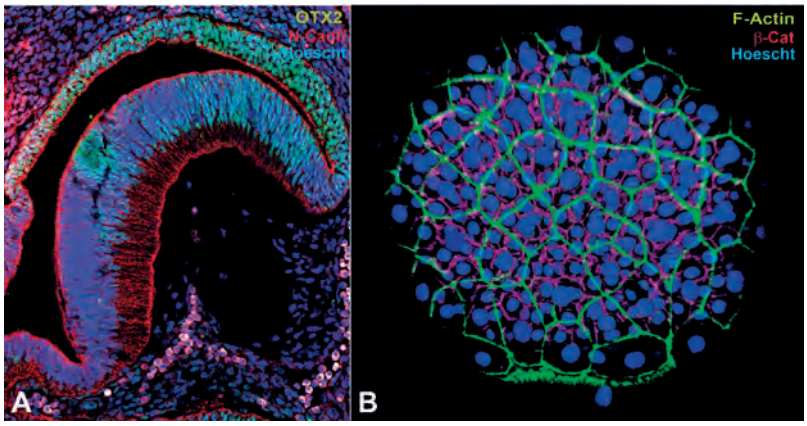


***Figure.*** *A) Section of a human embryonic optic cup with a thick RPE stained in green. B) Gastrulating zebrafish embryo showing nuclear (blue), cytoskeletal (green) and plasma-membrane (magenta) epithelial organization.*

## Publications


Esteve P, Rueda-Carrasco J, Inés Mateo M, Martin-Bermejo MJ, Draffin J, Pereyra G, Sandonís Á, Crespo I, Moreno I, Aso E, Garcia-Esparcia P, Gomez-Tortosa E, Rábano A, Fortea J, Alcolea D, Lleo A, Heneka MT, Valpuesta JM, Esteban JA, Ferrer I, Domínguez M, Bovolenta P. (2019) Elevated levels of Secreted-Frizzled-Related-Protein 1 contribute to Alzheimer's disease pathogenesis. *Nat. Neurosci*. **22**(8):1258-1268.

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Varilh M, Acquatella-TranVan Ba I, Silhol M, Nieto-Lopez F, Moussaed M, Lebart MC, Bovolenta P., Verdier JM, Rossel M, Marcilhac A, and Trousse F. (2020) REG-1α promotes differentiation of cortical progenitors via its N-terminal active domain. *Front Cell Dev Biol* **8**:681.

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Bovolenta P., Manzanares M, Buceta J (2020). Origins, (Co)Evolution and Diversity of Life. CSIC Scientific Challenges: towards 2030. Topic coordinators. Vol 2.


## Awards and recognition

- Paola Bovolenta is member of the ERC Scientific Council, Chair of the Open Access Working Group of the ERC; Member of the Scientific Advisory Board ERA-NET NEURON; Senior Editor of the Eur. J. Neurosci; President Elected SENC, among other duties.

- Coorganized the International Symposium Fundación Ramón Areces: "Understanding and reprogramming developmental visual disorders: from anophthalmia to cortical impairments. January 30-31, 2020. Madrid.

- Several members our group participate in outreach activities, i.e. Semana de la Ciencia 2019, 4ºESO+Empresa.

## Doctoral Theses

**Tania Moreno Mármol.** (2019). Morphogenesis of the zebrafish retinal pigment epithelium and its involvement in optic cup formation. Universidad Autónoma de Madrid. Supervisors: Paola Bovolenta and Florencia Cavodeassi.

**Inés Mateos Ruiz.** (2019) Niveles elevados de SFRP1 en un modelo transgénico de raton desencadenan neuroinflamacion y pérdida de memoria. Facultad de Ciencias, Universidad Autónoma de Madrid. Supervisors: Paola Bovolenta and Pilar Esteve.

## International projects / Research networks

- RedDevNeural 2.0 2018-2019, RedDevNeural 3.0 2020-2021.

- Sfrp1 as a therapeutic target and diagnostic/prognostic factor in Alzheimer's disease. Cure Alzheimer's Fund. 2020-2022.

- Our group belongs to the Centro de Investigación Biomédica en Red de enfermedades Raras CIBERER).

## CELL ARCHITECTURE & ORGANOGENESIS

# CYTOSKELETON-PLASMA MEMBRANE INTERACTIONS

***Principal Investigator:***
Isabel Correas Hornero

***Postdoctoral Fellow:***
Laura Rangel Sánchez

***Technician:***
Susana Aguilar García

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## Research summary

Cell morphology and tissue organization are maintained through multiple interactions between lipids and proteins from the plasma membrane and the underlying cytoskeleton. The coordination between surface membranes and different cytoskeletal networks is essential for processes such as cell migration, polarity, epithelial and endothelial barrier stability, cell division or intracellular vesicular transport.

Our group is interested in investigating the role of proteins that regulate plasma membrane and/or cytoskeletal organization in different cellular events. We have focused our attention on the 4.1 family of proteins - 4.1R, 4.1B, 4.N y 4.1G - which connect subcortical cytoskeleton to lipids and membranes through FERM (four-point-one, ezrin, radixin, moesin) domains. We are also interested in deciphering the mechanisms involved in the generation of protein 4.1 diversity. We have determined that three groups of 4.1R proteins, containing a common C-terminal domain but differing in their N-terminal domain, are generated by initiation of translation in three different ATGs due to alternative splicing events. We have also showed that a new type of proteins 4.1 lacking the C-terminal region of prototypical proteins 4.1R are generated by alternative polyadenylation. Therefore, our studies have revealed the great complexity of working on proteins 4.1 due to the existence of a large number of 4.1 isoforms generated by different mechanisms.

We have recently initiated a new study focused on understanding the mechanisms and proteins involved in the formation of the primay cilium, a non-motile projection that is present as a single copy in the plasma membrane of most mammalian cells. We have observed an increase in the length of the primary cilium of the cells lacking the protein caveolin 1 (Cav1). Our work revealed that Cav1 regulates ciliary length by modulating the activity of RhoA, which, in turn, controls the apical actin cytoskeleton through its effectors ROCK and DIA1. This signaling cascade allows the arrival of an excess of ciliary precursors to the pericentrosomal region for its incorporation into the ciliary structure. In a collaborative effort, we have performed studies on the biogenesis of primary cilium and the inheritance of the midbody remnant and on the regulation of VE-cadherin expression by ETS1 and the effects on endothelial barrier function in human endothelial cells.

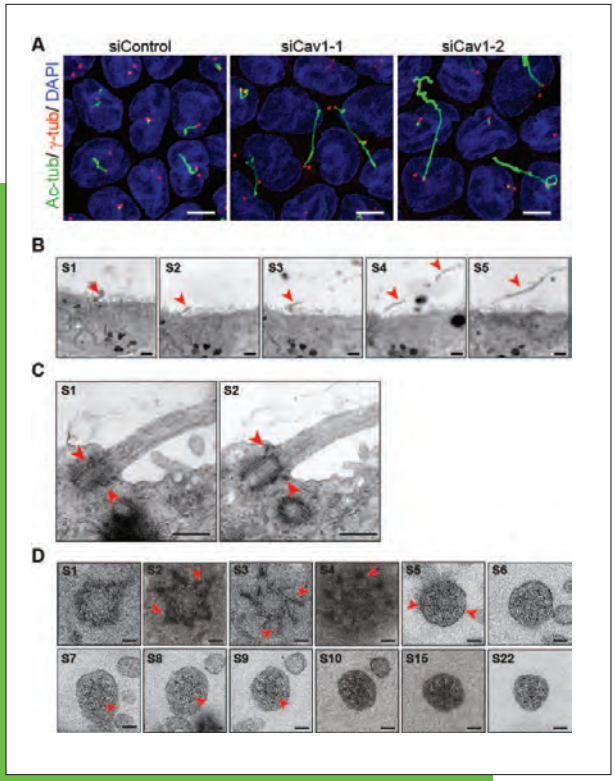


***Figure. Caveolin1 knockdown induces cilium lengthening.*** ***(A)*** *MDCK cells were transfected with control siRNA or siRNAs targeting Cav1 and stained for acetylated tubulin, gamma-tubulin, and nuclei (DAPI).* ***(B-D)*** *Ultrastructural analysis of a long primary cilium of caveolin1-α KO cells.* ***(B)*** *Representative TEM images of a longitudinally sectioned primary cilium (arrowhead) of Cav1-α KO cells.* ***(C)*** *Enlargements of representative sequential sections (S1 and S2) of a primary cilium of Cav1α KO cells. Arrowheads in S1 point to the basal feet, and in S2 indicate the transition fibers.* ***(D)*** *Representative TEM images of cross-sections of a primary cilium of Cav1α KO cells. Arrowheads indicate the basal feet in S2, the transition fibers in S3 and S4, Y-links in S5, and the microtubule doublet that moves to the center of the axoneme (S7 to S9).*

Rangel, L., Bernabé-Rubio, Fernández-Barrera, J, Casares-Arias, J., Millan, J., Alonso, M.A., Correas, I. (2019). Caveolin-1α regulates primary cilium length by controling RhoA GTPase activity. *Sci. Rep*. **9**, 1116.

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Colás-Algora N, García-Weber D, Cacho-Navas C, Barroso S, Caballero A, Ribas C, Correas I, Millán J. (2020). Compensatory increase of VE-cadherin expression through ETS1 regulates endothelial barrier function in response to TNFα. *Cell Mol Life Sci*. **77**:2125-2140.

## Awards and recognition

- Member of the TOMOXLIVER-CM Consortium of the Comunidad de Madrid (2018-2021).

## CELL ARCHITECTURE & ORGANOGENESIS

# CELL SIGNALING DURING IMAGINAL DEVELOPMENT IN *DROSOPHILA*

***Principal Investigator:***
Jose F. de Celis

***Scientific Staff:***
Ana Ruiz Gómez
Diego Pulido Vega

***Predoctoral Fellows:***
Cristina Martínez Ostalé
Patricia Vega Cuesta

***Technician:***
Ana López Varea

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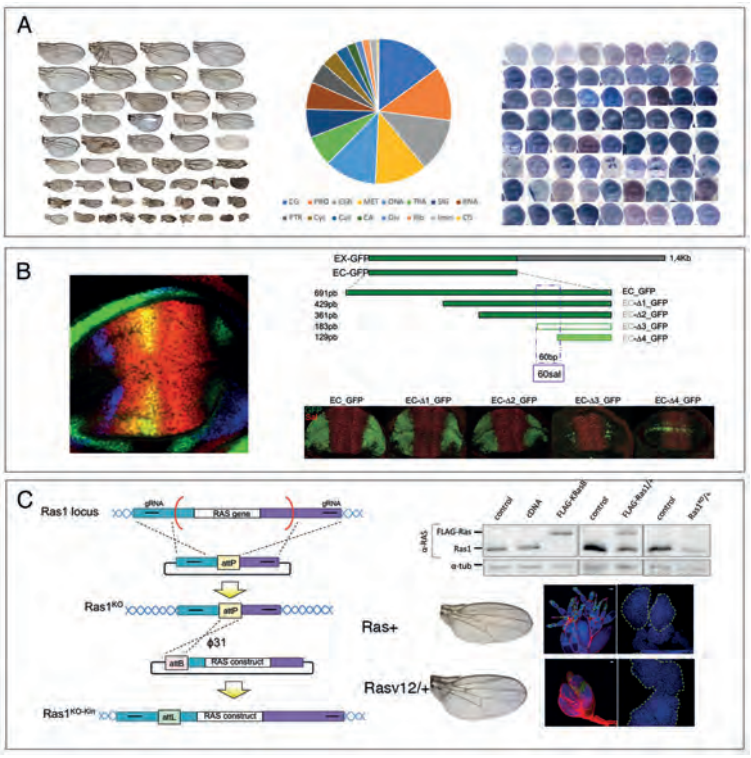
## Research summary

The *Drosophila* wing originates from an epithelial tissue that grow by proliferation during larval development and differentiate in the pupal stage into a wing and part of the thorax of the fly. Cell proliferation and differentiation are common processes in multicellular tissues, and they are directed by evolutionary conserved batteries of genes. The genetic and developmental analysis of the disc allow us to address experimentally different aspects of epithelial biology.

We are carrying out three research projects. The first project involves the analysis of the requirements of *Drosophila* genes in the wing. We grouped the 14.000 *Drosophila* genes into 16 functional groups (Fig. 1A) and screened UAS-RNAi lines targeting 10918 of these genes. We classified the resulting phenotypes into morphological classes affecting the size, pattern or differentiation of the wing (Fig. 1A), and correlate each mutant phenotype with the expression of the corresponding gene. Wing phenotypes reveal functional requirements, either in basic cellular functions impinging on cell viability or in wing-specific functions related to its growth and patterning, and together with gene expression patterns (Fig. 1A) constitute an optimal entry point to undertake detailed functional analysis. The second project is the analysis of the transcriptional effects of one *Drosophila* transcription factor (Spalt) that has a prominent role in the development of the wing disc (Fig. 1B). Spalt is a nuclear protein containing three pairs of Zn fingers and its human orthologs are involved in Towles-Brokes disease and Okihiro syndrome. We have identified a minimal DNA response element for Spalt through the analysis of the regulatory region of one of its downstream genes (Fig. 1B). Now we are defining the effects of Spalt on chromatin conformation as well as searching for Spalt co-repressors with the objective of understanding the Spalt mechanism of action. The third project concerns the Ras gene (Fig. 1C). Mutations in human Ras are common in multitude of cancers, and the *Drosophila* Ras gene has been used to model cancer progression in flies. Using Crisper/Cas9 and homologous recombination we have generated *Drosophila* transgenic lines carrying altered versions of the fly and human Ras genes (Fig. 1C). We are characterizing the consequences of activating Ras mutations when the gene is expressed at normal levels in the wing, the ovary and the lymph gland (Fig. 1C). We expect to generate genetic combinations in a background of endogenous activated Ras allowing us to model the formation and progression of tumors.




## Publications

Jiménez-Martínez, M., Ostalé C.M., van der Burg, L., Galán-Martínez, J., Hardwick, J C. H., López-Pérez, R., Hawinkels, L., Stamatakis, K.and Fresno, M.. (2019) DUSP10 Is a Regulator of YAP1 Activity Promoting Cell Proliferation and Colorectal Cancer Progression. *Cancers* **11**, 1767.

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Vega-Cuesta, P., Ruiz-Gómez, A., Molnar, C., Organista, M. F., Resnik-Docampo, M., Falo-Sanjuan, J., López-Varea, A. and de Celis, J.F. (2020). Ras2, the TC21/R-Ras2 *Drosophila* homologue, contributes to insulin signaling but is not required for organism viability. *Dev. Biol.* **461**,172-183.

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## CELL ARCHITECTURE & ORGANOGENESIS

# GENE EXPRESSION CONTROL, PATTERNING AND GROWTH DURING APPENDAGE DEVELOPMENT

***Principal Investigator:***
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David Blom-Dahl
Clara Agudo Rios

***Undergraduate and Master Students:***
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Raúl González Aroca
María Ruiz Azpiroz
Pablo Pérez Martínez

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## Research summary

The formation of an adult organism from a single fertilized egg is a highly regulated and complex process. It involves the generation of large number of cells with specialized functions that need to be assembled together in tissues and organs. This process, named morphogenesis, requires the specification within developmental fields of territories with the ability to acquire different fates and coordinated cell behaviors. Territorial specification depends on the precise modification of gene expression in both time and space and is mediated by a specific set of transcription factors encoded by selector and selector-like genes.

Territorial specification is coupled to a finely tuned control of cell proliferation, apoptosis, cell shape changes and cell adhesion, to allow organs to reach their proper final size and form. Defects on any of these processes could lead to inadequate cells that must be eliminated to prevent undesirable consequences such as malformations or tumorigenesis. In addition, genomes are under continuous assault by the exposure to environmental mutagenic agents as well as the byproducts of normal intracellular machinery. If DNA damage cannot be repaired, cells will undergo apoptosis rather than continue to divide and propagate a damaged genome. Therefore, a correct balance between cell fate specification, cell proliferation and apoptosis is essential to maintain tissue homeostasis.

Our main general aim is to decipher the molecular mechanisms that control cell fate specification and tissue homeostasis during development. To this end, we use the development of *Drosophila* appendages as our model.

We have two objectives in the lab:

1) **Specification, patterning and morphogenesis of appendages**: We study how legs and wings are specified in the embryo and how they are later subdivided in territories with different cell fates to generate the characteristic adult pattern. At the molecular level, a small number of signaling pathways and transcription factors are repeatedly used throughout development to specify and pattern the appendages in Drosophila and in vertebrates.

2) **Coordination between cell proliferation and apoptosis during tissue homeostasis**: Understanding the molecular basis of p53 pro-apoptotic activity and its coordination with the cell cycle could have important implications in our understanding of tumor formation and cancer treatment.

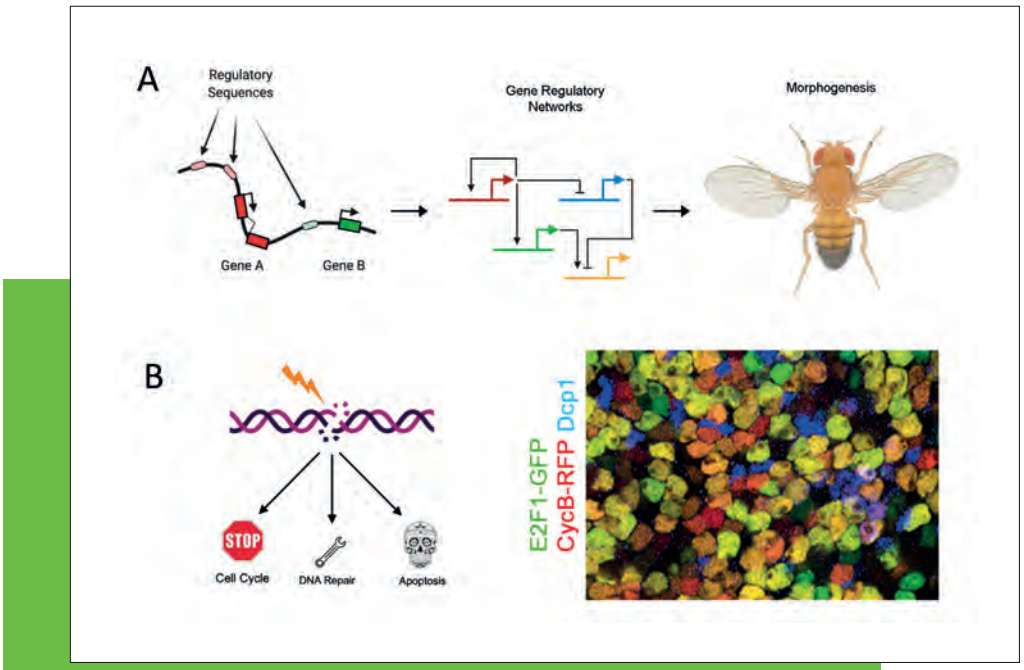


***Figure.*** *A) Integration of spatial and temporal information by regulatory sequences of evolutionary conserved genes that control the patterning and morphogenesis of Drosophila. B) Scheme of the cellular responses after DNA damage. Confocal image of cells marked at the different phases of the cell cycle and the apoptotic marker Dcp1 after irradiation.*

## Publications

Blom-Dahl D, Córdoba S, Gabilondo H, Carr-Baena P, Díaz-Benjumea FJ, Estella C. (2020) *In vivo* analysis of the evolutionary conserved BTD-box domain of Sp1 and Btd during *Drosophila* development. *Dev Biol*. **466**(1-2):77-89.

Córdoba S, Estella C. (2020) Role of Notch Signaling in Leg Development in *Drosophila melanogaster*. *Adv Exp Med Biol*. **1218**:103-127.

## CELL ARCHITECTURE & ORGANOGENESIS

# INTESTINAL MORPHOGENESIS AND HOMEOSTASIS


***Principal Investigator:***
Fernando Martín Belmonte

***Postdoctoral Fellows:***
Covadonga Díaz
Catalina Grabowski
Tatiana Alfonso

***Predoctoral Fellows:***
Gabriel Baonza
Gonzalo Herranz
Sergio Gómez-López

***Technicians:***
Tamara Gonzalez
Estefanía Vazquez de Oro

***Undergraduate and Master Students:***
Irene Pino Sáez (TFM)
Elenea Sánchez (TFG)

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## Research summary

Our main scientific interest is the understanding of intestinal morphogenesis and cellular polarity during morphogenesis, homeostasis, and regeneration, as well as their implications in human diseases, such as intestinal bowel diseases (IBD), obesity, diabetes, and cancer. Our research is based in the organotypic culture model of three-dimensional epithelial cells growing in micropatterns “organ-on-a-chip”, which are becoming one of the best *in vitro* models systems for the investigation of epithelial morphogenesis. Moreover, with this system, we are obtaining essential information about the molecular mechanisms that regulate epithelial morphogenesis. However, this model cannot reconstitute the complexity of the architecture given *in vivo*, which includes different cell types, dynamic remodeling, and tissue homeostasis. For this reason, the use of *in vivo* systems should serve to validate and better characterize the phenotypes observed *in vitro*. We used the zebrafish and mouse intestine as models systems to elucidate epithelial morphogenesis and intestinal homeostasis.

We are focused on the analysis of genes that regulate epithelial polarity during morphogenesis, and intestinal homeostasis, and particularly those controlling the following processes: Signaling, membrane trafficking, mechanical forces, and metabolic remodeling. Therefore, our lab is focused on:

- Characterization of signaling pathways for epithelial morphogenesis, differantiation and patterning
- Analysis of cellular mechanics controlling lumen formation in epithelial cells.
- Study of the metabolic reprogramming that takes place along intestinal development and homeostasis.
- Characterization of the metabolic crosstalk among microbiota, epithelial cells, and immune cells controlling the intestinal homeostasis.

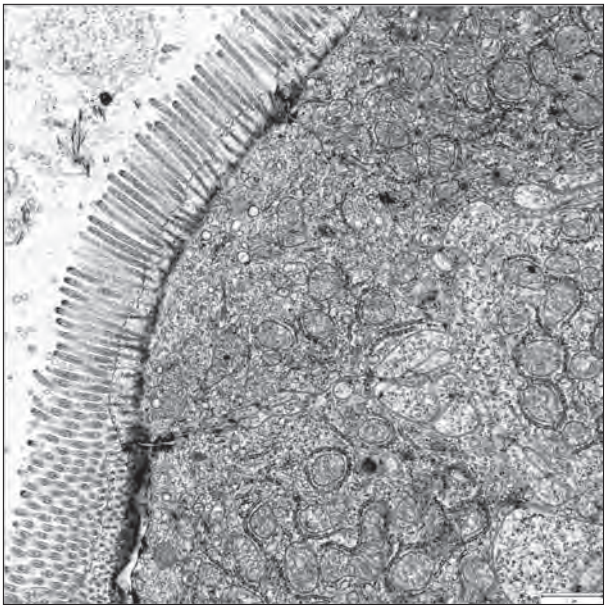

***Figure. TEM of an Intestinal epithelial cell (IEC) in the intestine.*** *TEM of a seven-day mice gut showing the enrichment of mitochondria in IECs.*

## Publications

Hachimi M, Grabowski C, Campanario S, Herranz G, Baonza G, Serrador JM, Gomez-Lopez S, Barea MD, Bosch-Fortea M, Gilmour D, Bagnat M, Rodriguez-Fraticelli AE, Martin-Belmonte F. (2020) Smoothelin-like 2 Inhibits Coronin-1B to Stabilize the Apical Actin Cortex during Epithelial Morphogenesis. *Curr Biol*. S0960-**9822**(20)31685-7.

Bosch-Fortea M, Martín-Belmonte F. (2020) Methods to Generate Tube Micropatterns for Epithelial Morphogenetic Analyses and Tissue Engineering. *Methods Mol Biol*.;**2179**:227-242.

Díaz-Díaz C, Martín-Belmonte F. (2020) Apical poles without neighbouring cells. *Nat Mater*. **19**(9):935-937.

Díaz-Díaz C, Baonza G, Martín-Belmonte F. (2020) The vertebrate epithelial apical junctional complex: Dynamic interplay between Rho GTPase activity and cell polarization processes. *Biochim Biophys Acta Biomembr*.**1862**(10):183398.

Bosch-Fortea M, Rodriguez-Fraticelli AE, Herranz G, Hachimi M, Barea MD, Young J, Ladoux B, Martin-Belmonte F. (2019). Micropattern-based platform as a physiologically relevant model to study epithelial morphogenesis and nephrotoxicity. *Biomaterials*. **218**:119339.

Bernascone I, González T, Barea MD, Carabaña C, Hachimi M, Bosch-Fortea M, Santamaria S, Martin R, Tarnick J, Garcia-Sanz JA, Martín-Belmonte F. (2019) Sfrp3 modulates stromal-epithelial crosstalk during mammary gland development by regulating Wnt levels. *Nat Commun*. **10**(1):2481.

## Awards and recognition

- Associated editor of Nephron (Journal).

- Scientific **vice deputy** of Spanish agency of research (Agencia Estatal Investigación (AEI-MINECO-Area BFS).

- Master professor: Coordination of sorting, routing and distribution of proteins in polarized cells, Universidad Autonoma de Madrid/ Madrid/ Spain.

- Master professor –Molecular Biology of the Cell course at Pasteur, Institute Pasteur.

- Master professor –Cytoskeleton course at Institute Curie/ Paris/ France.

## Doctoral Theses

**María D. Barea**. (2019). The roles of major signaling pathways in epithelial morphogenesis. Universidad Autónoma de Madrid. Supervisor: Fernando Martín-Belmonte. *Cum Laude* and International Mention.

## International projects / Research networks

- Novel Enabling Tools and Models Supporting Development of Interventions for Enteric Dysfunction. Co-proposer (three collaborative teams with M. Bagnat and J. Stosh). Goblal Grand Challenges- Bill and Melinda Gates Foundation (US). 2016-2019.

- Red CONSOLIDER COAT. Dr. Fernando Martin-Belmonte. Coordinador Vivek Malhotra. MINECO (BFU2016-81912-REDC). 2017-2019.

## CELL ARCHITECTURE & ORGANOGENESIS

### INTEGRATED METABOLISM IN IMMUNITY

***Principal Investigator:***
Nuria Martínez Martín

***Predoctoral Fellow:***
Marta Iborra Pernichi
(from October 2020)

***Technician:***
María Velázquez de la Esperanza
(June 2020)

www.cbm.csic.es/nmartinez

## Research summary

Inflammatory Bowel Disease (IBD) is a multifactorial condition that results from an intricate interplay of genetic predisposition, an altered immune response, changes in the intestinal microbiota and environmental factors. IBD courses with chronic inflammation of the intestine and, although its prevalence in Europe is high -and increasing-, there is not a cure for it yet due to the lack of a complete description of its etiology.

Intestinal homeostasis is established along development through the equilibrium between three cellular components: microbiota, the mucosal immune system, and the epithelial barrier. It has been proposed that IBD is a collapse of intestinal homeostasis.

Genome-wide association studies have revealed Autophagy (canonical and non-canonical) as a critical molecular pathway involved in intestine homeostasis and, therefore, as a possible therapeutic target to treat IBD. However, its precise function remains elusive still. The role of canonical and non-canonical autophagy has been addressed separately, as an isolated mechanism, giving place to misleading and contradictory conclusions. According to our previous results (N. Martinez-Martin *et al., Science* **355**, 641 (2017)) canonical and non-canonical coexist in splenic B cells, and its fine-tune regulation is strictly needed to mount an effective systemic humoral response. In this regard, and focusing on the mucosal immune system, in our laboratory we are characterizing the ¨Autophagy Fingerprint¨ (AF) -the precise amount of canonical and non-canonical autophagy- in each B cell type, paying particular attention to the role of microbiota on AF imprinting along development.

We propose that AF exerts a cell-intrinsic function targeting mitochondria content and, therefore, affecting cell metabolism. However, AF might also play a paracrine mechanism, turning B cells into a source of signaling molecules that would act on neighboring cells and controlling in this way the intestine homeostasis. An aberrant AF might then give place to a breakdown of intestinal homeostasis, being the likely origin of IBD.

Therefore, the main aims of our group are to characterize the AF in the B cell compartment and to describe its role in the establishment and maintenance of the intestinal homeostasis and correlate it with IBD.

Our results will offer new ways to understand the molecular mechanisms controlling intestine homeostasis and their relationship with human pathologies. We expect the results derived from this study would serve for the improvement of current treatments of IBD and the development of new treatment strategies.

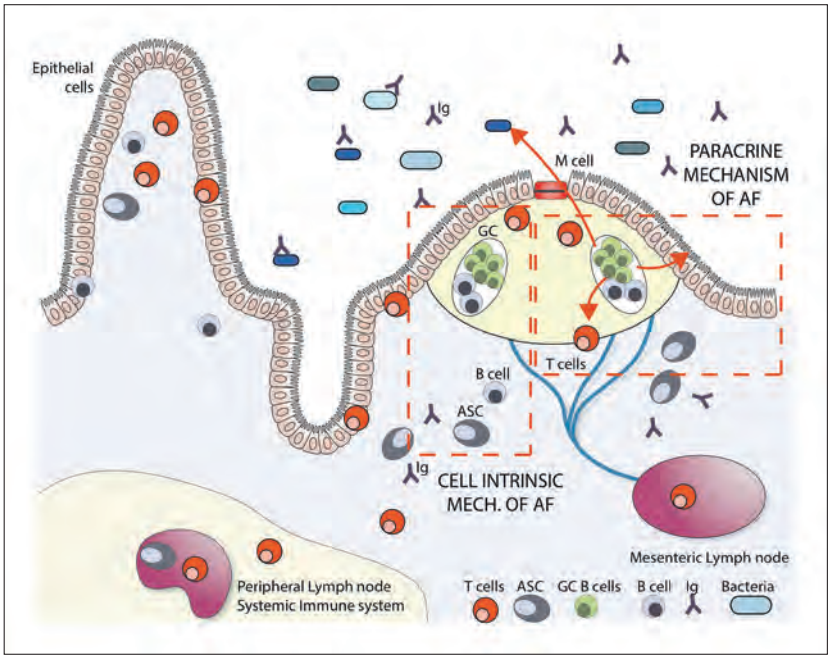


***Figure.*** *Study of the cell-intrinsic and paracrine mechanism of AF (Autophagy Fingerprint) in B cells and its role in the intestine homeostasis (ASC: Antibody Secreting cells; GC: Germinal Center).*

## Publications

Ortega-Molina A, Deleyto-Seldas N, Carreras J, Sanz A, Lebrero-Fernández C, Menéndez C, Vandenberg A, Fernández-Ruiz B, Marín-Arraiza L, de la Calle Arregui C, Plata-Gómez AB, Caleiras E, de Martino A, Martínez-Martín N, Troulé K, Piñeiro-Yáñez E, Nakamura N, Araf S, Victora GD, Okosun J, Fitzgibbon J, and Efeyan A. (2019). Oncogenic Rag GTPase signalling enhances B cell activation and drives follicular lymphoma sensitive to pharmacological inhibition of mTOR. *Nature Metabolism*. **1**(8);775-789.

# CELL ARCHITECTURE & ORGANOGENESIS

## BIOPHYSICS AND SYSTEMS BIOLOGY


***Principal Investigator:***
David Míguez

***Predoctoral Fellows:***
Mario Ledesma Terrón
Antonio López Izquierdo
(hasta 30/07/2019)
Diego Pérez Donés
(desde 01/04/2019)

***Undergraduate and Master Students:***
Blanca María Pozuelo
(until september 2020)
Laura Delgado Rojo
(until October 2020 )

***Technicians:***
Estefanía Vazquez del Oro
(from January 2020)
Nuria Peralta Cañadas
(until June 2019)

URL: https://www.cbm.uam.es/dmiguez

## Research summary

**• Regulation of stem cell differentiation during developmental processes**

The cellular machinery is governed by interacting proteins, genes and metabolites that form complex and highly interconnected networks of interactions. This way, extracellular stimuli triggers pathways of biological events that regulate gene expression, protein activity, and ultimately, cell response. We combine in vivo and in silico approaches to understand how the wiring of the pathways determines the role of the proteins that regulate the differentiation of neurons. We also study how the interplay between mode and rate of division of stem cells is orchestrated. To do that, we use in toto microscopy combined with theoretical tools and algorithms developed in the lab to quantify the effect of key regulators in the balance between proliferation and differentiation during vertebrate neurogenesis.

**• Nonlinear regulation in pathways and its impact on disease treatment**

Small molecule inhibitors display significant potential as treatment for diseases that involve the deregulation of signal transduction pathways. These inhibitors are developed based on their target specificity and binding affinity. We focus on the fact that the numerous signaling proteins and feedback loops in signaling pathways strongly influence the efficiency of pharmacological treatment. The existence of several regulatory positive and negative feedback loops either creates complex dose-responses, desensitization to periodic treatments, or modulation of the drug effect in combinatorial treatments. Our experiments show that the effect of inhibitors strongly depends on the architecture of the targeted pathway, and a detailed characterisation of these nonlinear effects can be useful when designing optimal treatment strategies.

**• Stochasticity and effect of perturbations in biological networks**

Biological networks control cellular behaviour both at the intracellular and at intercellular level. These highly interconnected networks need to perform in the continuously fluctuating and changing cellular microenvironment. Disruption of these networks often leads to aberrant cell behaviour and disease. This leads to some broad questions that we try to address in the lab at different levels: How can these highly nonlinear biological networks integrate and process information? How can they operate robustly in the presence of noise and fluctuations? What are the mechanisms at the network level underlying the adaptation to the different sources of extrinsic and intrinsic noise ? To answer these questions, we use a synthetic biology approach to analyse experimental and computationally the dynamics of fluctuations in minimal networks motifs.

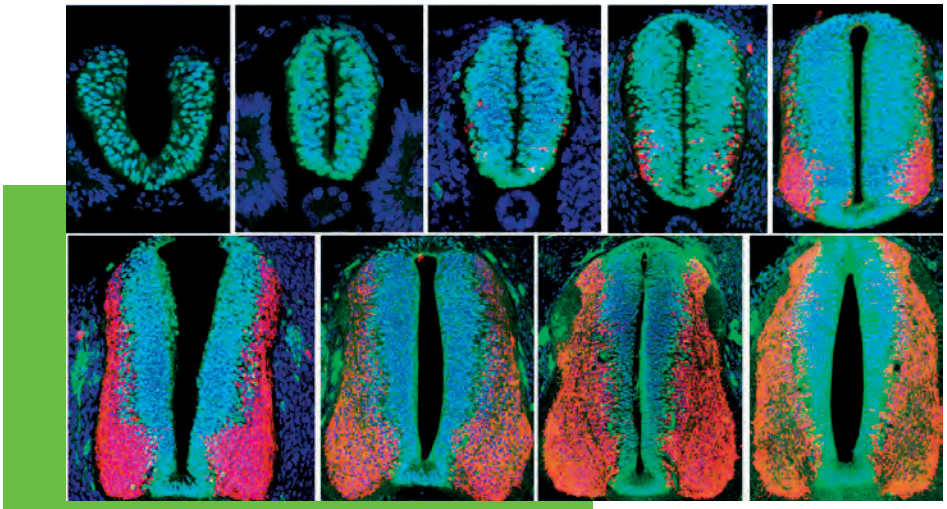

***Figure.*** *Chick Neural tube at different developmental times.*

## Publications

Ledesma-Terrón, M., Peralta-Canadas, N. , Míguez, D. G, (2020) FGF2 modulates simultaneously the mode, the rate of division and the growth fraction in cultures of Radial Glia, *Development* **147**: dev189712.

Javier Casares-Arias, et al., (2020) Midbody remnant inheritance is regulated by the ESCRT subunit CHMP4C, *iScience* **23**, 101244.

E. Giacomelli, et al., (2020) Human-iPSC-Derived Cardiac Stromal Cells Enhance Maturation in 3D Cardiac Microtissues and Reveal Non-cardiomyocyte Contributions to Heart Disease, Cell *Stem Cell* **26**, 862-879.e11.

Míguez*, D. G. , Garcia-Morales, D., Casares*, F., (2020) Control of size, fate and time by the Hh morphogen in the eyes of flies, *Current Topics in Developmental Biology*: Volume **137**, 307-332 , (*shared corresponding authorship).

Garcia-Morales, D., Míguez*, D. G., Casares, F., (2019). Dynamic Hh signaling can generate temporal information during tissue patterning. *Development,* **146**, dev176933 (*shared corresponding authorship).

## CELL ARCHITECTURE & ORGANOGENESIS

### CELL BIOLOGY OF INFLAMMATION

***Principal Investigator:***
Jaime Millán

***Predoctoral Fellows:***
Cristina Cacho Navas
Natalia Colás Algora
Gema Cerro Tello

***Technicians:***
Susana Barroso Fernández
Gema de Rivas Hidalgo

***Undergraduate and Master Students:***
Beatriz Queipo López
Pablo Muñoz Pinillos

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## Research summary

Complex organisms organize many tissues as a set of cellular barriers that compartmentalize functions, such as absorption, secretion, nutrition and protection. During the inflammatory response, cellular barriers transiently alter the expression of surface adhesion receptors and increase their permeability to guide immune cells towards the inflammatory focus. The main goal of our research group is to investigate the molecular mechanisms underlying the role of cellular barriers in the human inflammatory response.

The vascular endothelium controls the passage of cells and solutes between the blood and the parenchyma in the inflamed tissue (Figure ). During these two years we have identified new molecular mechanisms whereby human endothelial cells control the expression of VE-cadherin during the inflammatory response. Compensatory VE-cadherin expression prevents endothelial barrier collapse during the transient increase of permeability induced by proinflammatory cytokines such as TNF. Despite VE-cadherin is essential for controlling vascular permeability, we have also found that the human vascular endothelium significantly expresses 24 members of the cadherin superfamily and that most of them have not been yet investigated in these cells. We are also addressing the role of the RhoA subfamily of GTPases in regulating VE-cadherin expression levels and endothelial barrier function. We are exploring the role of this subfamily as a therapeutic target to prevent organ edema during systemic inflammation, which has led us to register a patent. Finally, we are applying our expertise to try to prevent pulmonary endothelial barrier disruption caused by the cytokine storm induced by SARS-Cov-2 in seriously ill COVID19 patients.

Once leukocytes traverse the endothelial barrier, they establish adhesions with parenchymal cells, searching for the inflammatory focus and for dysfunctional cells. The liver is a paradigm of complex organ in which leukocyte infiltration into the tissue is essential for immune-surveillance, control of cancer and infections, and tissue regeneration. ICAM-1 is the main adhesion receptor mediating leukocyte haptotaxis in hepatocytes. We found that polarized hepatocytes confine ICAM-1 in their apical domains, which are not accessible to immune cells, and that hepatic ICAM-1 polarization regulates hepatocyte-leukocyte interactions. We are currently investigating the molecular mechanisms determining ICAM-1 polarity in hepatic barriers and the role of ICAM-1 regulating bile canalicular morphogenesis. To do so, we have set up experimental procedures to generate hepatic organoids obtained from bipotent precursors isolated from human and murine livers and grown in 3D.

Finally, these two years we have initiated a partnership with the company Cornea SL to initiated an “industrial doctorate” to carry out a project in which we search for new inflammatory prognostic markers in the corneal epithelial barrier of patients with keratoconus, a disease of unknown etiology, which causes dramatic changes in the corneal shape that eventually requires corneal transplant.

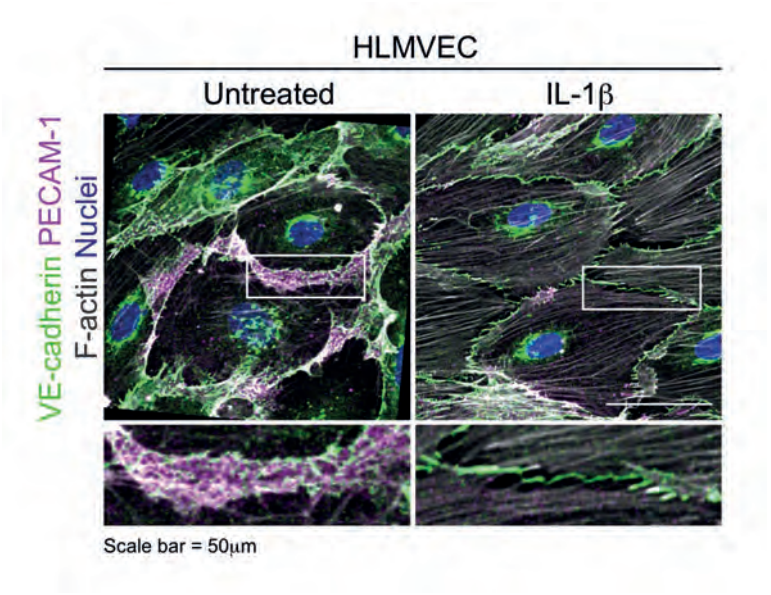



*Figure. Human lung microvascular endothelial cells (HLMVECs) form a barrier stabilized by cell-cell junctions containing VE-cadherin (green), PECAM-1 (purple) and F-actin (grey). Inflammatory cytokines such as IL1β (right images) induce F-actin stress fibers, junctional remodeling and endothelial barrier disruption. Nuclei are shown in blue. Bottom images show a two-fold size increase of the squared areas in the top images.*

## Publications

Colás-Algora N, García-Weber D, Cacho-Navas C, Barroso S, Caballero A, Ribas C, Correas I, Millán J. (2020). Compensatory increase of VE-cadherin expression through ETS1 regulates endothelial barrier function in response to TNFα. *Cell. Mol. Life Sci*. **77**(11):2125-2140.

Santaterra VAG, Fiusa MML, Hounkpe BW, Chenou F, Tonasse WV, da Costa LNG, Garcia-Weber D, Domingos IF, de Lima F, Borba-Junior IT, Araújo ADS, Lucena-Araújo AR, Bezerra MAC, Dos Santos MNN, Costa FF, Millán J, De Paula EV. (2020).Endothelial Barrier Integrity Is Disrupted *In Vitro* by Heme and by Serum From Sickle Cell Disease Patients.Front. *Immunol*. **11**:535147.

Colás-Algora N and Millán J. (2019). How many cadherins do human endothelial cells express? *Cell. Mol. Life Sci.* **76**(7):1299-1317.

Gómez-Escudero J, Clemente C, García-Weber D, Acín-Pérez R, Millán J, Enríquez JA, Bentley K, Carmeliet P, Arroyo AG. (2019). PKM2 regulates endothelial cell junction dynamics and angiogenesis via ATP production. *Sci Rep.* **21**;9(1):15022.

## Patents

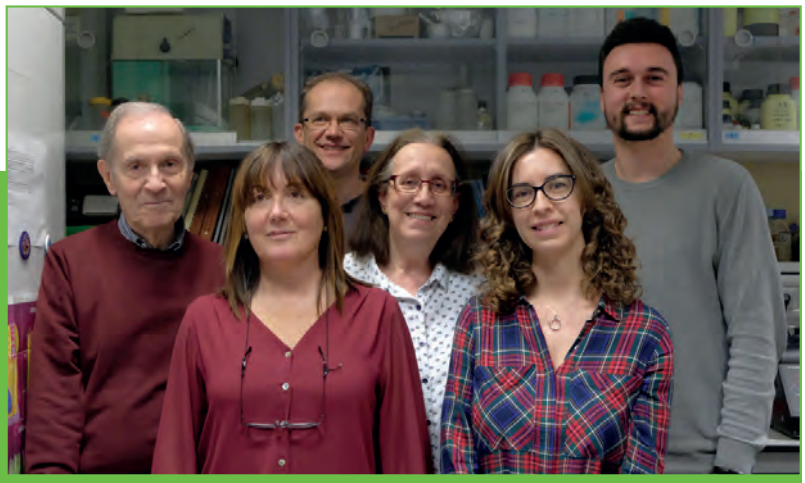
Inventors: Colás.Algora, N, Caballero, A; Ribas, C and Millán, J. Ref. ES1641.1450. 2019. Aplicants: CSIC, UAM, FJD. (Spain). Title: COMPUESTOS PARA SU USO EN EL TRATAMIENTO Y/O LA PREVENCIÓN DE SEPTICEMIA.

## Doctoral Theses

**Cristina Cacho Navas** (2020). Estudio de los mecanismos moleculares que median la polarización apical y función de ICAM-1 en células epiteliales hepáticas. Universidad Autónoma de Madrid. Director: Jaime Millán. Outstanding *Cum Laude*.

## CELL ARCHITECTURE & ORGANOGENESIS

# GENETIC AND FUNCTIONAL ANALYSIS OF THE RENAL FILTRATION DIAPHRAGM IN HEALTH AND DISEASE



***Principal Investigators:***
Mar Ruiz Gómez
Joaquim Culí Espigul

***Research Professor Ad Honorem:***
Juan Modolell Mainou

***Scientific Staff:***
Sonsoles Campuzano Corrales
(since June 2020)

***Postdoctoral Fellow:***
Marta Carrasco Rando

***Predoctoral Fellows:***
Alexandra Atienza Manuel
(until July 2019)
Vicente Castillo Mancho

***Technician:***
Roberto Menchén de Ochoa
(until March 2019)

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## Research summary

The podocyte slit diaphragm (SD) is a modified cell junction that plays a key role in the maintenance of the glomerular filtration barrier, being a major target of injury in many renal diseases, including chronic kidney disease (CKD), a prevalent pathology affecting more than 20% of the elderly population. The scarcity of early markers of kidney damage has hampered CKD early detection and its effective prevention and/or treatment. Hence the challenge and the main goal of our research is to advance in understanding the molecular mechanisms involved in SD formation and stability, and to identify major signalling events regulating SD dynamics in normal and pathological conditions.

A decade ago we and others described that nephrocytes, the fly excretory cells involved in haemolymph ultrafiltration, have SDs that share with those of vertebrates a striking conservation at the molecular, structural, functional and regulatory level, validating their use to study SD dynamics. To achieve our goal, in our laboratory we combine the use of the model organism *Drosophila melanogaster,* whose versatility of genetic techniques allows the rapid identification and functional characterisation of novel SD components and regulators, with that of zebrafish Danio rerio, utilised to validate the role of these genes in the vertebrate kidney. In the last two years we have focused in dissecting the requirement of the endocytic receptor CUBAM for the correct positioning of SDs, and in the functional characterisation of two novel genes essential for the assembly of the nephrocyte's SD, emphasising in the possible medical implications that may derive from our research. Our studies revealed that *Drosophila* CUBAM is a tripartite complex formed by Amnionless (dAMN) and two cubilin orthologues, Cubn and Cubn-2. It is responsible for most clathrin-mediated endocytosis in nephrocytes and its endocytic activity is crucial for SD positioning and nephrocyte global cytoarchitecture. Furthermore, our results indicate that the phenotypes of CUBAM deficient nephrocytes are consequence of an imbalance between endocytosis and exocytosis in these cells, and point to a hitherto unsuspected function of CUBAM in SD recycling.

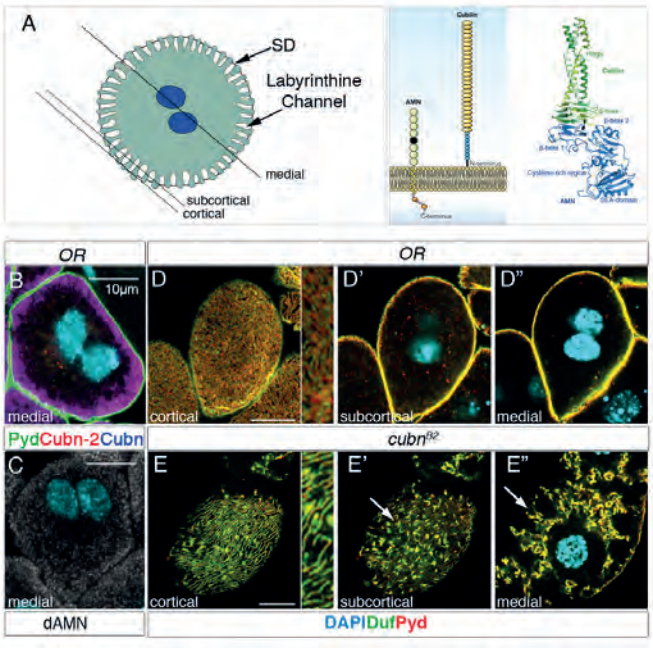



***Figure.*** *The CUBAM endocytic receptor in nephrocytes is formed by Amnionless (dAMN) and two cubilin orthologues Cubn and Cubn-2, it accumulates at the labyrinthine channel membrane (A, B and C) and its function is required for correct positioning of slit diaphragms (SD) at the nephrocyte periphery and to maintain global nephrocyte cytoarchitecture (D-E"). Duf and Pyd, the main components of the nephrocyte SD, co-localise at the external nephrocyte membrane displaying a fingerprint-like distribution in the wild-type.*

## Publications

Carrasco-Rando, M., Prieto-Sánchez, S., Culi, J., Tutor, A. S. and Ruiz-Gómez, M. (2019) A specific isoform of Pyd/ZO-1 mediates junctional remodelling and formation of slit diaphragms. J. *Cell Biol*. **218** (7), 2294-2308.

## Awards and recognition

- Several members of our group participate in scientific outreach activities, e.g. the Semana de la Ciencia workshop "De moscas, peces y otros seres: buscando el origen de enfermedades congénitas en el hombre" (2019).

## Doctoral Theses

**Alexandra Atienza Manuel** (2019). Función del complejo endocítico Cubilin-Amnionless y de la escramblasa de fosfolípidos Scramb1 en la biología del diafragma de filtración de *Drosophila*. Universidad Autónoma de Madrid. Director: M. Ruiz Gómez. International Mention.

## CELL ARCHITECTURE & ORGANOGENESIS

# TRANSCRIPTIONAL CONTROL OF SEXUAL DIFFERENTIATION OF THE NERVOUS SYSTEM


***Principal Investigator:***
Esther Serrano Saiz

***Predoctoral Fellows:***
Ana Bermejo Santos
Rafael Casado Navarro

***Technician:***
Rodrigo Torrillas de la Cal

***Undergraduate and Master Student:***
Denisa Lupu

http://www.cbm.csic.es/eserranosaiz

## Research summary

Identifying and understanding the genetic factors linked to neuropsychiatric disorders is a fundamental goal in neuroscience. The nervous system of male and females is sexually dimorphic at the molecular and structural levels and these differences lead to sex biases in the age of onset, prevalence, symptomatology and treatment for nearly every neuropsychiatric disorder. The general aim of our laboratory is to decipher the genetic and molecular mechanisms that control sex linked configurations of the nervous system. Most sex differences are caused by sex hormones, however there is robust evidence that points to genetic factors also contributing to sexual dimorphisms.

The family of Dmrt transcription factors (TFs) has been identified as a common theme in the specification of sex-specific traits across distant animal species, including nematodes, flies and vertebrates. Our previous work and others in *C. elegans* and *Drosophila*, have established that Dmrt genes control the sexual differentiation of the nervous system. However, the role of Dmrts in the vertebrate nervous system has not been profusely investigated. We are addressing this question by characterizing the expression of the Dmrt genes in the mouse brain. We aim to discover novel sexual-dimorphisms - linked to the Dmrt expression - and to understand the function of these TFs in the establishment and maintenance of mouse nervous system sex-specific layouts.

In summary, we will follow a multidisciplinary approach that will have important implications in the field: 1) it will reveal the high level of conservation of Dmrts as “master regulators of sexual dimorphisms” from nematodes to mouse; 2) it will identify novel mechanisms in the generation of sex-specific configurations of the mouse brain that may be independent of sex hormones; 3) it will define novel brain dimorphic regions that could only be revealed by the analysis of molecular profiles in restricted cell types and 4) it could have clinical implications by uncovering novel genetic components linked to mental disorders.

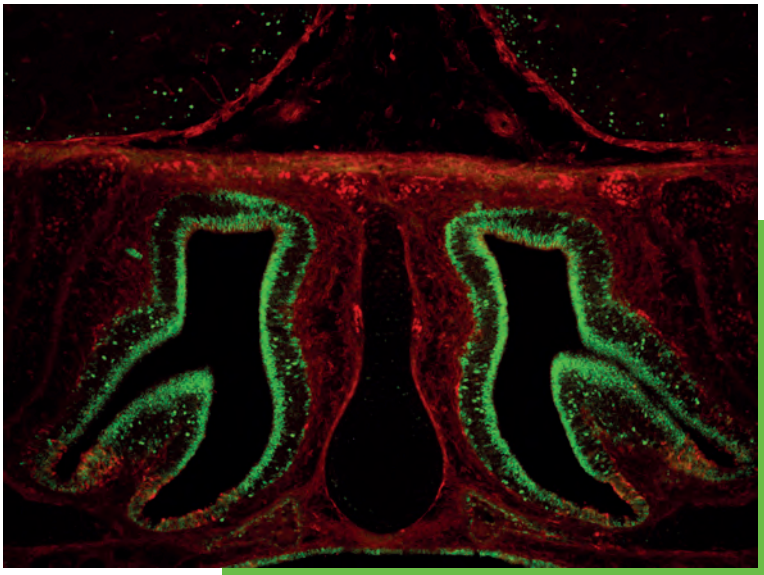


***Figure.*** *DMRT5 expression (green) in the main olfactory epithelium of a mouse embryo.*

## Publications

Yuste R et al (consortium). (2020). A community-based transcriptomics classification and nomenclature of neocortical cell types. *Nat Neurosci*. **23**(12):1456-1468.

Leyva-Díaz E, Masoudi N, Serrano-Saiz E, Glenwinkel L, Hobert O. (2020).Brn3/POU-IV-type POU homeobox genes-Paradigmatic regulators of neuronal identity across phylogeny. *Wiley Interdiscip Rev Dev Biol*. **9**(4):e374.

Serrano-Saiz E, Vogt MC, Levy S, Wang Y, Kaczmarczyk KK, Mei X, Bai G, Singson A, Grant BD, Hobert O. (2020). SLC17A6/7/8 Vesicular Glutamate Transporter Homologs in Nematodes. *Genetics.* **214(**1):163-178.

Serrano-Saiz E, Gulez B, Pereira L, Gendrel M, Kerk SY, Vidal B, Feng W, Wang C, Kratsios P, Rand JB, Hobert O.(2020). Modular Organization of Cis-regulatory Control Information of Neurotransmitter Pathway Genes in Caenorhabditis elegans. *Genetics*. **215**(3):665-681.

Serrano-Saiz E and Santesmanses M. Jesús. The mosaic brain: sex/gender & the neurosciences. In Brain, mind and behavior. (book chapter).

## International projects / Research networks

- Red REDEVNEURAL 3.0: *Un enfoque integrador para entender la logica del desarrollo neural* (RED2018-102553-T).

UNIT

MARÍA YÁÑEZ-MO

# Cell-Cell Communication & Inflammation

TISSUE AND ORGAN HOMEOSTASIS

***Cell-cell communication*** is essential for differentiation during development and maintenance of tissue homeostasis. Secretion of ***extracellular vesicles*** has emerged as a new mechanism of intercellular communication with great potential in the discovery of non-invasive biomarkers and in the therapeutic control of many diseases. Several groups in the ***Cell-Cell communication & Inflammation Unit*** are investigating the molecular mechanisms of extracellular vesicles production, secretion and uptake and their connection with the cellular metabolism, using *Drosophila* tissues during development or human tumours as model systems.

The extracellular matrix network in which cells are integrated constitutes an additional level of cell-cell communication. During aging, there is a progressive loss of tissue homeostasis, deregulated intercellular communication and increased ***inflammation and fibrosis***. The ***Cell-Cell communication & Inflammation Unit*** combines efforts of several groups studying cell-matrix interactions, matrix remodelling components and stress granules with especial focus in the cardiovascular system, muscle diseases and epithelial or mesothelial to mesenchymal transition. Moreover, some groups are studying the potential of targeting immune-metabolism to dampen inflammation to improve clinical interventions in age-associated diseases.

Main contributions of the groups in the unit include:

- A report in Science revealing that metabolic alteration in T-cells instigate multiple aging-related features resulting in premature death. T-cell metabolic failure induced the accumulation of circulating cytokines, resembling the chronic inflammation characteristic of aging ("inflammaging")(*Desdin-Mico et al., Science 2020*).

- The description of the vesicular trafficking of the Hedgehog receptor Patched to synapse-like contact sites between presenting and receiving cytonemes (filopodia-like structures involved in long-distance signaling processes in epithelial tissues during development) (*González-Méndez et al., EMBO J. 2020*).

- The implication of RIAM and its partner VASP in the outside-in signaling downstream of β2 integrins during complement-mediated phagocytosis (*Torres-Gómez et al., Cells 2020*)

- The relevance of tetraspanin CD9 in Human Papilloma Virus-16 infection (*Mikulicic et al., Med.Microbiol.Immunol. 2020*)

- The biotechnological potential of tetraspanins in the generation of synthetic exosomes *(Lozano-Andres et al., J.Extracell. Ves. 2019*); methods to quantitate exosome uptake (*Toribio et al., Sci.Rep. 2019*) or to detect them from human biofluids (*Campos Silva et al., Sci.Rep. 2019*)

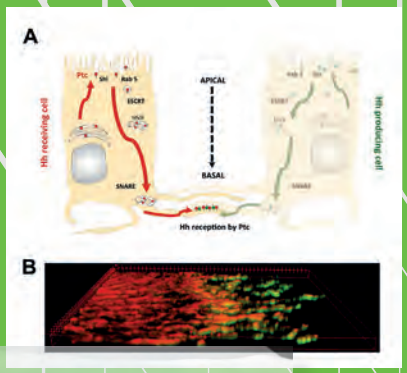
- The role of tetraspanin molecules in Herpes viral infection (Benayas et al., Med.Microbiol.Immunol. 2020) or matrix metalloproteinase regulation (*Suarez et al., Cells 2020*)

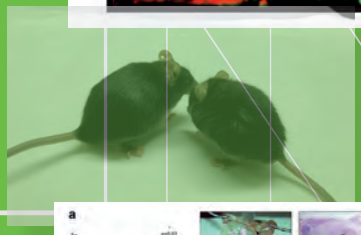
- The development of a heterologous cellular model to study Welander distal myopathy (WDM), representing a simple bioassay to study WDM-associated pathology and develop therapeutic strategies (*Carrascoso et al., Mol.Cell.Biol. 2019*).

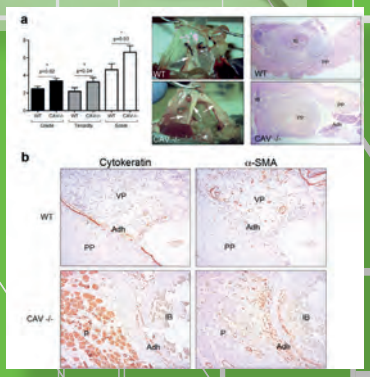
- The delineation of the landscape of proteases regulating lysyl oxidase activity (Rosell-García et al., J.Biol.Chem. 2019). Novel understanding of the transcription factors involved in hypoxia regulation of several extracellular matrix genes (*Rosell-García et al., J.Biol.Chem. 2019*).

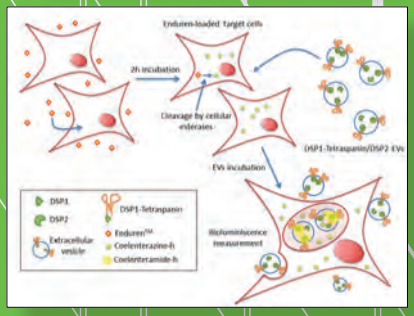
- A patented kit of biomarkers for the diagnosis and prognosis of both local peritoneal damage and systemic cardiovascular disease, to identify patients on peritoneal dialysis with the highest risk of cardiovascular disease.

- The characterization of molecular mechanisms involved in mechanical transduction behind the mesothelial to mesenchymal transition (*Strippoli R, et al., Cell Death. Dis. 2020*).






## CARLOS CABAÑAS

FUNCTIONAL INTERACTIONS BETWEEN TETRASPANINS AND CELL ADHESION MOLECULES

## ISABEL GUERRERO

MECHANISMS OF CELL-CELL SIGNALING DURING DEVELOPMENT

## JOSÉ MARÍA IZQUIERDO

MOLECULAR AND CELLULAR BASIS OF THE PHYSIOPATHOLOGY ASSOCIATED WITH THE EXPRESSION OF INTRACELLULAR ANTIGENS

## MANUEL LÓPEZ CABRERA

MOLECULAR PATHOPHYSIOLOGY OF PERITONEAL INFLAMMATION AND FIBROSIS (PERINFIB)

## MARÍA MITTELBRUNN

IMMUNOMETABOLISM AND INFLAMMATION LAB

## FERNANDO RODRÍGUEZ PASCUAL

EXTRACELLULAR MATRIX REMODELING IN THE CARDIOVASCULAR SYSTEM

## MARÍA YÁÑEZ-MO

TETRASPANIN-ENRICHED MEMBRANE MICRODOMAINS IN EXTRACELLULAR VESICLES AND CELL ADHESION AND MIGRATION

## CELL-CELL COMMUNICATION & INFLAMMATION

# FUNCTIONAL INTERACTIONS BETWEEN TETRASPANINS AND CELL ADHESION MOLECULES

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***Undergraduate and Master Students:***
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## Research summary

Tetraspanins are highly conserved proteins that function as membrane-organizers and control fundamental cell functions, including adhesion, migration and proliferation. The tetraspanin CD9 associates specifically with different cell adhesion receptors of the immunoglobulin and integrin families in tetraspanin-enriched microdomains (TEMs). Through these interactions, CD9 exerts important regulatory effects on the function of associated adhesion molecules. Our group has reported that CD9 associates directly with LFA-1 (integrin αLβ2 or CD11a/CD18) and with the metalloproteinase ADAM17 on the surface of different types of cells, exerting an inhibitory effect on the adhesive activity of LFA-1 and on the ADAM17 sheddase activity against a variety of its substrates. Interactions of a different integrin, α5β1, with ADAM17 may occur among molecules expressed on the same cell (cis) or on different cells (trans), with the latter reported to support cell-cell adhesion events. Interestingly, the interaction between integrin α5β1 and the disintegrin domain of ADAM17 has been shown to cause the inhibition of both the adhesive capacity of the integrin (i.e. its ability to bind its ligands) as well as of ADAM17 metalloproteinase activity due to steric hindrance leading to decreased accessibility of its catalytic site for the substrate. In contrast, stimuli that promote the dissociation of the α5β1-ADAM17 complex induce the activation of ADAM17 sheddase activity and increase integrin-mediated cell adhesion. Interestingly, the expression of CD9 on the cell surface abrogates integrin α5β1-mediated cell adhesion to its ligands fibronectin and ADAM17. In situ proximity ligation assays (PLA) and biochemical experiments based on co-immunoprecipitation collectively revealed that the mechanism by which CD9 inhibits α5β1-mediated cell adhesion involves the reinforcement of cis interactions between ADAM17 and α5β1 on the cell surface, which takes place without alteration in α5β1 integrin affinity but is evidenced by changes in the organization of integrin molecules at the plasma membrane.

Exosomes are a type of extracellular vesicles (EVs) of endocytic origin which serve important intercellular communication functions. Exosomes are produced and released by a large variety of donor cells in the organism and contain a specific cargo of lipids, proteins, miRNAs, mRNAs and DNA, which upon their selective binding and/or uptake can modify the phenotype and trigger a functional response in target cells. Tetraspanins are very abundantly expressed on the surface of different types of extracellular vesicles (EVs), including exosomes, and many research groups consider these proteins as the best markers for these EVs. Our group is currently investigating the role of CD9 as a regulator of adhesion molecules of the integrin and immunoglobulin families and of metalloproteinases, which are involved in the binding and/or uptake of exosomes by target cells.

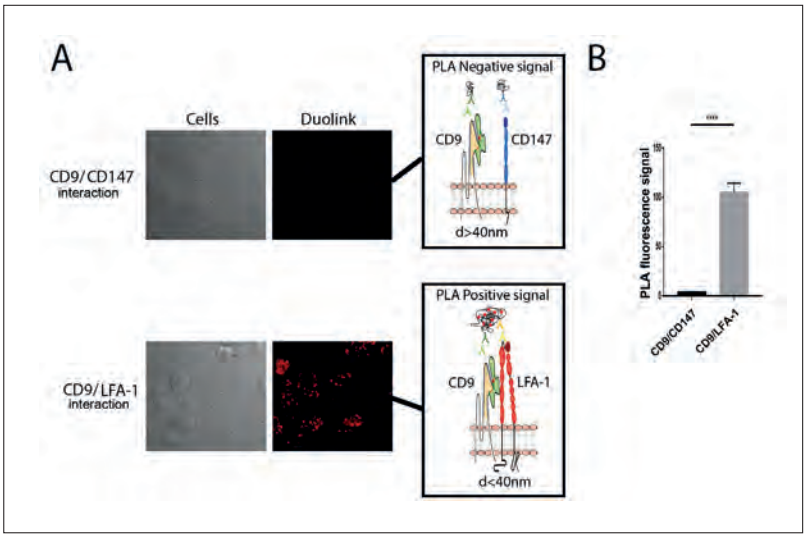


***Figure. Direct interaction between CD9 and integrin LFA-1 revealed by PLA. (A)*** *Proximity Ligation Assay (PLA) of the molecular association of CD9 with CD147 (negative control) and of CD9 with integrin LFA-1 (positive control) on THP1 macrophage-like cells.* ***(B)*** *The graph represents the quantification PLA signal from panel (A).*

## Publications

Torres-Gómez A , Cardeñes B, Díez-Sainz E, Lafuente EM, & Cabañas C. (2020) Functional Integrin Regulation Through Interactions with Tetraspanin CD9. *Methods in Molecular Biology* **2217**:47-56.

Torres-Gomez A, Cabañas C, & Lafuente EM. (2020). Phagocytic Integrins: Activation and Signaling. *Frontiers in Immunology* **11**:738.

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Mikuličić S, Fritzen A, Scheffer K, Strunk J, Cabañas C, Sperrhacke M, Reiss K, & Florin L. (2020). Tetraspanin CD9 affects HPV16 infection by modulating ADAM17 activity and the ERK signalling pathway. *Medical Microbiology and Immunology* **209**:461-471

Cabañas, C, Yáñez-Mó, M, & van Spriel, A. (2019). Functional relevance of tetraspanins in the immune system. *Frontiers in Immunology* **10**:1714.

Toribio V, Morales S, López-Martín S, Cardeñes B, Cabañas C, & Yáñez-Mó M. (2019). Development of a quantitative method to measure EV uptake. *Scientific Reports* **9**:10522.

## CELL-CELL COMMUNICATION & INFLAMMATION

# MECHANISMS OF CELL-CELL SIGNALING DURING DEVELOPMENT

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## Research summary

Cell-to-cell communication is a key event during development and its misregulation can cause diseases such as cancer, malformations and neurological disorders. In this inter-cellular communication, several signaling molecules act over long distances, having a gradient form distribution in a morphogenetic field. Some of these signals (called morphogens) are modified by lipids, which confer them a high affinity for membranes, making their free propagation through tissues difficult. Direct contact between cell membranes by filopodia-like structures (also known as cytonemes) could be a simple explanation for their dispersal. In *Drosophila*, we have demonstrated that cytonemes are required for the establishment of Hedgehog (Hh) morphogen gradient and that vesicles are the Hh carriers in cytoneme-mediated transport for its secretion in exovesicles. The ability of the receptor cells to respond specifically to different ligand concentrations is also mediated by cytonemes. Our hypothesis is that during morphogenesis cells exchange signaling proteins at sites of direct contact between cytoneme membranes in a way similar to neuronal synapses.

The research of the group is related to the mechanisms of cell signaling communication, analyzing the implication of cytoskeleton and vesicular trafficking in the process of signal presentation and reception and the crosstalk between signaling pathways. The specific objetives are: 1) To study the mechanisms of interaction between cytonemes from Hh-receiving and Hh-sending cells during the reception. To this aim we are investigating the possibility that the process of Hh signalling resembles a synaptic contact. 2) To explore the molecular mechanisms for the formation, guidance and dynamic regulation of cytonemes involved in Hh signaling during normal development. 3) To decipher the role of the EGFR pathway in cytoneme formation and to analyze the crosstalk between pathways in normal and pathological development.

Our research is interdisciplinary; we use diverse experimental systems in a variety of *Drosophila* tissues, state-of-the-art methodologies for genetic, cellular and molecular analyses, superresolution and 4D *in vivo* imaging confocal microscopy and electron microscopy. The quantitative information generated is allowing the development of mathematical models for the signalling processes that, in turn, let us explore different hypotheses.

The knowledge of the mechanisms underlying cell-cell signaling and cell signaling integration during normal development will also contribute to understand other cell communication processes such as the maintenance of adult homeostasis and tumor progression. These findings could be the base to design specific therapies since they will ultimately permit the identification of potential new drug targets.

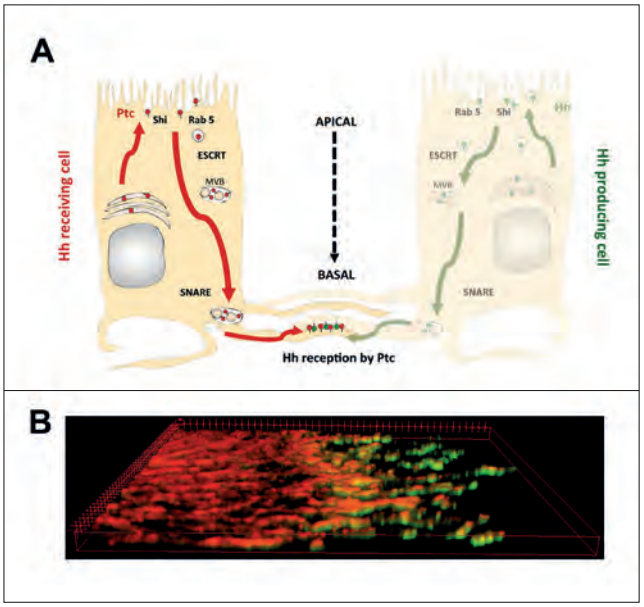


***Figure. Polarized sorting of Hedgehog and its receptor Patched enables cytoneme-mediated reception in the Drosophila wing disc epitheium.*** *A) Scheme depicting the Hedgehog (Hh) (right side of the figure) vesicular trafficking mediated by the ESCRT and SNARE components that enables Hh to be transported along cytonemes at the basolateral surface of the Drosophila wing disc epithelia. The Hh receptor Patched (Ptc) (left side of the figure) also undergoes similar ESCRT and SNARE mediated trafficking to be externalize to interact with Hh at discrete, synapse-like, cytoneme contact sites. B) Basal confocal section in the area of reception of the wing imaginal disc epithelium; the receiving cell cytonemes (red) accumulate Hh (green) presented by the producing cell cytonemes.*

## Publications

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González-Méndez L, Gradilla AC, Sánchez-Hernández D, González E, Aguirre-Tamaral A, Jiménez-Jiménez C, Guerra M, Aguilar G, Andrés G, Falcón-Pérez JM, Guerrero I. (2020) Polarized sorting of Patched enables cytoneme-mediated Hedgehog reception in the *Drosophila* wing disc. *EMBO J*. **39**(11):e103629.

Simon E, de la Puebla SF, Guerrero I. (2019). *Drosophila* Zic family member odd-paired is needed for adult post-ecdysis maturation. *Open Biol.* **9**(12):190245.

González-Méndez L, Gradilla AC, Guerrero I. (2019). The cytoneme connection: direct long-distance signal transfer during development. *Development.* **146**(9):dev174607.

## Awards and recognition

- Congress organization: EMBO Workshop in Long distance cell-cell signalling in development and disease communication. Exeter. UK, delayed by Covid19 to Sept. 2021. Co-organizer. https://meetings.embo.org/event/21-cell-signalling.

## Doctoral Theses

**Adrián Aguirre Tamaral**. (2020). *In silico* modeling of cytoneme-mediated Hedgehog signaling in *Drosophila*. Universidad Autónoma de Madrid. Supervisor: Isabel Guerrero Vega. Outstanding *cum Laude*.

## International projects / Research networks

- Red traslacional para aplicación clínica de vesiculas extracelulares (TENTACLES). RED18-102411-T. Ministerio de Ciencia Innovación y Universidades. Coordinated Project. 2019-2021. PI: Isabel Guerrero Vega.

## CELL-CELL COMMUNICATION & INFLAMMATION

# MOLECULAR AND CELLULAR BASIS OF THE PHYSIOPATHOLOGY ASSOCIATED WITH THE EXPRESSION OF INTRACELLULAR ANTIGENS

***Principal Investigator:***
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***Technician:***
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***Undergraduate and Master Students:***
Andrea Fernández
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The human T-cell intracellular antigens TIA are involved in the regulation of gene expression on different aspects of the metabolism of cell RNA, such as: i) transcription through its interaction with DNA and RNA polymerase II; ii) splicing of pre-mRNA through the selection of atypical 5'/3' splicing sites; iii) localization, stability and/or translation of eukaryotic mRNAs through the interaction with 5' and/or 3' untranslatable regions and/or microRNAs; and iv) modulation of pivotal biological programs (inflammation, proliferation, autophagy, apoptosis, embryogenesis, cell stress and infections by viruses). Our Hypothesis is that these master regulators play a central role in controlling gene expression by regulating the dynamics of human transcriptome and proteome, their expression and function, to prevent situations which put aberrant cell viability at risk in patho-physiological situations. Thus, today and the next future, our main Goal is to characterize the cellular processes and molecular mechanisms at early and long-term in which they are involved and how they contribute to cell survival via preventing the development and/or progression of deleterious cell phenotypes. Welander distal myopathy (WDM) is a muscle dystrophy characterized by adult-onset distal muscle weakness, prevalently impacting the distal long extensors of the hands and feet. WDM is an autosomal dominant disorder caused by a missense mutation (c.1362G>A; p.E384K) in the TIA1 (T-cell intracellular antigen 1) gene, which encodes an RNA-binding protein basically required for the posttranscriptional regulation of RNAs. We have developed a heterologous cell model of WDM to study the molecular and cellular events associated with mutated TIA1 expression. Specifically, we have analyzed how this mutation affects three regulatory functions mediated by TIA1: (i) control of alternative SMN2 (survival motor neuron 2) splicing; (ii) formation, assembly, and disassembly of stress granules; and (iii) mitochondrial dynamics and its consequences for mitophagy, autophagy, and apoptosis. Our results show that whereas WDM-associated TIA1 expression had only a mild effect on SMN2 splicing, it led to suboptimal adaptation to environmental stress, with exacerbated stress granule formation that was accompanied by mitochondrial dysfunction and autophagy. Our observations indicate that some aspects of the cell phenotype seen in muscle of patients with WDM can be recapitulated by ectopic expression of WDM-TIA1, highlighting the potential of this model to investigate the pathogenesis of this degenerative disease and possible therapeutics.

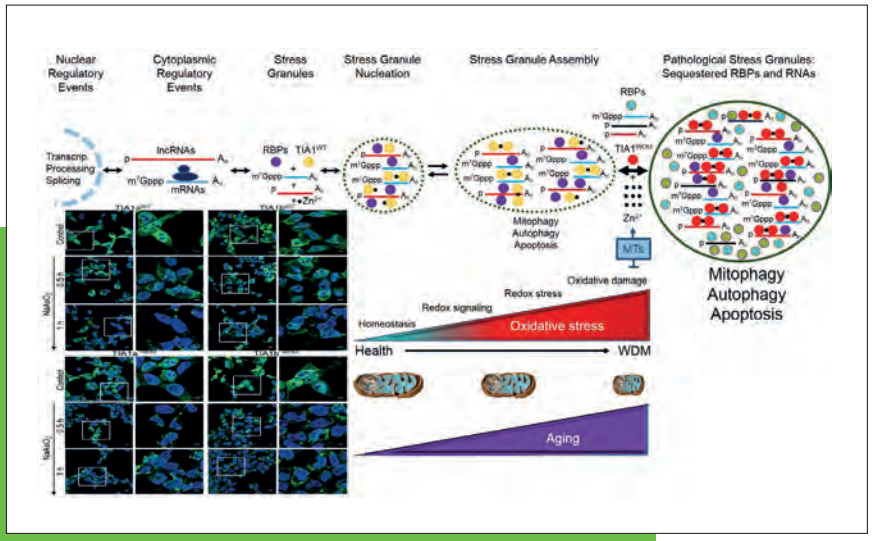


***Figure.*** *Working model to explain the pathophysiological processes of the dynamics of TIA1-dependent stress granule associated with proteostasis and Welander distal myopathy (WDM). Time-course of stress granule (SG) formation in NaAsO2 (arsenite)-treated FT293 cells. Fluorescence images of GFP-TIA1a/b$^{WT}$ (upper panels) or GFP-TIA1a/b$^{WDM}$ (lower panels) FT293 cells (green). Nuclei were stained with To-Pro3 (blue). Scale bars bars represent 10 $\mu$m. LncRNAs, long noncoding RNAs; RBPs, RNA-binding proteins; MTs, metallothioneins.*

## Publications

Carrascoso, I., Sánchez-Jiménez, C., Silion, E., Alcalde, J. and Izquierdo, J. M. (2019) A heterologous cell model for studying the role of T-cell intracellular antigen 1 in Welander distal myopathy. *Mol. Cell. Biol*. **39**, e0029918.

## CELL-CELL COMMUNICATION & INFLAMMATION

# MOLECULAR PATHOPHYSIOLOGY OF PERITONEAL INFLAMMATION AND FIBROSIS (PERINFIB)

***Principal Investigator:***
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***Postdoctoral Fellows:***
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Guadalupe González Mateo

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(Marie Curie, IMPROVE-PD)
Eva Arriero País
(Marie Curie, IMPROVE-PD)

***Technician:***
Javier Carrero Liroa
(Project Manager, IMPROVE-PD)

***Undergraduate and Master Students:***
Cristina Benítez García
(TFM 2019)

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## Research summary

Used to treat advanced chronic kidney disease, peritoneal dialysis (PD) consists of using the peritoneum as a semipermeable membrane across which diffusion and ultrafiltration take place. During PD, the peritoneum is exposed to bio-incompatible solutions that cause inflammation, angiogenesis and fibrosis, resulting in membrane failure. Our group has shown that mesothelial cells undergo an epithelial-mesenchymal transition (EMT) in response to the peritoneal insult. During the last 17 years, there have been reports in the literature that mesothelial EMT (also termed Mesothelial to Mesenchymal Transition, MMT) is a good marker for membrane failure and a therapeutic target for preventing PD-induced fibrosis and/or angiogenesis.

Recently, we considered whether the EMT plays a role in other peritoneal pathologies such as peritoneal metastasis and post-surgical adhesions. Peritoneal metastasis is a complication of abdominal carcinomas (e.g. ovarian carcinoma) for which there is no effective therapy. Progression of the metastatic implants is affected by Carcinoma-Associated Fibroblasts (CAFs), which can derive from several cell types. We have shown in human peritoneal implant biopsies that a subpopulation of CAFs derives from mesothelial cells through EMT. Our results also suggest that this EMT renders the peritoneum more receptive to implantation of tumor cells, contributes to the growth and vascularization of secondary tumors and that EMT is a therapeutic target in peritoneal carcinomatosis.

Adhesions are areas of fibrotic tissue that bind tissues and organs that would normally not be connected, and can be seriously life-threatening. Most adhesions are post-surgical. Histological analysis of human post-surgical adhesions has demonstrated that the mesothelial cells adjacent to the fibrotic tissue show signs of an EMT, suggesting that this could be an initial step in their development. The physiopathological processes involved in their formation remain unknown. Recent results have shown that mechanical injury is the main inducer of EMT during the formation of adhesions. Consequently, molecules involved in mechano-transduction, such as caveolin 1 (CAV-1), could play a role in the modulation of mechanical EMT and adhesion formation (Figure 1).

The aim of our work is to expand the knowledge of the pathological implications of the EMT of mesothelial cells and the molecular mechanisms that regulate this process, and to identify molecular targets for the design of therapeutic strategies, with possible applications in diseases associated with peritoneal fibrosis/angiogenesis, and in peritoneal metastasis.

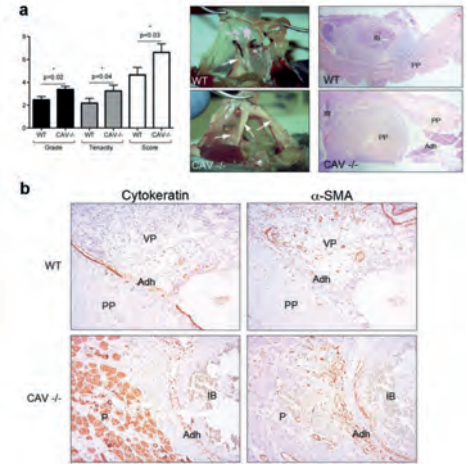


***Figure. a)*** *Peritoneal adhesion experiments, based on ischemic buttons, showed that Cav-1 KO mice have increased capacity to develop adhesions compared with WT mice.* ***b)*** *Immunohistochemical analysis on serial sections of peritoneal biopsies, showed that Cav-1 KO mice have exacerbated signs of mesothelial to mesenchymal transition, as demonstrated by the co-expression of cytokeratin and α-SMA in stromal fibroblast-like cells.*

## Publications


Díaz R, Sandoval P, Rodrigues-Diez RR, Del Peso G, Jiménez-Heffernan JA, Ramos-Ruíz R, Llorens C, Laham G, Alvarez-Quiroga M, López-Cabrera M, Ruiz-Ortega M, Bajo MA, Selgas R. (2020). Increased miR-7641 Levels in Peritoneal Hyalinizing Vasculopathy in Long-Term Peritoneal Dialysis Patients. *Int J Mol Sci*. **21**(16):5824.

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Selgas, R.*, Honda, K., López-Cabrera, M., Hamada, C., Gotloib, L. (2020) Peritoneal Structure and Changes as a Dialysis Membrane After Peritoneal Dialysis, in Nolph and Gokal's Textbook of Peritoneal Dialysis, 4th Edition, Springer Nature Switzerland (In press).

Jiménez-Heffernan, J.A., Del Peso Gilsanz, G., López-Cabrera, M., Selgas Gutierrez, R. (2020) De la histología a la función: El peritoneo como membrana dializante y biológicamente activa, en Tratado de Diálisis Peritoneal 3ª Edicion, Elsevier España. Jesús Montenegro Martínez, Ricardo Correa Rotter y Miguel Carlos Riella (Eds). ISBN: 978-84-9113-471-8.


## Awards and recognition

- Member of the External Scientific Advisory Board of "Instituto de Investigación del Hospital Ramón y Cajal (Madrid).

- Guest Editor of Special Issue of Frontiers in Physiology entitled "Peritoneal Dialysis and Its Local and Systemic Complications: From the Bench to the Clinic".

- Guest Editor of Special Issue of Cancers entitled "Molecular Biology of Ovarian Cancer: From Mechanisms of Intraperitoneal Metastasis to Therapeutic Opportunities.

## Patents

Inventors: Manuel López-Cabrera, Abelardo Aguilera, Rafael Selgas, Jutta Passlick-Deetjen, Janine Buechel, Sonja Steppan. Method and kit for diagnosing epithelial to mesenchymal transition of the peritoneum (EMT-Chip). German Patent Number: DE 102015115158.8. (Granted). Country of Priority: Germany, Date of Priority: 09/09/2015 Date of granting: 13/07/2017. International applications: PCT/EP2016/071149 WO2017042253 (A1). Extended Countries:USA patent: US 2018/0246098 A1. China patent: CN108449998 (A)-2018-08-24. European Patent (Granted): EP 3 347 715 B1 (Date of Granting: 26-08-2020). Ownership: Fresenius Medical Care AG & Co. KGaA.

Inventors: Abelardo Aguilera, Manuel López-Cabrera, Rafael Selgas, Jutta Passlick-Deetjen, Janine Büchel, Sonja Stephan. Pharmaceutical Compositions Containing Steviosides. Priority application EP 14 191 301.2 Date of Priority: October 31st, 2014. International applications: PCT/EP2015/074955 WO2016066672 (A1) Extended Countries: USA patent: US 2017/0304335 A1. Chinese Patent (Granted): CN 107073022 B (Date of Granting: 29-12-2020). Ownership: Fresenius Medical Care AG & Co. KGaA.

## International projects / Research networks

- Identification and Management of Patients at Risk – Outcome and Vascular Events in Peritoneal Dialysis (Acrómimo: IMPROVE-PD). H2020-MSCA-ITN-2018 (Marie S-Curie Innovative Training Networks) (Grant # 812699). EUROPEAN UNION, 2019-2022 Coordinator: Manuel López Cabrera (CSIC).

## CELL-CELL COMMUNICATION & INFLAMMATION

# IMMUNOMETABOLISM AND INFLAMMATION LAB

***Principal Investigator:***
María Mittelbrunn

***Postdoctoral Fellows:***
Elisa Carrasco Cerro
Jorge-Oller
Enrique Gabandé

***Predoctoral Fellows:***
Gabriela Grisel Desdín Micó
Gonzalo Soto Herrero
Manuel Montero

***Technician:***
Eva María Blanco Ruiz

***Undergraduate and Master Students:***
Pedro Acuña

***Visiting Scientists:***
Omar Dominguez-Amorocho

http://www.cbm.uam.es/mittelbrunn

## Research summary

The metabolism of immune cells needs to be reprogrammed to fulfill the specific energy demands that occur during the different phases of the inflammatory responses. In the case of T cells, naïve T cells are metabolically quiescent and have a low energy demand, which is mainly obtained from mitochondrial respiration. However, upon activation by antigen, T cells proliferate and differentiate into the different effector subsets. Their metabolism is profoundly rewired to accommodate the high energetic and biosynthetic demands of proliferation and effector function, by engaging preferentially glycolysis and glutaminolysis. Recently, Immunometabolism has emerged as a new field to boost immune responses for cancer immunotherapies, as well as to dampen autoimmune diseases. Pioneer studies by us and others have begun to elucidate the potential of targeting immunometabolism to prevent the onset of age-associated diseases and multimorbidity.

Interestingly, as in many other cells and tissues, T cells present mitochondrial decline with age losing their metabolic plasticity . Our working hypothesis is that age-associated mitochondrial decline in T cells enhances glycolytic metabolism over time, promoting the acquisition of a proinflammatory phenotype, that contributes to inflammaging, an state of low-grade chronic inflammation that appears in association with aging. To understand the contribution of T cell immunometabolism to inflammaging and age-associated disorders, my group has previously generated a mouse model of mitochondrial dysfunction in T lymphocytes by specifically deleting the mitochondrial transcription factor A (TFAM) in T cells.

We found that T lymphocyte-specific knockout of TFAM, does not only cause an immunometabolic dysfunction that drives T cell senescence, but actually causes a general, body-wide deterioration of health with multiple aging-related features, including metabolic, musculoskeletal, cardiovascular and cognitive alterations, altogether resulting in premature death (Desdin-Mico et al Science 2020). Our results place the metabolism of T cells at the crossroad between inflammation, senescence and aging, highlighting that immunometabolism can be a therapeutic target to delay aging and aging-associated diseases.

The second line of research is aim to investigate how changes in vascular metabolism during aging predispose to develop aortic aneurysms (Oller et al, Circulation, 2021) and neuroinflammation (Gabandé-Rodriguez, in preparation). Mitochondrial function of vascular smooth muscle cells is controlled by the extracellular matrix and drives the development of aortic aneurysms. We have investigated novel treatments that boosts vascular metabolism to reverse aortic aneurysm.

***Figure.*** *Compared with a normal mice (left), a mouse with defective mitochondria in its T cells , appears old.*

## Publications

Desdín-Micó G, Soto-Heredero G, Aranda JF, Oller J, Carrasco E, Gabandé-Rodríguez E, Blanco EM, Alfranca A, Cussó L, Desco M, Ibañez B, Gortazar AR, Fernández-Marcos P, Navarro MN, Hernaez B, Alcamí A, Baixauli F, Mittelbrunn M. (2020). T cells with dysfunctional mitochondria induce multimorbidity and premature senescence. *Science*. **368**(6497):1371-1376.

Soto-Heredero G, Gómez de Las Heras MM, Gabandé-Rodríguez E, Oller J, Mittelbrunn M. (2020). Glycolysis - a key player in the inflammatory response. *FEBS J*. **287**(16):3350-3369.

Gabandé-Rodríguez E, Gómez de Las Heras MM, Mittelbrunn M. (2019). Control of Inflammation by Calorie Restriction Mimetics: On the Crossroad of Autophagy and Mitochondria. *Cells*. **9**(1):82.

Carrasco E, Soto-Heredero G, Mittelbrunn M.(2019). The Role of Extracellular Vesicles in Cutaneous Remodeling and Hair Follicle Dynamics. *Int J Mol Sci.* **2019** 20(11):2758.

## Awards and recognition

- Invited Speaker, 1st Eurogeroscience conference: Madrid, 2019.

- Invited Speaker a BSCB/BSDB Spring Meeting University of Warwick (Birmingham), UK, Abril 2019.

- Keynote Speaker 1stBirmingham Inflammation, Repair and Ageing Conference,Univ of Birmingham, UK, 2020 (postponed 2021).

- President, Scientific Committee Fundación Alsmtrom.

- Member of Scientific advisory board of Fundacion Gadea Ciencia.

- PINP 2020 Award for CBMSO PhD students: Gabriela Desdín Micó.

## Patents

P202030906, 04/09/2020. Precursores de nad+ para uso en la prevención y/o tratamiento de aneurismas aórticos hereditarios. María Mittelbrunn and others.

## International projects / Research networks

- “Endolysosomal Mitochondria Crosstalk in Cell and organism homeostasis” 715322 EndoMitTalk . European Research Council (ERC). Funding: 1.498.000 € (2017-2022). Principal investigator.

- “Systemic and cellular interactions between cancer and metabolic signaling” METABOCANCER.Ministerio de Economía y Competitividad (Red de Excelencia). 2020-2021. Investigator.

## CELL-CELL COMMUNICATION & INFLAMMATION

# EXTRACELLULAR MATRIX REMODELING IN THE CARDIOVASCULAR SYSTEM

***Principal Investigator:***
Fernando Rodríguez Pascual

***Predoctoral Fellows:***
Tamara Rosell García

***Undergraduate and Master Students:***
Latifa Chenaina
(since September 2020)

http://www.cbm.uam.es/frodriguez

## Research summary

The extracellular matrix (ECM) constitutes an intricate molecular network that surrounds and integrates cells and tissues in multicellular organisms. Far beyond the traditional concept of static organic material, today it is widely accepted that the ECM is a highly dynamic biomaterial that provides tissues with their mechanical properties, serves as point of interaction for the anchorage and movement of cells, and regulates the bioavailability of several growth factors, among other functions. Therefore, proper synthesis and assembly of the components of the ECM is essential for cell and tissue homeostasis, and defects or alterations in these processes are associated with the development of several human disorders, particularly in the cardiovascular system. The biosynthesis of collagen, the main component of the ECM, is a complex and highly regulated process involving numerous steps, including chain association and folding, extracellular secretion, proteolytic processing and cross-linking. A set of prolyl- and lysyl-hydroxylases, glycosidases, isomerases and lysyl oxidases catalyze an extensive series of post-translational modifications, most of which are unique to collagen protein. In the last few years our group has investigated different aspects of this biosynthetic pathway. On one hand, we have focused an important part of our studies on lysyl oxidases (LOX), the enzymes responsible for the collagen cross-linking, where we have analyzed their proteolytic regulation and their biotechnological applications in regenerative medicine. Results from our group have shown the existence of a complex pattern for the proteolysis of the LOX enzymes, with multiple proteases contributing to the extracellular processing of these enzymes. Based on this knowledge, we have developed patent-protected protocols to enhance the capacity of cells to produce and deposit collagen in the ECM. On the other hand, it has been reported that the expression of several ECM components is upregulated by low oxygen levels. Within this context, we have investigated the molecular requirements for the hypoxia to increase the expression of ECM components. So far, our results have provided novel knowledge to understand the interplay of different transcription factors such as hypoxia inducible factors (HIF) and Smad proteins contributing to the effect of hypoxia on the ECM.

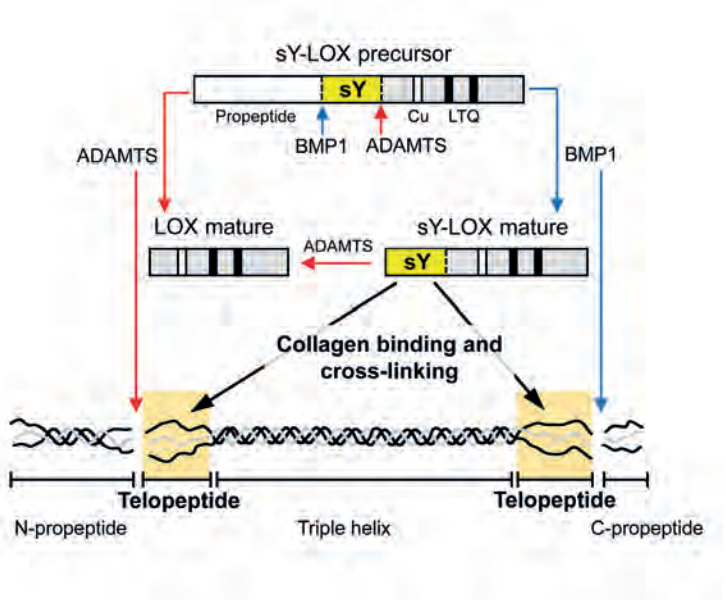



**Figure.** *Schematical model depicting the regulation of lysyl oxidase (LOX) by the proteases BMP1 and ADAMTS in the context of collagen processing. The diagram summarizes the ability of these proteases to cleave LOX into two different locations yielding mature LOX species with different affinities to bind collagen telopeptides based on the presence of a sulfotyrosine domain.*

## Publications

Rosell-García, T., Rodríguez-Pascual, F. (2020) Boosting collagen deposition with a lysyl oxidase/bone morphogenetic protein-1 cocktail. Methods in Cell Biology, **156**,259-270. Cell-derived Matrices-Part A. Academic Press Inc.

Rosell-García, T., Palomo-Álvarez, O., Rodríguez-Pascual, F. (2019) A hierarchical network of hypoxia-inducible factor and SMAD proteins governs procollagen lysyl hydroxylase 2 induction by hypoxia and transforming growth factor β1. *Journal of Biological Chemistry,* **294** (39),14308-14318.

Rosell-García, T., Paradela, A., Bravo, G., Dupont, L., Bekhouche, M., Colige, A., Rodriguez-Pascual, F. (2019) Differential cleavage of lysyl oxidase by the metalloproteinases BMP1 and ADAMTS2/14 regulates collagen binding through a tyrosine sulfate domain. *Journal of Biological Chemistry,* **294** (29)11087-11100.

Rodríguez-Pascual, F. (2019) How evolution made the matrix punch at the multicellularity party. *Journal of Biological Chemistry*, **294** (3)770-771.

## Awards and recognition

- Fernando Rodríguez Pascual. Member of the Editorial Board of Scientific Reports (Molecular Biology Section).

- Fernando Rodríguez Pascual. Member of the Editorial Board of Asia-Pacific Journal of Ophthalmology (Visual Sciences Section).

## Doctoral Theses

**Tamara Rosell García** (2020). Contribución de las enzimas lisil hidroxilasa 2 y lisil oxidasa al remodelado de la matriz extracelular. Implicaciones en la biosíntesis del colágeno fibrilar y su aplicación en la ingeniería de tejidos. Universidad Autónoma de Madrid. Supervisor: Fernando Rodríguez Pascual. *Cum laude.*

## International projects / Research networks

- The Glaucoma Foundation 2019 Research Grants. “Unraveling the Proteolytic Landscape Regulating LOXL1. Implications in the Development of Pseudoexfoliation Syndrome”. PI Fernando Rodríguez Pascual. 01/08/2019-31/07/2020.

## CELL-CELL COMMUNICATION & INFLAMMATION

# TETRASPANIN-ENRICHED MEMBRANE MICRODOMAINS IN EXTRACELLULAR VESICLES AND CELL ADHESION AND MIGRATION

***Principal Investigator:***
María Yáñez-Mó

***Predoctoral Fellows:***
Henar Suárez Montero
(until October 2019)
Victor Toribio Serrano
Beatriz Benayas López
(from March 2019)

***Technician:***
Soraya López-Martín

***Undergraduate and Master Students:***
Blanca Lacruz Pleguezuelos

***Visiting Scientists:***
Charles Williams
Aldo Emmanuel Pérez Rivera

http://www.cbm.uam.es/myanez

## Research summary

Our group is focused on the functional characterization of membrane microdomains based on tetraspanin proteins, which are involved in cell-cell adhesion and migration as well as in the biogenesis and cargo selection of extracellular vesicles (EVs). EVs represent a novel mechanism of intercellular communication and have a great potential as carriers of therapeutics or biomarkers for diagnosis in the liquid biopsy. Our translational efforts aim at exploiting the tools against tetraspanin molecules to develop new isolation, detection and quantification devices, as well as to develop synthetic exosome mimetics as reference materials or vaccination strategies.

In addition, tetraspanin enriched microdomains are connected to different cytoskeletal components and signalling pathways and have been shown to regulate different steps from the infectious cycle of several viruses, ranging from viral entry to budding but including also other aspects that are not so evidently linked to cell membranes such as viral replication. Therefore, a parallel research line in our group aims at stablishing the anti-viral potential of tetraspanin-targeted reagents as broad-spectrum therapeutics.

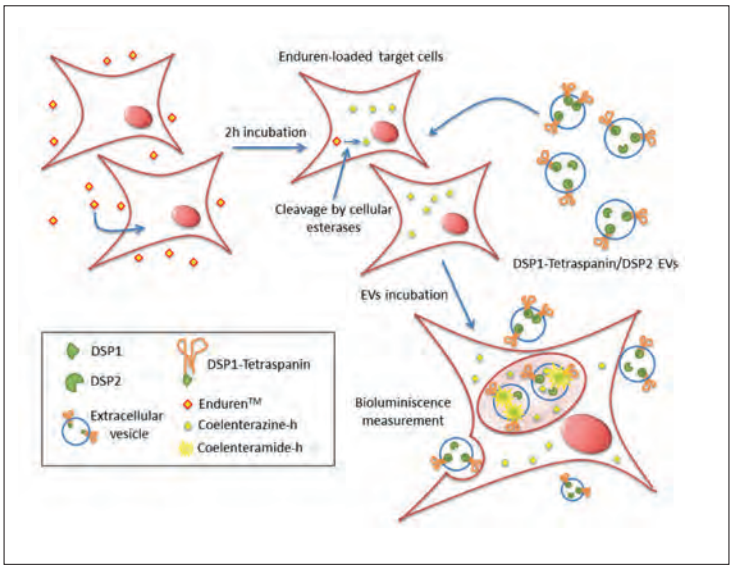



***Figure.*** *Scheme of the EV uptake assay based on Renilla-tagged tetraspanins. This assay provides a dynamic and very sensitive readout of EV uptake by the target cell allowing to discriminate between binding and uptake. Extracted from Toribio et al., Sci Reports (2019).*

## Publications

Benayas B, Sastre I, López-Martín S, Oo A, Kim B, Bullido MJ, Aldudo J, Yáñez-Mó M. (2020) Tetraspanin CD81 regulates HSV-1 infection. *Med Microbiol Immunol.* **209**(4):489-498.

García-Manrique P, Serrano-Pertierra E, Lozano-Andrés E, López-Martín S, Matos M, Gutiérrez G, Yáñez-Mó M, Blanco-López MC. (2020) Selected Tetraspanins Functionalized Niosomes as Potential Standards for Exosome Immunoassays. *Nanomaterials (Basel).* **10**(5):E971.

Torres-Gomez A, Sanchez-Trincado JL, Toribio V, Torres-Ruiz R, Rodríguez-Perales S, Yáñez-Mó M, Reche PA, Cabañas C, Lafuente EM. (2020) RIAM-VASP Module Relays Integrin Complement Receptors in Outside-In Signaling Driving Particle Engulfment. *Cells*. **9**(5):1166.

Salsano S, González-Martín R, Quiñonero A, López-Martín S, Gómez-Escribano AP, Pérez-Debén S, Yañez-Mo M, Domínguez F. (2020) Novel nonclassic progesterone receptor PGRMC1 pulldown-precipitated proteins reveal a key role during human decidualization. *Fertil Steril.* **113**(5):1050-1066.e7.

Suárez H, López-Martín S, Toribio V, Zamai M, Hernández-Riquer MV, Genís L, Arroyo AG, Yáñez-Mó M. (2020) Regulation of MT1-MMP Activity through Its Association with ERMs. *Cells*. **9**(2):348.

Rattila S, Dunk CEE, Im M, Grichenko O, Zhou Y, Yanez-Mo M, Blois SM, Yamada KM, Erez O, Gomez-Lopez N, Lye SJ, Hinz B, Romero R, Cohen M, Dveksler G. (2019) Interaction of Pregnancy-Specific Glycoprotein 1 With Integrin A5β1 Is a Modulator of Extravillous Trophoblast Functions. *Cells*. **8**(11). pii: E1369.

Cabañas C, Yáñez-Mó M, van Spriel AB. (2019). Editorial: Functional Relevance of Tetraspanins in the Immune System. *Front Immunol.* **10**:1714.

Toribio V, Morales S, López-Martín S, Cardeñes B, Cabañas C, Yáñez-Mó M. (2019) Development of a quantitative method to measure EV uptake. *Sci Rep*. **9**(1):10522.

Lozano-Andrés E, Libregts SF, Toribio V, Royo F, Morales S, López-Martín S, Valés-Gómez M, Reyburn HT, Falcón-Pérez JM, Wauben MH, Soto M, Yáñez-Mó M. (2019) Tetraspanin-decorated extracellular vesicle-mimetics as a novel adaptable reference material. *J Extracell Vesicles*. **8**(1):1573052.

Campos-Silva C, Suárez H, Jara-Acevedo R, Linares-Espinós E, Martinez-Piñeiro L, Yáñez-Mó M, Valés-Gómez M. (2019) High sensitivity detection of extracellular vesicles immune-captured from urine by conventional flow cytometry. *Sci Rep.* **9**(1):2042.

Calle A, López-Martín S, Monguió-Tortajada M, Borràs FE, Yáñez-Mó M, Ramírez MÁ. (2019) Bovine endometrial MSC: mesenchymal to epithelial transition during luteolysis and tropism to implantation niche for immunomodulation. *Stem Cell Res Ther.* **10**(1):23.

Jara-Acevedo R, Campos-Silva C, Valés-Gómez M, Yáñez-Mó M, Suárez H, Fuentes M. (2019) Exosome beads array for multiplexed phenotyping in cancer. *J Proteomics*. **198**:87-97.

## Awards and recognition

- Member of the Scientific committee of the 5th International GEIVEX Symposium 2019 (Granada November 6-8th 2019)

- Member of the organizing committee of the GEIVEX-UFV/TeNTaCLES 2020 Minisymposium (December 17th 2020, online)

- Moderator at the ISEV Workshop on Extracellular Vesicle Targeting to Cells and Tissues (Philadelphia, Feb 27th 2019)

- Speaker at the Web EV-Talks Series (https://www.youtube.com/watch?v=s6qnVRlNxsM&t=2686s)

- Teacher at the 1st online Specialization Course on Extracellular Vesicles (GEIVEX/UFV)

- Member of the Board of the non-profit association “Grupo español de innovación e investigación en vesículas extracelulares GEIVEX”

- Member of the Research Commission at IIS-IP

- Member of the Editorial Board of Scientific Reports

- Guest Editor for Frontiers in Immunology

- Research and Development contract between the CSIC, the FUAM and IMMUNOSTEP S.L.

## Patents

Valés Gómez, Mª Mar; Campos Silva, Carmen, Cáceres Martell, Yaiza; Reyburn, Hugh Thomson; Yáñez-Mó, María; Jara Acebedo, Ricardo. “Method for the detection and/or quantification of extracelular vesicles in fluid biological samples”. EP20382602.9. ES. 06/07/2020. CSIC/UAM.

## Doctoral Theses

**Henar Suárez Montero** (2019). “Papel de las tetraspaninas en la internalización y el tráfico de moléculas asociadas en modelos tumorales y de infección viral” UAM. Supervisor: María Yáñez-Mó. Outstanding “*cum laude*”.

## International projects / Research networks

- PI of a consolidated group in the Instituto de Investigaciones Sanitarias Princesa (IIS-IP).

- Translational NeTwork for the CLinical application of Extracellular VesicleS (TeNTaCLES). RED2018-102411-T. Universidad Autónoma de Madrid. Principal Investigator: Dr Yáñez-Mó. 01/01/2020-31/12/2021.

UNIT

GINÉS MORATA

# Specification, Reprogramming & Regeneration

TISSUE AND ORGAN HOMEOSTASIS

The Morata/Azpiazu group is investigating in *Drosophila* the role of cell competition as a tumour suppressing mechanism, with special emphasis in the cases in which oncogenic cells that evade cell competition, for they can provide insights on how tumours develop in animal tissues. Two mechanisms have been identified that allow oncogenic cells to escape cell competition; one is “group protection”; when oncogenic cells are grouped, some cells are beyond the reach of cell competition. The second is the acquisition by oncogenic cells of distinct cell-adhesion properties that preclude the cell-cell interactions required for cell competition. The group is also studying the mechanisms of molecular reprogramming during regeneration, in particular the acquisition of epigenetic marks in the chromatin and the transcription of retrotransposons.

The Baonza group is studying the regenerative response of the nervous system of *Drosophila* to various stress conditions and the role of glial cells in the response. Their results suggest that glial cells have intrinsic mechanisms that attenuate the apoptotic response to irradiation; a possible reason why glioma cells are radioresistant. One of the goals is to define the genetic and molecular mechanisms responsible of the insensitivity of glial cells to irradiation. Understanding those mechanisms is key for developing new strategies to repair neural damage.

The Martinez Serrano group has focused on understanding the biology of stem cells with the overall goal of targeting their therapeutic potential in Parkinson’s disease. *In vitro* studies reveal the importance of physical/topographical cues for generation of dopaminergic neurons while transplantation studies reveal better outcome in Parkinsonian mice. To understand the particular nature of the human brain, studies have been done on the development and maturation of brain tissue from stem cells; the generation of brain organoids.

The Sanchez-Herrero group is characterizing the function of Hox genes in the determination of the body patterns in *Drosophila*. A research line is the analysis of the role of the Abd-B gene in the development of the testis. The male gonads show a spherical shape in the larva but elongate and coil during pupal stages to achieve their adult form. This process depends on the interaction between the testis and muscle cells that migrate from the genital disc. The group has described the interactions between Abd-B, the Notch pathway and the contribution of MyosinID during the muscle migration and the appropriate coiling of the testes.

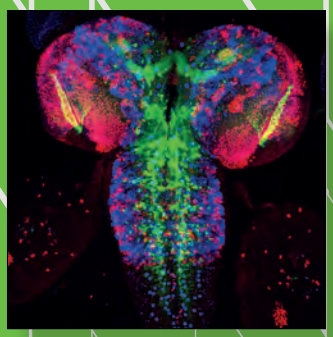
Highlights:

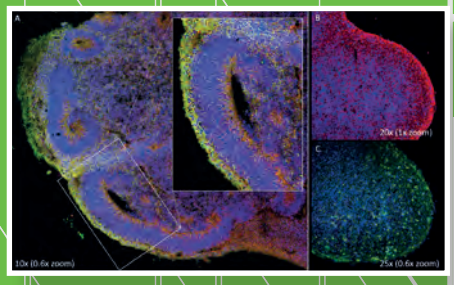
- Demonstration of the role of the JNK pathway in tumorigenesis and regeneration (*Open Biology* 2019).

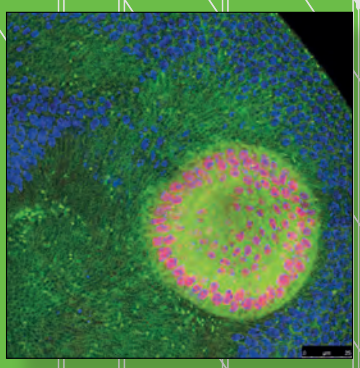
- Proposal of the “group protection” phenomenon as a pro-tumorigenic mechanism (*Seminars in Cancer Biology* 2020).

- Role of glial cells in the neuronal damage in the *Drosophila* visual system (*BioRxiv* 2020, in revision in *Plos Biology*).

- Genes implicated in the formation of the *Drosophila* testes (*Develop. Biol*, 2019).







**ANTONIO BAONZA CUENCA /**
**FERNANDO J. DÍAZ BENJUMEA**

MECHANISMS OF STRESS RESPONSE IN THE *DROSOPHILA* NERVOUS SYSTEM

**ALBERTO MARTÍNEZ SERRANO**

BIOLOGY OF HUMAN NEURAL STEM CELLS. POTENTIAL FOR CELL AND GENE THERAPY IN NEURODEGENERATION

**GINÉS MORATA / NATALIA AZPIAZU**

TUMOROGENESIS AND REGENERATION IN *DROSOPHILA*

**ERNESTO SÁNCHEZ-HERRERO**

SEGMENTAL SPECIFICATION AND PATTERN FORMATION IN *DROSOPHILA*

## SPECIFICATION, REPROGRAMMING & REGENERATION

# MECHANISMS OF STRESS RESPONSE IN THE *DROSOPHILA* NERVOUS SYSTEM

***Principal Investigators:***
Antonio Baonza Cuenca
Fernando J. Díaz-Benjumea

***Scientific Staff:***
Pilar Herrero Solans

## Research summary

Nervous system cells can be expose to a wide range of stress conditions, such as infection, hypoxia or DNA damaged by ionizing radiation (IR). Cells can response to stressful stimuli in various ways, from the activation of survival pathways, to the induction of apoptosis that eventually eliminates damaged cells. When insults result in neural damage, a regenerative response aims to preserve the structural integrity and function of the nervous system is induced. Glial cells mediate this response. Therefore, understanding the underlying mechanisms that control glial response after damage in terms of first, number and cell types, and secondly, signalling pathways that control their response, is key for developing strategies to repair the damaged tissue.

Although our knowledge about the genetic factors promoting glial regenerative response has greatly improved over the past decade, there are still many important issues about this process that remains largely unknown. To obtain a complete understanding of how these processes are regulated, it is essential to use model organisms that allow us in vivo studies, in the context of the complex interactions that take place among the different cell types that are involved. To address these issues, we take advantage of the unique developmental features of the *Drosophila* eye imaginal disc.

Glial-derived tumors (gliomas) are the most prevalent and malignant brain tumours in humans. Radiotherapy is considered the treatment of choice for gliomas, nonetheless, the efficacy of this therapeutic approach is often hampered by a marked radio-resistance of tumoural cells. In this sense, the identification of the molecular mechanisms that attenuate or block the resistance to radiation of glioma cells is essential to generate an effective treatment against this type of tumors.

Data from our lab using *Drosophila* melanogaster as a model system suggest that normal glial cells have intrinsic mechanisms that attenuated the apoptotic response after irradiation. This could be one of the underlying causes of why glioma cells are radioresistant

Our goal is to define the genetic and molecular mechanisms that block or attenuate the apoptotic response to IR in glial cells. This is relevant for understanding the mechanisms that confer high intrinsic resistance of glioma cells to irradiation.

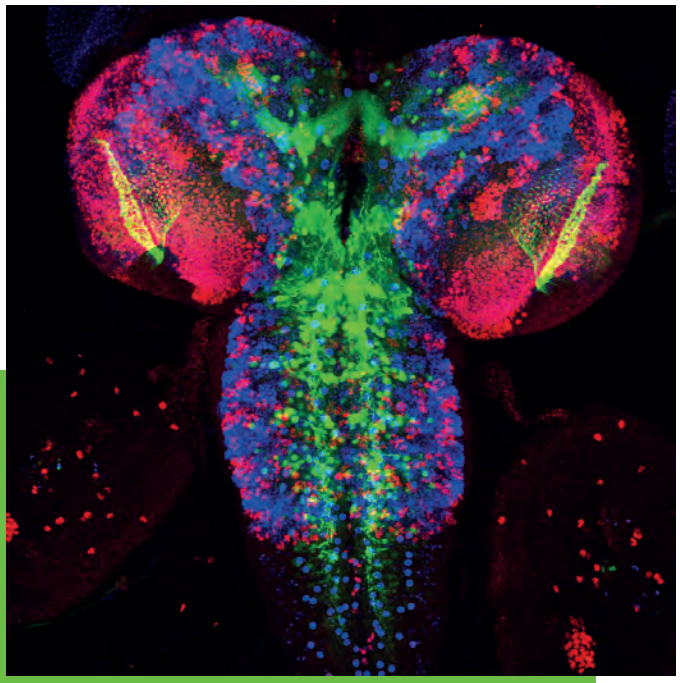


***Figure.*** *Expression of Elav (green; neurons), Asense (red; neuroblasts) and Prospero (blue; GMCs) in a ganglio of third instar larva.*

## Publications

Velarde SB, Quevedo A, Estella C, Baonza, A (2020). Dpp and Hedgehog promote the Glial response to neuronal damage in the developing *Drosophila* Visual system. *BioRxiv* (Preprint).

Diaz-de-la-Peña, L., Maestro-Paramio, L., Diaz-Benjumea, F.J., Herrero, P (2020). Temporal groups of lineage-related neurons have different neuropeptidergic fates and related functions in the *Drosophila melanogaster* CNS. *Cell Tissue Res* **381**, 381–396.

Blom-Dahl, D., Córdoba, S., Gabilondo, H., Carr-Baena, P.M., Díaz-Benjumea, F.J., Estella, C., (2020). *In vivo* analysis of the evolutionary conserved BTD-box domain of Sp1 and Btd during *Drosophila* development. *Dev Bio*l **466**, 70-89.

## Doctoral Theses

**Javier Barrio Perez** (2020). Caracterizacion de nuevas funciones de altered disjunction/monopolar spindle 1 en *Drosophila melanogaster.* Universidad Autónoma de Madrid. Septiembre 2019. Supervisor:Antonio Baonza.

**Sergio Benjamin Velarde Rangel** (2019). Respuesta regenerativa Glial en el disco imaginal de ojo de *Drosophila melanogaster.* Universidad Autónoma de Madrid. Septiembre 2019. Supervisor:Antonio Baonza.

## SPECIFICATION, REPROGRAMMING & REGENERATION

# BIOLOGY OF HUMAN NEURAL STEM CELLS. POTENTIAL FOR CELL AND GENE THERAPY IN NEURODEGENERATION

***Principal Investigator:***
Alberto Martínez Serrano

***Scientific Staff:***
Marta Pérez Pereira

***Postdoctoral Fellows:***
Silvia García-López
Anna Nelke

***Predoctoral Fellows:***
Marina Rodriguez Rubio
(since March 2019)
Brina Stančič
(since October 2019)
Miguel Esteban Lucía
(since October 2019)
Camille Baumlin
(until October 2020)
Sandrina Campos Maçãs
(until October. 2020)
Theresa SP Rothenbücher
(until July 2020)
Judith Estengre Pérez
(until July 2020)

***Visiting Scientists:***
Mª Teresa Alameda
(IMDEA Nanociencia)

https://www.cbm.uam.es/amserrano

## Research summary

The concept of treating diseases with replacement cells in not new; blood transfusions, skin grafts and organ transplantation are all forms of cell replacement therapy. Many neurological diseases, like PD, are the result of cell death or degeneration. The exponentially growing impairment/death rate of dopaminergic neurons (DAn) in the midbrain's *substantia nigra* (the A9 subgroup) in PD limits the therapeutic window of the treatments available that are known to increase the quality of life of patients although none can prevent the progression of PD.

Consequently, repairing damaged tissue becomes the goal; when cell loss cannot be prevented, cell replacement holds the key to recovery. Cell replacement therapy for PD is based on the concept that DAn implanted ectopically may functionally restore and maintain the DA levels lost in the disease. Clinical research using human fresh fetal ventral mesencephalic (VM) tissue (hfVM, containing some DAn precursors and many other cell types) provided proof of principle of the therapeutic efficacy of dopaminergic transplants on a long-term basis. However, limitations in hfVM supply, along with the variability of results of different clinical trials and the appearance of graft-induced dyskinesias in some patients, have precluded the implantation of tissue transplantation as a clinical therapy. In this context, research on the basic biology of human stem cells acquires special relevance. Our research group is interested in the basic biology of stem cells and the developmental events leading to maturation of neuronal derivatives of use in the study of the human brain and the development of novel cell-based therapies for neurodegenerative diseases (e.g. Parkinson's and Alzheimer's disease).

We have studied the trophic actions of human neural and mesenchymal stem cells in experimental in vivo models of PD focusing on the parallelism between pathological changes occurring in the brain vs. neurological and motor alterations. With a multidisciplinary approach, we have worked in the development of the technology for externally controllable bioimplants of therapeutic cells on-demand. These bioimplants consisting in multifunctional leaky optoelectrical fiber for potential neuromodulation and as a cell substrate for application in combined optogenetic stem cell therapy.

With the aim of minimizing the number of laboratory animals used for basic research while increasing the body of knowledge on the biology human neural tissue we have developed a research line devoted to the generation of human cerebral organoids with improved features facilitating patterning studies and useful for improving current preclinical research testing.

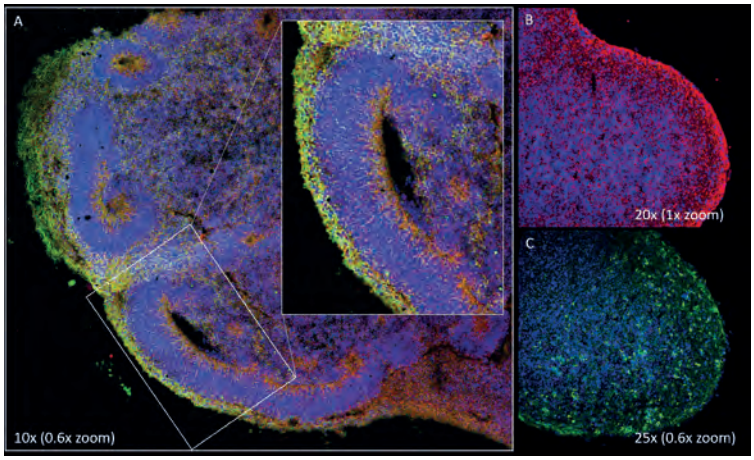



*Figure. Immunohistochemistry of a cerebral organoid (A) compared to a 3D neural aggregate (B, C).*

*A. IHC for nestin (red, labelling NSCs) and beta-III-tubulin (green, labelling neurons). The inset illustrates the spatial organization of the tissue (40 days maturation; xeno-free h-iPSCs)); B and C) Details of a 3D neural tube (3 mm total length, one month maturation in culture) prepared from human forebrain NSCs. Staining in B corresponds to nestin (red) and, in panel C, beta-III-tubulin (green). All tissues were counterstained with DAPI to identify nuclei. Magnification is indicated in the individual panels.*

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Okarmus J, Bogetofte H, Schmidt SI, Ryding M, García-López S, Ryan BJ, Martínez-Serrano A, Hyttel P, and Meyer M. (2020). Lysosomal perturbations in human dopaminergic neurons derived from induced pluripotent stem cells with PARK2 mutation. *Sci Rep*. **10**(1):10278.

Kajtez J, Buchmann S, Vasudevan S, Birtele M, Rocchetti S, Pless CJ, Heiskanen A, Martinez-Serrano A, Parmar M, Lind JU and Emnéus J. (2020). 3D-Printed soft lithography for complex compartmentalized microfluidic neural devices. *Adv Sci* (Weinh);**7**(16):2001150.

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Hey SM, Jensen P, Ryding M, Martínez Serrano A, Kristensen BW, Meyer M. (2019). Nonhypoxic pharmacological stabilization of Hypoxia Inducible Factor 1α: Effects on dopaminergic differentiation of human neural stem cells *Eur J Neurosci*. **49**(4):497-509.

Coronel R, Lachgar M, Bernabeu-Zornoza A, Palmer C, Domínguez-Alvaro M, Revilla A, Ocaña I, Fernández A, Martínez-Serrano A, Cano E and Liste I. (2019) Neuronal and glial differentiation of human neural stem cells is regulated by Amyloid Precursor Protein (APP) *Levels Mol Neurobiol.* **56**(2):1248-61.

Bernabeu-Zornoza A, Coronel R, Palmer C, Calero M, Martínez-Serrano A, Cano E, Zambrano A and Liste I. (2019) Aβ42 peptide promotes proliferation and gliogenesis in human neural stem cells. *Mol Neurobiol*. **56**(6):4023-36.

## Doctoral Theses

**Anna Nelke** (2019) "Differential effects of neural stem cell therapy in adult and middle-aged Parkinsonian mice". UAM. Supervisors: Marta P. Pereira and Alberto Martínez Serrano.

**Theresa SP Rothenbücher** (2020) "Engineered brain organoids. Developing an improved and larger human brain model *in vitro".* UAM. Supervisors: Alberto Martínez Serrano and Marta P. Pereira.

## International projects / Research networks

- European Training Network for Cell-based Regenerative Medicine_Training4CRM. Marie Sklodowska-Curie Innovative Training Networks (H2020-MSCA-ITN-2016). Desde 01/11/2016 hasta 31/10/2020

- Training for Advanced Stem Cell Technologies in Neurology (ASCTN-Training). Marie Sklodowska-Curie Innovative Training Networks (H2020-MSCA-ITN-2018). Desde 01/10/2018 hasta 30/09/2022

- Red Temática de Investigación en Terapia Celular. TerCel Instituto de Salud Carlos III. Programa RETICS, (Ref. RD16/0011/0032)

## SPECIFICATION, REPROGRAMMING & REGENERATION

# TUMOROGENESIS AND REGENERATION IN *DROSOPHILA*

***Principal Investigators:***
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Natalia Azpiazu Torres

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***Postdoctoral Fellow:***
Noelia Pinal Seoane

***Predoctoral Fellows:***
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Juan Manuel García Arias
(since October 2020)

***Technicians:***
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Rosa M ª González Herrera

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Celia Contreras Andrés (TFG)
Alexandra Rico Gil (TFG)

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## Research summary

Following the experimental line initiated some years ago, during the 2019-2020 period a principal focus of our research has been the analysis of the role of the Jun N-Terminal Kinase (JNK) pathway in processes like tumorigenesis and regeneration, using *Drosophila* imaginal discs as a model system. Previous work from our lab (Martin et al 2017; Pinal et al 2018) has demonstrated a direct involvement of JNK in both processes. Moreover, in collaboration with the Igaki group in Kyoto we have recently shown that it plays also a role in wound repair by extruding damaged cells (Iida et al 2019).

Our studies have been extended to investigate the role of JNK in various cases on tumorigenesis caused by mutations in Tumour Suppressing Genes (TSG) like *scribble, erupted* and *polyhomeotic.* In the cases of scribble and *polyhomeotic* we have shown that isolated mutant cells suffer oxidative stress (Pinal et al 2019 and unpublished observations) and generate high levels of Reactive Oxygen Species (ROS), which lead to the activation of JNK and eventually their elimination by apoptosis. The conclusion from all these experiments is the JNK is the key tool used by the cell competition process to remove undesirable cells from the imaginal discs (reviewed in Morata and Calleja 2020. New observations, still unpublished, about the behavior of *polyhomeotic* mutant cells, forcefully support this view.

Because of the critical importance of the JNK pathway in the processes we are investigating, we have performed a RNA screen to identify JNK target genes that are expressed differentially in tumorigenesis. Since JNK is also associated with human cancers, we searched for target genes that are conserved and their expression is altered in human tumours. So far this screen has identified eight new conserved JNK target genes that are required for the development of tumours in *Drosophila*. These genes are being characterized at the functional and molecular level.

We are also interested in the mechanisms that govern regeneration in *Drosophila*. Our previous studies have shown that ablated domains are recovered by cells from nearby domains. This process implies a resetting of the already determined fate of the regenerating cells, that we are analyzing at the level of their chromatin. Our previous results show that open chromatin states precede the formation of close heterochromatic regions, and that transcription of retro-transposons is needed for the chromatin to be remodeled.

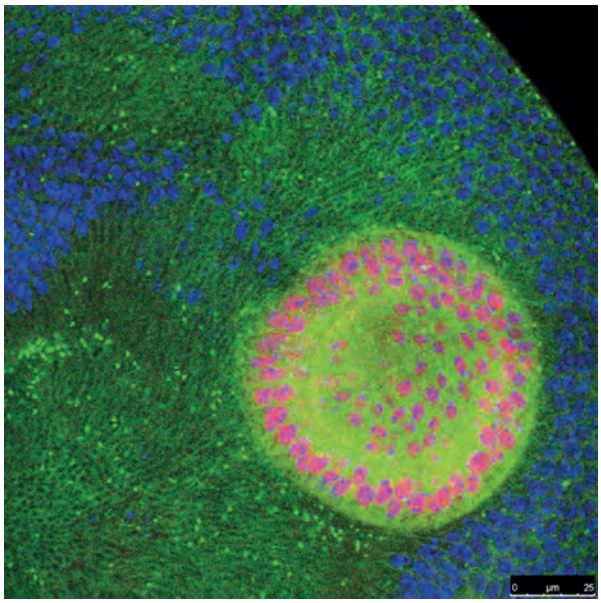

***Figure.*** *Mutant polyhomeotic clone in the wing imaginal disc of Drosophila, marked red by the Red Fluorescent Protein (RFP). The clone shows a high level of myosin expression (green) and forms a round vesicle, indicating it is sorting out from surrounding tissue. Nuclei stained with TOPRO.*

## Publications

Lawrence, PA, Casal, J. de Celis J and Morata, G. “A refutation to Abbasi R, Marcus JM. (2019). A new A-P compartment boundary and organizer in holometabolous insect wings.” *Scientific Reports* **9**(1):7049.

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Morata, G. and Calleja, M. (2020) “Cell competition and tumorigenesis in the imaginal discs of *Drosophila*” *Seminars in Cancer Biology* **63**, 19-26.

## SPECIFICATION, REPROGRAMMING & REGENERATION

# SEGMENTAL SPECIFICATION AND PATTERN FORMATION IN *DROSOPHILA*

***Principal Investigator:***
Ernesto Sánchez-Herrero Arbide

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(until June 2020)
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(until September 2019)

***Technician:***
María Paloma Martín Fernández

***Undergraduate and Master Students:***
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(until June 2019)
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(until June 2019)
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(October 2019 - June 2020)
Jesús Díaz Gamero
(October 2019 - June 2020)

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## Research summary

The Hox genes are a group of genes required for the specification of the antero-posterior axis in bilaterians. We have studied the role of two of these genes, Ultrabithorax (Ubx) and Abdominal-B (Abd-B), in organ formation in *Drosophila melanogaster*. Ubx regulates the shape of the haltere, a small dorsal appendage of the third thoracic segment homologous to the wing, which is present on the second thoracic segment. Halteres have a globular shape, in contrast to the flat one of wings, but both shapes seem to be important for a correct flight. We have previously shown that Ubx down-regulates Metalloproteinase expression and activity to prevent degradation of the Extracellular Matrix (ECM) in the haltere disc, and in this way Ubx maintains the haltere globular form by blocking adhesion of dorsal and ventral surfaces. We have also demonstrated that Ubx down-regulates in the haltere disc the expression of myospheroid, which encodes a β subunit of the integrin dimer, of blistery, coding for the integrin-associated protein Tensin, and of the *Drosophila* Serum Response Factor (DSRF), coded by the blistered gene. The down-regulation of integrin expression and activity, as well as the prevention of ECM elimination, both controlled by Ubx, determines the globular shape of the haltere as opposed to the flat one of the wing.

We have also analyzed the role of the Hox gene Abdominal-B (Abd-B) in *Drosophila* testes development. The male gonads show a spherical shape in larva but elongate and coil during pupal stages to achieve their adult size and shape. This change depends on the interaction between the testis and muscle cells from the genital disc, a structure that develops at the back of the larva. These muscle cells migrate and surround the gonad in pupa, and at the same time the testis elongate and coil. We have found that Abd-B regulates muscle cell migration, which also needs the activity of the Notch and sex determination pathways. Testes coil dextrally, as viewed from their tip to their base, and the coiling direction depends on the expression of MyosinID in the muscles surrounding the testis. Abd-B is also needed for this dextral direction of coiling, possibly by regulating the expression of MyosinID, and in the absence of Abd-B testes barely elongate and coil abnormally. The results with the Ubx and Abd-B genes help to understand how Hox genes contribute to the formation of different organs.

***Figure.*** *Drosophila male testes and internal genitalia, stained with Tropomyosin (in green), marking muscle tissue, and Topro (in purple), marking nuclei.*

## Publications

Rice, G., David, J. R., Kamimura, Y., Masly, J. P., Mcgregor, A. P., Nagy, O., Noselli, S., Nunes, M. D. S., O'Grady, P., Sánchez-Herrero, E., Siegal, M. L., Toda, M. J., Rebeiz, M., Courtier-Orgogozo, V. and Yassin, A. (2019). A standardized nomenclature and atlas of the male terminalia of *Drosophila melanogaster*. *Fly* **13**, 51-64.

Romero-Pozuelo, J., Foronda, D., Martín, P., Hudry, B., Merabet, S., Graba, Y. and Sánchez-Herrero, E. (2019). Cooperation of axial and sex specific information controls *Drosophila* female genitalia growth by regulating the Decapentaplegic pathway. *Dev Biol*. **454**, 145-155.

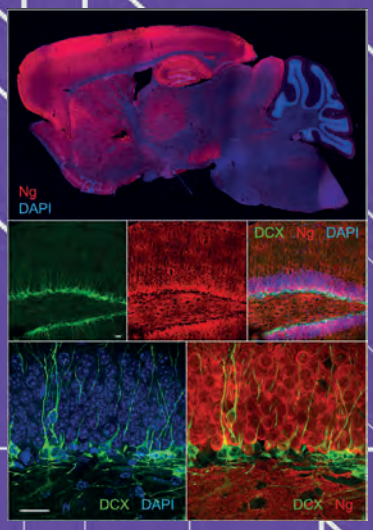
## Doctoral Theses

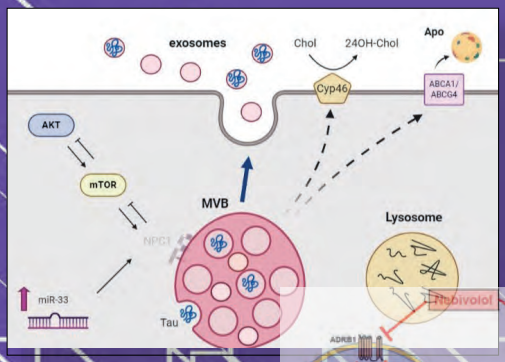
**Celia García Cortés** (2020). "Estudio de la función del gen Hox Ultrabithorax en la morfogénesis del halterio de *Drosophila melanogaster*". Autónoma University, Madrid. Supervisor: Ernesto Sánchez-Herrero. International mention.

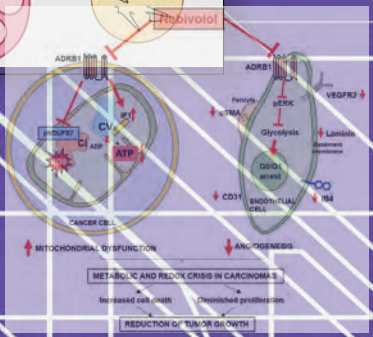
**Rafael Alejandro Juárez Uribe** (2019). "Estudio de la expresión de genes Hox y de la función de la proteína "Homeodomain interacting protein kinase" en procesos de regeneración en *Drosophila melanogaster*"- Autónoma University, Madrid. Supervisor: Ernesto Sánchez-Herrero.

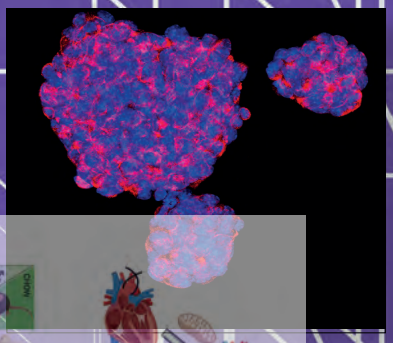
# Physiological and Pathological Processes

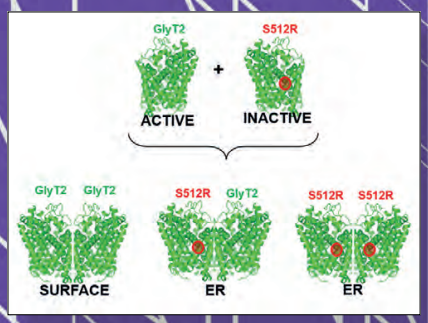
## PROGRAM



# UNITS

## MOLECULAR NEUROPATHOLOGY



## METABOLIC AND SIGNALING NETWORKS IN DISEASE

UNIT

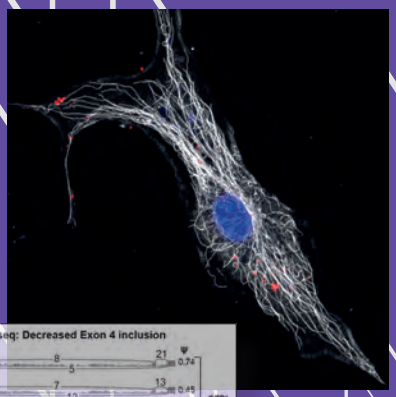
BEATRIZ LÓPEZ-CORCUERA

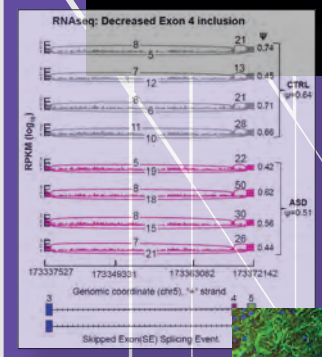
# Molecular Neuropathology

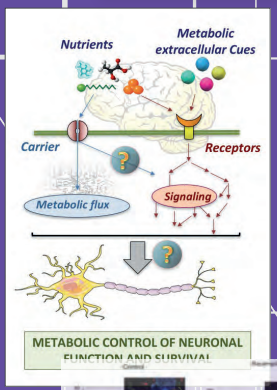
PHYSIOLOGICAL AND PATHOLOGICAL PROCESSES

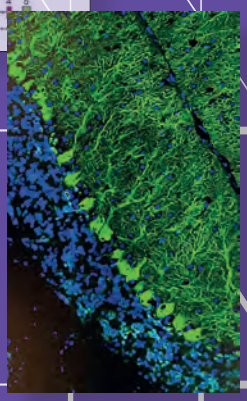
At the **Molecular Neuropathology Unit**, we carry out basic research aimed at understanding the most intimate mechanisms of healthy brain function, in order to then identify the processes that lead to the occurrence of human nervous system disorders. Our studies take place at molecular, cellular -both neuronal and non-neuronal- and circuit levels and are aimed to generate knowledge connecting brain activities to whole brain function leading to complex behavior. For these purposes, the scientific expertise of the members of this unit covers all areas of neurobiology, including genetics, biochemistry, cell and molecular biology, electrophysiology, animal behavior, computational biology, and the research is properly assisted by expert technical staff driving cutting edge facilities comprising animal facility, bioinformatics, flow cytometry, fermentation, genomics and mass sequencing, electron, optical and confocal microscopy, proteomics, transgenesis, which permit using complex techniques and carrying out demanding experiments. Hence, our recent research has made valuable contributions addressing fundamental neurobiological questions. We have made important advances to the understanding of the physiological bases of neuronal differentiation, survival and plasticity not only during nervous system development but also in the adulthood and at the old age. We have importantly contributed to the comprehension of the processes of cell regeneration and replacement. All these issues are crucial for the comprehension of the basis of memory, learning and human behavior.

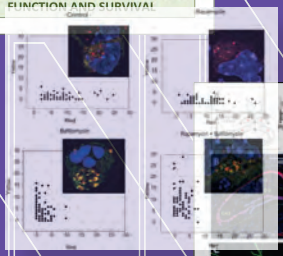
In addition to our normal brain physiology studies, in our unit we emphasize on the transfer to the clinics and to the pharmaceutical and biotechnology industry the scientific information we obtain in physiological and pathological systems. Some of our studies are dedicated at understanding the molecular mechanisms underlying brain pathologies both hereditary and sporadic, neurological and psychiatric such as Ataxias, Hyperekplexia, Alzheimer's disease, Huntington's and Parkinson's disease, Niemann Pick A and C disease, Autism, Bipolar disorder and Schizophrenia. In addition, from the alterations detected in physiological studies, we obtain clinically relevant information to several pathologies. This is the case for the analysis of defects in synaptic plasticity, which have been appreciated in multiple neurological diseases such as Schizophrenia, various forms of mental retardation or Alzheimer's disease. The study of nervous system myelination, altered in demyelinating diseases such as Multiple Sclerosis or Leukodystrophies, currently orphans of an effective treatment, gives clues for therapy. The study of the adult hippocampal neurogenesis has an emerging therapeutic potential in the treatment of neurodegenerative diseases, such as Alzheimer's disease and related tauopathies, and psychiatric diseases. The advances in the knowledge of the molecular mechanisms of pain offers a research avenue for the development of pathological pain treatments. Understanding the laws that govern these aspects is crucial for pursuing and revealing the fundamental principles and processes underlying memories, learning, thoughts and complex behaviors.

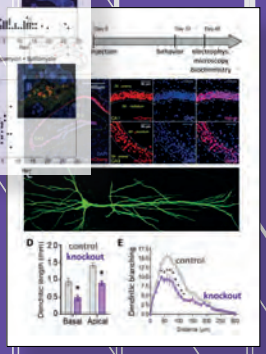


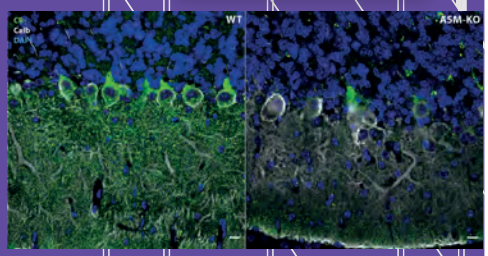







**JESÚS ÁVILA / FÉLIX HERNÁNDEZ**

TAU FUNCTION AND DYSFUNCTIONS IN ALZHEIMER DISEASE

**MARÍA JESÚS BULLIDO**

PATHOGENIC MECHANISMS OF ALZHEIMER'S DISEASE

**JAVIER DÍAZ-NIDO**

PHYSIOPATHOLOGY AND THERAPY OF NEURODEGENERATIVE DISEASES: FRIEDREICH'S ATAXIA

**FCO. JAVIER DÍEZ GUERRA**

MOLECULAR BASES OF NEURONAL PLASTICITY

**CARLOS DOTTI**

LABORATORY OF SURVIVAL AND PLASTICITY IN THE AGING BRAIN

**JOSÉ ANTONIO ESTEBAN**

MECHANISMS OF SYNAPTIC PLASTICITY, AND CONTRIBUTION TO COGNITIVE FUNCTION

**ALFREDO GIMÉNEZ-CASSINA**

INTEGRATION OF CELL SIGNALING AND ENERGY METABOLISM IN THE BRAIN

**MARÍA DOLORES LEDESMA**

LIPIDS IN NEURONAL PHYSIOLOGY AND PATHOLOGY

**MARÍA LLORENS-MARTÍN**

ADULT NEUROGENESIS AND NEURODEGENERATIVE DISEASES

**BEATRIZ LÓPEZ-CORCUERA**

FISIOPATHOLOGY OF GLYCINE TRANSPORTERS IN GLYCINERGIC NEUROTRANSMISSION: HYPEREKPLEXIA AND PAIN

**JOSÉ LUCAS**

MOLECULAR BASIS OF HUNTINGTON'S DISEASE AND OTHER CENTRAL NERVOUS SYSTEM DISORDERS

**CLAUDIO TOMA**

THE GENETICS OF PSYCHIATRIC DISEASES

**FRANCISCO WANDOSELL / BEATRIZ CUBELOS**

MOLECULAR MECHANISMS OF NEURODEGENERATION

**FRANCISCOP ZAFRA / EVA PORLAN**

MOLECULAR BASIS OF NEUROPROTECTION AND NEUROREPAIR

## MOLECULAR NEUROPATHOLOGY

# TAU FUNCTION AND DYSFUNCTIONS IN ALZHEIMER DISEASE

***Principal Investigators:***
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Alejandro Antón

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Juan R. Perea
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***Technicians:***
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Nuria de la Torre

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## Research summary

Tau is a neuronal protein involved, directly or indirectly, in different neuronal functions. Tau dysfunction could result in neurodegenerative disorders, tauopathies, being Alzheimer disease the most relevant one. Tau functions depend on its subcellular localization and binding to specific neuronal components. Our objective is to analyze these tau functions and possible new ones.

During 2019 and 2020 relevant results were obtained in the group, leaded by Dr. Llorens-Martín, on Adult Hippocampal Neurogenesis. Now Dr. Llorens-Martín's group is independent and is working on that area. Thus, we have not included Dr. María Llorens-Martín and her group here, since now they are working in an independent way.

Also, during that 2019-2020 period, we have analyzed the structural and functional differences between human and tau proteins by focusing in an extra aminoacid sequence only present in human tau, that has a function in axonal transport of some proteins, also we have studied the secretion mechanism that results in the presence of extracellular tau, or the role of tau protein in the localization of NMDA extrasynaptic receptors. Since tau is a phosphoprotein mainly modified by GSK3β kinase, we have studied the consequences of GSK3β overexpression under CaMKII promotor in neuronal cells, or under GFAP promotor, in neuronal precursor cells and in astrocytes. Different and, in overexpression under those different promoters.

Mainly, in the group headed by Dr. Hernández, the role of tau presence in glia cells (oligodendrocytes, astrocytes and microglia) was analyzed, and it was also found that different cell surface receptors could be involved in the interaction of extracellular tau with neurons or with glia cells.

Also, a protective role of tau protein was described in some types of gliomas, in which that presence could decrease the proliferation of tumor cells.

Finally, our group has done several collaborations with other groups located inside or outside of the CBMSO, in aspects related to Alzheimer disease or other neurodegenerative disorders or aging. About aging, we have described the rejuvenation of some old granule neurons, located at hippocampal area upon expression of the reprogramming (Yamanaka) factors.

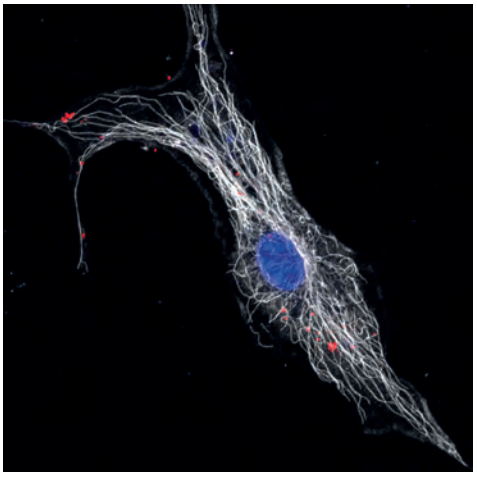

***Figure.*** *Microglia internalizing extracellular tau protein (red). The white signal corresponds to the microtubules of the cell.*

## Publications


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## Doctoral Theses

**Jesús Merchán Rubira** (2020). Caracterización del modelo murino de tauopatia P301S: alteraciones en el sistema nervioso periférico y central. Félix Hernández. Universidad Autónoma de Madrid.

**Juan Ramón Perea Úbeda-Portugués** (2020). Efecto de tau en la respuesta inflamatoria de la microglía. Marta Bolós y Jesús Avila. Universidad Autónoma de Madrid.

**Alberto García Rodríguez** (2020). Envejecimiento, Alzheimer y reprogramación celular *in vivo*. Efecto de la sobreexpresión de GSK-3B y de los factores de Yamanaka en el sistema nervioso central. Félix Hernández. Universidad Autónoma de Madrid.

## International projects / Research networks

- Pertenencia a CIBERNED

## MOLECULAR NEUROPATHOLOGY

# PATHOGENIC MECHANISMS OF ALZHEIMER'S DISEASE

***Principal Investigator:***
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María Recuero Vicente

***Postdoctoral Fellows:***
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(March 2019-Sept 2020)

***Technicians:***
Isabel Sastre Merlín
Paula Pérez González
(Feb. 2020- )

***Undergraduate and Master Students:***
Víctor Mejías Pérez
(Nov.2019- )
Jaime Morales García
(Oct. 2020- )
Jorge Vindel Alfageme
(Nov. 2019-May 2020)
Laura Zamorano Horcajada
(Oct. 2019-Jun 2020)

http://www.cbm.uam.es/mjbullido

## Research summary

To identify mechanisms mediating the neurodegenerative cascade leading to Alzheimer's disease (AD), we perform genomic and functional analyses in neuronal cell models, which present AD characteristic neurodegeneration markers induced by herpes simplex 1 virus (HSV-1) infection. We have identified the endo-lysosomal route as the main process altered by HSV-1. This alteration is accompanied by an intracellular accumulation of cholesterol in endolysosomal membranes. The data show that lysosomal dysfunction is involved in the degeneration present in the models, so we are studying the role of selected candidates of this functional route.

In this last period, we have found that cells deficient in the lysosomal membrane protein LAMP2 develop a less efficient infection and a partial reversion of HSV-1-induced neurodegeneration markers. As for the possible mechanisms responsible, recent co-immunoprecipitation results point to several HSV-1 proteins capable of interacting with LAMP2, which through the interaction could modify its function triggering a degenerative cascade. Moreover, we have found that MMP14 metalloproteinase participates in the proteolysis of the β-amyloid precursor protein and modulates lysosomal function and HSV-1 infection, which points to MMP14 as another possible target for inhibiting the infection or the associated neurodegeneration. Finally, preliminary results suggest that the pharmacological modulation of intracellular cholesterol level modifies the efficiency of HSV-1 infection and the intracellular accumulation of β-amyloid peptide induced by the virus.

To validate the involvement of these candidates in AD, we are analyzing genes and markers related to HSV-1 infection, lysosomal function and cholesterol metabolism in biological samples of controls and patients diagnosed with AD at different clinical stages of the dementia prodromic, mild and moderate/severe . Analysis of cholesterol level in peripheral blood leukocytes suggests a reduction in patients' leukocytes, which is more pronounced in cells with myeloid lineage markers and in patients at prodromic stages of the disease. These data, which we are replicating in an independent sample, supports the involvement of cholesterol homeostasis in AD and encourages us to continue exploring these biomarkers.

The sum of our current results, coming from functional and gene expression studies in the cell models and from genetic association and biomarkers analysis in patients, continues supporting that failures of lysosomal function and of cholesterol homeostasis are mechanisms relevant in neurodegeneration, and that the candidates we are studying could constitute early biomarkers and pharmacological targets in AD.

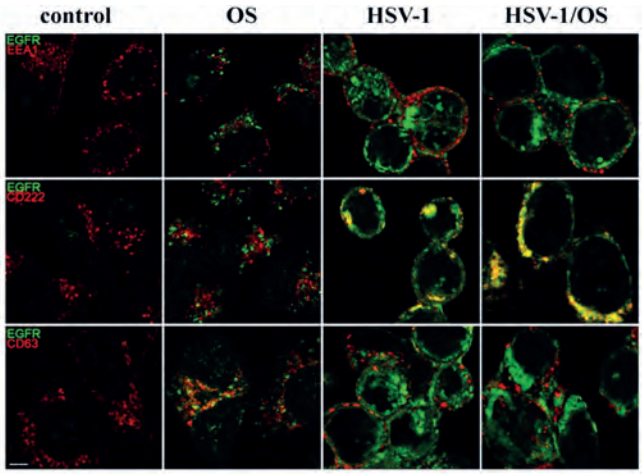


***Figure.*** *Herpes simplex 1 virus impairs the lysosomal trafficking/ degradation of the EGF receptor.*

## Publications

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## International projects / Research networks

- CIBER de Enfermedades Neurodegenerativas- CIBERNED. IP MJ Bullido (https://ciberned.es/grupo-bullido)

- Instituto de Investigación Sanitaria IdiPaz. (http://idipaz.es/DefaultEN.aspx) Grupo "Neurología y Enfermedades Cerebrovasculares", IP Exuperio Díez-Tejedor

- Consorcio Español de Genética de las Demencias-DEGESCO (https://ciberned.es/proyectos/degesco.html)

- EU Joint Program on Neurodegenerative Disease: European Alzheimer's Disease Bank. (JPND-EADB) Coord JC Lambert. Associated group. 2017-20.

## MOLECULAR NEUROPATHOLOGY

# PHYSIOPATHOLOGY AND THERAPY OF NEURODEGENERATIVE DISEASES: FRIEDREICH'S ATAXIA

***Principal Investigator:***
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Saúl Herranz Martín

***Predoctoral Fellows:***
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Andrés Vicente Acosta

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## Research summary

Our research group is interested in the study of Friedreich's ataxia, which is the most common hereditary ataxia in the Spanish population. We try to elucidate the molecular basis of this disease and develop novel therapies.

Friedreich's ataxia is caused by a deficiency of frataxin, a protein that mainly localizes to mitochondria. In addition to the neurodegenerative process, which mainly affects to the spinal cord and the cerebellum, many patients also develop a hypertrophic cardiomyopathy and diabetes. For this reason, and despite of being a very early onset disease, Friedreich's ataxia may also serve as a useful model for the study of degenerative diseases associated with aging in which mitochondrial dysfunction plays an important role.

We have developed distinct neural cell models to study the molecular mechanisms underlying the degenerative process triggered by the frataxin deficiency both in neurons and in astrocytes. These cell models are also being used to test potential therapeutic strategies, particularly those focused on identifying molecules (drugs or genes) capable of compensating for the functional defects induced by the loss of frataxin, or that are capable of efficiently increasing the expression of frataxin. In this context we are now characterizing the effect of an agonist of the Sonic Hedgehog signalling pathway on the neurotoxicity of frataxin-deficient astrocytes.

Currently we are also characterizing a novel mouse model for Friedreich's ataxia. Our group is also working on the development of a gene therapy approach for Friedreich's ataxia trying to combine frataxin gene delivery with the delivery of neurotrophic factors.

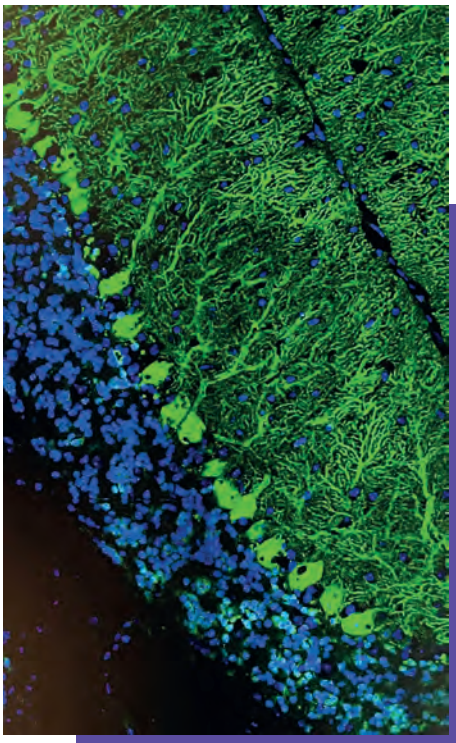

***Figure.*** *Mouse cerebellar cortex.*

## Publications

Fernández-Frías, I., Pérez-Luz, S. and Díaz-Nido, J. (2020) Enhanced Production of Herpes Simplex Virus 1 Amplicon Vectors by Gene Modification and Optimization of Packaging Cell Growth Medium. *Mol Ther Methods Clin Dev*. **17**:491-496.

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Agrò, M. and Díaz-Nido, J. (2020) Effect of Mitochondrial and Cytosolic FXN Isoform Expression on Mitochondrial Dynamics and Metabolism. *Int J Mol Sci*. **21**(21):8251.

## Awards and recognition

- Our Research Group also belongs to the "Instituto de Investigación Sanitaria Puerta de Hierro Majadahonda IDIPHIM" (Health Research Institute "Puerta de Hierro Majadahonda").

- In addition to our research activity, we are strongly committed with educational and outreach activities of advances in biomedical research. Javier Diaz-Nido is involved in the teaching of different courses at the Bachelor in Biochemistry and the Master in Molecular Biomedicine at UAM. He is also Director of the Doctoral School at UAM.

## Doctoral Theses

**Mauro Agró** (2019). Functional characterization of Frataxin isoforms and mechanisms of regulation of frataxin expression. Universidad Autónoma de Madrid. Supervisors: Javier Díaz-Nido, Alfredo Giménez-Cassina.

## MOLECULAR NEUROPATHOLOGY

# MOLECULAR BASES OF NEURONAL PLASTICITY

***Principal Investigator:***
Fco. Javier Díez Guerra

***Postdoctoral Fellows:***
Sahba Mobini
(until June 30, 2020)

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Sara Muñoz López
(since Sept 2020)
José Carlos Martínez Santamaría
(Sept 1, 2019 to Aug 31, 2020)

***Technician:***
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***Undergraduate and Master Students:***
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(2019-2020)
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(2019)
Anaïs Notario Reinoso
(2019)

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## Research summary

Memories are encoded by long-term changes in synaptic efficiency and connectivity among neurons. An in-depth knowledge of the molecular basis of synaptic regulation is fundamental to decipher the mechanisms involved in the formation, storage and retrieval of memories. Our group studies the cellular and molecular mechanisms that modulate the plasticity of neural networks, with the aim of finding molecular targets and effective strategies to improve cognitive performance. Synaptic activity triggers intracellular calcium ($Ca^{+2}$) oscillations that locally modulate a number of signaling pathways. Calmodulin (CaM), a protein that binds calcium, translates these oscillations into intracellular signaling events. Its availability and activity are locally regulated by proteins such as Neurogranin (Ng), very abundant in the post-synaptic compartment, which sequesters CaM in a $Ca^{+2}$ and phosphorylation dependent manner. Since Ng levels and cognitive performance are positively correlated in the human brain, we aim to understand the mechanisms underlying the regulation of Ng transcription and its local translation in dendrites. We use *in vitro* models including primary cultures of dissociated neurons to understand the role of Ng in events of synaptic plasticity, such as those associated with Hebbian plasticity (Long-Term Potentiation -LTP- and Long-Term Depression -LTD) and homeostatic plasticity. As an *in vivo* model, we use adult newborn neurons of the Dentate Gyrus in the mouse Hippocampus. Here we try to establish causal correlations between the integration of newborn neurons in existing neuronal networks and the regulation of Ng expression. For that, we use a combination of biochemical, molecular biology, advanced microscopy, and bioimage analysis techniques. We propose Ng as a potential target for strategies designed to prevent, alleviate or cure pathologies associated to impaired cognitive function. We justify this objective on the basis that Ng function is quite restricted to its action in the brain. For example, its knock-out in mice does not cause apparent anatomical or physiological abnormalities, but severe cognitive impairments. Further, physiological expression of Ng is restricted to the postnatal forebrain. Thus, manipulating Ng expression to improve cognition will be very likely devoid of side-effects. In summary, a deeper understanding of the role of Ng and other CaM-sequestering proteins in the mechanisms of neuronal plasticity will contribute to the development of newer therapies to improve the cognitive function and quality of life of aging individuals and patients suffering from neurological diseases.

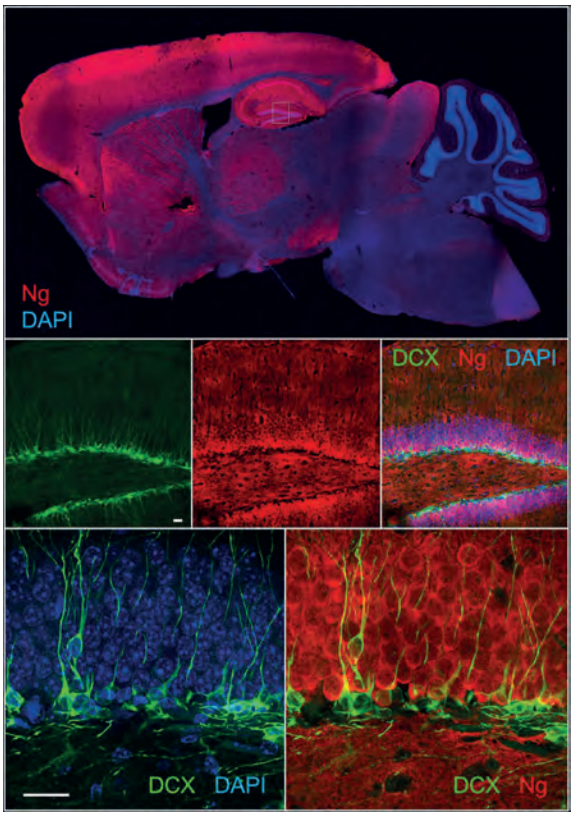


***Figure.*** *Adult neurogenesis as a model to study Neurogranin expression. Upper row: Sagittal section of an adult mice brain stained for Neurogranin and cellular nuclei (DAPI). Middle row: Detail of the Hippocampal Dentate Gyrus stained for Doublecortin (DCX, green), Neurogranin (Ng, red) and cellular nuclei (DAPI, blue). Lower row: Zoom into the granular layer of the dentate gyrus showing neurons positive for Doublecortin (DCX, green) and Neurogranin (Ng, red). Neurogranin expression is not tuned up in this early stage of newborn neuron differentiation (bar = 100 µm).*

## Publications

Garrido-García, A., de Andrés, R., Jiménez-Pompa, A., Soriano, P., Sanz-Fuentes, D., Martínez-Blanco, E. and Díez-Guerra, F. J. (2019) Neurogranin Expression Is Regulated by Synaptic Activity and Promotes Synaptogenesis in Cultured Hippocampal Neurons. *Molecular Neurobiology* **56** (11): 7321-7337.

## Patents

"Imaging compatible, miniaturized device for *in vitro* electrical stimulation", Spanish Patent Application (SPTO) number P202030626, by Dr Sahba Mobini, Dr. José Miguel García Martín, Dr. María Ujué González Sagardoy, Dr. Mª Soledad Martín González, Dr. Olga Caballero Calero, Dr. Jorge M. García Martínez, Dr. Fco Javier Díez Guerra and Dr. Erin E. Patrick. The patent application as well as the patent once it has been granted, belong to the applicants in the following proportion: CSIC: 80%, UAM: 10% and UFRF (USA):10%.

## International projects / Research networks

- "Inducing Neural Plasticity Using Electrical Stimulation Delivered by Nano-Structured Electrodes: A Critical Step Toward Post-Stroke Recovery. NeuPES (793102)" Individual fellowship awardee: Dr. Sahba Mobini. Supervising labs: Dr. José Miguel García Martín (Instituto de Micro y Nanotecnología – CSIC) y Dr. Fco. Javier Díez-Guerra (Centro de Biología Molecular Severo Ochoa (CSIC-UAM). Project duration: 24 months (starting from 1st Nov, 2018).

## MOLECULAR NEUROPATHOLOGY

# LABORATORY OF SURVIVAL AND PLASTICITY IN THE AGING BRAIN

***Principal Investigator:***
Carlos Dotti

***Postdoctoral Fellows:***
Francesc Guix Rafols
Inés Berenguer López
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Marta Carus Cadavieco
(since Jan. 2020)

***Predoctoral Fellows:***
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Silvia de Vidania
(Until April 2020)

***Technicians:***
Irene Palomares Perez
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Mercedes Hernández del Cerro

***Undergraduate and Master Students:***
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Ana Marrero Capitán
Inés López del Castillo
Elisa Fernández Martínez

https://www.cbm.uam.es/cdotti.

## Research summary

Aging is associated with several physiological modifications occurring in multiple organs. With advancing age, brain cells, especially neurons, suffer the consequences of having to function throughout the individual's entire lifespan without being able to be replaced, as occurs with cells in other tissues. And this characteristic causes neurons to accumulate different types of damage over time, in their plasma membrane, in the different organelles, proteins, lipids and in nucleic acids. And although as a consequence of these alterations there are deficits in certain brain functions, something evident for any individual who is getting older, neuronal death does not occur. However, if one considers that aging is the main risk factor for the appearance of diseases like Alzheimer´s and that Alzheimer's symptoms are the consequence of the gradual but progressive loss of neurons it then comes that it is crucial to guarantee the survival of neurons as we age. In order to guarantee neuronal survival, we must know more about the underlying mechanisms, especially in conditions of accumulation of damage. This is the main objective of our laboratory, and for this we use different experimental strategies. One of the strategies we use is based on the study of the molecular pathways that are activated to compensate for the loss of function of organelles involved in the cleaning of old material, a source of deadly by-products. Another strategy that we use is the identification of the molecular pathways that are activated in response to trophic factors that participate in neuronal survival in the old brain but participate in learning and memory function in the young animal. A third strategy used in the laboratory is the search for genes that would be counteracting the harmful effects of mutations in the APP gene, using mice expressing bearing familial forms of Alzheimer's disease cause disease in humans.

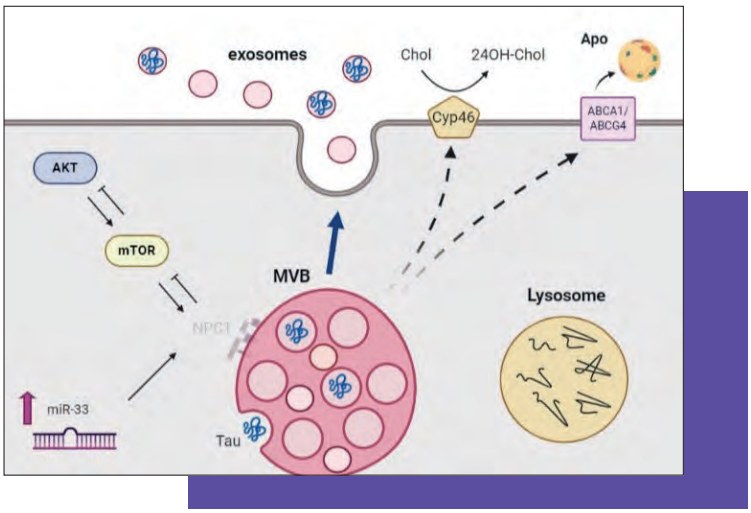


***Figure.*** *Chronological aging is accompanied by numerous defects at the molecular level, which affect functions such as the removal of toxic material. Recent work from our laboratory demonstrated that part of this toxic material g (exemplified in the figure as Tau) is counterweighed by an increased capacity of the old cells to build and release of vesicles of endocytic origin (exemplified as EVs, for extracellular vesicles) to the extracellular milieu, and that this is due to the downregulation of the cholesterol transporter protein NPC1 due to a double mechanism: increased degradation via activation of the Akt/mTOR pathway and decreased synthesis due to the upregulation of the micro RNA miR-33.*

## Publications

Martín-Segura, A., Casadomé-Perales, Á., Fazzari, P., Mas, J. M., Artigas, L., Valls, R., Nebreda, A. R. and Dotti, C. G. (2019) Aging Increases Hippocampal DUSP2 by a Membrane Cholesterol Loss-Mediated RTK/p38MAPK Activation Mechanism. *Front Neurol.* **10**:675.

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Martín-Segura, A., Ahmed, T., Casadomé-Perales, Á., Palomares-Perez, I., Palomer, E., Kerstens, A., Munck, S., Balschun, D., Dotti, C. G. (2019) Age-associated cholesterol reduction triggers brain insulin resistance by facilitating ligand-independent receptor activation and pathway desensitization. *Aging Cell.* **18**:e12932.

de Vidania, S., Palomares-Perez, I., Frank-García, A., Saito, T., Saido, T. C., Draffin, J., Szaruga, M., Chávez-Gutierrez, L., Calero, M., Medina, M., Guix, F. X. and Dotti C. G. (2020) Prodromal Alzheimer's Disease: Constitutive Upregulation of Neuroglobin Prevents the Initiation of Alzheimer's Pathology. *Front Neurosci*. **14**:562581.

## Awards and recognition

- Co-organizer of Mini-Symposium Brain-Body Connection, Madrid May 13-14 2019.

## Doctoral Theses

**Silvia de Vidania Ballesteros** (2019). Título: Estudio de los mecanismos neuroprotectores frente a la toxicidad del péptido ß amiloide en un modelo preclínico de la enfermedad de Alzheimer. Universidad Autónoma de Madrid. Carlos Dotti.

## International projects / Research networks

- EU JPND (Joint Project Neurodegenerative Disease).

Effect of early and adult-life stress on the brain epigenome: relevance for the occurrence of Alzheimer's disease and Diabetes-related dementia.

Acronym: EpiAD.

Duration: 01/01/2018-31/12/2020 (extended to 31/12/2021).

## MOLECULAR NEUROPATHOLOGY

# MECHANISMS OF SYNAPTIC PLASTICITY, AND CONTRIBUTION TO COGNITIVE FUNCTION

***Principal Investigator:***
José Antonio Esteban García

***Postdoctoral Fellows:***
María Isabel Cuartero Desviat
(until January 2020)
Víctor Briz Herrezuelo

***Predoctoral Fellows:***
Carla Sánchez Castillo
Alba Fernández Rodrigo
Sergio López García
Esperanza López Merino

***Technicians:***
Raquel Jiménez Sánchez
Silvia Gutiérrez Eisman

***Undergraduate and Master Students:***
Gloria Cabañas Pujadas
(TFG) (June 2019–Feb 2020)
Cristina Boers Escuder
(TFG) (from June 2020)
Pablo Zamorano González
(TFM) (from Sept 2020)

http://www.cbm.uam.es/estebanlab

## Research summary

My research group has a longstanding interest in the molecular and cellular mechanisms of synaptic plasticity, and their contribution to cognitive processes such as learning and memory. Using electrophysiological, imaging and molecular techniques, we have made important contributions to understand how the membrane trafficking machinery of the neuron controls synaptic function by shuttling neurotransmitter receptors in and out of the synaptic membrane. We are also particularly interested in how these processes are altered in human pathologies associated to cognitive disorders. Indeed, using mouse genetics and behavioral assays, we have found that some signaling cascades controlling this trafficking machinery are defective in Alzheimer's disease and some forms of autism.

In recent years, our research is concentrated on two intersecting directions. On the one hand, we continue uncovering the molecular components and signaling cascades that control the remodeling of synapses during plasticity. We are dedicating particular effort to the signaling pathway controlled by phosphoinositide 3-kinases (PI3Ks) and the small GTPase Ras. The PI3K-Ras pathway is crucial during embryonic development, when it controls cell proliferation and differentiation. Nevertheless, it is also very active in postnatal and adult life. In particular, in the brain, it participates in several forms of synaptic plasticity and in this manner is thought to contribute to cognitive function. However, the mechanisms that control PI3K activity in neurons, and how this activity is relayed into downstream intracellular signaling, such as the Ras-mTOR cascade, are far from clear. We are investigating these mechanisms both in neurons and in glial cells, as well as their impact on cognitive function and behavioral aspects such as anxiety and sociability.

On the other hand, we are investigating how environmental contaminants shift some of these signaling mechanisms, altering neurodevelopmental programs with long-lasting consequences in the adult life. In particular, using rats as animal model, we are finding that chronic exposure to low concentrations of common pesticides during embryonic development and early postnatal life changes the activation of intracellular signaling and impairs synaptic plasticity. We believe these changes are responsible for behavioral deficits observed in these animals later in life. We are now exploiting this information to design and test potential therapeutic approaches to correct some of these deficits.

In summary, our combined application of *in vitro* and *in vivo* approaches is allowing us to explore how individual molecules and signaling pathways control synaptic function and determine our cognitive abilities in health and disease.

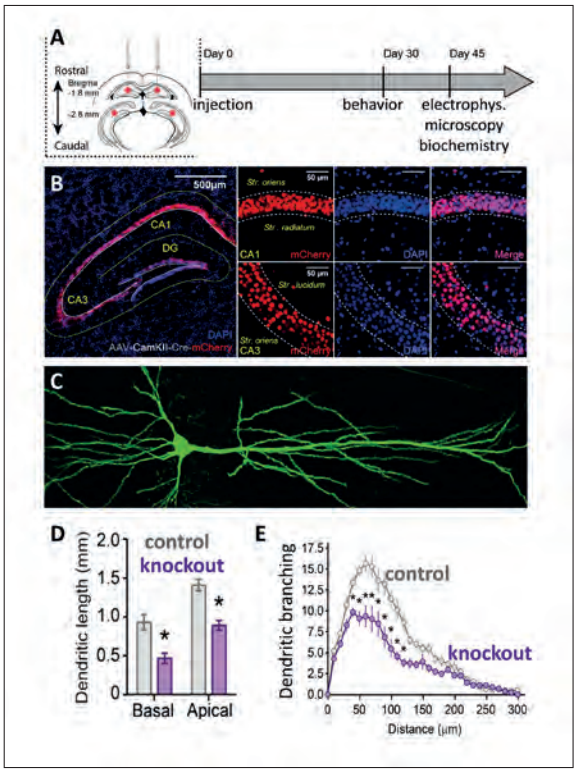



***Figure.*** *A. Experimental strategy for knocking-out specific genes using in vivo viral injection to express the cre recombinase, followed by behavioral assays, and terminated with electrophysiological, biochemical and morphological analysis. B. Representative hippocampal section showing the extent of viral infection (red) together with nuclear staining (DAPI). C. Representative confocal image of an infected CA1 pyramidal neuron for morphological analysis. D, E. Quantification of total dendritic length (D) and Sholl analysis (E) from uninfected (control) and infected (knockout) neurons.*

## Publications


Draffin, J. E., Sánchez-Castillo, C., Fernández-Rodrigo, A., Sánchez-Sáez, X., Ávila, J., Wagner, F. F. and Esteban, J. A. (2020) GSK3α, not GSK3β, drives hippocampal NMDAR-dependent LTD via tau-mediated spine anchoring. *EMBO J*. **40**, e105513.

Sánchez-Puelles, C., Calleja-Felipe, M., Ouro, A., Bougamra, G., Arroyo, A., Díez, I., Erramuzpe, A., Cortés, J., Martínez-Hernández, J., Luján, R., Navarrete, M., Venero, C., Chan, A., Morales, M., Esteban. J. A.* and Knafo, S.* (2020) PTEN activity defines an axis for plasticity at cortico-amygdala synapses and influences social behavior. *Cereb. Cortex* **30**, 505-524. *Co-corresponding authors.

Navarrete, M., Cuartero, M. I., Palenzuela, R., Draffin, J. E., Konomi, A., Serra, I., Colié, S., Castaño-Castaño, S., Hasan, M. T., Nebreda, A. R. and Esteban, J. A. (2019) Astrocytic p38α MAPK drives NMDA receptor-dependent long-term depression and modulates long-term memory. *Nat. Commun*. **10**:2968.

Royo, M., Gutiérrez, Y., Fernández-Monreal, M., Gutiérrez-Eisman, S., Jiménez, R., Jurado, S. and Esteban, J. A. (2019) A retention-release mechanism based on Rab11-FIP2 for AMPA receptor synaptic delivery during long-term potentiation. *J. Cell Sci*. **132**: jcs234237.

Mitroi, D. N., Pereyra-Gómez, G., Soto-Huelin, B., Senovilla, F., Kobayashi, T., Esteban, J. A., Ledesma, M. D. (2019) NPC1 enables cholesterol mobilization during long-term potentiation that can be restored in Niemann-Pick disease type C by CYP46A1 activation. *EMBO Rep*. **20**,e48143.

Esteve, P., Rueda-Carrasco, J., Mateo, M. I., Martin-Bermejo, M. J., Draffin, J., Pereyra, G., Sandonís, A., Crespo, I., Moreno, I., Aso, E., Garcia-Esparcia, P., Gomez-Tortosa, E., Rábano, A., Fortea, J., Alcolea, D., Lleó, A., Heneka, M. T., Valpuesta, J. M., Esteban, J. A., Ferrer, I., Domínguez, M., Bovolenta, P. (2019) Elevated levels of Secreted-Frizzled-Related-Protein 1 contribute to Alzheimer's disease pathogenesis. *Nat. Neurosci*. **22**, 1258-1268.

Casadomé-Perales, A., De Matteis, L., Alleva, M., Infantes-Rodríguez, C., Palomares-Pérez, I., Saito, T., Saido, T. C., Esteban, J. A., Nebreda, A. R., de la Fuente, J. M. and Dotti, C. G. (2019) Inhibition of p38 MAPK in the brain through nasal administration of p38 inhibitor loaded in chitosan nanocapsules. *Nanomedicine* **14**, 2409-2422.

Pallas, N., Draffin, J. E., Cuadros, R., Esteban, J. A. and Ávila, J. (2019) Tau is required for the function of extrasynaptic NMDA receptors. *Sci Rep* **9**:9116.

Pereda-Pérez, I., Valencia, A., Baliyan, S., Núñez, Á., Sanz-García, A., Zamora, B., Rodríguez-Fernández, R., Esteban, J. A. and Venero, C. (2019) Systemic administration of a fibroblast growth factor receptor 1 agonist rescues the cognitive deficit in aged socially isolated rats. *Neurobiol Aging* **78**, 155-165.


## International projects / Research networks

- European Union JPI HDHL Joint Action, PCIN-2016-095 (2017-2019). "MiTyrAge: Targeting the mitochondria-tyr kinase axis to prevent age-associated neuronal decline".

## MOLECULAR NEUROPATHOLOGY

# INTEGRATION OF CELL SIGNALING AND ENERGY METABOLISM IN THE BRAIN

***Principal Investigator:***
Alfredo Giménez-Cassina Sendón

***Postdoctoral Fellows:***
Mauro Agró
(since July 2019)

***Predoctoral Fellows:***
Mauro Agró
(until July 2019 – Co-supervised with Javier Díaz-Nido)

***Undergraduate and Master Students:***
Darío García Rodríguez
(2019: TFG; 2020. TFM)

https://www.cbm.uam.es/agcasina

## Research summary

The brain has a very high metabolic demand, and compelling evidence over the last years has evidenced that the source of energy that neurons and glial cells utilize contributes to shape neuronal function and survival. However, little is known on how brain cells adapt to changes in metabolic situations, such as “energy fuel” availability and other extracellular cues. Furthermore, how the utilization of select fuels contribute to modulate neuronal excitability and survival remains poorly understood.

Our current research focuses on understanding how signaling cascades regulate energy metabolism in the brain, with a special focus on mitochondria. Mitochondrial metabolism plays a key role in cellular physiology, which is underscored by the fact that mitochondrial dysfunction and metabolic decline have been linked to several neurological disorders. Our previous studies have shown that manipulation of the metabolic program in the brain constitutes a promising therapeutic strategy to treat disorders in which metabolic dysfunction is a central feature. Therefore, understanding the molecular mechanisms that modulate energy metabolism and mitochondrial function will contribute to gain insight into the underpinnings responsible for the pathogenesis of such diseases.

In particular, we are interested in:

1) Identification and characterization of signaling mechanisms that regulate the choice of fuel in brain cells.

2) Study the molecular underpinnings that link metabolic flow with neuronal function and viability.

3) Ultimately, we plan to utilize this knowledge to design and evaluate potential therapeutic strategies against mitochondrial dysfunction-induced neurological disorders.

To address these questions, we use a multidisciplinary approach that combines cellular biology, metabolic flux analysis, biochemistry, proteomics, molecular biology and *in vivo* studies. Our ultimate goal is to identify integrative mechanisms of regulation of energy metabolism and to develop novel therapeutic strategies for neurological disorders in which aberrations in metabolism play a prominent role. In this regard, we conduct part of our research in close collaboration with the group of Dr. Javier Díaz Nido.

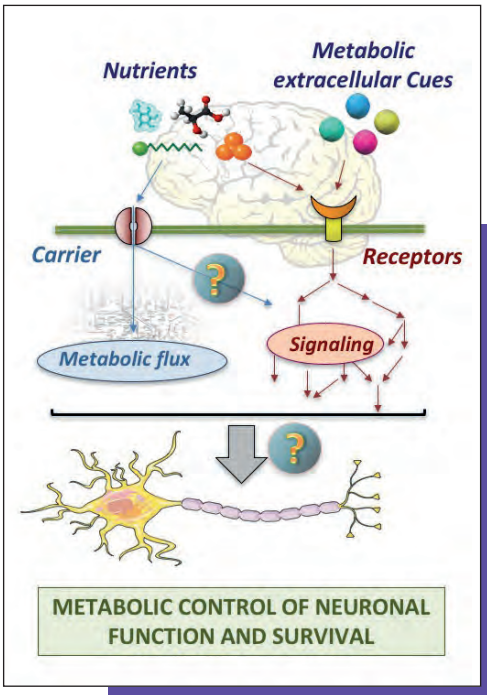



***Figure.*** *Nutrients and other metabolic cues impinge on neurons and crosstalk with signaling networks to modulate neuronal function and survival.*

## Publications

Alcover-Sánchez, B., García-Martín, G., Escudero-Ramírez, J., González-Riano, C., Lorenzo, P., Giménez-Cassina, A., Formentini, L., de la Villa, P., Wandosell, F., Cubelos, B. (2020) Absence of R-Ras1 and R-Ras2 causes mitochondrial alterations that trigger axonal degeneration in a hypomyelinating disease model. *Glia* **69**:619-637.

Katsu-Jiménez, Y., Giménez-Cassina, A. (2019) Fibroblast Growth Factor-21 (FGF21) promotes ketone body utilization in neurons through activation of AMP-dependent Kinase (AMPK). *Mol. Cell. Neurosci.* **101**:103415,

Katsu-Jimenez, Y., Vázquez-Calvo, C., Maffezzini, C., Halldin, M., Peng, X., Freyer, C., Wredenberg, A., Giménez-Cassina, A.§, Wedell, A.§ and Arnér, E. S. J.§ (2019) Absence of TXNIP in humans leads to lactic acidosis and low serum methionine linked to deficient respiration on pyruvate. *Diabetes* **68**:709-723 (§AGC, AW & ESJ: Co-senior authors).

## Awards and recognition

- We are strongly committed with educational and outreach activities. Alfredo Giménez-Cassina is involved in the teaching of different courses at the Bachelor and Master programmes at UAM.

## Doctoral Theses

**Mauro Agró** (2019). Functional characterization of frataxin isoforms and mechanisms of regulation of frataxin expression. Universidad Autónoma de Madrid. Co-supervisors: Javier Díaz-Nido and Alfredo Giménez-Cassina. International mention.

## MOLECULAR NEUROPATHOLOGY

# LIPIDS IN NEURONAL PHYSIOLOGY AND PATHOLOGY

***Principal Investigator:***
María Dolores Ledesma

***Postdoctoral Fellows:***
Daniel Mitroi
(until June 2019)
Ángel Gaudioso
(since September 2019)
Marta Guerrero
(since January 2020)

***Predoctoral Fellows:***
Adrian Bartoll
(until May 2019)
Beatriz Soto
Ana Toledano
Sara Naya
(since October 2020)

***Technician:***
Mercedes Hernández

***Undergraduate and Master Students:***
Gema Muñoz
(January-June 2019)
Mario Díaz
(October 2019 –June 2020)
Elba Molpeceres
(January-June 2020)
Jaime Mulero
(since October 2020)

http://www.cbm.uam.es/dledesma

## Research summary

The key contribution of lipids to neuronal physiology and pathology is increasingly recognized. Still, the lack of suitable tools to analyse lipids leaves many questions unaddressed. In our laboratory we use mice in which the metabolism of certain lipids has been genetically altered and mimic human diseases. In particular, we have focused on the study of mice lacking the acid sphingomyelinase (ASMko) and mice mutant for the cholesterol transport protein NPC1. These mice accumulate sphingomyelin (SM) and cholesterol, respectively, in neurons and synapses and mimic Niemann Pick types A (NPA) and C (NPC) diseases, which lead to cognitive and psychiatric alterations, neurodegeneration and early death. During 2019-2020 our studies in these mouse models have unveiled the existence of a feedback loop between SM and the endocannabinoid system by which the levels of this lipid and the CB1 receptor control each other in neurons (Bartoll et al., EMBO Mol Med 2020). We have also described the key contribution of the NPC1 protein to synaptic plasticity by promoting LTP-induced cholesterol release through the surface delivery of the cholesterol-degrading enzyme CYP46 (Mitroi et al., EMBO Rep 2019). These findings prompted us to assess pharmacological strategies using inhibitors of the endocannabinoid-degrading enzyme FAAH in ASMko mice or the CYP46-activator Efavirenz in the NPC1 mutant mice. The cognitive and psychiatric benefits and the extended life span observed in the mouse models after these treatments have led to assess them in on-going clinical trials in NPA and NPC patients. We have also validated preclinically a gene therapy strategy for NPA. Cisterna magna injection of adenoviral-9 vectors expressing the acid sphingomyelinase promotes a mild and broad brain expression of the missing enzyme in ASMko mice, which diminishes SM levels and neurodegeneration avoiding inflammation (Samaranch et al., Sci Transl Med 2019). All these strategies also ameliorated the aberrant phenotype of the microglia we have described in NPA and NPC. This cell type, which at early disease stages has a beneficial effect by clearing myelin debris, become toxic since accumulating SM permeabilizes the lysosomal membrane promoting lysosomal exocytosis and Cathepsin B release to the extracellular milieu (Gabande-Rodriguez et al., EMBO J 2019). We believe the results obtained during 2019-2020 contribute to the understanding of SM and cholesterol roles in brain cells, of the pathological mechanisms in NPA and NPC and open new therapeutic perspectives for these fatal lipidosis.

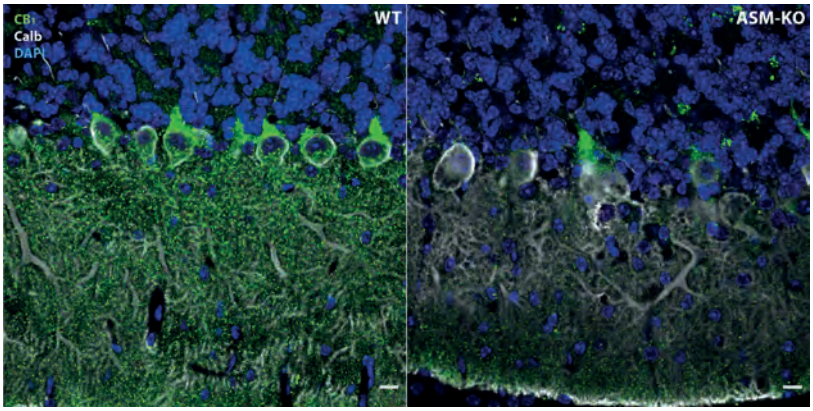



***Figure.*** *Reduced CB1 levels in neurons of a mouse model for Niemann Pick type A disease. Immunofluorescene images of the endocannabinoid receptor CB1 and the Purkinje cell marker Calbindin in the cerebellum of wt mice and of mice lacking the acid sphingomyelinase (ASMko), which mimic Niemann Pick disease type A. DAPI in blue stains cell nuclei. Scale bar= 10μm.*

## Publications

Gabandé-Rodríguez, E., Pérez-Cañamás, A., Soto-Huelin, B., Mitroi, D. N., Sánchez-Redondo, S., Martínez-Sáez, E., Venero, C., Peinado, H., and Ledesma, M. D. (2019) Lipid-induced lysosomal damage after demyelination corrupts microglia protective function in lysosomal storage disorders. *EMBO J*. **38**, e99553.

Samaranch, L., Pérez-Cañamás, A., Soto-Huelin, B., Sudhakar, V., Jurado-Arjona, J., Hadaczek, P., Ávila, J., Bringas, J. R., Casas, J., Chen, H., He, X., Schuchman, E. H., Cheng, S. H., Forsayeth, J., Bankiewicz, K. S., and Ledesma M. D. (2019) Adeno-associated viral vector serotype 9-based gene therapy for Niemann-Pick disease type A. *Sci Transl Med*. **11**, pii: eaat3738.

Mitroi, D. N., Pereyra-Gómez, G., Soto-Huelin, B., Senovilla, F., Kobayashi, T., Esteban, J. A., and Ledesma, M. D. (2019) NPC1 enables cholesterol mobilization during long-term potentiation that can be restored in Niemann-Pick disease type C by CYP46A1 activation. *EMBO Rep*. **20**, e48143.

Toledano-Zaragoza, A., and Ledesma, M. D. (2020) Addressing neurodegeneration in lysosomal storage disorders: Advances in Niemann Pick diseases. *Neuropharmacology*. **171**, 107851.

Albi, E., Alessenko, A. V., Elahi, F. M., and Ledesma, M. D. (2020) Lipids in the brain. *Front Neurol* **11**, 7.12.

Bartoll, A., Toledano-Zaragoza, A., Casas, J., Guzmán, M., Schuchman, E. H., and Ledesma, M. D. (2020) Inhibition of fatty acid amide hydrolase prevents pathology in neurovisceral acid sphingomyelinase deficiency by rescuing defective endocannabinoid signaling. *EMBO Mol. Med.* **12**, e11776.

## Awards and recognition

- Organization of the international Symposium “Brain-Body Connection”, 13-14th May 2019, CBMSO

## Doctoral Theses

**Adrián Bartoll Andrés** (2019). El sistema endocannabinoide en la patología y terapia de la enfermedad de Niemann Pick tipo A. Departamento Biología Molecular, Universidad Autónoma Madrid. Directora: María Dolores Ledesma.

# MOLECULAR NEUROPATHOLOGY

## ADULT NEUROGENESIS AND NEURO-DEGENERATIVE DISEASES


***Principal Investigator:***
María Llorens-Martín

***Postdoctoral Fellows:***
Berenice Márquez Valádez
(start date: Nov/01/2020)

***Predoctoral Fellows:***
Julia Terreros Roncal
Elena Moreno Jiménez
Miguel de la Flor García

***Technician:***
Carla Rodríguez Moreno

***Undergraduate and Master Students:***
Héctor Cañeque Rufo
(Jan/01/2019 – July/31/2020)
Laura Álvarez Méndez
(starting date Oct/01/2020)

https://www.cbm.uam.es/llorenslab

## Research summary

The hippocampus is a brain region that hosts one of the most striking forms of neural plasticity, namely the generation of new neurons throughout life, or adult hippocampal neurogenesis (AHN). AHN participates in hippocampal-dependent learning and mood regulation, and it encompasses the birth and functional integration of newborn neurons. The generation of new neurons is compromised during physiological aging and under neurodegenerative conditions in rodents. Our group demonstrated that AHN persists during physiological aging until the tenth decade of human life. Moreover, we showed that AHN is critically impaired in patients with one of the most prevalent neurodegenerative diseases, namely Alzheimer´s disease (Moreno-Jiménez et al., Nature Medicine, 2020). Progress in the field of human AHN has been hindered by technical limitations related to the quality of available human brain samples. In this regard, our group overcame these technical difficulties and demonstrated that specific tissue processing methodologies are necessary to observe the presence of new neurons in the adult human hippocampus (Flor-García et al., Nature Protocols, 2020).

Supported by the recent award of the ERC Consolidator Grant "ERC-CoG-2020-101001916-HumAN", our research group will investigate the basic biology of neural stem cells (the cell population that gives rise to new neurons in the adult mammalian hippocampus) as well as the mechanisms that control the modulation of adult hippocampal neurogenesis under physiological and pathological conditions in humans. We also perform *in vivo* and *in vitro* studies on animal models of different diseases. In particular, we are interested in determining the therapeutic potential of increasing adult hippocampal neurogenesis for the treatment of neurodegenerative and psychiatric diseases. Importantly, in most of these disorders, the hippocampus is one of the most affected areas. Therefore, other research lines of our lab are focused on developing strategies capable of increasing the functionality of newborn neurons. For that purpose, we use novel viral tools and distinct non-pharmacological approaches aimed at increasing hippocampal plasticity.

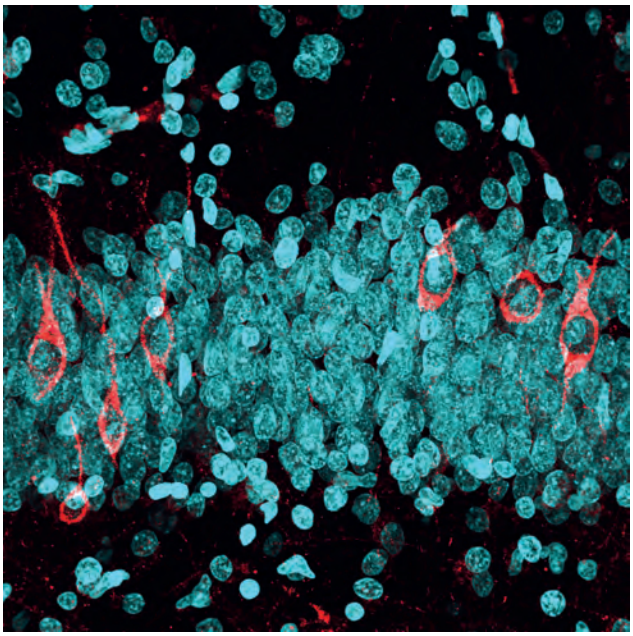


***Figure.*** *Image of the human dentate gyrus showing immature (in red) and mature (blue) neurons in the hippocampal granule cell layer.*

## Publications

Llorens-Martín, M. (2020) A new player in the beneficial effects of exercise on the aged brain. Signal Transduction and Targeted Therapy. *Sep* **3**;5(1):184.

Flor-García, M., Terreros-Roncal, J., Moreno-Jiménez, E. P., Ávila, J., Rábano, A., and Llorens-Martín, M. (2020) Unraveling human adult hippocampal neurogenesis. *Nature Protocols.* **15**(2):668-693.

Terreros-Roncal, J., Flor-García, M.*, Moreno-Jiménez, E. P.*, Pallas-Bazarra, N.*, Rábano, A., Sah, N., van Praag, H., Giacomini, D., Schinder, A. F., Ávila, J., and Llorens-Martín, M. (2019) Activity-dependent reconnection of adult-born DGCs in a mouse model of frontotemporal dementia. *Journal of Neuroscience* **39**:5794-5815.

Llorens-Martín, M. (2019) Adult-born neurons in brain circuitry. *Science* **364**:530.

Moreno-Jiménez, E. P., Flor-García, M., Terreros-Roncal, J., Rábano, A., Cafini, F., Pallas-Bazarra, N., Ávila, J., and Llorens-Martín, M. (2019) Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in Alzheimer´s disease patients. *Nature Medicine* **25**:554-60.

Moreno-Jiménez, E. P., Jurado-Arjona, J., Ávila, J., and Llorens-Martín, M. (2019) The Social Component of Environmental Enrichment Is a Pro-neurogenic Stimulus in Adult c57BL6 Female Mice. *Front. Cell. Dev. Biol*. **7**:62.

Bolós, M., Terreros-Roncal, J., Perea, J. R., Pallas-Bazarra, N., Ávila, J., and Llorens-Martín, M. (2019) Maturation dynamics of the axon initial segment (AIS) of newborn dentate granule cells in young adult C57BL/6J mice. *Journal of Neuroscience* **39**:1605-1620.

## Awards and recognition

- María Llorens-Martín (2019). 'Miguel Catalán' Young Investigator Award for Scientific Research, Comunidad de Madrid, Spain.

- María Llorens-Martín (2019). Young Female Talent in Biology, Spain's Royal Academy of Science, Spain.

- Elena Moreno-Jiménez (2019). Young Investigators in Neuroscience 2019, Instituto de Neurociencias Federico Olóriz, University of Granada (Spain).

- Miguel Flor-García (2020). Young Investigator Award: CIBERNED (Spain).

## International projects / Research networks

- ERC Consolidator Grant 2020 (European Commission), ERC-CoG-2020-101001916-HumAN. PI: María Llorens-Martín. Sept/01/2021 – Aug/30/2026.

- The Alzheimer´s Association 2017 Research Grant, AARG-17-528125. (USA). PI: María Llorens-Martín. Jan/01/2018 – June/30/2021.

- Our group belongs to CIBERNED.

## MOLECULAR NEUROPATHOLOGY

# FISIOPATHOLOGY OF GLYCINE TRANSPORTERS IN GLYCINERGIC NEUROTRANSMISSION: HYPEREKPLEXIA AND PAIN

***Principal Investigator:***
Beatriz López Corcuera

***Scientific Staff***
Carmen Aragón Rueda
(until September 2019)

***Postdoctoral Fellows:***
Cristina Benito Muñoz
(until December 2019)

***Predoctoral Fellows:***
Andrés de la Rocha Muñoz
Raquel de Felipe Mendía

***Technician:***
Enrique Núñez Balbuena
(50% dedication)

***Undergraduate and Master Students:***
Elena Melgarejo de la Peña
(2019)
Alejandro Ferrando Muñoz
(2020)

http://www.cbm.uam.es/blopez

## Research summary

The group studies the $Na^+$-and $Cl^-$-dependent glycine transporters (GlyTs), which are essential modulators of the glycine-mediated neurotransmission in the Central Nervous System. We focus on the physiology and pathologies associated to the glycinergic transmission such as hyperekplexia and pain. Hyperekplexia is a rare sensorimotor disorder provoked by the interruption of the glycinergic inhibition that may have severe consequences in neonates. The neuronal glycine transporter GlyT2, which removes and recycles synaptic glycine to supply neurotransmitter for synaptic vesicle refilling, is nonfunctional in the presynaptic defect causing the disease. Our aim is to identify new mutations in the human GlyT2 gene (*SLC6A5*) found in hyperekplexia patients and analyze their pathogenic mechanisms. By the help of 3D computational models, the effects of the mutations on 3D structure, biogenesis, intracellular trafficking, oligomerization, interactoma and, eventually, the function of the transporter will be analyzed. We have localized small molecules and regions in the transporter structure that modulate folding, trafficking and activity, and have reach to rescue two trafficking-defective variants using chemical chaperones. This may help developing specific pharmacopherones as candidate therapeutic tools for hyperekplexia.

In addition, we have discovered the first E3 ubiquitin ligase (LNX2) that regulates GlyT2 by interacting, ubiquitinating and promoting transporter degradation in primary neurons under the activation of protein kinase C.

We have also identified a second regulatory mechanism of GlyT2 by the Hedgehog pathway whose activation reduces the functional expression of the transporter both in vitro and in vivo. We will pursue this modulation to analyze the role of GlyT2 in the nervous system developmental failures during hyperekplexia.

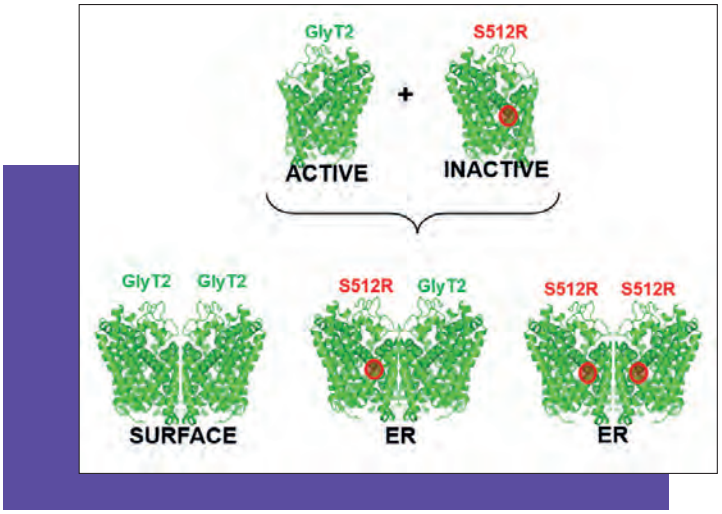



***Figure.*** *Schematic diagram of how a hyperekplexia mutation alters expression levels of GlyT2.*

## Publications

de la Rocha-Muñoz, A., Núñez, E., Gómez-López, S., López-Corcuera, B., de Juan-Sanz, J., and Aragón, C. (2020) The presynaptic glycine transporter GlyT2 is regulated by the Hedgehog pathway in vitro and *in vivo*. *bioRxiv* **2020**.07.28.224659.

da Silva, V. D., Silva, R. R., Neto, J. G., López-Corcuera, B., Guimarães, M. Z., Noël, F., and Buarque, C. D. (2020) New α-hydroxy-1,2,3-triazoles and 9H-fluorenes-1,2,3-triazoles: synthesis and evaluation as Glycine Transporter 1 Inhibitors. *J. Braz. Chem. Soc*. **31**(6): 1258-1269.

de la Rocha-Muñoz, A., Núñez, E., Arribas-González, E., López-Corcuera, B., Aragón, C., and de Juan-Sanz, J. (2019) E3 ubiquitin ligases LNX1 and LNX2 are major regulators of the presynaptic glycine transporter GlyT2. *Sci Rep* **9**:14944.

López-Corcuera, B., Arribas-González, E. and Aragón, C. (2019) Hyperekplexia-associated mutations in the neuronal Glycine Transporter 2. *Neurochemistry International* **123**:95-100.

## Awards and recognition

- The group belongs to the Institute of Investigación Biosanitaria IdiPAZ from November 2010 as research group “Implication of glycinergic and glutamatergic systems in pathologies of the Central Nervous System”.

## Doctoral Theses

**Andrés de la Rocha Muñoz** (2020). Estudio del tráfico intracelular, regulación e interactoma de mutantes de GlyT2 asociados a hiperplexia humana. Universidad Autónoma de Madrid. Supervisor: B. López Corcuera and C. Aragón.

## MOLECULAR NEUROPATHOLOGY

# MOLECULAR BASIS OF HUNTINGTON'S DISEASE AND OTHER CENTRAL NERVOUS SYSTEM DISORDERS

***Principal Investigator:***
José J. Lucas Lozano

***Postdoctoral Fellows:***
Ainara Elorza Peregrina
Ivó Hernández Hernández

***Technicians:***
María Santos Galindo
Miriam Lucas Santamaría

***Predoctoral Fellows:***
Sara Picó del Pino
Ivana Ollá
Claudia Rodríguez López
(incorp: june 2019)
David Lozano Muñoz
(incorp: january 2020)

www.cbm.uam.es/lineas/lucasgroup.

## Research summary

Huntington's disease (HD), the most prevalent genetic neurodegenerative disease, is caused by a CAG trinucleotide expansion in the huntingtin gene. We study the molecular basis of HD through *in vitro* and *in vivo* approaches, the latter generating and characterizing transgenic mouse models. This way, we discovered a key role of tau, a protein related with Alzheimer's disease and other dementias, and a new histopathological hallmark (the Tau Nuclear Rods or TNRs) in HD. Furthermore, the splicing factor SRSF6, involved in tau splicing and capable to bind CAG-repeats, is altered in HD and sequestered in the mutant huntingtin inclusion bodies (Nat Med. 20(8):881-5 2014). SRSF6 alteration opened a new research line on our lab, focusing on splicing factors. We have been able to relate Huntington's disease to another genetic disease of the basal ganglia called XDP that is caused by a mutation in TAF1. This link was observed through the alteration of another splicing factor, SREK1, whose levels drop in HD and lead to diminished TAF1 levels. SREK1 restoration in the HD model R6/1 is able to partially correct HD-like phenotype (Brain 143(7):2207-2219 2020).

Another research line that we have conducted relates to the role of the unfolded protein response (UPR) in neurodegeneration. We described for the first time the expression of the UPR-related protein ATF5 in adult mouse neurons (Brain 136(Pt4):1161-76 2013) and its neuroprotective induction in a mouse model of epilepsy. More recently, we were able to demonstrate ATF5 expression in adult human neurons and found decreased levels and sequestration into huntingtin inclusion bodies in HD, rendering neurons more vulnerable to mutant huntingtin-induced apoptosis (Acta Neuropathol 134(6):839-850 2017).

Another recent and important finding has relied in a serendipitous finding which has related HD with idiopathic Autism Spectrum Disorder (ASD). Studying the polyadenylation-related proteins CPEBs in HD, we found that CPEB4 binds transcripts of ASD-risk genes. In ASD cases, CPEB4 splicing is imbalanced and an equivalent alteration of CPEB4 isoforms in mice mimics the polyadenylation and protein alterations found in ASD and gives rise to ASD-like phenotype (electrophysiological, neuroanatomical and behavioral), identifying CPEB4 as regulator of ASD risk genes (Nature 560(7719):441-446 2018). Further studying polyadenylation in CNS disorders, we have found its alteration in an epilepsy model (Brain 143(7):2139-2153 2020).

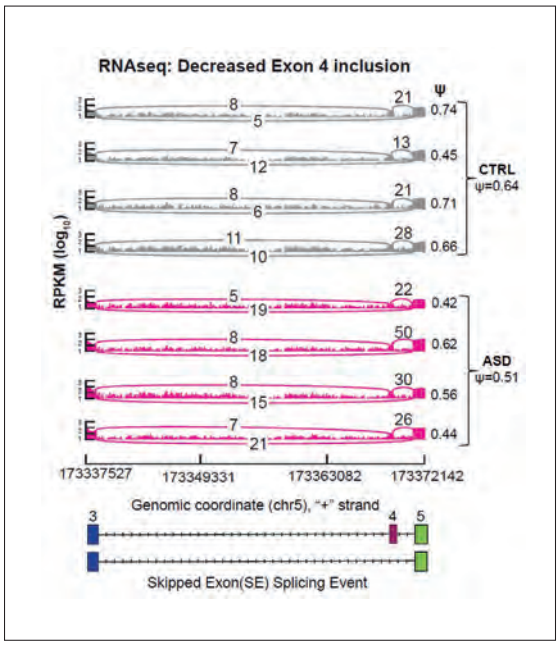



***Figure.*** *Sashimi plots representing CPEB4 splicing in control and ASD cases.*

## Publications

Arranz, J., Balducci, E., Arató, K., Sánchez-Elexpuru, G., Najas, S., Parras, A., Rebollo, E., Pijuan, I., Erb, I., Verde, G., Sahun, I., Barallobre, M. J., Lucas, J. J., Sánchez, M. P., de la Luna, S., and Arbonés, M. L. (2019) Impaired development of neocortical circuits contributes to the neurological alterations in DYRK1A haploinsufficiency syndrome. *Neurobiol Dis*. **127**:210-222.

Sebastián-Serrano, Á., Simón-García, A., Belmonte-Alfaro, A., Pose-Utrilla, J., Santos-Galindo, M., Del Puerto, A., García-Guerra, L., Hernández, I. H., Schiavo, G., Campanero, M. R., Lucas, J. J. and Iglesias, T. (2019) Differential regulation of Kidins220 isoforms in Huntington's disease. *Brain Pathol.* **30**(1):120-136.

Fernández-Nogales, M, and Lucas, J. J. (2020) Altered Levels and Isoforms of Tau and Nuclear Membrane Invaginations in Huntington's Disease. *Front Cell Neurosci*. **13**:574.

Hernández, I. H., Cabrera, J. R., Santos-Galindo, M., Sánchez-Martín, M., Domínguez, V., García-Escudero, R., Pérez-Álvarez, M. J., Pintado, B. and Lucas JJ (2020) Pathogenic SREK1 decrease in Huntington's disease lowers TAF1 mimicking X-linked dystonia parkinsonism. *Brain* **143**(7):2207-2219.

Parras, A., de Diego-Garcia, L., Alves, M., Beamer, E., Conte, G., Jimenez-Mateos, E. M., Morgan, J., Ollà, I., Hernandez-Santana, Y., Delanty, N., Farrell, M. A., O'Brien, D. F., Ocampo, A., Henshall, D. C., Méndez, R., Lucas, J. J.* and Engel, T.* (2020) *Corresponding authors. mRNA polyadenylation as a novel regulatory mechanism of gene expression in temporal lobe epilepsy. *Brain* **143**(7):2139-2153.

Ollà, I., Santos-Galindo, M., Elorza, A. and Lucas, J. J. (2020) P2X7 Receptor Upregulation in Huntington's Disease Brains. *Front Mol Neurosci.* **13**:567430.

Conte, G., Parras, A., Alves, M., Ollà, I., De Diego-Garcia, L., Beamer, E., Alalqam, R., Ocampo, A., Mendez, R., Henshall, D. C., Lucas, J. J.* and Engel, T.* (2020) *Corresponding authors. High concordance between hippocampal transcriptome of the mouse intra-amygdala kainic acid model and human temporal lobe epilepsy. *Epilepsia* **61**(12):2795-2810.

Elorza, A., Márquez, Y., Cabrera, J. R., Sánchez-Trincado, J. L., Santos-Galindo, M., Hernández, I. H., Díaz-Hernández, J. I., García-Escudero, R., Irimia, M. and Lucas, J. J. (2020) Huntington's disease-specific mis-splicing unveils key effector genes and altered splicing factors. Accepted in Brain. BioRxiv preprint: doi.org/10.1101/2020.05.11.086017.

## Awards and recognition

- 2019: José Lucas elected member of Academia Eur opaea.

- 2019: XV Award "Ciencias de la Salud de la Fundación - Caja Rural de Granada" to José Lucas.

- 2019: Award "Vanguardia de la Ciencia 2018" - La Vanguardia y Fundació La Pedrera (2nd award) to José Lucas.

## Patents

Patents. Inventors: José J. Lucas, Sara Picó, Alberto Parras y María Santos
Title: Combined use of thiamine and biotin in the treatment of Huntington's disease
Application N.: P201930825 / PCT/ES2020/070570
Priority country: Spain
Priority date: 24-09-2019
Holder: CSIC/CiberNed

## Doctoral Theses

**Ivó Hernández Hernández** (2019). Análisis del papel de ATF5 en la enfermedad de Huntington. Universidad Autónoma de Madrid. José J. Lucas Lozano y María José Pérez.

## International projects / Research networks

- Part of the H2020 ITN Consortium PurinesDX.

- Part of the Networking Research Center on Neurodegenerative Diseases (CIBERNED) (http://www.ciberned.es/grupo-lucas-lozano.html).

- José Lucas is Member of the National Royal Academy of Pharmacy (RANF).

## MOLECULAR NEUROPATHOLOGY

# THE GENETICS OF PSYCHIATRIC DISEASES

***Principal Investigator:***
Claudio Toma

***Undergraduate and Master Students:***
José Ignacio Gómez Blanco
(TFG, 2020-2021)
Nerea Regueira Acebedo
(TFG, 2020-2021)

http://www.cbm.uam.es/claudio.toma

## Research summary

The identification of genes in psychiatric diseases is our front-line research. Family studies have established a strong genetic contribution to psychiatric diseases, but the specific genes involved still remain largely unknown. Psychiatric disorders are caused by a combination of common variants, each with a small effect, and multiple rare variants of higher penetrance. We adopt several genomic approaches, and a combination of them, to identify susceptibility genes implicated in autism spectrum disorder (ASD), bipolar disorder (BD), and schizophrenia. Our studies include the analysis in ASD and BD of rare inherited variants or *de novo* variants via next-generation sequencing (whole-exome sequencing or whole-genome sequencing), the examination of copy number variants, and the investigation of common variants through case-control and family-based association studies.

In the first exome sequencing study in multiplex autism families, and a recent follow-up, we showed that truncating variants of genes (alleles that disrupt proteins) negatively correlate with IQ in autistic patients. In a separate study we found that these disrupting alleles correlate also with age of onset (proxy for disease severity) in bipolar disorder. Our findings suggest that psychiatric patients with severe symptoms accumulate a higher number of these deleterious mutations.

We also presented a novel genetic approach for gene discovery by combining our expertise in linkage analysis with exome sequencing. In extended families with BD we applied non-parametric linkage analyses and found a linkage peak on chromosome 10, which is explained by rare coding variants, including those from the BD-associated *ANK3* gene.

We identified promising genes likely to be involved in different psychiatric phenotypes: i) We found a stop mutation in the X-linked insulin receptor substrate (*IRS4*) gene which mapped in a linkage peak for a multiplex BD family. The mutation was segregating in five siblings with schizoaffective disorder; ii) In an autistic patient, we identified a *de novo* variant in a canonical splice site of *LRP1* gene, a post-synaptic density gene. We proved that this mutation leads to an in-frame skipping of *exon-29*, likely to lead a B-propeller domain to collapse. Using large genetic data sets we showed that *LRP1* rare variants are implicated in autism (1,778 patients), whereas common variants are associated to schizophrenia (33,640 patients); iii) By combining functional studies, association studies, and sequencing we pinpointed the 14-3-3 gene family to be implicated in ASD and schizophrenia.

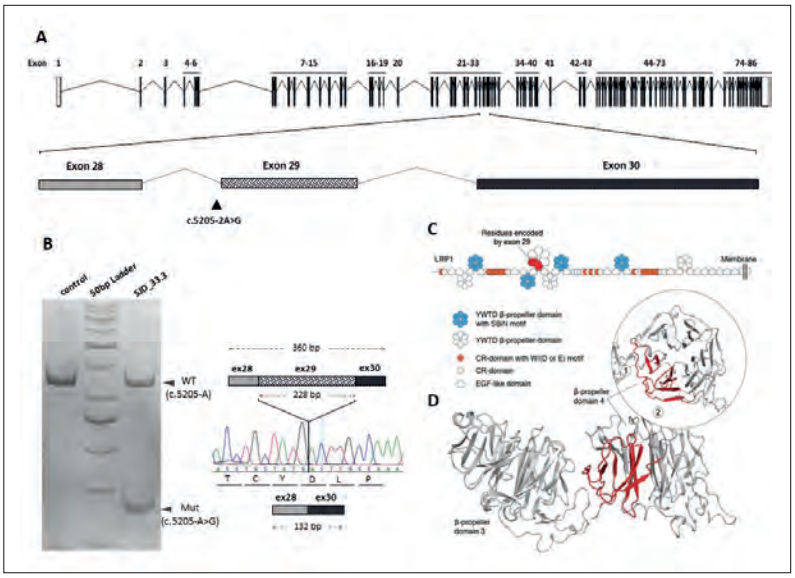



***Figure.*** *Whole-exome sequencing identified a de novo variant (c.5205–2A>G) with splicing effect on LRP1 in an autistic patient (A), which generated an in-frame transcript lacking exon 29 (76 amino acids) (B). The region encoded by exon 29 in β-propeller 4 is in red (C), and the skipping of exon 29 lead to the removal of the first 2 blades of 6 from β-propeller 4 (D).*

## Publications

**Publications with CBM affiliation:**

Toma, C. (2020) Genetic Variation across Phenotypic Severity of Autism. *Trends Genet*, **36**, 228-231.

Torrico, B., Anton-Galindo, E., Fernandez-Castillo, N., Rojo-Francas, E., Ghorbani, S., Pineda-Cirera, L., Hervas, A., Rueda, I., Moreno, E., Fullerton, J. M., Casadó, V., Buitelaar, J. K., Rommelse, N., Franke, B., Reif, A., Chiocchetti, A. G., Freitag, C., Kleppe, R., Haavik, J., Toma, C.*, Cormand, B.* (2020) Involvement of the 14-3-3 Gene Family in Autism Spectrum Disorder and Schizophrenia: Genetics, Transcriptomics and Functional Analyses. *J Clin Med*, **9**.

Jamshidi, J., Williams, L. M., Schofield, P. R., Park, H. R. P., Montalto, A., Chilver, M. R., Bryant, R. A., Toma, C., Fullerton, J. M. and Gatt, J. M. (2020) Diverse phenotypic measurements of wellbeing: Heritability, temporal stability and the variance explained by polygenic scores. *Genes Brain Behav,* **19**, e12694.

Psychiatric Genomics Consortium Bipolar Disorder (PGC-BD3) (2020) Genome-wide association study of over 40,000 bipolar disorder cases provides novel biological insights. *MedRxiv* doi: 10.1101/2020.09.17.20187054. (Accepted in *Nat Genet*).

**Other publications:**

Torrico, B., Shaw, A. D., Mosca, R., Vivo-Luque, N., Hervas, A., Fernandez-Castillo, N., Aloy, P., Bayes, M., Fullerton, J.M., Cormand, B.*, and Toma, C.* (2019) Truncating variant burden in high functioning autism and pleiotropic effects of *LRP1* across psychiatric phenotypes. *J. Psychiatry Neurosci*. **44**, 350-359.

Toma, C., Diaz-Gay, M., Soares de Lima, Y., Arnau-Collell, C., Franch-Exposito, S., Munoz, J., Overs, B., Bonjoch, L., Carballal, S., Ocana, T., Cuatrecasas, M., Castells, A., Bujanda, L., Cubiella, J., Balaguer, F., Rodríguez-Alcalde, D., Fullerton, J. M., Castellví-Bel, S. (2019) Identification of a Novel Candidate Gene for Serrated Polyposis Syndrome Germline Predisposition by Performing Linkage Analysis Combined With Whole-Exome Sequencing. *Clin Transl Gastroenterol*, **10**, e00100.

Toma, C., Diaz-Gay, M., Franch-Exposito, S., Arnau-Collell, C., Overs, B., Munoz, J., Bonjoch, L., Soares de Lima, Y., Ocana, T., Cuatrecasas, M., Castells, A., Bujanda, L., Balaguer, F., Cubiella, J., Caldés, T., Fullerton, J. M., Castellví-Bel, S. (2020) Using linkage studies combined with whole-exome sequencing to identify novel candidate genes for familial colorectal cancer. *Int J Cancer*, **146**, 1568-1577.

Putt, S., Yanes, T., Meiser, B., Kaur, R., Fullerton, J. M., Barlow-Stewart, K., Schofield, P. R., Toma, C., Peay, H. and Mitchell, P. B. (2020) Exploration of experiences with and understanding of polygenic risk scores for bipolar disorder. *J Affect Disord*, **265**, 342-350.

Toma, C., Shaw, A. D., Overs, B. J., Mitchell, P. B., Schofield, P. R., Cooper, A. A. and Fullerton, J.M. (2020) De Novo Gene Variants and Familial Bipolar Disorder. *JAMA Netw Open*, **3**, e203382.

## Awards and recognition

- University of New South Wales Sydney Publication Excellence Award (2019; to Toma).
- Associate editor for *Frontiers in Psychiatry*.
- Associate editor for *Frontiers in Genetics*.
- Associate editor for *Frontiers in Neuroscience*.
- Topics editor for *Genes*.
- Editorial board member for *Journal of Clinical Medicine* (section psychiatry).
- Honorary appointment at Neuroscience Research Australia (NeuRA) (2020-23).
- Adjunct senior lecturer at School of Medicine, University of New South Wales Sydney (2020-23).

## International projects / Research networks

- Psychiatric Genomics Consortium Bipolar Disorder (PGC-BD).
- Bipolar Sequencing Consortium (BSC).

## MOLECULAR NEUROPATHOLOGY

# MOLECULAR MECHANISMS OF NEURODEGENERATION

***Principal Investigators:***
Francisco Wandosell
Beatriz Cubelos

***Postdoctoral Fellows:***
Lara Ordoñez
(CIBERNED)
Carolina Melero
(RETOS COLABORACIÓN)

***Predoctoral Fellows:***
Mario Villa
Assist. Contract–UAM
(from 2018)
Sergio Rivas
(CIBERNED 2018-2021)
Marta García Juan
(FPI)
Alba Orantes
(CIBERNED 2020 & FPI)
Berta Alcover
(Contract)

***Undergraduate and Master Students:***
Elena Anaya
(2018-2019, TFM)
Pedro Arroyo
(2019-2020, TFM)
Andrea Arnanz
(2019-2020, TFM)
Germán Benito
(2018-2019, TFG)
Juan Escudero
(2018-2019, )TFM
Gonzalo García Martín
(2019-2021, TFG-TFM)

http://www.cbm.uam.es/fwandosell

## Research summary

Our group is mostly interested in neurodegenerative disorders is now focusing in a group of brain disorders associated with aging, such as Alzheimer disease (AD) and some type of brain tumours. In 2016, Dr. Beatriz Cubelos (Assist. Prof) has been incorporated as Project Leader in our lab. Dr. Cubelos' group is interested in the analysis of demyelination pathologies. We are interested in describing common molecular mechanisms underlying the age-dependency of these central nervous system disorders. Data from different animal models strongly support the idea that PI3K-Akt-mTORC1 pathway is deregulated in these brain pathologies.

Thus we are analysis some of the molecular mechanisms altered in AD. The accumulation of beta amyloid support the hypothesis that proteostasis is altered in AD. We just analyse the role of mTORC1 activity in the generation of amyloid in AD transgenic models. This protein complex controls general aspects of protein synthesis and autophagy; our data indicated mTORC1 inhibition reduced that beta amyloid accumulation in AD mouse model brains, ex vivo and *in vivo*.

Our second goal is the analysis of a new oncogenic pathway that we recently described in glioma: Akt/WIP/YAP/TAZ. Our data indicated that Akt and WIP are responsible of the cell division of cancer-stem cells and the maintenance of its stem-like phenotype. Our current work is about defining the elements of this path that are between Akt and WIP and WIP and YAP/TAZ that regulate the conversion from astrocyte-astrocytoma-glioma [Colaboration: F. Wandosell (CBM) &. Dr. I. Anton (CNB)].

On the other hand, Dr. B. Cubelos' group is interested in elucidating the molecular mechanisms responsible for the processes of myelination in the CNS. Hypomyelination is a progressive and complex disease phenotype in Multiple Sclerosis and orphan leukodystrophies with no effective treatment. In principle, therapies could either stimulate regeneration of new oligodendrocytes, or boost the capacity of the remaining oligodendrocyte (OL) pool to produce more myelin.

Dr. B. Cubelos' group previous reported that R-Ras1 and/or R-Ras2 loss function reduces activation of PI3K/Akt and MAPK signaling pathways, causing a decrease in OL differentiation and survival. This group probed the differential hypomyelination phenotypes of R-Ras1 and R-Ras2 in greater depth, and in the context of metabolic adaptations.

Dr. F Wandosell and Dr. B. Cubelos' groups have initiated a collaborative work analyzing the defective mechanism/s that produce the myelin defects in several mutants' mice; and second trying to determine if similar mechanisms are modify in human demyelination pathologies.

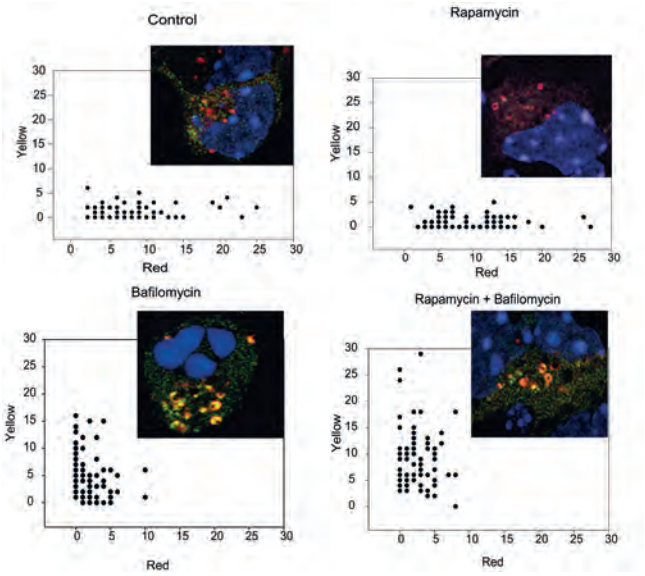



***Figure.*** *Primary neurons + FUW mCherry-GFP-LC3 treated with rapamycin and/or bafilomycin.*

## Publications

Joussain, C., Le Coz, O., Pichugin, A., Marconi, P., Lim, F., Sicurella, M., Salonia, A., Montorsi, F., Wandosell, F., Foster, K., Giuliano, F., Epstein, A. L., and Aranda Muñoz, A. (2019) Botulinum neurotoxin light chains expressed by defective herpes simplex virus type 1 vectors cleave SNARE proteins and inhibit CGRP release in rat sensory neurons. *Toxins* **11**(2).

Cespedes, A., Villa, M., Benito-Cuesta, I., Perez-Alvarez, M. J., Ordoñez, L., and Wandosell, F. (2019). Energy-Sensing Pathways in Ischemia: The Counterbalance Between AMPK and mTORC. *Current Pharmaceutical Design* **25**, 45:4763-70.

Herrera, J. L., Ordoñez-Gutierrez, L., Fabrias, G., Casas, J., Morales, A., Hernandez, G., Acosta, N. G., Rodriguez, C., Prieto-Valiente, L., Garcia-Segura, L. M.*, Wandosell, F.G.* and Alonso, R.* (2019) Ovarian Hormone-Dependent Effects of Dietary Lipids on APP/PS1 Mouse Brain. *Frontiers in Aging Neuroscience*. **11**:346.

Benito-Cuesta, I., Ordoñez, L. and Wandosell, F. (2020) AMPK activation does not enhance autophagy in neurons in contrast to MTORC1 inhibition: different impact on β-amyloid clearance. *Autophagy* **17-3**:656-71.

Ordonez-Gutierrez, L. and Wandosell, F. (2020) Nanoliposomes as a Therapeutic Tool for Alzheimer's Disease. *Frontiers in Synaptic Neuroscience* **12**-20.

Anton, I. M., Gomez-Oro, C., Rivas, S., and Wandosell, F. (2020) Crosstalk between WIP and Rho family GTPases. *Small GTPases* **11**-3:160-166.

Escoll, M., Lastra, D., Robledinos-Anton, N., Wandosell, F., Anton, I. M. and Cuadrado, A. (2020) WIP Modulates Oxidative Stress through NRF2/KEAP1 in Glioblastoma Cells. *Antioxidants* **9**-9.

Alcover-Sanchez, B., Garcia-Martin, G., Wandosell, F. and Cubelos, B. (2020) R-Ras GTPases Signaling Role in Myelin Neurodegenerative Diseases. *International Journal of Molecular Sciences* **21**-16.

Alcover-Sanchez, B., Garcia-Martin, G., Escudero-Ramirez, J., Gonzalez-Riano, C., Lorenzo, P., Gimenez-Cassina, A., Formentini, L., de la Villa-Polo, P., Pereira, M. P., Wandosell, F. and Cubelos, B. (2020) Absence of R-Ras1 and R-Ras2 causes mitochondrial alterations that trigger axonal degeneration in a hypomyelinating disease model. *Glia* **69**:619–637.

## Awards and recognition

- CIBERNED group - PI- F Wandosell: http://ciberned.es/grupo-wandosell.htlm

- Editorial Board of Apoptosis (from 2000).

- Editorial Board of Frontiers in Aging Neuroscience.

- Editorial Board of Cancers (from 2018).

- Editorial Advisory Board of the Journal Recent Patents on CNS Drug Discovery.

- Guest Associated Editor of Frontiers in Neuroscience.

- Comité Científico Editor de la Revista Médica de Risaralda, Colombia.

- Editor of International Journal of Molecular Science (IMS) (B. Cubelos).

## International projects / Research networks

- ERA-NET NEURON 2016 (PCIN-2016-108) "*Regulación del reflejo de micción después de lesión medular: abolición por silenciamiento de los aferentes de las fibras C de la vejiga hiper-excitados mediante terapia génica para restaurar la continencia y la micción*"-ELPIS. Coordinator: Pr. François Giuliano (Francia); PI-(CBM-CSIC): F Wandosell (Spain). May 2016-April 2019.

## MOLECULAR NEUROPATHOLOGY

# MOLECULAR BASIS OF NEUROPROTECTION AND NEUROREPAIR

***Principal Investigators:***
Francisco Zafra
Eva Porlan

***Postdoctoral Fellows:***
Dolores Piniella
Ana Laura Barrios
Coral López Fonseca

***Technician:***
Enrique Núñez

***Undergraduate and Master Students:***
Marta de Juan García
Ania Canseco Rodríguez
Carla Galiana Pérez
Btissam Ben Said

http://wwww.cbm.uam.es/zafra-porlan

## Research summary

The work of our laboratory is dedicated to the understanding of the molecular and cellular mechanisms of neuroprotection and neuroregeneration of the brain against injuries such as cerebral ischemia. For us, the characterization of the neuroreparative mechanisms activated during the so-called ischemic tolerance is of special interest. These mechanisms are induced in response to moderate brain lesions and make this organ resistant to more severe damage that may subsequently suffer. These plastic responses of the brain involve mechanisms ranging from modifications in the activity of the glutamatergic pathways (main executors of toxic damage), to the activation of antioxidant mechanisms and, probably, the generation of new cells through neurogenesis. In relation to glutamatergic pathways, we have investigated the role of glutamate transporters and the potential cross talk with other pathways (like glycinergic and dopaminergic). We have found that the potassium channels regulate the activity of glutamate and dopamine transporters under different circumstances. Moreover, we have found that some microRNAs produced by neurons (mir-96 and mir-137) might impact on the activity of the glycine transporter GLYT1, present in glial cells. We are investigating how these and other microRNAs are packaged into exosomes and are transferred between neurons and glia and vice versa, as neuroprotective agents. Relative to the neurogenic response, we are interested first, in the basic mechanisms by which this process is activated in the adult brain, and second, how this can be modified is a response to situations of stress. In particular, Eva Porlan's group main focus is the potential of druggable targets for enhancing adult neural stem proliferation and neurogenic output with focus in two experimental paradigms: regeneration of the adult supependymal zone after depletion of progeny, and hipoxia. Dr. Porlan's leading interest is the elucidation of the mechanisms underlying the maintenance of the adult neural stem and progenitor cell pools, their implications in pathological conditions and their potential for regenerative processes and therapeutic interventions.

During this time, we have maintained collaborations with the groups of Dr. F.J. Díez-Guerra, in exosome transfer and imaging studies, Drs. B. López-Corcuera (CBM) and C. Avendaño (UAM) on the role of glycinergic pathways, Dr. A. Rodríguez Artalejo (UCM) and D. Bartolomé-Martín in the study of the activity of the sodium and potassium channels. Also with Dr. I. Fariñas (UV, CIBERNED), Dr. M. Malumbres (CNIO) and Dr. T. Iglesias (IIBM Alberto Sols, CSIC) for the adult neurogenesis studies.

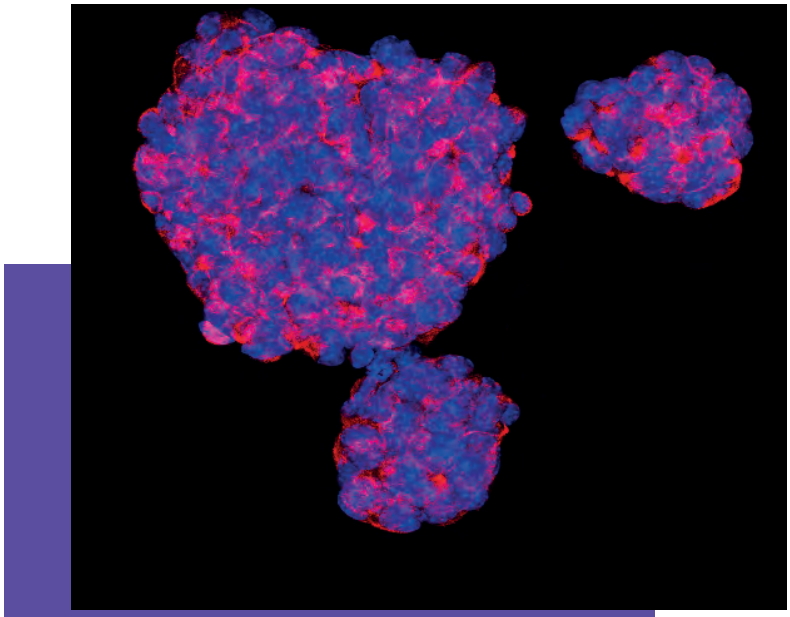


***Figure.*** *Mouse neurospheres from the adult subependymal neurogenic niche stained with nestin (red) and DAPI (blue).*

## Publications

Bartolomé-Martín, D., Ibáñez, I., Piniella, D., Martínez-Blanco, E., Pelaz, S.G. and Zafra, F. (2019) Identification of potassium channel proteins Kv7.2/7.3 as common partners of the dopamine and glutamate transporters DAT and GLT-1. *Neuropharmacology* **161**,107568.

Morante-Redolat, J.M., Porlan, E. (2019) Neural Stem Cell Regulation by Adhesion Molecules Within the Subependyma Niche. *Front Cell Dev Biol*. **7**, 102.

UNIT

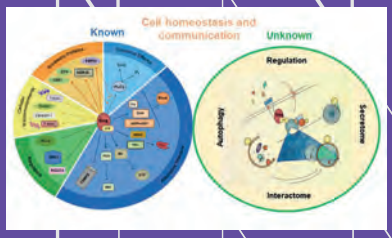
EVA RICHARD

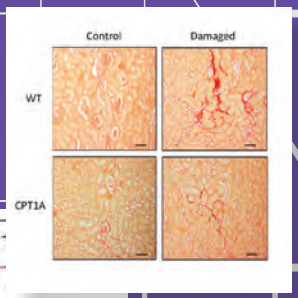
# Metabolic and Signaling Networks in Disease

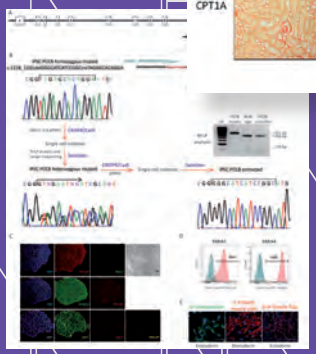
PHYSIOLOGICAL AND PATHOLOGICAL PROCESSES

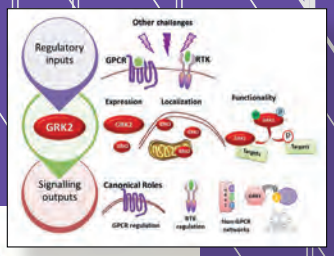
At the **Metabolic and Signaling Networks in Disease Unit**, we are interested in deciphering the various cellular and signaling pathways involved in metabolic homeostasis to understand the pathogenic mechanisms of several human diseases, including both complex and rare monogenic diseases, with the aim of identifying new therapeutic targets. Using diverse cellular and animal models, as well as patient samples, we have contributed to the elucidation of the role of membrane signaling pathways during the onset or progression of metabolic, cardiovascular and inflammatory diseases and in cancer; and the cellular processes that promote the dysregulation of mitochondrial function and other underlying pathophysiological events in cancer, metabolic syndrome, kidney fibrosis, cardiovascular and rare metabolic diseases. In addition to generation of knowledge, our studies place a strong emphasis on the transfer of our results to the clinics to advance in the diagnosis, prognosis and treatment of these pathologies as evidenced by the recent repurposing of drugs for the treatment of cancer and insulin resistance. Some of our highlights are the following:

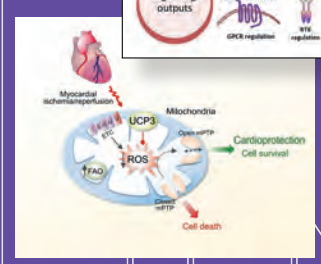
- Mechanistic implication of IF1 overexpression in suppressing metastatic disease in colon cancer.
- Repurposing of the β-adrenergic blocker nebivolol for the treatment of cancer.
- Elucidation of the role of muscular mitochondrial perturbations in metabolic disorders and of the therapeutic potential of edaravone.
- Development of new iPSC-derived and CRISPR-Cas gene edited disease models.
- Protection from kidney fibrosis by recovery of FAO and mitochondrial function in different models of renal injury.
- Role of microRNAs in fibrogenesis and cardiomyopathies.
- Modulation by GRK2 of adipose-liver crosstalk in high fat diet-induced obesity.
- Protection by UCP3 against cardiac ischemia-reperfusion injury and its involvement in the protective phenomenon of ischemic preconditioning.




**EDUARDO BALSA**

MOLECULAR AND METABOLIC MECHANISM UNDERLYING MITOCHONDRIAL DYSFUNCTION

**PEDRO BONAY**

FUNCTIONAL GLYCOGENOMICS

**SUSANA CADENAS**

MITOCHONDRIAL PATHOPHYSIOLOGY

**SARA COGLIATI**

MOLECULAR MECHANISMS OF SEX-DIFFERENCES IN METABOLISM PHYSIOLOGY AND DISEASE

**JOSÉ MANUEL CUEZVA**

THE ROLE OF MITOCHONDRIA IN HUMAN PATHOLOGY

**LAURA FORMENTINI**

ROLE OF MITOCHONDRIAL METABOLISM ON THE PATHOPHYSIOLOGY OF SKELETAL MUSCLE

**SANTIAGO LAMAS**

MOLECULAR PATHOPHYSIOLOGY OF FIBROSIS

**FEDERICO MAYOR JR**

PATHO-PHYSIOLOGICAL IMPLICATIONS OF G PROTEIN-COUPLED RECEPTORS SIGNALING NETWORKS

**CRISTINA MURGA**

SIGNALING PATHWAYS IN MOUSE MODELS OF METABOLIC AND CARDIOVASCULAR PATHOLOGIES

**PETRONILA PENELA**

CELLULAR SIGNALING NETWORKS IN CANCER (ONCO-RESECEL)

**BELÉN PÉREZ / PILAR RODRÍGUEZ-POMBO**

TRANSLATIONAL MEDICINE IN INBORN ERRORS OF METABOLISM AND OTHER RARE GENETIC DISEASES

**CATALINA RIBAS**

REGULATORY FUNCTIONS AND MECHANISMS OF CELL SIGNALING PATHWAYS THROUGH G PROTEINS: A NEW INTERACTOME

**LOURDES RUIZ DESVIAT / EVA Mª RICHARD**

PHYSIOPATHOLOGY STUDIES AND THERAPEUTICAL APPROACHES IN ANIMAL AND CELLULAR MODELS OF NEUROMETABOLIC DISEASES

**BEATRIZ PARDO / ARACELI DEL ARCO / JORGINA SATRÚSTEGUI / CAYETANO VON KOBBE**

CALCIUM SIGNALING IN MITOCHONDRIA AND INSULIN/LEPTIN SIGNALING DURING AGEING

**JAVIER TRABA**

MITOCHONDRIAL BIOLOGY IN IMMUNE MODULATION

## METABOLIC AND SIGNALING NETWORKS IN DISEASE

# MOLECULAR AND METABOLIC MECHANISM UNDERLYING MITOCHONDRIAL DYSFUNCTION

***Principal Investigator:***
Eduardo Balsa Martínez

https://www.cbm.uam.es/balsalab

## Research summary

Mitochondria are unique and complex organelles that carry out critical metabolic functions within the cells. Once considered to be mere sites of ATP generation, it is now evident that these organelles participate in a wide range of cellular processes including calcium homeostasis, apoptosis, redox balance or cell fate. Because of this multifaceted contribution of mitochondria to key biologic and metabolic pathways it is not surprising that mitochondrial dysfunction has been linked to many human disorders including neurodegeneration, diabetes, cancer or aging.

Specifically, our lab focuses on defects in the oxidative phosphorylation system (OXPHOS) occurring from mitochondrial disease mutations that compromise cellular fitness and survival. This biochemical failure is thought to underlie pathologies associated with mitochondrial dysfunction. However, the precise metabolic processes, signaling pathways and compensatory responses resulting from a defective mitochondrial Electron Transport Chain (ETC) that drive these fatal disorders are not entirely understood. Although diminished ATP production has been considered a hallmark of mitochondrial dysfunction, our recent discoveries highlighted that other metabolic failures such as disturbed redox hemostasis due to accumulated levels of NADH can be equally detrimental. Moreover, which cell types contribute the most to the disease and whether disease-carrying cells negatively impact the function of its surrounding wild-type neighbors or distant organs remain poorly characterized.

The long-tern goal of our lab is to understand the molecular components that regulate mitochondrial metabolism, in the context of physiology and diseases, and use this knowledge to develop successful therapies. To accomplish these goals, we are employing cutting-edge technologies such CRISPR/Cas9-based genetic screenings, multi-omics platform and preclinical mouse models to elucidate the molecular mechanisms whereby mitochondrial dysfunction compromise cellular fitness and leads to organ failure in the context of human diseases.

So far therapeutic interventions to combat diseases caused by mitochondrial dysfunction have been limited by poor definition of molecular targets. In this regard, the outcomes of this studies will yield novel information about potential targets that would help to initiate drug development programs for clinical studies.

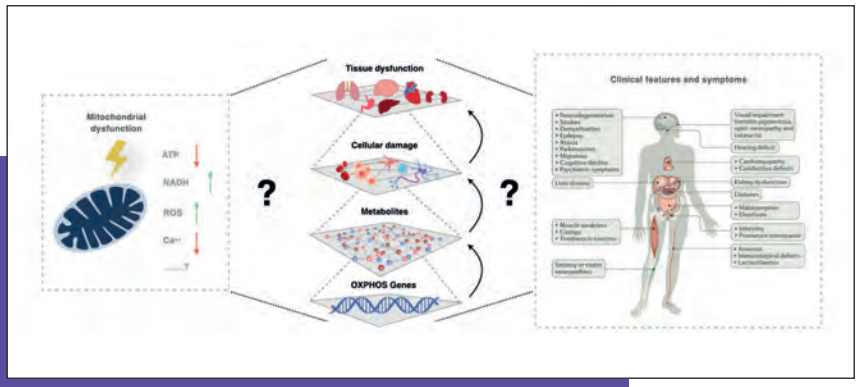



***Figure.*** *Mitochondrial dysfunction leads to biochemical abnormalities such decreased ATP levels, overproduction of Reactive Oxygen Species (ROS) or accumulation of NADH that drive human pathologies. However, the precise metabolic processes, signalling pathways and compensatory responses resulting from these biochemical abnormalities are not entirely understood. Our work focused on developing a holistic understanding of the molecular and metabolic components that contribute to cell and tissue deterioration in the context of Mitochondrial dysfunction.*

## Publications

**Articles without CBM-UAM affiliation:**

Balsa E, Perry EA, Bennett CF, Jedrychowski M, Gygi SP, Doench J, Puigserver P. 2020. Defective NADPH Production in Mitochondrial Disease Complex I Causes Inflammation and Cell Death. *Nature Communications* volume **11**, Article number: 2714 (2020)

Luo C, Balsa E, Perry EA, Liang J, Tavares CD, Vazquez F, Widlund HR, Puigserver P. 2020. H3K27me3-mediated PGC1α gene silencing promotes melanoma invasion through WNT5A and YAP. *J Clin Invest*. **3**;130(2):853-862.

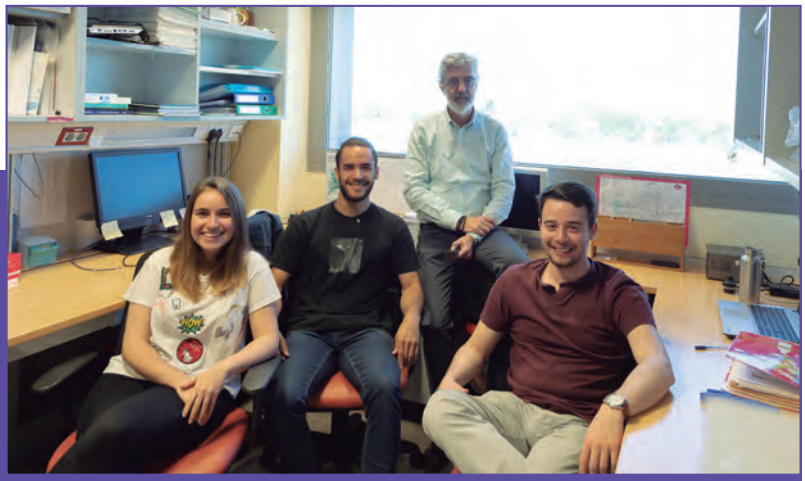
Balsa E, Soustek MS, Thomas A, Cogliati S, Garcia-Poyatos C, Martin-Garcia E, Jedrychowski M, Gygi SP, Enríquez JA, Puigserver P. 2019. ER and Nutrient Stress Promote Assembly of Respiratory Chain Supercomplexes Through PERK/eIF2α Axis. *Mol Cell*. Volume **74**, Issue 5, Pages 877-890.

## International projects / Research networks

- ERC Starting Grant (2020 ERC-Stg) 948478 -Mito-Cure-. Funded by the EC-European Research Council. 01/01/2021 - 31/12/2025. Coordinator/PI: Eduardo Balsa Martinez.

## METABOLIC AND SIGNALING NETWORKS IN DISEASE

# FUNCTIONAL GLYCOGENOMICS



***Principal Investigator:***
Pedro Bonay Miarons

***Predoctoral Fellow and Technician:***
Laura Corvo Villén

***Visiting Scientist:***
Marcello Rossi

***Undergraduate and Master Students:***
Jose Carlos Paredes
Irene Serrano (TFG)
Jorge Mondéjar (TFG)
Cristian González (TFG)
Sergio Marín Portugués (TFG)
Laura Noguera (TFG)

## Research summary

The Glycosylation is the most abundant, diverse and dynamic post-translational modification in nature, generating one of the most complex biological molecules found in nature, the glycans. Those are covalent conjugates of an oligosaccharide to certain amino acid residues on the protein backbone, resulting in a plethora of glycoforms potentially exhibiting a wide spectrum of functional and biological proteins for a single gene product. Almost all secreted and membrane proteins are glycosylated and hence almost all plasma and serum proteins are glycoproteins. This co-translational modification widens the functional spectra of proteins at least one magnitude order. Glycan biosynthesis is more significantly affected by disease states than by protein production. Glycomics, therefore holds considerable promise specifically as disease markers. The nonlinear and non-template based biosynthesis of glycans make head to head compare glycomics to proteomics is not technically possible, and complex structural analysis of glycome is necessary in order to get a glycomic profile.

The group has devoted the last five years to assemble, implement and validate a novel technological platform that allows us to analyze the N-glycome from minute amounts of biological samples: sera, plasma or tissues, unique at the UAM campus and second in Spain, and fourth in Europe behind Croacia and Ireland. The group has curated one of the largest collections of clinically well characterized biological samples of American tripanosomiasis biological samples (around 5000), leishmaniasis visceral and Neurocisticercosis from all stages of the diseases, before and after chemotherapeutic treatment.

The glicomic evaluation of individuals (not populations) allows to establish associations to disease progression, therapeutic efficacy or failure and reinfections. The system has been used to analyze samples form three defined infectious disease from which we have clinically defined cohorts (Chagas disease, Leishmaniasis and Neurocysticercosis). From our previous studies on tyiortal sera N-glycome we have moved to study the effector profile of human Immunoglobiulin G derived from its glycosylation profile. By using this novel approach, we have been allowed to identify some molecular markers for efficacy during the treatment with Benznidazole for acute Chagas disease patients, and able to discriminate the latent form active form of neurocysticercosis, previously only possible by using classical image systems like NMR or PET-TAC.

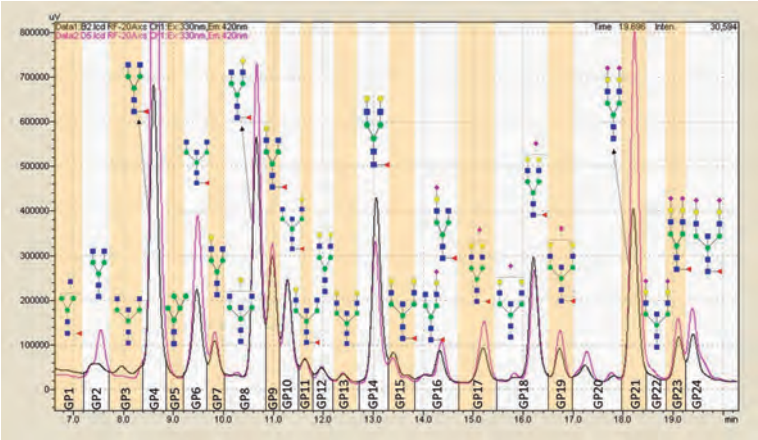



***Figure.*** *HILIC-UPLC chromatograms stack showing the robustness of glycan resolution from 0.2 uG total IgG isolated from (pink) American trypanosomiasis (Chagas´ disease) patient and (black) control healthy subject*

## Publications

Pineda M, Corvo L, Callejas-Hernández F, Fresno M, Bonay P. (2020). Trypanosoma cruzi cleaves galectin-3 N-terminal domain to suppress its innate microbicidal activity *Clin Exp Immunol*. **199**(2):216-229.

## Awards and recognition

- The IP has been awarded one more year as the national representative from Spain at the International Glycoconjugate Organization (IGO, associated to IUB-MB since 1989).

The roles inside the IGO are to further international collaboration for the study of glycoconjugates, survey the academic activities on Glycosciences around the globe and to define the roadmap to integrate the glycosciences as a significant part of the science curriculum in the high-school, undergraduate and graduate education.

## METABOLIC AND SIGNALING NETWORKS IN DISEASE

# MITOCHONDRIAL PATHOPHYSIOLOGY


***Principal Investigator:***
Susana Cadenas Álvarez

***Predoctoral Fellows:***
Patricia Sánchez Pérez
(until November 2019)
May-Kristin Torp
(July-November 2019)
Ana Mata Villanueva
(since July 2020)

***Visiting Scientists:***
Jürgen Prasch
(May-December 2019)
Visiting predoctoral fellow,
Medical University of Graz,
Austria
Iswarya Sreeram
(October-December 2019)
Erasmus+ student

http://www.cbm.uam.es/scadenas

## Research summary

Research in our lab is focused on the role of mitochondria in cell physiology and in the development of pathological conditions. The oxidation of fuels in mitochondria produces most of the energy that cells need to survive. However, it also produces free radicals and other reactive oxygen species (ROS) that, when generated in excess, cause oxidative stress and a wide range of maladies, including cancer, cardiovascular and neurodegenerative diseases. We are particularly interested in ischemia-reperfusion (IR) injury, which consist of the paradoxical exacerbation of cellular damage following restoration of blood flow to previously ischemic tissues. Myocardial IR contributes to adverse cardiovascular outcomes after myocardial ischemia, cardiac surgery or circulatory arrest. IR injury is mediated by several factors including excessive ROS production, which are generated mainly at reperfusion. The underlying mechanism involves succinate accumulation during ischemia and superoxide production at complex I by reverse electron transfer driven by succinate oxidation upon reperfusion. The combined effects of ROS and elevated calcium concentration lead to the opening of the mitochondrial permeability transition pore (mPTP), which plays a critical role in reperfusion damage. Non-mitochondrial sources of ROS such as xanthine oxidase and NADPH oxidases (NOX) contribute to secondary tissue damage and subsequent inflammation.

The mitochondrial uncoupling protein 3 (UCP3) has been proposed to play a role in the control of the production of mitochondrial ROS and in the protection against oxidative damage. We have previously shown that UCP3 is upregulated in response to oxidative stress in mouse cardiomyocytes and this in turn increases cell survival under these conditions. Our recent studies have focused on the protective role of UCP3 against cardiac IR injury and its involvement in ischemic preconditioning. We have found that UCP3 protein levels increase in isolated perfused mouse hearts in response to myocardial ischemia followed by reperfusion. Moreover, hearts subjected to IR from mice lacking UCP3 have larger infarct size than those from wild-type mice, as determined by tetrazolium chloride staining and creatine kinase activity. In addition, ischemic preconditioning is lost in UCP3 knockout mice. We are currently investigating the mechanisms underlying this protection. Our main hypothesis is that UCP3 protects from IR injury by decreasing mitochondrial superoxide production and by affecting cardiac metabolism. The results of these studies will help understand the mechanism through which UCP3 confers cardioprotection. These studies have potential clinical value as they might help develop compounds aimed at decreasing this type of cardiac damage.

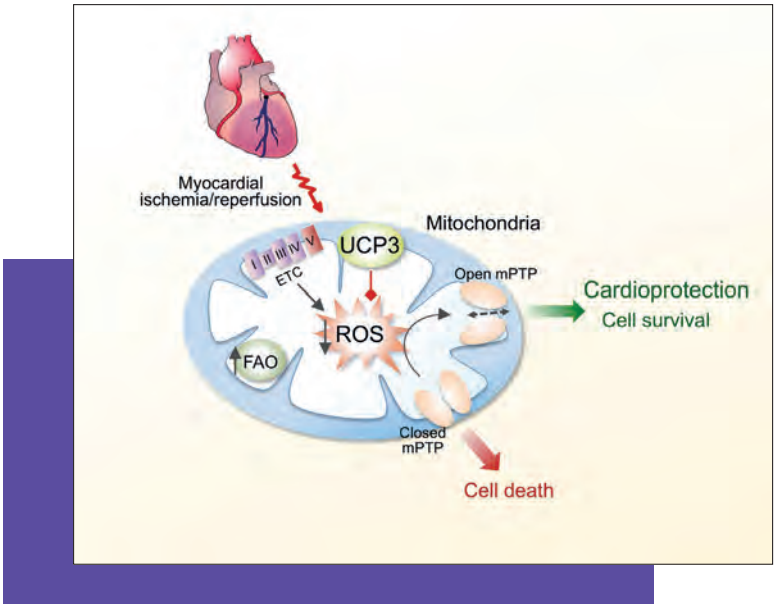



***Figure.*** *Mitochondrial uncoupling protein 3 (UCP3) protects against cardiac ischemia-reperfusion injury through effects on energy metabolism and redox balance. ETC, electron transport chain; FAO, fatty acid oxidation; mPTP, mitochondrial permeability transition pore; ROS, reactive oxygen species.*

## Publications

González-Tajuelo R., de la Fuente-Fernández M., Morales-Cano D., Muñoz-Callejas A., González-Sánchez E., Silván J., Serrador J.M., Cadenas S., Barreira B., Espartero-Santos M., Gamallo C., Vicente-Rabaneda E.F., Castañeda S., Pérez-Vizcaíno F., Cogolludo Á., Jiménez-Borreguero L.J., Urzainqui A. (2020) Spontaneous pulmonary hypertension associated with systemic sclerosis in P-selectin glycoprotein ligand 1-deficient mice. *Arthritis Reumatol*. **72**, 477-487.

Rubio-Navarro A., Vázquez-Carballo C., Guerrero-Hue M., García-Caballero C., Herencia C., Gutiérrez E., Yuste C., Sevillano Á., Praga M., Egea J., Cannata P., Cortegano I., de Andrés B., Gaspar M.L., Cadenas S., Michalska P., León R., Ortiz A., Egido J., Moreno J.A. (2019) Nrf2 plays a protective role against intravascular hemolysis-mediated acute kidney injury. *Front Pharmacol*. **10**: 740.

## Awards and recognition

- Our group belongs to the Instituto de Investigación Sanitaria del Hospital Universitario de La Princesa (IIS-IP). http://www.iis-princesa.org.

## Doctoral Theses

**Patricia Sánchez Pérez (2019)** Protective role of the mitochondrial uncoupling protein UCP3 and the transcription factor Nrf2 against cardiac ischemia-reperfusion injury and their involvement in ischemic preconditioning. Universidad Autónoma de Madrid. Supervisor: Susana Cadenas Álvarez. International PhD Mention.

## International projects / Research networks

- We participate in the COST Action MITOEAGLE CA15203 (2016-2020).

## METABOLIC AND SIGNALING NETWORKS IN DISEASE

# MOLECULAR MECHANISMS OF SEX-DIFFERENCES IN METABOLISM PHYSIOLOGY AND DISEASE

***Principal Investigator:***
Sara Cogliati

https://www.cbm.uam.es/scogliati

## Research summary

As part of the progress towards personalized medicine, there is an urgent need to understand biological sex-differences. However, the progress in the understanding of the sex-specific physio-pathology is still marginal.

Our laboratory aims to understand the molecular mechanisms of metabolic sex-differences in health and disease, explicitly exploring mitochondria's role. Indeed, mitochondria are the central hub of metabolism and targets of sexual-hormones, with a suggested role in modulating sex-specific differences in many physio-pathological conditions.

Currently, we are running two projects: one considering Type 2-diabetes (T2D) and another heart failure (HF).

Type 2-diabetes (T2D), one of the most important medical challenges of the 21st century, shows important clinical sex-differences inferring that the molecular mechanisms responsible for its pathophysiology in males and females would be different. Nevertheless, these mechanisms are almost unknown. Our preliminary data show that mitochondrial ATP synthase rate is higher in female mice that coincides with better glucose tolerance suggesting that mitochondria have sex-specific characteristics that could shape glucose metabolism differently. Interestingly, when we modulate mitochondrial activity in mouse models, only males show differences in glucose tolerance. At the same time, females are barely affected, supporting the hypothesis that females have strategically developed metabolic rescue pathways to compensate for potential mitochondrial dysfunctions. This project aims to define the molecular components of mitochondrial sex-differences and decipher their role in glucose metabolism response in both male and female mice comparing normal and diabetic conditions.

HF is the inability of the heart to sustain the energy needs of the organism. It presents crucial sex-differences: men have a reduced ejection fraction with dilatated ventricles, while women have preserved ejection fraction with smaller and stiffer ventricles, which has a more unfavourable prognosis. Unfortunately, the molecular mechanisms responsible for these sexual dimorphisms are still unknown, and their knowledge is essential to identify specific therapeutic targets for men and women. It has recently been demonstrated that mitochondria have a crucial role in HF development, but how they contribute to generating sex-differences in HF is still unknown. We are currently studying the mitochondrial role after trans-aortic-constriction in male and female mice and further analyse the cardiac functions by echocardiography in collaboration with the Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid. This project is supported by L'Oreal-Unesco, For Women in Science prize.

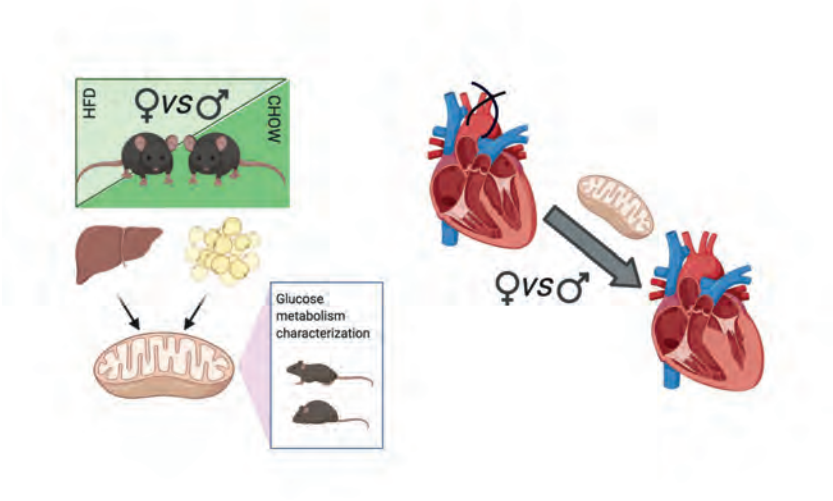



***Figure.*** *Graphical representation of the on-going projects.*

*Sex-Differences in glucose metabolism: characterization of the mitochondrial role (on the left). This project aims to identify the mitochondrial functions responsible for the sex-differences in glucose metabolism, considering liver and white adipose tissue of male and female mice under normal conditions (chow diet) and high-fat diet (HFD) as a model for diabetes. Characterization of the mitochondrial role in the sex-differences of heart failure (on the right). After inducing heart failure by trans-aortic constriction technique in mice, we analyze the mitochondrial role in the sex-specific molecular mechanisms leading to heart failure. (Figure created with Biorender.com).*

## Publications

**Articles with CBM-UAM affiliation:**

Cogliati* S. , Herranz F., Ruiz-Cabello J., Enríquez J.A.* (*co-correspondent authors) (Epub 2020). Digitonin concentration is determinant for mitochondrial supercomplexes analysis by BlueNative Page. *BBA-Bioenergetics,* **1**,1862:1148332.

**Articles without CBM-UAM affiliation:**

Alvarez-Franco A, Rouco R, Ramirez RJ, Guerrero-Serna G, Tiana M, Cogliati S, Kaur K, Saeed M, Magni R, Enriquez JA, Sanchez-Cabo F, Jalife J, Manzanares M. (2020) Transcriptome and proteome mapping in the sheep atria reveal molecular features of atrial fibrillation progression. Cardiovascular Res. Epub 2020 Oct.29; cvaa307.

Calvo E*, Cogliati S*, Hernansanz-Agustín P*, Loureiro-López M*, Guarás A, Casuso RA, García-Marqués F, Acín-Pérez R, Martí-Mateos Y, Silla-Castro JC, Carro-Alvarellos M, Huertas JR, Vázquez J, Enríquez JA. (* co-authors). (2020). *Sci.Adv.* **6**(26):eaba7509.

García-Poyatos C, Cogliati S, Calvo E, Hernansanz-Agustín P, Lagarrigue S, Magni R, Botos M, Langa X, Amati F, Vázquez J, Mercader N, Enríquez JA. (2020). Scaf1 promotes respiratory supercomplexes and metabolic efficiency in zebrafish *EMBO Rep*. **21**(7):e50287.

Huertas JR, Casuso RA, Agustín PH, Cogliati S. Stay Fit, Stay Young: Mitochondria in Movement: The Role of Exercise in the New Mitochondrial Paradigm (2019) *Oxid Med Cell Longev*. 2019 Jun 19;2019:7058350.

Balsa E, Soustek MS, Thomas A, Cogliati S, García-Poyatos C, Martín-García E, Jedrychowski M, Gygi SP, Enriquez JA, Puigserver P. (2019) ER and Nutrient Stress Promote Assembly of Respiratory Chain Supercomplexes through the PERK-eIF2α Axis. *Mol Cell.* **74**(5):877-890.e6.

## Awards and recognition

- 2019 L'Oreal-Unesco For women in Science Prize.

## METABOLIC AND SIGNALING NETWORKS IN DISEASE

# THE ROLE OF MITOCHONDRIA IN HUMAN PATHOLOGY

***Principal Investigator:***
José M. Cuezva

***Postdoctoral Fellows:***
Fulvio Santacatterina
Ana García Aguilar
Cristina Nuevo Tapioles
Pau B. Esparza Moltó
Alba Roca Portoles
Jesús Vallejo Diaz

***Predoctoral Fellows:***
Laura Torresano Cicuendez
Inés Romero Carramiñana
Sonía Dominguez Zorita

***Technicians:***
Cristina Núñez de Arenas
Brenda Sánchez Garrido

***Undergraduate and Master Students:***
Beatriz Cicuende Salazar (2019)
Teresa Manchón Campillo (2020)

http://www.cbmso.es/jmcuezva

## Research summary

Mitochondria play key roles in cellular metabolism, bioenergetics, the execution of cell death and intracellular signaling. Consistent with its prime physiological roles mitochondrial dysfunction is involved in the genesis and progression of ageing and of a plethora of human pathologies including cancer, metabolic syndrome, neurodegeneration and rare disorders. The mitochondrial ATP synthase is a key transducer in energy conservation by oxidative phosphorylation (OXPHOS), in the execution of cell death and in intracellular signaling by calcium and reactive oxygen species (ROS). Previously, we documented the mechanisms and role-played by the ATP synthase in metabolic reprogramming during liver development and in human carcinomas. More recently, we demonstrated that the inhibitor of the ATP synthase, named ATPase Inhibitory Factor 1 (IF1), is highly overexpressed in carcinomas playing a pivotal role in metabolic reprogramming of cancer and stem cells. We showed that binding of IF1 to the ATP synthase inhibits the enzyme under normal physiological conditions and this binding is prevented by phosphorylation of IF1-S39 through the activity of a cAMP-dependent protein kinase A like activity. Inhibition of the ATP synthase is required for adaptation to hypoxia, cell cycle progression and in cancer. Contrariwise, dephosphorylation of IF1 is required to increase the mitochondrial output of ATP in response to an increase in energy demand. Moreover, the IF1-mediated inhibition of the ATP synthase triggers a ROS signal that promotes the activation of nuclear programs of proliferation and resistance to cell death. Hence, IF1 is a most relevant mitochondrial protein that participates in defining the cellular phenotype.

A main objective of our group is to deepen into the cellular biology and role of the ATP synthase/IF1 axis in cancer and other metabolic disorders, neuronal and immune functions and in ageing. To cover these aims, we have developed transgenic mice (Tg-IF1) that conditionally overexpress human IF1 in neurons, liver, colon, heart or skeletal muscle, and generated the *ATP5IF1* lox/lox mouse which has been successfully used to knock-out IF1 (IF1-KO) in neurons, enterocytes and immune cells. With these models, we have demonstrated in vivo the role of the ATP synthase/IF1 in metabolic reprogramming and in signaling adaptive cellular and tissue responses in normal and pathophysiological situations. Moreover, we have developed (i) the PROTEOmAb Platform for the identification of metabolic proteins as biomarkers of disease and (ii) identified FDA-approved small molecules that regulate OXPHOS for targeting mitochondria and effective bedside translation of the drugs to patients affected by mitochondrial dysfunction (Fig).

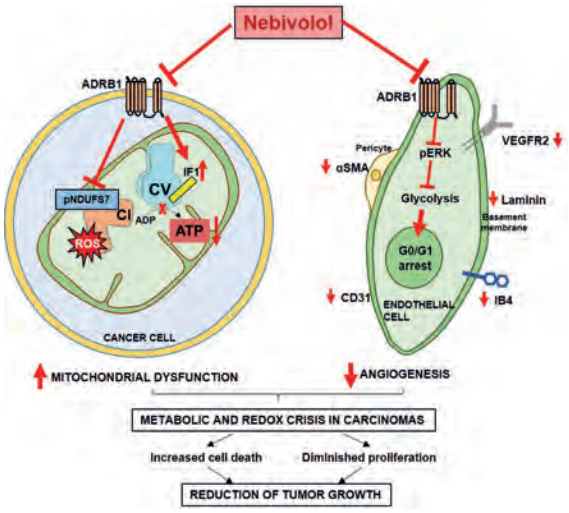



***Figure.*** *A drug repurposing strategy identifies the β1-adrenergic blocker nebivolol as a promising drug to treat cancer. Nebivolol targets OXPHOS both at Complex I and the IF1/ATP synthase axis and in addition prevents tumor angiogenesis, what results in the inhibition of tumor growth due to a metabolic and redox crisis in carcinomas.*

## Publications


Nuevo-Tapioles C, Santacatterina F, Stamatakis K, Núñez de Arenas C, Gómez de Cedrón M, Formentini L, Cuezva JM. (2020) Coordinate β-adrenergic inhibition of mitochondrial activity and angiogenesis arrest tumor growth. *Nat Commun.* **11**:3606.

Povo-Retana A, Mojena M, Stremtan AB, Fernández-García VB, Gómez-Sáez A, Nuevo-Tapioles C, Molina-Guijarro JM, Avendaño-Ortiz J, Cuezva JM, López-Collazo E, Martínez-Leal JF, Boscá L. (2020) Specific Effects of Trabectedin and Lurbinectedin on Human Macrophage Function and Fate-Novel Insights. *Cancers* (Basel). **12**:3060.

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García-Aguilar A, Martínez-Reyes I, Cuezva JM. (2019) Changes in the Turnover of the Cellular Proteome during Metabolic Reprogramming: A Role for mtROS in Proteostasis. *J Proteome Res.* **18**:3142-3155.

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## Patents

*Co-inventors*: Cristina Nuevo-Tapioles, Jorgina Satrústegui, Francisco Palau y José M. Cuezva. " Pharmaceutical composition for the treatment of Charcot-Marie-Tooth disease". P202031320. Priority: Spain, 30 December 2020. Propietarios: 68% Universidad Autónoma de Madrid; 20% consorcio público estatal CIBER y 12% Hospital Sant Joan de Deu.

## Doctoral Theses

**Cristina Nuevo Tapioles** (2019). "Regulación de la OXPHOS mediada por IF1 y su potencial como diana terapéutica en cáncer". Universidad Autónoma de Madrid. Supervisors: José M. Cuezva and Laura Formentini. *Cum laude.*

**Pau B. Esparza- Moltó** (2020). "Tissue-specific expression of the ATPase inhibitory factor 1 and its role in neuronal function" Universidad Autónoma de Madrid. Supervisor: José M. Cuezva. *Cum laude* and international mention.

## International projects / Research networks

- José M. Cuezva is leader of unit "U713" of the CIBER of Enfermedades Raras (CIBERER) and of the Research Group "Metabolismo Energético Traslacional" of the Instituto Universitario Hospital 12 de Octubre (i+12) , both are initiatives of the "Instituto de Salud Carlos III". In addition, forms part of the Network of Excellence RED2018-102379-T METABOCANCER.

## METABOLIC AND SIGNALING NETWORKS IN DISEASE

# ROLE OF MITOCHONDRIAL METABOLISM ON THE PATHOPHYSIOLOGY OF SKELETAL MUSCLE


***Principal Investigator:***
Laura Formentini

***Predoctoral Fellows:***
Cristina Sánchez González
Juan Cruz Herrero Martín

https://www.cbm.uam.es/lformentini

## Research summary

Laura Formentini (ORCID: 0000-0001-5641-821X) is Professor "Contratado Doctor" at UAM University. Previously, she was supported by Juan de Cierva, AECC and Ramon y Cajal competitive fellowships. Nowadays, she has produced 30 publications (92% Q1, 70% D1) with more than 1300 citations and an h index of 19 (Web of Science, 2020), has supervised 3 PhD thesis (2 in progress) and different research students.

She started her research career in Italy at the School of Medicine of the University of Firenze. Since then, her investigation was focused on understanding how mitochondrial energy metabolism participates in the integration of different cellular functions. Complex regulatory mechanisms enable mitochondrial metabolism to match cell demands, which extend beyond the production of ATP: during the last years she demonstrated that mitochondrial oxidative phosphorylation (OXPHOS) plays further roles in controlling cell death (Formentini L. et al., *EMBO J*, 2014, 33(7):762-78); immunity (Formentini L. et al., *Cell Reports*, 2017, 19(6):1202-1213) and oncogenesis (Formentini L et al., Mol Cell, 2012, 45(6):731-42; Nuevo-Tapioles, C. et al., *Nat Comm*, 2020, 11:3606). Impaired mitochondrial function also deeply alters lipid species and metabolism (Formentini L et al., *Diabetologia*, 2017, 60(10):2052-2065) and is emerging as a pivotal hallmark of metabolic disorders. Understanding which products of metabolism are limiting for correct cell function, and how cells obtain or transform them in physiological tissue environments, is crucial to exploit mitochondrial metabolism for therapy.

A main purpose of her research line (supported by PID2019-104241RB-I00 and SAF2016-76028-R national funding) is to deepen into the knowledge of mitochondrial metabolism in the pathophysiology of skeletal muscle, the highest oxidative tissue in mammals. Nowadays, her group is defining how mitochondria dysfunctions, environmental factors and diet impact metabolism at the cell, tissue, and organism level (Sanchez-Gonzalez C et al., *EMBO J.* 2020, e103812), and identifying aspects of mitochondria activity that are limiting for cell homeostasis in different contexts. She is part of the editorial board of Frontiers in Physiology (Q1) and members of RAC, SEBBM and EFSD societies. She started and strengthened international collaborations with EU and USA partners and national institutions (CIBERER, i+12 Institute) for translating the understanding of muscle cell metabolism into novel therapy approaches. Ultimately, she aims to provide knowledge based on new mitochondrial aspects for better prevention, diagnosis and therapy of metabolic and rare diseases that have skeletal muscle as a target organ.

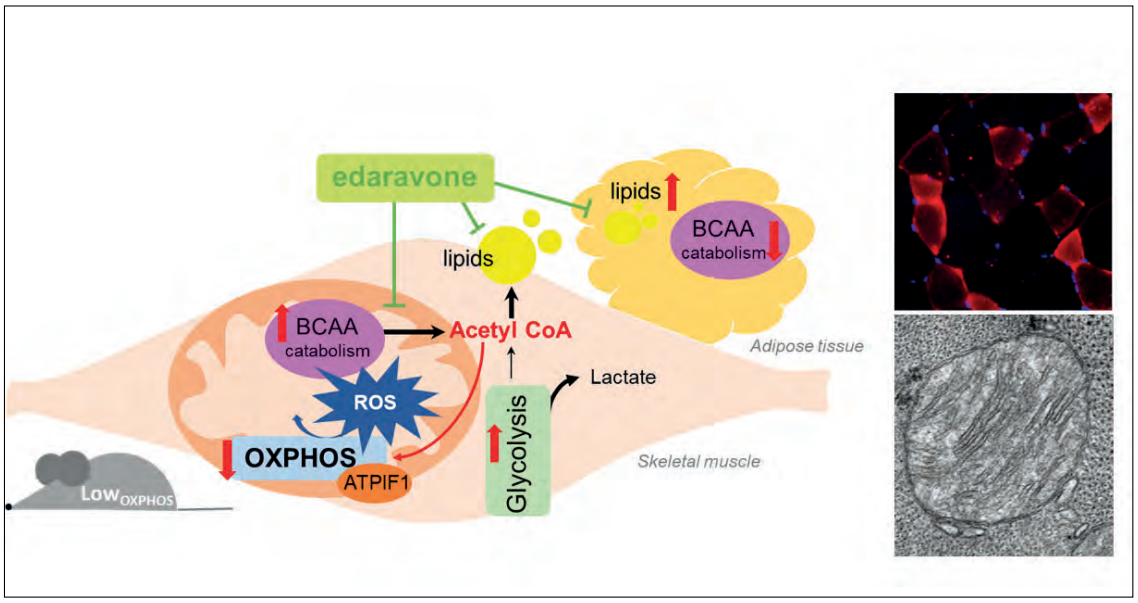


***Figure.*** *We are defining how skeletal muscle mitochondria dysfunctions act in an autocrine, paracrine and endocrine manner to regulate lipid, glucose and amino acid metabolism. Our final aim is to identify aspects of muscle mitochondria activity that are limiting for whole-body homeostasis in different contexts.*

## Publications

Sanchez-Gonzalez C., Nuevo-Tapioles C, Herrero-Martín J, Pereira MP, Cuezva JM, Formentini L* (*corresponding author). (2020). Dysfunctional muscle oxidative phosphorylation shunts BCCA catabolism onto lipogenesis in skeletal muscle. *EMBO J.*;**39**(14):e103812.

Nuevo-Tapioles C, Santacatterina F, Stamatakis K, Nuñez de Arenas C, Gomez de Cedron, M, Formentini L and Cuezva JM. (2020). Coordinate β-adrenergic inhibition of mitochondrial activity and angiogenesis arrest tumor growth. *Nat Commun.* **11**(1):3606.

Alcover-Sanchez B, Garcia-Martin G, Escudero-Ramirez J, Gonzalez-Riano C, Lorenzo P, Gimenez-Cassina A, Formentini L, de la Villa-Polo P, Pereira MP, Wandosell F, Cubelos B. (2021). Absence of R-Ras1 and R-Ras2 causes mitochondrial alterations that trigger axonal degeneration in a hypomyelinating disease model. *Glia*. **69**(3):619-637.

## Doctoral Theses

**Cristina Nuevo Tapioles** (2019). Regulación de la fosforilación oxidativa mediada por IF1 y su potencial como diana terapéutica en cáncer. UAM. Directors: J.M. Cuezva and Laura Formentini. *Cum laude.*

## METABOLIC AND SIGNALING NETWORKS IN DISEASE

# MOLECULAR PATHOPHYSIOLOGY OF FIBROSIS

***Principal Investigator:***
Santiago Lamas Peláez

***Postdoctoral Fellows:***
Verónica Miguel Herranz

***Predoctoral Fellows:***
Carlos Rey Serra

***Technicians:***
Jessica Paola Tituaña Fajardo
José Ignacio Herrero Lahuerta

***Undergraduate and Master Students:***
Arturo López López
Until June 2020 (co-supervisor Verónica Miguel Herranz)

Irene Ranz Fernández
From September 2020 (co-supervisor Verónica Miguel Herranz)

Laura Fernández Hernández
From September 2020 (co-supervisor Verónica Miguel Herranz)

Belén Sirera Conca
From October 2020 (co-supervisor Carlos Rey Serra)

http://www.cbm.uam.es/lamaslab

## Research summary

Organ fibrosis is a final common outcome for many diseases such as diabetic nephropathy, liver cirrhosis, scleroderma or myocardial sclerosis. It involves the replacement of cellular living tissue by extracellular matrix, with the subsequent functional derangement. Thus, it is crucial to understand the underlying molecular pathways leading to fibrogenesis in human pathology. MicroRNAs are essential post-transcriptional regulators of gene expression for multiple physiological and pathophysiological pathways, including fibrosis. During the past years we have focused on renal fibrosis regarding two questions: a) the role of metabolism in the genesis of renal injury and repair and b) the role of miRNAs involved in specific metabolic pathways critical for tubular epithelial cell function. We aim to prevent, defer or revert fibrosis in the kidney by understanding underlying metabolic changes associated to kidney fibrosis. To this end we use animal models with specific gain-of-function for critical enzymes involved in fatty acid oxidation, such as carnitine palmitoyltransferase 1A (CPT1A). These studies are complemented by cellular models and biochemical approaches directed towards the study of mitochondrial biogenesis and function. We have found that overexpression of the enzyme Cpt1a in kidney tubules promotes enhanced fatty acid oxidation, restores mitochondrial homeostasis and protects from fibrosis. In addition, we have identified specific microRNAs that are important to regulate the genesis of fibrosis by targeting specific metabolic routes.

Circadian regulation is essential for almost every living organism. The circadian rhythm governs many physiological functions in humans and its alteration is also responsible for the genesis of pathophysiological processes. In the kidney, hormonal action and electrolyte transport, among other functions, are regulated by the circadian rhythm. By dissecting the role of specific components of the circadian clock through the employment of genetically modified mouse models, we are investigating the cross-regulation between the circadian rhythm and kidney inflammation and fibrosis, with a particular emphasis on the metabolic component. We are complementing this approach by evaluating the impact of circadian-related metabolically healthy diets, such as time restriction feeding, on kidney fibrosis and function.

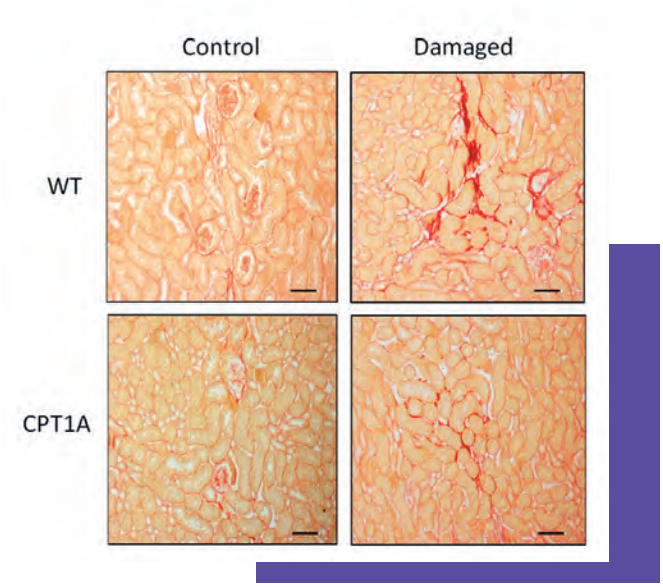


***Figure.*** *Animal model of kidney fibrosis. Collagen staining with sirius red of kidneys from mice overexpressing Cpt1a in an animal model of experimental fibrosis. Intensity of red color reflects the degree of fibrosis.*

## Publications

Miguel V, Tituaña J, Herrero JI, Herrero L, Serra D, Cuevas P, Barbas C, Rodríguez-Puyol D, Márquez-Exposito L, Ruiz-Ortega M, Castillo C, Sheng X, Susztak K, Ruiz-Canela M, Salas-Salvadó J, Hu FB, Martínez Gonzalez MA, Ortega S, Ramos R, Lamas S. (2021). Renal tubule Cpt1a overexpression mitigates kidney fibrosis by restoring mitochondrial homeostasis. *Journal of Clinical Investigation.* **131**(5):e140695.

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## Awards and recognition

- Coordination of the NOVELREN-CM consortium of the Biomedicine programme from the Comunidad de Madrid for the study on chronic renal failure. 2018-2021.

- Group leader in the research network RedinRen, RETICS programme, Instituto de Salud Carlos III.

- Chair of the Biomedicine Area, Spanish State Research Agency until October 2020.

- Collaboration with M2R laboratories.

## Doctoral Theses

**Verónica Miguel Herranz**. (2019). The metabolic basis of renal fibrosis: Role of microRNAs and insight from genetic models targeting lipid metabolism. UAM. Supervisor: Santiago Lamas Peláez. International PhD Mention. Awarded with Extraordinary Prize.

**Alba Sanz Hipólito**, (2020). Targeting Durotaxis in Pulmonary Fibrosis and Pancreatic Cancer. UAM. Supervisors: David Lagares Salto and Santiago Lamas Peláez.

## International projects / Research networks

- REDinREN. RD16/0009/0016. (2017-2021).
- NOVELREN-CM. S2017/BMD-3751. (2018-2021).
- CSIC-COV19-096/PIE 202020E160 (2020-2021).
- FIBROMET. EIN2020-112282. (2020-2022).
- RENFIBMET. PID2019-104233RB-I00. (2020-2023)

## METABOLIC AND SIGNALING NETWORKS IN DISEASE

# PATHO-PHYSIOLOGICAL IMPLICATIONS OF G PROTEIN-COUPLED RECEPTORS SIGNALING NETWORKS

***Principal Investigator:***
Federico Mayor Jr

***Scientific Staff:***
Irene García Higuera (from May 2019)

***Postdoctoral Fellows:***
María Sanz Flores (co-supervised with Catalina Ribas)
Alba Concepción Arcones (co-supervised with Cristina Murga)

***Predoctoral Fellows:***
Alejandro Asensio (co-supervised with Catalina Ribas)
Alvaro Caballero (until November 2019, co-supervised with Catalina Ribas)
Maria Margarida Martins Neves (co-supervised with Petronila Penela)
Angela Albitre (co-supervised with Petronila Penela)
Viviana Marolda (from Sept 2020, co-supervised with Petronila Penela)

***Technicians:***
Alba Ortega
Paula Ramos (until November 2020)
Susana Rojo-Berciano (until September 2019)

***Undergraduate and Master Students:***
Mercedes Grima (co-supervised with Catalina Ribas)

http://www.cbm.uam.es/fmayor

## Research summary

Our group is interested in understanding the maladaptive rewiring of cell signaling networks that takes place during the onset or progression of metabolic, cardiovascular and inflammatory diseases and in cancer. G protein-coupled receptor kinase2 (GRK2) is a versatile protein that modulates signalling mediated by many GPCRs and via phosphorylation or scaffolding interactions with a growing array of cellular partners. Our laboratory has pioneered the research on such complex “interactome” and on GRK2 regulation mechanisms. We revealed that GRK2 acts as an oncomodulator, with a relevant role in breast tumor progression and in the safeguard of the epithelial phenotype in stratified epithelia, by interacting with signalling networks related to the hallmarks of cancer in a tumor and cell type-dependent way. GRK2 levels are also altered in **cardiovascular pathologies and in obesity and insulin resistance-related contexts**, which are frequent clinical co-morbidities, being convergently upregulated in the heart and in tissues key for metabolic control. Our group has contributed to discern the role of GRK2 in cardiac and systemic insulin resistance and found that myeloid GRK2 plays a relevant role in inter-organ coordination, pointing to pleiotropic effects in the modulation of metabolic homeostasis and inflammation.

Our aim is to elucidate **how canonical and non-canonical GRK2 interaction networks are engaged and integrated in a cell type or context-specific manner and how these interactomes contribute to the physiological and pathological roles of this kinase**. In close collaboration with other members of our CBMSO Programme, we use cellular and animal models with altered GRK2 levels or functionality to study:

**-The participation of GRK2 in signaling crosstalk within the tumor microenvironment**. We want to discern the connections among tumor microenvironment stresses, chemokine and growth factor-receptors and GRK2-governed networks in the rewiring of breast cancer cell metabolic and invasive features to promote metastasis. We collaborate with the group of P. Penela and are part of the EU consortium Oncornet2.0.

**- The role of GRK2 in epidermal homeostasis and in keratinocyte-immune cells crosstalk** and its potential pathological implications in skin inflammatory diseases and squamous cell carcinomas, in collaboration with the group of C. Ribas.

**-The impact of altered of GRK2 GRK2 levels in obesity and insulin resistance-related** cardiometabolic conditions. In collaboration with the group of C. Murga and as part of CIBER cardiovascular network, we investigate the role of GRK2 signalling networks in the maladaptive reshaping of metabolic networks in different tissues and on inflammatory features of myeloid **cells leading to altered inter-organ crosstalk**.

## Publications


Smit MJ, Schlecht-Louf G, Neves M, den Bor JV, Penela P, Siderius M, Bachelerie F, Mayor F Jr.* (2021) The CXCL12/CXCR4/ACKR3 Axis in the Tumor Microenvironment: Signaling, Crosstalk, and Therapeutic Targeting. *Annu Rev Pharmacol Toxicol*. **61**:541-56.

Arcones AC, Murga C, Penela P, Inserte J and Mayor F Jr* (2021) G protein–coupled receptor kinase 2 at crossroads of metabolic and cardiovascular diseases. *Current Op Endo Metabol Research* **16**:75-85

Vila-Bedmar R, Cruces-Sande M, Arcones AC, Willemen HLDM, Prieto P, Moreno-Indias I, Díaz-Rodríguez D, Francisco S, Jaén RI, Gutiérrez-Repiso C, Heijnen CJ, Boscá L, Fresno M, Kavelaars A, Mayor F*, Murga C* (*, corresponding authors) (2020) GRK2 levels in myeloid cells modulate adipose-liver crosstalk in high fat diet-induced obesity. *Cell. Mol. Life Sci*. **77**:4957-4976.

Reglero C, Lafarga V, Rivas V, Albitre Á, Ramos P, Berciano SR, Tapia O, Martínez-Chantar ML, Mayor F Jr*, Penela P* (*, corresponding authors) (2020) GRK2-Dependent HuR Phosphorylation Regulates HIF1α Activation under Hypoxia or Adrenergic Stress. *Cancers (Basel)* **12**(5):1216.

Cruces-Sande M, Arcones AC, Vila-Bedmar R, Mayor F*, Murga C*. (*, corresponding authors) (2020) Autophagy mediates hepatic GRK2 degradation to facilitate glucagon-induced metabolic adaptation to fasting. *FASEB J*. **34**(1):399-409.

González-Amor M, Vila-Bedmar R, Rodrigues-Díez R, Moreno-Carriles R, Arcones AC, Cruces-Sande M, Salaices M, Mayor F Jr, Briones AM, Murga C. (2020) Myeloid GRK2 Regulates Obesity-Induced Endothelial Dysfunction by Modulating Inflammatory Responses in Perivascular Adipose Tissue. *Antioxidants*, **9**(10):953.

Palacios-García J, Sanz-Flores M, Asensio A, Alvarado R, Rojo-Berciano S, Stamatakis K, Paramio JM, Cano A, Nieto MÁ, García-Escudero R, Mayor F Jr*, Ribas C*. (*, corresponding authors). (2020) G-protein-coupled receptor kinase 2 safeguards epithelial phenotype in head and neck squamous cell carcinomas. *Int J Cancer*.**147**(1):218-229.

Neves M, Perpiñá-Viciano C, Penela P, Hoffmann C, Mayor F Jr * (2020) Modulation of CXCR4-Mediated Gi1 Activation by EGF Receptor and GRK2. *ACS Pharmacol Transl Sci*. **3**(4):627-634.

Penela P, Inserte J, Ramos P, Rodriguez-Sinovas A, Garcia-Dorado D, Mayor* F Jr (2019) Degradation of GRK2 and AKT is an early and detrimental event in myocardial ischemia/reperfusion. *EBioMedicine* **48**:605-618.

Arcones AC, Cruces-Sande M, Ramos P, Mayor F Jr*, Murga C* (*, corresponding authors) (2019). Sex Differences in High Fat Diet-Induced Metabolic Alterations Correlate with Changes in the Modulation of GRK2 Levels. *Cells* **8**(11):1464.

Ruso-Julve F, Pombero A, Pilar-Cuéllar F, García-Díaz N, Garcia-Lopez R, Juncal-Ruiz M, Castro E, Díaz Á, Vazquez-Bourgón J, García-Blanco A, Garro-Martinez E, Pisonero H, Estirado A, Ayesa-Arriola R, López-Giménez J, Mayor F Jr, Valdizán E, Meana J, Gonzalez-Maeso J, Martínez S, Vaqué JP, Crespo-Facorro B. (2019) Dopaminergic control of ADAMTS2 expression through cAMP/CREB and ERK: molecular effects of antipsychotics. *Transl Psychiatry* **9**(1):306.

Oliver E, Mayor F Jr, D'Ocon P (20199 Beta-blockers: Historical Perspective and Mechanisms of Action. *Rev Esp Cardiol (Engl Ed)*. **72**(10):853-862.

Aluja D, Inserte J, Penela P, Ramos P, Ribas C, Iñiguez MÁ, Mayor F Jr, Garcia-Dorado D. (2019) Calpains mediate isoproterenol-induced hypertrophy through modulation of GRK2.*Basic Res Cardiol.* **26**:114(3).

Fumagalli A, Zarca A, Neves M, Caspar B, Hill SJ, Mayor F Jr, Smit MJ, Marin P. (2019) CXCR4/ACKR3 Phosphorylation and Recruitment of Interacting Proteins: Key Mechanisms Regulating Their Functional Status. *Mol Pharmacol*. **96**(6):794-808.

Avila J, Mayor F jr and Ruiz-Desviat L (2019) Margarita Salas (1938-2019) *Obituary Nature* **576**, 208

Murga C, Arcones AC, Cruces-Sande M, Briones AM, Salaices M, Mayor F Jr* (2019) G Protein-Coupled Receptor Kinase 2 (GRK2) as a Potential Therapeutic Target in Cardiovascular and Metabolic Diseases *Front Pharmacol*. **10:**112.

Neves M, Fumagalli A, van den Bor J, Marin P, Smit MJ, Mayor F *. (2019) The Role of ACKR3 in Breast, Lung, and Brain Cancer. *Mol Pharmacol*. **96**(6):819-825.

Penela P, Ribas C, Sánchez-Madrid F, Mayor F Jr*. (2019) G protein-coupled receptor kinase 2 (GRK2) as a multifunctional signaling hub. *Cell Mol Life Sci*. **76**(22):4423-4446.


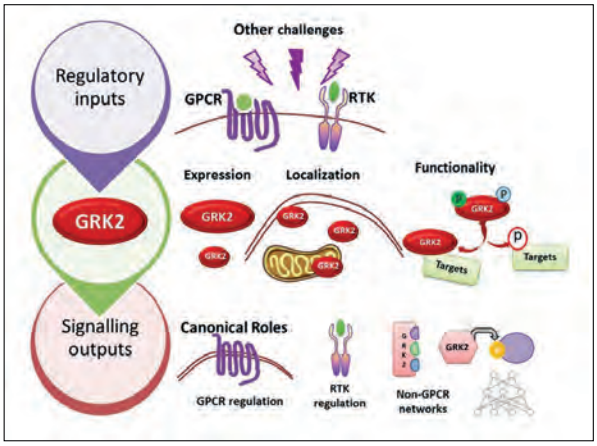


***Figure. The GRK2 signaling hub integrates multiple upstream inputs and trigger diverse and pleiotropic downstream outputs via complex cell-type specific interactomes**. GRK2-governed networks undergo maladaptive rewiring in cardiometabolic/ inflammatory diseases and in certain tumors, playing a relevant tole in these pathological conditions. Scheme modified from Nogués et al., Sem Cancer Biol. (2018).*

## Awards and recognition

- Federico Mayor: Director, Institute of Molecular Biology, Universidad Autónoma, Madrid (from 2017). Member of Scientific advisory boards of the Lilly Foundation Spain, IDIBAPS-Clinic (Barcelona, Spain) and Instituto de Investigación Sanitaria "Fundación Jiménez Díaz" (Madrid, Spain).

## Doctoral Theses

**Alba Concepción Arcones** (2020) "Influence of GRK2 levels in the modulation of glucose homeostasis in health and disease". Departamento de Biología Molecular. Universidad Autónoma de Madrid. Supervisors: Cristina Murga and Federico Mayor. *"Cum laude"* and International Mention.

## International projects / Research networks

- European Union:H2020-MSCA Programme, Grant agreement 860229-ONCORNET2.0 (ONCOgenic Receptor Network of Excellence and Training) -Coordinator: Martine Smit, Amsterdam, F. Mayor Network group PI, 2020-2024.

- CIBER CARDIOVASCULAR, Instituto de Salud Carlos III, Group CB16/11/00278 (PI, F Mayor) 2017-2021.

- Comunidad de Madrid–Programa de Actividades I+D en BIOMEDICINA. INFLAMUNE-B2017/BMD-3671/ New molecular and cellular mechanisms in immune physiopathology and inflammatory diseases. 2018 -2021.

- European Union: H2020-MSCA Programme, Grant agreement 64183-ONCORNET. (ONCOgenic Receptor Network of Excellence and Training), Coordinator: Martine Smit, Amsterdam, F. Mayor Network group PI, 2015 -2019.

- Instituto de Investigación Sanitaria Hospital La Princesa. Group 11 (PI: F. Mayor).

- Participation in ERNEST (European Research Network on Signal Transduction) COST ACTION.

## METABOLIC AND SIGNALING NETWORKS IN DISEASE

# SIGNALING PATHWAYS IN MOUSE MODELS OF METABOLIC AND CARDIOVASCULAR PATHOLOGIES

***Principal Investigator:***
Cristina Murga

***Postdoctoral Fellows:***
Alba Concepción Arcones
(co-supervised with Federico Mayor)
Almudena Val Blasco
(until January 2020)
Marta Cruces-Sande
(until May 2019)

***Undergraduate and Master Students:***
Claudia Yáñez
(Biology MSc, UAM)

http://www.cbm.uam.es/cmurga

## Research summary

Our group has been involved in the past two years in analyzing some of the cellular processes and intracellular signal transduction pathways that control physiological and pathological responses. In particular, those able to regulate metabolic adaptation to both nutrient overload situations and also to physiological conditions such as fasting, refeeding or calorie restriction. We have focused our efforts on characterizing some of the signaling nodes and also the molecular and cellular mechanisms that allow for the integration of concerted responses among different organs (**inter-organ crosstalk**) in mouse models of obesity, lipotoxicity, vascular and cardiac function, insulin resistance and glucose intolerance. We have also published results on how the **sterile inflammation**, cytokine production and immune infiltrates observed in different tissues such as the perivascular adipose tissue, adipose depots or the liver can impact both vascular and whole body insulin signalling and glucose handing in genetically-modified mice. On the other hand, we have also studied the **metabolic rewiring** that occurs during **physiological situations** such as **fasting and refeeding**, or **calorie restriction** protocols. We have characterized some of the molecular cues and cellular mechanisms responsible for an efficient and rapid metabolic adaptation to these conditions including hepatic autophagy, transcriptional reprogramming, selective protein degradation or changes in key nodal signalling pathways.

We have paid particular attention to some molecular determinants that are key to understanding how **aged animals** may handle these physiopathological responses differently than young mice. Specifically, we focused on the molecular and cellular events that differ between aged and young animals in relationship with glucose homeostasis, lipotoxicity, adiposity and cardiac steatosis among others. In this line, we have also initiated a research line (and have some already published work) aimed at deciphering some of the tissue, cellular and molecular aspects that differentiate **female and male metabolic responses** to nutrient overload and cardio-metabolic insults with particular attention to **postmenopausal female animals**, a mouse model that integrates the effects of sexual hormones and age. In this ongoing area, we have found that a **sexual dimorphism** exists in the regulation of the levels and expression of proteins representing key signalling nodes in important tissues. We have characterized their dynamic changes depending on the **age of onset** of the metabolic insult particularly in female animals. The results obtained may contribute to explain the differential profile in the **development of cardiometabolic risk** observed between male and female patients and also in mouse models of disease.

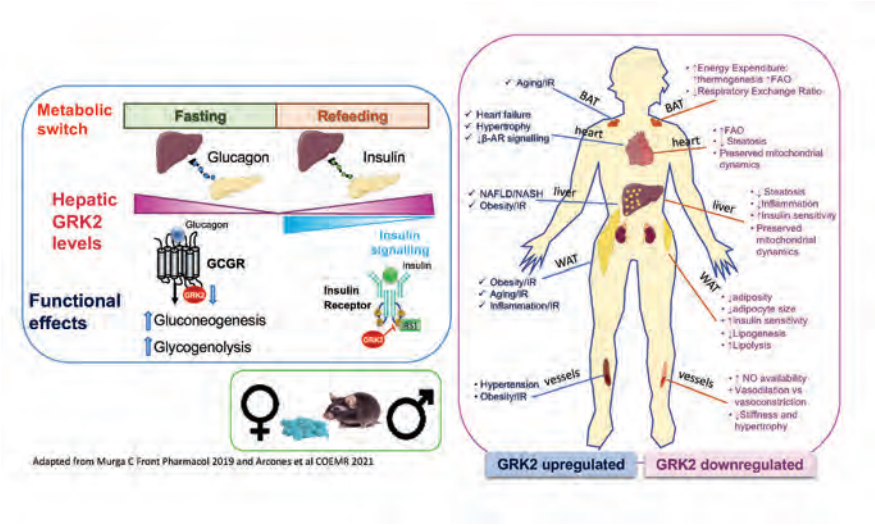


***Figure.*** *Graphical summary of recent contributions of our group regarding the influence of signaling cascades in the regulation of cellular and tissue processes involved in human physiopathology (mostly performed in animal models of human disease). BAT, Brown adipose tissue; BAR, beta adrenergic receptor; FAO, fatty acid oxidation; GRK2, G protein-coupled receptor kinase; GCGR, glucagon receptor; IR, insulin resistance; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NO nitric oxide; WAT, White adipose tissue.*

## Publications

Arcones AC, Murga C, Penela P, Inserte J and Mayor F Jr* (2021) G protein–coupled receptor kinase 2 at crossroads of metabolic and cardiovascular diseases. *Current Op Endo Metabol Research* **16**:75-85.

Vila-Bedmar R, Cruces-Sande M, Arcones AC, Willemen HLDM, Prieto P, Moreno-Indias I, Díaz-Rodríguez D, Francisco S, Jaén RI, Gutiérrez-Repiso C, Heijnen CJ, Boscá L, Fresno M, Kavelaars A, Mayor F* and Murga C*. (2020) GRK2 levels in myeloid cells modulate adipose-liver crosstalk in high fat diet-induced obesity. *Cell. Mol. Life Sci.* **77**:4957-4976.

Cruces-Sande M, Arcones AC, Vila-Bedmar R, Mayor F* and Murga C*. (2020) Autophagy mediates hepatic GRK2 degradation to facilitate glucagon-induced metabolic adaptation to fasting. *FASEB J.* **34**(1):399-409.

González-Amor M, Vila-Bedmar R, Rodrigues-Díez R, Moreno-Carriles R, Arcones AC, Cruces-Sande M, Salaices M, Mayor F Jr, Briones AM, Murga C. (2020) Myeloid GRK2 Regulates Obesity-Induced Endothelial Dysfunction by Modulating Inflammatory Responses in Perivascular Adipose Tissue. *Antioxidants,* **9**(10):953.

Arcones AC, Cruces-Sande M, Ramos P, Mayor F Jr*, Murga C* (2019). Sex Differences in High Fat Diet-Induced Metabolic Alterations Correlate with Changes in the Modulation of GRK2 Levels. *Cells* **8**(11):1464.

Murga C, Arcones AC, Cruces-Sande M, Briones AM, Salaices M, Mayor F Jr* (2019) G Protein-Coupled Receptor Kinase 2 (GRK2) as a Potential Therapeutic Target in Cardiovascular and Metabolic Diseases Front *Pharmacol.* **10**:112.

## Awards and recognition

- Cristina Murga is member of the Rector Commitee of the Instituto de Investigación Sanitaria “La Princesa” (Madrid, Spain) and also elected member of the “Claustro” of the UAM.

## Doctoral Theses

**Alba Concepción Arcones** (2020) “Influence of GRK2 levels in the modulation of glucose homeostasis in health and disease”. Departamento de Biología Molecular. Universidad Autónoma de Madrid. Supervisors: Cristina Murga and Federico Mayor. “*Cum laude*” and International Mention.

## International projects / Research networks

- European Union: H2020-MSCA Programme, Grant agreement 64183-ONCORNET. (ONCOgenic Receptor Network of Excellence and Training), Coordinator: Martine Smit, Amsterdam, F. Mayor Network group PI, 2015 -2019.

- CIBER CARDIOVASCULAR, Instituto de Salud Carlos III, Group CB16/11/00278 (PI, F Mayor) 2017-2021.

- Comunidad de Madrid–Programa de Actividades I+D en BIOMEDICINA. INFLAMUNE-B2017/BMD-3671/ New molecular and cellular mechanisms in immune physiopathology and inflammatory diseases. 2018 -2021.

- Instituto de Investigación Sanitaria Hospital La Princesa.

## METABOLIC AND SIGNALING NETWORKS IN DISEASE

# CELLULAR SIGNALING NETWORKS IN CANCER (ONCO-RESECEL)

***Principal Investigator:***
Petronila Penela

***Postdoctoral Fellows:***
Verónica Rivas
(until December 2020)

***Predoctoral Fellows:***
Belén Ortiz del Castillo
Maria Margarida Martins Neves
(co-supervised with Federico Mayor)
Ángela Albitre (co-supervised with Federico Mayor)
Viviana Marolda
(from Sept 2020, co-supervised with Federico Mayor)

***Undergraduate and Master Students:***
Andrea De Castro
(2020)
Ángela Albitre
(2019)

https://www.cbm.uam.es/ppenela

## Research summary

Tumor heterogeneity and versatility of cancer cells to circumvent the mechanisms of anti-tumor therapies foster tumor progression and the emergence of resistances. Moreover, lifestyle factors also affect the incidence of cancer and the efficacy of treatments. Therefore, it is urgent to find out new molecular dependencies of the tumor for better tackling with cancer treatment. Dynamic rewiring of cell signaling networks is a common strategy ensuring tumoral transformation and rapid adaptive resistance, which might become susceptible to being therapeutically targeted. The objective of our group is to identify, as potential multifunctional therapeutic targets, signaling nodes that work as molecular switchers cooperating with oncogenic-signaling routes or reshaping normal signaling compensatory pathways to trigger transformation or cope with intrinsic tumor-derived vulnerabilities.

Results of our laboratory showed that G protein-coupled receptor kinase2 (GRK2) is a versatile protein that modulates signalling mediated by many GPCRs and a growing array of cellular partners. GRK2 is emerging as a relevant player of oncogenic signalling networks by its ability to alter RNA regulation and posttranslational control of manifold proteins in transformed cells via complex regulatory loops affecting stress-related RNA binding proteins, E3 ubiquitin ligases or cytosolic protein deacetylases, and concurrent up-regulation of these activities emerges as a functional signature in breast cancer types beyond hormonal dependent tumours.

Our research aims are to identify a) GRK2-governed signalling circuits involved in cancer progression and resistance, deciphering the relevant modified proteome by phosphorylation, acetylation, ubiquitination, responsible for pro-tumoral signalling rewiring which subverts normal cellular behaviour, impairing cell cycle control and cell division, differentiation, energy metabolism, DNA damage response and repair or senescence; b) influence of hormonal (adrenergic, estrogenic), metabolic stresses and micro-environmental conditions on GRK2 intertwinement with relevant partners for genomic stability and stroma remodelling, analysing the pathological angiogenesis and fibrosis that facilitate tumor growth and dissemination.

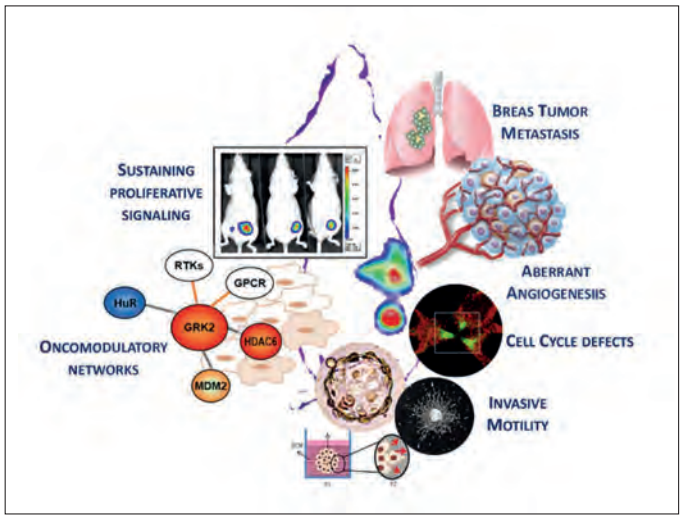


***Figure. Overall integrative effect of GRK2 in the acquisition of tumoural features and cancer progression.*** *Cell type- and context-specific GRK2-regulated partners and substrates have a wide impact on proteome functionality altering the signal transduction network of the cell and reshaping basic cellular processes in a tumoral way.*

## Publications

Smit MJ, Schlecht-Louf G, Neves M, den Bor JV, Penela P, Siderius M, Bachelerie F, Mayor F Jr.* (2021) The CXCL12/CXCR4/ACKR3 Axis in the Tumor Microenvironment: Signaling, Crosstalk, and Therapeutic Targeting. *Annu Rev Pharmacol Toxicol*. **61**:541-56.

Arcones AC, Murga C, Penela P, Inserte J and Mayor F Jr* (2021) G protein–coupled receptor kinase 2 at crossroads of metabolic and cardiovascular diseases. *Current Op Endo Metabol Research* **16**:75-85.

Reglero C, Lafarga V, Rivas V, Albitre Á, Ramos P, Berciano SR, Tapia O, Martínez-Chantar ML, Mayor F Jr, Penela P* (2020) GRK2-Dependent HuR Phosphorylation Regulates HIF1α Activation under Hypoxia or Adrenergic Stress. *Cancers (Basel)* **12**(5):1216.

Neves M, Perpiñá-Viciano C, Penela P, Hoffmann C, Mayor F Jr * (2020) Modulation of CXCR4-Mediated Gi1 Activation by EGF Receptor and GRK2. ACS *Pharmacol Transl Sci*. **3**(4):627-634.

Penela P, Inserte J, Ramos P, Rodriguez-Sinovas A, Garcia-Dorado D, Mayor* F Jr (2019) Degradation of GRK2 and AKT is an early and detrimental event in myocardial ischemia/reperfusion. *EBioMedicine* **48**:605-618.

Aluja D, Inserte J, Penela P, Ramos P, Ribas C, Iñiguez MÁ, Mayor F Jr, Garcia-Dorado D. (2019) Calpains mediate isoproterenol-induced hypertrophy through modulation of GRK2. *Basic Res Cardiol*. **26**:114(3).

Fumagalli A, Zarca A, Neves M, Caspar B, Hill SJ, Mayor F Jr, Smit MJ, Marin P. (2019) CXCR4/ACKR3 Phosphorylation and Recruitment of Interacting Proteins: Key Mechanisms Regulating Their Functional Status. *Mol Pharmacol*. **96**(6):794-808.

Neves M, Fumagalli A, van den Bor J, Marin P, Smit MJ, Mayor F *. (2019) The Role of ACKR3 in Breast, Lung, and Brain Cancer. *Mol Pharmacol*. **96**(6):819-825.

Penela P, Ribas C, Sánchez-Madrid F, Mayor F Jr*. (2019) G protein-coupled receptor kinase 2 (GRK2) as a multifunctional signaling hub. *Cell Mol Life Sci*. **76**(22):4423-4446.

## International projects / Research networks

- European Union:H2020-MSCA Programme, Grant agreement 860229-ONCORNET2.0 (ONCOgenic Receptor Network of Excellence and Training) -Coordinator: Martine Smit, Amsterdam/ P Penela, Ethical Board Network and trainee supervisor, 2020-2024.

- CIBER CARDIOVASCULAR, Instituto de Salud Carlos III, Group CB16/11/00278 (Researcher, P Penela 2017-2021).

- Comunidad de Madrid–Programa de Actividades I+D en BIOMEDICINA. INFLAMUNE-B2017/BMD-3671/ New molecular and cellular mechanisms in immune physiopathology and inflammatory diseases (Researcher, P Penela, 2018 -2021).

- European Union: H2020-MSCA Programme, Grant agreement 64183-ONCORNET. (ONCOgenic Receptor Network of Excellence and Training), Coordinator: Martine Smit, Amsterdam/ P. Penela, trainee supervisor 2015 -2019.

-Instituto de Investigación Sanitaria Hospital La Princesa. Group 13 (PI: P. Penela)

## METABOLIC AND SIGNALING NETWORKS IN DISEASE

# TRANSLATIONAL MEDICINE IN INBORN ERRORS OF METABOLISM AND OTHER RARE GENETIC DISEASES

***Principal Investigators:***
Belén Pérez
Pilar Rodríguez-Pombo

***Scientific Staff:***
Alejandra Gámez

***Postdoctoral Fellows:***
Alvaro Briso-Montiano
(from July 2020)

***Predoctoral Fellows:***
Laura Arribas
Irene Bravo
(until March 2020)
Álvaro Briso-Montiano
(until July 2020)
Diana Gallego
Alicia Vilas
Obdulia Sánchez-Lijarcio
Cristina Segovia

***Technicians:***
Rosa Navarrete
Fatima Leal

***Undergraduate and Master Students:***
Alejandro Soriano

https://tinyurl.com/y5dygyp7

## Research summary

The group belongs to the Biomedical Network Research Centre for Rare Diseases (CIBERER), the Hospital La Paz Institute for Health Research (IdiPAZ) and to the Centro de Diagnóstico de Enfermedades Moleculares (CEDEM-UAM), a reference national Lab for the study of inborn errors of metabolism (IEM).

We are currently involved in transferring the research knowledge for improving the diagnosis and management of these rare diseases. Our research goal is focused on the elucidation of the genetic and molecular basis of IEM to develop therapeutic strategies based on the studies of the pathophysiology and/or pathogenicity mechanism of the mutations identified.

Using an orthogonal analysis that combines data from different omics (metabolomic, proteomic, transcriptomic, and genomic) and functional genomic analysis (protein traffic studies, enzyme assays, mitochondrial respiration analysis, cell ultrastructure, respiration complex analysis, splicing studies, etc.), we have elucidated the genetic basis of different defects and analysed aspect of the pathophysiological mechanisms.

We have identified new congenital disorders of glycosylation, and some others affecting mitochondrial function. In all cases, we have identified specific genetic and biochemical biomarkers that improve the diagnosis and management of these rare defects. For some of that and based on pathophysiology studies, we have been able to propose tailored therapies in the era of the Translational Precision Medicine. This is the case of new cases with pathogenic variants in BCAT2, SLC7A2 or DNAJC12. In all three cases, we provided pathogenic evidence for considering new defects.

Regarding therapies, we are working on folding therapies including pharmacological chaperones (PCs) and proteostasis regulators. Indeed, we have provided a proof-of-concept of the potential treatment of PMM2-CDG by proteostasis regulators, either alone or in combination with pharmacological chaperones. The treatment with celastrol increases the concentration and activity of mutant PMM2 protein to occur through Hsp90-driven modulation of the proteostasis network. Due to their common pathogenicity mechanism, this promising approach based on the rescue of misfolded proteins is being applied to the analysis of other IEM (such as Methylmalonic aciduria or Nonketotic hyperglycinemia).

Besides, we have done an exercise of drug repurposing for PMM-CDG and reported a mutation-specific treatment based on the overexpression of the co-chaperone DNAJC12. Our results suggest that the proteostasis network could be a genetic modifier of PAH deficiency and a potential target for developing mutation-specific treatments for PKU. We have generated specific cellular models based in the reprogramming of patient-derived fibroblasts to iPS and differentiation to hepatocyte, neural progenitors, astrocytes, or neuron-like cells as platforms for drug development and pathophysiology studies of metabolic disorders.

## Publications


Martínez-Monseny, A.F et al (2019) AZATAX: Acetazolamide safety and efficacy in cerebellar syndrome in PMM2 congenital disorder of glycosylation (PMM2-CDG). *Annals of Neurology* (2019) **85**(5): 740-751

Bravo-Alonso,I., et al. (2019) Genes and variants underlying human congenital lactic acidosis: from genetics to personalized treatment. *Journal of Clinical Medicine* **1**;8(11):1811.

Meléndez-Rodríguez, F et al. (2019) HIF1α Suppresses Tumor Cell Proliferation through Inhibition Of Aspartate Biosynthesis. *Cell Rep*. **26**(9):2257-2265.e4.

Martinez-Monseny, A., Cuadra, D., Bolasell, M., Muchart, J., Arjona, C., Borregan, M., et al. (2019) From gestalt to gene: early predictive dysmorphic features of PMM2-CDG. *J Med Genet.* **56**(4):236-245.

Navarrete,R., et al. (2019) Value of genetic analysis for confirming inborn errors of metabolism detected through the Spanish neonatal screening program. *Eur J Hum Genet.* **27**(4):556-562.

Yahyaoui, R., et al. (2019) A new metabolic disorder in human cationic amino acid transporter-2 mimicking arginase 1 deficiency in newborn screening. *Inherit Metab Dis.* **42**(3):407-413.

Medrano, C., et al. (2019) Clinical and molecular diagnosis of non-phosphomannomutase 2 N-linked congenital disorders of glycosylation in Spain. *Clin Genet.* **95**(5):615-626.

López-Márquez, A., et al. (2019) Generation and characterization of a human iPSC line (UAMi004-A) from a patient with propionic acidemia due to defects in the PCCB gene. Stem Cell Res. Jul;38: 101469. Erratum in: *Stem Cell Res*. 2019 Aug; **39**: 101513.

Arribas-Carreira, L., et al. 2019) Generation and characterization of a human iPSC line (UAMi005-A) from a patient with nonketotic hyperglycinemia due to mutations in the GLDC gene. *Stem Cell Res.* **39**:101503.

Knerr, I., et al. (2019) Expanding the genetic and phenotypic spectrum of branched-chain amino acid transferase 2 deficiency. *JIMD J Inherit Metab Dis.* **42**(5): 809-817.

Stanescu, S., et al. (2020) Beneficial Effect of N-Carbamylglutamate in a Neonatal Form of Multiple Acyl-CoA Dehydrogenase Deficiency. *Case Reports in Paediatric*. ID 1370293.

Correa-Vela,M., et al. (2020) Impaired proteasome activity and neurodegeneration with brain iron accumulation in FBXO7 defect. *Annals of clinical and translational neurology* **7**(8): 1436-1442.

Yahyaoui,R., et al. (2020) Metabolic Serendipities of Expanded Newborn Screening. *Genes*, **11**(9), 1018.

Vaes,L., et al. (2020) PMM2-CDG caused by uniparental disomy: Case reportand literature review. *JIMD Reports* **54**;16-19.

Fulgencio-Covián, A., et al. (2020) Pathogenic implications of dysregulated miRNAs in propionic acidemia related cardiomyopathy. *Transl Res*. Apr; **218**:43-56.

Gallego, D., et al. (2020) Pathogenic variants of DNAJC12 and evaluation of the encoded co-chaperone as a genetic modifier of hyperphenylalaninemia. *Human Mutation* **41**(7): 1329-1338.

Vilas, A., et al. (2020) Proteostasis regulators as potential rescuers of pmm2 activity. *Biochim Biophys Acta Mol Basis Dis*.**1866**(7):165777.

Hillert, A., et al. (2020) The Genetic Landscape and Epidemiology of Phenylketonuria. *Am J Hum Genet*. **107**(2):234-250.

Fulgencio-Covián, A., et al. (2020) Generation of a gene-corrected human isogenic line (UAMi006-A) from propionic acidemia patient iPSC with an homozygous mutation in the PCCB gene using CRISPR/Cas9 technology. *Stem Cell Res*. **49**:102055.

Gámez, A., et al.. (2020) New and potential strategies for the treatment of PMM2-CDG. *Biochim Biophys Acta Gen Subj*. **1864**(11):129686.


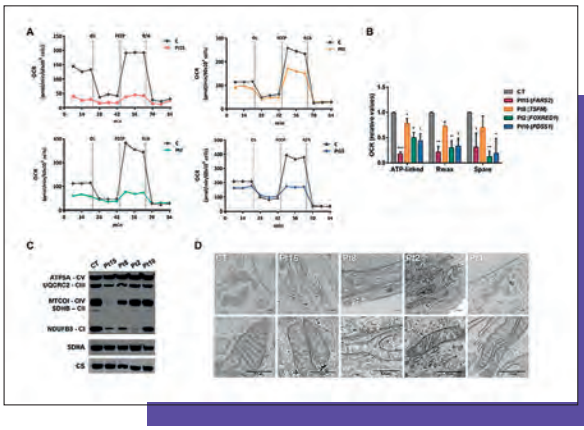

***Figure.*** *Mitochondrial dysfunctional analysis in Congenital Lactic Acidosis patient-derived fibroblasts: Respiratory profile, oxygen consumption rates, respiratory complexes and electron microscopy images. Modified figure from Bravo-Alonso I. et al. (2019) J Clin Med.*

## Doctoral Theses

**Alvaro Briso-Montiano** (2020). Desarrollo de modelos para la evaluación de terapias con chaperonas farmacológicas en aciduria metilmalónica y deficiencia en fosfomanomutasa. Universidad Autónoma de Madrid. Supervisor: Belén Pérez. Mención internacional.

## International projects / Research networks

- Belen Pérez Head of a CIBERER group (CB06/07/0004) and the IdiPAZ group, “Investigación y diagnóstico de enfermedades metabólicas hereditarias”.

- Proyecto Comunidad de Madrid B2017/BMD3721. “Red de recursos genómicos, funcionales, clínicos y terapéuticos para el estudio de las enfermedades raras neurológicas”. Group PI: Belén Pérez

- “Towards a new era for the identification and characterisation of inborn errors of glycosylation”. European project. E-Rare-3 Joint Transnational Call 2018. Coordinator: Gert Matthijs (PI: Belen Pérez).

## METABOLIC AND SIGNALING NETWORKS IN DISEASE

# REGULATORY FUNCTIONS AND MECHANISMS OF CELL SIGNALING PATHWAYS THROUGH G PROTEINS: A NEW INTERACTOME

***Principal Investigator:***
Catalina Ribas

***Scientific Staff:***
Inmaculada Navarro
(from December 2020)

Alvaro Caballero
(until November 2019, co-supervised with Federico Mayor)

***Postdoctoral Fellows:***
Sofía Cabezudo
(until May 2019)
María Sanz Flores
(co-supervised with Federico Mayor)

***Undergraduate and Master Students:***
Lara García Merino
Mercedes Grima
(co-supervised with Federico Mayor)

***Predoctoral Fellows:***
Alejandro Asensio
(co-supervised with Federico Mayor)

https://www.cbm.uam.es/cribas

## Research summary

Our laboratory is investigating key nodes in signaling networks involved in both physiological and pathological conditions and the molecular mechanisms involved. G-protein-coupled receptors (GPCRs) are a family of membrane proteins with great physiological and pharmacological importance. In particular, Gq protein-coupled receptors (Gq-GPCR) are increasingly involved in pathologies such as cardiovascular/metabolic diseases and cancer. In recent years, the Gαq interactome (and the homologous Gα11 isoform) has expanded considerably with the description of new effectors, helping to improve our understanding of the cellular and physiological events controlled by this Gα subunit. Recently, our group has described a new adapter role for Gq, important for the activation of MAPK ERK5 by Gq-coupled receptors as a new PLCβ-independent signaling axis, which is based on the interaction between this G protein and two new effectors (PKCζ and MEK5) through a NEW INTERACTION REGION in Gαq, different from the classical effector-binding region, and PB1 protein domains. **Our recent results reveal an unforeseen connection between non-canonical Gαq/11 signaling and cell homeostasis** via interaction of Gαq with the PB1 domain proteins. Furthermore, Gαq is known to interact with various components of the cytoskeleton, as well as with important membrane microdomain organizers, suggesting the existence of signaling complexes that could be limited to specific subcellular environments.

The main objective of our group is to understand how changes in Gq-GPCR signaling (involving different types of cells and tissues) are integrated at the cellular and organism level, and how they can promote the progression of pathologies, through the use of cell and animal models with altered expression/activity of this protein, as well as samples from patients or animal models of disease. **We will focus particularly on the functional impact of the new interactions of Gαq with proteins** containing PB1 domains, and their modulation by accessory proteins (such as GRKs, Caveolins, AGS, RGS, EBP50, Ric8), in cell death processes, integration of detection signals of nutrients/autophagy/exosome trafficking and oxidative stress in the development of cardiovascular/metabolic diseases and cancer. The identification of new signaling pathways that relate Gαq to the crosstalk between different cell homeostasis and communication machineries will provide a better understanding of the impact of maladaptive Gq-coupled GPCR activation in pathological conditions.

In addition, and in close collaboration with other members of our CBMSO Programme, we use cellular and animal models with altered GRK2 levels or functionality to study:

- **The role of GRK2 in epidermal homeostasis and in keratinocyte-immune cells crosstalk** and its potential pathological implications in skin inflammatory diseases and squamous cell carcinomas, in collaboration with the group of F. Mayor.

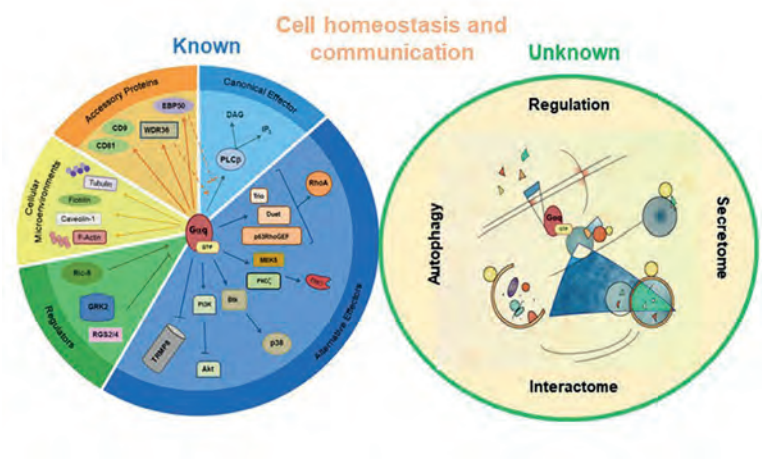


***Figure. Unveiling Gαq signaling:*** *Gαq versatile binding potential allows for diverse and novel functional activities. Scheme modified from "Galphaq signalling: the new and the old", Cell signaling (2014) and Thesis Sofia Cabezudo (2018).*

## Publications

Palacios-García J, Sanz-Flores M, Asensio A, Alvarado R, Rojo-Berciano S, Stamatakis K, Paramio JM, Cano A, Nieto MÁ, García-Escudero R, Mayor F Jr*, Ribas C*. (*, corresponding authors). (2020) G-protein-coupled receptor kinase 2 safeguards epithelial phenotype in head and neck squamous cell carcinomas *Int J Cancer.***147**(1):218-229.

Aluja D, Inserte J, Penela P, Ramos P, Ribas C, Iñiguez MÁ, Mayor F Jr, Garcia-Dorado D. (2019) Calpains mediate isoproterenol-induced hypertrophy through modulation of GRK2. *Basic Res Cardiol.* **26**:114(3).

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Colás-Algora N, García-Weber D, Cacho-Navas C, Barroso S, Caballero A, Ribas C, Correas I, Millán J. (2020). Compensatory increase of VE-cadherin expression through ETS1 regulates endothelial barrier function in response to TNFα. *Cell Mol Life Sci.* **77**(11):2125-2140.

## Awards and recognition

- Catalina Ribas: Academic Secretary of Molecular Biology Department, Universidad Autónoma, Madrid (from 2019).

## Patents

Millán J; Colás-Algora N.; Ribas C; Caballero A. 201930571. Use a RhoA subfamily activating toxin for the treatment of sepsis. Spain. Consejo Superior de Investigaciones Científicas.

## International projects / Research networks

- Member of the Instituto de Investigación Sanitaria Hospital La Princesa. Group 11:

"Animal models of inflammatory diseases and tissue remodeling"

- Participation in ERNEST (European Research Network on Signal Transduction) COST ACTION (European cooperation in Science and Technology)

- Participation in TRANSAUTOPHAGY COST ACTION (European cooperation in Science and Technology)

- European Union:H2020-MSCA Programme, Grant agreement 860229-ONCORNET2.0 (ONCOgenic Receptor Network of Excellence and Training) -Coordinator: Martine Smit, Amsterdam, F. Mayor Network group PI, 2020-2024.

- CIBER CARDIOVASCULAR, Instituto de Salud Carlos III, Group CB16/11/00278 (PI, F Mayor) 2017-2021.

- Comunidad de Madrid–Programa de Actividades I+D en BIOMEDICINA. INFLAMUNE-B2017/BMD-3671/ New molecular and cellular mechanisms in immune physiopathology and inflammatory diseases. 2018 -2021.

## METABOLIC AND SIGNALING NETWORKS IN DISEASE

# PHYSIOPATHOLOGY STUDIES AND THERAPEUTICAL APPROACHES IN ANIMAL AND CELLULAR MODELS OF NEUROMETABOLIC DISEASES

***Principal Investigators:***
Lourdes Ruiz Desviat
Eva Mª Richard

***Postdoctoral Fellows:***
Arístides López-Márquez
Ainhoa Martínez-Pizarro

***Predoctoral Fellows:***
Esmeralda Alonso-Barroso
Alejandro Fulgencio Covián

***Technicians:***
Mar Álvarez
Elena Montalvo

***Undergraduate and Master Students:***
Rubén Vicente
Pablo González Jabalera
Gema Cerro Tello

***Visiting Scientist:***
Ulrika S. Spangsberg Petersen
(University of Southern Denmark)

https://www.cbm.uam.es/lab220

## Research summary

Our research is focused in neurometabolic diseases, propionic acidemia (PA) and hyperphenylalaninemias (HPAs) among others, enzymatic deficiencies of autosomal recessive inheritance, characterized by the toxic accumulation of precursors and lack of downstream metabolites.

Our work during this period represents a translational study with the aim of generating and characterizing relevant animal and cellular models, to be used as research tools to understand the molecular and physiopathological mechanisms responsible for disease, to analyse potential biomarkers for prognosis and follow-up, and to identify new therapeutical targets. The ultimate aim is to develop personalized RNA targeted therapies (antisense oligonucleotides) as well as pharmacological therapies with antioxidant compounds and mitochondrial activators, performing preclinical studies in the corresponding disease models.

We have generated and characterized patient-derived iPSCs generated from PA patients' fibroblasts and have successfully differentiated them to cardiomyocytes, in which we are studying the biochemical phenotype and different signaling pathways related to mitochondrial function and cardiac alterations, which are one of the major life-threatening complications in patients with the disease. In relation to this, we have used gene editing CRISPR/Cas9 technology for the generation of gene corrected isogenic iPS cells from a PA patient to serve as controls in the research of physiopathological mechanisms and for therapeutic compound testing.

Moreover, using a murine model of PA, we have revealed alterations in $Ca^{2+}$ mishandling, associated to elevated ROS levels and higher SERCA2a oxidation rate, as mechanisms involved in the development of arrhythmias and cardiac dysfunction, frequent in PA patients. In addition, in heart tissue of the PA mouse we have identified a series of upregulated cardiac-enriched miRNAs (cardiomiRs), some of them also altered as circulating miRNAs in PA patients' plasma samples. Several of these miRNAs regulate signaling pathways that we also found altered; notably, we have found an activation of the mammalian mTOR pathway and a decrease in autophagy, which are reverted by rapamycin treatment. These alterations potentially contribute to cardiac remodelling and dysfunction and can be further explored as therapeutic targets in the disease.

We have also generated using CRISPR/Cas novel cellular and animal models with specific splicing mutations in the PAH gene, responsible for phenylketonuria, to characterize splicing regulatory mechanisms and to identify and test candidate therapeutical antisense oligonucleotides.

Funding: MINECO SAF2016-76004-R; MICINN PID2019-105344RB-I00; EU-COST Action CA17103; Propionic Acidemia Foundation PAF107.

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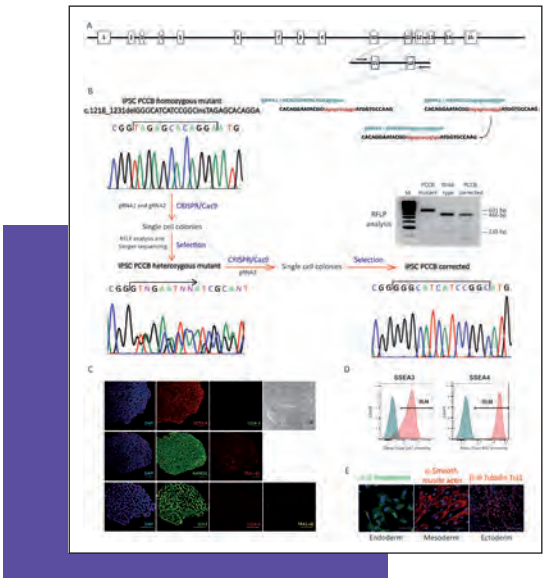

***Figure. Characterization of the CRISPR/Cas-mediated corrected PCCB iPSC line.*** *A) RNA guides designed in this study. B) Schematics of the mutation correction workflow. C) Immunofluorescence analysis. D) Flow cytometry analysis for pluripotency markers. E) In vitro differentiation analysis by immunofluorescence. (adapted from Fulgencio-Covian et al. Stem Cell Res 2020).*

## Doctoral Theses

**Alejandro Fulgencio-Covián** (2020). Investigación traslacional en las cardiomiopatías asociadas a la acidemia propiónica. UAM. Supervisors: Lourdes Ruiz Desviat, Eva Mª Richard. International Mention.

**Esmeralda Alonso Barroso** (2020). Estudios fisiopatológicos para la búsqueda de nuevas dianas terapéuticas en acidemia propiónica mediante la caracterización del modelo murino y el desarrollo de nuevos modelos celulares humanos basados en iPSCs. UAM. Supervisors: Lourdes Ruiz Desviat, Eva Mª Richard. International Mention.

**Ana Rivera Barahona** (2019) Estudios genéticos y fisiopatológicos para la búsqueda de nuevos biomarcadores y terapias en acidemia propiónica. UAM. Supervisor: Lourdes Ruiz Desviat. International Mention. Premio Extraordinario de Doctorado UAM.

**Ainhoa Martínez Pizarro** (2019) Identificación y caracterización de mutaciones de splicing en pacientes con hiperfenilalaninemia; aproximaciones terapéuticas específicas de RNA. UAM. Supervisor: Lourdes Ruiz Desviat. International Mention.

## International projects / Research networks

- European cooperation in Science and Technology (COST) Action CA17103. Delivery of RNA therapy (2018-2022). LR Desviat, Management Committee member Spain.

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## METABOLIC AND SIGNALING NETWORKS IN DISEASE

# CALCIUM SIGNALING IN MITOCHONDRIA AND INSULIN/LEPTIN SIGNALING DURING AGEING

***Principal Investigators:***
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Eduardo Herrada Soler
(from February 2020)

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## Research summary

Our interests are understanding calcium regulation of mitochondrial function by way of the calcium-dependent mitochondrial carriers of aspartate-glutamate/AGCs, components of the malate aspartate shuttle (MAS), or ATP.$Mg^{2+}$-Pi/SCaMCs. These carriers have $Ca^{2+}$-binding motifs facing the intermembrane space and are not activated by matrix calcium. We also aim at learning the role of these carriers in health and disease.

In neurons, calcium is thought to regulate neuronal activation, by adjusting ATP production to ATP consumption. This occurs thanks to stimulation of glycolysis and OXPHOS. The mitochondrial calcium uniporter (MCU) was thought to play a major role by increasing mitochondrial calcium and OXPHOS in response to activation. We have tested this possibility in neurons using glucose and have found that MCU is dispensable for the increase in respiration in response to neuronal stimulation. Instead, using intracellular sensors of glucose, pyruvate and lactate, we find that Aralar-MAS is required to stimulate glycolysis, pyruvate production and respiration, revealing a calcium dependent mechanism essential to boost glycolysis and respiration in neurons using glucose. Our aim is to study the role of citrin/AGC2 in liver in the mitochondrial response to $Ca^{2+}$- mobilizing agonists.

Deficiency in Aralar/AGC1 is a rare disease with impaired neurodevelopment, epilepsy and hypomyelination. We have explored treatments for Aralar deficiency and found that β-hydroxybutyrate (βOHB), the main metabolic product of ketogenic diets, is able to overcome the defect in basal and workload-stimulated respiration in Aralar-deficient neurons and partially reverts their failure to produce aspartate and NAA. *In vivo* administration of βOHB to Aralar-KO mice increases myelin protein levels and dopaminergic markers in these mice, suggesting βOHB administration as a potential treatment in Aralar deficiency.

However, whether the defect in myelination of the Aralar-KO mouse is due to the lack of aralar in neurons or oligodendrocytes is unclear. Our present aim is to generate neuron or oligodendrocyte-specific Aralar KO to address these issues.

Citrin deficiency is a urea cycle disorder with different manifestations. Citrin/AGC2 is mainly expressed in liver. In the frame of the Citrin Foundation, we are exploring the exogenous expression of Aralar, which has low expression in normal liver, as possible therapy for Citrin deficiency. We have generated Citrin-KO mice carrying liver-specific Aralar transgene and are studying the effect of the transgene in recovering liver MAS activity and other traits of Citrin deficiency reproduced in Citrin-KO mice.

An ongoing COVID-19 project (CvK), is aimed at developing a therapy against COVID-19 symptoms with the use of senolytics, which would selectively eliminate organisms' senescent cells.

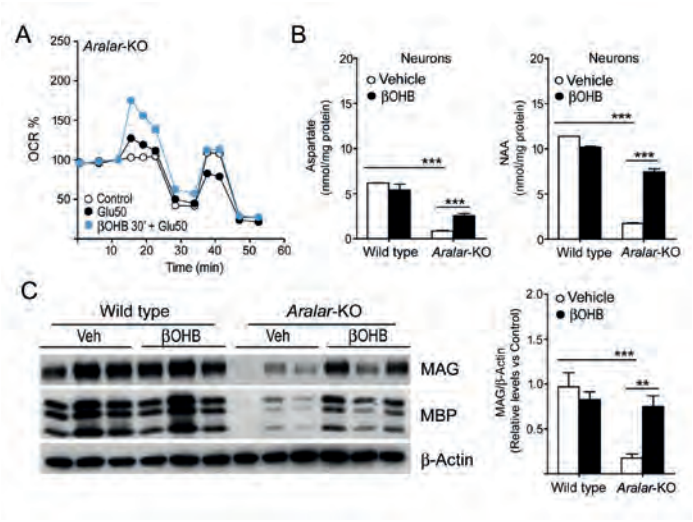


***Figure.*** *βOHB Protective roles in Aralar-KO Neurons and Brain: An Alternative to Ketogenic Diet. βOHB increases glutamate-stimulated respiration (A) and the content of Aspartate and NAA (B) in Aralar-KO neuronal cultures, and the levels of cortical myelin proteins in Aralar-KO mice (C) when administered in vivo.*

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## Doctoral Theses

**Inés Juaristi Santos** (2019) “Regulation of mitochondrial respiration in astrocytes: role of $Ca^{2+}$, ATP demand and pyruvate production”. Universidad Autónoma de Madrid. Supervisors: Jorgina Satrústegui Gil-Delgado and Araceli del Arco Martínez.

**Irene Pérez Liébana** (2020) “The mitochondrial aspartae-glutamate carrier Aralar/AGC1 controls neuronal respiration and (β)-Hydroxybutyrate rescues brain defects caused by AGC1 deficiency”. Universidad Autónoma de Madrid. Supervisors: Jorgina Satrústegui Gil-Delgado y Beatriz Pardo Merino.

## International projects / Research networks

- This group is Member of the Instituto de Investigación Sanitara Fundación Jiménez Díaz (IIS FJD) (from 2013) as “Señalización mitocondrial del calcio” unit.

## METABOLIC AND SIGNALING NETWORKS IN DISEASE

# MITOCHONDRIAL BIOLOGY IN IMMUNE MODULATION

***Principal Investigator:***
Javier Traba
(from September 2020)

***Undergraduate and Master Students:***
Alejandra Carrancho Arroyo (Master Student, TFM), from December 2020

Rocío Moreno Palomares (Master Student, TFM), from December 2020

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## Research summary

Mitochondria are considered the powerhouses of the cell, and yet they are also involved in heat production, calcium signaling, detoxification of reactive oxygen species (ROS), synthesis of heme and other molecules, and regulation of cell death. Emerging functions include their role as damage-associated molecular patterns, which are important in immune activation. In this context, release of mitochondrial components—such as mitochondrial DNA (mtDNA)—to the cytosol may activate several pathways that lead to the secretion of pro-inflammatory cytokines. Among them is the NLRP3 inflammasome, a complex that senses mtDNA in the cytosol (and thus is a sensor for mitochondrial dysfunction) and in turn activates caspase-1, an enzyme that cleaves cytokines interleukin-1β and -18, and the pyroptosis inducer Gasdermin D, into active peptides. This inflammasome thus plays a central role in immunity and is associated with a broad range of degenerative diseases, including Alzheimer's, asthma, ischemia/reperfusion, diabetes or psoriasis.

Mitochondria are also important sensing organelles which functionally adapt in a nutrient-dependent manner. Nutrient restriction leads to activation of several pathways and to higher levels of nicotinamide adenine dinucleotide (NAD+), which activate sirtuins, enzymes possessing deacetylase activity that require NAD+ as a cosubstrate.

We have shown that nutrient restriction blunts the activation of the inflammasome, and that this depends partially on the activation of the mitochondrial deacetylase Sirtuin 3 (SIRT3), which acts through a very intriguing mechanism: by modulating the acetylation status and activity of mitochondrial superoxide dismutase—and thus mitochondrial ROS levels—it finely controls the extrusion of mtDNA into the cytosol, where it acts as an NLRP3 agonist. In addition, we have found that nicotinamide riboside (NR), a precursor of NAD+, functions as a fasting mimetic and blunts monocyte/macrophage interleukin-1β production and reduces T helper 1 (Th1) and 17 (Th17) cell activation. Interestingly, a mouse model of psoriasis, a chronic skin disease linked to hyperactivation of Th17 cells, displayed downregulation of SIRT3, which might be involved in the hyperinflammatory phenotype. It is also known that NLRP3 activation is associated with psoriasis progression. Given our findings of the role of SIRT3 in immune modulation, a question arising is whether NAD+ precursors could mimic caloric restriction and ameliorate inflammatory diseases. Psoriasis appears to be an appealing candidate to test this hypothesis.

The lines of research of our group are:

- Expand our studies into the fundamental role of mtDNA in inflammatory pathways regulated by SIRT3.

- Evaluate whether NAD+ precursors blunt inflammation in a psoriatic mouse model via augmentation of mitochondrial function, fidelity and quality control programs.

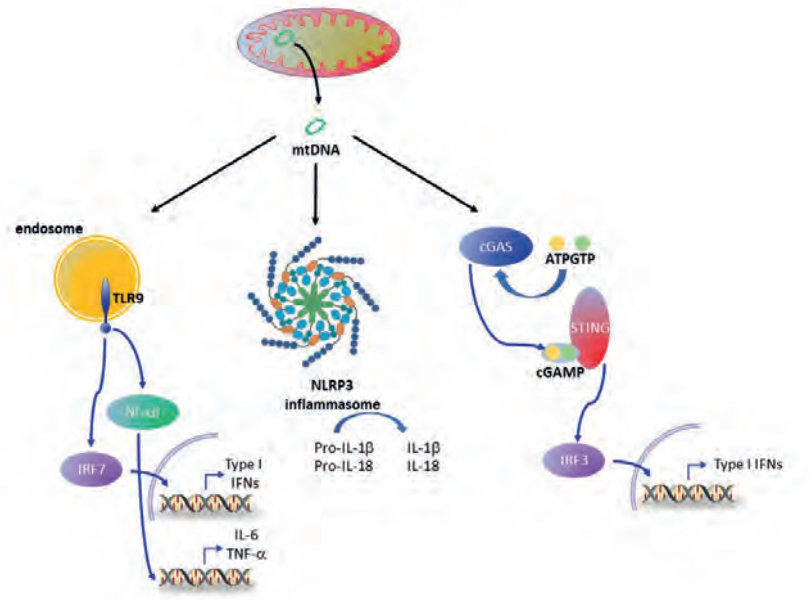


***Figure.*** *Release of mtDNA during mitochondrial dysfunction triggers at least three distinct pathways linked to inflammation: the endosomal TLR9 pathway, the NLRP3 inflammasome and the cGAS-STING pathway.*

## Publications

**Articles with CBM affiliation:**

Traba, J., Waldmann, T. A. and Anton, O. M. (2020) Analysis of Human Natural Killer Cell Metabolism. *J Vis Exp* **160**, e61466.

**Articles without CBM affiliation:**

Anton, O. M., Peterson, M. E., Hollander, M. J., Dorward, D. W., Arora, G., Traba, J., Rajagopalan, S., Snapp, E. L., Garcia, K. C., Waldmann, T. A. and Long, E. O. (2020) Trans-endocytosis of intact IL-15Rα-IL-15 complex from presenting cells into NK cells favors signaling for proliferation. *Proc. Natl. Acad. Sci.* U. S. A. **117**, 522-531.

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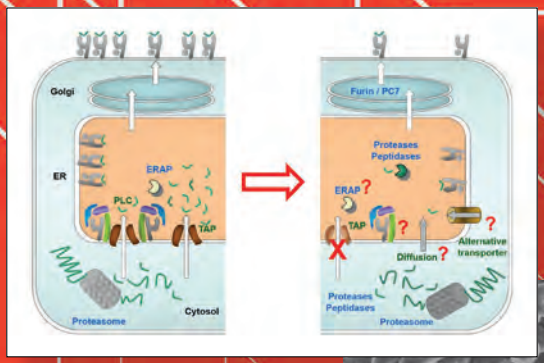
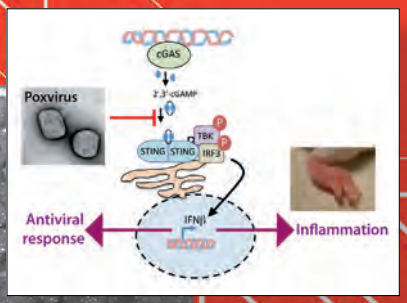
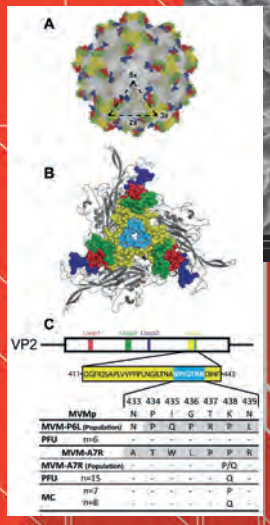
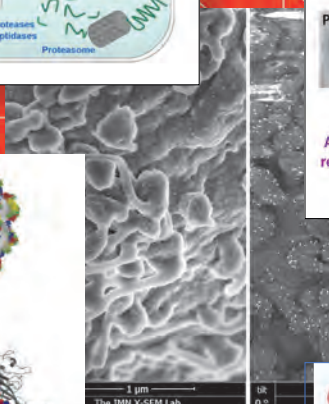
# Interactions with the Environment

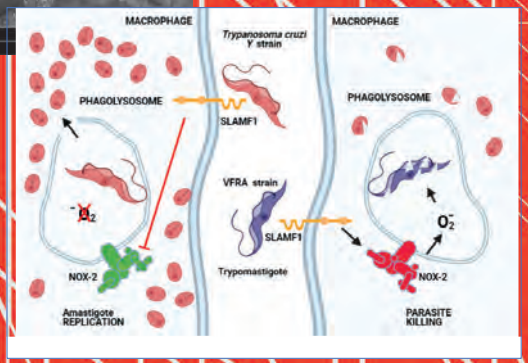
PROGRAM



# *UNITS*

## IMMUNE SYSTEM DEVELOPMENT AND FUNCTION



## MICROBES IN HEALT AND WELFARE

UNIT

CÉSAR COBALEDA

# Immune System Development & Function

INTERACTIONS WITH THE ENVIRONMENT

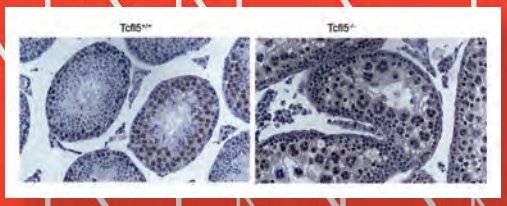
Our Unit is composed of basic and translational scientists focused on the study of the immune system in health and disease, in order to understand how the immune system dynamically maintains the integrity of living organisms protecting them from endogenous alterations of tissue homeostasis and from environmental pathogens, and how malignances of the immune system itself appear. Our final goal is to understand and treat infectious diseases, cancer, immunodeficiencies and autoimmunity. To this aim, we investigate the molecular, cellular, genetic and epigenetic mechanisms regulating the development and function of the cells of the immune system and the bases of their interactions with (and response against) self-components and pathogens, as a key requirement for developing novel therapeutic strategies.

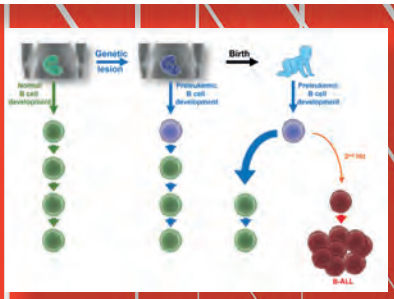
In the past two years, in the field of leukemias, our groups have identified critical physiological pathways of T-cell development with a direct implication in T-cell leukemia development, and have also shown that these pathways are suitable targets for novel immunotherapies. It has also been discovered that exposure to common infections could be involved in the appearance of childhood B-cell leukemias, and it has been found that the transcription factor TCFL5 has a crucial role in lymphocyte differentiation and survival, explaining its correlation with leukemia severity and making it a potential therapeutic target. In myeloid leukemias, a new chimeric receptor against the CD33 protein, a marker associated with this disease, has been generated and optimized. Finally, our groups have also shown that overexpression of wild type RRas2 can induce the generation of chronic lymphocytic leukemias while, on the other side, in the absence of this small GTPase, mice have a reduced repertoire of autoimmune T cells.

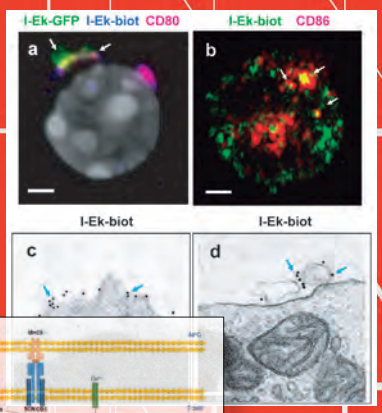
Important advances have also been made in our understanding of lymphocyte activation and its role in normal and aberrant immune responses. We have discovered how interleukin-23 promotes pathogenic T-cell migration to inflamed sites, and we are using proteomics approaches to decipher cytokine receptor signaling in autoimmunity, and how metabolic routes can be used to treat inflammatory disorders. Also, focusing on the study of bioactive lipids that regulate T-cell activation, novel targets of nitroalkylation have been found involved in controlling transcriptional induction and production of proinflammatory cytokines.

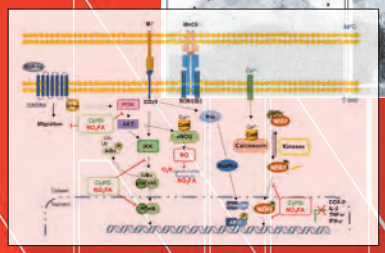
In the context of the response against pathogens, omics studies have unraveled the mechanisms of host-parasite interaction in the context of *Trypanosoma cruzi* infections and its SLAMF1 immune receptor. Also, our groups have identified new properties of virus-encoded cytokine receptors that enhance their anti-inflammatory activity, and have demonstrated that a viral inhibitor of DNA sensing controls the initiation of the inflammatory response, pointing at new strategies to control autoimmune and inflammatory conditions. Other groups are also studying the immune response to viral infections, focusing primarily on the CD8+ T-lymphocyte response, with the ultimate goal of developing strategies that can circumvent immune defects and thus contribute to the vaccine toolkit.

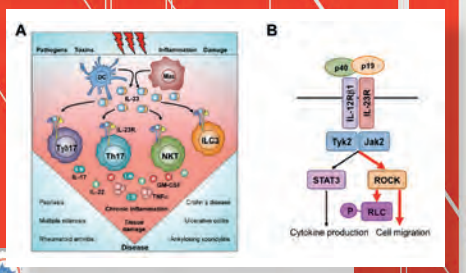
Finally, since the COVID-19 pandemic started, several groups of the Unit have been actively involved in the study of SARS-CoV-2 from several perspectives, ranging from the characterization of virus aerosol transmission in hospitals, to the development of new assays for quantitative detection of antiviral antibodies in patient's blood, the determination of ACE2 receptor levels in patients as a risk biomarker, or the characterization of the nature and degree of protection conferred by infection or vaccination.

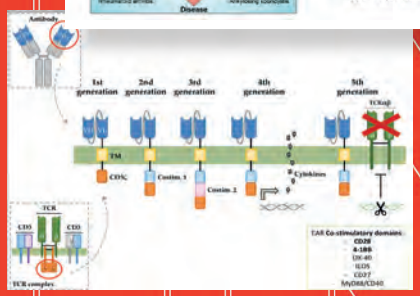





**BALBINO ALARCÓN**

SIGNAL TRANSDUCTION BY THE T-CELL ANTIGEN RECEPTOR

**ANTONIO ALCAMÍ**

IMMUNITY AND VIROMICS

**CÉSAR COBALEDA**

CELLULAR PLASTICITY IN DEVELOPMENT AND CANCER

**MARGARITA DEL VAL**

VIRAL IMMUNOLOGY

**MANUEL FRESNO**

IMMUNE DEVELOPMENT AND INFLAMMATORY-MEDIATED DISEASES

**NÚRIA GIRONÉS**

IMMUNOREGULATORY MECHANISMS IN THE DEVELOPMENT OF CHAGAS DISEASE: TRANSLATIONAL APPLICATIONS

**JUAN MANUEL SERRADOR / MIGUEL ÁNGEL ÍÑIGUEZ**

NITRIC OXIDE AND BIOACTIVE LIPIDS IN THE IMMUNE RESPONSE

**MARÍA N. NAVARRO**

INTRACELLULAR SIGNALLING IN INFLAMMATORY PROCESSES

**MARÍA LUISA TORIBIO**

DEVELOPMENT OF THE HUMAN LYMPHOHEMATOPOIETIC SYSTEM

**HISSE MARTIEN VAN SANTEN**

TCR DOMAINS IN T CELL DIFFERENTIATION AND (PATHO)PHYSIOLOGICAL AND THERAPEUTIC RESPONSES

## IMMUNE SYSTEM DEVELOPMENT AND FUNCTION

# SIGNAL TRANSDUCTION BY THE T-CELL ANTIGEN RECEPTOR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## Research summary

T-lymphocyte activation requires recognition of antigenic peptides on MHC (pMHC) by the T cell antigen receptor (TCR). The TCR contacts the antigen extracellularly through its variable α and β chains, which have very short cytoplasmic tails, so that signal initiation is passed on to the CD3 subunits, which contain longer tails that can contact intracellular interactors. We have found that the TCR is organized in the plasma membrane of resting T cells as oligomers of up to 20 TCRs that we have named as TCR nanoclusters. We believe that the organization of the TCR in nanoclusters can explain in part the high sensitivity of T cells for antigen in spite the low affinity of the monovalent TCR for pMHC. Indeed, we have recently found that TCR nanoclustering allows cooperativity phenomena between pMHC-engaged and non-engaged TCRs. In addition, we have proposed that conformational changes mediate the conversion of pMHC-TCR contacts into CD3-driven intracellular signals, thus triggering the TCR. One of the consequences of the conformational change in the TCR is the exposure of a proline-rich sequence (PRS) of CD3ε that becomes available for binding to the adaptor protein Nck. We have developed small molecular weight inhibitors of the recruitment of Nck to the TCR as immunomodulatory agents. These inhibitors are orally available and show a potent prophylactic and therapeutic effect in different models of autoimmune diseases while sparing the T cell response to pathogens. Another direct effector of the TCR is the small GTPase RRas2 (also known as TC21), which binds constitutively to the non-phosphorylated TCR and plays important roles in homeostatic signaling through PI3K. RRas2 is also a direct effector of the B cell antigen receptor (BCR). We are studying the role of RRas2 in physiological processes of T and B lymphocytes such as homeostatic control of the populations, formation of the immunological synapse, thymic selection, germinal center formation, as well as in pathological processes such as formation of T and B cell lymphomas and leukemias. Our most exciting recent findings suggest that RRas2 is an oncogenic driver in the generation of chronic lymphocytic leukemias and some types of breast cancer. RRas2 drives the generation of cancer in the absence of activating mutations which are common in KRas and other classic Ras GTPases. We are trying to figure out the mechanisms and the relevance of RRas2 for human cancer.

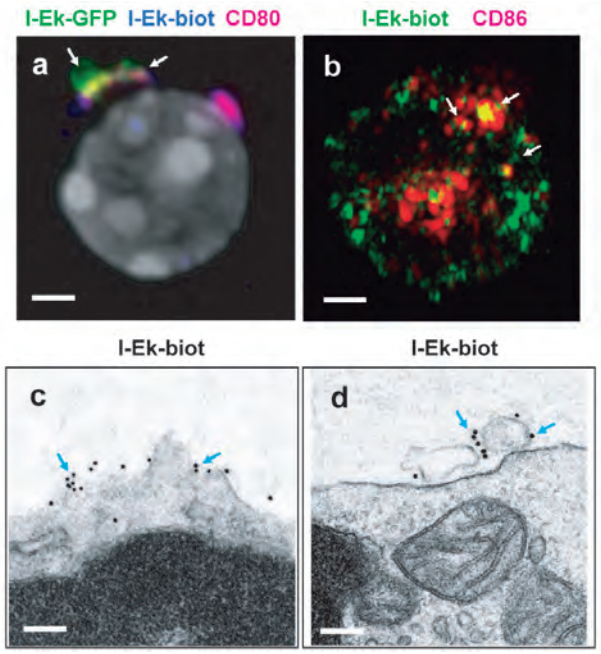


***Figure.*** *T cells acquire and express complexes of antigen and MHC-II and display them on their own cell surface. In this way, T cells become antigen-presenting cells themselves. Activation of other T cells by antigen-presenting T cells results in the generation of pro-inflammatory Th17 cells. a, midplane confocal section showing the nucleus in gray and acquired molecules as indicated. b, a z-axis projection of confocal sections acquired by ELYRA super-resolution microscopy. c and d, MHC-II (I-Ek) expression on the T cell's plasma membrane analyzed by pre-embedding immunogold electron microscopy after labelling with 10 nm streptavidin-gold particles. Blue arrows mark the presence of gold particles associated to the plasma membrane or to surface-bound microvesicles. Bocassavia et al, Cell Reports, in press.*

## Publications

Martínez-Riaño, A., Bovolenta, E. R., Boccasavia, V. L., Ponomarenko, J., Abia, D., Oeste, C. L., Fresno, M., van Santen, H. M. and Alarcon B. (2019) RRAS2 shapes the TCR repertoire by setting the threshold for negative selection. *J Exp Med.* **216**(10):2427-2447.

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Alarcon, B. and Borroto, A. (2020) Small molecule AX-024 targets T cell receptor signaling by disrupting CD3ε-Nck interaction. J Biol Chem. 2020 295(29):10076.

Schamel, W. W., Alarcon, B. and Minguet, S. (2019) The TCR is an allosterically regulated macromolecular machinery changing its conformation while working. *Immunol Rev*. **291**:8-25.

## Patents

Balbino Alarcón Sánchez, Lydia Horndler Gil, Pilar Delgado Cañaveras, Ivaylo Balabanov, Hisse Martien van Santen. Flow cytometry multiplexed method for the detection of SARS-CoV-2 antibodies. EP20382667.2. País de prioridad: España. Fecha de prioridad: 24-07-2020. Propietario: CSIC. Licenciatario: Vitro SA.

# IMMUNE SYSTEM DEVELOPMENT AND FUNCTION

## IMMUNITY AND VIROMICS

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Carolina Sánchez Fernández
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Javier Alvarez de Miranda
(TFM 01/2020-07/2020)
Lorena Mejías
University of Surrey, UK
(10/2019-07/2020)

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## Research summary

We are characterizing viral mechanisms to modulate the host anti-viral immune response and the inflammatory response. We work on herpesvirus and poxvirus proteins that are secreted from infected cells and interact with cytokines or chemokines, and modulate their activity. The contribution of viral proteins to pathogenesis is tested in mouse models of infection. We are characterizing glycoprotein G from herpes simplex virus, a protein that interacts with both chemokines and neurotrophic factors to facilitate viral invasion of the nervous system. We have further characterized unique anti-inflammatory properties of soluble tumour necrosis factor receptors encoded by poxviruses. We have shown that addition of a viral chemokine binding domain to the human tumour necrosis factor receptor used in the clinic to treat autoimmune diseases enhances its anti-inflammatory activity, and a mutation in the human receptor restricts its ligand specificity. We also described that the Schlafen poxvirus protein is a potent inhibitor of the DNA sensing pathway, which controls the early production of type I interferon and limits the activation of protective anti-viral responses. The DNA sensing pathway has a major role in the initiation of the inflammatory response, and the viral protein has the potential to inhibit uncontrolled inflammatory responses leading to autoimmune diseases. Understanding how viruses have optimized their immune modulatory activities may help us to identify new therapeutic approaches to treat human inflammatory diseases.

Viral metagenomics is offering us the opportunity of identifying complex viral communities present in natural environments. Using next generation sequencing we are characterizing at the genetic level complex viral communities adapted to the extreme environmental conditions of from Polar regions, Antarctica and the Arctic. We are expanding this research to the study of Alpine ecosystems in the Ordesa and Monte Perdido National Park in the Pyrenees.

Lastly, our group has optimized the methodology to capture microorganisms and viruses in the air (airbiota) to identify at the genetic level the presence of airborne pathogens. Since the COVID-19 pandemic started, we have adapted this methodology to identify SARS-CoV-2 in aerosols. In collaboration with clinicians we characterized the aerosol transmission of SARS-CoV-2 in hospitals and the impact of air transmission in the COVID-19 pandemic.

## Publications


Pontejo, S. M., Sánchez, C., Ruiz-Argüello, B. and Alcamí, A. (2019) Insights into ligand binding by a viral tumor necrosis factor (TNF) decoy receptor yield a selective soluble human type 2 TNF receptor. *J. Biol. Chem*. *294*(13):5214-5227.

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Yaron, J. R., Zhang, L., Guo, Q., Burgin, M., Schutz, L. N., Awo, E., Wise, L., Krause, K. L., Ildefonso, C. J., Kwiecien, J. M., Juby, M., Rahman, M. M., Chen, H., Moyer, R. W., Alcami, A., McFadden, G. and Lucas, A. R. (2020) Deriving Immune Modulating Drugs from Viruses-A New Class of Biologics. *J. Clin. Med*. **9**(4):972.

Hernáez, B. and Alcamí, A. (2020) Virus-encoded cytokine and chemokine decoy receptors. *Curr. Opin. Immunol*. **66**:50-56.

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Kropp, K. A., Srivaratharajan, S., Ritter, B., Yu, P., Krooss, S., Polten, F., Pich, A., Alcamí, A. and Viejo-Borbolla, A. (2020) Identification of the Cleavage Domain within Glycoprotein G of Herpes Simplex Virus Type 2. *Viruses* **12**(12):1428.

Al Rumaih, Z., Tuazon Kels, M. J., Ng, E., Pandey, P., Pontejo, S. M., Alejo, A., Alcamí, A., Chaudhri, G. and Karupiah, G. (2020) Poxvirus-encoded TNF receptor homolog dampens inflammation and protects from uncontrolled lung pathology during respiratory infection. *Proc. Natl. Acad. Sci.U. S. A*. **117**(43):26885-26894.

Alcamí, A. (2020) Was smallpox a widespread mild disease? *Science* **369**(6502):376-377.


## Awards and recognition

- Organizer. 3rd European Chemokine and Cell Migration Conference. Salamanca, Spain. 06/2019.

- Advisor to the World Health Organization Advisory Committee on Variola Virus Research.

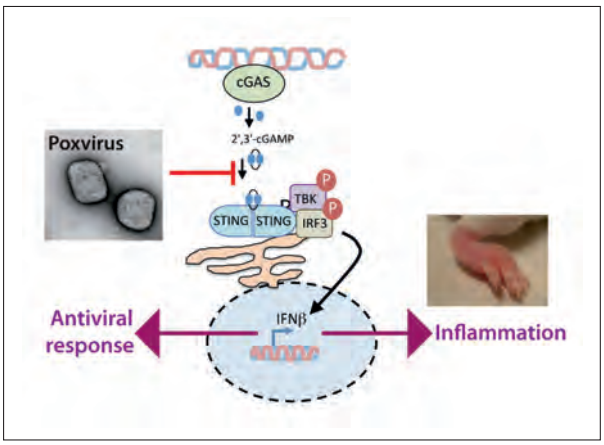


***Figure.*** *Inhibition of the DNA sensing pathway by the poxvirus protein Schlafen. The blockade of this pathway prevents the early production of type IFN leading to inhibition of the anti-viral and inflammatory responses.*

## Doctoral Theses

**Graciela Alonso** (2019). Relevancia de la actividad viral antilinfotoxina y el gen viral Schalfen en la patogénesis del virus ectromelia. Universidad Autónoma de Madrid. Co-supervisada: Antonio Alcamí y Bruno Hernáez.

**Alberto Domingo López** (2019). Papel de la glicoproteína G del virus herpes simplex en patogénesis. Universidad Autónoma de Madrid. Co-supervisada: Antonio Alcamí y Alberto Rastrojo.

## Patents

S. M. Pontejo, C. Sánchez and A. Alcamí. A variant form of etanercept with increased specificity for TNF. PCT/EP2019/079492. International Patent Application 29/10/2019. Owner: CSIC.

A. Rastrojo and A. Alcamí. Device and method for capturing and analysing airborne organisms. EP20382510.4. European Patent Application 12/06/2020. Owner: CSIC.

## International projects / Research networks

- Ancient viruses and microorganisms in polar lakes. EU Horizon 2020 EASIGenomics (PID10643 POLARPALEOVIRUS). PI A. Alcamí. 07/2020-06/2022.

- Diversity of DNA and RNA viruses in Antarctica. Joint Genome Institute, U.S. Department of Energy (JGI Proposal ID: 505820; Contract No. DE-AC02- 05CH11231). PIs: A. Alcamí and C. Cary 01/2020-12/2022.

## IMMUNE SYSTEM DEVELOPMENT AND FUNCTION

# CELLULAR PLASTICITY IN DEVELOPMENT AND CANCER

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(co-IP: María Gómez)

***Undergraduate and Master Students:***
Silvia Alemán Arteaga
(January-June 2019)
Blanca Gálvez García
(January-June 2019)

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## Research summary

The goal of our research group is to understand how cellular identity is determined and how it is deregulated in pathological conditions affecting hematopoietic development, such as leukemias and immunodeficiencies. As experimental tools we use: (1) genetically engineered mouse models (GEMMs) in which we modify the expression of transcription factors and epigenetic regulators—either normal or oncogenic—and (2) patient samples.

Cancer is, after accidents, the most frequent cause of death of children in developed countries, and B-cell acute lymphoblastic leukemia (B-ALL) is the most common form of childhood cancer. Almost 5% of healthy newborn children present an inborn genetic predisposition to develop B-ALL. Luckily, very few (<1%) of these predisposed children will progress to B-ALL, by suffering a second hit that leads to full-blown disease (Figure 1). The causes that trigger this progression are still unclear, but B-ALL incidence seems to be increasing in parallel with the adoption of modern lifestyles. A stress on the immune system could be involved in the progression to overt B-ALL, and this stress could be triggered by exposure to common infections under certain circumstances, or by other stressors like antibiotics, diet, or alterations in the microbiota. Therefore, B-ALL could be a preventable disease, and understanding the interaction between preleukemic cells and immune stress would provide us with strategies aimed at preventing childhood B-ALL development.

We have generated GEMMs expressing the oncogenic lesions responsible for the initiation of childhood B-ALLs, and we are investigating the role that the exposure to different agents might play in B-ALL development. So far, we have demonstrated that infections are one of the triggering factors of B-ALLs while, for example, exposure to low-frequency electromagnetic fields does not seem to cause any effect. Also, we have shown that the production of inflammatory cytokines can act in an autocrine fashion to promote leukemia growth in mice and in human patients.

Wolf Hirschhorn Syndrome (WHS) is a complex disease, and affected patients present many severe problems, including life-threatening immunodeficiencies. One of the genes affected in WHS is WHS-Candidate-1 (WHSC1), an epigenetic regulator involved in many processes affecting genome function and that, interestingly, is also mutated in childhood B-ALLs and other malignancies of B-cell origin. We are using GEMMs with deregulated WHSC1 function to gain insight into the molecular biology of WHS-associated hematopoietic pathologies, in order to better understand the role of epigenetics in normal and aberrant hematopoietic development, and to develop future prospective therapeutic interventions.

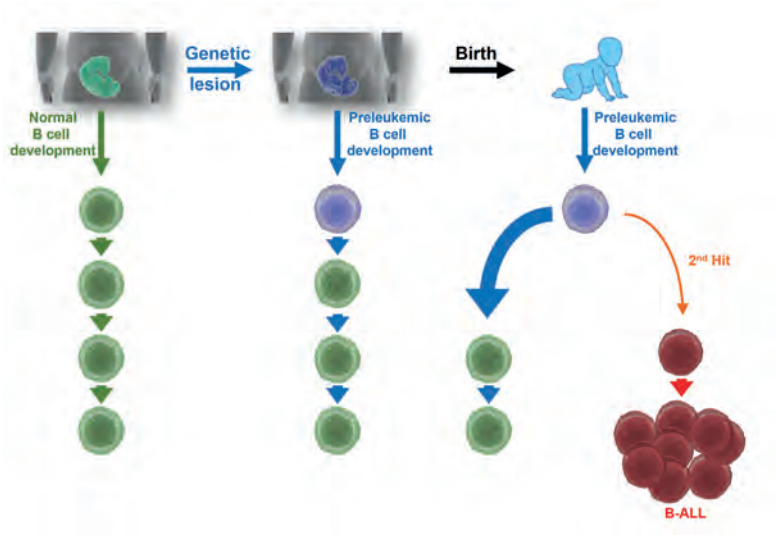


***Figure.*** *Prenatal origin of the pre-leukemic clone. In more than 5% of healthy newborns, an oncogenic mutation arises in utero, leading to the appearance of a pre-leukemic B-cell clone, which nevertheless allow normal B cell development to take place, and is therefore clinically silent. Most children carrying this alteration will never develop B-ALL but, in a small percentage of them, under conditions triggering an immune stress (environmental exposures, etc.), this pre-leukemic clone will acquire a second hit, therefore giving rise to B-ALL.*

## Publications

Sánchez-García, I. and Cobaleda, C. (2020) Leukemia Stem Cells: Concept and Implications. *Methods Mol Biol*. **2185**, 25-37.

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Isidro-Hernández, M., Mayado, A., Casado-García, A., Martínez-Cano, J., Palmi, C., Fazio, G., Orfao, A., Ribera, J., Ribera, J. M., Zamora, L., Raboso-Gallego, J., Blanco, O., Alonso-López, D., De Las Rivas, J., Jiménez, R., García Criado, F. J., García Cenador, M. B., Ramírez-Orellana, M., Cazzaniga, G., Cobaleda, C., Vicente-Dueñas, C. and Sánchez-García, I. (2020) Inhibition of inflammatory signaling in Pax5 mutant cells mitigates B-cell leukemogenesis. *Sci Rep*. **10**, 19189.

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Martínez-Cano, J., Campos-Sánchez, E. and Cobaleda, C. (2019) Epigenetic Priming in Immunodeficiencies. *Front Cell Dev Biol*. **7**:125.

Campos-Sanchez, E., Vicente-Dueñas, C., Rodríguez-Hernández, G., Capstick, M., Kuster, N., Dasenbrock, C., Sánchez-García, I. and Cobaleda, C. (2019) Novel ETV6-RUNX1 Mouse Model to Study the Role of ELF-MF in Childhood B-Acute Lymphoblastic Leukemia: a Pilot Study. *Bioelectromagnetics* **40**, 343-353.

Campos-Sanchez, E., Martínez-Cano, J., Del Pino Molina, L., López-Granados, E., Cobaleda, C. (2019) Epigenetic Deregulation in Human Primary Immunodeficiencies. *Trends Immunol*. **40**, 49-65.

## Awards and recognition

- Editor: "Leukemia Stem Cells: Methods and Protocols" (2021), Volume 2185 of the "Methods in Molecular Biology Series" (Springer Nature, US). doi: 10.1007/978-1-0716-0810-4.

- Member of the Scientific Advisory Board of "Fundación Unoentrecienmil".

- Ad hoc member of Advisory Committees for the German Federal Office for Radiation Protection. (Bundesamt für Strahlenschutz, BfS).

- Member of the Working Group on Non-Ionizing Radiation of the Spanish "Plataforma Nacional de I+D en Protección Radiológica" (PEPRI) [of the Spanish "Consejo de Seguridad Nuclear" (CSN)].

# IMMUNE SYSTEM DEVELOPMENT AND FUNCTION

## VIRAL IMMUNOLOGY


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Beatriz Tejedor Sáez-Bravo

***Technician:***
Beatriz Mena Romero
(Since April 1st 2019)

***Undergraduate and Master Students:***
Andrés Soto Zaragoza
(Since September 1st 2020)

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## Research summary

Our field of interest is aimed at improving the control of chronic and opportunistic infections by the immune system, for which the cellular immune response, as opposed to antibodies alone, plays a leading role, with the long-term goal of contributing to an improved design of new vaccines. Although much is known, vaccines inducing a potent and long-lasting T-cell immunity are still not available. Beyond childhood-oriented vaccines, and due to recent demographic changes and medical advances, the focus of vaccine development is shifting to the new needs of protecting a growing number of adult patients with diverse congenital (primary immunodeficiencies) or iatrogenic levels of immunosuppression (cancer, organ transplantation, autoimmunity) or in specific stages of life (pregnancy, elderly). In order to adapt basic research to these changing needs, we are analyzing basic issues of antiviral cellular immune responses and vaccination in two models of partially immunocompromised mice.

Our first model are mice deficient in the transporter associated with antigen processing (TAP), a crucial player in the MHC class I antigen presentation pathway for recognition by CD8+ T lymphocytes, which are the effector cells responsible for the elimination of infected cells in vivo. These mice are a model for TAP-deficient patients, who suffer from severe recurrent bacterial infections, and have a strong quantitative defect in CD8 cellular immunity, although the remaining CD8+ T lymphocytes are functional. We are currently identifying MHC-I presented viral peptide epitopes in TAP-deficient cells and animals, the proteases and the antigen processing pathways generating them in infected cells and trying to elucidate their contribution to the CD8 immune response in these animals.

The second model is represented by mice deficient for the Nras signaling protein, which we have shown to have a qualitative Eomes defect that precludes development of anti-viral memory CD8+ T lymphocytes, and thus prevents vaccination. We are currently evaluating vaccination procedures that could overcome this defect and that might be applied to vaccine design.

The viral infection models we are using are vaccinia virus and mouse cytomegalovirus. Vaccinia virus, a poxvirus, is the vaccine vector behind the first and major success of vaccinology in public health: the eradication of smallpox, officially declared in 1980. Cytomegalovirus is relevant for vaccine design, as it remains latent and continuously presents antigen without pathology, which leads for life to a massive number of functional virus-specific T lymphocytes in the infected organisms, called inflationary memory.

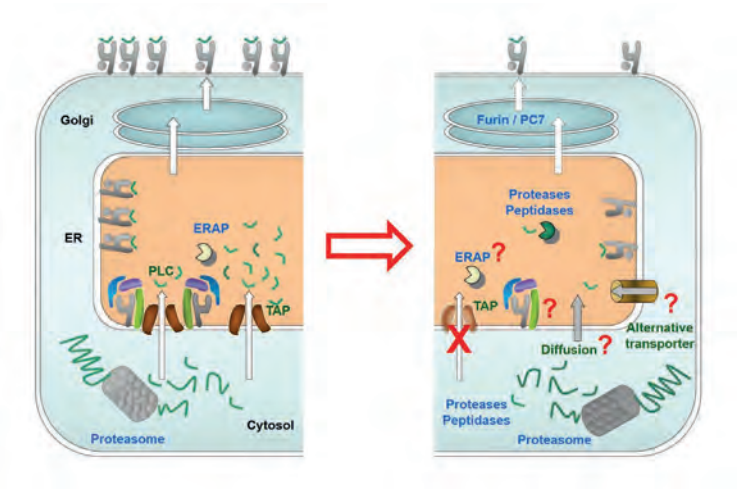


***Figure.*** *MHC class I viral antigen processing and presentation pathway. The left panel shows the central role played by TAP transporter in this pathway, translocating peptides as well as serving as the hub for the assembly of the peptide loading complex (PLC). The right panel shows the impact of TAP inactivation on MHC class I antigen presentation. The panel also points to potential routes that may mediate the processing of MHC class I antigenic peptides that, although at low levels, are presented by MHC class I in these cells.*

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González-Fernández, Á., Bermúdez Silva, F. J., López-Hoyos, M., Cobaleda, C., Montoliu, L., Del Val, M. and Leech, K. (2020) Non-animal-derived monoclonal antibodies are not ready to substitute current hybridoma technology. *Nat. Methods*. **17**(11), 1069-1070.

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Ramírez de Arellano, E., Díez-Fuertes, F., Aguilar, F., de la Torre Tarazona, H. E., Sánchez-Lara, S., Lao, Y., Vicario, J. L., García, F., González-Garcia, J., Pulido, F., Gutierrez-Rodero, F., Moreno, S., Iribarren, J. A., Viciana, P., Vilches, C., Ramos, M., Capa, L., Alcamí, J. and Del Val, M. (2019) Novel association of five HLA alleles with HIV-1 progression in Spanish long-term non progressor patients. *PLoS One*. **14**(8), e0220459.

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## Awards and recognition

- Margarita del Val and Luis C. Antón: Editors of the September 2019 Special Issue of Molecular Immunology (vol. 113) on Antigen Processing and Presentation (Elsevier, ISSN: 0161-5890).

## IMMUNE SYSTEM DEVELOPMENT AND FUNCTION

# IMMUNE DEVELOPMENT AND INFLAMMATORY-MEDIATED DISEASES

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Javier Galán Martínez

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## Research summary

Cyclooxygenase 2 (Cox2) inhibitors reduce cancer but their therapeutic use has been hampered by their side effects. As an alternative, we have analyzed genes regulated by Cox2 or prostaglandins (PG) that may provide a protooncogenic advantage. Among those mPGES-1, DUSP10, Trop2 and many from the TGFβ pathway, as PMEPA1, that interestingly were induced by PGF2α. DUSP10 controls stress response to serum deprivation and confluence arrest and binds and dephosphorylates Yes-associated protein 1 (YAP) in colorectal cancer and PGF2α TGFβ-PMEPA1 pathway is a critical mediator of epithelial plasticity and ovarian carcinoma progression Those molecules are amenable to drug discovery, lacking the unwanted side effects of COX-2 pharmacological inhibitors.

TCFL5 is a member of the bHLH transcription factor family with multiple isoforms in both humans and mice Complete deletion of the isoforms drastically reduces the tumor properties of the colon cancer cells Interestingly, The 2 major isoforms, TCFL5 and CHA showed a reciprocal regulation and showed different functions in the cell, with CHA exercising a protumoral TCFL5 / CHA expression is essential for NFKB2 activity regulating the expression of anti-apoptotic genes such as BCL2 and also controls the expression of the pluripotency markers SOX2, NANOG and KLF4.

We have also defined a set of genes regulated by TCFL5/CHA in leukemia cell lines and established its role in the prognosis and development of B-and T-acute lymphoblastic leukemia and in normal hematopoiesis. The expression of TCFL5 and CHA was associated with greater severity in lymphoma and myeloma samples from patients. Last but not least, Using Tcfl5 deficient mice, we found *Tcfl5* is involved in the formation of germinal centers and in the stage of differentiation of pro-B to pre-B cells, two processes that are not only characterized by a high proliferation rate being MYC dependent The lack of TCFL5 caused a radical decrease in the levels of the most important molecules in BCR signalling, such as SYK and BLNK, resulting in an inability to respond to stimuli, an increase in cell death and an abnormal cell cycle.

Finally using *Tcfl5* deficient mice, we found that TCFL5 is expressed in early mouse embryonic development during the preimplantation period and plays a role in the differentiation of embryonic cells to germline precursors by controlling the expression of genes important in the differentiation

The protozoan parasite, *Trypanosoma cruzi* causes Chagas' disease. We have been also working on many aspects of this disease (see N. Gironès research group).

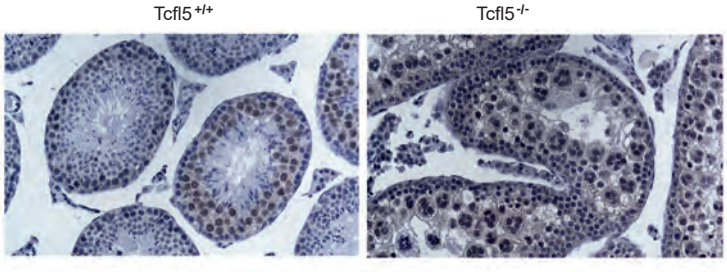


***Figure.*** *Immunohistochemistry analysis of Tcfl5 expression in Tcfl5+/+ and Tcfl5-/- testis. Tcfl5-/- mice showed a destructuring of seminiferous tubules.*

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## Doctoral Theses

**Sara Isabel Vaz Francisco**. 2019. Toll-like receptor 2 and 4: Differential signaling, dimerization and the outcome in inflammation. Dtors: Manuel Fresno y Alicia Arranz. Outstanding "*Cum Laude*". UAM.

**Javier Galán Martínez**. 2019. Papel de las isoformas del factor de transcripción TCFL5 en procesos tumorales de cáncer de colon, pluripotencia y desarrollo. Directores: Manuel Fresno Escudero y Núria Gironés Pujol. Outstading-*Cum Laude*". UAM. International Mention.

**Francisco Callejas Hernández**. 2019- Análisis de la diversidad genómica y transcriptómica de *T. cruzi* y su relación con la enfermedad de Chagas. Directores: Manuel Fresno y Núria Gironés. Outstanding "*Cum Laude*". UAM.

## International projects / Research networks

- Fresno Escudero, Manuel. Collaborative Research Network on Tropical Diseases (RICET). RD16/0027/0006.: ISCIII. 2017-2021.

- Manuel Fresno (Coordinador). B2017/BMD-3671. INFLAMUNE-CM. New molecular and cellular mechanisms involved in immune pathophysiology and disease. Comunidad de Madrid. 2018-2021.

- Fresno Escudero, Manuel. Validation of neuroinflammatory drug targets for the treatment of chronic pain. RETOS COLABORACIÓN RTC-2017-6292-1. mMINECO. 2018-2021.

- Manuel Fresno. Head of Group 12 of the "La Princesa" Health Research Institute.

## IMMUNE SYSTEM DEVELOPMENT AND FUNCTION

# IMMUNOREGULATORY MECHANISMS IN THE DEVELOPMENT OF CHAGAS DISEASE: TRANSLATIONAL APPLICATIONS


***Principal Investigator:***
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***Postdoctoral Fellows:***
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***Predoctoral Fellows:***
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Alfonso Herreros Cabello
Inés Sánchez Gómez
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## Research summary

Chagas disease caused by *Trypanosoma cruzi* affects approximately 10 million people in Latin America. Cardiac pathology is the most severe and characteristic manifestation.

Infection can be naturally transmitted by insect Reduviidae vectors of the endemic areas, but also by blood transfusion, organ transplantation, oral ingestion, vertical transmission, and laboratory accidents. Thus, blood transfusion and organ transplantation are a sanitary problem in countries receptors of migrants from endemic areas. In Spain it is estimated a future incidence between 6.000 y 30.000 cases of chagasic cardiomyopathy.

The disease is characterized by an acute phase that lasts about two months during which myocarditis can occur. Mortality in the acute phase is low, mostly in children. After parasite clearance disease remains asymptomatic for decades, and cardiomyopathy progressively develops that resembles Idiopathic Dilated Cardiomyopathy. Arrhythmias are characteristic in the chronic phase that can lead to sudden death.

There are only two drugs available for the treatment of the patients, which is most effective in the acute phase. In the chronic phase, drug effectiveness has not been demonstrated and causes important side effects. Nowadays, asymptomatic chronic patients are treated, but there is a need to identify new biomarkers to follow disease progression.

There are many parasite isolates (strains) that show a high degree genomic diversity and different virulence. Because of this diversity, the immune response against infection is very complex. Our working hypothesis is that the development of pathology depends on a combination of factors: host genetic background, a different parasite infecting capacities with different genetic backgrounds, and the regulatory immune response will affect the inflammatory process.

During the 2019-2020 period, we studied the infection of different strains in macrophages and mice focusing on the role of the SLAMF1 receptor as a sensor of infection in the immune response. We found an association between SLAMF1 and reactive oxygen species (ROS) production by NADPH oxidase (NOX-2) that was parasite-strain dependent.

Finally, to deepen the understanding of this pathogen we performed genomic, transcriptomic, and proteomic analysis using different T. cruzi strains. Future studies will focus on the identification and characterization of microRNAs in the regulation of the immune response against the parasite and its usefulness as biomarkers of the disease.

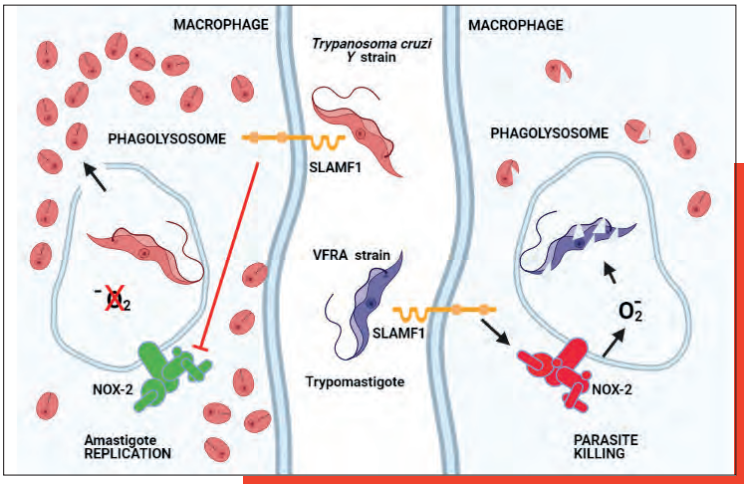


***Figure.*** *Trypanosoma cruzi affects SLAMF1 receptor-dependent ROS production mediated by NOX-2 in the phagolysosome and controls parasite replication in macrophages in a parasite strain-dependent manner (created in BioRender.com).*

## Publications

Herreros-Cabello, A., Callejas-Hernández, F., Gironès, N., and Fresno, M. (2020) *Trypanosoma Cruzi* Genome: Organization, Multi-Gene Families, Transcription, and Biological Implications. *Genes (Basel)* **11**(10):1196.

Poveda, C., Herreros-Cabello, A., Callejas-Hernández, F., Osuna-Pérez, J., Maza, M. C., Chillón-Marinas, C., Calderón, J., Stamatakis, K., Fresno, M., and Gironès, N. (2020) Interaction of Signaling Lymphocytic Activation Molecule Family 1 (SLAMF1) receptor with *Trypanosoma cruz*i is strain-dependent and affects NADPH oxidase expression and activity. *PLoS Negl Trop Dis*. **14**(9):e0008608.

Sánchez-León, E., Bello-Morales, R., López-Guerrero, J. A., Poveda, A., Jiménez-Barbero, J., Gironès, N., and Abrusci, C. (2020) Isolation and characterization of an exopolymer produced by Bacillus licheniformis: In vitro antiviral activity against enveloped viruses. *Carbohydr Polym*. **248**:116737.

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Callejas-Hernández, F., Gutierrez-Nogues, Á., Rastrojo, A., Gironès, N., and Fresno, M. (2019) Analysis of mRNA processing at whole transcriptome level, transcriptomic profile and genome sequence refinement of *Trypanosoma cruzi. Sci Rep.* **9**(1):17376.

Herreros-Cabello, A., Callejas-Hernández, F., Fresno, M., and Gironès, N. (2019) Comparative proteomic analysis of trypomastigotes from *Trypanosoma cruzi* strains with different pathogenicity. *Infect Genet Evol*. **76**:104041.

Santi-Rocca, J., Gironès, N., and Fresno, M. (2019) Multi-Parametric Evaluation of *Trypanosoma cruzi* Infection Outcome in Animal Models. *Methods Mol Biol*. **1955**:187-202.

## Doctoral Theses

**Javier Galán Martínez** (2019). Papel de las isoformas del factor de transcripción TCFL5 en procesos tumorales de cáncer de colon, pluripotencia y desarrollo. Universidad Autónoma de Madrid. Directores/as: Manuel Fresno Escudero y Núria Gironès Pujol.

**Francisco Callejas Hernández** (2019). Análisis de la diversidad genómica y transcriptómica de Trypanosoma cruzi y su relación con la enfermedad de Chagas. Universidad Autónoma de Madrid. Directores/as: Manuel Fresno Escudero y Núria Gironès Pujol.

## International projects / Research networks

- Funding agency: Comunidad de Madrid/FEDER
Title: *Redes Moleculares y celulares en enfermedades inflamatorias* (INFLAMUNE)
Affiliation: Centro de Biología Molecular Severo Ochoa, CSIC-UAM.
Responsible: Núria Gironès Pujol
From: January 1st 2018 to: December 31st 2021
Coordinator: Manuel Fresno Escudero

- Funding agency: ISCIII
Title: *Red de Investigación Colaborativa en Enfermedades Tropicales (RICET)* (0027/0006)
Affiliation: Centro de Biología Molecular Severo Ochoa, CSIC-UAM.
From: January 1st 2017 to: December 31st 2021
Coordinator UAM-B: Manuel Fresno Escudero

- Funding agency: CYTED
Title: *Red Iberoamericana de Medicina Genómica en Enfermedad De Chagas (RIMGECH)*
Affiliation: Centro de Biología Molecular Severo Ochoa, CSIC-UAM.
From: January 1st 2017 to: December 31st 2020
Coordinator: Javier Martín Ibáñez (IPBLN, CSIC)

- Participant of Instituto Sanitario Princesa (ISCIII)

## IMMUNE SYSTEM DEVELOPMENT AND FUNCTION

# NITRIC OXIDE AND BIOACTIVE LIPIDS IN THE IMMUNE RESPONSE


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## Research summary

Nitric oxide (NO) and bioactive lipids as nitro-fatty acids ($NO_2$-FA) or prostaglandins, are key mediators for maintaining cellular homeostasis, with an essential role in inflammation. Our research lines are dedicated to the study the role played by NO as well as nitro and oxo modified fatty acids in inflammation and in the activation and differentiation of T lymphocytes. We are currently studying the actions exerted by these agents on the activation of human T lymphocytes, analysing their involvement in the regulation of gene expression and activation of transcription factors. We are also interested in the analysis of chemotaxis, intercellular adhesion and the organization of adhesion and signalling receptors at the immune synapse. In addition, we are also examining the potential actions of these compounds on the selection of the adaptive immune response in human T lymphocytes.

NO is a key messenger in the pathogenesis of inflammation. In the immune system, NO has been considered to be a cytotoxic molecule associated with the response of phagocytic cells to pathogens as part of the first line of host defence against infection. However, NO can also regulate the adaptive immune response, linking innate and adaptive immunity. NO affects T helper cell differentiation and the effector functions of T lymphocytes, and is a potential target for therapeutic manipulation. In the last years, our group has been interested in the study of the regulatory actions exerted by NO in T cell functions, focusing on protein S-nitrosylation and fatty acid nitro-alkylation, leading to the formation $NO_2$-FA, as important post-translational modifications by which NO can act as a signalling molecule during T cell-mediated immunity.

Fatty acid oxidative modifications result in the production of bioactive lipids including prostaglandins and $NO_2$-FA, important signalling molecules that can modulate the inflammatory process and the immune response. We are interested in the analysis of their influence on diverse parameters of T lymphocyte function, focusing on their effects on transcriptional activation and gene expression and their consequences on cell activation and differentiation. Their anti-inflammatory and immunomodulatory effects take place mainly through their ability to covalently modify transcriptional regulatory proteins and enzymes and to activate various nuclear and membrane receptors, finally modifying protein function and altering patterns of gene expression. Research on the molecular and cellular basis of the actions of electrophilic fatty acids in inflammation and the immune response, will contribute to the understanding of the potential therapeutic benefits of these compounds.

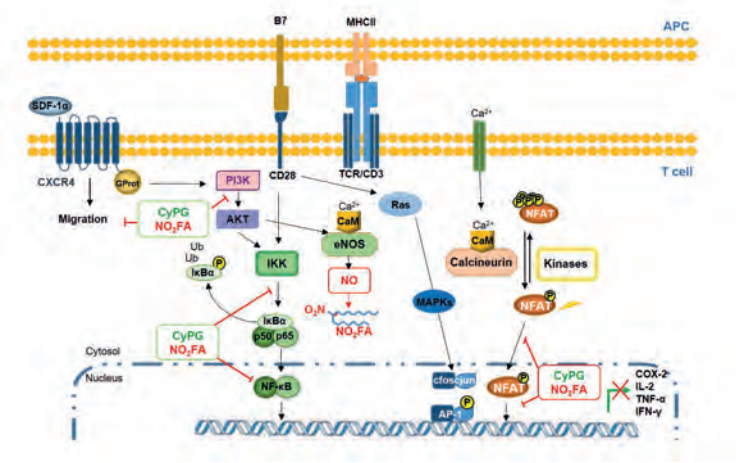


***Figure.*** *Model of NO, $NO_2$-FA and CyPG actions on T cell activation. Upon signaling through the T cell receptor (TCR), different signaling pathways are activated, that ultimately promote the activation of various transcription factors, including NF-κB, AP-1 and NFAT. Activation of these factors is central for the transcription of essential genes in the immune and inflammatory response, such as IL-2, TNF-α and IFN-γ, among others. Nitric Oxide (NO) production by eNOS, as well as production of electrophilic fatty acids such as Cyclopentenones (CyPG) and Nitro fatty acids ($NO_2$FA), regulate different steps in T cell activation process, resulting in changes on gene expression, cell migration, intercellular adhesion and organization of the immune synapse, which accounts for some of their immunoregulatory properties.*

## Publications

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## Awards and recognition

- Member of the Instituto de Investigación Sanitaria Hospital La Princesa. Group 18.

## IMMUNE SYSTEM DEVELOPMENT AND FUNCTION

# INTRACELLULAR SIGNALLING IN INFLAMMATORY PROCESSES

***Principal Investigator:***
María N. Navarro

***Predoctoral Fellows:***
Gloria Pastor Fernández

***Undergraduate and Master Students:***
Andrea Sánchez de la Cruz
Isabel Rodríguez Mariblanca

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## Research summary

The incidence of chronic inflammatory diseases has significantly increased among developed countries in the past 30 years. Therefore, there is a need for development of novel treatments for this type of conditions. The chronic inflammatory diseases are initiated by an uncontrolled immune response that triggers the hyper-activation of signalling cascades, promoting the accumulation of pro-inflammatory mediators and finally, the manifestation of clinical symptoms. In different chronic inflammatory diseases, the axis formed by interleukin 23 and interleukin 17 (IL-23/IL-17) has emerged as a key signalling hub in pathologies such as psoriasis, inflammatory bowel diseases, multiple sclerosis or rheumatoid arthritis in murine models and more importantly, in humans. The pathogenic effects of IL-23 have been linked to its ability to promote the production of inflammatory mediators, mainly interleukin 17 and 22 (IL-17/IL-22). These secreted mediators amplify intercellular communication networks, causing a chronic inflammation and finally, clinical symptoms. IL-23 pathogenic actions are mostly restricted to distinct subpopulations of T lymphocytes: the CD4 helper subset Th17 and the TCRγδ subpopulation Tγδ17. Therapeutic strategies aimed at inhibiting the intracellular signalling networks triggered by different receptors have been successfully applied for treatment of inflammatory diseases and cancer. Despite the prominent role of IL-23 in diseases, these therapeutic approaches have not been fully exploited in the context of inflammatory pathologies since the IL-23 signalling cascade remains largely unknown.

Our lab is interested in the characterisation of the signalling network triggered by IL-23 and other pro-inflammatory cytokines, as a strategy to uncover novel mediators of cytokine signalling for the development of therapeutic tools based on the interference with intracellular signalling pathways. Recently, the lab has discovered how interleukin 23 promotes pathogenic T cell migration to the inflamed site. In the next years, we will address how the pharmacological manipulation of metabolic routes in immune cells can be used for treatment of chronic inflammatory diseases. Moreover, a screening platform developed in the lab will identify novel drugs and bioactive compounds to interfere with pro-inflammatory signalling cascades for management of autoimmunity.

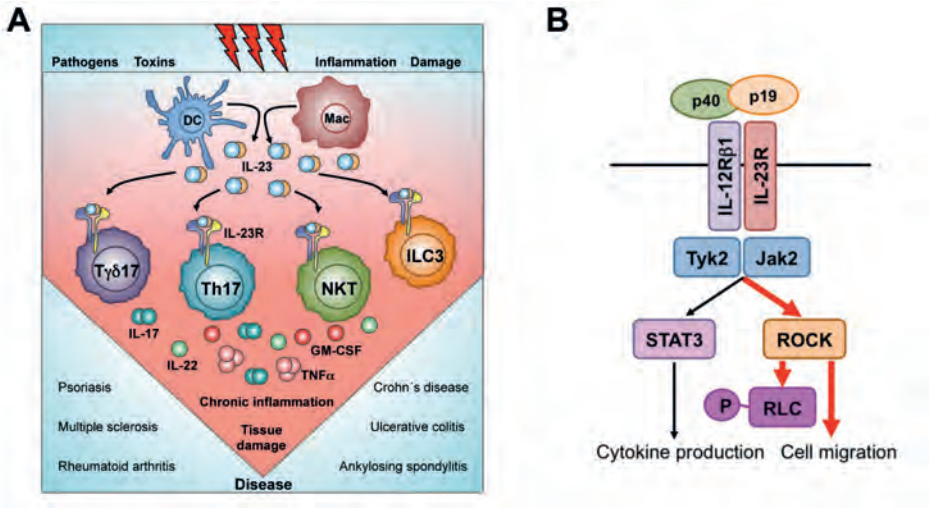


***Figure.*** *A. Role of IL-23 in chronic inflammatory diseases (Cells 9(9):2044, 2020). B. Lab working model for the role of IL-23 signaling pathway in inflammatory diseases (PLoS Biol 18(3):e3000646, 2020).*

## Publications

Álvarez-Salamero, C., Castillo-González, R., Pastor-Fernández, G., Mariblanca, I. R., Pino, J., Cibrian, D., and Navarro, M. N. (2020) IL-23 signaling regulation of pro-inflammatory T-cell migration uncovered by phosphoproteomics. *PLoS Biol.* **18**(3):e3000646.

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## IMMUNE SYSTEM DEVELOPMENT AND FUNCTION

# DEVELOPMENT OF THE HUMAN LYMPHO-HEMATOPOIETIC SYSTEM

***Principal Investigator:***
María Luisa Toribio García

***Postdoctoral Fellows:***
Marina García Peydró
Patricia Fuentes Villarejo
Sara González García

***Predoctoral Fellows:***
Alba Murcia Ceballos
Fátima Bayón Calderón
Carmela Cela Rodríguez
(September 2020)

***Undergraduate and Master Students:***
Isabel Torres Romón
(February-July 2019)
María Bajo Fernández
(September 2019-June 2020)
Carmela Cela Rodríguez
(September 2019-July 2020)

***Technician:***
Juan Alcain Sánchez

http://www.cbm.uam.es/toribiolab

## Research summary

Our group studies the cellular and molecular mechanisms that control the commitment and development along the T-cell lineage of hematopoietic progenitors seeding the human thymus. Our goal is to obtain mechanistic information about developmental pathways whose deregulation leads to T-cell acute lymphoblastic leukemia (T-ALL), with the final aim of identifying key molecular targets for therapeutic intervention. In the last years, we focused on the interleukin-7 receptor (IL-7R), a signaling pathway critically involved in T-cell development that is transcriptionally regulated by NOTCH1 and is commonly expressed in T-ALL. Given that NOTCH1 is a major driver of T-ALL, we developed a novel *in vivo* model of NOTCH1-induced human T-ALL and investigated the significance of IL-7R/IL-7 signaling in T-ALL pathogenesis. Our study showed that IL-7R is an early hallmark of human T-ALL that is expressed at sequential stages of T-ALL development. Using Il7r-deficient mice, and loss-of-function genetic approaches, we provided formal evidence that IL-7R signaling is essential for NOTCH1-induced T-cell leukemogenesis, and demonstrated that IL-7R expression is a functional biomarker of T-ALL cells with leukemia-initiating cell (LIC) potential, as impaired IL-7R function hampered engraftment and progression of patient-derived T-ALL xenografts. Notably, the key role of IL-7R in LIC activity and T-ALL tumor progression was extended to human B-cell ALL (B-ALL). These results have important therapeutic implications, highlighting the relevance that targeting normal IL-7R signaling may have in future therapeutic interventions, particularly for preventing T-ALL and B-ALL relapse.

Regarding the development of novel therapeutic strategies for acute leukemias, recent advances in targeted immunotherapies for B-cell malignancies involving CAR T cells have engendered unprecedented successful results. However, shared expression of targeted antigens by healthy and malignant T cells, is a main drawback of immunotherapies for T-ALL and other T-cell malignancies, and no effective targeted immunotherapies for these diseases exist. Based on our model of human T-ALL pathogenesis, our current studies are focused on identifying specific biomarkers of T-ALL LICs that are optimal targets for immunotherapy with CAR T cells. Several human T-cell engineering CAR strategies are being studied with the goal of developing optimal CAR T cells for T-ALL.

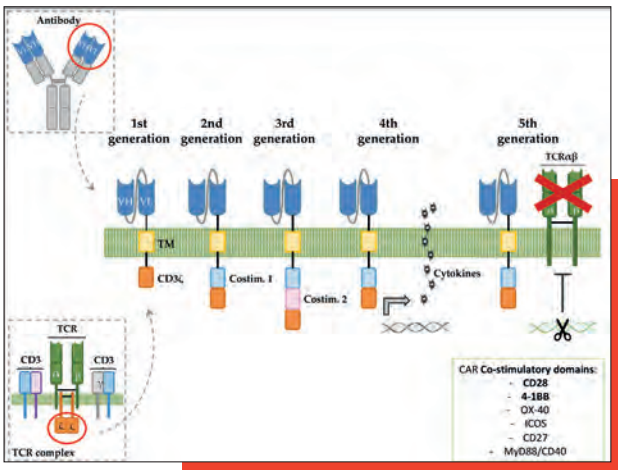


***Figure.*** *Schematic representation of different generations of chimeric antigen receptors (CARs). CARs are conformed by an extracellular antigen recognition domain derived from the VH and VL antibody regions, a transmembrane domain (TM) and an intracellular signaling domain from the CD3ζ component of the TCR (first-generation), or from CD3ζ in combination with signaling domains from one (second-generation) or two (third-generation) costimulatory molecules. Fourth-generation CAR T cells co-express the CAR together with a stimulatory cytokine, while fifth-generation CAR T cells express a second-generation CAR, but are genetically engineered to no longer express the endogenous TCR (and/or MHC molecules).*

## Publications


Bayón-Calderón, F., Toribio, M. L.*, and González-García, S. * (2020) Facts and Challenges in Immunotherapy for T-Cell Acute Lymphoblastic Leukemia. *Int J Mol Sci*. **21**(20), 7685.

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## IMMUNE SYSTEM DEVELOPMENT AND FUNCTION

# TCR DOMAINS IN T CELL DIFFERENTIATION AND (PATHO)PHYSIOLOGICAL AND THERAPEUTIC RESPONSES

***Principal Investigator:***
Hisse Martien van Santen

***Project Managers***
Estefanía Martínez
(until 31/10/2019)
Sofía Navarro
(as of 01/01/2020)

***Predoctoral Fellows:***
Ivaylo Balabanov
Lydia Horndler
(co-director Balbino Alarcón)

***Postdoctoral Fellows:***
Elena R. Bovolenta
(until 30/11/2019)

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## Research summary

T cells use their T cell receptor (TCR) to continuously scan Major Histocompatibility Complex (MHC) molecules presenting the immense repertoire of peptides derived from the proteome present in the host. Presentation of pathogen-derived peptides gives rise to beneficial immune responses, while responses against peptides derived from the host itself can lead to auto-immune manifestations. Our research focuses on understanding how the organization of the TCR into nanoclusters allows T cells to become activated upon recognition of only a few peptide-bound MHC molecules. We use transgenic mouse models to study the role of TCR nanoclusters during early T cell differentiation, where a selection process takes place that filters out potentially autoimmune T cells, and to understand how this organization impacts on the ability to mount protective memory and undesired autoimmune responses. We also develop approaches to improve T cell responses against cancer, focusing on the signaling domains of recombinant tumor-specific receptors. Our research shows that the identity and topology of TCR-derived signaling domains of these recombinant receptors determines their efficiency with respect to activating the T cells. These results have a direct implication for improving cellular cancer immunotherapy. They also provide new inroads into understanding the mechanisms underlying TCR function, which we are currently following-up upon. In a third, clinically-oriented project, we aim to generate CAR-T cells against Acute Myeloid Leukemia (AML). Cellular Immunotherapy against this type of cancer has been severely hampered by unacceptable toxicity against the patient's myeloid precursors that express the same antigen as the ones targeted on the AMLs. As no better target antigens have been defined, we are generating CAR-T cells that use a logical gate formed by an activating CAR recognizing the shared antigen on precursors and AMLs and an inhibitory immune receptor that recognizes a ligand only expressed by the myeloid precursors. If successful, this concept can be applied to other type of cancers using appropriate combinations of activating and inhibiting receptors.

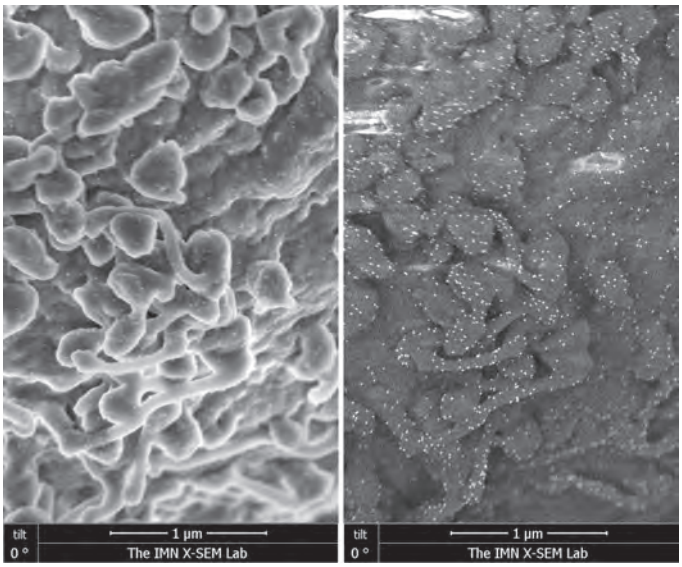



***Figure.*** *Scanning electron microscopy images (left panel: secondary electrons; right panel: back scatter electrons) of the Jurkat human T cell leukemia cell line labeled with an antibody against the TCR complex and 10nm-gold conjugated protein A (white dots). Images obtained in collaboration with Drs. Gonzalez and San Pablo of the Institute of Micro and Nanotechnology (CSIC).*

## Publications

Martín-Leal, A., Blanco, R., Casas, J., Sáez, M. E., Rodríguez-Bovolenta, E., de Rojas, I., Drechsler, C., Real, L. M., Fabrias, G., Ruíz, A., Castro, M., Schamel, W. W. A., Alarcón, B., van Santen, H. M., and Mañes, S. (2020) CCR5 deficiency impairs CD4+ T cell memory responses and antigenic sensitivity through increased ceramide synthesis. *EMBO J* **39**: e104749.

Mirones, I., Moreno, L., Patiño-García, A., Lizeaga, G., Moraleda, J. M., Toribio, M. L., Pérez-Martínez, A., en representación del Grupo de Inmunoterapia y Terapias Avanzadas de la Sociedad Española de Hematología y Oncología Pediátricas (26/24) (2020) Inmunoterapia con células CAR-T en hematooncología pediátrica. *An Pediatr (Barc)* **93**: 59.e1-59.e10.

Martínez-Riaño, A., Bovolenta, E. R., Boccasavia, V. L., Ponomarenko, J., Abia, D., Oeste, C. L., Fresno, M., van Santen, H. M.*, and Alarcon, B.* (2019) RRAS2 shapes the TCR repertoire by setting the threshold for negative selection. *J. Exp. Med*. **216**, 2427-2447. (*: co-corresponding authors).

## International projects / Research networks

- European Network on Anti-Cancer Immuno-Therapy Improvement by modification of CAR and TCR Interactions and Nanoscale Geometry (EN-ACTI2NG) 2017 – 2021. Coordinator: HM van Santen. Participation of 13 research groups and companies from Spain, Austria, Germany, The Netherlands and the United Kingdom; H2020-MSCA-ITN-2016, 721358; funding agency: European Commission.

UNIT

JOSÉ BERENGUER

# Microbes in Health and Welfare

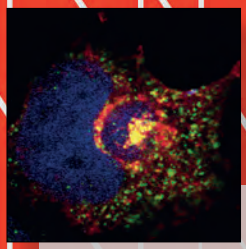
INTERACTIONS WITH THE ENVIRONMENT

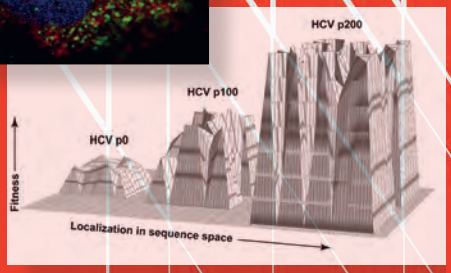
As evidenced with COVID-19, infectious diseases remain an important cause of human morbidity and mortality, and are responsible for huge economic losses all over the world. Also, cellular and acellular microbes are suitable models to gain insight in eukaryotic cell biology and also as tools and source of biotechnological products that improve economy and health. Accordingly, the overall scientific objective of the MHW unit is to study basic and applied aspects of the interactions of microbes with their hosts and the environment.

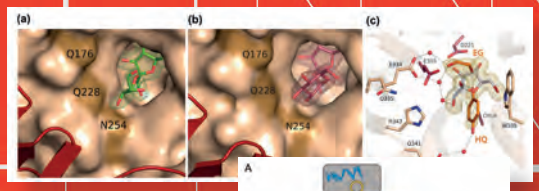
Regarding basic science, virologists of the unit have got evidences in the last two years of how individual proteins build a viral capsid lattice by high-speed atomic force microscopy. They have also implemented methods to understand capsid assembly and to study the evolutionary capacity of viruses, identified new viruses in the oral cavity, and studied the way viruses evade the host immune system. On the other hand, the bacteriologists of the unit have studied environmental adaptation like the discovery of microbial life in a polyextreme environment where life was though impossible, or the mechanisms for cold adaptation in a bacterial pathogen. In addition, studies on lateral gene transfer have allowed the description of new surface exclusion system and regulators interactions controlling conjugation and natural competence in a model bacterium, and revealed the existence of unusual DNA-scavenging genetic mobile elements and new mechanism of defense against invading DNA in extreme thermophiles.

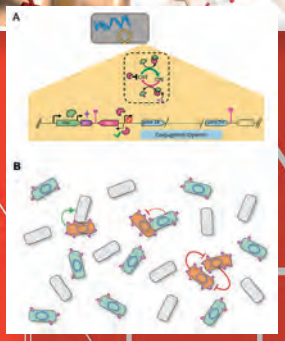
On more applied fields, biomedical aspects such as the use of modified viruses as tumor lytic agents, and the development of new vaccines, either attenuated viral forms or synthetic peptide vaccines against animal and human viruses are being addressed. New therapeutic strategies focused on the discovery of HIV antivirals, the antiviral potential of innate immune inducers and cell-targeted compounds, the exploration of a wide spectrum antiviral strategy based on increased mutational rates, or the development of new molecular dynamics methods for the in-silico discovery of cell division inhibitors, have been also relevant achievements within the last two years.

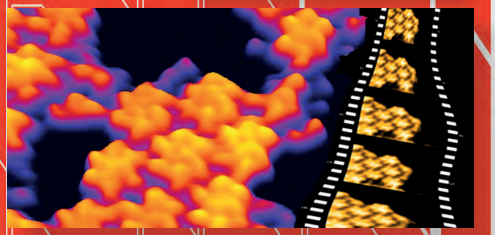
Finally, the unit has been also involved in biotechnology-focused research with applications also outside the biomedical field, like new thermostable reverse transcriptase variants, new thermostable enzyme variants to obtain building blocks for the (bio)chemical synthesis, or the description of new and improved glycolytic enzymes for the production of sugar derivatives and for the transformation of chitin wastes into high valuable products for the food and medical fields.

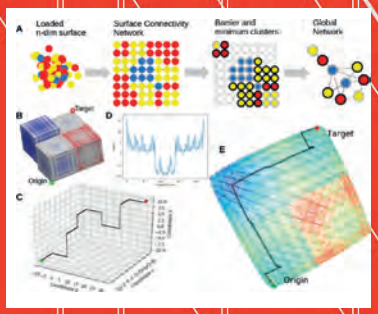







**JOSÉ M. ALMENDRAL**

ssDNA VIRUS EVOLUTION, PATHOGENESIS, AND ANTI-CANCER POTENTIAL

**RICARDO AMILS**

MOLECULAR ECOLOGY OF EXTREME ENVIRONMENTS

**JOSÉ BERENGUER**

BIOTECHNOLOGY ANF GENETICS OF EXTREME THERMOPHILES

**ESTEBAN DOMINGO / CELIA PERALES**

GENETIC VARIABILITY OF RNA VIRUSES

**MARÍA FERNÁNDEZ LOBATO**

YEAST ENZYME BIOENGINEERING TO GENERATE BIOACTIVE COMPOUNDS

**MAURICIO G. MATEU**

VIRUS ENGINEERING AND NANOBIOTECHNOLOGY

**PAULINO GÓMEZ-PUERTAS**

MOLECULAR MODELLING

**AURELIO HIDALGO**

ULTRAHIGH-THROUGHPUT DISCOVERY AND ENGINEERING OF ENZYMES FOR BIOTECHNOLOGICAL APPLICATIONS (HT DISCOVERY LAB)

**WILFRIED J.J. MEIJER**

PLASMID CONJUGATION IN GRAM-POSITIVE BACTERIA

**LUIS MENÉNDEZ ARIAS**

HUMAN IMMUNODEFICIENCY VIRUS REPLICATION AND ANTIRETROVIRAL THERAPY

**MARÍA GRACIELA PUCCIARELLI**

RNA-BASED CONTROL OF *LISTERIA* ADAPTATION TO STRESS AND VIRULENCE

**YOLANDA REVILLA**

VIRUS-CELL INTERACTION AND VACCINES DEVELOPMENT: THE ASFV MODEL

**MARGARITA SÁIZ**

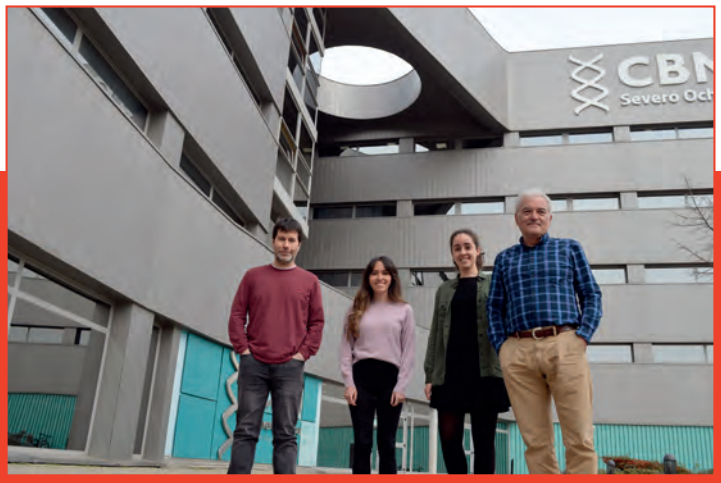
MODULATION OF INNATE IMMUNE RESPONSES BY VIRAL PROTEASES AND RNAS DERIVED FROM VIRAL GENOMES. BIOTHERAPEUTIC APPLICATION

**FRANCISCO SOBRINO**

NEW STRATEGIES FOR PREVENTION AND CONTROL OF VIRAL DISEASES: FOOT-AND-MOUTH DISEASE VIRUS AS A MODEL

## MICROBES IN HEALTH AND WELFARE

# ssDNA VIRUS EVOLUTION, PATHOGENESIS, AND ANTI-CANCER POTENTIAL



***Principal Investigator:***
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***Scientific Staff:***
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***Postdoctoral Fellow:***
Luisa F. Bustamante-Jaramillo
(until February 28th, 2019)

***Predoctoral Fellows:***
Tania Calvo López
(until February 28th, 2019)
Violeta Lara Aguilar
(since March 1st, 2020)

***Technician:***
Josefa González-Nicolás
(until August 1st, 2019)

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## Research summary

We are pursuing the study of ssDNA virus biology, particularly virus members of the *Parvoviridae*, toward developing effective novel anticancer therapeutics as well as to better understand their pathogenicity and evolutionary patterns. In our most recent efforts, the parvovirus minute virus of mice (MVM) was targeted to the tumour vasculature for oncolytic purposes by engineering an antibody foot-print located at the three-fold axis of the capsid (see Figure 1) with heterologous peptides blocking the vascular endothelial growth factor (VEGF). Infectious chimeric viruses exposing these peptides on the capsid surface induced anti-native VEGF antibodies, contributed to better understand MVM assembly, and illustrated the evolutionary capacity of ssDNA viruses to overcome engineered structural restrictions. Our immediate research aims will extend this approach to other functional domains of MVM capsids, and the resulting VEGF-chimeric viruses will be tested for infectivity and tropism to human cancer cells. Other experimental approaches are being conducted to understand the molecular basis of the newly found heterogeneous innate responses that primary human glioblastoma cells mount to block parvovirus infection. We are further attempting physical and chemotherapeutic treatments to overcome these innate responses. In addition, a significant part of our research has recently focused to explore different evolutionary strategies to eventually develop viruses with improved anticancer properties by optimizing their cytotoxicity and replication capacity in human tumour cells.

Metagenomic studies. We are also interested in understanding viral assemblages in the human oral cavity. By using metagenomics, we have sequenced the genome of new human viruses from the *Anelloviridae, Papillomaviridae* and *Redondoviridae* families as well as hundreds of new bacteriophages. The bioinformatic prediction of their host interactions will contribute to a better understanding of this microbial ecosystem.

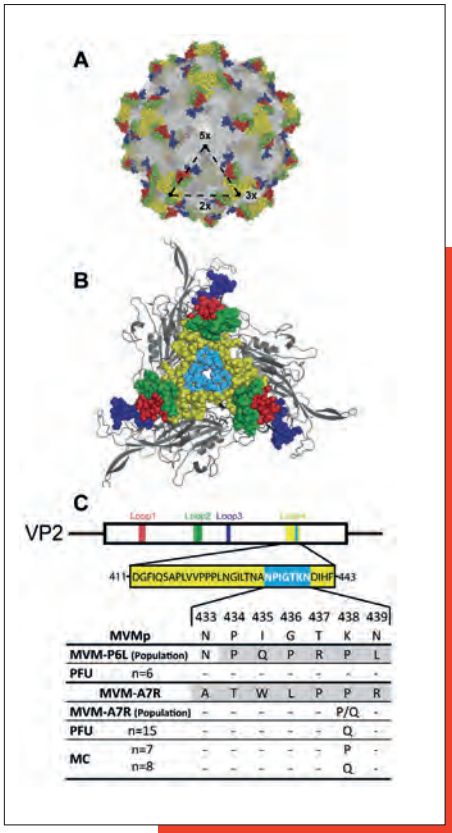


***Figure.*** *A. Icosahedral structure of the MVMp capsid with the spike shown as spheres coloured at loop 1 (red), loop 2 (green), loop 3 (dark blue), and loop 4 (yellow). The locations of the 5x, 3x and 2x symmetry axes are highlighted. B. Closer top view of the topology of the loops conforming the MVM spike. Residues substituted by VEGF blocking peptides within loop 4 are shown in blue. C. Genetic analysis of chimeric virus stability across loop 4. The residues substituted by the VEGF binding peptides are highlighted. The average sequence of chimeric virus populations was obtained after two weeks in culture. PFU: plaque forming unit. MC: molecular clone.*

## Publications

Grueso, E., Sánchez-Martínez, C., Calvo-López, T., de Miguel, F. J., Blanco-Menéndez, N., Fernández-Estévez, M., Elizalde, M., Sánchez, J., Kourani, O., Martin, D., Tato, A., Guerra, M., Andrés, G., and Almendral, J. M. (2019) Antiangiogenic vascular endothelial growth factor-blocking peptides displayed on the capsid of an infectious oncolytic parvovirus: assembly and immune interactions. *Journal of Virology* **93**(19):e00798-19.

## Doctoral Theses

**Marcos Parras Moltó** (2019) “Estudio Metagenómico de la comunidad de virus y de su interacción con la microbiota en la cavidad bucal humana”. Director: Alberto López-Bueno. Universidad Autónom de Madrid.

## MICROBES IN HEALTH AND WELFARE

# MOLECULAR ECOLOGY OF EXTREME ENVIRONMENTS


***Principal Investigator:***
Ricardo Amils Pibernat

***Postdoctoral Fellows:***
Cristina Escudero (until september 2019)
Kary G. Haro Pérez

***Predoctoral Fellows:***
José Manuel Martínez
Enrique Marín
Mahshid Sedghi

***Technician:***
Nuria Rodríguez González

***Undergraduate and Master Students:***
Adrián Martínez (2020)
Guillermo Mateos (2020)
Andrea Silva (2020)
Sofía de Francisco (2019)
Gonzalo Ibáñez (2019)

***Visiting Scientists:***
David Fernández-Remolar (Space and Science Institute, Macao University)
Erik Zetller (NIOZ, Netherlands)
Linda Amaral (NIOZ, Netherlands)

http://www.cbm.uam.es/ramils

## Research summary

Geomicrobiology of the Iberian Pyrite Belt (IPB): subsurface characterization of the bioreactor responsible of the extreme conditions existing in the Río Tinto basin. This work is done in collaboration with the group of professor J.L. Sanz from the Department of Molecular Biology (UAM) and different scientists from the Centro de Astrobiología. The development of this objective allowed to identify the microorganisms involved in the functional operation of the H, C, N, S and Fe cycles in the deep subsurface of the IPB, their isolation and genomic and physiological characterization to evaluate their capacity to oxidize metal sulfides under strict anaerobic conditions.

Acidophiles: conventional microbial ecology, molecular ecology, molecular biology and biotechnology of extreme acidic environments (biomining, specific metal sequestering, biomineralization and phytoremediation). This objective is mainly devoted to the exploration of the biotechnological applications of acidophiles inhabiting the Tinto basin.

Characterization of extreme environments of astrobiological interest: Río Tinto and Iberian Pyrite Belt (Mars analogues), Uyuni Salt Lake (Bolivia, Europa analogue), Dallol in the Danakil depression (Ethiopia, Mars analogue). This objective aims to characterize different extreme environments to evaluate the limits of life and the habitability in different planets and moons from the Solar System and from other extrasolar systems.

***Figure.*** *Dallol, a polyextreme environment.*

## Publications

Ramos-Barbero, M. D., Martínez, J. M., Almansa, C., Rodríguez, N., Villamor, J., Gomariz, M., Escudero, C., Rubin, S., Antón, J., Martínez-García, M., and Amils R. (2019) Viral seasonal dynamics in the chaotropic salt lake Salar de Uyuni. *Environ. Microbiol.*, **21**(6): 2029-2042.

Mariñán, N., Martínez, J. M., Leandro, T., and Amils, R. (2019) Draft genome sequence of *Rhodoplanes* sp. strain T2.26MG-98, isolated from 492.6 meter deep on the subsurface of the Iberian Pyrite Belt. *Microbiol. Resour. Announc*. **8**: e00070.

Gómez, F., Cavalazzi, B., Rodríguez, N., Amils, R., Ori, G. G., Olsson-Francis, K., Escudero, C., Martínez, J. M., and Mirtus, H. (2019) Ultra-small microorganisms in the polyextreme conditions of the Dallol volcano, Northen Afar, Ethiopia*. Sci. Rep*., **9**: 7907e.

García-Descalzo, L., Parro, V., García-Villadangos, M., Cockell, C. S., Moissl-Eichinger, C., Perras, A., Rettberg, P., Beblo-Vranesevic, K., Bohmeier, M., Rabbow, E., Westall, F., Gaboyer, F., Amils, R., Malki, M., Marteinsson, V., Vannier, P., Ehrenfreund, P., Monaghan, E., Riedo, A., Cabezas, P., Walter, N., and Gómez Gómez, F. (2019) Microbial markers profile in anaerobic Mars analogue environments using the LDChip (Life Detector Chip) antibody microarray core of the SOLID (Signs Of Life Detector) platform. *Microorganisms*, **7**, 365.

Rodríguez-Robles, E., Martínez, J. M., Leandro, T., and Amils, R. (2019) Draft genome sequence of *Brevundimonas* sp. strain T2.26MG-97, isolated from a rock core simple from 492.6 m eep on the subsurface of the Iberian Pyrite Belt. *Microbiol. Resour*. *Announc*., **8**(32): e00375-19.

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Abramov, S. M., Tejada, J., Grimm, L., Schädler, F., Bulaev, A., Tomaszewski, E. J., Byrne, J. M., Straub, D., Thorwarth, H., Amils, R., Kleindienst, S., and Kappler, A. (2020) Role of biogenic Fe(III) minerals as a sink and carrier of heavy metals in the Río Tinto river, Spain. *Science Total Environment* **718**: 137294.

de Francisco-Polanco, S., Martínez, J. M., Leandro, T., and Amils, R. (2020) Draft genome sequence of *Shewanella* sp. strain T2.3D-1.1 isolated from 121.8 meters deep in the subsurface of the Iberain Pyrite Belt. *Microbiol. Resour. Announ*. **9**: e00190-20.

Escudero, C., del Campo, A., Ares, J. M., Sánchez, C., Martínez, J. M., Gómez, F., and Amils, R. (2020) Analysis of microorganism-mineral interaction in the Iberian Pyrite Belt subsurface: the *Acidovorax* case. *Frontiers Microbiology*, **11**: 572104.

Redrejo, M. J., Friedrich E. J., Rodríguez, N., Amils, R., and Fernández-Ruiz, R. (2020) Suspension assisted analysis of sulfur in petroleum coke by Total-reflection X-Ray Fluorescence. *Spectrochimica Acta Part B* **174**: 105997.

Martínez, J. M., Escudero, E., Leandro, T., Mateos, G., and Amils, R. (2020) Draft genome of *Psudomonas* sp. strain T2.31D-1 isolated from a drilling core simple at 414 meters below surface in the Iberian Pyrite *Belt. Microb. Res. Announc*.**10**: e01165-20.

Book chapters:

Beblo-Vranesevic, K., Bohmeier, M., Schleumer, S., Rabbow, E., Perras, A., Moissi-Eichinger, C., Schwendner, P., Cockell, C. S., Vannier, P., Marteinsson, V. T., Monoghan, E. P., Riedo, A., Ehrenfreund, P., García Descalzo, L., Gómez, F., Malki, M., Amils, R., Gaboyer, F., Hickman-Lewis, K., Westall, F., Cabezas, P., Walter, N., Rettberg, P. (2020) Impact of simulated Martian conditions on (facultative) anaerobic bacterial strains from different Mars analogue sites. In “Astrobiology: Current, Evolving, and Emerging Perspectives”. Current Issues in Molecular Biology Vol 38, Caister Academic Press, pp 103-121.

Amils, R., Fernández-Remolar, D. (2020) Río Tinto: an extreme acidic environmental model of astrobiological interest. In “Extremophiles as Astrobiological models”, Seckbach, J. and Stan-Lotter, H. (eds.), Wiley and Scrivener (Beverley, MA, USA), pp 21-44.

## Doctoral Theses

**Jose Jordan** (2019) Geomicrobiología de revestimiento de rocas en un ambiente ácido extremo: Río Tinto. Universidad Autónoma de Madrid. Felipe Gómez and Ricardo Amils.

## International projects / Research networks

- The physicochemical nature of water on early Mars (MarsFirstWater). ECR Consolidator Grant 818602 (2019-2023).

- Red Nacional de Microorganismos Extremófilos (RedEx, 2019, 2020).

## MICROBES IN HEALTH AND WELFARE

# BIOTECHNOLOGY ANF GENETICS OF EXTREME THERMOPHILES

***Principal Investigator:***
José Berenguer Carlos

***Scientific Staff:***
Mario Mencía Caballero
Alba Blesa Esteban

***Postdoctoral Fellows:***
Nieves García Quintans
(until 30-11-2019)
Dione Sanchez Hevia
(until 31-12-2019)

***Predoctoral Fellows:***
Ignacio Baquedano Mozos
(until 31-08-2019)
Carlos Verdú Cano
Mercedes Sanchez Costa

***Technician:***
Elena Trapero Jiménez
(until 15-05-2020)

***Undergraduate and Master Students:***
Anna Yasinskaya

http://www.cbm.uam.es/jberenguer

## Research summary

The main objective of our group during this period has been to analyse the mechanisms of DNA transfer and those acting as defence barriers in thermophilic bacteria that could render biotechnological applications.

DNA acquisition and exchange is enhanced in thermal environments due to the strong selective factor appointed by high temperatures against the maintenance of large genomes. For *Thermus thermophilus* (Tth), this selection has led to the evolution of the most efficient natural competence apparatus (NCA) so far described. In addition, Tth can exchange DNA by direct cell contacts using “transjugation”, a process in which a DNA donation apparatus (DDA) allows the ejection of DNA from a “donor” strain with the concomitant incorporation by a competent recipient mate through its NCA. The main focus of our research in the last two years has been the analysis of the mechanism underlying transjugation, and those involved in protection against the integration external DNA in the genome.

The DDA is encoded by a mobile genetic element (ICETh1). ICETh1 functions as a DNA-scavenging element, allowing its host to capture DNA from mating partners generating genomic mosaicity among the donor-derived progeny (Fig1A). To be efficient, ICETh1 has to excise from the genome, using a excisionase encoded by other mobile element (ICETh2), showing the coevolution of both elements for transjugation. ICETh2 encodes a DNA primase-polymerase (PrimPol), an enzyme already commercialized for isothermal amplification. We found that PrimPol deletion increases the transformability of the cells by orders of magnitude (Fig1), a defensive role that is inactive against DNA scavenged from mating pairs.

This differential protective activity of PrimPol is similar to that provided by ThAgo a Tth homologue of the human Argonaute. It has been found that ThAgo uses DNA-DNA interference to cleave exogenous DNA entering the cells by transformation, a mechanism reminiscent to the RNA-DNA interference used by the Cas9/12 CRISPR proteins, that recently has emerged as a new DNA edition tool. In this respect, we have characterized a less thermophilic prokaryotic Ago protein (CbcAgo) for its putative use as gene edition tool at 37ºC.

Currently, and because the mechanisms to generate the ssDNA guides used by ThAgo are far from understood, we are studying the putative involvement of PrimPol and other proteins in this process *in vivo*.

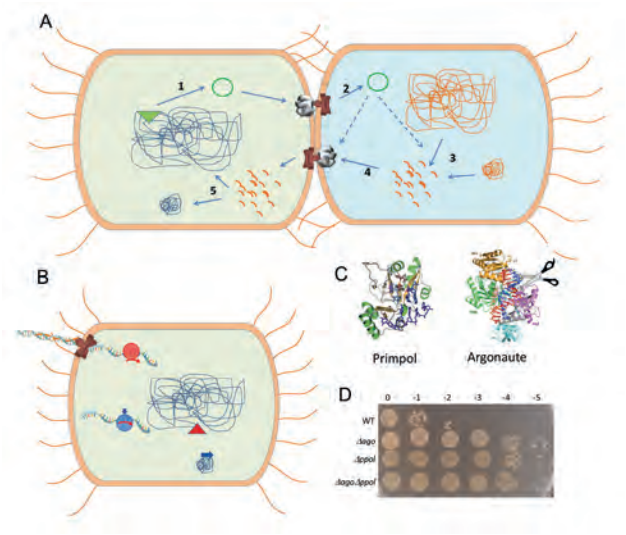


***Figure.*** *Gene scavenging and defence in T. thermophilus. A) Scheme showing gene ICETh1-encoded (Geen trinagle) DNA scavenging: excision from the chromosome and transfer to the mating pair (1,2); fragmentation of the mate genome by the nuclease it encodes (3), transfer back to the donor of DNA fragments through the ICETh1 encoded DDA (4); and further fragments integration into the genome (5), generating mosaicity in the progeny. B) PrimPol (red ball), encoded by ICETh2 (red triangle), synthesizes primers on ssDNA entering cells through the NCA, that could be used by the megaplasmid-encoded ThAgo (blue ball) as guides to cleave the invading DNA. C) Structural models for PrimPol and ThAgo (taken from Nat Commun. 7:13296 and PNAS 11:652). D) Decimal dilutions of cultures of the wild type, and single and double deletion mutants in the PrimPol and ThAgo genes (labelled) transformed with identical amounts of a plasmid conferring thermostable resistance to kanamycin.*

## Publications


Sanchez, M., Blesa, A., Sacristan-Horcajada, E., and Berenguer, J. (2019) Complete genome sequence of *Mycolicibacterium hassiacum* DSM 44199. *Microbiol. Resour. Announc.* **8**(4)e01522-18.

Verdú, C., Sanchez, E., Ortega, C., Hidalgo, A., Berenguer, J., and Mencía, M. (2019) A modular vector toolkit with a tailored set of thermosensors to regulate gene expression in *Thermus thermophilus*. *ACS Omega*. **4**, 14626-14632.

Rocha-Martin, J., Sanchez-Murcia, P. A., Lopez-Gallego, F., Hidalgo, A., Berenguer, J., and Guisán, J. M. (2019) Functional characterization and structural analysis of NADH oxidase mutants from *Thermus thermophilus* HB27: Role of residues 166, 174, and 194 in the catalytic properties and thermostability. *Microorganisms.* **7**(11), 515.

Garcia-Quintans, N., Bowden, L., Berenguer, J., and Mencía, M. (2019) DNA interference by a mesophilic argonaute protein, Cbcago. F1000Res. 8, 321.

Blesa, A., Sanchez, M., Sacristan-Horcajada, E., Gonzalez-de la Fuente, S., Peiro, R., and Berenguer, J. (2019) Into the *Thermus* mobilome: Presence, diversity and recent activities of insertion sequences across *Thermus spp*. *Microorganisms*. **7**(1), 25.

Acosta, J., Del Arco, J., Del Pozo, M. L., Herrera-Tapias, B., Clemente-Suarez, V. J., Berenguer, J., Hidalgo, A., and Fernandez-Lucas, J. (2020) Hypoxanthine-guanine phosphoribosyltransferase/adenylate kinase from zobellia galactanivorans: A bifunctional catalyst for the synthesis of nucleoside-5'-mono-, di- and triphosphates. *Front. Bioeng. Biotechnol*. **8**, 677.

Baquedano, I., Mencía, M., Blesa, A., Burrus, V., and Berenguer, J. (2020). ICETh1 & ICETh2, two mobile genetic elements coordinated in *Thermus thermophilus* transjugation. *Environ. Microbiol.* **22**, 158–169.

Blesa, A., Baquedano, I., Gonzalez-de la Fuente, S., Mencía, M., and Berenguer, J. (2020) Integrative and conjugative element ICETh1 functions as a pangenomic DNA capture module in *Thermus thermophilus. Microorganisms*. **8**(12),2051,1-14.

Blesa, A., and Berenguer, J. (2020) Methods to identify and analyze vesicle-protected DNA transfer. *Methods Mol. Biol*. **2075**, 209-221.

Consolati, T., Bolivar, J. M., Petrasek, Z., Berenguer, J., Hidalgo, A., Guisan, J. M., and Nidetzky, B. (2020) Intraparticle ph sensing within immobilized enzymes: Immobilized yellow fluorescent protein as optical sensor for spatiotemporal mapping of ph inside porous particles. *Methods Mol. Biol*. **2100**, 319-333.

Mate, D. M., Rivera, N. R., Sanchez-Freire, E., Ayala, J. A., Berenguer, J., and Hidalgo, A. (2020) Thermostability enhancement of the *Pseudomonas fluorescens* esterase I by *in vivo* folding selection in *Thermus thermophilus. Biotechnol. Bioeng*. **117**, 30-38.

Mencía, M. (2020) The archaeal-bacterial lipid divide, could a distinct lateral proton route hold the answer? *Biol. Direct*.**15**, 7.

Donsbach, P., Yee, B. A., Sanchez-Hevia, D., Berenguer, J., Aigner, S., Yeo, G. W., and Klostermeier, D. (2020) The *Thermus thermophilus* DEAD-box protein Hera is a general RNA binding protein and plays a key role in tRNA metabolism. *RNA.* **26**,1557-1574.

Garcia-Quintans, N., Baquedano, I., Blesa, A., Verdu, C., Berenguer, J., and Mencía, M. (2020) A thermostable DNA primase-polymerase from a mobile genetic element involved in defence against environmental DNA. *Environ. Microbiol.* **22**, 4647-4657.

Sanchez-Costa, M., Blesa, A., and Berenguer, J. (2020) Nitrate respiration in *Thermus thermophilus* NAR1: From horizontal gene transfer to internal evolution. *Genes (Basel)* **11**(11),1038,1-16


## Awards and recognition

- José Berenguer: Editor in Chief of International Microbiology, the journal of the Spanish Society for Microbiology (Ed, Springer-Nature group).

## Doctoral Theses

**Ignacio Baquedano Mozos** (2019). ICETh1 & ICETh2, two mobile genetic elements coordinated in *Thermus thermophilus* transjugation. Universidad Autónoma de Madrid. José Berenguer & Mario Mencía. International mention.

**Mercedes Sanchez Costa** (2020). Estudio y desarrollo de nuevas herramientas para la exploración de ambientes extremos. José Berenguer & Aurelio Hidalgo.

## International projects / Research networks

- MetaFluidics H2020-LEIT-BIO-2015-1 GA(685474-2). Coordinator: A. Hidalgo.

- National Network of Extremophilic Microorganisms REDEX2019. Coordinator: J. Gonzalez-Grau.

## MICROBES IN HEALTH AND WELFARE

# GENETIC VARIABILITY OF RNA VIRUSES

***Principal Investigators:***
Esteban Domingo Solans
Celia Perales Viejo

***Predoctoral Fellows:***
Carlos García Crespo
(since July 2019)
Victoria Castro Illana
Brenda Martínez González
(since January 2020)
Rebeca Lobo Vega
(since February 2020)

***Undergraduate and Master Students:***
Brenda Martínez González
(November 2018-July 2019)
Lucía Vazquez Sirvent
(October 2019-September 2020)
Miriam Herráez Moncusí
(since November 2020)

***Technicians:***
Ana Isabel de Ávila Lucas
Isabel Gallego Jiménez
María Eugenia Soria Benito

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## Research summary

Four related aspects have focused the work of our group: lethal mutagenesis as a broad-spectrum antiviral strategy, viral population dynamics in constant biological environments, new mechanisms of antiviral resistance, and comparison of residue conservation at different levels of analysis. The main model virus used has been hepatitis C virus (HCV), with further analyses of earlier results obtained with foot-and-mouth disease virus (FMDV). The projects have been carried in collaboration with the group of Dr. Celia Perales at Fundación Jiménez Díaz (Madrid). The main developments and implications of our research have been:

- Demonstration of a new mechanism of antiviral synergy between the two lethal mutagens favipiravir and ribavirin, consisting on the two analogues having a different preference for the HCV genome sites where the mutations that drive the virus towards extinction are introduced. This enhances prospects of broad-spectrum antiviral treatments for RNA viruses.

- Identification of mutational waves (continuous changes in mutation frequency) during prolonged HCV replication in cell culture that suggests persistent population disequilibrium with supportive evidence also with FMDV. This has led to the proposal of broad diversification as a new positive selection mechanism (Figure).

- Discovery of a new group of amino acid substitutions in HCV from infected patients who fail current therapies, and that confer resistance to multiple treatments in absence of the standard, treatment-specific, resistance-associated substitutions. This implies that HCV can find alternative mutational pathways for antiviral resistance.

- Incongruence of nucleotide and amino acid conservation scores calculated from HCV mutant spectra, and those derived from the corresponding consensus sequences or from sequence alignments in the Los Alamos National Laboratory data bank. The disagreement applies to HCV quasispecies in cell culture and in infected patients, thus complicating considerably current research whose aim is to develop universal vaccines or pan-genotypic antiviral agents.

The teams that have collaborated to these results are those of Jordi Gómez, Carlos Briones, Alejandro Brun, Ignacio Gadea, Josep Quer, Josep Gregori, and Nuria Verdaguer.

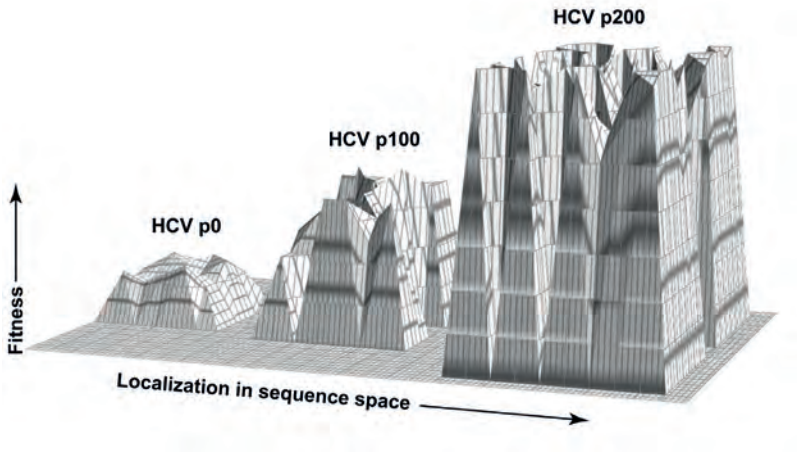



***Figure.*** *A mountain-valley depiction of broadly diversifying selection of hepatitis C virus.*

## Publications

Domingo, E. and Perales, C. (2019) Viral quasispecies. *PLoS Genetics*. **15**(10), e1008271.

Domingo, E. and Perales, C. (2019) Virus evolution. *Encyclopedia of Microbiology.* 4th edition, 558-568.

Ariza-Mateos, A., Briones, C., Perales, C., Domingo, E., and Gómez, J. (2019) The archaeology of coding RNA. *Ann N Y Acad Sci.* **1447**(1):119-134.

Domingo, E., de Avila, A. I., Gallego, I., Sheldon, J. and Perales, C. (2019) Viral fitness: history and relevance for viral pathogenesis and antiviral interventions. *Pathog Dis*. **77**(2): ftz021.

Altan-Bonnet, N., Perales, C., and Domingo, E. (2019) Extracellular vesicles: Vehicles of en bloc viral transmission. *Virus Res*. **265**:143-149.

Borrego, B., de Avila, A. I., Domingo, E., and Brun, A. (2019) Lethal Mutagenesis of Rift Valley Fever Virus Induced by Favipiravir. *Antimicrob Agents Chemother* **63**:e00669-19.

Gallego, I., Soria, M. E., Gregori, J., de Ávila, A. I., García-Crespo, C., Moreno, E., Gadea, I., Esteban, J., Fernández-Roblas, R., Esteban, J. I., Gómez, J., Quer, J., Domingo, E., and Perales, C. (2019) Synergistic lethal mutagenesis of hepatitis C virus. *Antimicrob Agents Chemother* **63**:e01653-19.

Perales, C., Gallego, I., de Ávila, A. I., Soria, M. E., Gregori, J., Quer, J., Domingo, and E. (2019) The increasing impact of lethal mutagenesis of viruses. *Future Med. Chem*. **11**(13), 1645–1657.

Gallego, I., Soria, M. E., García-Crespo, C., Chen, Q., Martínez-Barragán, P., Khalfaoui, S., Martínez-González, B., Sánchez-Martin, I., Palacios-Blanco, I., de Ávila, A.I., García-Cehic, D., Esteban, J. I., Gómez, J., Briones, C., Gregori, J., Quer, J., Perales, C., and Domingo, E. (2020) Broad and dynamic diversification of infectious hepatitis C virus in a cell culture environment. *J. Virol*. **94**:e01856-19.

Chen, Q., Perales, C., Soria, M. E., García-Cehic, D., Gregori, J., et al. (2020) Deep-sequencing reveals broad subtype-specific HCV resistance mutations associated with treatment failure. *Antiviral Research* **174**: 104694.

Domingo, E., Soria, M. E., Gallego, I., de Ávila, A. I., García-Crespo, C., Martínez-González, B., Gómez, J., Briones, C., Gregori, J., Quer, J., and Perales, C. (2020) A new implication of quasispecies dynamics: broad virus diversification in absence of external perturbations. *Infection, Genetics and Evolution*. **82**:104278.

Soria, M. E., García-Crespo, C., Martínez-González, B., Vazquez-Sirvent, L., Lobo-Vega, R., de Ávila, A. I., Gallego, I., Chen, Q., García-Cehic, D., Llorens-Revull, M., Briones, C., Gómez, J., Ferrer-Orta, C., Verdaguer, N., Gregori, J., Rodríguez-Frías, F., Buti, M., Esteban, J., Domingo, E., Quer, J., Perales, C. (2020) Amino acid substitutions associated with treatment failure of hepatitis C virus infection. *J. Clin. Microbiol.* **58**:e01985-20.

García-Crespo, C., Soriam M, E., Gallego, I., De Ávila A. I., Martínez-González, B., Vázquez-Sirvent, L., Gómez, J., Briones, C., Gregori, J., Quer, J., Perales, C., and Domingo, E. (2020) Dissimilar conservation pattern in hepatitis C virus mutant spectra, consensus sequences, and data banks. *J. Clin. Med*., **9**, 3450

## Awards and recognition

- Member of the Spanish Academy of Science (Real Academia de Ciencias Exactas, Físicas y Naturales) (since 2012). Vicepresident since 2019.

- International member of National Academy of Sciences (USA) (2020).

- Associated editor Virus Research, since 2012.

## International projects / Research networks

- Combination of antiviral agents for SARS-CoV-2. CSIC-COV19-014. Funding: Agencia Estatal CSIC. (2020-2021).

- Platform for the development of strategies for animal health. PLATESA2, S2018/BAA-4370. Funding: Comunidad de Madrid/ FEDER. (2019-2022).

- Study of molecular sequelae in the host cell following eradication of hepatitis C virus in cell culture. Funding: Ministerio de Economía, Industria y Competitividad. SAF2017-87846-R. (2018-2020).

- Search of cDNA of hepatitis C virus. Funding: Ministerio de Ciencia, Innovación y Universidades. BFU2017-91384-EXP. (2018-2020). Coordinator: Celia Perales.

- The group is a member of a network for the study of hepatic and digestive diseases. [Red Centro de Investigación Biomédicas en Red de Enfermedades Hepáticas y Digestivas (CIBERehd); group CB06/04/0086].

## MICROBES IN HEALTH AND WELFARE

# YEAST ENZYME BIOENGINEERING TO GENERATE BIOACTIVE COMPOUNDS

***Principal Investigator:***
María Fernández Lobato

***Scientific Staff:***
Miguel Remacha Moreno

***Predoctoral Fellows:***
David Piedrabuena Estrada
Peter Elias Kidibule
Zoran Merdzo Kunovac
Martín García González
Marina Minguet Lobato
Eglè Narmontaite

***Technicians:***
Mª Asunción Martín Redondo (to 2019)
Gloria Roa Felipe (to 2019)

***Undergraduate and Master Students:***
Pablo Ortega Martínez (2019)
Miguel Arribas Tiemblo (2019)
Ignacio Sastre Cejudo (2020)
Dariia Radchenko (2020)
Paula Ramiro Martínez (2020)

http://www.cbm.uam.es/MFernandezLobato

## Research summary

We work with microorganisms, mainly fungi and yeasts, producing bioactive compounds for application in functional and nutraceutical food. We try to connect the generation of knowledge to the development of biotechnological applications. Basically we focus on the characterization of enzymes producing new compounds, the analysis of their structural-functional determinants, their operational improvement using molecular biology tools and in the purification and characterization of the new molecules produced that may have potential biological activity of industrial utility. We have patented in different countries the industrial applicability of some of the proteins characterized and designed methods to simplify protocols as the biocatalysts attachment to solid supports.

During the last few years, we have continued with the characterization and study of proteins from non-conventional yeast showing glycosyltransferase activity, applicable in the production of new heterooligosaccharides and glycoconjugate derivatives that may have prebiotic or antioxidant properties as well as fungal chitinases producing chitooligosaccharides with bioactive properties. In general, most of the characterized proteins are glycosyl hydrolases (GH) structurally included in families GH32, 31, 13 or 18. In fact, we resolved the 3D structure of the first yeast protein including in family GH32, assigned a function to the beta-sandwich domain that is present in all members of this family and proved that the oligomerization is directly involved in the substrate recognition and specificity. Recently we have characterized new enzymes, obtained numerous protein variants showing increased or altered pattern of synthesized products, isolated and characterized new products, optimized biosynthetic reactions, and deciphered the molecular specificity of some phenolic compounds acting on GHs. We have also been working on the production and characterization of chitinolytic derivatives with antioxidant or antibacterial properties that could be used in the functionalization of new materials. We intend to extend our study to hydrolases included in other structural families acting on industrial wastes, increase and modify the biosynthetic activity of the enzymes studied, design more stable biocatalysts, scale up the enzyme production and the products generated, as well as validate the biological activity of the molecules obtained. Objectives included in those of the Glicoenz consortium (http://www.glicoenz.org/p/glicoenz.html).

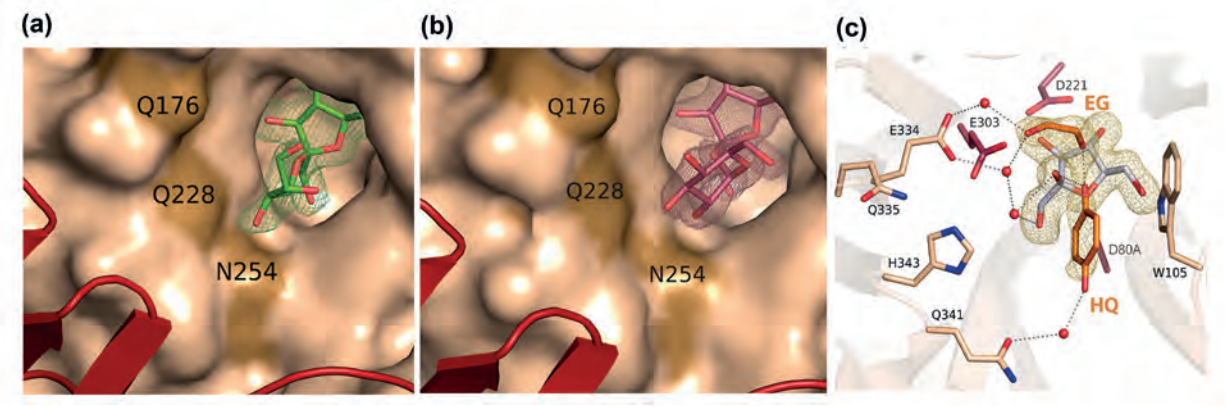



***Figure.*** *Some alditols and phenolic compounds are used in numerous pharmaceutical, food and domestic products. Surface representation of a Ffase-D50A subunit showing its active site complexed with (a) fructosyl-erythritol (green) or (b) sucrose (prune). A small part of the second subunit is shown as red cartoon. (c) Enzymatic glycosylation of polyphenols is a tool to improve their physicochemical properties and bioavailability. Representation of the Xd-INVD80A mutant soaked into fructose and hydroquinone as acceptor substrate with a molecule of ethylene glycol (EG).*

## Publications

Rodrigo-Frutos, D., Piedrabuena, D., Sanz-Aparicio, J. and Fernández-Lobato, M. (2019) Yeast cultures expressing the Ffase from *Schwanniomyces occidentalis*, a simple system to produce the potential prebiotic sugar 6-kestose. *Appl. Microbiol Biotechnol.***103**, 279-289.

Gutiérrez, M. S., Campusano, S., Gonzalez, A. M., Gómez, M., Barahona, S., Sepúlveda, D., Espenshade, P. J., Fernández-Lobato, M., Baeza, M., Cifuentes, V. and Alcaíno, J. (2019) Sterol Regulatory Element-Binding Protein (Sre1) promotes the synthesis of carotenoids and sterols in *Xanthophyllomyces dendrorhous*. *Front. Microbiol.* **10**,586.

Santos-Moriano, P., Kidibule, P., Míguez, N., Fernández-Arrojo, L. Ballesteros, A. O., Fernández-Lobato, M. and Plou, F.J. (2019) Tailored enzymatic synthesis of chitooligosaccharides with different deacetylation degree and their anti-inflammatory activity. *Catalysts,* **9**(5), 405.

Garcia-Gonzalez, M., Plou, F. J., Cervantes, F. V., Remacha, M., Poveda, A., Jiménez-Barbero, J. and Fernandez-Lobato, M. (2019) Efficient production of isomelezitose by a glucosyltransferase activity in *Metschnikowia reukaufii* cell extracts. *Microbial Biotechnology,* **12**, 1274-1285.

Ramírez-Escudero, M., Miguez, N., Gimeno-Perez, M., Ballesteros, A. O., Fernández-Lobato, M., Plou, F. J. and Sanz- Aparicio, J. (2019) Deciphering the molecular specificity of phenolic compounds as inhibitors or glycosyl acceptors of β-fructofuranosidase from *Xanthophyllomyces dendrorhous*. *Scientific Reports,* **9**, 17441.

Cervantes, F. V., Neifar, S., Merdzo, Z., Viña-Gonzalez, J., Fernandez-Arrojo, L., Ballesteros, A. O., Fernandez-Lobato, M., Bejar, S. and Plou, F. J. (2020) A three-step process for the bioconversion of whey permeate into a glucose D-free tagatose syrup. *Catalysts,* **10**, 647.

García-Gonzalez, M., Minguet-Lobato, M., Plou, F. J. and Fernández-Lobato, M. (2020) Molecular characterization and heterologous expression of two α-glucosidases from *Metschnikowia* spp, both producers of honey sugars. *Microbial Cell Factories,* **19**, 140.

Kidibule, P. E., Santos-Moriano, P., Plou, F.J. and Fernández-Lobato, M. (2020) Endochitinase Chit33 specificity on different chitinolytic materials allows the production of unexplored chitooligosaccharides with antioxidant activity. *Biotechnology Reports,* **27**:e00500.

## Doctoral Theses

**María Gimeno-Pérez** (2019). Estudio estructural de la β-fructofuranosidase de *Xanthophyllomyces dendrorhous* y su empleo para la producción de oligosacáridos prebióticos y otros dereivados fructosilados. Universidad Autónoma de Madrid. Director: María Fernández Lobato.

## International projects / Research networks

- H2020-BG-2014, Proposal Nº 634486-1-INMARE: Industrial Applications of Marine Enzymes: Innovative screening and expression platforms to discover and use the functional protein diversity from the sea. Coordinator Peter Golyshin (Bangor University, UK). 2015-2019.

- EMFF-BlueEconomy-2018. Proposal number: 863697. FISH chitinolytic biowastes FOR FISH active and sustainable packaging material (FISH4FISH). Coordinator: Prof. Rebecca Pogni (University Degli Studi di Siena, Italia). Nov-2019-Nov-2022. Role: Partner

## MICROBES IN HEALTH AND WELFARE

# VIRUS ENGINEERING AND NANOBIOTECHNOLOGY

***Principal Investigator:***
Mauricio G. Mateu

***Postdoctoral Fellow:***
Alejandro Valbuena Jiménez

***Predoctoral Fellows:***
Santos Domínguez Zotes
Luis Valiente Martínez-Sicluna
Judith Escrig Traver

***Technicians:***
Miguel A. Fuertes Villadangos
Alicia Rodríguez Huete

***Undergraduate and Master Students:***
Bram Cremers

http://www.cbm.uam.es/mgmateu

## Research summary

Major research goals: We use protein engineering techniques and biochemical, biophysical and virological analyses to study assembly, conformational stability and dynamics and physical properties of viruses, and their biological relevance (Mateu (ed.) (2013) *Structure and Physics of Viruses*, Springer 2013). Based on these studies, we aim also at the design and analysis of genetically and/or structurally modified viral particles for the development of biomedical and bionanotechnological applications (Mateu (2016). In *Protein-based Engineered Nanostructures*, Springer 2016, pp.83-120).

Scientific relevance and technological implications: In-depth knowledge of certain key processes for viral infection, including virus morphogenesis, structural rearrangements and uncoating; application of this knowledge for the design of vaccines, antiviral drugs, biomaterials and modified nanoparticles for biomedical or bionanotechnological uses.

Some recent results: i) In collaboration with Dr. W.H. Roos (University of Groningen) we have used high-speed atomic force microscopy (HS-AFM) to visualize in real time single viral protein molecules following multiple, stochastic pathways to build the mature capsid lattice of the human immunodeficiency virus (Figure; see video in: https://www.youtube.com/watch?v=QmSLGOwNV6c). ii) We have used protein engineering, AFM and the minute virus of mice capsid to determine in atomic detail structural determinants of the mechanical strength of a protein-based spherical nanoparticle with biomedical and nanotechnological implications. iii) We have found a relationship between changes in capsid thermostability and virus viability by analyzing the effects of lethal and viability-restoring mutations in the capsid of foot-and-mouth disease virus. These and other studies by our group have implications for a better understanding of processes essential for viral infection, the design of new antivirals that may inhibit these processes, and the development of nanoparticles and bidimensional biomaterials with improved mechanical properties for applications such as targeted drug delivery or tissue regeneration.

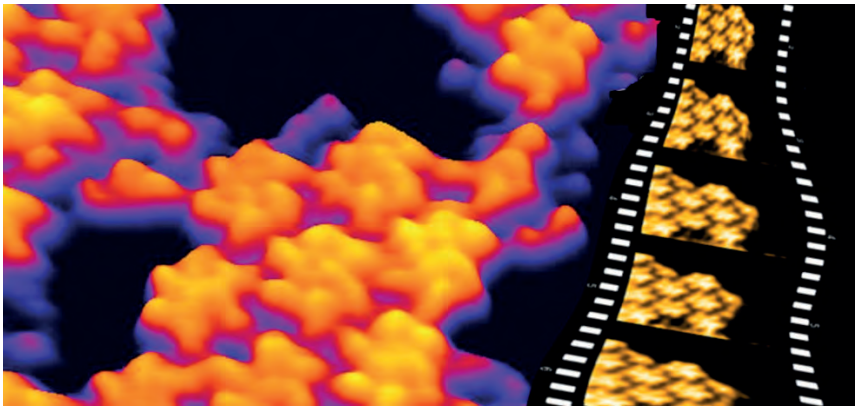


***Figure.*** *Viral protein molecules building the mature human immunodeficiency virus capsid, visualized in real time by high-speed atomic force microscopy (see: https://www.youtube.com/watch?v=QmSLGOwNV6c).*

## Publications

López-Argüello, S., Rincón, V., Rodríguez-Huete, A., Martínez-Salas, E., Belsham, G.J., Valbuena, A., Mateu, M.G. (2019). Thermostability of the foot-and-mouth disease virus capsid is modulated by lethal and viability-restoring compensatory amino acid substitutions. *J. Virol*. **93**, e02293-18.

Medrano, M., Valbuena, A., Rodríguez-Huete, A., Mateu, M.G. (2019). Structural determinants of mechanical resistance against breakage of a virus-based protein nanoparticle at a resolution of single amino acids. *Nanoscale* **11**, 9369-9383.

Caridi, F., López-Argüello, S., Rodríguez-Huete, A., Torres, E., Bustos, M.J., Cañas-Arranz, R., Martín-Acebes, M.A., Mateu, M.G., Sobrino, F. (2020). Negatively charged amino acids at the foot-and-mouth virus capsid reduce the virion-destabilizing effect of viral RNA at acidic pH. *Sci. Rep*. **10**, 1657.

Valbuena, A., Maity, S., Mateu, M.G.,* Roos, W.* (2020). Visualization of single molecules building a viral capsid protein lattice through stochastic pathways. *ACS Nano* **14**, 8724-8734. (*:corresponding authors).

## Awards and recognition

- Mauricio G. Mateu, member of the Editorial Board of Virus Research.

## International projects / Research networks

- Red Temática Nacional de Excelencia en Física Virológica.

- ARBRE-Mobieu European Biophysics Network.

- Global Virology Network.

- Global Foot-and-Mouth Disease Research.

# MICROBES IN HEALTH AND WELFARE

## MOLECULAR MODELLING

***Principal Investigator:***
Paulino Gómez-Puertas

***Scientific Staff***
Íñigo Marcos-Alcalde

http://www.cbm.csic.es/bioweb

## Research summary

Computational biology lab. The work is devoted to the integration of evolutive and structural information to study the function of proteins, the simulation of dynamic processes of protein-protein and protein-ligand interaction, the development of novel “in silico” drug design systems and the generation of new quantitative methods for computational biology.

Current projects:

A. Analysis by computational simulation of enzymatic reactions catalyzed by enzymes of biomedical interest. Design of specific inhibitors.

- Cohesin: Analysis of the molecular interactions among the protein components of the cohesin ring and the interaction of the protein complex with DNA

- FtsZ: Simulation by molecular dynamics of the processes of polymerization and depolymerization of the bacterial septum protein FtsZ, associated with the GTPase activity of its active center.

- Carbapenemases. Dynamic simulation of the interaction of bacterial carbapenemases (VIM-2, KPC-2, OXA-48) with substrates and known inhibitors.

B. Development of a new and efficient drug design system based on computational dynamic simulation of macromolecular structures. Based on the analysis of enzyme active centers, the method developed by our group consists of simulating these protein structures through molecular dynamics for several hundred nanoseconds, selecting different representative structures and filtering a database of 3D compounds for each one of them:

- Molecules able to inhibits the cell cycle of human tumor cells, with the potential to be used as an anti-tumor drugs.

- Bacterial cell division cycle inhibitor molecules that can be used as potential antimicrobials.

- New carbapenemases inhibitors ready to enter into clinical trials.

C. Development of a new and efficient method (MEPSAnd) that natively calculates minimum energy paths across energy surfaces of any number of dimensions.

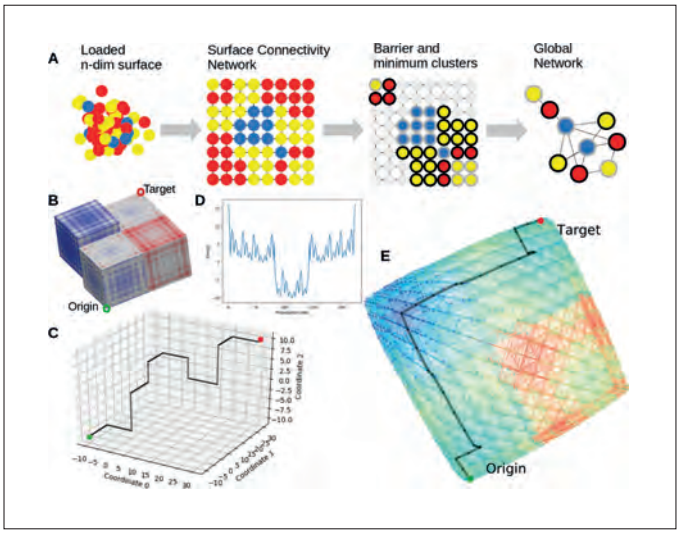



***Figure.*** *Overview of MEPSAnd, a new and efficient method developed in the group that natively calculates minimum energy paths across energy surfaces of any number of dimensions. The figure shows a diagram of the MEPSAnd algorithm steps and some examples and applications [doi: 10.1093/bioinformatics/btz649].*

## Publications

Marcos-Alcalde, I., Lopez-Viñas, E. and Gómez-Puertas, P. (2020). MEPSAnd: Minimum Energy Path Surface Analysis over n-dimensional surfaces. *Bioinformatics* **36**, 956–958.

Latorre-Pellicer, A., Ascaso, A., Trujillano, L., Gil-Salvador, M., Arnedo, M., Lucia-Campos, C., Antoñanzas-Pérez, R., Marcos-Alcalde, I., Parenti, I., Bueno-Lozano, G., Musio, A., Puisac, B., Kaiser, F. J., Ramos, F. J., *Gómez-Puertas, P. and *Pié, J. (*Corresponding authors) (2020). Evaluating Face2Gene as a Tool to Identify Cornelia de Lange Syndrome by Facial Phenotypes. *International Journal of Molecular Sciences* **21**, 1042.

Lazo, P. A., García, J. L., Gómez-Puertas, P., Marcos-Alcalde, I., Arjona, C., Villarroel, A., González-Sarmiento, R. and Fons, C. (2020). Novel dominant KCNQ2 exon 7 partial in-frame duplication in a complex epileptic and neurodevelopmental delay syndrome. *International Journal of Molecular Sciences* **21**, 4447.

Marcos, A. T., Martín-Doncel, E., Morejón-García, P., Marcos-Alcalde, I., Gómez-Puertas, P., Segura-Puimedon, M., Armengol, L., Navarro-Pando, J. M. and Lazo, P. A. (2020). VRK1 (Y213H) homozygous mutant impairs Cajal bodies in a hereditary case of distal motor neuropathy. *Annals of Clinical and Translational Neurology* **7**, 808-818.

Ruiz-Márvez, E., Ramírez, C. A., Rodríguez, E. R., Flórez, M. M., Delgado, G., Guzmán, F., Gómez-Puertas, P., Requena, J.M. and Puerta, C. J. (2020). Molecular characterization of Tc964, a novel antigenic protein from Trypanosoma cruzi. International *Journal of Molecular Sciences* **21**, 2432.

Krab, L. C., Marcos-Alcalde, I., Assaf, M., Balasubramanian, M., Andersen, J. B., Pedersen, A-M. B., Cefle, K., Fitzpatrick, D., Gudmundsson, S., Huisman, S., McKee, S., Maas, S. M., Menke, L. A., Mulder, P. A., Martínez, F., Mokry, J., Murch, O. D., Parker, M., Pie, J., Ramos, F., Rieubland, C., Scarano, E., Shinawi, M., Gómez-Puertas, P., Tümer, Z. and Hennekam, R.C. (2020). Delineation of phenotypes related to cohesin structural protein RAD21. *Human Genetics* **139**, 575–592.

Arnedo, M., Latorre-Pellicer, A., Lucia-Campos, C., Gil-Salvador, M., Antoñanzas-Pérez, R., Gómez-Puertas, P., Bueno-Lozano, G., Puisac, B. and Pié, J. (2019). More Than One HMG-CoA Lyase: The Classical Mitochondrial Enzyme Plus the Peroxisomal and the Cytosolic Ones. *International Journal of Molecular Sciences* **20**, 6124.

Gudmundsson, S., Annéren, G., Marcos-Alcalde, I., Wilbe, M., Melin, M., Gómez-Puertas, P. and Bondeson, M.-L. (2019). A novel RAD21 p.(Gln592del) variant expands the clinical description of Cornelia de Lange syndrome type 4 - review of the literature. *European Journal of Medical Genetics* **62**, 103526.

Reis, F. P., Bárria, C., Gómez-Puertas, P., Gomes, C. M. and Arraiano, C. M. (2019). Identification of temperature-sensitive mutations and characterization of thermolabile RNase II variants. *FEBS Letters* **593**, 352-360.

## Awards and recognition

- Paulino Gomez-Puertas, member of the editorial board of “International Journal of Molecular Sciences”, sections “Molecular Biology” and “Molecular Biophysics”.

## International projects / Research networks

- CONNECT: inCreasing cOmmunicatioN, awareNEss and data sharing in a global approaCh against resisTance. EU JPIAMR Virtual Research Institute Network. 2018-2020.

- COMPUDRUG: Development of a new and efficient drug design system based on computational dynamic calculation of macromolecular structures. State Research Agency / EU-ERDF. 2018-2021

- CARE: Combating Antibiotic-Resistant Enterobacteriaceae; structure-based discovery of clinical trial-ready inhibitors. Carlos III Health Institute. 2020-2022.

- DRUGCOHESIN: New drugs for chromosome biology. Design of specific inhibitors of the Cohesin complex”. State Research Agency. 2019-2022

- AEPIC: Alliance for the Exploration of Pipelines for Inhibitors of Carbapenemases. Joint Programming Initiative On Antimicrobial Resistance - EU-JPIAMR Network Plus 2020-2022.

- Dr. Gomez-Puertas, associated researcher to the Institute for Health Research of La Paz University Hospital (IdiPAZ) since 2012.

## MICROBES IN HEALTH AND WELFARE

# ULTRAHIGH-THROUGHPUT DISCOVERY AND ENGINEERING OF ENZYMES FOR BIOTECHNOLOGICAL APPLICATIONS (HT DISCOVERY LAB)

***Principal Investigator:***
Aurelio Hidalgo

***Postdoctoral Fellows:***
Davide Cecchini
Alejandro Herrera
(until 11/2020)
María Gimeno
(from 08/2020)
Diana Maté
Ana L. Ribeiro
(until 3/2019)
Dione L. Sánchez
(until 9/2019)
Mercedes Sánchez
(from 8/2020)

***Predoctoral Fellows:***
Sandra Bosch
Jorge Bravo
Mercedes Sánchez
(José Berenguer, until 7/2020)

***Technicians:***
Carmen Ortega
Esther Sánchez
(until 11/2020)

***Undergraduate and Master Students:***
Vanessa Rondón
(from 2/2020 until 12/2020)
Laura Blas
(from 11/2020)

***Visiting Scientist:***
Rosanna Mattosovich
(from 9/2020 until 6/2020)

***Management Staff:***
Nargisse Nejda

http://www.cbm.uam.es/ahidalgo

## Research summary

Microbial diversity is a vast reservoir of genetic information that can be valorized through industrial application, from biosynthetic gene clusters to novel enzyme catalysts. The synergy between new experimental discovery tools based on biology and those based on nanotechnologies are instrumental to find relevant genes faster and more efficiently, enabling especially academic labs to undertake screening campaigns until now costly and limited to large enterprises.

In the HT Discovery lab, we develop methods to find and engineer industrially relevant enzymes and biosynthetic gene clusters in the natural or artificially created genetic diversity. Biological selections are inexpensive methods to find enzymes that couple the improved fitness of a protein to the survival of a biological host under selective pressure. We investigate the accuracy of biological selections to enhance the stability of enzymes to withstand harsh conditions of industrial processes, such as the presence of organic cosolvents or high temperatures. Using such methods, we have developed stable esterases, dehalogenases and aldolases in the EU-funded project CarbaZymes.

However, the complexity of cellular metabolism limits the applicability of biological selections. For this reason, we also work on screening methods, which involve individual enzymatic assays *in vitro* of each enzyme variant generated. To shorten this long, tedious and expensive process, droplet microfluidics enables the miniaturization of assays with throughput of kHz rates as well as a 1000x reduction of volume and assay costs. Moreover, microfluidics enables the conversion of general lab operations (additions, aliquoting, detection of a given property) into a particular chip design. Using ultraHT, we have discovered ancestral and promiscuous esterases, KREDs, glycosidases and other enzymes in the EU-funded projects HotDrops and MetaFluidics.

Finally, after suitable enzymes are found, we study the underlying rationale for their improved fitness, often using bioinformatic approaches, thus uncovering how and why enzymes function and ultimately, learning the language of proteins.

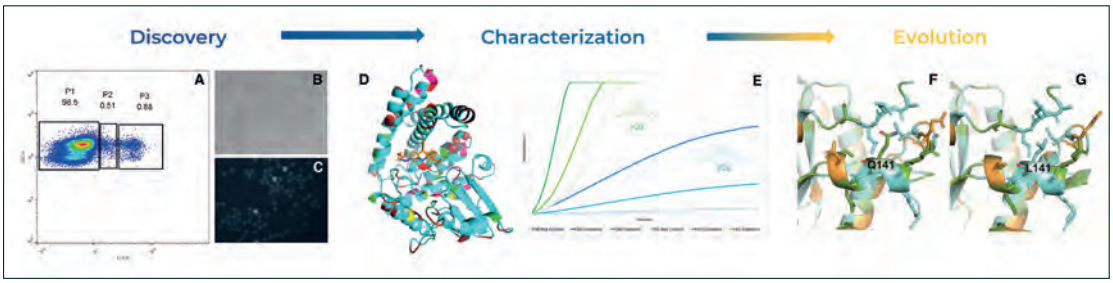



***Figure.*** *From nature to application. A: FACS assay of metagenomic KREDs; B and C: sorted fraction; D: Thal0063, a kinase discovered by ultrahigh-throughput functional metagenomic screening; E: promiscuous esterase activity of Thal0063; F and G: improvement of the operational stability of the Yfau aldolase from E. coli by protein engineering.*

## Publications

Mate, D. M., Rivera, N. R, Sanchez-Freire, E., Ayala, J. A, Berenguer, J., and Hidalgo, A. (2020) Thermostability enhancement of the *Pseudomonas fluorescens* esterase I by *in vivo* folding selection in *Thermus thermophilus*. *Biotechnol. Bioeng.*,**117**, 30-38,

Acosta, J., Del Arco, J., Del Pozo, M. L., Herrera-Tapias, B., Clemente-Suárez, V. J., Berenguer, J., Hidalgo, A., and Fernández-Lucas, J. (2020) Hypoxanthine-Guanine Phosphoribosyltransferase/adenylate Kinase From *Zobellia galactanivorans*: A Bifunctional Catalyst for the Synthesis of Nucleoside-5′-Mono-, Di-and Triphosphates. *Front. Bioeng. Biotechnol*. **8**, 677.

Verdú, C., Sanchez, E., Ortega, C., Hidalgo, A., Berenguer, J., and Mencía, M. (2019). A modular vector toolkit with a tailored set of thermosensors to regulate gene expression in *Thermus thermophilus*. *ACS Omega,* **4**, 14626-14632.

Rocha-Martin, J., Sánchez-Murcia, P. A., López-Gallego, F., Hidalgo, A., Berenguer, J., and Guisan, J. M. (2019) Functional characterization and structural analysis of NADH oxidase mutants from *Thermus thermophilus* HB27: Role of residues 166, 174, and 194 in the catalytic properties and thermostability. *Microorganisms*, **7**, 515.

Book chapters:

Consolati, T., Bolivar, J. M., Petrasek, Z., Berenguer, J., Hidalgo, A., Guisan, J. M., and Nidetzky, B. (2020) Intraparticle pH Sensing Within Immobilized Enzymes: Immobilized Yellow Fluorescent Protein as Optical Sensor for Spatiotemporal Mapping of pH Inside Porous Particles. In: Immobilization of Enzymes and Cells. Springer. pp 319-333.

## Awards and recognition

- Scientific committee of the Functional Metagenomics Conference 2019.

- Participation in the DG Research stand, EU Day, Brussels 2019.

- Participation in the European Night of Researchers, Zaragoza 2019.

## Doctoral Theses

**Mercedes Sánchez Costa** (2020). Estudio y desarrollo de nuevas herramientas para la exploración de ambientes extremos. Universidad Autónoma de Madrid. José Berenguer y Aurelio Hidalgo.

## International projects / Research networks

- Sustainable industrial processes based on a C-C bond-forming enzyme platform (CarbaZymes), 2015-2019

- Advanced toolbox for rapid and cost-effective functional metagenomic screening -microbiology meets microfluidics (MetaFluidics), 2016-2020, Coordinator.

- Red Nacional de Microorganismos Extremófilos (RedEx), 2020-2021

## MICROBES IN HEALTH AND WELFARE

# PLASMID CONJUGATION IN GRAM-POSITIVE BACTERIA

***Principal Investigator:***
Wilfried J.J. Meijer

***Predoctoral Fellows:***
Andrés Miguel Arribas
(till January 2020)
César Gago Córdoba
(till January 2020)
Jorge Val Calvo
(till January 2020)

***Visiting Scientist:***
Nuria Quiles

***Undergraduate and Master Students:***
Sandra Boldu Fernández
(January-July 2019, till January 2020)
Laura Toribio Celestino
(February –September 2019)

http://www.cbm.uam.es/wmeijer

## Research summary

Most bacteria contain, in addition to their chromosome, autonomously replicating units called plasmids. Plasmids have a modular structure. Besides essential modules required for DNA replication and stable maintenance, many plasmids contain a conjugation module, which contains all the genes required for transfer of the plasmid from a donor to a recipient cell via a connecting pore. These are named conjugative plasmids. Often, conjugative plasmids contain toxin, virulence and/or antibiotic resistance genes, which is why these genes are efficiently spread to other bacteria. In fact, of the various horizontal gene transfer routes present in bacteria, conjugation is the one that is mainly responsible for the spread of antibiotic resistance (AR), which is an increasing global problem affecting the health of humans and animals and causing large economic losses. Understanding different aspects of the conjugation process is essential for designing strategies or drugs to combat conjugation-mediated spread of antibiotic resistance. Some conjugative plasmids of Gram-negative (G-) bacteria have been studied in considerable detail, but little is known about the process of conjugation in Gram-positive (G+) bacteria. This is a main reason why we study conjugation in G+ bacteria in our lab. We use the related conjugative plasmids pLS20 and p576 of *Bacillus subtilis* and *Bacillus pumilus,* respectively, as model systems. *B. subtilis* forms part of the microbiome of humans and animals. Another incentive for studying these plasmids is that many G+ bacteria of scientific, industrial or clinical importance are reluctant to genetic manipulation. Conjugation is a natural method to introduce genes into a recipient bacterium. Hence, insights in aspects of the conjugation process serve two goals: (i) it can be used for constructing tools to modify Gram+ bacteria, and (ii) it may be used for the design of strategies to conjugation-mediated spread of antibiotic resistance. In the last two years, progress has been made particularly in two stages of the conjugation process: (i) recognition between donor and recipient cell and avoiding futile transfers between donor cells, and (ii) insights in the way the conjugation genes are regulated in a very strict manner.

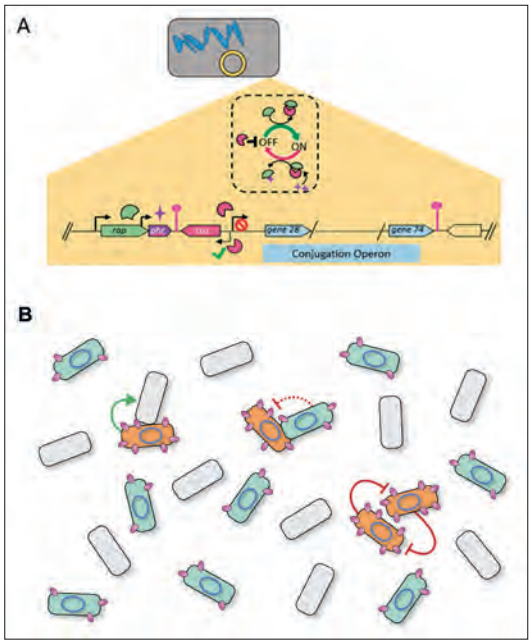



***Figure.*** *Schematic representation of the mechanism regulating expression of the pLS20 conjugation genes, and functioning of the pLS20 surface exclusion system. Panel A shows regulation of the main conjugation promoter. The transcriptional regulator Rco (pink) represses the conjugation promoter and also controls its own promoter. The anti-repressor Rap (green) activates expression of the conjugation genes by relieving Rco-mediated repression. The activity of Rap is controlled by the signalling peptide Phr (purple). Panel B shows how the surface–located Ses protein encoded by pLS20 favours transfer of pLS20 to plasmid free cells, without fully blocking plasmid transfer between two donor cells. Donor cells (rectangles containing blue ellipse) that have not (green rectangles) and those that have activated the conjugation pathway (orange rectangles) produce low and high levels of Ses (small purple ellipses), respectively. The level of inhibition of plasmid transfer is directly related to the level of Ses, as indicated by the arrow and T-shaped ending lines.*

## Publications

Singh, P. K, Serrano, E., Ramachandran, G., Miguel-Arribas, A., Gago-Cordoba, C., Val-Calvo, J., López-Pérez, A., Alfonso, C., Wu, L. J., Luque-Ortega, J. R., and Meijer, W. J. J. (2020) Reversible regulation of conjugation of *Bacillus subtilis* plasmid pLS20 by the quorum sensing peptide responsive anti-repressor $Rap_{pLS20}$. *Nucleic Acids Res*. **48**(19), 10785-10801.

Crespo, I., Bernardo, N., Miguel-Arribas, A., Singh, P. K., Luque-Ortega, J. R., Alfonso, C., Malfois, M., Meijer, W. J. J., and Boer, D. R. (2020) Inactivation of the dimeric $Rap_{pLS20}$ anti-repressor of the conjugation operon is mediated by peptide-induced tetramerization. *Nucleic Acids Res*. **48**(14), 8113-8127.

Gago-Córdoba, C., Val-Calvo, J., Miguel-Arribas, A., Serrano, E., Singh, P. K., Abia, D., Wu, L. J., Meijer, and W. J. J. (2019) Surface exclusion revisited: Function related to differential expression of the surface exclusion system of *Bacillus subtilis* plasmid pLS20. *Front Microbiol.***10**:1502.

Val-Calvo, J., Miguel-Arribas, A,. Gago-Córdoba, C., López-Pérez, A., Ramachandran, G., Singh, P. K., Ramos-Ruiz, R., Meijer, W. J. J. (2019) Draft genome sequences of sporulation-impaired *Bacillus pumilus* strain NRS576 and its native plasmid p576. *Microbiol Resour Announc.* **8**(16):e00089-19.

## Doctoral Theses

**Andrés Miguel Arribas** (2020). Discovery of a novel antitermination system present on many conjugative plasmids of Gram-positive bacteria. Universidad Autónoma Madrid. Director: Wilfried J.J. Meijer. International recognition.

## MICROBES IN HEALTH AND WELFARE

# HUMAN IMMUNODEFICIENCY VIRUS REPLICATION AND ANTIRETROVIRAL THERAPY

***Principal Investigator:***
Luis Menéndez Arias

***Postdoctoral Fellows:***
Estrella Beltrán Frutos
(Feb 2019 – Sept 2020)

***Predoctoral Fellows:***
Samara Martín Alonso
Javier Martínez del Río
(Oct 2019 – Jun 2020; since Nov 2020)

***Undergraduate and Master Students:***
Miguel Barbero Cascón
(Oct 2019 – June 2020)
David Borrego García
(since Oct 2020)
Óscar Herrera Chacón
(until July 2019)
Adrián Sánchez Ibáñez
(since Sept 2020)
Víctor A. Torre González
(Feb – July 2019)

***Visiting Scientists:***
Moisés A. Árquez Mendoza
(June 2019 – Jan 2020), Ph. D. student, from Universidad Simón Bolívar, Barranquilla, Colombia
Cagil Urhan
(Jun – Sept 2019), undergraduate, from Middle East Technical University, Ankara, Turkey

http://www.cbm.csic.es\retrovir

## Research summary

Infections caused by human immunodeficiency viruses type 1 and type 2 (HIV-1 and HIV-2, respectively) constitute a major burden to human health worldwide. Despite significant advances in antiretroviral therapy, HIV still causes around 700,000 deaths each year. The HIV genome is composed of two copies of single-stranded RNA. The viral reverse transcriptase (RT) is responsible for the replication of the HIV genome. RT inhibitors constitute the backbone of current therapies against HIV-1 and HIV-2.

For years, our efforts have been directed towards the elucidation of molecular mechanisms involved in RT inhibitor resistance; and understanding the role of different amino acids in nucleotide specificity and fidelity of DNA synthesis of retroviral RTs. We have engineered HIV-1 RT variants with increased thermal stability and DNA polymerization accuracy, currently marketed as biotechnological tools with many applications in basic and translational research. Present and future research focuses on understanding the molecular basis of template switching and strand displacement, as well as obtaining novel RTs with high nucleic acid binding affinity, suitable for RNA amplification from single cells.

Although nucleoside RT inhibitors (NRTIs) constitute the backbone of antiretroviral therapies, HIV-1 and HIV-2 differ in their mutational pathways of NRTI resistance. Unlike in HIV-1 RT, thymidine analogue resistance mutations (TAMs) such as M41L, D67N, K70R and S215Y do not confer resistance to AZT and other NRTIs when found in HIV-2, due to amino acid differences located in the fingers subdomain of its RT. We have recently demonstrated that the accumulation of TAMs in HIV-2 RT renders an enzyme with defective strand displacement activity. In a related study, we have shown that in HIV-1, nucleocapsid protein precursors NCp9 and NCp15 can suppress the effects of TAMs in NRTI resistance, suggesting a link between NRTI and protease inhibitor resistance.

Despite the remarkable advances in antiretroviral therapy, prevalence of drug-resistant HIV strains is increasing around the World, particularly in less-developed countries. In this scenario, the search for unexploited targets of antiretroviral intervention is important for the future clinical management of the infection. To address coming challenges in antiretroviral research, we are extending our biochemical studies to other catalytic activities of the RT (e.g. ribonuclease H). Collaborations with medicinal chemists have been established to identify potentially useful RNase H inhibitors (binding both active or allosteric sites). These drugs, specific for HIV-1 and HIV-2, could also be developed into broader spectrum inhibitors blocking infection of other viruses (e.g. hepatitis B virus).

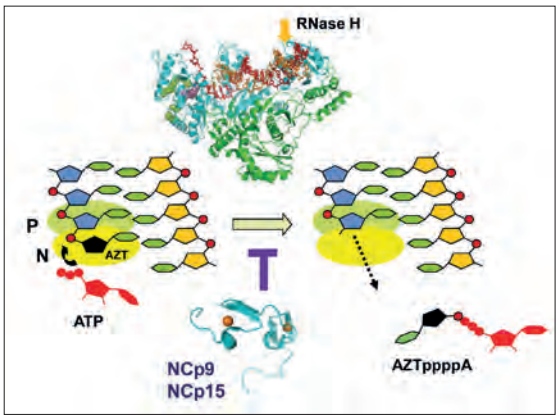



***Figure.*** *HIV-1 RTs containing TAMs confer resistance to AZT and other NRTIs due to their ability to excise the inhibitor from the 3' end of the blocked DNA chain. In vitro, this activity can be inhibited by NCp9 and NCp15 only when the RNA is used as template. For details see Árquez et al. (2020) Antimicrob. Agents Chemother.* ***64,*** *e00958-20.*

## Publications

Gao, P., Wang, X., Sun, L., Cheng, X., Poongavanam, V., Kongsted, J., Álvarez, M., Luczkowiak, J., Pannecouque, C., De Clercq, E., Lee, K.-H., Chen, C.-H., Liu, H., Menéndez-Arias, L., Liu, X. and Zhan, P. (2019) Design, synthesis and biological evaluation of novel galloyl derivatives as HIV-1 RNase H inhibitors. *Chem. Biol. Drug Des.* **93**, 582-589.

Gao, P., Cheng, X., Sun, L., Song, S., Álvarez, M., Luczkowiak, J., Pannecouque, C., De Clercq, E., Menéndez-Arias, L., Zhan, P. and Liu, X. (2019) Design, synthesis and biological evaluation of 3-hydroxyquinazoline-2,4(1H,3H)-diones as dual inhibitors of HIV-1 reverse transcriptase-associated RNase H and integrase. *Bioorg. Med. Chem.* **27**, 3836-3845.

Luczkowiak, J., Álvarez, M., Sebastián-Martín, A. and Menéndez-Arias, L. (2019) DNA-dependent DNA polymerases as drug targets in herpesviruses and poxviruses. In: Gupta, S. P. (ed) Viral polymerases: Structures, functions and roles as antiviral drug targets. *Elsevier-Academic Press*, pp. 95-134.

Tramontano, E., Corona, A. and Menéndez-Arias, L. (2019) Ribonuclease H, an unexploited target for antiviral intervention against HIV and hepatitis B virus. *Antiviral Res*. **171**, 104613.

Akkina, R., Garry, R., Bréchot, C., Ellerbrok, H., Hasegawa, H., Menéndez-Arias, L., Mercer, N., Neyts, J., Romanowski, V., Segalés, J. and Vahlne, A. (2019) 2019 meeting of the Global Virus Network. *Antiviral Res.* **172**, 104645.

Sánchez-Murcia, P. A., de Castro, S., García-Aparicio, C., Jimenez, M. A., Corona, A., Tramontano, E., Sluis-Cremer, N., Menéndez-Arias, L., Velazquez, S., Gago, F. and Camarasa, M.-J. (2020) Peptides mimicking the β7/β8 loop of HIV-1 reverse transcriptase p51 as "hotspot-targeted" dimerization inhibitors. *ACS Med. Chem. Lett*. **11**, 811-817.

Martín-Alonso, S., Alvarez, M., Nevot, M., Martinez, M. A. and Menéndez-Arias, L. (2020) Defective strand-displacement DNA synthesis due to accumulation of thymidine analogue resistance mutations in HIV-2 reverse transcriptase. *ACS Infect. Dis*. **6**, 1140-1153.

Árquez, M. A., Martín-Alonso, S., Gorelick, R. J., Scott, W. A., Acosta-Hoyos, A. J. and Menéndez-Arias, L. (2020) Nucleocapsid protein precursors NCp9 and NCp15 suppress ATP-mediated rescue of AZT-terminated primers by HIV-1 reverse transcriptase. *Antimicrob. Agents Chemother.* **64**, e00958-20.

Wei, F., Kang, D., Menéndez-Arias, L., Liu, X. and Zhan, P. (2020) Recent developments in small molecular HIV-1 and hepatitis B virus RNase H inhibitors. In: Islam S, Hashmi, A. A. and Khan, S. A. (eds.) Advances in Metallodrugs: Preparation and Applications in Medicinal Chemistry. Series: Emerging trends in medicinal and pharmaceutical chemistry. *Scrivener Publishing LLC*, pp. 273-292.

Kellner, M. J., Ross, J. J., Schnabl, J., Dekens, M. P. S., Heinen, R., Grishkovskaya, I., Bauer, B., Stadlmann, J., Menéndez-Arias, L., Fritsche-Polanz, R., Traugott, M., Seitz, T., Zoufaly, A., Foedinger, M., Wenisch, C., Zuber, J., Vienna Covid-19 Diagnostics Initiative (VCDI), Pauli, A. and Brennecke, J. (2020) A rapid, highly sensitive and open-access SARS-CoV-2 detection assay for laboratory and home testing. *bioRxiv* 2020.06.23.166397.

Ren, Y., Ma, Y., Cherukupalli, S., Tavis, J. E., Menéndez-Arias, L., Liu, X. and Zhan, P. (2020) Discovery and optimization of benzenesulfonamides-based hepatitis B virus capsid modulators via contemporary medicinal chemistry strategies. *Eur. J. Med. Chem.* **206**, 112714.

Martín-Alonso, S., Frutos-Beltrán, E. and Menéndez-Arias, L. (2020) Reverse transcriptase: from transcriptomics to genome editing. *Trends Biotechnol*. doi:10.1016/j.tibtech.2020.06.008.

## Awards and recognition

- Luis Menéndez Arias is Academic Editor of *PLoS ONE,* and member of the Editorial Boards of *Antimicrobial Agents and Chemotherapy, Antiviral Research, Antiviral Therapy, Journal of Biological Chemistry, Viruses, Virus Research and World Journal of Translational Medicine*.

- Member of the Global Virus Network (www.gvn.org).

## Doctoral Theses

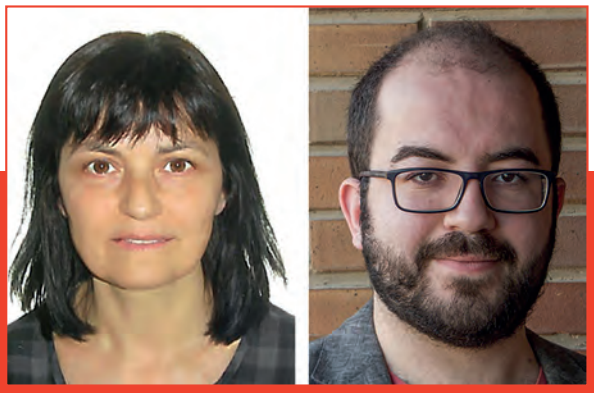
**Alba Sebastián Martín** (2019). Fidelity of human immunodeficiency viruses type 1 and type 2 reverse transcriptases in DNA synthesis reactions using DNA and RNA templates. Faculty of Sciences, Autonomous University of Madrid. Director: Luis Menéndez Arias. Thesis with International Mention.

## International projects / Research networks

- Member of the 'Network for antivirals against arboviral diseases' (REARBOVIR, 2017-2020). National excellence network.

## MICROBES IN HEALTH AND WELFARE

# RNA-BASED CONTROL OF *LISTERIA* ADAPTATION TO STRESS AND VIRULENCE



***Principal Investigator:***
María Graciela Pucciarelli Morrone

***Predoctoral Fellows:***
Marcos Peñalver Medina

https://www.cbm.uam.es/mg.pucciarelli

## Research summary

Our group aims to understand the molecular mechanisms that the intracellular bacterial pathogen *Listeria monocytogenes* uses to cope with different stresses, especially the adaptation to cold and the ability to proliferate at refrigeration temperatures. This capacity is one of the main factors that allow this pathogen to survive in food processing facilities and to be transmitted to humans by the food chain. The investigation of the functions of the pathogen that support growth in the cold may facilitate the design of new antimicrobials specifically designed to arrest survival and growth in the food.

Our group has been pioneered in proteomic analysis of the *Listeria* cell wall and the characterization of regulatory processes in which small RNA molecules control gene expression at the post-transcriptional level. We are currently undergoing new studies in bacteria growing at 4ºC to identify, based on transcriptomic and proteomic data, new regulators that promote adaptation at this low temperature. In parallel, we are carrying out virulence assays with mutants defective in growth at 4ºC to define the cross-talk between the virulence program and cold adaptation. An enigmatic aspect that we want also to explore is how the same RNA regulatory molecules can function at so different temperatures (37ºC in the host and 4ºC in food), considering that such dramatic change affects RNA folding at large extent.

We are also analyzing the composition of a macromolecular complex, the stressosome, which activates the alternative sigma factor SigB to respond to stress. The stressosome has been extensively studied in responses to acid or osmotic stresses but, to date, not yet explored regarding cold adaptation. The availability of mutants lacking specific stressosome proteins or deficient in the signaling cascade will facilitate to dissect the underlying regulatory mechanisms.

We hope these studies will pave the way to design new strategies to control survival and growth of *Listeria* in food and, consequently, to reduce the impact of listeriosis in human health.

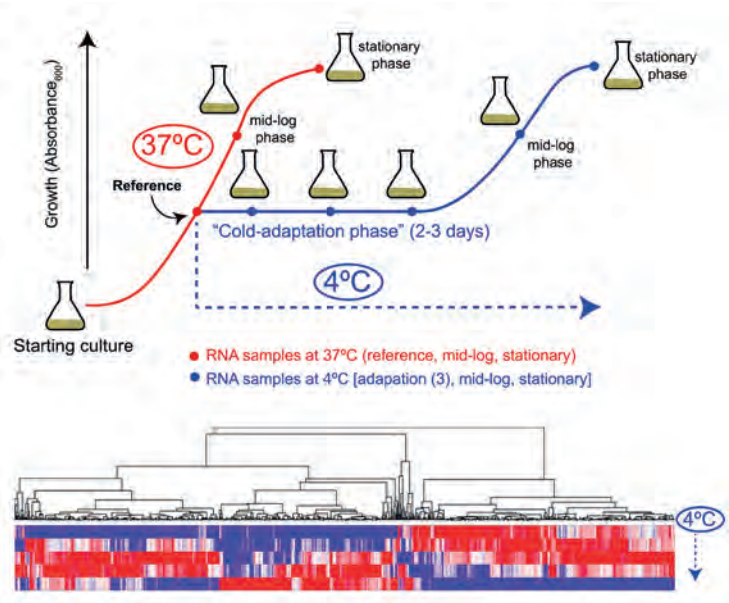



***Figure.*** *Gene expression analysis during Listeria monocytogenes adaptation to cold.*

## Publications

Giner-Lamia, J., Vinuesa, P., Betancor, L., Silva, C. Bisio, J., Soleto, L., Chabalgoity, J.A., Puente, J.L., The Salmonella CYTED Network (Pucciarelli, M. G.), and García-del Portillo, F. (2019) Genome analysis of *Salmonella enterica* subsp. diarizonae isolates from invasive human infections reveals enrichment of virulence-related functions in lineage ST1256. *BMC Genomics* **20**, 99.

Dessaux, C., Guerreiro, D. N., Pucciarelli, M. G., O'Byrne, C. P., and García-Del Portillo, F. Impact of osmotic stress on the phosphorylation and subcellular location of *Listeria monocytogenes* stressosome proteins. (2020) *Scientific Reports* **10**(1): 20837.

Castanheira, S., López-Escarpa, D., Pucciarelli, M. G., Cestero, J. J., Baquero, F., and García-Del Portillo, F. (2020) An alternative penicillin-binding protein involved in *Salmonella* relapses following ceftriaxone therapy. *EBioMedicine* **55**:102771.

## Patents

Inventors: García-del Portillo, F., Rico-Pérez, G., Ramón-Marquès, E, San Félix, A.R., Velázquez, S., De La Puente, S., García, E., De Castro, S., Pucciarelli, M.G.

Title: Peptides containing D-Alanine (D-Ala) or related amino alcohols.

Reference: PCT1641.1371

Date: 10-11-2020

Owners: CSIC-UAM

Inventors: García-del Portillo, F., Castanheira, S., Cestero, J.J., López, D. Pucciarelli, M.G.

Title: Inhibitors of intracellular bacterial growth and persistence as antibiotics.

Reference: EP1641.1498.

Date: 20-01-2020.

Owners: CSIC-UAM

## Doctoral Theses

**Charlotte Dessaux** (2020). Dynamics of the *Listeria monocytogenes* stressosome protein in response to osmotic stress and the eukaryotic intracellular environment. Universidad Autónoma de Madrid. Supervisors: Francisco García-del Portillo and M. Graciela Pucciarelli Morrone.

## MICROBES IN HEALTH AND WELFARE

# VIRUS-CELL INTERACTION AND VACCINES DEVELOPMENT: THE ASFV MODEL

***Principal Investigator:***
Yolanda Revilla Novella

***Postdoctoral Fellows:***
Daniel Pérez Núñez
Elena García Sánchez

***Predoctoral Fellows:***
Raquel García Belmonte
Elena Riera Laguna

***Undergraduate and Master Students:***
Gonzalo Vigara Astillero (TFM)
David Marín (TFM)

***Technician:***
Carmen Sánchez Valdepeñas

http://www.cbm.uam.es/yrevilla

## Research summary

As a consequence of the pressure through evolution, mammalians develop refined mechanisms to neutralize viral infections. In turn, viruses have adapted multiple tricks to disrupt host defense pathways and hijack host mechanisms for their advantage.

The prompt induction of type I interferon (IFN-I), is a central event of the innate immune defense against viral infection. Interferons are cytokines playing a key role against viral infections. Specifically, type I IFNs are involved in induction of antimicrobial state both in infected and neighboring cells, activating the innate immune response and leading to the adaptive immune response. In order to ensure an efficient replication and viral dissemination in the host, viruses have developed several mechanisms to counteract the immune response by targeting type I IFN expression. This is particularly relevant since a complete understanding of the molecular strategies by which viruses disrupt the host response will be involved in the rational design of vaccines.

African Swine Fever Virus (ASFV) is the etiological agent of African swine fever (ASF), a serious disease affecting both wild boar and domestic pigs. Endemic in Africa, an outbreak in the Caucasus in 2007 started its spread across Russia and Eastern Europe. A recent outbreak Belgium in 2018 probably produced the first case detected in wild boar in Germany during 2020. Importantly, in August 2018 the first ASFV outbreak was reported in China, one of the most important pork producers in the world, and since then the virus has spread to neighboring countries as Vietnam, Laos, Myanmar, Korea and Philippines. An outbreak was also declared in India during the current year. The situation is economically dramatic causing a serious unbalance of the food chain.

Despite efforts to develop an effective vaccine against ASFV, only control and eradication measures have been implemented so far, based on the early diagnosis, regionalization and eradication. However, the rapid spread of the disease shows these measures insufficient to control the current pandemic situation, and the development of a vaccine is urgently required.

Molecular mechanisms leading to virulence are of great importance to understand viral pathogenesis in the rational design of vaccines. Our recent work has established the different ability to control the host IFN-β production as a key element for virulence. Functional screenings -some of them already developed “in house”- will identify new ASFV genes which will be deleted from the virulent genomes for the construction of vaccine prototypes.

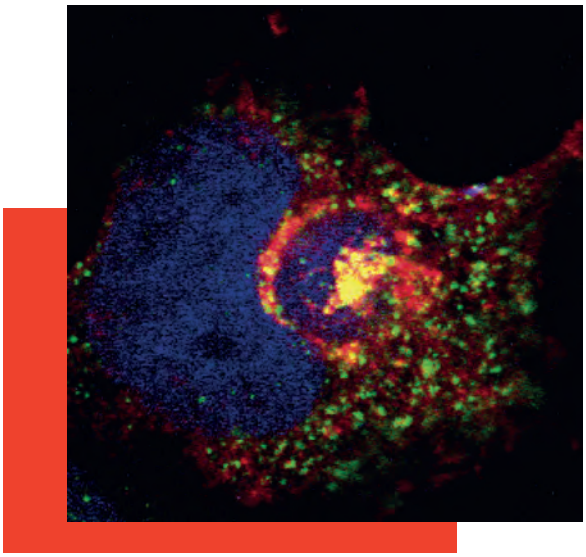


***Figure.*** *COS cells infected with ASFV-E70. At 16 hpi, cells were fixed and immunostained with Abs anti adaptin (AP-1) (green) and anti -CD2v (red). Cellular nucleus and viral factory were stained with DAPI (blue). Scale bar represents 5 µm.*

## Publications


Pérez-Núñez, D., Castillo-Rosa, E., Vigara-Astillero, G., García-Belmonte, R., Gallardo, C., and Revilla, Y. (2020) Identification and Isolation of Two Different Subpopulations Within African Swine Fever Virus Arm/07 Stock Vaccines. *Vaccines. MDPI.* **8**-625.

Meekins, DA., Trujillo, J. D., Gaudreault, N. N., Morozov, I., Pérez-Núñez, D., Revilla, Y., and Richt, J. A. 2020. Long amplicon sequencing for improved genetic characterization of African Swine Fever Virus. *Journal of Virological Methods. Elsevier.* Volume-**285**, pp.113946.

Karger, A., Pérez-Núñez, D., Urquiza, J., Hinojar, P., Alonso, C., Freitas, F. B., Revilla, Y., Le Potier, M. F., and Montoya, M (2019) An Update on African Swine Fever Virology. *Viruses*. **11**(9):864.

Sánchez, E. G., Pérez-Núñez, D., and Revilla, Y. (2019) Development of vaccines against African swine fever virus. *Virus Research*. **265**, pp. 150-155.

García-Belmonte, R., Pérez-Nuñez, D., Pittau, M., Richt, J., and Revilla, Y. African swine fever virus Armenia/07 virulent strain controls IFN-β production through cGAS-STING pathway. *Journal of Virology* **93**:e02298-18.

Pérez-Núñez, D., Sunwoo, S. Y., Sánchez, E. G., Haley, N., García-Belmonte, R., Nogal, M., Morozov, I., Madden, D., Gaudreault, N. N., Mur, L., Shivanna, V., Richt, J. A., and Revilla, Y. (2019). Evaluation of a viral DNA-protein immunization strategy against African swine fever in domestic pigs. Vet Immunol Immunopathol. *Elsevier*. **208**, pp.34-43.

Sunwoo, S. Y., Pérez-Núñez, D., Morozov, I., Sánchez, E. G., Gaudreault, N. N., Trujillo, J. D., Mur, L., Nogal, M., Madden, D., Urbaniak, K., Kim, I. J., Ma, W., Revilla, Y., and Richt, J. A. (2019). DNA-Protein Vaccination Strategy Does Not Protect from Challenge with African Swine Fever Virus Armenia 2007 Strain. *Vaccines (Basel)*. **28**;7(1):12.


## Patents

Coinventors: Yolanda Revilla and Daniel Pérez-Núñez. Title: RECOMBINANT AFRICAN SWINE FEVER VIRUS AS LIVE ATTENUATED VACCINE AGAINST AFRICAN SWINE FEVER. Number: 904 443 Applicant Consejo Superior de Investigaciones Científicas. Country: ES (Spain).

## International projects / Research networks

- ERA-NET CO-FUNDED CALL FOR PROPOSALS, International Coordination of Research on Infectious Animal Diseases (ICRAD). Grant Agreement N° 862605. Project: “Characterization of virus- and host-specific modulation of type I IFN in African Swine Fever Virus virulence or attenuation” (“IFNASF”), 2020-2023. Coordinator CBMSO, PI Yolanda Revilla, Daniel Pérez-Núñez co-PI.

- Transference of technology to develop vaccines against ASF Chinese strains. PI: Yolanda Revilla. Center for Animal Health Emerging diseases China (CAHEC), 2018-2021.

## MICROBES IN HEALTH AND WELFARE

# MODULATION OF INNATE IMMUNE RESPONSES BY VIRAL PROTEASES AND RNAS DERIVED FROM VIRAL GENOMES. BIOTHERAPEUTIC APPLICATION

***Principal Investigator:***
Margarita Sáiz Zalabardo

***Scientific Staff:***
Miguel Ángel Sanz Hernández
(since September 2019)

***Postdoctoral Fellows:***
Miguel Ramón Rodríguez Pulido

***Undergraduate and Master Students:***
Miryam Polo Hernández
(Master Student, academic year 2020-2021)
Lucía Camacho Pulido
(Undergraduate Student, academic year 2020-2021)

***Visiting Scientist:***
Ranjitha Huildore
(Indian Council of Agricultural Research, November 9, 2019 to February 4, 2020)

https://www.cbm.uam.es/msaiz

## Research summary

Our research interest is focused on i) the interplay between the host innate immunity system and foot-and-mouth disease virus (FMDV), a highly infectious and worldwide distributed RNA virus. Our studies include the identification of proteins involved in detection of the viral RNA genome, as well as the characterization of the immune evasion mechanisms exerted by the virus to counteract the host antiviral response based on triggering of the type-I interferon pathway in infected cells. The outcome of the balance between the antiviral response and viral antagonism may determine the onset of disease and pathogenesis and we are actively working on the identification of host innate effector proteins which are targets for the viral proteases. We have recently reported a novel mechanism of viral evasion based on the dual cleavage of LGP2 and MDA5, immune sensors of the RIG-I-like receptors family, at a conserved helicase motif by the FMDV Leader protease ($L_{pro}$). The proteolytic activity of the two virally encoded proteases ($L_{pro}$ and $3C_{pro}$) is being tested for interference with different signaling routes known to respond to infection by RNA viruses (RIG-I-like receptors, *Toll*-like receptors, cGAS/STING, RNA interference); ii) the biotherapeutic applications of synthetic non-coding RNAs derived from the FMDV genome (ncRNAs), known to induce a broad spectrum antiviral activity and to enhance specific B- and T-cell mediated immune responses elicited after vaccination. We have reported the inhibitory effect of the ncRNAs against infection by pathogens of different viral families including relevant zoonotic viruses (FMDV, West Nile virus, Rift Valley fever virus, African swine fever virus) and shown their biological activity in cells from different species including farm and wild animals, as well as *in vivo* in mice and swine. The use of these ncRNAs as antiviral molecules against human coronaviruses, including SARS-CoV-2, is being currently addressed. The results derived from these studies will contribute to gain knowledge on the interaction between viral pathogens and the innate immune system of the host cell and to design new and more effective strategies against viruses and likely other infectious diseases. Also, learning from how viruses counteract host immune responses through specific targeting of relevant effector proteins may help in the design of new therapeutic strategies for the treatment and prevention of diseases that involve the immune system.

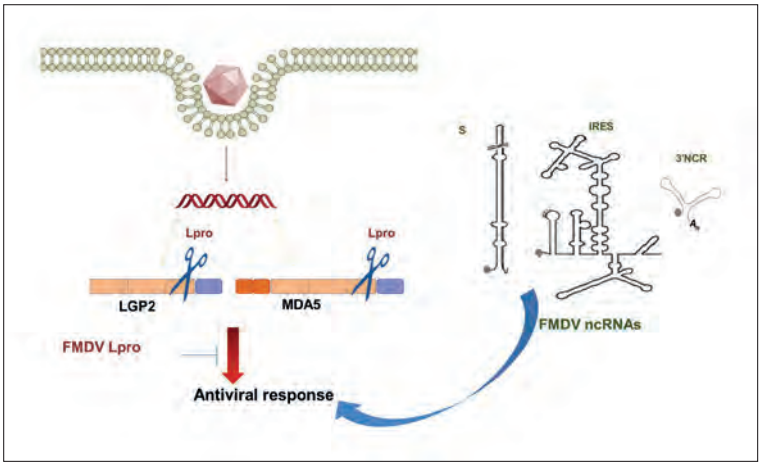



***Figure.*** *Schematic representation of the role of the FMDV Leader protease (Lpro) in disruption of the RIG-I-like receptors mediated antiviral response and the use of FMDV non coding RNAs (ncRNAs) as IFN-I inducers eliciting the host antiviral defense.*

## Publications

Rodriguez Pulido, M., Martinez-Salas, E., Sobrino, F., and Saiz, M. (2020) MDA5 cleavage by the Leader protease of foot-and-mouth disease virus reveals its pleiotropic effect against the host antiviral response. *Cell Death Dis*. **11**:718.

Rodríguez Pulido, M., Ranjitha, H. B., and Saiz, M. (2020) A wide-ranging antiviral response in wild boar cells is triggered by non-coding synthetic RNAs from the foot-and-mouth disease virus genome. *Front. Vet. Sci*. **7**:495.

Cañas-Arranz, R., Forner, M., Defaus, S., Rodríguez-Pulido, M., de León, P., Torres, E., Bustos, M. J., Borrego, B., Saiz, M., Blanco, E., Andreu, D., and Sobrino, F. (2020) A bivalent B-cell epitope dendrimer peptide can confer long-lasting immunity in swine against foot-and-mouth disease Transbound. *Emerg. Dis*. **67**, 1614-1622. .

Defaus, S., Forner, M., Cañas-Arranz, R., de León, P., Bustos, M. J., Rodríguez-Pulido, M., Blanco, E., Torres, E., Sobrino, F., and Andreu, D., Designing functionally versatile, highly immunogenic peptide-based multiepitopic vaccines against Foot-and-Mouth Disease Virus (2020) *Vaccines* **8**:406.

Sanz, M. A., Almela, E. G., García-Moreno, M., Marina, A. I., and Carrasco, L. A viral RNA motif involved in signaling the initiation of translation on non-AUG codons (2019) *RNA* **25**, 431-452.

Garcia-Moreno, M., Noerenberg, M., Ni, S., Järvelin, A. I., González-Almela, E., Lenz, C. E., Bach-Pages, M., Cox, V., Avolio, R. Davis, T., Hester, S., Sohier, T. J. M., Li, B., Heikel, G., Michlewski, G., Sanz, M. A., Carrasco, L., Ricci, E. P., Pelechano, V., Davis, I., Fischer, B., Mohammed, S. and Castello, A. System-wide Profiling of RNA-Binding Proteins Uncovers Key Regulators of Virus Infection (2019) *Molecular cell*. **74**, 196-21.

Sobrino, F., Caridi, F., Cañas-Arranz, R. and Rodríguez-Pulido, M. (2019) Foot-and-Mouth Disease Virus. In: Zakaryan, H (ed) Porcine Viruses: From Pathogenesis to Strategies for Control. *Caister Academic Press*, pp. 43-80.

## Awards and recognition

- Associate editor of Frontiers in Molecular and Infection Microbiology-Virus and Host section (since 2018).

- Associate editor of Virology Journal (BioMed Central, UK) (2012 - 2019).

## Doctoral Theses

**Esther María González Almela** (2019). Factores de iniciación implicados en la traducción de mRNAs virales. Importancia de los motivos estructurales en el RNA. Universidad Autónoma de Madrid. Co-directors: Miguel Ángel Sanz and Luis Carrasco. International Mention.

## International projects / Research networks

- Plataforma para el desarrollo de estrategias de control de Salud Animal (PLATESA2CM). Comunidad de Madrid/ FEDER S2018/BAA-4370 (2019-2021).

- Global Foot-and-mouth Research Alliance (GFRA) http://www.ars.usda.gov/GFRA.

## MICROBES IN HEALTH AND WELFARE

# NEW STRATEGIES FOR PREVENTION AND CONTROL OF VIRAL DISEASES: FOOT-AND-MOUTH DISEASE VIRUS AS A MODEL

***Principal Investigator:***
Francisco Sobrino

***Postdoctoral Fellows:***
Patricia de León
Elisa Torres
Flavia Caridi

***Predoctoral Fellows:***
Rodrigo Cañas

***Technician:***
María José Bustos

***Visiting Scientists:***
Gema Marin Royo

http://www.cbm.uam.es/fsobrino

## Research summary

Development of new, effective vaccines and antivirals are key aspects for animal and human health control. Foot-and-mouth disease virus (FMDV) is one of the major concerns for animal health. It is also an interesting model system for understanding the interactions of a highly variable RNA virus and its natural hosts and the implications of these interactions on disease control. We are working in the development of new FMDV peptide marker vaccines that can induce protective humoral and cellular immune responses, using pig and cattle, important domestics hosts, as animal models. In particular, we are further understanding the role of different FMDV epitopes in evoking protective responses, whose main known effectors are specific neutralizing antibodies, and using this information to optimize dendrimer peptide constructions displaying FMDV B- and T- cell epitopes as feasible field vaccines. We have also analyzed the functional role of FMDV proteins on the viral particle stability and internalization, the replication cycle and the mechanisms mediating the pathogenesis of FMDV and other related viruses causing vesicular diseases, such as swine vesicular disease virus (SVDV), and vesicular stomatitis virus (VSV). The role of different cellular lipids in the multiplication of these and other zoonotic viruses such as West Nile virus have also been addressed. As part of these studies, we have continued collaborating in the characterization of the inhibitory effect of valproic acid and other antiviral compounds targeting cellular metabolism such as lauryl gallate and cerulenin on the multiplication of FMDV and SVDV, as well as different enveloped viruses, like African swine fever virus (ASFV), VSV and type I herpesvirus.

The expertise gained in our group is being applied to explore the potential of the modular approach based on dendrimer peptides vaccines against SARS-CoV-2 as well as of the antiviral effect against different coronaviruses of compounds such as valproic acid and lauryl gallate.

## Publications

Jiménez de Oya, N., Sler, W. P., Kim Huard, K. El-Kattan, A., Karamanlidis, G., Blázquez, A. B., Ramos-Ibeas, P., Escribano-Romero, E., Louloudes-Lázaro, A., Casas, J., Sobrino, F., Hoehn, K., James, D. E., Gutiérrez-Adán, A., Saiz, J. C. and Martín-Acebes, M. A. (2019). Targeting host metabolism by inhibition of acetyl-Coenzyme A carboxylase reduces flavivirus infection in mouse models. *Emerging Microbes & Infections*, **8**:1, 624-636.

de León, P., Bustos, M. J., Torres, E., Cañas-Arranz, R., Sobrino, F*. and Carrascosa, A. L. (2019). Inhibition of porcine viruses by different cell-targeted antiviral drugs. *Frontiers Microbiol.* **10**, 1853.

Gil, M. J., González-González, R., Alvarez-Gutiérrez, A., Martín-Acebes, M. A., Vázquez-Calvo, A., Sáiz, J. C., López-Guerrero, J.A., Tabarés, E. and Sobrino, F. (2019). Herpes virus infections in patients treated with valproic Acid: A nested case-control study in the Spanish primary care database, BIFAP. *J. Clin. Med*. **8**, 1442.

Caridi F., López-Arguello, S., Rodriguez-Huete, A., Torres, E., Bustos, M. J., Cañas-Arranz, R., Martín-Acebes, M. A., Mateu, M. G. and Sobrino, F. (2020). Introduction in foot-and-mouth disease virus of a negatively charged group at the capsid internal surface reduces the virion-destabilizing effect of the viral RNA at acidic pH. *Sci. Rep*. **10**:1657.

Cañas-Arranz, R., Forner, M., Defaus, de León, P., Bustos, M. J., Torres, E., Andreu, D., Sobrino, F*. and Blanco, E. (2020). A single dose of dendrimer B2T peptide vaccine partially protects pigs against foot-and-mouth disease virus infection. *Vaccines* 2020, 8,19.**10**;8(1):19.

Cañas-Arranz, R., Forner, M., Defaus, S., Rodrigez-Pulido, M., de León, P., Torres, E., Bustos, M. J., Borrego, B., Sáiz, M., Blanco, E., Andreu, D. and Sobrino, F. (2020). A bivalent B-cell epitope dendrimer peptide can confer long lasting protective immunity in swine against foot-and-mouth disease. *Transbound. Emerg. Dis*. 67:1614-1622.

Cañas-Arranz, R., de León, P., Forner, M., Defaus, S., Bustos, M. J., Torres, E., Andreu, D., Blanco, E. and Sobrino, F. (2020). Immunogenicity of a dendrimer B2T peptide harboring a T-cell epitope from FMDV non-structural protein 3D. *Frontiers Vet. Sci.* **7**:498.

Defaus, S., Forner, R., Cañas-Arranz, R., de León, P., Bustos, M. J., Rodríguez-Pulido, M., Blanco, E, Sobrino, F*. and Andreu, D. (2020). Designing functionally versatile, highly immunogenic peptide-based multiepitopic vaccines against foot-and-mouth disease virus. *Vaccines* **8**(3):406.

Rodríguez-Pulido, M. Martínez-Salas, E., Sobrino, F. and Sáiz, M. (2020). MDA5 cleavage by the Leader protease of foot-and-mouth disease virus reveals its pleiotropic effect against the host antiviral response. Cell Death Dis. 11:718.

de León, P., Cañas-Arranz, R., Saez, Y., Forner, M., Defaus, S., Bustos, M. J., Torres, E., Cuadra, D., Andreu, A. Blanco, E., Sobrino, F*. and Hammer, S. (2020). Association of porcine SLA alleles with B- and T-cell immune response to foot-and-mouth disease virus peptides. *Vaccines* **8**, 513.

Orden, J. A., García-Meniño, I., Flament-Simon, S. C., Blanco, J., de la Fuente, R., Martínez-Rodrigo, A., Masa, A., Carrión, J., Sobrino, F. and Domínguez-Bernala, G. (2020). Raccoons (Procyon lotor) in Madrid region (Spain) are an important reservoir of antimicrobial resistant Escherichia coli and enteropathogenic E. coli. *Zoonoses and Public Health* **68**:69–78.

* F. Sobrino corresponding author.

Edited books:

Sobrino, F.*, Caridi, F., Cañas-Arranz, R. and Rodríguez-Pulido, M. (2019) Foot-and-mouth disease virus. In: Porcine viruses: from pathogenesis to strategies for control. H. Zakaryan (ed.). ISBN: 978-1-910190-91. doi.org/10.21775/9781910190913. Caister Academic Press, Norfolk, UK.

Escribano-Romero, E., Martín-Acebes, M. A., Vázquez-Calvo, A., Brocchi, E., Pezzoni, G., Sobrino, F.*, and Borrego, B. (2019) Swine vesicular disease virus. In: Porcine viruses: from pathogenesis to strategies for control. H. Zakaryan (ed.). ISBN: 978-1-910190-91-3. doi.org/10.21775/9781910190913. Caister Academic Press, Norfolk, UK.

## Patents

Peptide vaccines for the prevention of foot-and-mouth disease. PCT. Inventors: Sira Defaus, Mar Forner, Rodrigo Cañas, Patricia de León, María J. Bustos, Elisa Torres, Francisco Sobrino. Application EP20382406.5. Date of presentation: 14/05/2020.

Active Patents

Use of esters derived from gallic acid as antivirals. Inventores: de León, A. L. Carrascosa, M.J. Bustos, F. Sobrino. E. Torres, R. Cañas. PCT. Nº ES1641.1421. Date of presentation: 12-11-2018.

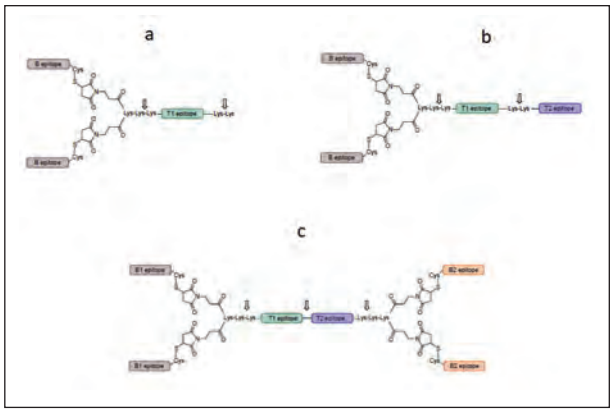



***Figure.*** *Dendrimer peptide vaccines: a modular approach. Scheme of the prototypes of FMDV dendrimeric peptide vaccine that conferred solid protection against homologous virus challenge in the pig. Bivalent-branched B-cell epitope immunogens (B2T) conjugated to one (a) or two (b) T-cell epitopes in tandem via thiol-maleimide linkages at both α- and ε-amino ends of a branched Lys core. (c) Tail-to-tail fusion of two B2T molecules via orthogonal chemical ligation (Click chemistry), leading to a B2T-TB2 multivalent platform. The arrows indicate a target for cathepsine D, a lysosomal protease suggested to be involved in MHC class II antigen processing. This vaccine approach can be extended to other pathogens provided continuous B- cell epitopes and efficient T-cell epitopes are identified.*

## Doctoral Theses

**Rodrigo Cañas Arranz** (2020). Synthetic dendrimer peptide vaccines against foot-and-mouth disease virus. Universidad Autónoma de Madrid. Co-supervised by F. Sobrino and E. Blanco (CISA-INIA). International Mention.

## International projects / Research networks

- EU funding

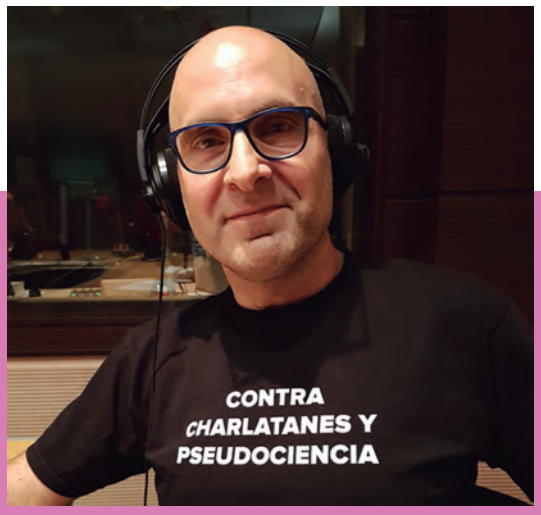
- VetBioNet Project (EU). REF: VBN_20_45. Characterization of the protection conferred and the B- and T-cell responses elicited in the MHC homozygous Babraham pigs by a dendrimeric peptide (B2T) FMD vaccine. (2020-2022). CBMSO, INIA, UPF. Grant coordinator: F. Sobrino.

# Science and Society

# SCIENCE AND SOCIETY

JOSÉ ANTONIO LÓPEZ GUERRERO

## SCIENCE AND SOCIETY DEPARTMENT




***Director:***
José Antonio López Guerrero

***Technical Director:***
Almudena Hernando

***Scientific Staff:***
Raquel Bello-Morales

***Collaborators:***
Begoña Aguado
Mar Ruiz
Noemí Tabanera
Beatriz Praena

http://www.cbm.uam.es/ccientifica

## Activities

Within the framework of the CBMSO Scientific Culture Program, The Department of Scientific Culture (DCC) of the CBMSO has collaborated in the following activities 2019-2020: 1) Scientific Weeks in Madrid (workshop and seminars in 2019 and webinars in 2020). 2) Annual program of 30 (2019-2020) guided visits of Secondary School students –about 1000 students- to our Research Centre. 3) Weblog "Bio(Ciencia+Tecnología)" of the Madri+d Foundation. 4) Social Networks: Facebook and Twitter. 5) Weekly-updated Institutional Website about Social Communication of Science (until June 2020). 6) Organization and coordination of the "Biotechnology Explorer" courses for secondary school teachers (Webinar in 2020). 7) Educational Program "4ESO + EMPRESA" of the Community of Madrid. 8) Stay at the CBMSO of the Students "Finalist of the Spanish Biology Olympiad". 9) Participation in Mass Media about Scientific Dissemination and Disclosure: Spanish National Radio (Radio 5 –"Entre Probetas" and "El Laboratorio de JAL"-, Radio 1 –"A Hombros de Gigantes- and Radio Exterior –"Marca España"-), Spanish National TV -TVE-1-, Spanish TV -La Sexta-; Collaborations with the magazines of scientific culture "El Cultural".

*2019: Plaque of Honor from the AEC (Spanish Association of Scientists).*

## Research

The NeuroVirology (UAM) group aims at the study of the effect of HSV-1 on neurodegeneration, demyelinating disease and new antiherpetic compounds in both immature and differentiated myelin-producing oligodendrocytic cells. Several cell receptors for viral entry have been described, but other observations suggest that more receptors for HSV-1 might exist. A novel role for the proteolipid protein (PLP) in HSV-1 entry into the human oligodendrocytic cell line HOG has been proposed. In addition, the UAM NeuroVirology group has describe the features of shedding microvesicles released by HOG cells infected with HSV-1 and their participation in the viral cycle. It has been detected for the first time microvesicles containing HSV-1 virions. Finally, we have started a new project on the effect of new antivirals and viricides on coronavirus infection.

## Publications

López Guerrero, J.A. (2019 2ºEd) VIRUS: Ni vivos ni muertos. *Ed. Guadalmazán. Spain* ISBN: 978-84-94778-62-9

Praena B, Bello-Morales R, de Castro F, López-Guerrero JA. "Amidic derivatives of valproic acid, valpromide and valnoctamide, inhibit HSV-1 infection in oligodendrocytes". *Antiviral Res*. 2019 Aug;**168**:91-99.

Gil M, González-González R, Vázquez-Calvo A, Álvarez-Gutiérrez A, Martín-Acebes MA, Praena B, Bello-Morales R, Saiz JC, López-Guerrero JA, Tabarés E, Sobrino F.J. (2019). "Clinical Infections by Herpesviruses in Patients Treated with Valproic Acid: A Nested Case-Control Study in the Spanish Primary Care Database, BIFAP". *Clin Med*. 2019 Sep **11**;8(9):1442.

Bello-Morales R, Ripa I, López-Guerrero JA. " Extracellular Vesicles in Viral Spread and Antiviral Response". *Viruses*. 2020 Jun **8**;12(6):623.

López-Guerrero JA, Ripa I, Andreu S, Bello-Morales R. "The Role of Extracellular Vesicles in Demyelination of the Central Nervous System". *Int J Mol Sci.* 2020 Nov 30;**21**(23):9111.

Bello-Morales R, López-Guerrero JA. "Isolation/Analysis of Extracellular Microvesicles from HSV-1-Infected Cells". *Methods Mol Biol*. 2020;**2060**:305-317.

Andreu S, Ripa I, Bello-Morales R, López-Guerrero JA. "Valproic Acid and Its Amidic Derivatives as New Antivirals against Alphaherpesviruses". *Viruses.* 2020 Nov 26;**12**(12):1356.

Bello-Morales R, Andreu S, López-Guerrero JA. "The Role of Herpes Simplex Virus Type 1 Infection in Demyelination of the Central Nervous System". *Int J Mol Sci*. 2020 Jul 16;**21**(14):5026.

Sánchez-León E, Bello-Morales R, López-Guerrero JA, Poveda A, Jiménez-Barbero J, Gironès N, Abrusci C. "Isolation and characterization of an exopolymer produced by Bacillus licheniformis: In vitro antiviral activity against enveloped viruses". *Carbohydr Polym*. 2020 Nov 15;**248**:116737.

López-Guerrero JA, de la Nuez C, Praena B, Sánchez-León E, Krummenacher C, Bello-Morales R. "Herpes Simplex Virus 1 Spread in Oligodendrocytic Cells Is Highly Dependent on MAL Proteolipid". *J Virol.* 2020 Jan 31;**94**(4):e01739-19.

Praena B, Bello-Morales R, López-Guerrero JA. "Hsv-1 Endocytic Entry into a Human Oligodendrocytic Cell Line is Mediated by Clathrin and Dynamin but Not Caveolin". *Viruses*. 2020 Jul **7**;12(7):734.

## Social communication of science

López-Guerrero, J.A. El Cultural:

- 08/2/2019 "¿Por qué vacunarse? La ciencia responde
- 13/11/2019 "Margarita Salas, mentora de grandes científicos"
- 30/12/2019 "Hitos del 2019"
- 22/01/2020 "La nueva emergencia viral se llama Wuhan"
- 17/03/2020-03/06/2020 "Diario de una cuarentena"
- 29/09/2020 "¿Una vacuna bajo presión?"
- 20/11/2020 "Vacunas… ¡y dos huevos duros!"
- 02/01/2021 "Hitos del 2020"

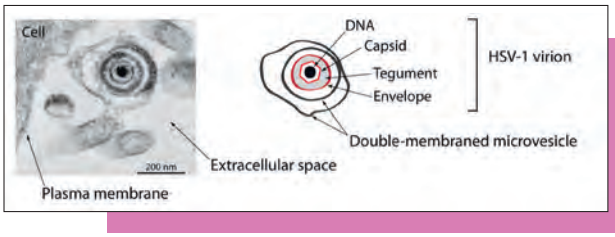
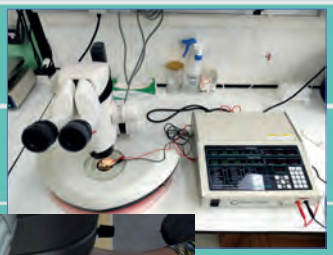


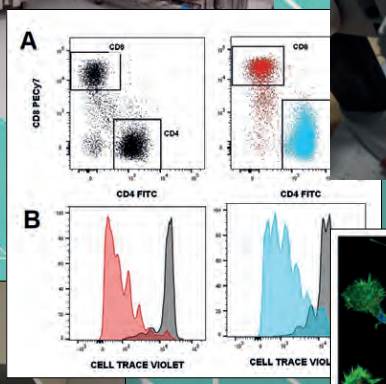

***Figure.*** *Transmission electron microscopy image showing a microvesicle enclosing a viral particle. Int.J.Mol.Sci, 2020.*

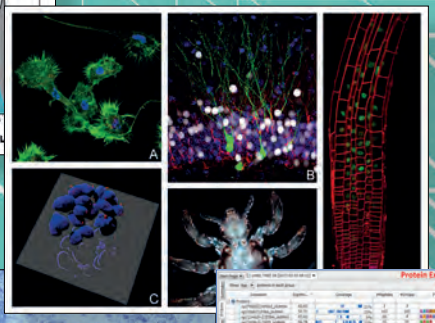
## Awards and recognition

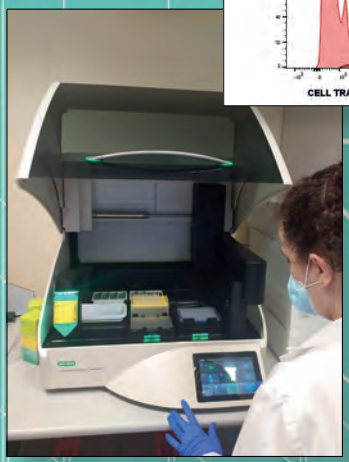
- 2019: Plaque of Honor from the AEC (Spanish Association of Scientists).
- 2020: Prize "Lupa Scéptica" of the ARP-SAPC.

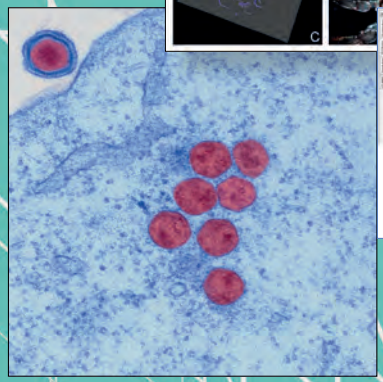
# Scientific Facilities







## SCIENTIFIC FACILITIES

# ANIMAL FACILITY

***Technical Director:***
Dra. Elena Hevia Hernández

***Scientific Supervisor:***
Dr. César Cobaleda Hernández

***Animal Health Veterinary Advisor:***
Dra. Elena Hevia Hernández

***Animal Welfare Supervisor:***
Fernando Núñez Martín

***Technical Personnel:***
Laura López Martínez
Beatriz García Martínez
José Mª Sedano Torres
Marta González Mella
Virginia González de la Torre
Blanca Sánchez Novillo
Amanda Abad Serrano
(VIVOTÉCNIA)

***Administrative Support:***
Miguel A. Bordallo Martín-Fontecha

***Animal Keepers:***
Alfonso Gutiérrez García
Staff from VIVOTÉCNIA

http://www.cbm.uam.es/animalario

## Description

It is a modern facility, designed and equipped with the latest advances in this type of facilities and its purpose is the production and maintenance of rodents and aquatic species (zebrafish, medakas and xenopus) under optimum conditions for use in the projects developed in the Center.

The installation also includes:

- SPF (Specific Pathogen Free) zone where the lines of genetically modified mice are produced
- Conventional or experimental zone
- Zones with Biocontainment Level 2 (BSL2), for mice and rats
- Zone with Biocontainment (BSL3), for mice
- Zone for the maintenance of aquatic animals
- Laboratories / Operating Rooms / Behavior Rooms/ Quarantines

On a regular basis, seminars are organized on subjects related to laboratory animals for the continuous training of both users and staff

Our goal is good health and animal welfare

## Services

- Rederivation of genetically modified lines of mice for introduction into the barrier zone
- Cryopreservation of mouse embryos and gametes.
- Maintenance and production of mice under SPF, BSL2, BSL3 and conventional conditions
- Maintenance of rats under conventional conditions and BSL2
- Maintenance and production of zebrafish and medakas
- Maintenance of Xenopus frogs
- Management of colonies of transgenic mice
- Production of pregnant females of rodents with known date
- Various types of administrations and extractions
- Support and technical advice to researchers
- Quarterly animal health checks externally, following the recommendations of FELASA
- Training of animal staff and users

# BIOINFORMATICS FACILITY

***Technical Director:***
David Abia Holgado

***Scientific Supervisor:***
Dr. Ugo Bastolla Bufalini

http://ub.cbm.uam.es

## Description

The mission of the Bioinformatics Facility is to support scientists, mainly from research groups of the CBMSO, in the bioinformatics analysis of their data, as well as to perform predictions that can guide their research and to advise them on the computational aspects of their research projects. In addition, the personnel in the Facility teach courses in CSIC training programs and participate in bioinformatics training of master´s students. Nowadays, biological experiments generate large amount of data that needs to be analyzed through bioinformatics tools to extract the information of interest, and experimental results must be complemented with bioinformatics analyses and predictions that help to rationalize them and to maximize the knowledge that they provide. In addition, “in silico” predictions may help researchers to optimize their experiments instead of relying uniquely on intuition or exhaustive search. These analyses and predictions are performed through computer programs developed by third parties or by the staff of the facility to tackle specific scientific problems and are run on personal computers or in the computing infrastructure of the Bioinformatics Facility.

The bioinformatics facility was involved in 3 research projects dealing with the Covid-19 pandemics. The first project, in collaboration with Balbino Alarcón’s group, consisted in the computational design and experimental validation of antibodies against the SARS-CoV2 Spike protein, and it generated a highly efficient method for detecting specific antibodies against Spike protein in blood serum, which was patented and licensed. The second one, in collaboration with Ugo Bastolla, Manuel Fresno and Laura García Bermejo from the Ramon y Cajal hospital, aims at investigating the relationship between ACE2 levels in the blood of patients and the severity of symptoms of SARS-CoV2 infection. The third project, in collaboration with the CNB-CBMSO transgenesis facility, was an in-silico study for humanizing the mouse ACE2 receptor using the CRISPR technique in order to generate a murine model of Covid-19...

## Services

The main functions of the Facility are the analysis of proteins at the sequence level (homologous search, family characterization, prediction of properties) and structure level (modeling of non-crystallized or mutant proteins and characterization of interactions using molecular dynamics).

## Publications

Gago-Córdoba, C., Val-Calvo, J., Miguel-Arribas A, Serrano, E., Singh, P.K., Abia, D., Wu, L.J. and Meijer, W.J.J. (2019) Surface exclusion revisited: Function related to differential expression of the surface exclusion system of Bacillus subtilis plasmid pLS20. *Front Microbiol.* **10**, 1502.

Martínez-Riaño, A., Bovolenta, E.R., Boccasavia, V.L., Ponomarenko, J., Abia, D., Oeste, C.L., Fresno, M., van Santen, H.M. and Alarcón, B (2019) RRAS2 shapes the TCR repertoire by setting the threshold for negative selection. *J Exp Med*. **216**, 2427-2447.

# FLOW CYTOMETRY FACILITY

***Technical Director:***
Dra. Berta Raposo Ponce

***Flow Cytometry Scientific Supervisor:***
Dra. María Luisa Toribio

***Cell Metabolism Scientific Supervisor:***
Nuria Martínez Martín

***Personnel:***
Silvia Andrade Calvo
Raquel Nieto Pintado

http://www.cbm.uam.es/scf

## Description

The flow cytometry facility provides access to state-of-the-art multiparametric flow cytometry and cell sorting. Available techniques range from classic cell immunophenotype, cell cycle and apoptosis determinations, or quantifications of cytokine production, cell signaling and metabolic pathways, to analyses of prokaryote organisms, dissociated discs of Drosophila larvae, or protoplasts and various plant cell types. Cell sorting provides high-purity cell separations, single-cell sorting, and particle enrichment. Determinations of protein-protein interactions based on FRET are also provided. Highly experienced and dedicated personnel offer training and advice on flow cytometry principles and applications, cell-sorting operation and equipment calibration and maintenance. Equipment includes two analogical cytometers with two lasers and four fluorescence detectors (FACScalibur), three digital cytometers, one with two lasers and six fluorescence detectors (FACSCanto A), and two with three lasers and eight fluorescence detectors (FACSCanto II), one equipped with High Throughput Sampler. The facility also includes two cell sorters, a FACSVantage SE with three lasers and six fluorescence detectors and a FACS Aria Fusion integrated inside a Class II biosafety cabinet, equipped with four lasers and sixteen fluorescence detectors. A Seahorse metabolic analyzer enables the detection of discrete changes in cellular bioenergetics in real-time using living cells in a 96-well platform.

## Services

- Training and advice on instrument operation.
- Advice on new experimental designs and adaptive setups.
- Training on postacquisition data analysis using “FlowJo” and “FACSDivA” softwares.
- Equipment operation and maintenance.
- Availability to commonly used reagents.

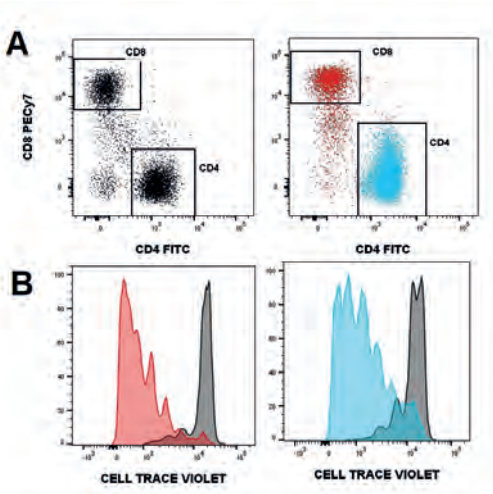



***Figure.*** *Tracking human T cell division by flow cytometry. Human peripheral blood lymphocytes, either non-stimulated or stimulated with anti-human CD3 antibody for 4 days in culture, were labeled with anti-CD3-PE, anti-CD4-FITC and anti-CD8-PECy7 antibodies and Cell Trace Violet. (A) Flow cytometry analysis of CD8 and CD4 expression in CD3-gated non- stimulated (left plot) and stimulated (right plot) T cells. (B) Successive divisions of CD8+ (blue) and CD4+ (red) stimulated cells. Black peaks represent CD8+ and CD4+ non-stimulated cells.*

# FERMENTATION FACILITY

***Technical Director:***
Dionisio Ureña Rodríguez

***Scientific Supervisor:***
Dr. José Berenguer Carlos

***Personnel:***
María Isabel Carrascal Blanco

http://www.cbm.uam.es/fermentacion

## Description

The Fermentation facility plays two main roles on the CBMSO scientific activities, one focused on the growth of natural and recombinant microorganisms, and the other centered on the production of biological consumables. At the level of microorganism growth, the Fermentation Facility provides advice on the appropriate bacterial strains, plasmid expression systems, and conditions for the overproduction of recombinant proteins and the scale up of the production process from Erlenmeyer to larger fermenters, allowing also the isotopic labeling of the expressed proteins when required for structural analysis. The facility also allows for the cultivation of a great variety of non-recombinant microorganisms in large volumes. In all cases, the growth in fermenters is monitored for the main parameters (temperature, stirring, pH, oxygen concentration, foam and biomass, in compliance with cGMP rules (current Good Manufacturing Practice).

On the other hand, the Fermentation Facility provides ready-to-use competent preparations of different strains of E.coli suitable for gene cloning or expression of recombinant proteins. It also produces DNA size markers for the internal use by most of the research groups in the CBMSO.

## Services

- Cultures of microorganisms in 4, 10 or 30 liters reactors
- Improvement and production of recombinant proteins in cultures of bacteria, yeast or filamentous fungi
- Scale up for overproduction in cultures from Erlenmeyer to high capacity fermenter
- Isotope labeling of expressed proteins in *Escherichia coli* for structural analysis
- Ready-to-use *Escherichia coli* competent cells
- DNA molecular weight markers
- Disruption of cell cultures by French Press

# GENOMICS AND NEXT GENERATION SEQUENCING FACILITY (GENGS)

***Technical Director:***
Fernando Carrasco Ramiro

***Scientific Supervisor:***
Dra. Begoña Aguado Orea

***Genomics Section Coordinator***
Dra. Laura Tabera Moreno

***NGS Section Coordinator***

Ramón Peiró Pastor (until August 2020)

***Personnel:***

Sandra Gonzalo Flores
Manuel Belda Ávila
María José López Sánchez
Sandra González de la Fuente
Eva Sacristán Horcajada
Eva Castillo Rosa (until November 2020)

http://www.cbm.uam.es/genomica

## Description

The Genomics and Next-Generation Sequencing (NGS) Facility (GENGS) is responsible for the implementation and development of molecular biology, genomic and NGS technologies and provides advice and technical supervision on these methodologies to research groups. In particular, it offers technical advice and support in the experimental design, undertaking, and data analysis of PCR, RT-PCR, qPCR, RT-qPCR and, more recently, digital PCR experiments. Related to NGS, the facility offers advice on the experimental design, mediates between diverse NGS platforms and the users, and is responsible for monitoring the development of the projects. Very appreciated is the provided tailored computational analysis of data obtained from NGS, microarrays, or proteomics experiments. In addition, the facility organises specialised seminars, meetings and courses and is involved in different universities teaching programs. It also contributes to different science dissemination activities through the Center. From March 2020 GENGS is actively contributing to SARS-Cov-2 research projects, such as the “Centinela” led by Dr Margarita del Val, and the “Air-COVID” directed by and Dr Antonio Alcamí. The GENGS Facility has developed four very sensitive in-house SARS-Cov-2 RT-qPCR assays, currently protected as trade secrets by CSIC.

## Organised courses

NGS data analysis. CSIC, FSO, 2019.

Genomics: past, present and future. Secondary school teachers training courses. Comunidad de Madrid-CSIC 2019.

## Publications


Sánchez M, Blesa A, Sacristán-Horcajada E, Berenguer J. Complete Genome Sequence of Mycolicibacterium hassiacum DSM 44199. *Microbiol Resour Announc*,2019.

Blesa, A.; Sánchez, M.; Sacristán-Horcajada, E.; González-de la Fuente, S.; Peiró, R.; Berenguer, J. Into the Thermus Mobilome: Presence, Diversity and Recent Activities of Insertion Sequences Across Thermus spp. *Microorganisms*, 2019.

Sequeira-Mendes J, Vergara Z, Peiró R, Morata J, Araguez I, Costas C, Mendez Giraldez R, Casacuberta JM , Bastolla U, Gutierrez C. Differences in firing efficiency, chromatin and transcription underlie the developmental plasticity of the Arabidopsis DNA replication origins. *Genome Research*, 2019.

Camacho E, González-de la Fuente S, Rastrojo A, Peiró-Pastor R, Solana JC, Tabera L, Gamarro F, Carrasco-Ramiro F, Requena JM, Aguado B. Complete assembly of the Leishmania donovani (HU3 strain) genome and transcriptome annotation. *Sci Rep.*, 2019.

Peiró-Pastor R, Carrasco-Ramiro F, Ramos-Ruiz R, Aguado B: Genomics Data Treatment in the Era of Next Generation Sequencing. In: Alejandro Cifuentes (Ed.) *Comprehensive foodomics*, Elsevier, 2019.

García-Olmo DC, Peiró-Pastor R, Picazo MG, Olmedillas-López S, García-Arranz M, Aguado B, García-Olmo D: Liquid biopsy by NGS: Differential presence of exons (DPE) is related to metastatic potential in a colon-cancer model in the rat. *Translational Oncology*, 2020.

Sanchiz Á, Morato E, Rastrojo A, Camacho E, González-de la Fuente S, Marina A, Aguado B, Requena J M. The Experimental Proteome of Leishmania infantum Promastigote and Its Usefulness for Improving Gene Annotations. *Genes*, 2020.

Pérez-Núñez D, Castillo-Rosa E, Vigara-Astillero G, García-Belmonte R, Gallardo C, Revilla Y. Identification and Isolation of Two Different Subpopulations Within African Swine Fever Virus Arm/07 Stock. *Vaccines*, 2020

Blesa A, Baquedano I, González-de la Fuente S, Mencía M, Berenguer J. Integrative and Conjugative Element ICETh1 Functions as a Pangenomic DNA Capture Module in *Thermus thermophilus*. *Microorganisms*, 2020.

Sacristán-Horcajada E, González-de la Fuente S, Peiró-Pastor R, Carrasco-Ramiro F, Amils R, Requena JM, Berenguer J, Aguado B. ARAMIS: From systematic errors of NGS long reads to accurate assemblies. *Brief. Bioinf.*, 2021.


## Services

Nanodrop spectrophotometers.

Maxwell16 and 48 (Promega) automated nucleic acid extractors.

Real-time thermocyclers: Lightcycler (3 units). ABI 7900HT.

Ingenuity Pathway Analysis (IPA) software.

RNA integrity (Agilent bioanalyzer).

Nucleic acid concentration by fluorometry (Promega Quantus).

Eppendorf epMotion pipetting robot, GenEx analysis software.

PCR, RT-PCR, qPCR, RT-qPCR and digital PCR: experimental design, performance and analysis of experiments.

- qPCR, RT-qPCR: ABI 7900HT, Bio-Rad CFX384
- digital PCR: Bio-Rad QX200 AutoDG Droplet Digital PCR

NGS: DNA-seq, RNA-seq, ChIP-seq, metagenomics, amplicons, etc. Advice on grant proposals, experimental design, mediation with external sequencing platforms and follow-up of projects. Access to different technologies: Illumina, PacBio and Oxford Nanopore.

NGS data computational analysis: alignments, assemblies, genome annotation, differential expression studies, biological diversity, etc. using different software packages as well as in-house specific developed software. Analysis of data from any NGS platform.

Other: microarray & statistical data analysis, primer design, training in computational and genomics techniques, software development.

## Patents

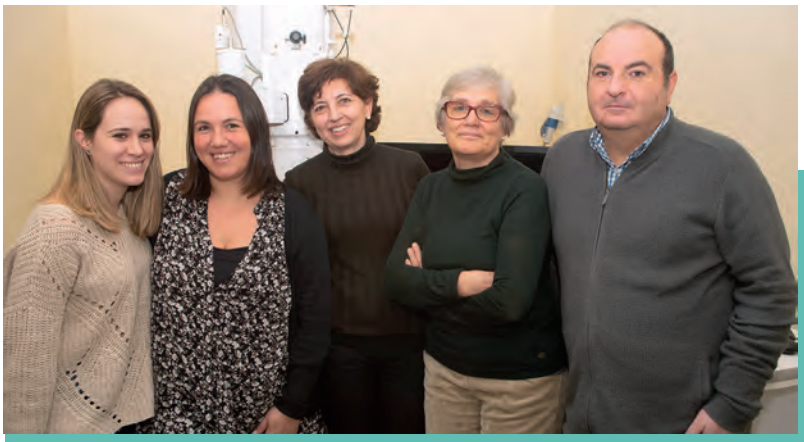
RT-qPCR SARS-CoV-2 assays registered as trade secret: 5721/2020, 5722/2020, 5723/2020 and 5724/2020.

# ELECTRON MICROSCOPY FACILITY (EMF)

***Technical Director:***
Dra. María Teresa Rejas Marco

***Scientific Supervisor:***
Dr. Germán Andrés Hernández

***Personnel:***
Milagros Guerra Rodríguez
Dra. Tamara Matamoros Grande
Lidia Romo Patiño



http://www.cbm.uam.es/sme

## Description

The electron microscopy facility (EMF) provides scientific and technical support to research teams interested in using transmission EM to analyze macromolecular assembles, viruses, bacteria, eukaryotic cells, multicellular specimens and tissues. Available methods for sample processing include conventional techniques and cryo-techniques such as freeze-substitution, cryosectioning and freeze-etching. We also offer methods for in situ localization of nucleic acids and proteins, including correlative light-electron microscopy (CLEM). The EMF equipment includes two transmission electron microscopes of 100kV and 120kV, equipped with 4k x 4k CMOS cameras, an inverted fluorescence microscope devoted to CLEM techniques and the following instruments for sample processing: a plunge-freezing unit, an automatic freeze-substitution system, a conventional ultramicrotome, a cryoultramicrotome and a freeze-etching unit. During the 2019-2020 period, the EMF has been used by more than 50 research groups and has contributed to 18 publications.

## Services

1. Technical and scientific supervision on experimental design and data analysis
2. Training and supervision of EM facility users
3. Negative staining of macromolecular complexes, nanoparticles and viruses
4. Chemical fixation and resin-embedding of biological specimens
5. Cryofixation, freeze-substitution and low temperature embedding
6. Ultramicrotomy of resin embedded samples
7. Tokuyasu´s cryosectioning
8. Immunolectron microscopy with gold conjugates
9. Correlative light-electron microscopy
10. Freeze-fracture, freeze-etching and Pt-C replication
11. Electron microscopy of nucleic acids

## Publications

Grueso E, Sánchez-Martínez C, Calvo-López T, de Miguel FJ, Blanco-Menéndez N, Fernandez-Estevez M, Elizalde M, Sanchez J, Kourani O, Martin D, Tato A, Guerra M, Andrés G, Almendral JM. Antiangiogenic Vascular Endothelial Growth Factor-Blocking Peptides Displayed on the Capsid of an Infectious Oncolytic Parvovirus: Assembly and Immune Interactions. *J Virol*. 2019 Sep 12;**93**(19):e00798-19.

Andrés G, Charro D, Matamoros T, Dillard RS, Abrescia NGA. The cryo-EM structure of African swine fever virus unravels a unique architecture comprising two icosahedral protein capsids and two lipoprotein membranes. *J Biol Chem*. 2020 Jan 3;**295**(1):1-12. (editor´s pick).

González-Méndez L, Gradilla AC, Sánchez-Hernández D, González E, Aguirre-Tamaral A, Jiménez-Jiménez C, Guerra M, Aguilar G, Andrés G, Falcón-Pérez JM, Guerrero I. Polarized sorting of Patched enables cytoneme-mediated Hedgehog reception in the Drosophila wing disc. *EMBO J*. 2020 Jun 2;**39**(11):e103629. (journal cover).

Matamoros T, Alejo A, Rodríguez JM, Hernáez B, Guerra M, Fraile-Ramos A, Andrés G. African Swine Fever Virus Protein pE199L Mediates Virus Entry by Enabling Membrane Fusion and Core Penetration. *mBio*. 2020 Aug 11;**11**(4):e00789-20.

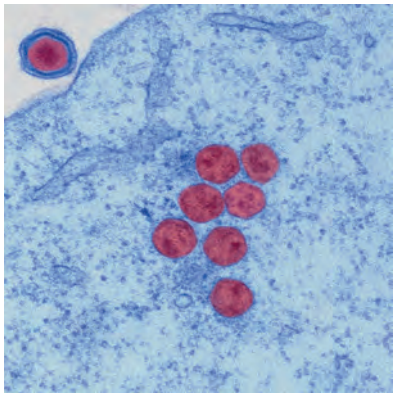


***Figure.*** *The electron micrograph shows an African swine fever virus particle (upper left) and a cluster of cytosolic "naked" cores (in red) delivered into the cytoplasm after virus entry (See related article: Matamoros et al. 2020 mBio. 11:e00789-20.).*

# OPTICAL AND CONFOCAL MICROSCOPY CORE FACILITY (SMOC)

***Technical Director:***
María Ángeles Muñoz Alcalá

***Scientific Supervisor:***
Dr. Javier Díez Guerra

***Personnel:***
Teresa Villalba Villacorta
Carmen Sánchez Jiménez
Alfonsa Díaz Torres (until December 2019)
Carlos Gallego García (since September 2019)
Francisco José Vega Sabugo (since September 2019)
Elena Calvo Cazalilla (since February 2020)

http://www.cbm.uam.es/confocal

## Description

The SMOC facility was created in 1999 being the responsible of maintaining and managing the advanced-optical-microscopy equipment in the CBMSO. It offers 6 laser scanning confocal microscopes, 1 spinning disk confocal microscope and 6 wide-field microscopes, with different levels of automation allowing studies for long-term in vivo experiments and fixed samples. In addition, users have at their disposal 2 workstations for image analysis, a vibratome and a stereomicroscope. The SMOC staff offers support, advice and training in optical microscopy techniques and image analysis, distributes reagents and other materials useful for microscopy, and is responsible for finding resources to acquire new equipment that suits the requests of CBMSO researchers. The daily work is organized through an online platform to schedule and manage the equipment, control the user rights and do the invoicing (PPMS). Usually, the SMOC staff organizes theoretical and practical training seminars.

The SMOC facility has implemented a quality management system ISO 9001:2015 certified by AENOR since March 2009. It is a member of the Spanish Network of Advanced Optical Microscopy (REMOA), the “Red de Laboratorios e Infraestructuras (RedLab)” in “Comunidad de Madrid” (CM) (Laboratory nº216) since 2007, and the “Plataforma de Microscopía para Biociencias” in CM created in 2012.

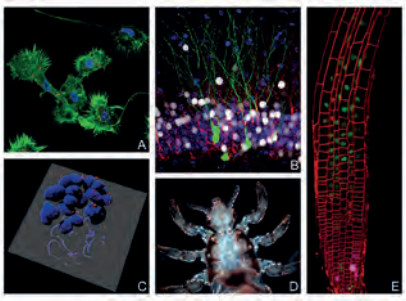



***Figure**. Different kind of images and representations in microscopy. (A) Fluorescent microglial cells and (B) mouse brain tissue acquired by confocal microscopy. (C) 3D rendering of Cysts. (D) A louse adquired by dark field microscopy. (E) Tile scan of a fluorescent Arabidopsis thaliana root.*

## Services

1. Controlled access and use of the microscopy equipment
2. Management of online reservations and invoicing
3. User assistance and training
4. Organization of seminars and development of tutorials and guides
5. Maintenance and update of the facility web page (http://www.cbm.uam.es/confocal)
6. Stock and distribution of common use reagents for optical microscopy applications
7. Forecast future needs of the Institute in microscopy through user surveys

## Publications

Moreno-Jiménez EP, Flor-García M, Terreros-Roncal J, Rábano A, Cafini F, Pallas-Bazarra N, Ávila J, Llorens-Martín M. (2019) Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer’s disease. *Nat. Med*. **25**(4):554-560.

Bernascone I, González T, Barea MD, Carabaña C, Hachimi M, Bosch-Fortea M, Santamaria S, Martin R, Tarnick J, Garcia-Sanz JA, Martín-Belmonte F. (2019) Sfrp3 modulates stromal–epithelial crosstalk during mammary gland development by regulating Wnt levels. *Nat. Commun*. **10**(1):2481.

Navarrete M, Cuartero MI, Palenzuela R, Draffin JE, Konomi A, Serra I, Colié S, Castaño-Castaño S, Hasan MT, Nebreda ÁR, Esteban JA. (2019) Astrocytic p38α MAPK drives NMDA receptor-dependent long-term depression and modulates long-term memory. *Nat. Commun*. **10**(1):2968.

Flor-García M, Terreros-Roncal J, Moreno-Jiménez EP, Ávila J, Rábano A, Llorens-Martín M. (2020) Unraveling human adult hippocampal neurogenesis. *Nat. Protoc*. **15**(2):668-693.

Desvoyes B, Arana-Echarri A, Barea MD, Gutierrez C. (2020) A comprehensive fluorescent sensor for spatiotemporal cell cycle analysis in Arabidopsis. *Nat. Plants*. **6**(11):1330-1334.

# PROTEOMICS AND PROTEIN CHEMISTRY CORE FACILITY

***Technical Director:***
Dra. Anabel Marina Ramírez

***Scientific Supervisor:***
Dr. José María Cuezva

***Personnel:***
Dr. Carlos García García
Dra. Esperanza Morato López
Nuria Sánchez López
Laura Peláez Aguado

www.cbm.uam.es/servicios/proteomica.htm
www.proteored.org.

## Description

In 1993 the Protein Chemistry Facility was created in the CBMSO. The trajectory of the Proteomics Facility has ever since paralleled the evolution of the scientific community in the study of proteins and its objectives are to provide technical support and advice to researchers on the appropriate methods of sample preparation and design of the appropriate work-flow for each objective. The laboratory is equipped with systems to run electrophoresis and two mass spectrometers: a LTQ-VELOS (2D-Ion Trap in tandem) and a LTQ-ORBITRAP-VELOS-PRO (2D-Ion Trap in tandem coupled to Orbitrap analyzer) both from Thermo-Scientific. Our service has been used for numerous research projects in a broad range of scientific fields.

**Training courses:**

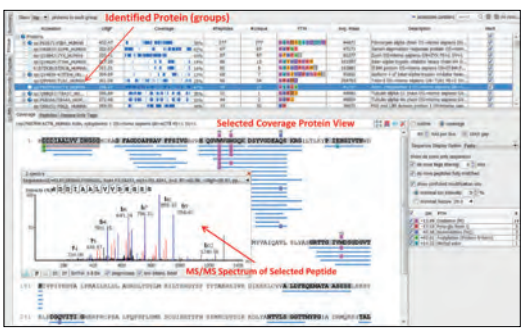
"EuPA Summer School on PostTranslational Modifications", French Proteomics Society, Sète, Francia.
"Introducción a Tissue MALDI Imaging Mass Spectrometry" Plataforma de Proteómica, CIC bioGUNE. Derio (Bizkaia).
"Cromatografía de Líquidos Acoplada a la Espectrometría de Masas", AET CSIC.
"IX Curso de Proteómica Cuantitativa" CNB, Madrid.

**Participation in national and international meetings on proteomics.**

"VI Jornadas de Jóvenes Investigadores en Proteómica". Madrid.
"XVIII Congreso Nacional de Biotecnología y Bioingeniería", México.
"PROTEOMIC FORUM 2019 XIII". Annual Congress of the European Proteomics Association: From Genes via Proteins and their Interactions to Functions. Alemania.
"X Reunión Científica sobre Proteómica Clínica" Hospital Clínico Universitario Lozano Blesa, Zaragoza.
"PROTEOVIEDO"_"Online Meeting".
•Conference at "Tercer coloquio en Biotecnología", Universidad Tecnológica de Corregidora, México.

**Guests:**

Carlos Álvarez_ Universidad Austral de Chile.
Inés Zapico_U.C.M.
Martín Hugo_ Instituto Investigación Princesa.
Silvia Vega_Instituto Ciencias Agrarias.
Juan San Francisco_Universidad de Antofagasta, Chile.




## Services

- SDS-PAGE electrophoresis.
- Gel staining.
- Sample preparation (precipitation, digestion, desalting).
- Protein Identification by LC-MS/MS (ESI-LTQ-VELOS/ORBITRAP).
- Protein and proteome characterization (analysis of post-traslational modifications).
- Relative quantification of differences in protein abundance by isobaric methods (TMT, iTRAQ, SILAC) and Label Free methods.
- De novo sequencing.

## Publications

Sanz MA, Almela EG, García-Moreno M, Marina AI, Carrasco L. A viral RNA motif involved in signaling the initiation of translation on non-AUG codons. *RNA*. 2019 Apr;**25**(4):431-452.

Fernández-Puente P, González-Rodríguez L, Calamia V, Picchi F, Lourido L, Camacho-Encina M, Oreiro N, Rocha B, Paz-González R, Marina A, García C, Blanco FJ, Ruiz-Romero C. Analysis of Endogenous Peptides Released from Osteoarthritic Cartilage Unravels Novel Pathogenic Markers. *Mol Cell Proteomics*. 2019 Oct;**18**(10):2018-2028.

Hernández F, Cuadros R, Ollá I, García C, Ferrer I, Perry G, Avila J. Differences in structure and function between human and murine tau. *Biochim Biophys Acta Mol Basis Dis*. 2019 Aug 1;**1865**(8):2024-2030.

Sanchiz Á, Morato E, Rastrojo A, Camacho E, González-de la Fuente SG, Marina A, Aguado B, Requena JM. The Experimental Proteome of Leishmania infantum Promastigote and Its Usefulness for Improving Gene Annotations. *Genes (Basel)*. 2020 Sep 2;**11**(9):1036.

Durán D, Albareda M, Marina A, García C, Ruiz-Argüeso T, Palacios J. Proteome analysis reveals a significant host-specific response in Rhizobium leguminosarum bv viciae endosymbiotic cells. *Mol Cell Proteomics*. 2020 Nov 19:mcp.RA120.002276.

# *DROSOPHILA* TRANSGENESIS FACILITY

***Technical Directors:***
Eva Caminero Jiménez
Mar Casado García

***Scientific Supervisor:***
Dra. Mar Ruiz Gómez

www.cbm.uam.es/transgenesisdro

## Description

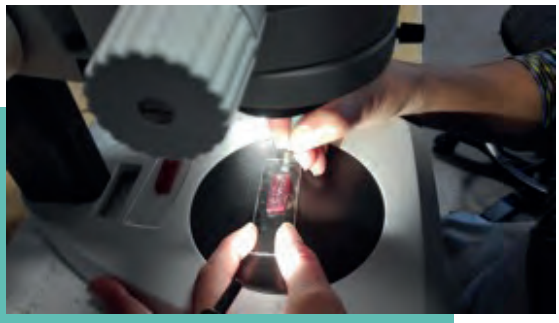
The *Drosophila* Transgenesis Facility was established in 2014 to provide technical support to research teams at CBMSO, CSIC and other national and international groups, by generating genetically modified *Drosophila* strains.

It is equipped with two microinjection setups including microinjectors, stereomicroscopes with cold light source units and one micropipette puller as well as several incubators for desiccating embryos and for maintenance of *Drosophila* stocks.

The main activity of the transgenesis facility is to generate *Drosophila* transgenic lines and to inject cocktails for CRISPR/Cas genome editing. The facility also maintains a collection of over 500 Drosophila stocks and a *Drosophila* cDNA plasmid collection, available to researchers upon request.

## Services

1. Generation of transgenic strains mediated by the transposon P (random insertion into *yw* or *w* strains)
2. Helper DNA is provided by the facility, that also prepares the injection mixture
3. Targeted insertion of transgenes mediated by the PhiC31 integrase into the following acceptor strains: ZH-attP-22A, ZH-attP-51D, ZH-attP-68E, ZH-attP-86Fb
4. Transgenesis with BAC vectors [P(acman) collection]
5. Injection of DNA cocktails for gene editing using CRISPR/Cas technology in the strains: 25C, 68A, Nos-cas9 and Vasa-cas9
6. Insertion of DNA into specific strains provided by the costumer
7. Shipment of injected embryos or generation and shipment of transgenic lines according to the requested service
8. Shipment of *Drosophila* stocks from the collection
9. Plating and shipment of cDNA plasmids



# CBM-CNB TRANSGENESIS FACILITY

***Scientific Supervisor and Technical Director:***
Dra. Mª Belén Pintado Sanjuanbenito
(CNB)

***Personnel:***
Verónica Domínguez Plaza
(ES cell and CRISPR/Cas9 based models)
CBMSO

Mª José Palacios Barea
(embryo collection and transfer,
colony management)

http://www.cbm.uam.es/transgenesis

## Description

The Transgenesis Service is a joint scientific service shared by CBMSO and CNB that provides support to research groups in the creation, interchange and management of genetically modified mouse models. The service covers all the required steps: from founder generation, to breeding and management of lines. Models can be generated with traditional technology, transgenesis or ES cell derived gene targeting, and also with CRISPR/Cas9 based genome edition trough embryo microinjection or electroporation. The Transgenesis Service offers technical and scientific support complementing the expertise of our customers, advising on the best strategy to obtain the desired model. The service counts with two fully equipped microinjection settings, one electroporator specifically designed for embryo edition, a standard molecular biology laboratory and a laboratory for ES cells. With full access to the animal facilities of CBMSO and CNB we deliver the newly created animal models in the barrier of both centers. The service is integrated in the scientific-technological platform INNOTEK (UAM+CSIC). The service is also involved in the production and characterization of two new mouse models for the study of COVID-19. The general activity of the service is complemented with the organization of specific workshops and the participation in specialization courses and master programs.

## Services

- Rederivation of genetically modified mouse lines from external animal facilities
- Technical and scientific support in the design of target vectors, transgenes and guides for genome edition
- Pronuclear microinjection of plasmidic, BAC and YAC DNA
- Gene edition based on CRISPR/Cas9 technology, from guides design and validation to embryo microinjection and electroporation
- Generation of KO and KI models based on ES cell lines
- Derivation of new murine ES cell lines
- Support in establishment and management of genetically altered mouse lines
- Specific training in collection, handling and culture of mouse embryos in preimplantational stages

## Publications

Hernández, I.H., Cabrera, J.R , Santos-Galindo, M., Sánchez-Martín, M, Domínguez, V., García-Escudero, R. Pérez-Álvarez, M.J., Pintado, B., Lucas J.J.. (2020) Pathogenic SREK1 decrease in Huntington's disease lowers TAF1 mimicking X-linked dystonia parkinsonism. *Brain*, Volume **143**, Issue 7, July 2020, Pages 2207–2219.

# General Services

## GENERAL SERVICES

**ADMINISTRATION**

**BIOLOGICAL SECURITY**

**CELL CULTURE, WASHING AND STERILIZATION**

**COMPUTING**

**GRAPHIC DESIGN, PHOTOGRAPY**

**INSTITUTIONAL RELATIONS**

**INSTRUMENTS AND EQUIPMENT**

**LIBRARY**

**MAINTENANCE**

**MANAGEMENT**

**MANAGEMENT BOARD**

**NATIONAL AND INTERNATIONAL PROGRAMS**

**PERSONNEL MANAGEMENT**

**PURCHASE DEPARTMENT**

**QUALITY MANAGEMENT**

**SAFETY AND OCCUPATIONAL RISK PREVENTION**



### ADMINISTRATION

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David Arjona Lara
Carmen Arroyo Martín
Montserrat Barbero Maturana
Celia Conde Mateos
Miguel Pérez Pulido
Sonia Rubio Lago
Rosa Sánchez Caballero
Gregorio Javier Sánchez García
Belén Villar Pérez

### LIBRARY

María Luisa Gorines López

### PERSONNEL MANAGEMENT

Lucía Horrillo Muñoz
Jessica Martínez Santos
Isabel de la Rosa Santos

### CELL CULTURE, WASHING AND STERILIZATION

Mercedes Dávila Cerrato
Mª Carmen Alonso Barba
Mª Ángeles Blanco Ferreras
Irene Bustos Sánchez
María Cazorla Plaza
Antonia Cerrato Gómez
Marta Fierro Fernández
Anunciación Gaceo Esteban
Miriam García Carrascal
Lorena García Murillo
Mª Teresa Gómez Buendía
Josefina González-Nicolás
Mª Nieves Martín Bermejo
F. Borja Mirasol Burgos
Ana Mª Pérez Colmenar
Juan Antonio Rebelles Vicente

### MANAGEMENT BOARD

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José Antonio Pérez Gracia

### QUALITY MANAGEMENT

Amalia Aneiros López

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Diego Díaz Rodilla
Ángel J. Gonzalo Gonzalo
María Peña Pérez García


**MANAGEMENT**

Germán Lerma Rodrigo

**NATIONAL AND INTERNATIONAL PROGRAMS**

Volga del Castillo Domingo

**PURCHASE DEPARTMENT**

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José Miguel Celestén Martín
Mª José Fernández Martín
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Herminio E. Jiménez Bassalo
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Angeles Sánchez Sánchez
Gema Caparrós de la Jara
Sandra Núñez Egido
Mª Cruz Valladares Bartolomé

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Almudena Hernando

**INSTRUMENTS AND EQUIPMENT**

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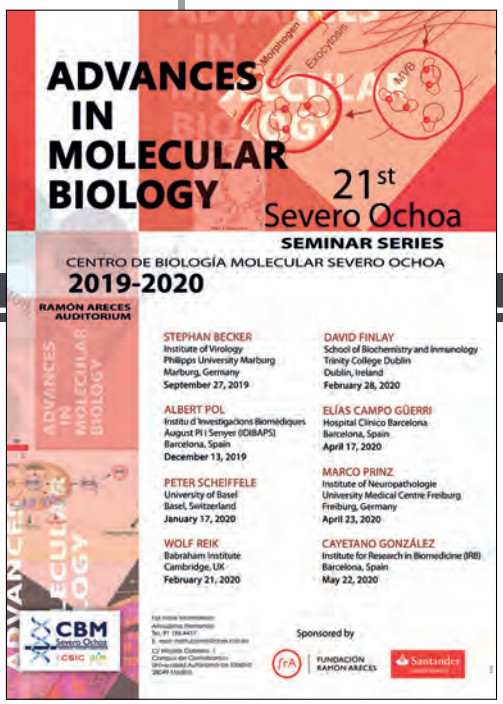
Fernando Muñoz Maqueda
Lenin Germán Tipán Basantes

**SAFETY AND OCCUPATIONAL RISK PREVENTION**

Fernando García Muñoz

# Seminars and Lectures


*SEVERO OCHOA SEMINARS CYCLE*

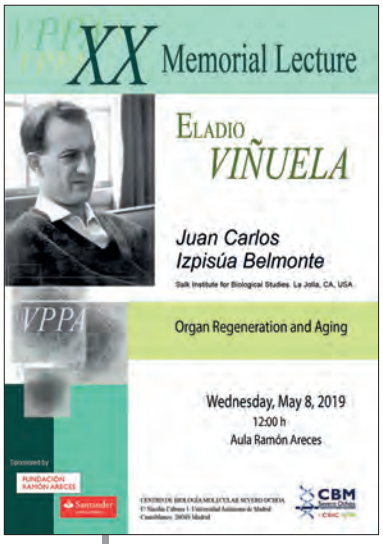




*ADVANCES IN BIOMEDICINE SEMINAR CYCLE*

## SEMINARS AND LECTURES

# MEMORIAL LECTURES (2019-2020)




***XX ELADIO VIÑUELA MEMORIAL LECTURE 2019***

**JUAN CARLOS IZPISÚA BELMONTE**

Salk Institute for Biological Studies, La Jolla, CA, USA

***Organ Regeneration and Aging***

May 8, 2019


***13 DAVID VÁZQUEZ MEMORIAL LECTURE***

**ERNESTO GARCÍA**

Centro de Investigaciones Biológicas (CIB), Madrid

***La colina y las proteínas de unión a colina de neumococo: más de un siglo de historia***

March 15, 2019

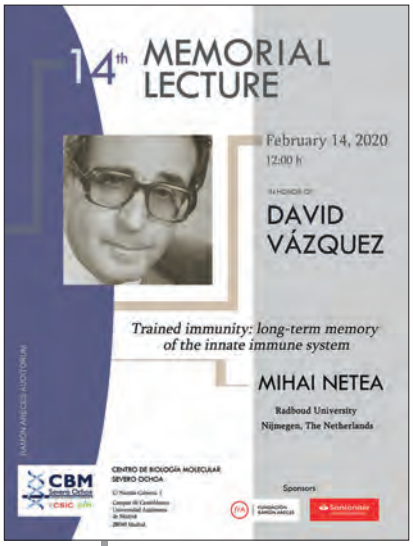

***26th SEVERO OCHOA MEMORIAL LECTURE***

**ÁNGELA NIETO**

Instituto de Neurociencias de Alicante, Alicante, Spain

***Cell plasticity in health and disease***

November 22, 2019




***14th DAVID VÁZQUEZ MEMORIAL LECTURE***

**MIHAI NETEA**

Radboud University Nijmegen, The Netherlands

***Trained immunity: long-term memory of the innate immune system***

February 14, 2020


***1st MARGARITA SALAS MEMORIAL LECTURE***

**MARISOL SOENGAS**

Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid

***Bringing light to melanoma metastasis: animal models and therapeutic alternatives***

December 2, 2020

# SEVERO OCHOA SEMINARS CYCLE *ADVANCES IN MOLECULAR BIOLOGY* (2019-2020)


## *2019*

| | *SPEAKER* | *CENTER* | *TITLE OF SEMINAR* |
|---|---|---|---|
| 01/02/2019 | **SHARON TOOZE** | The Francis Crick Institute, London, UK | Molecular mechanisms of mammalian autophagy |
| 22/02/2019 | **ISABEL FARIÑAS** | Universidad de Valencia, Valencia, España | Systemic control of adult stem cells in the adult brain: the niche extended |
| 07/06/2019 | **ANA POMBO** | MDC foro Molecular Medicine. Berlin, Germany | Genome architecture mapping: exploring cell-specific 3D genome topologies in the brain |
| 17/05/2019 | **JAMES BERGER** | John Hopkins School of Medicine. Baltimore, MD, USA | (Un?)structural mechanisms for initiating DNA replication |
| 24/05/2019 | **ANA FERNÁNDEZ-SESMA** | Icahn School of Medicine. Mount Sanai, New York, NY, USA | Modulation of innate immunity by dengue virus: beyond the STING |
| 31/05/2019 | **EILEEN FURLONG** | EMBL Heidelberg. Heidelberg, Germany | Transcriptional regulation during developmental transitions: new views from 3D and single-cells |
| 27/09/2019 | **STEPHAN BECKER** | Institute of Virology, Philipps University Malburg, Germany | Intracellular transport and replication of filoviruses |
| 13/12/2019 | **ABERT POL** | (ICREA Research Professor. Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS) | Mammalian lipid droplets: from metabolism to immunity |

## *2020*

| | | | |
|---|---|---|---|
| 17/01/2020 | **PETER SCHEIFFELE** | University of Basel, Basel, Switzerland | Synapse Specification: From Alternative RNA Splicing to Autism |
| 21/02/2020 | **WOLF REIK** | Babraham Institute, Cambridge, UK | Single cell epigenome landscape of development and ageing |
| 28/02/2020 | **DAVID FINLAY** | Trinity College Dublin (Dublin, Ireland | Nutrients and metabolites in the control of immune responses |
| 01/10/2020 | **VÍCTOR CORCÉS** | Department of Human Genetics, EMORY University School of Medicine, Atlanta, GA, USA | Mechanisms of transgenerational inheritance of obesity epiphenotypes |
| 16/10/2020 | **LUISA COCHELLA** | IMP - Research Institute of Molecular Pathology, Vienna. AUSTRIA | Combinatorial action of transcription factors over time: a mechanism for cell diversification |
| 23/10/2020 | **ROTEM SOREK** | Weizmann institute of Science, Rehovot, Israel | The immune system of bacteria: Beyond CRISPR |

# SPECIAL SEMINARS 2019-2020


| 2019 | SPEAKER | CENTER | TITLE OF SEMINAR |
|---|---|---|---|
| 09/01/2019 | **JAVIER TRABA** | NIH-Bethesda US | Mitochondrial control of immune activation: nutrients, sirtuins and disease |
| 21/01/2019 | **JOSÉ LUIS GÓMEZ SKARMETA** | Centro Andaluz de Biología del Desarrollo (CABD) , Sevilla | Evolution of regulatory landscapes |
| 01/03/2019 | **JUAN LASHERAS** | Institute of Engineering in Medicine, University of California, San Diego. La Jolla, CA, USA) | A Strain-Accumulation Visco-Elastic hypothesis for the onset of idiopathic Normal Pressure Hydrocephalus (iNPH): in search of the molecular culprits |
| 18/03/2019 | **BENEDIKT BERNINGER** | Centre for Developmental Neurobiology, MRC Centre for Neurodevelopmental Disorders, Institute of Psychiatry, Psychology & Neuroscience. King's College London, UK | Engineering neurogenesis for the postnatal brain |
| 20/03/2019 | **ADOLFO LÓPEZ DE MUNAIN** | Instituto de Investigación Sanitaria Biodonostia | Etiopatogenia de la ELA: evidencias clínico-epidemiológicas y experimentales de una hipótesis metabólica |
| 03/04/2019 | **RICHARD S. MANN** | Columbia University of New York | The transcription factor specificity paradox: Lessons from Hox proteins |
| 08/04/2019 | **MAURO COSTA-MATTIOLI** | Departments of Neuroscience, Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas | The gut-microbiome-brain axis in neurodevelopmental disorders |
| 06/05/2019 | **VALERIE WALLACE** | Krembil Research Institute. Department of Ophthalmology and Vision Sciences, University Health Network, Toronto, ON, Canada | Material Exchange Between Photoreceptors: A New Way of Thinking About Retinal Cell Transplantation |
| 09/05/2019 | **KENNETH BIRNBAUM** | Center for Genomics and Systems Biology, New York University, USA) | Act locally, assay globally: single-cell RNA-seq approaches to mapping positional signaling effects in plants |
| 16/05/2019 | **Mª DOLORES MARCOS** | Universidad Autónoma de Madrid | Conociendo Internet de las Cosas. Las posibilidades de una tecnología en desarrollo |
| 27/05/2019 | **HÉCTOR GARCÍA SEISDEDOS** | gWeizmann Institute of Science, Department of Structural Biology, Rehovot, Israel | Supramolecular assemblies in evolution, disease and design |
| 03/06/2029 | **CLAUDIO CUELLO** | Department of Pharmacology, McGill University, Montreal, Quebec, Canadá | Discovery of a novel NGF metabolic pathways and its significance in Alzheimer's disease |
| 07/06/2019 | **ALEJANDRO SCHINDER** | Instituto Leloir, Buenos Aires, Argentina | Remodeling of adult hippocampal circuits by neurogenesis and experience |
| 02/07/2019 | **DANIEL L. GARAULET** | Sloan-Kettering Institute, New York) | Post-transcriptional processing of behavior: a view from the female fly |
| 16/07/2019 | **JULIEN DUXIN** | Novo Nordisk Foundation Center for Protein Research, Faculty of Health and Medical Sciences, University of Copenhagen | Mechanisms of DNA-protein crosslink repair |
| 24/07/2019 | **CHENG ZHU** | Wallce H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, USA | Mechanical Regulation of T cell Antigen Receptor |

# SPECIAL SEMINARS 2019-2020


| 2019 | SPEAKER | CENTER | TITLE OF SEMINAR |
|---|---|---|---|
| 03/09/2019 | **PAULA GUTIÉRREZ-MARTÍNEZ** | Intellia Therapeutics, Cambridge, MA, USA | From DNA damage and cancer to heamatopoietic stem cells and beyond |
| 13/09/2019 | **RAFAEL RADI** | Departamento de Bioquímica, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay | Protein Tyrosine Nitration in Biological Systems: Mechanisms, consequences and impact in Molecular Medicine |
| 24/09/2019 | **EDUARDO BALSA MARTÍNEZ** | Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA, USA | Molecular and metabolic mechanisms underlying mitochondrial dysfunction |
| 03/10/2019 | **BARUCH I. KANNER** | Hebrew University Hadassah Medical School, Jerusalem, Israel | Molecular Mechanism of Gating by the Sodium-Coupled GABA Transporter GAT-1 |
| 14/10/2019 | **MICHEL BAGNAT** | Department of Cell Biology, HHMI Faculty Scholar, Duke University Medical Center, Durham, NC, USA | Mechanical and metabolic roles of large lysosomes in vertebrates |
| 17/10/2019 | **ALINE PROBST** | GReD, Université Clermont Auvergne | Role of histone chaperones in plant histone dynamics |
| 24/10/2019 | **AURA CARREIRA** | Institut Curie, Paris, France | BRCA2: a model to understand genome integrity mechanisms and cancer predisposition |
| 24/10/2019 | **JOSÉ CARLOS REYES ROSA** | Centro Andaluz de Biología Molecular y Medicina Regenerativa (CABIMER) CSIC, Sevilla | Regulation of enhancers and coexpression domains by TGFbeta |
| 31/10/2019 | **WOLF-DIETRICH HEYER** | Professor and Chair of the Department of Microbiology & Molecular | Homologous recombination and Genome Instability |
| 13/11/2019 | **TOBÍAS RUFF** | Max-Planck-Institute of Neurobiology, Munich-Martinsried, Germany | Less FLiRTing favours a folded cortex |

| 2020 | SPEAKER | CENTER | TITLE OF SEMINAR |
|---|---|---|---|
| 27/02/2020 | **ANU SUOMALAINEN-WARTIOVAARA** | Biomedicum Helsinki, Univeristy of Helsinki, Finland | Mechanisms of tissue specificity – lessons from mitochondrial diseases |
| 08/07/2020 | **DAVID VILCHEZ** | CEDAD Research Center, Cologne, Germnay | Proteostasis of aging and stem cells |

# ADVANCES IN BIOMEDICINE SEMINAR CYCLE (2019-2020)


## 2019

| | SPEAKER | CENTER | TITLE OF SEMINAR |
|---|---|---|---|
| 08/02/2019 | **ALEJO EFEYAN** | CNIO, Madrid, Spain | Nutrient Signaling in Health and Disease |
| 22/03/2019 | **STEPHEN CUSACK** | EMBL outstation, Grenoble, France | Snapshots of actively transcribing influenza polymerase |
| 20/06/2019 | **SALVADOR AZNAR BENITAH** | Institute for Resarch in Biomedicine, Barcelona, Spain | Adult stem cells in homeostasis, aging and cancer: why time and what we eat matters |
| 05/04/2019 | **FRANK VAN KUPPEVELD** | Utrecht University, Utrecht, Netherlands | A novel mechanism of stress response antagonism by viral accessory proteins |
| 11/04/2019 | **BORIS PFANDER** | Max-Plank Institute of Biochemistry. Martinsried, Germany | DNA double strand break repair – control by chromatin and the cell cycle |
| 10/05/2019 | **HANNS ULRICH ZEILHOFER** | University of Zurich, Zurich, Switzerland | Neurons and circuits of descending pain control |
| 25/10/2019 | **ANTHONY UNDERWOOD** | Wellcome Trust Sanger Institute, Cambridge, UK | DNA double strand break repair – control by chromatin and the cell cycle |
| 29/11/2019 | **FERNANDO CASARES** | CABD - Sevilla, Spain | The colonization of space |
| 27/09/2019 | **STEPHAN BECKER** | Institute of Virology Philipps-University of Marburg Marburg, Germany) | Intracellular transport and replication of filoviruses |
| 25/10/2019 | **ANTHONY UNDERWOOD** | Centre for Genomic Pathogen Surveillance, Wellcome Genome Campus, Hinxton, Cambridge, UK | Science, surveillance and software. Bringing together multi disciplinary teams to tackle genomic surveillance of pathogens |
| 29/11/2019 | **FERNANDO CASARES** | Unidad GEM-DMC2, Centro Andaluz de Biología del Desarrollo, Sevilla | The colonization of space |
| 13/12/2019 | **ALBERT POL** | ICREA Research Professor. Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona | Mammalian lipid droplets: from metabolism to immunity |

## 2020

| | | | |
|---|---|---|---|
| 07/02/2020 | **KARL KADLER** | University of Manchester | Circadian clock control of the secretory pathway and tissue homeostasis |
| 21/02/2020 | **Mª ELENA TORRES-PADILLA** | Institute of Epigenetics & Stem Cells, München, Germany | Epigenetic mechanisms in early mammalian development |

# TRANSVERSAL INTER-PROGRAM (TRiP) SEMINARS (2019-2020)


## 2019

| | SPEAKER | CENTER | TITLE OF SEMINAR |
|---|---|---|---|
| 14/10/2019 | **JOSÉ F. DE CELIS** | Tissue and organ homeostasis | Functional analysis of *Drosophila Ras* and *Sal* genes |
| 21/10/2019 | **ARÍSTIDES LÓPEZ** | Phisiological and Pathological Processes | Challenges in CRISPR/Cas9 gene editing for generation of cellular and murine models of disease |
| 28/10/2019 | **ÍÑIGO MARCOS ALCALDE** | Interactions with the enviroment | MEPSAnd: a path-finding tool for n-dimensional energy |
| 04/11/2019 | **ALICIA DEL PRADO** | Genome Dynamics and Function | Structural and functional studies of Phi29 DNA polymerase |
| 11/11/2019 | **DAVID MÍGUEZ GÓMEZ** | Tissue and organ homeostasis | Systems approaches to study the development of the neocortex and the retina in vertebrates |
| 18/11/2019 | **Mª DOLORES LEDESMA** | Physiological and Pathological Processes | Lipids in the brain: from physiology to pathology while searching for therapies for lipid storage |
| 25/11/2019 | **BRUNO NERNÁEZ** | Interactions with the enviroment | Role of DNA sensing and IFN in the protection against acute lethal virus infection |
| 02/12/2019 | **JOSÉ ANTONIO TERCERO** | Genome Dinamics and Function | Preventing genomic instability at damaged replication forks |
| 16/12/2019 | **NURIA MARTÍNEZ MARTÍN** | Tissue and organ homeostasis | Autophagy Fingerprint: a transversal concept |

## 2020

| | | | |
|---|---|---|---|
| 10/02/2020 | **ERNESTO SÁNCHEZ-HERRERO** | Tissue and organ homeostasis | The *Drosophila* Hox gene *Abdominal-B* and organogenesis: segment elimination and genitalia |
| 02/03/2020 | **JOSÉ J. LUCAS** | Physiological and Pathological Processes | Regulated mRNA polyadenylation affects gene expression in neurodevelopment and neurodegeneration |

# DOCTORAL THESES 2019-2020


| 2019 | DOCTORAL STUDENT | DEPARTMENT | DIRECTOR | TITLE |
|---|---|---|---|---|
| 14/01/2019 | **ALBA SEBASTIÁN MARTÍN** | Virology and Microbiology | Luis Menéndez Arias | Fidelity of Human Immunodeficiency Viruses Type 1 and Type 2 Reverse Transcriptases in DNA Synthesis Reactions using DNA and RNA Templates |
| 23/01/2019 | **INÉS JUARISTI SANTOS** | Molecular Neuropathology | Jorgina Satrústegui y Araceli de Arco | Regulation of mitochondrial respiration in astrocytes: role of $Ca^{2+}$, ATP demand and pyruvate production |
| 30/01/2019 | **IVÓ HERNÁNDEZ HERNÁNDEZ** | Molecular Neuropathology | José Javier Lucas Lozano y Mª José Pérez Álvarez | Papel Del Factor De Transcripción Atf5 En La Enfermedad De Huntington |
| 15/02/2019 | **JOSÉ LUIS MARÍN RUBIO** | Cell Biology and Immunology | José Fernández Piqueras y María Villa Morales | Alterations of FADD expression and phosphorylation in T-cell lymphoblastic lymphoma |
| 22/02/2019 | **GRACIELA ALONSO CASTRO** | Virology and Microbiology | Antonio Alcamí Pertejo | Relevancia de la actividad anti-linfotoxina y el gen viral Schlafen en la patogénesis del virus ectromelia |
| 22/02/2019 | **GRACIELA ALONSO CASTRO** | Virology and Microbiology | Antonio Alcamí Pertejo | Relevancia de la actividad anti-linfotoxina y el gen viral Schlafen en la patogénesis del virus ectromelia |
| 28/02/2019 | **MARÍA GIMENO PÉREZ** | Virology and Microbiology | María Fernández Lobato | Estudio estructural de la β-fructofuranosidasa de Xanthophyllomyces dendrorhous y su empleo para la producción de oligosacáridos prebióticos y otros derivados fructosilados |
| 27/03/2019 | **ANA RIVERA BARAHONA** | Molecular Neuropathology | Lourdes Ruiz Desviat | Estudios genéticos y fisiopatológicos para la búsqueda de nuevos biomarcadores y terapias en acidemia propiónica |
| 12/04/2019 | **AINHOA MARTÍNEZ PIZARRO** | Molecular Neuropathology | Lourdes Ruiz Desviat | Identificación y caracterización de mutaciones de splicing en pacientes con hiperfenilalaninemia; aproximaciones terapéuticas específicas de RNA |
| 24/04/2019 | **PATRICIA ALEJANDRA CALVO FERNÁNDEZ** | Genome Dynamics and Function | Luis Blanco Dávila y María Isabel Martínez Jiménez | Structure-function analysis of human PrimPol |
| 26/04/2019 | **ALBERTO JIMÉNEZ MARTÍN** | Genome Dynamics and Function | José Antonio Tercero Orduña | La AAA+ ATPasa Mgs1/WRNIP1 y su relación con la tolerancia al daño en el DNA durante la replicación cromosómica |
| 26/04/2019 | **CRISTINA NUEVO TAPIOLES** | Cell Biology and Immunology | José María Cuezva y Laura Formentini | Regulación de la Oxphos Mediada por If1 y su Potencial como Diana Terapéutica en Cáncer |
| 30/04/2019 | **SILVIA DE VIDANIA BALLESTEROS** | Molecular Neuropathology | Carlos Dotti y Francesc Xavier Rafols | Estudio de los mecanismos neuroprotectores frente a la toxicidad del péptido β-amiloide en un modelo preclínico para la enfermedad de Alzheimer |
| 24/05/2019 | **ADRIÁN BARTOLL ANDRÉS** | Molecular Neuropathology | María Dolores Ledesma | Endocannabinoide en la patología y terapia de la enfermedad de Niemann Pick tipo A |
| 12/06/2019 | **ALEXANDRA ATIENZA MANUEL** | Development and Regeneration | Mar Ruiz Gómez | Función del complejo endocítico Cubilin-Amnionless y de la escramblasa de fosfolípidos Scramb1 en la biología del diafragma de filtración de Drosophila |

# DOCTORAL THESES 2019-2020


| 2019 | DOCTORAL STUDENT | DEPARTMENT | DIRECTOR | TITLE |
|---|---|---|---|---|
| 26/06/2019 | **FRANCISCO DEL CAÑO OCHOA** | Genome Dynamics and Function | Santiago Ramón Maiques | Functional characterization of CAD, an antitumoral target controlling the de novo pyrimidine biosynthesis |
| 27/06/2019 | **PABLO GUASP** | Cell Biology and Immunology | José Antonio López de Castro | The peptidome of the Behçet's disease associated HLA-B*51 molecule and its shaping by endoplasmic reticulum aminopeptidases. Redundancy and complementarity between ERAP1 and ERAP2 |
| 28/06/2019 | **MARCOS PARRAS MOLTÓ** | Virology and Microbiology | Alberto López Bueno | Estudio metagenómico de la comunidad de virus y de su interacción con la microbiota en la cavidad bucal humana |
| 28/06/2019 | **TANIA MORENO MÁRMOL** | Development and Regeneration | Paola Bovolenta y Florencia Cavodeassi | Morfogénesis del epitelio pigmentario en pez cebra y su papel en la formación de la copa óptica |
| 04/07/2019 | **MAURO AGRÒ** | Molecular Neuropathology | Javier Díaz-Nido | Functional characterization of Frataxin isoforms and mechanisms of regulation of Frataxin expression |
| 05/07/2019 | **ALBERTO DOMINGO LÓPEZ MUÑOZ** | Virology and Microbiology | Antonio Alcamí Pertejo y Alberto Rastrojo Lastras | Contribution of herpes simplex virus glycoprotein G to viral pathogenesis |
| 12/07/2019 | **SARA ISABEL VAZ FRANCISCO** | Cell Biology and Immunology | Manuel Fresno y Alicia Arranz | Toll-like receptor 2 and 4: differential signaling, dimerization and the final outcome in inflammation |
| 25/07/2019 | **JAVIER GALÁN MARTÍNEZ** | Cell Biology and Immunology | Manuel Fresno y Núria Gironés | Role of the Transcription Factor TCFL5 isoforms in colon cancer tumoral processes, pluripotency and development |
| 26/07/2019 | **Mª INÉS MATEO RUIZ** | Development and Regeneration | Paola Bovolenta y Pilar Esteve | Niveles elevados de Sfrp1 en un modelo transgénico de ratón desencadenan neuroinflamación y pérdida de memoria |
| 03/09/2019 | **ESTHER MARÍA GONZÁLEZ ALMELA** | Genome Dynamics and Function | Luis Carrasco | Factores de Iniciación implicados en la Traducción de mRNAs virales. Importancia de los motivos estructurales en el RNA |
| 12/09/2019 | **SERGIO BENJAMÍN VELARDE RANGEL** | Cell Biology and Immunology | Antonio Baonza Cuenca | Respuesta Regenerativa Glial en el disco imaginal de ojo de *Drosophila melanogaster* |
| 12/09/2019 | **VERÓNICA MIGUEL HERRANZ** | Cell Biology and Immunology | Santiago Lamas | The metabolic basis of renal fibrosis: role of microRNAs and insight from genetic models targeting lipid metabolism |
| 20/09/2019 | **RAFAEL ALEJANDRO JUÁREZ URIBE** | Development and Regeneration | Ernesto Sánchez-Herrero Arbide | Estudio de la expresión de genes Hox y de la función de la proteína "Homeodomain interacting protein kinase" en procesos de regeneración en *Drosophila melanogaster* |
| 11/10/2019 | **HENAR SUÁREZ MONTERO** | Development and Regeneration | María Yáñez-Mó | Papel de las tetraspaninas en la internalización y el tráfico de moléculas asociadas en modelos tumorales y de infección viral |
| 11/10/2019 | **JOSÉ JORDÁN SORIA** | Cell Biology and Immunology | Ricardo Amils y Felipe Gómez | Geomicrobiología del revestimiento de rocas en un ambiente ácido extremo: Río Tinto |

# DOCTORAL THESES 2019-2020


| 2019 | DOCTORAL STUDENT | DEPARTMENT | DIRECTOR | TITLE |
|---|---|---|---|---|
| 14/10/2019 | **MARÍA DELGADO BAREA** | Development and Regeneration | Fernando Martín Belmonte | Exploring the roles of major signaling pathways in epithelial morphogenesis |
| 15/10/2019 | **IRENE DÍAZ LÓPEZ** | Genome Dynamics and Function | Iván Ventoso Bande y René Toribio López | Involvement of ES6S region of 40S subunit in mRNA threading and scanning during translation initiation |
| 16/10/2019 | **ANNA NELKE** | Development and Regeneration | Marta Pérez Pereira y Alberto Martínez Serrano | Differential effects of neural stem cell therapy in adult and middle-aged Parkinsonian mice |
| 18/10/2019 | **ANA SOFIA MATOS MADEIRA** | Genome Dynamics and Function | Crisanto Gutiérrez y Bénédicte Desvoyes | Role of histone H3 variant, HTR6, during stress response |
| 25/10/2019 | **JOSÉ MIGUEL FERNÁNDEZ JUSTEL** | Genome Dynamics and Function | María Gómez Vicente-franqueira | Characterization of the regulatory roles of histone H1 in the homeostasis of the mammalian genome |
| 20/11/2019 | **FRANCISCO CALLEJAS HERNÁNDEZ** | Cell Biology and Immunology | Manuel Fresno Escudero/Nuria Gironés Pujol | Análisis de la diversidad genómica y transcriptómica de *Trypanosoma cruzi* y su relación con la enfermedad de Chagas |
| 28/11/2019 | **PATRICIA SÁNCHEZ PÉREZ** | Development and Regeneration | Susana Cadenas Álvarez | Protective role of mitochondrial uncoupling protein UCP3 and the transcription factor Nrf2 against cardiac ischemia-reperfusion injury and their involvement in ischemic preconditioning |
| 13/12/2019 | **IGNACIO BAQUEDANO MOZOS** | Cell Biology and Immunology | José Berenguer Carlos y Mario Mencía | ICETh1 & ICETh2, two mobile genetic elements coordinated in Thermus thermophilus transjugation |
| 12/09/2019 | **VERÓNICA MIGUEL HERRANZ** | Cell Biology and Immunology | Santiago Lamas | The metabolic basis of renal fibrosis: role of microRNAs and insight from genetic models targeting lipid metabolism |

| 2020 | DOCTORAL STUDENT | DEPARTMENT | DIRECTOR | TITLE |
|---|---|---|---|---|
| 26/02/2020 | **GUSTAVO CARVALHO DIAS** | Genome Dynamics and Function | Luis Blanco Dávila y María I Martínez-Jiménez | Characterization of murine PrimPol as a robust DNA primase |
| 28/02/2020 | **TAMARA JIMÉNEZ SAUCEDO** | Dinámica y Función del Genoma | Miguel A. Rodríguez y Juan José Berlanga | Role of eIF2alfa-dependent translational regulation in aging |
| 28/02/2020 | **PAU B. ESPARZA MOLTÓ** | Physiological and Pathological Processes | José M. Cuezva | Tissue-specific expression of the ATPase inhibitory factor 1 and its role in neuronal function |
| 24/04/2020 | **JAVIER CASARES ARIAS** | Tissue and Organ Homeostasis | Miguel Ángel Alonso Lebrero | Caracterización del remanente del cuerpo medio y la regulación de su herencia |
| 23/06/2020 | **ALBERTO GARCÍA RODRÍGUEZ** | Physiological and Pathological Processes | Félix Hernández | Envejecimiento, Alzheimer y reprogramación celular *in vivo*. Efecto de la sobreexpresión de GSK-3β y de los factores de Yamanaka en el sistema nervioso central |
| 24/06/2020 | **CELIA GARCÍA CORTÉS** | Tissue and Organ Homeostasis | Ernesto Sánchez-Herrero | Estudio de la función del gen Hox Ultrabithorax en la morfogénesis del halterio de *Drosophila melanogaster* |
| 25/06/2020 | **CRISTINA CACHO NAVAS** | Tissue and Organ Homeostasis | Jaime Millán | Estudio de los mecanismos moleculares que median la polaridad apical y función de ICAM-1 en células epiteliales hepáticas |
| 24/06/2020 | **RODRIGO CAÑAS ARRANZ** | Interactions with the Environment | Francisco Sobrino Castelló y Esther Blanco Lavilla | Synthetic Dendrimer Peptide Vaccines Against Foot-and-Mouth Disease Virus |

# DOCTORAL THESES 2019-2020


| 2020 | DOCTORAL STUDENT | DEPARTMENT | DIRECTOR | TITLE |
|---|---|---|---|---|
| 25/06/2020 | **CRISTINA CACHO NAVAS** | Tissue and Organ Homeostasis | Jaime Millán | Estudio de los mecanismos moleculares que median la polaridad apical y función de ICAM-1 en células epiteliales hepáticas |
| 26/06/2020 | **JAVIER BARRIO PÉREZ** | Tissue and Organ Homeostasis | Antonio Baonza Cuenca | Caracterización de nuevas funciones de la quinasa altered disjunction / monopolar spindle 1 en *Drosophila melanogaster* |
| 26/06/2020 | **JUAN RAMÓN PEREA ÚBEDA-PORTUGUÉS** | Physiological and Pathological Processes | Jesús Ávila y Marta Bolós | Efecto de tau en la respuesta inflamatoria de la microglía |
| 30/06/2020 | **IRENE PÉREZ LIÉBANA** | Interactions with the Environment | Jorgina Satrústegui, Beatriz Pardo | El transportador mitocondrial de aspartato-glutamato Aralar/AGC1 controla la respiración neuronal y β-Hidroxibutirato recupera los daños cerebrales causados por la deficiencia en AGC1 |
| 10/07/2020 | **ADRIÁN AGUIRRE TAMARAL** | Tissue and Organ Homeostasis | Isabel Guerrero Vega | In silico modeling of cytoneme-mediated Hedgehog signaling in *Drosophila* |
| 08/09/2020 | **ALBERTO DÍAZ TALAVERA** | Genome Dynamics and Function | Luis Blanco Dávila | Human PrimPol: a TLS-DNA primase for alleviating DNA replication stress, and a potential target in cancer |
| 11/09/2020 | **ANDRÉS MIGUEL ARRIBAS** | Interactions with the Environment | Wilfried JJ Meijer | Discovery of a novel antitermination system present on many conjugative plasmids of Gram-positive bacteria |
| 06/10/2020 | **ANDRÉS DE LA ROCHA MUÑOZ** | Physiological and Pathological Processes | Beatriz López Corcuera y Carmen Aragón Rueda | Fisiopatología del transportador neuronal de glicina GlyT2: regulación y estudio de mutantes asociados a hiperplexia |
| 29/10/2020 | **ESMERALDA ALONSO BARROSO** | Physiological and Pathological Processes | Lourdes Ruiz Desviat | Estudios fisiopatológicos para la búsqueda de nuevas dianas terapéuticas en acidemia propiónica mediante la caracterización del modelo murino y el desarrollo de nuevos modelos celulares humanos basados en iPSCs |
| 29/10/2020 | **ALBA CONCEPCIÓN ARCONES** | Physiological and Pathological Processes | Cristina Murga Montesinos y Federico Mayor Menéndez | Influence of GRK2 levels in the modulation of glucose homeostasis in health and disease |
| 29/10/2020 | **THERESA ROTHENBÜCHER** | Tissue and Organ Homeostasis | Alberto Matínez Serrano y Marta Pérez Pereira | Engineered brain organoids: Developing and improved and larger human brain model *in vitro* |
| 05/11/2020 | **AZMAN EMBARC BUH** | Genome Dynamics and Function | Encarnación Martínez Salas | Gemin5, a multifunctional RNA-binding protein involved in translation control |
| 03/12/2020 | **TAMARA ROSELL GARCÍA** | Tissue and Organ Homeostasis | Fernando Rodríguez Pascual | Contribution of the lysyl hydroxylase 2 and lysyl oxidase enzymes to the remodeling of the extracellular matrix. Implications in the biosynthesis of collagen and its application in tissue engineering |
| 04/12/2020 | **ALEJANDRO FULGENCIO COVIÁN** | Physiological and Pathological Processes | Lourdes Ruiz Desviat y Evar Richard | Investigación traslacional en las cardiomiopatías asociadas a la acidemia propiónica |
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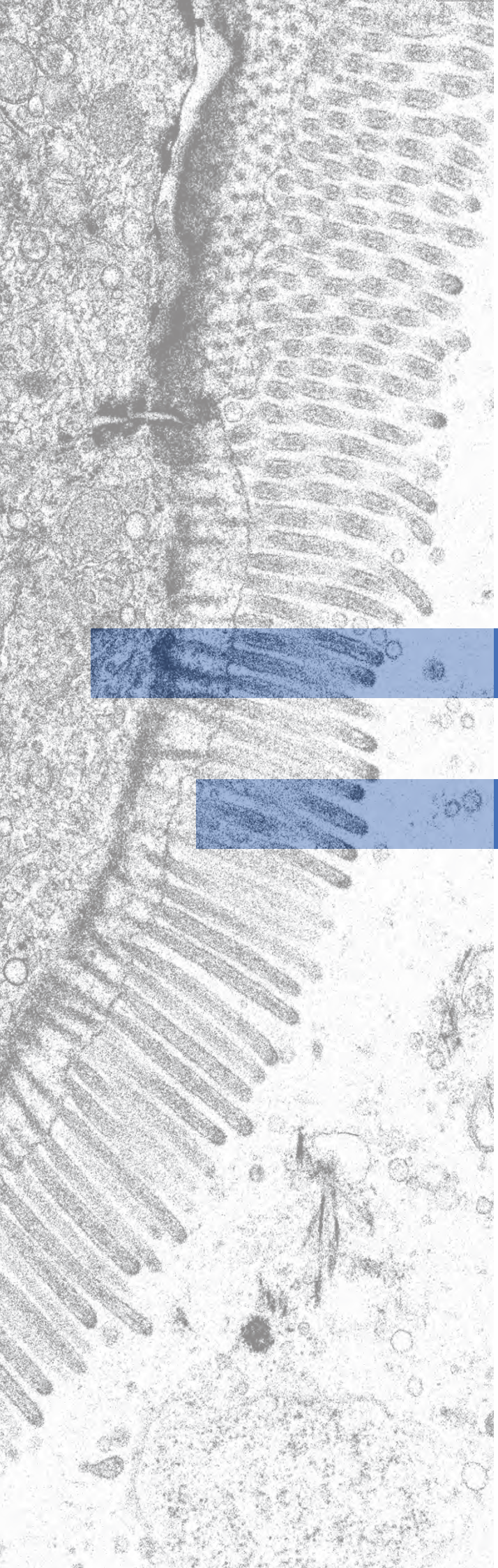

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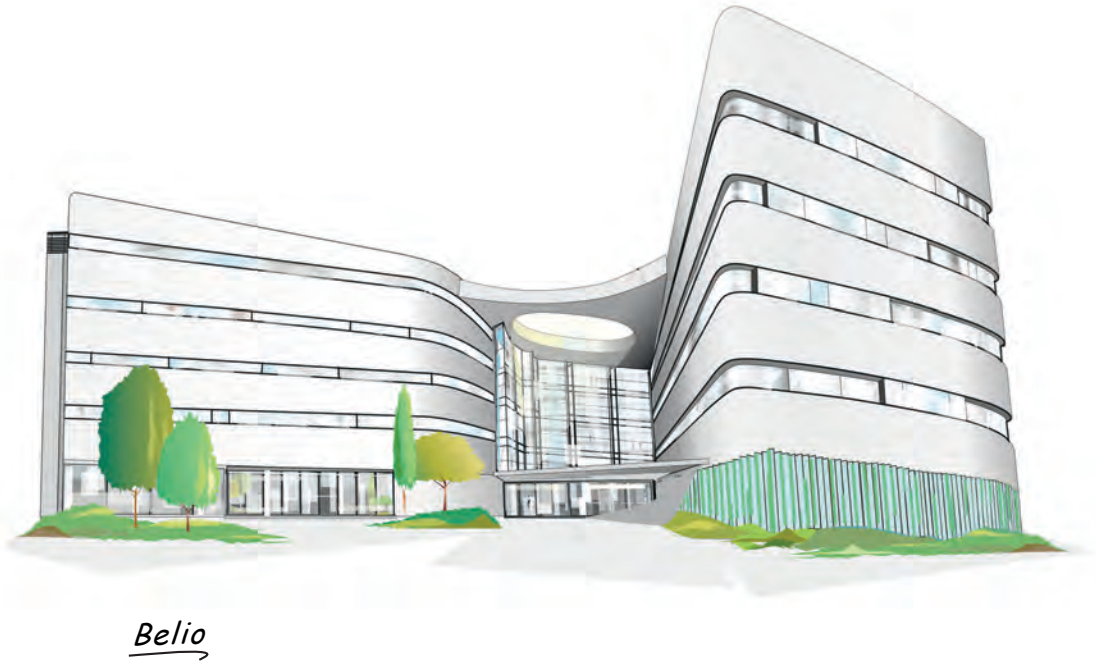


(2003) First sketch of the CBM building prior to its construction

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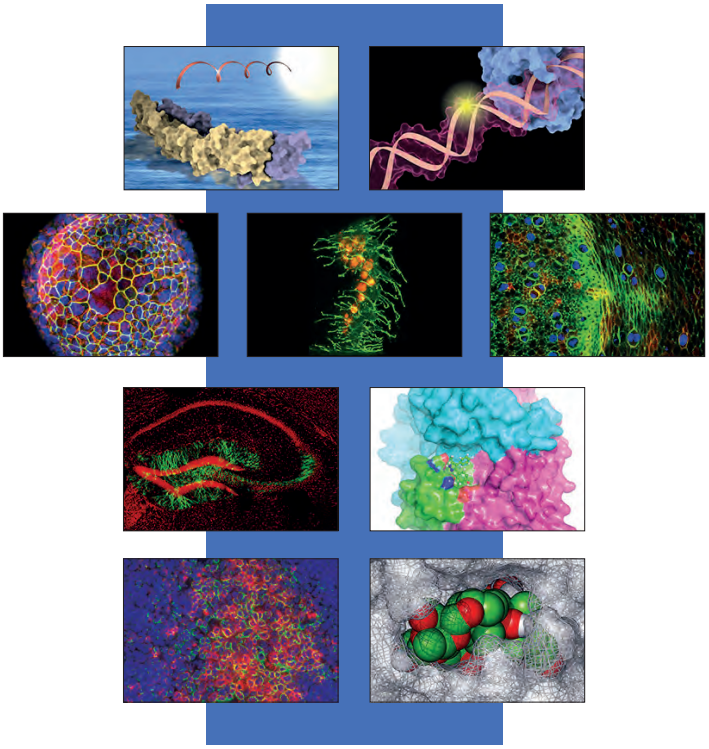

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