



SCIENTIFIC REPORT 2023 - 2024

Director Report	6	Tissue and organ homeostasis
Obituary	10	Cell-cell communication and inflammation
The CBM in a nutshell	12	Carlos Anerillas Aljama. Damage response
Scientific organization of the CBM	32	and senescence
Genome dynamics and function	34	Carlos Cabañas Gutiérrez. Functional interactions between tetraspanin CD9 and cell
Genome decoding	36	adhesion molecules
<b>Ugo Bastolla.</b> Computational biology and Bioinformatics	38	<b>Miguel R. Campanero.</b> Molecular characterization of vascular pathogenesis and lymphomagenesis
<b>Crisanto Gutierrez</b> . DNA replication, chromatin and cell division	40	Isabel Guerrero Vega. Mechanisms of cell-
<b>Encarnación Martinez Salas.</b> RNA-binding proteins and translation control in eukaryotes	42	cell signaling during development  José María Izquierdo Juárez. Molecular
Carlos Perea Resa. Dynamic and recycling of the transcriptional machinery across mitosis	44	and cellular basis of the physio(patho)logy associated with the expression of intracellular antigens
Jose María Requena Rolanía and Begoña Aguado Orea. Regulation of gene expression in Leishmania	46	<b>Manuel López Cabrera.</b> Molecular Pathophysiology of Peritoneal Inflammation and Fibrosis
Javier Santos Hernández and María Villa	48	Jaime Millán. Cell Biology of Inflammation
<b>Morales.</b> Genetics and cell biology of cancer: T-cell lymphoblastic neoplasms		María Mittelbrunn Herrero. Immunometabolism and inflammation
<b>Iván Ventoso.</b> Regulation of mRNA translation in eukaryotes and its implications for organismal life	50	Ginés Morata Pérez and Natalia Azpiazu.  Mecanismos de tumorigénesis en <i>Drosophila</i>
Genome maintenance and instability	52	Juan Miguel Redondo Moya. Molecular and
Luis Blanco Dávila. Genome maintenance	54	translational research in vascular pathologies
and variability: enzymology of DNA replication and repair		<b>Fernando Rodriguez Pascual.</b> Extracellular matrix remodeling in the cardiovascular system
<b>Aura Carreira Moreno.</b> Genome Instability and Cancer Predisposition	56	María Yáñez-Mó. Tetraspanin-enriched
<b>Ignacio Flores.</b> Telomeres in cancer and regeneration	58	membrane microdomains in extracellular vesicles and cell adhesion and migration
María Gómez Vicentefranqueira. Functional	60	Cell architecture and organogenesis
Organization of the Mammalian Genome		Miguel Ángel Alonso and Isabel Correas Hornero. Cell Polarity
<b>Emilio Lecona Sagrado.</b> Chromatin, cancer and the ubiquitin system	62	Luis Alberto Baena. Molecular
<b>Jose Antonio Tercero Orduña.</b> Chromosome replication and genome stability	64	characterisation of caspase biology <b>Leonardo Beccari.</b> Transcriptional regulation
<b>Miguel de Vega José.</b> Maintenance of bacterial genome stability	66	of brain cortex development

Paola Bovolenta. Morphogenesis and neurodegeneration of the vertebrate Central	104	Carlos Dotti. Survival and plasticity in the aging brain	140
Nervous System  Carlos Estella and Antonio Baonza. Cellular response to stress and morphogenesis	106	<b>José Antonio Esteban.</b> Mechanisms of synaptic plasticity, and contribution to cognitive function	142
<b>José Félix de Celis.</b> Cell signalling during imaginal development in <i>Drosophila</i>	108	<b>María Dolores Ledesma.</b> Lipids in neuronal physiology and pathology	144
Miguel Manzanares. Functional genomics	110	María Llorens-Martín. Adult neurogenesis	146
<b>Fernando Martín Belmonte.</b> Intestinal morphogenesis and homeostasis	112	and neurodegenerative diseases  Beatriz López-Corcuera and Francisco	148
<b>Nuria Martínez Martín.</b> Metabolism and B cell function	114	<b>Zafra.</b> Physiopathological aspects of glycine transporters in glycinergic neurotransmission: hyperekplexia and pain	
<b>David Míguez.</b> Biophysics and systems biology	116	<b>José Javier Lucas Lozano.</b> Molecular basis of Huntington's disease and other central	150
Vicente Pérez García. Placenta biology	118	nervous system disorders	
<b>lar Ruíz Gómez y Joaquim Culí.</b> Genetic nd functional analysis of the renal filtration		<b>Ernest Palomer.</b> Neuroepigenetics of brain physiology and pathology	152
diaphragm in health and disease		Marta Pérez Pereira. Biology of human stem cells in translational neuroscience	154
Ernesto Sánchez-Herrero. Segmental specification and pattern formation in Drosophila	122	<b>Eva Porlan.</b> Neural stem cells in the adult brain	156
<b>Esther Serrano.</b> Transcriptional control of sexual differentiation of the nervous system	124	<b>Claudio Toma.</b> The genetics of psychiatric diseases	158
Physiological and pathological processes	126	Francisco Wandosell. Molecular mechanisms	160
Molecular neuropathology	128	of Neurodegeneration	
Jesús Ávila and Félix Hernández. Tau	130	Metabolic and signaling networks in disease	162
function and dysfunction in Alzheimer disease		<b>Eduardo Balsa.</b> Mitochondrial dysfunction in metabolic diseases	164
<b>María Jesús Bullido.</b> Pathogenic mechanisms of Alzheimer's disease	132	Pedro Bonay. Functional Glycogenomics	166
<b>Beatriz Cubelos.</b> Molecular mechanisms of Oligodendrocyte-Neuron interaction &	134	<b>Susana Cadenas.</b> Mitochondrial pathophysiology	168
pathologies associated with myelin		Sara Cogliati. Molecular mechanisms of sex-	
<b>Javier Díaz Nido.</b> Physiopathology and Therapy of Neurodegeneration: Friedreich's	136	differences in metabolism physiology and disease	
Ataxia	138	<b>Laura Formentini.</b> Translational Energy Metabolism	172
<b>Francisco Javier Díez Guerra.</b> Molecular Bases of Neuronal Plasticity		<b>Santiago Lamas.</b> Molecular Pathophysiology of Fibrosis	174

Patho- physiological implications of G protein- coupled receptors signaling networks	176	María N. Navarro. Intracellular Signalling in Inflammatory Processes	
Petronila Penela. Cellular signaling networks in cancer	178	<b>Ana Ortega Molina.</b> Metabolism in cancer and aging	212
Belén Pérez and Pilar Rodríguez Pombo.	180	<b>Felipe X. Pimentel-Muiños.</b> Unconventional autophagy in health and disease	214
Translational medicine in inborn errors of metabolism and other rare genetic diseases		María Luisa Toribio. Development of the human lymphohematopoietic system	216
<b>Natalia Reglero.</b> Vascular inflammation and Autophagy	182	<b>Hisse Martien van Santen.</b> TCR domains in T cell differentiation and pathophysiological	218
Catalina Ribas. Cell communication in	184	and therapeutic responses	
homeostasis and disease through new Gq- GPCR signaling nodes		Microbes in health and welfare	220
Lourdes Ruiz Desviat and Eva María Richard. Physiopathology studies and therapeutical	186	<b>José María Almendral.</b> ssDNA virus evolution, pathogenesis and anti-cancer potential	222
approaches in animal and cellular models of neurometabolic diseases		<b>Ricardo Amils.</b> Molecular ecology of extreme environments	224
<b>Beatriz Pardo and Araceli del Arco.</b> Calcium signaling in mitochondria: metabolic control and mitochondria physiopathology	188	<b>Esteban Domingo.</b> Genetic variability of RNA viruses	226
Javier Traba. Mitochondrial biology in immune modulation	190	María Fernandez Lobato. Yeast enzymes bioengineering to generate bioactive compounds	228
Interactions with the environment	192	Mauricio García Mateu. Virus engineering	230
Immune system development and function	194	and Nanobiotechnology	
Balbino Alarcón. Signal transduction by the	196	Paulino Gómez-Puertas. Molecular Modeling	232
T-cell antigen Receptor  Antonio Alcamí. Immunity & Viromics	198	Aurelio Hidalgo. Ultrahigh-throughput discovery and engineering of enzymes for biotechnological applications	234
<b>César Cobaleda.</b> Cellular plasticity in development and cancer	200	Wilfried J. Meijer. Conjugation in Grampositive bacteria	236
Margarita del Val. Viral Immunology	202	Mario Mencía. Biotechnology and genetics of	238
Manuel Fresno. Regulation and function	204	extreme thermophiles	
of proinflammatory mediators and their involvement in immune and inflammatory mediated diseases		<b>Luis Menéndez-Arias.</b> Human immunodeficiency virus reverse transcriptase and antiretroviral therapy	240
<b>Núria Gironés.</b> Immunoregulatory mechanisms in the development of Chagas disease: translational applications	206	Manuel Pazos. Bacterial cell envelope during preseptal growth	242
Miguel Angel Íñiguez and Juan Manuel Serrador. Nitric oxide and bioactive lipids signalling in the immune response	208	<b>María Graciela Pucciarelli.</b> RNA-based control of Listeria adaptation to stress and virulence	244

ASFV model	246
<b>David Ruano.</b> Biotechnology of probiotics and intestinal pathogens	248
Margarita Sáiz. Modulation of antiviral immunity by viral proteases and noncoding RNAs	250
<b>Francisco Sobrino.</b> New strategies for prevention and control of viral diseases: footand-mouth disease virus as a model	252

Scientific facilities	254
Advanced light microscopy facility	256
Animal facility	258
Biocomputational analysis facility	260
Bioinformatics facility	262
<b>Drosophila</b> transgenesis facility	264
Electron microscopy facility	266
Flow cytometry facility	268
Genomics facility	270
Mouse gene editing facility	272
Preclinical biomedicine facility	274
Protein biotechnology facility	276
Proteomics facility	278

Technical services and operations teams	280
Committees	284
Train@CBM committee	286
Equality committee	288
Predoc & postdoc committee	290
Help@hand	292
Sustainability committee	294
Scientific outreach	296
Training activities	310
Seminars and lectures	318

# **Director Report**



Paola Bovolenta CBM Director

In

March 2023, I assumed the role of Director of the Centro de Biología Molecular Severo Ochoa (CBM), succeeding Lourdes Ruiz-Desviat, who led the centre with dedication for more than four years. I would like to express my sincere gratitude to her for her commitment and contributions during this period, including the first two months of 2023.

It is now my turn and my pleasure to introduce this 2023–2024 report, covering a period of significant changes and new activities at the CBM. These two years have been marked by an important reorganization and a variety of scientific and institutional milestones. All of these initiatives have been made possible thanks to the constant support of the CBM Deputy Director, Beatriz López-Corcuera, the guidance of the Internal Scientific Advisory Committee, the commitment of the CBM Manager, German Lerma, and the generous, dedicated contributions of many CBM faculty members. Equally essential has been the continuous and efficient support of the CBM's administrative and technical services, whose professionalism and dedication have been fundamental to the successful implementation of many of the activities outlined in this report, in addition to their daily responsibilities that are critical for sustaining research at CBM

A key development has been the implementation of a new collaboration agreement between our parent institutions, the CSIC and the UAM. This agreement introduces internal regulations specific to the CBM, which have led to important updates in our governance structure. These include the establishment of a Supervisory Board, a revised Steering Committee, and a new Internal Scientific Advisory Committee. We also began the implementation of the scientific program proposed in our Centre of Excellence project, launching several associated initiatives. Another highlight of this period was the visit, in May 2023, of our External Scientific Advisory Board (SAB). The visit included interviews with the majority of CBM principal investigators, and culminated in a detailed report. This report offered valuable general recommendations regarding the organization and scientific activity of the CBM, as well as individual feedback for PIs.

As a result of these developments, and with the support of the Internal Scientific Advisory Committee, the Direction has undertaken a major reorganization of laboratory space. This redistribution follows newly established internal criteria, aiming to support the growth of research groups expanding their activities, while promoting interaction and mentorship between senior researchers and recently appointed PIs. We have also made a concerted effort to optimize the use of shared spaces and equipment, enhancing access and efficiency for all researchers across the centre.

A second major area of focus has been the optimization of our scientific and technical services, through the acquisition of new equipment and—perhaps more importantly—the incorporation of new staff. While I cannot mention every change in this introduction, I will highlight the most significant ones. Both the Electron Microscopy and Protein Biotechnology services have welcomed new facility heads, and several other services have added staff, where possible, to enhance their operations. A particularly noteworthy achievement has been the establishment of a joint Proteomics Service in collaboration with the neighbouring CNB, physically located within the CNB facilities. This joint service now benefits from the support of CBM staff, whose expertise has been essential in managing the high volume of requests. Both the CBM and CNB leadership are especially proud of this collaborative effort, which we see as a model for future service optimizations. We have also restructured the former Genomics/NGS Service into two independently managed units: Functional Genomics and Biocomputational Analysis. This division has significantly improved efficiency and specialization. The Functional Genomics service has implemented several new technologies, including RNA-seq and ChIP-seq library preparation, as well as various approaches to singlecell transcriptomics—supported by the acquisition of dedicated equipment. Meanwhile, the Biocomputational Analysis service has enhanced its computational pipelines and developed custom software solutions tailored to specific user needs. In addition, the Advanced Light Microscopy service has expanded its microscopy capabilities with the incorporation of a lightsheet system, broadening the range of imaging techniques available at the centre. Other important improvements include the expansion and reorganization of services offered by the Protein Biotechnology and Preclinical Biomedicine platforms, further strengthening our technical infrastructure.

The CBM has a long-standing tradition in scientific outreach, and in recent years, we have taken a significant step forward by formally establishing a dedicated Communications and Science Outreach office. This initiative has greatly enhanced both our internal and external communication efforts and significantly expanded the number and scope of outreach activities in which the CBM participates or leads. A full list of activities is included in this report, but I wish to highlight the tremendous success of the "Ciencia Contigo" events—particularly the 2024 edition focused on "The Microlife". Held at the Caixaforum in Madrid, the event attracted more attendees than the auditorium could seat, demonstrating the growing public interest in our initiatives.

The creation of this office has also had a positive impact on our predoctoral and postdoctoral communities, encouraging early-career researchers to take an active role in organizing events and engaging with institutional life. One outstanding outcome of this empowerment is the formation of a strong and dynamic Predoc-Postdoc Committee, which has significantly enriched the scientific and social life of the centre. It is a pride to have such an active collective of early career scientists. Congratulations to all involved!

The work of early-career researchers at the CBM has been further supported by the creation of the Train@CBM program, which includes a variety of activities designed to complement the PhD training provided by the University. Each doctoral student is also assigned a PhD Thesis Advisory Committee to help ensure the successful development and completion of their research project. An associated innovation has been the establishment of the Help@ Hand office, which serves as a first point of contact for addressing academic or workplace-related concerns of PhD students and postdoctoral researchers. Training the next generation of scientists remains a core mission of the CBM. Many of our faculty members actively participate in undergraduate and graduate teaching, and the centre regularly hosts a large number of summer as well as bachelor and master's students, who engage in hands-on research and complete their final degree projects (TFG and TFM) within our laboratories.

I wish to take this opportunity to express the CBM's—and my own—gratitude to the members of the Equality and Sustainability Committees. The first, active since 2021, continues to position the CBM at the forefront of innovation in this area through meaningful initiatives and educational activities. The Sustainability Committee, although less than a year old, has already made a significant impact by raising awareness of the critical importance of sustainability in both everyday life and scientific practice.

The past reorganization of the CBM into four scientific programs and eight research units, along with the incorporation of highly promising tenure-track scientists—many of whom have already secured permanent positions—had already significantly revitalized the scientific life of the centre. To maintain this momentum and foster collaboration, we launched an "Intramural Exploratory Grants Program Severo Ochoa" and a dedicated "Postdoctoral Call" aimed at attracting international talent. Both initiatives were supported through funding from the Severo Ochoa Centre of Excellence grant and awarded based on the evaluation and recommendations of our SAB, to whom we are deeply grateful for their continued support. During this period, five new tenure-track scientists joined the CBM.



As previously noted, eight non-tenured researchers secured permanent positions, and five additional tenured researchers at various career stages were incorporated into the CBM. Scientific accomplishments include securing highly competitive national and international grants, such as ERC Grants. In total, the CBM produced over 500 scientific publications during this time, many of which appeared in leading international journals, as detailed in the individual team reports. A substantial number of CBM researchers have received national and international awards, distinctions, and have taken on key leadership and management roles in the scientific community. Their efforts have also translated into successful knowledge transfer activities, establishing valuable connections with both the clinical and industrial sectors, as highlighted in the following pages.

I would like to close this introduction by acknowledging those who have left the CBM over the past period. Many have been students or postdoctoral researchers who have moved on to continue their careers at other research institutions or have chosen to pursue new directions in related fields. To all of them, we extend our best wishes for the journey ahead. Several more senior colleagues have entered retirement. Both the CBM and I are deeply grateful for their dedication, time, and passion throughout the years. We will miss them all, but we also wish them the best in this new—and hopefully more relaxed—chapter of their lives. Sadly, a few members of our community have passed away. Their absence is deeply felt, and we have highlighted their invaluable contributions to the CBM and to Spanish science in the following pages.



## **Obituaries**

## FEDERICO MAYOR ZARAGOZA



Federico Mayor Zaragoza passed away in Madrid on Thursday, December 19, 2024. Don Federico was a universal Catalan, born in Barcelona, whose childhood coincided with the civil war. His life and professional career took him to Tarragona, Madrid, Oxford, Granada, Paris, and back to Madrid. Wherever he went, he left a deep mark, promoting improvements and founding institutions useful for the progress of society.

In Granada, he established a pioneering research group for the analysis of hereditary metabolic diseases, whose contributions he later transferred to Madrid. This work was crucial for the prevention and treatment of multiple diseases, as reflected in the documentary "La vida en una gota" (Life in a Drop), in which he appears discreetly.

In Madrid, he co-founded the Centro de Biología Molecular Severo Ochoa (CBM) and served as its first director. Aware of the importance of providing the center with an efficient structure and financial support, he promoted the creation of the Severo Ochoa Foundation. In addition, he carried out fruitful teaching work at the Autonomous University of Madrid (UAM) and, on a temporary basis, assumed the presidency of the Spanish National Research Council (CSIC). His management left a deep mark on Spanish science.

He later served as Minister of Education and Science in the Spanish government. Later, during his time in Paris as Director-General of UNESCO, he worked tirelessly for the cultural and economic development of the most disadvantaged countries, often facing resistance from the major powers.

Upon his return to Madrid, he continued to support the CBM and played a prominent role in the Ramón Areces Foundation. Finally, he founded the Culture of Peace Foundation, promoting the values of dialogue and understanding.

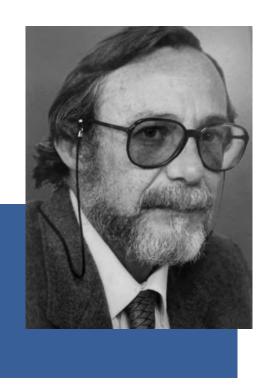
Don Federico was a person of extraordinary brilliance, generosity, and good faith. In his talks and classes, he was known for his clarity, wit, and quick wit. At work, he always put the common good before individual interests. He was a person of integrity and trustworthiness, whose human qualities and dedication to his work leave an indelible mark.

Thanks to his work, the CBM is today one of the most important research centers in Spain. His absence will be felt not only at the center, but also in all the areas where he displayed his commitment and talent.

Don Federico Mayor Zaragoza was an exemplary professional, an exceptional human being, and someone who faithfully followed the principle that Santiago Ramón y Cajal established for scientists: "Be wise, not arrogant."

Jesús Ávila de Grado, CBM emeritus senior researcher Paola Bovolenta, CBM director

## PEDRO RIPOLL QUINTAS



With these few words, we would like to remember our colleague Pedro Ripoll, who passed away in January 2025. Pedro was a man of few words, but of action, with an intense gaze that said it all.

Pedro was an important figure in the history of the CBM, where he served as director in 1985. His work during the 1970s to 1990s contributed significantly to the international reputation of our center and to creating a tradition of scientific excellence in the field of developmental biology. Several prominent scientists in the discipline were trained by him.

Pedro was one of the first doctoral students of our colleague Antonio García-Bellido and from the beginning stood out for his understanding of the genetic approach and his creativity. After a postdoctoral period at the University of California in San Diego with Dan Lyndsley, the most renowned Drosophila geneticist of the time, Pedro returned to Spain and joined the CBM, where he remained for the rest of his professional life. Pedro was an exceptional geneticist; his knowledge and handling of the devilish genetic combinations of Drosophila were legendary, as was his relentless critical spirit, from which many of us benefited.

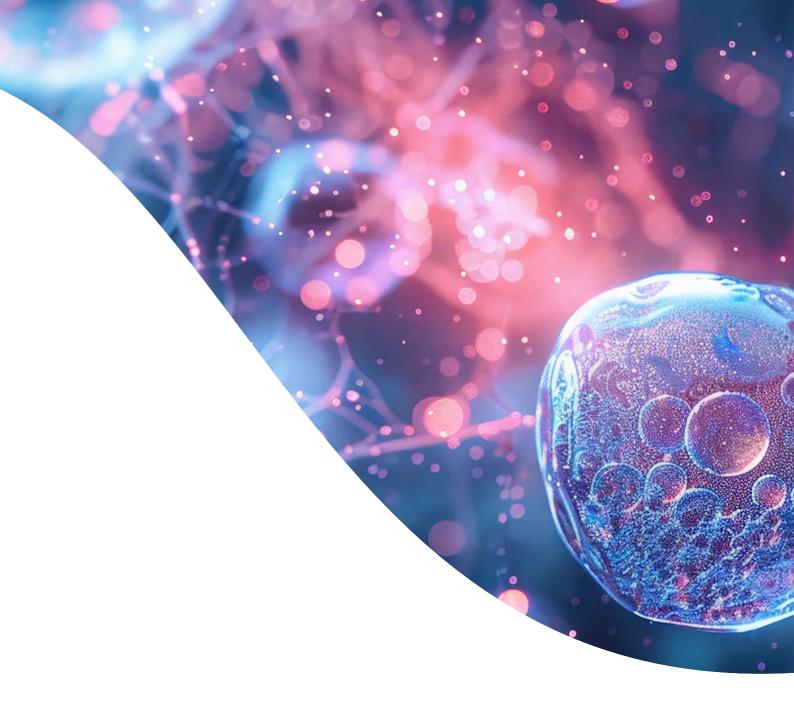
He was Director General of Research in 1989-90. This period was brief—Pedro was not cut out for management—but very effective, as he resolved several long-standing issues, such as the regularization of scholarship holders' payroll.

He spent the last years of his professional life in Isabel Guerrero's laboratory. Those of us who worked on the 4th floor saw him until a few weeks ago with his trays of tubes and bottles of flies on his way to do his crosses, as we did in the 1970s, and which he continued to do with the same enthusiasm and rigor. In his last project, Pedro found a new mutation that he named Bavaglio (Bav, gag) in homage to his Italian collaborators. The name of the mutation is very significant, as mutations in Bav silence the expression of some mini-genes in Drosophila.

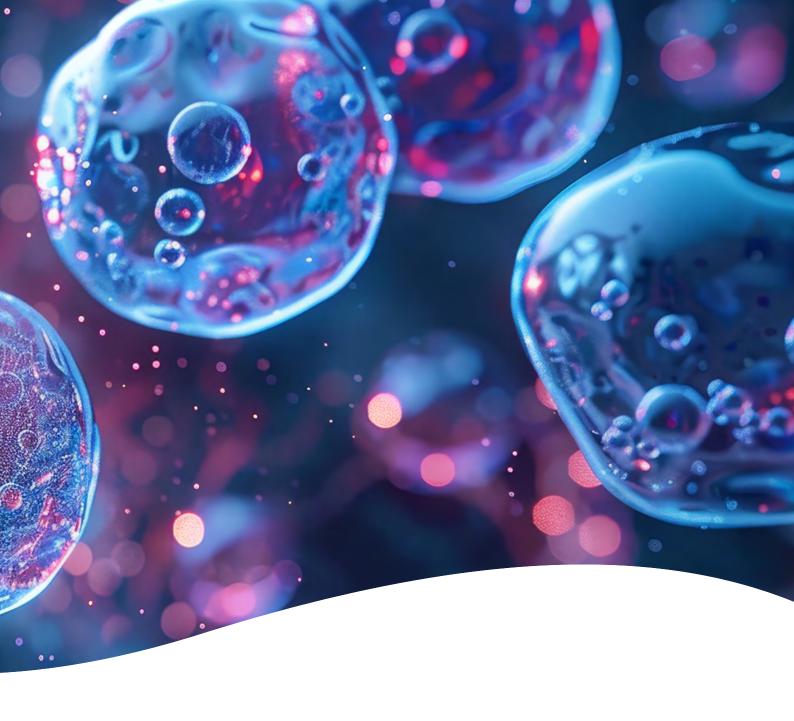
In recent decades, the way of working in experimental biology has undergone a radical change, with the introduction and widespread use of high-definition molecular methods and a strictly mechanistic approach. Pedro was an old-school geneticist, more interested in phenomena than mechanisms. Both approaches, phenomenological and mechanistic, should be complementary.

With Pedro's passing, an important part of the history of the CBM is lost.

Ginés Morata Research Professor at the CBM



# The CBM in a nutshell



## **ORGANIGRAM**

## **BOARD**



Carlos Closa Montero
CSIC VP for Organization
and Institutional Relation



Enrique Sastre de Andrés CSIC Institutional Delegate at the Community of Madrid



Daniel Jaque García

UAM Vice-Rector for Scientific

Policy



Ernesto Fernández Bofill **UAM General Manager** 

## DIRECTORATE AND MANAGEMENT



Paola Bovolenta Nicolao **Director** 



Beatriz López Corcuera

Vicedirector



Germán Lerma Rodrigo

Manager

## SCIENTIFIC COMMITTEEE



Paola Bovolenta Nicolao

President



Beatriz López Corcuera



Crisanto Gutiérrez



Fernando Martín-Belmonte



Federico Mayor Menéndez



Maria Luisa Toribio



Luis Blanco Dávila



Aurelio Hidalgo



Lola Ledesma



María Yáñez-Mó

## STEERING COMMITTEEE



Paola Bovolenta Nicolao **Director** 



Beatriz López Corcuera

Vicedirector



Germán Lerma Rodrigo

Manager



Crisanto
Gutiérrez

Genome dynamics
and function program
director



Fernando Martín-Belmonte Tissue and organ homeostasis program director



Mayor Menéndez

Physiological and
pathological processes
program director

Federico



María Luisa Toribio Interactions with the environment program director



Aura Carreira

Representative of the scientific staff



Carlos Gallego

Representative of the research support staff



Konstantinos Stamatakis Representative of nonpermanent research staff

#### SCIENTIFIC EXTERNAL ADVISORY BOARD



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of Basel
Switzerland



Maria-Elena Torres-Padilla Helmholtz Munich Germany



Juan Valcárcel

Centro de Regulación
Genómica
Spain

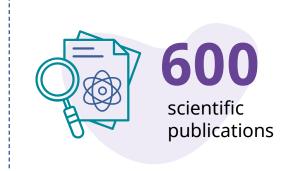


Matthijs Verhage
Vrije Universiteit
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Fiona M. Watt
King's College London
& EMBO
United Kingdom
& Germany

## **2023-24 IN NUMBERS**

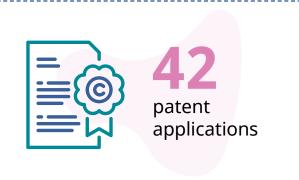














+4500

attendees to our science outreach activities & events



+19.000 followers gained across social media platforms



55.547 website visits



PhD theses completed





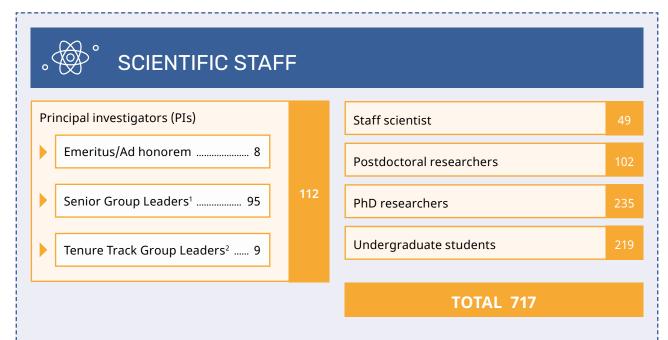
outreach volunteers



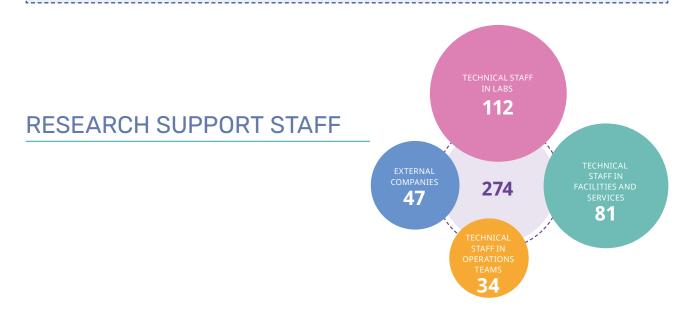
projects with industry

## PEOPLE AT THE CBM

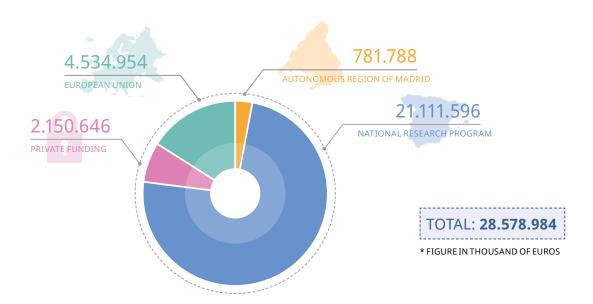




- <sup>1</sup> Includes the following categories: Profesor de Investigación, Científico Titular, Investigador Científico, Doctor FC1, Catedrático, Profesor Titular, Profesor Contratado Doctor, Profesor Permanente Laboral, Profesor Ayudante Doctor
- <sup>2</sup> Includes the following categories: Ramón y Cajal, Talento, La Caixa Junior, Profesor Contratado Doctor, Profesor Permanente Laboral, Profesor Ayudante Doctor



## PROJECTS AND FUNDING



## **SCIENTIFIC FACILITIES**



Advanced light microscopy

Animal facility

Biocomputational analysis

**Bioinformatics** 

Drosophila transgenesis

Electron microscopy

Flow cytometry

Genomics

Library

Mouse gene editing

Preclinical biomedicine Protein biotechnology

Proteomics

## **TECHNICAL SERVICES**

Biosafety and radioprotection

Cell culture, glass wash and media preparation

Facilities Maintenance

**Information Technology and Services** 

Occupational Health and Safety

**Technical Maintenance** 

Photography

## **OPERATIONS TEAMS**

Communications & Scientific Outreach

Finance and grants

**Grant support** 

**Human resources** 

National and international programs

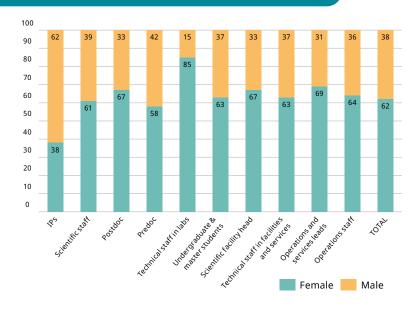
Institutional relations

Procurement

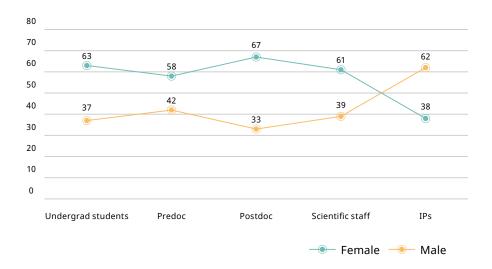
Quality management

## **GENDER**

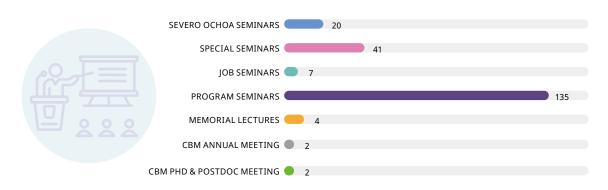
## **GENDER BY STAFF GROUP (%)**



## GENDER IN ACADEMIC CAREER PATH (%)



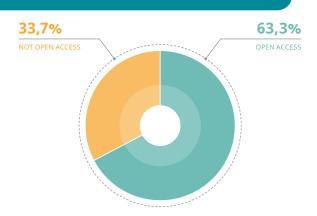
## **SEMINARS AND LECTURES**



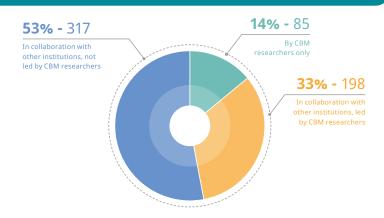
## **PUBLICATIONS**

# 600 PUBLICATIONS

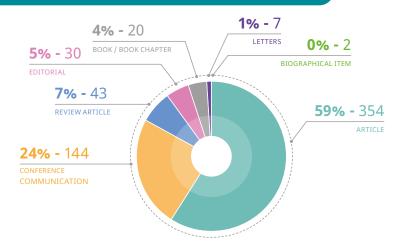
### **OPEN ACCESS**



## **COLLABORATIONS IN SCIENTIFIC PUBLICATIONS**



## **PUBLICATION TYPES**



## TRAINING ACTIVITIES

DOCTORAL THESES

67

223

MASTER AND DEGREE THESES

## **KNOWLEDGE TRANSFER**

During the 2023-2024 period, the Centro de Biología Molecular Severo Ochoa has intensified its knowledge transfer activities, with results that reflect the center's commitment to applying research for the benefit of society and the economy.

#### 1. PATENTS





During this period, 42 new patent applications have been filed covering a wide range of áreas. These applications reflect the CBM's dynamism in protecting research results with industrial application potential, with a special emphasis on technologies that provide solutions to challenges in health and biotechnology.

#### 2. LICENSED PATENTS

Eight patents have been licensed to companies in the biotechnology sector. These licenses consolidate the collaboration between the CBM and the productive sector, reinforcing the social impact of the research carried out at the center.

#### **List of licensed patents:**

- 17/687066, APPARATUS AND METHODS FOR SAMPLING AIR-BORNE PARTICLES ANTONIO ALCAMÍ PERTEJO, 2023
- PCT/ES22/070527, ATTENUATED AFRICAN SWINE FEVER VIRUS AND USES THEREOF ISABEL YOLANDA REVILLA NOVELLA, 2023
- 22382815, ATTENUATED AFRICAN SWINE FEVER VIRUS AND USES THEREOF ISABEL YOLANDA REVILLA NOVELLA, 2023
- PCT/ES22/070574, ATTENUATED AFRICAN SWINE FEVER VIRUS AND USES THEREOF ISABEL YOLANDA REVILLA NOVELLA, 2023
- 17/2024, A NON-HUMAN ANIMAL MODEL OF MMSET-ASSOCIATED MULTIPLE MYELOMA CESAR JOSE COBALEDA HERNANDEZ, JORGE MARTINEZ CANO, ELENA CAMPOS SANCHEZ, 2024
- 324/2024, HIBRIDOMA LINEA DE CELULAS 2H6F11 PRODUCTORA DE ANTICUERPOS MONOCLONALES CONTRA ENDOGLINA HUMANA (CD105) CARLOS CABAÑAS GUTIERREZ, CARMELO BERNABEU QUIRANTE, 2024
- 107/2024, TITLE UNAVAILABLE ISABEL YOLANDA REVILLA NOVELLA, DANIEL PEREZ NUÑEZ, 2024
- 156/2024, TITLE UNAVAILABLE ISABEL YOLANDA REVILLA NOVELLA, DANIEL PEREZ NUÑEZ, 2024

#### 3. PROJECTS WITH INDUSTRY

The CBM has developed 13 R&D projects in collaboration with industry, with funding exceeding €750,000, working with companies in the pharmaceutical and biotechnology sectors to accelerate technology transfer to the productive sector, enhance industrial competitiveness, and generate innovative solutions. These projects have involved and been funded by entities such as Careside, BrightFocus Foundation, Ionis Pharmaceutical Inc, One Chain Immunotherapeutics, Fundación UAM, Sener Mobility, HIPRA Scientific, Exbio Praha, 4Basebio, Fundación BBVA, Tragsatec, and MIMO Biosciences, ensuring that outcomes address real market needs. The projects have covered topics including the development of attenuated African Swine Fever Virus, the study of accelerated brain aging mechanisms in patients, replacement of TAU mRNAs in Alzheimer's disease, scalable platforms for allogeneic off-the-shelf cell therapies, biological systems for environmental control, virus stability in aerosol chambers, and exclusive licenses for trade secrets and patents related to anti-CD3 epsilon monoclonal antibodies, HIV-1 retrotranscriptases active at high temperatures, and phi29 phage DNA polymerase chimeras. The CBM has also advanced projects under Fundación BBVA Leonardo Grant programs, engaged in wildlife DNA detection and sequencing projects with Tragsatec, and developed animal models for multiple myeloma, consolidating CBM's role as a key player in technology transfer applied to industrial and clinical challenges.

#### 4. 2023 CSIC EBTON AWARD FOR THE PHAGE-PHI PROJECT

The CSIC's EBTon program awarded the Phage-Phi Project in 2023, developed by María Isabel Martínez Jiménez and Alicia del Prado Díaz. Phage-Phi is an integrated system for the early detection of specific bacteriophages in fermentation processes in the food industry, based on improved variants of the Phi29 polymerase. This technology seeks to prevent economic losses due to contamination, optimizing safety and quality in industrial fermentation processes and improving production efficiency in the food sector. The recognition at EBTon CSIC reinforces the value of the innovation developed at CBM aimed at solving specific industrial challenges.

#### 5. 2024 CSIC EBTON AWARD FOR THE TARMEMET PROJECT

In the final of EBTon CSIC 2024, researcher Manuel López Cabrera received an award for the Tarmemet project, an innovative therapeutic strategy against ovarian cancer. This project is based on the development of an antibody-drug conjugate (ADC) specifically targeting cells in the tumor microenvironment that feed tumor growth, with the aim of blocking their support function and promoting tumor regression. This approach seeks to reduce the side effects of conventional treatments and offer new, safer, and more effective therapeutic alternatives for ovarian cancer patients.

#### 6. BEST NATIONAL PATENT AWARD

As part of the center's technology transfer achievements, Manuel López Cabrera, received the First Prize for the patent "Solutions for peritoneal dialysis containing a natural flavonoid as an osmotic agent" (PCT/ES2023/070430; WO2024/008987 A1) in the 2024 Call for the Best National Patent Award by the University of Alcalá on November 27, 2024. This recognition highlights the innovative capacity of the CBM in generating patents with high clinical and industrial relevance, reinforcing the center's commitment to transferring research results to address real healthcare challenges. The patent is co-owned by CSIC (50%) and the University of Alcalá (50%).



In summary, the 2023-2024 period has been particularly productive in terms of knowledge transfer at the CBM, consolidating new patents, licenses, industrial collaborations, and external recognition that boost the social and economic impact of the research carried out at the center.

## 2023-24 AWARDS TO OUR STAFF

## SELECTED AWARDS 2023-2024







 CSIC Margarita Salas Medal to Crisanto Gutiérrez and Jesús Ávila



 Boehringer Ingelheim FENS Research Award 2024 to María Llorens-Martín



 María Mittelbrunn named Corresponding Member of the Royal Academy of Exact, Physical and Natural Sciences of Spain



 Luis Blanco named Member of the Academia Europaea 2024



 Ana Ortega named Full Member of the Young Academy of Spain



Jesús Serra Research Award 2024 to María Mittelbrunn



XXIX Carmen and Severo
 Ochoa Award for Research
 in Molecular Biology to
 María Llorens-Martín



Honorary Doctorate awarded to Santiago Lamas



Margarita Salas Scientific and Technological Research Award from the Madrid City Council, in the Life Sciences category, awarded to Elena Moreno



 Cavaliere dell'Ordine della Stella d'Italia awarded to Paola Bovolenta

## **COMPLETE LIST**

#### • 2023 •

- Crisanto Gutiérrez 2023 Margarita Salas Medal from the CSIC for Mentoring and Supervision
- Jesús Ávila 2023 Margarita Salas Medal from the CSIC for Mentoring and Supervision
- Margarita del Val Award for Added Value in Science and Technology, Fundación Transforma España, 2023
- Federico Mayor Menéndez Honorary Member of the SEBBM 2023
- Santiago Lamas Doctor Honoris Causa, Faculty of Medicine, University of the Republic (Uruguay) 2023
- Tatiana Alfonso-Pérez 3<sup>rd</sup> prize in the Huygens Image Contest

#### • 2024 •

- Julia Terreros Roncal 2023 Lilly Foundation Award for Best Doctoral Thesis in Biochemistry and Molecular Biology
- Julia Terreros Roncal VIU-NED Chair Award for Best Doctoral Thesis in Neuroscience
- Susana Cadenas and group SfRBM and SFRR-E Paper of the Year 2023
- María Llorens-Martín Boehringer Ingelheim FENS Research Award 2024
- María Llorens-Martín XXIX Carmen and Severo Ochoa Award for Research in Molecular Biology
- Ana Ortega Molina Full Member of the Young Academy of Spain
- Luis Blanco Member of the Academia Europaea 2024
- Luis Blanco 2024 Plaque of Honor from the Spanish Association of Scientists
- Elena Moreno Runner-up Prize for Best Doctoral Thesis SEBBM and Lilly Foundation
- Elena Moreno Margarita Salas Scientific and Technological Research Award from the Madrid City Council
- Marcos Jiménez Best Communication Award for Young Researchers, SEBBM and BBVA Foundation
- Diego Martín Sanz COBCM Award for Best Final Degree Project 2024
- María Mittelbrunn Jesús Serra Research Award 2024
- María Mittelbrunn Corresponding Member of the Spanish Royal Academy of Sciences
- Paola Bovolenta President-elect of FENS
- Paola Bovolenta Cavaliere dell'Ordine della Stella d'Italia
- Javier Álvarez de Miranda Runner-up for Best Talk SEV
- Samuel Donaire Plenary Session First Prize 19th Young European Scientist Meeting
- Lourdes Ruiz Desviat EPF Martínez-Pardo Award for Metabolic Diseases
- Alicia del Prado Díaz IV Margarita Salas Researcher of the Year Award, Margarita Salas Foundation and LeadinGirls
- José Antonio López Guerrero I "Research and Scientific Dissemination" Award, 5th Edition of the Código Sepsis Awards
- Manuel López Cabrera First Prize, University of Alcalá for Best National Patent
- Pilar Sandoval Correa 2024 CSIC Hackathon prize
- Tatiana Alfonso-Pérez Third Prize, Huygens Imagen International Competition
- Alicia del Prado Díaz Margarita Salas Award in the category of Science and Technology Researcher of the Year

# 2023-24 AWARDS TO OUR STAFF

### **CBM INTERNAL AWARDS**

#### 2023 •

#### **PINP Award for Best Original Paper**

Supported by IUBM (University Institute of Molecular Biology)

Carla Sánchez Castillo

Functional specialization of different PI3K isoforms for the control of neuronal architecture, synaptic plasticity, and cognition

#### **PINP Award for Best Doctoral Thesis**

Supported by IUBM (University Institute of Molecular Biology)

Carl Philip Lehman

Spatial regulation of the central DNA damage tolerance protein Rad5 in response to cellular stress

#### **SMOA Award, Optical Microscopy Image Contest**

First prize: Rafael Casado Navarro Second prize: Ángela Albitre Sanz Third prize: Cristina Clemente Toribio

#### **PhD & Postdoc Meeting**

#### **Best Talk Award**

Andrés Soto Zaragoza

Determination of the influence of the viral infection in the TAP-independent presentation of vaccinia virus epitopes

#### **Best Poster Award**

Jorge Galán Cruz

Exploring the neuroprotective effects of 7,8-dihydroxiflavone in a neuronal model of Friederich's Ataxia









#### 2024 •

#### **PINP Award for Best Original Paper**

Supported by IUBM (University Institute of Molecular Biology)

Claudia Cifuentes Castillo

Unmutated RRAS2 emerges as a key oncogene in post-partum-associated triple-negative breast cancer

#### **PINP Award for Best Doctoral Thesis**

Supported by IUBM (University Institute of Molecular Biology)

Elena Moreno Jiménez

Adult hippocampal neurogenesis process in humans. Alteration of the integration of new granular neurons in pathological conditions.

#### **SMOA Award, Optical Microscopy Image Contest**

First prize for image: Domnica Finis

Second prize for image: Nerea Murugarren and Miguel Hernández

Third prize for image: Cristina Cacho and Susana Barroso

Video prize: Elena Sánchez Bustamante

#### **PhD & Postdoc Meeting**

#### **Best Talk Award**

Javier Isoler Alcaraz

Addressing the role of the epigenetic regulator NSD2 in cellular plasticity.

#### **Best Poster Award**

José García-Consuegra Martínez

Sex Hormones Influence on Mitochondrial Function: Tissue-Specific Effects and Implications for Metabolic Health









## **ERC GRANTS 2023 AND 2024**





#### **CRISANTO GUTIERREZ ARMENTA**

833617-PLANTGROWTH-ERC-2018-ADG

# EXPLOITING GENOME REPLICATION TO DESIGN IMPROVED PLANT GROWTH STRATEGIES — PLANTGROWTH

DATES	BENEFICIARY	FUNDING
01/06/2019 • 31/05/2025	CSIC	2.497.800.00€



#### **MARÍA LLORENS-MARTÍN**

ERC-CoG-2020-101001916

'INTERROGATING HUMAN ADULT HIPPOCAMPAL NEUROGENESIS — HumAN' ('ACTION')

DATES	BENEFICIARY	FUNDING
01/10/2021 • 30/09/2026	CSIC/UAM	1.993.792,74 €



#### **EDUARDO BALSA MARTINEZ**

948478 MitoCure ERC-2020-STG

# MOLECULAR AND METABOLIC MECHANISMS UNDERLYING MITOCHONDRIAL DYSFUNCTION

DATES	BENEFICIARY	FUNDING
01/01/2021 • 31/12/2025	UAM	1.463.410,00€



#### **MARIA MITTELBRUNN HERRERO**

715322-EndoMitTalk-ERC-2016-STG

# ENDOLYSOSOMAL-MITOCHONDRIA CROSSTALK IN CELL AND ORGANISM HOMEOSTASIS

DATES	BENEFICIARY	FUNDING
01/03/2017 • 28/02/2023	UAM	1.199.875,00 €



# SCIENTIFIC ORGANIZATION OF THE CBM

SCIENTIFIC PROGRAMS

UNITS

GENOME DYNAMICS AND FUNCTION

Genome decoding

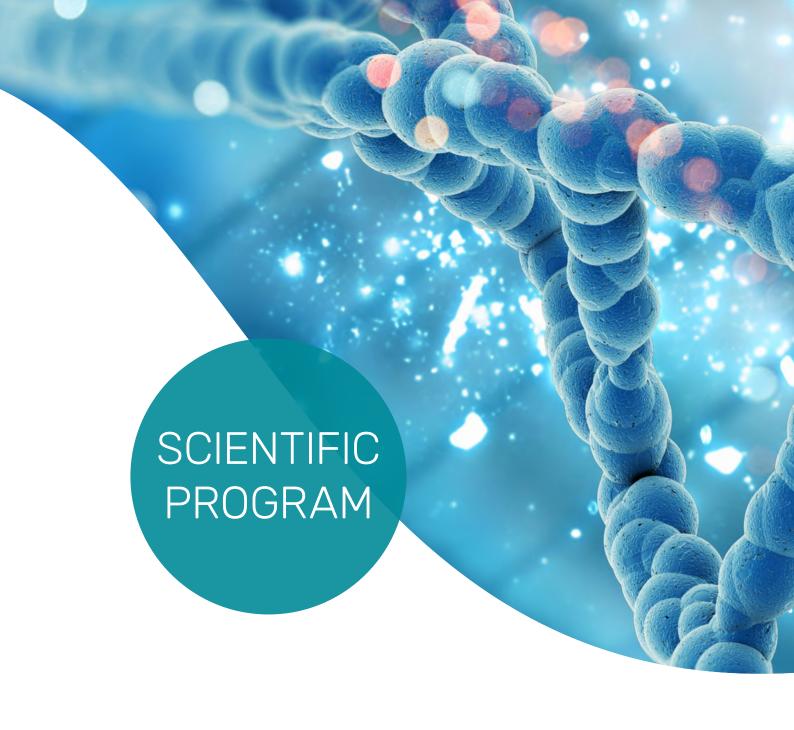
Genome maintenance and instability

TISSUE AND ORGAN HOMEOSTASIS

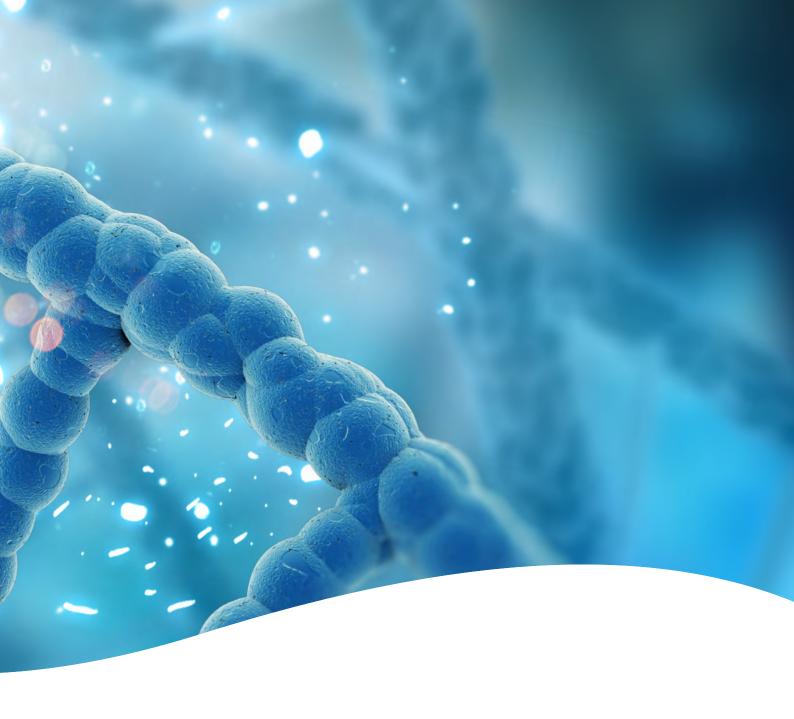
Cell-cell communication and inflammation

Cell architecture and organogenesis

**PHYSIOLOGICAL** INTERACTIONS AND WITH THE **PATHOLOGICAL ENVIRONMENT PROCESSES** Immune system Molecular development neuropathology and function Metabolic and Microbes in health signaling networks and welfare in disease



# Genome dynamics and function



Genome decoding unit

Genome maintenance and instability unit



# Genome dynamics and function

## Genome decoding



## Encarnación Martínez Salas

The research activities of members within the Genome Decoding UNIT are centered at deciphering the molecular basis of essential cellular processes to better understand how genomic information is interpreted in a wide variety of organisms, including viruses infecting animal cells, parasites, yeasts, plants, and mammalian cells. A critical task in gene expression studies is to establish a direct link between genomic data and proteome homeostasis. Achieving this goal may help to provide the basis for cellular and developmental processes as well as to find new diagnostic tools and therapeutic strategies.

Specific goals of the Unit are aimed at understanding fundamental questions on cell proliferation, gene expression and genome replication and how epigenetics affects these processes in the model plant *Arabidopsis thaliana*. In the last two years, this work led to the generation of the PlaCCI Arabidopsis line, currently used by >200 laboratories world-wide. This line combines three fluorescent markers allowing the identification of cell cycle phases in the whole organism, instrumental to decipher gene networks controlling proliferation of stem cells and their derivatives during organogenesis where the G1 duration is crucial. A related goal of a junior group that recently joined the Unit aims at understanding how transcription rewires as eukaryotic cells transit through mitosis.

Computational biology, inferring structural and physico-chemical properties of proteins is a main focus of the Unit. Research done led to the development of three interesting tools. PC\_ali combines protein structure and sequence similarity, and generates phylogenetic trees based on sequence and structure divergence. SSCPE allows the phylogenetic analysis of proteins with a substitution model considering protein folding stability, native dynamics, and structural response to mutations. ZPeaks\_U was generated to study GGN motifs and nucleosome organization for eukaryotic replication origins.



Linking transcription and protein synthesis, the generation of improved genome assemblies, transcriptome and gene annotations for pathogenic Leishmania species through the incorporation of proteomic data analysis allowed the discovery of new protein-coding genes, greatly improving the gene annotations in the *L. infantum* and *L. donovani* genomes. Furthermore, ribosome-protected mRNAs fragments (Ribo-Seq) data allowed the generation of gene models in the L. donovani genome and uncovering of small upstream open reading frames (uORFs) in a significant number of transcripts. Further establishing a relation between transcriptome data with gene expression programs, the analysis of precursor T-cell lymphoblastic neoplasms identified specific circRNAs that discriminate between different stages of thymocyte differentiation. Identification of alterations with a clinical potential for T-ALL/ LBL patients, like PIM1 and SEPTIN6::ABL2, which can be therapeutic a targets. Reduced expression of long non-coding MALAT1 alters ERK and AKT kinase pathways through up-regulation of IGFBP2 in precursor T-cell neoplasms.

An important goal of the Genome Decoding Unit is aimed at deciphering mechanisms of translation initiation through the functional and structural characterization of RNA structural elements and RNA-binding proteins (RBPs), as well as regulation of mRNA translation in eukaryotic cells and viruses, their implications for stress response and aging, the effect of arginine-rich peptides on translation, and the impact of protein quality control system on ageing and survival of fission yeast. Along this line, the RBP GEMIN5 was identified as a gene expression balancer, consistent with its dual role in snRNP assembly and modulator of translation. Importantly, GEMIN5 variants carrying pathological mutations are deficient in oligomerization capacity, ribosome binding and translation regulation. In line with this finding, the mRNAs associated with heavy polysomes in cells with high levels of GEMIN5 protein revealed that alternative splicing events undergo translation. Furthermore, detailed analysis of the differential effect of SARS-CoV-2 NSP1 on mRNA translation and stability, revealed new insights linking ribosome recruitment, codon usage and virus evolution.

## Research groups

#### **Ugo Bastolla**

Computational biology and Bioinformatics

#### **Crisanto Gutierrez**

DNA replication, chromatin and cell division

#### **Encarnación Martinez Salas**

RNA-binding proteins and translation control in eukaryotes

#### **Carlos Perea Resa**

Dynamic and recycling of the transcriptional machinery across mitosis

#### Jose María Requena Rolanía Begoña Aguado Orea

Regulation of gene expression in Leishmania

#### Javier Santos Hernández María Villa Morales

Genetics and cell biology of cancer: T-cell lymphoblastic neoplasms

#### Iván Ventoso

Regulation of mRNA translation in eukaryotes and its implications for organismal life



#### COMPUTATIONAL BIOLOGY AND BIOINFORMATICS

#### **Group members**

**Principal investigator:** Ugo Bastolla

**Undergraduate and Master students:** Fatemeh Saebi Armin Bahraminia de la Fuente Alvaro Ayala



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



#### Summary

Our work presents four main lines: (1) Developing structure-aware substitution models of protein evolution for phylogenetic inference, with selection on protein structure and its folding stability. (2) Developing and applying computational methods for predicting protein functional dynamics and response to mutations. (3) Characterizing the relation between chromatin structure and genome replication. (4) Investigating through mathematical models the ecological interactions between species in microbial and general communities.

Along line (1), we developed the structure and stability-constrained substitution model of protein evolution (SSCPE), based on our new predictions of the structural effect of mutations (https://www.biorxiv.org/content/10.1101/2023.01.22.525075v2). It greatly improves evolutionary inference with respect to our previous model that only takes into account protein folding stability. Based on this previous model, with Miguel Arenas we found that recombination events and point mutations produce similar loss of protein folding stability, suggesting that the requirement of folding does not particularly limit recombination.

Moreover, we developed the hybrid protein alignment tool PC\_ali based on sequence and structure similarity, which improves the alignment quality and the estimated divergence time with respect to methods that take into account only one type of information. From these divergences, we infer phylogenetic trees of protein superfamilies and classify their molecular functions by detecting candidate function change

events as branches of the phylogenetic tree where protein structure diverges more than expected based on sequence divergence, pointing at positive selection.

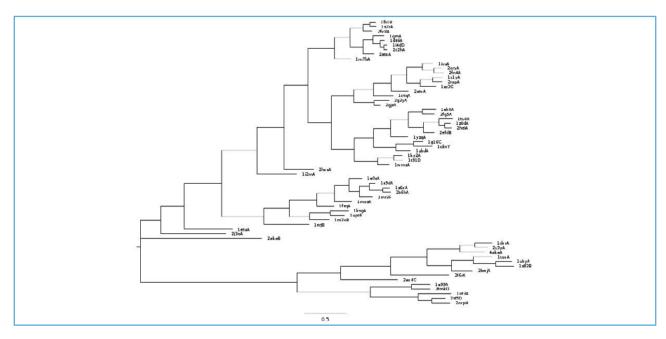
Along line (2), we studied the allosteric couplings and the structural effects of mutations of GABA receptors (collaboration with Francisco Zafra's group, CBMSO). We also contributed to develop a new method for estimating the change of stability due to mutations (collaborations with Pablo Chacón's group, IQFR).

Along line (3), we developed the algorithm ZPeaks\_U for calling replication origins and estimating their replication time in experiments with thymidine analogues and, in collaboration with the groups of Crisanto Gutierrez, María Gómez (CBMSO) and Carlos Gonzales (IQFR), we investigated the relationship between repeats of GGN triplets, genome replication origins and chromatin structure (nucleosome occupancy and G-quadruplex).

Along line (4), we studied how different types of ecological interactions influence the structural stability of mathematical models of species communities. In collaboration with Fenando Puente, Alberto Pascual, Javier Tamames and Carlos Pedrós (CNB), we discovered a negative relationship between the size of bacterial genomes and the number of taxa of the community they belong to, hinting at cooperative auxotrophic interactions.

Finally, we investigated Covid-19 infections in children (collaboration with M.Fresno's group).





Phylogenetic tree of the Ploop containing Nucleoside Triphosphate Hydrolases superfamily (one ot the largest superfamilies in the CATH and SCOP databases, which includes the kinase-GTPase class), obtained with our program PC\_ali using integrated sequence and structure information.



#### List of publications

- Puente-Sánchez F, Pascual-García A, Bastolla U, Pedrós-Alió C, Tamames J. (2024) Cross-biome microbial networks reveal functional redundancy and suggest genome reduction through functional complementarity. Commun Biol. 7(1):1046. doi: 10.1038/s42003-024-06616-5.
- Bastolla U, Abia D, Piette O. (2023) PC\_ali: a tool for improved multiple alignments and evolutionary inference based on a hybrid protein sequence and structure similarity score. Bioinformatics. 39(11):btad630. doi: 10.1093/bioinformatics/btad630.
- Úbeda M, Maza MDC, Delgado P, Horndler L, Abia D, García-Bermejo L, Serrano-Villar S, Calvo C, Bastolla U, Sainz T, Fresno M. (2023) Diversity of immune responses in children highly exposed to SARS-CoV-2. Front Immunol. 14:1105237. doi: 10.3389/fimmu.2023.1105237.
- Piniella D, Canseco A, Vidal S, Xiol C, Díaz de Bustamante A, Martí-Carrera I, Armstrong J, Bastolla U, Zafra F. (2023) Experimental and Bioinformatic Insights into the Effects of Epileptogenic Variants on the Function and Trafficking of the GABA Transporter GAT-1. Int J Mol Sci. 24(2):955. doi: 10.3390/ijms24020955.
- Hernández IM, Dehouck Y, Bastolla U, López-Blanco JR, Chacón P. (2023) Predicting protein stability changes upon mutation using a simple orientational potential. Bioinformatics. 39(1):btad011. doi: 10.1093/bioinformatics/btad011.
- Del Amparo R, González-Vázquez LD, Rodríguez-Moure L, Bastolla U, Arenas M. (2023) Consequences of Genetic Recombination on Protein Folding Stability. J Mol Evol. 91(1):33-45. doi: 10.1007/s00239-022-10080-2.



#### Research projects

- Integrated model of protein evolution in sequence, strucure and function for bioinformatics and phylogenetic applications. PID2023-151032NB-C21. Granting agency: Agencia Española de Investigación. 09/2024-12/2027. PI: U. Bastolla
- Theoretical and computational investigation of tuberculosis antimicrobial resistance development based on extensive experimental library of mycobacterium strains. H2020-MSCA-RISE-2018-823922. European Commission. 11 teams coordinated by Aston University (UK).
- Substitution processes with selection on protein structure and stability, for phylogenetic inference and prediction of the effects of mutations. PID2019-109041GB-C22. Spanish Research Agency (AEI). PI: Ugo Bastolla. 06/2020-05/2023.



#### DNA REPLICATION, CHROMATIN AND CELL DIVISION

#### **Group members**

**Principal investigator:** Crisanto Gutierrez

**Scientific staff:** Bénédicte Desvoyes Sara Diaz-Triviño

Postdoctoral fellows: Ivan del Olmo Montoro Miguel Hernández Sánchez-Rebato Diego Gómez Martínez Julia Emiliani María Sol Gómez Jorge Fung-Uceda Predoctoral fellows: Rocío Núñez-Vázquez Nerea Murugarren Garrido Adrián Pastor Solano Laura Rodríguez Casillas

**Technicians:** Miguel López Gestoso

**Undergraduate and Master students:**Juan Manuel Domínguez
Pacheco



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



#### 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 the establishment of complex gene regulatory networks. Our research is aimed at understanding fundamental questions on cell proliferation control, gene expression and genome replication in multicellular organisms and how epigenetics affects such coordination. To that end, we use the model plant Arabidopsis thaliana. Contrary to animals, plant development is postembryonic and occurs during the entire life of the organism, providing excellent experimental settings to study cell proliferation, arrest and differentiation. Additionally, Arabidopsis offers the possibility of combining molecular, cellular, genetic and genomic approaches.

A major advance has been finding the roles played by the two ORC1 proteins of *Arabidopsis*. ORC1a and ORC1b possess ~86% of amino acid identity but we were able to select single mutants with distinct phenotypes. ORC1a is required for proper deposition and maintenance of the typical heterochromatic mark H3K27me1 whereas ORC1b is required for genome replication (Fig. 1). The H3K27me1 mark is enriched not only in transposable elements but also in genes that are normally repressed. We have found a diurnal control of H3K27me1 deposition across genes involved in the DNA damage response. We

are also interested in understanding the relevance of chromatin dynamics on the stress response with particular interest in novel histone H3 variants specifically suited to respond to abiotic stress.

We are now deciphering the mechanisms controlling the expression pattern of ORC1 proteins as well as the requirements of specific protein domains and amino acid residues in heterochromatin dynamics and genome replication. Regarding the latter, we are focusing on the identification of molecular determinants of DNA replication origin (ORI) function. Based on previous genome-wide maps of ORIs we are exploring the role of individual modifications in ORI function using CRISPR-dCas9 tools.

The development of a line (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 has been received by the community with extraordinary interest and is now distributed to >200 laboratories world-wide. We are using these tools to decipher the gene networks and molecular basis controlling stem cell biology, proliferation of their derivatives and how dividing cells communicate with each other. In collaboration with various partners we have expanded these studies to other organs.



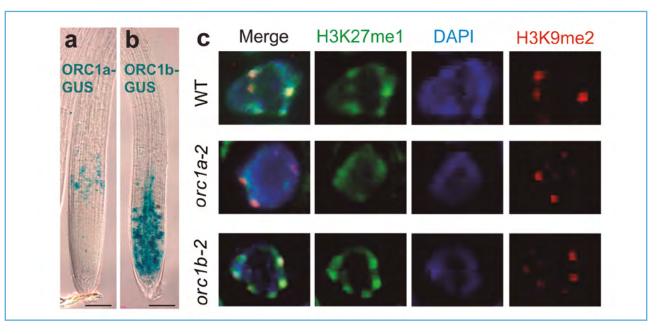


Figure 1. Expression of Arabidopsis ORC1a (a) and ORC1b (b) in the endoreplication zone of the root and in the proliferation zone of the root, respectively. (c) orc1a-2 mutants show defects in chromocenters (heterochromatin) with reduced H3K27me1 mark (green) whereas H3K9me2 (red) is not affected.



- Vergara, Z., Gomez. M. S., Desvoyes, B., Sequeira-Mendes, J., Masoud, K., Costas, C., Noir, S., Caro, E., Mora-Gil., V., Genschik, P. and Gutierrez, C. (2023) Distinct roles of Arabidopsis ORC1 proteins in DNA replication and heterochromatic H3K27me1 deposition. Nature Comm. 14(1):1270. doi: 10.1038/s41467-023-37024-8
- Hirt, H., et al. PlantACT! how to tackle the climate change. (2023) Trends Plant Sci. 28, 537-543 (2023) doi: 10.1016/j. tplants.2023.01.005
- Coloma, I., Cortijo, M., Mancheño, M.J., León-González, M.E., Gutierrez, C., Desvoyes, B. and Herrero, S. (2023) Diruthenium complexes as pH-responsive delivery systems: a quantitative assessment. Inorg. Chem. Frontiers 10, 4402-4413. doi: 10.1039/d3qi00399j
- Desvoyes, B. and Gutierrez, C. Recent advances in plant cell biology. (2023) Eur. J. Cell Biol. 151345. doi: 10.1016/j. ejcb.2023.151345.



#### Research projects

- PID2021-123319NB-I00. Ministerio de Ciencia e Innovación. Integration of cell cycle control and chromatin dynamics: the Arabidopsis model (ChromCC). Crisanto Gutierrez (PI). 2022-2025.
- ERC-2018-AdG\_833617. European Research Council. Exploiting genome replication to design improved plant growth strategies (PLANTGROWTH). Crisanto Gutierrez (PI). 2019-2024.
- Conexión GENOMA CSIC. Crisanto Gutierrez (Coordinator), Josefa Gonzalez (Adjunct Coordinator). 2024-2025.

- Voichek, Y., Hurieva, B., Michaud, C., Schmücker, A., Vergara, Z., Desvoyes, B., Gutierrez, C., Nizhynska, V., Jaegle, B., Borg, M., Berger, F., Nordborg, M. and Ingouff, M. Cell cycle status of male and female gametes during Arabidopsis reproduction. (2023) Plant Physiol (2023) 194, 412-421. doi: 10.1093/plphys/kiad512.
- Bahafid, E., Bradtmöler, I., Thies, A. M., Nguyen, T. T. O. N., Gutierrez, C., Desvoyes, B., Stahl, Y., Blilou, I. and Simon, R. (2923) The Arabidopsis SHORTROOT network coordinates shoot apical meristem development with auxin dependent lateral organ initiation. eLife 12, e83334. doi: https://doi. org/10.7554/eLife.83334
- Fung-Uceda, J., Gomez, M. S., Rodriguez-Casillas, L., Gonzalez-Gil, A. and Gutierrez, C. (2024) Diurnal control of H3K27me1 deposition shapes expression of a subset of cell cycle and DNA damage response genes. Plant J. 120, 2335-2336. doi: 10.1111/tpj.17114. See also front cover of the issue and highlight by Balcerowicz, M. (Plant J. 120, 2323–2324).

#### Other activities

- Member of the EMBO Council.
- Chair of the Cell and Developmental Biology Section of Academia Europaea.
- Member of the Scientific Advisory Board of Fundación Gadea - Ciencia.
- Member of Editorial Board of EMBO J., Eur. J. Cell Biol. and Plant J (until December 2024)
- Conexión GENOMA CSIC, coordinator.



# RNA-BINDING PROTEINS AND TRANSLATION CONTROL IN EUKARYOTES

#### **Group members**

**Principal investigator:** Encarnación Martinez Salas

Scientific staff:

Encarnación Martinez Salas

Postdoctoral fellows: Rosario Francisco Velilla

Predoctoral fellows:

Salvador Abellan Perez Azadeh Nahavandi

**Technicians:** Jorge Ramajo Alonso

Visiting scientists: Nazli Sevval Menemenli



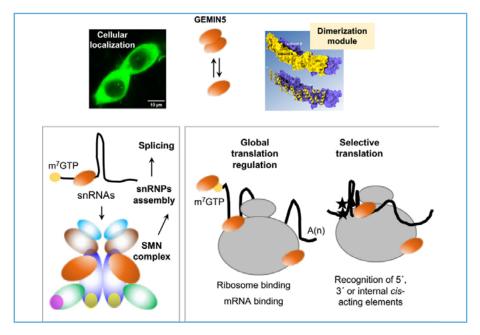
https://www.cbm.uam.es/encarna\_martinez-salas



#### Summary

Our main objective is focused to understand the principles guiding translation regulation in eukaryotes through the characterization of RNAbinding proteins (RBPs) interacting with mRNAs. Dysregulation of RBPs often produce widespread effects on multiple RNA-driven processes, challenging the identification of the molecular mechanisms contributing to disease. GEMIN5 is a predominant cytoplasmic protein, known as the RBP of the survival of the motor neurons (SMN) complex, directing the assembly of small nuclear RNAs (snRNAs) in ribonucleoproteins (snRNPs). In addition, GEMIN5 binds ribosomes and regulates translation (Figure 1). The protein is organized in structural and functional domains, including a WD40 repeats domain at the N-terminus, and a tetratricopeptide repeat (TPR) dimerization module and a non-canonical RNAbinding site at the half C-terminal region. The latter adopts a three-dimensional structure comprised of a dimer of pentamers, such that the intact architecture is critical to bind cognate RNA ligands and to regulate translation. The singular organization of GEMIN5 enables this protein to perform multiple functions through its ability to interact with distinct RNAs and proteins. GEMIN5 is an essential protein, as shown by the detrimental activity of biallelic mutants found in neurodevelopmental patients, and the lethal embryonic defects of the Drosophila homologue (Rigor mortis). Silencing of GEMIN5 in human cells revealed that this protein promotes polysome association of a selected subset of its targets, including ribosomal and histone mRNAs, consequently favoring translation of mRNAs that play a key role in cellular proliferation. We have found that patients carrying GEMIN5 biallelic variants, that display cerebellar atrophy, ataxia, cognitive delay, and hypotonia, harbor conformational changes within the dimerization domain. Molecular and structural studies of GEMIN5 mutants carrying pathological mutations led us to conclude that the GEMIN5 interactome depends upon its oligomerization capacity, strongly impacting in ribosome binding and translation regulation. Subsequently, by analyzing the properties of different regions of the protein, lacking or not the dimerization module, we have shown that protein dimerization failure strongly impinges on most of its activities, including protein interactome and regulation of translation, although the cytoplasmic localization is not altered. Recently, we have shown that upregulation of *GEMIN5* enhances alternative splicing (AS) events of genes involved in a broad range of cellular processes. Identification of the mRNAs associated with heavy polysomes in cells with high levels of *GEMIN5* reveals that a large fraction of the differential AS events undergo translation. Taken together, our data supports the notion that GEMIN5 has evolved as a gene expression balancer, consistent with its dual role in snRNP assembly and as a modulator of translation, ultimately impinging on cell homoeostasis.





Overview of the GEMIN5 involvement in snRNP assembly and translation control. The cellular localization, and the threedimensional structure of the dimerization module is shown.



#### List of publications

- Martinez-Salas E, Francisco-Velilla R. GEMIN5 and neurodevelopmental diseases: from functional insights to disease perception. Neural Regen Res. doi: 10.4103/ NRR.NRR-D-24-01010.
- Francisco-Velilla R, Abellan S, Garcia-Martin JA, Oliveros JC, Martinez-Salas E. 2024. Alternative splicing events driven by altered levels of GEMIN5 undergo translation. RNA Biol. 21(1):23-34. doi: 10.1080/15476286.2024.2394755.
- Francisco-Velilla R, Abellan S, Embarc-Buh A, Martinez-Salas E. 2024. Oligomerization regulates the interaction of Gemin5 with members of the SMN complex and the translation machinery. Cell Death Discovery. 10(1):306. doi: 10.1038/s41420-024-02057-5.



#### Research projects

- PID2023-148273NB-I00. Funcional studies of RBPs involved in translation control, antiviral inmunity and pathology (2024-2027)
- EU-COST OC-2021-1-25480 (2022-2025) TRANSLACORE. Translational control in Cancer.
- PID2020-115096RB-100 (2021-2024) Gemin5 function in RNA-driven processes: impact on neuromuscular diseases
- 2024AEP022. CSIC (2024) Role of Gemin5 in RNA-mediated events: impact on neuromuscular diseases
- Member of the networks: Conexion Genoma (CSIC), RiboRed, RNA-life, and RER-CSIC



#### Other activities

- 2023. Member of the ANR panel Genetics, genomics and RNA (CES12). France.
- 2023-2024. Reviewer: HFSP. Millenium Institute (Chile). SNF(Suiza), AEI (Spain).
- 2023. Member of CSIC human resources recruitment .
- 2023-2024. Member of the Editorial Board: Virology, Frontiers in Microbiology, Cellular and Infection Microbiology 2023. Invited talk, TRANSLACORE meeting, Lisbon, Portugal. Gemin5-driven mRNA association with polysomes enables selective translation of genes promoting cell growth.
- 2023. Invited talk, Intellia Therapeutics, Cambridge, MA, USA. RNA and RNA-binding proteins at the intersection of translation regulation".
- 2023. X Workshop Students&Postdocs. CBMSO, Madrid. Poster.

- 2024 Conexion Genoma. Invited talk. RNA and RNAbinding proteins at the intersection of translation regulation. Miraflores de la Sierra. Madrid
- 2024. RNA life 2024 meeting. El Escorial, Madrid. Talk.
- 2024. XI Workshop Students&Postdocs. CBMSO, Madrid. Talk



#### **Doctoral theses**

- Salvador Abellan Perez. Analysis of Gemin5 posttranslational modifications. In progress.
- Azadeh Nahavandi. Impact of the RBS1 domain of Gemin5 in RNA-binding. In progress.



# DYNAMIC AND RECYCLING OF THE TRANSCRIPTIONAL MACHINERY ACROSS MITOSIS

#### **Group members**

**Principal investigator:** Carlos Perea Resa

**Predoctoral fellows:** Estrella Isabel Sayago Aida Contreras

**Undergraduate and Master students:** 

Héctor Espinosa Alba Esteban Isabel Fernández



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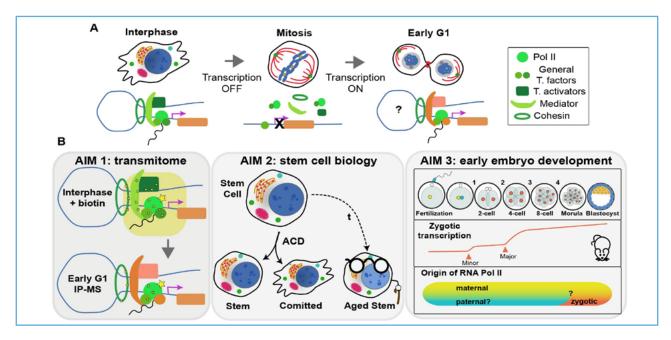


#### **Summary**

One of the most exciting aspects of our biology is the fact that all our cells share the same genetic information (handbook). The differential reading of this info is key to generate the diversity of cell types during the developmental program of a complex organism. In this frame, the transcription of DNA into RNA constitutes the first stage of that reading and is essential to establish differential gene expression patterns defining the distinct cell lineages. In addition to transcription, the process of mitosis is fundamental to obtain the precise number of cells to build a whole organism while the establishment of most of the cell lineages required the passage of cells through mitosis. Interestingly, transcription is widely silenced during mitosis due the release of RNA polymerases and transcription factors from the condensing chromatin. Once mitosis ends, transcription restart immediately to guarantee the viability and identity of daughter cells. The mechanisms behind transcription restart following mitosis are poorly understood. How daughter cells remember the transcriptional program of the mother or/and how those programs rewire during development are mostly unknown. In our laboratory we aim to answer these questions using the culture of mammalian cells as a model.

One of our goals is to understand the dynamic and recycling of the RNA polymerase 2, the enzyme transcribing all protein coding genes in eukaryotes, and the associated complex named Cohesin. In parallel, we investigate the potential implication of these processes in the etiology of developmental diseases as cohesinopathies. By combining the use of cell culture, genomic and proteomic approaches, CRISPR technology and live cell microscopy, we aim to understand how the transcriptional machinery works across the challenge of mitosis with the hope to understand how gene expression and cell division coordinates during development.





Scientific Interests and directions covered by the research at Perea-Resa laboratory



Contreras, A. and Perea-Resa C\*, Biochem Soc Trans, 2024, doi: 10.1042/BST20231071.



#### Other activities

- Co-organizador seminarios de programa.
- Miembro de la red Genoma del CSIC.



#### Research projects

- PID2022-137049NA-100. Ministry of Science and Innovation. Mechanisms and Function of Cohesin Recycling through Mitosis. Carlos Perea Resa.
- 01/09/2023-31/08/2026. CNS2023-144243. Ministry of Science and Innovation/ Recovery and Resilience Plan. Functional analysis of Pol II inheritance through mitosis in the identity, differentiation, and aging of stem cells. Carlos Perea Resa. 06/01/2024-05/31/2026.
- RYC2022-035585-I. Ministry of Science and Innovation/ European Union. Carlos Perea Resa. 01/09/2024-31/08/2029.



#### REGULATION OF GENE EXPRESSION IN LEISHMANIA

#### **Group members**

**Principal investigator:** Jose María Requena Rolanía (PI) Begoña Aguado Orea (co-PI)

Scientific staff: Manuel Soto Álvarez

**Postdoctoral fellows:** Jose Carlos Solana Morcillo

**Technicians:** Javier Adán Jiménez Alejandro Sánchez Salvador Ronny Pacheco Hinojosa

**Undergraduate and Master students:** Marina Rebollo Ortiz

Paula Alcain Arranz



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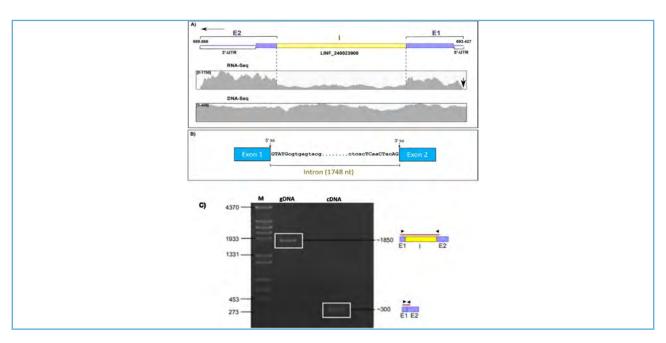


#### Summary

Leishmaniasis is caused by Leishmania, a unicellular protozoan parasite. Among parasitic diseases, leishmaniasis ranks second (after malaria) and affects several million people in many regions of the world, and Spain is an endemic area. No vaccine for preventing leishmaniasis exists and treatment options are limited. Leishmania is an atypical eukaryote regarding genome organization and gene expression regulation: genes are expressed as long polycistronic transcription units requiring extensive post-transcriptional processing. Leishmania is an adequate model for studying posttranscriptional regulation mechanisms without the interference of transcriptional regulation. Our research activity is aimed to generate information regarding genomics, transcriptomics and proteomics data for several prototypical Leishmania species. As a result, complete genome assemblies were obtained, and these are available at both general (NCBI/ENA and UniProt) and more specific (TriTrypDB) repositories. Moreover, we have delineated the poly(A)-transcriptomes and generated gene models for addressing differential gene expression based on RNA massive sequencing. Inaddition, we have incorporated proteomics analysis with the objective of improving gene annotations and uncovering new protein-coding genes based on data derived from proteogenomics approaches. Finally, ribosome-protected mRNAs fragments (Ribo-Seq) data have been analyzed to improve further gene model annotations. In sum, our main

objective has been to generate accurate genomics and transcriptomics annotations as a contribution to the field of leishmaniasis. This will advance our knowledge of the molecular biology of this parasite, which in turn will help us to develop more effective strategies to combat this deadly parasitosis. The participation of two scientific facilities at CBM, Biocomputational Analysis and Proteomics, have been paramount for progressing in our research activity. Another relevant contribution of our group was the building and maintenance of a web page (http://leish-esp.cbm.uam.es) for hosting the genomics/transcriptomics data generated by our group for four Leishmania species (L. major, L. infantum, L. donovani y L. braziliensis). Also, we are depositing curated datasets in the Mendeley data and Wikidata platforms to make publicly available the continuous improvements in gene/protein annotations we are introducing based on our experimental data and those derived from the scientific literature. As integrating of the leishmaniasis group in the CIBER of infectious diseases (CIBERinfec; https:// www.ciberinfec.es), we are engaged in collaborative research dealing with the molecular diagnosis and typing of Leishmania strains isolated from patients at the Centro Nacional de Microbiologia (ISCIII). In addition, we have active roles in the CSIC PTI\_Global Health, PTI\_Digital Science and Computational Biology and Bioinformatics Hub. Our group also belongs to the Conexión Genome CSIC Hub.





Events of cis-splicing are rare in Leishmania. Our group uncovered the third case described to date; this affects gene LINF\_240023900 (coding for a nuclear poly-A binding protein).



- Camacho, E., González-de la Fuente, S., Solana, J.C., Tabera, L., Carrasco-Ramiro, F., Aguado, B., and Requena, J.M. (2023). Leishmania infantum (JPCM5) Transcriptome, Gene Models and Resources for an Active Curation of Gene Annotations. Genes (Basel). 14, 866. doi: 10.3390/ GENES14040866
- Sánchez-Salvador, A., González-de la Fuente, S., Aguado, B., Yates, P.A., and Requena, J.M. (2023). Refinement of Leishmania donovani Genome Annotations in the Light of Ribosome-Protected mRNAs Fragments (Ribo-Seq Data). Genes (Basel). 14, 1637. doi: 10.3390/GENES14081637
- González-de la Fuente, S., Requena, J.M., and Aguado, B. (2023). Datasets of Iso-Seq transcripts for decoding transcriptome complexity in four Leishmania species. Data Br. 52, 109838. doi: 10.1016/J.DIB.2023.109838
- Callejas-Hernández, F., Robello, C., and Requena, J.M. (2023). Editorial: Protozoan parasites in the multi-omics era: present and future. Front. Cell. Infect. Microbiol. 13, 1281638. doi: 10.3389/FCIMB.2023.1281638
- Matiz-González, J.M., Pardo-Rodriguez, D., Puerta, C.J., Requena, J.M., Nocua, P.A., and Cuervo, C. (2024). Exploring the functionality and conservation of Alba proteins in Trypanosoma cruzi: A focus on biological diversity and RNA binding ability. Int. J. Biol. Macromol. 272, 132705. doi: 10.1016/J.IJBIOMAC.2024.132705
- Adán-Jiménez, J., Sánchez-Salvador, A., Morato, E., Solana, J. C., Aguado, B. and Requena, J. M. (2024). A Proteogenomic Approach to Unravel New Proteins Encoded in the Leishmania donovani (HU3) Genome. Genes 15, 775. doi: 10.3390/GENES15060775

- Requena, J. M. (2024). Genetic Mechanisms Involved in Microbial Stress Responses. Genes 15, 1265. doi: 10.3390/ GENES15101265
- Requena, J. M. (2024). The Leishmania ribosome: more than passive mRNA translating machinery. Trends in Biochemical Sciences 49, 754–756. doi: 10.1016/j. tibs.2024.06.008
- Aguado, B., Begeman, L., Günther, A., Iervolino, M., Soto, F., Ralph, E. T., Vanstreels, R.E.T., Reade, A. Coerper, A., Wallis, B., Alcamí, A. and Dewar M. (2024). Searching for high pathogenicity avian influenza virus in Antarctica. Nature Microbiol. 9, 3081–3083. doi: 10.1038/s41564-024-01868-7



#### Research projects

- PID2020-117916RB-I00. Proyecto de la Agencia Estatal de Investigación. Integrating OMICS data to decipher Leishmania gene organization and expression: clues for tackling leishmaniasis (Leish-OMICs). PIs: Jose M. Requena & Begoña Aguado (CSIC). September 1, 2021-August 31, 2025
- The group belongs to the CIBER en ÁREA ENFERMEDADES INFECCIOSAS (CIBERINFEC). Ref: CB21/13/00018.



#### Other activities

En preparación



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

#### **Group members**

Principal investigator: Javier Santos Hernández (PI) María Villa Morales (co-PI)

Scientific staff: Concepción Vaquero Lorenzo

Postdoctoral fellows: Laura vela Martín

**Predoctoral fellows:** Sara Ruíz García

**Technicians:** Isabel Sastre Merlín



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



#### Summary

T-cell lymphoblastic neoplasms (T-ALL/LBL) are a type of aggressive hematological cancer derived from immature T-cells. Current treatments consist of high-dose multi-agent chemotherapy followed by hematopoietic stem cell transplantation in standardhigh risk patients. Cure rates reach up to 90% among pediatric patients (48% in adults), and they are associated with acute and long-term toxicity. In addition, 20-40% of patients experience a relapse or do not respond to first-line treatments, showing a dismal prognosis with survival rates below 10% and very limited therapeutic options. The lack of molecular markers that can accurately allocate T-ALL/ LBL patients to a specific risk stage at diagnosis argues, at least in part, the toxicity associated with the treatment. On the other hand, no targeted treatments against the main alterations identified T-ALL/LBL are currently available in the clinical setting and since the approval of nelarabine in 2005 no pharmacological inhibitors have been approved for relapsed/refractory (R/R) T-ALL/LBL patients. All this highlights two unmet needs: 1) a more refined stratification of T-ALL/LBL patients according to their potential risk, which would lead to improved cure rates and reduced toxicity; and 2) the development of targeted treatments against the main alterations affecting T-ALL/LBL patients, which would contribute to lowering chemotherapy doses to avoid late effects and would offer therapeutic options for R/R patients. Our group is trying to contribute to solving these two issues. During the 2023-2024 period, we found that different oncogenic mutations

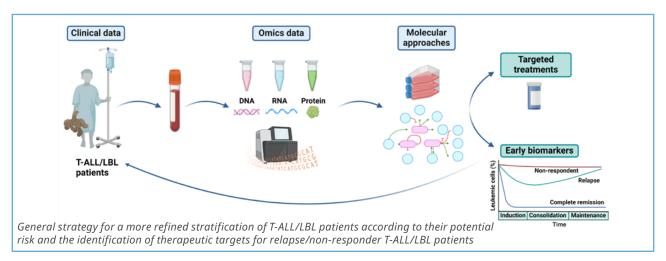
in members of the JAK/STAT pathway induce PIM1 overexpression and inhibition of PIM proteins with PIM447 is a therapeutic option to treat a broad group of T-ALL/LBL patients. We also identified the JAK3Q988P mutation in a T-ALL patient who did not achieve a durable response after the conventional treatment, and that ruxolitinib may not be efficient for future patients bearing the JAK3Q988P mutation who instead may benefit from tofacitinib. Furthermore, we identified a novel and oncogenic SEPTIN6::ABL2 fusion in a T-ALL/LBL patient and demonstrated the high efficacy of tyrosine kinase inhibitors (TKIs) in inhibiting the growth of cells expressing SEPTIN6::ABL2. To ameliorate the clinical management of T-ALL/LBL patients who relapse, it is urgent to identify biomarkers able to predict their outcomes, and we reported that high NRF2 levels may be a predictive biomarker of poor treatment response in T-ALL patients. Finally, we identified SOCS3 hypermethylation as a recurrent event in T-ALL/LBL and, therefore, SOCS3 emerges as a potential therapeutic target in these tumors.



#### Doctoral theses

■ Laura Vela Martín (2024). "Estudio de las bases moleculares subyacentes a las neoplasias de células T precursoras para aplicar la medicina personalizada: caracterización de la fusión SEPTIN::ABL2 como nueva diana terapéutica e identificación del gen AHR como biomarcador de la respuesta al tratamiento Universidad Autónoma de Madrid. Supervisors: María Villa Morales and José Luis Marín Rubio.







- Lahera A, Vela-Martín L, Fernández-Navarro P, Llamas P, López-Lorenzo JL, Cornago J, Santos J, Fernández-Piqueras J, Villa-Morales M. (2024). PIM1 is a potentialtherapeutic target for the leukemogenic effects mediated by JAK/ STAT pathway mutations in T-ALL/LBL. NPJ Precis Oncol. 8(1):152. doi:10.1038/s41698-024-00638-2
- Lahera A, Vela-Martín L, Fernández-Navarro P, Llamas P, López-Lorenzo JL, Cornago J, Santos J, Fernández-Piqueras J, Villa-Morales M. (2024). The JAK3<sup>Q988P</sup>mutation reveals oncogenic potential and resistance to ruxolitinib. Mol Carcinog. 63(1):5-10. doi: 10.1002/mc.23632.
- Lahera A, Vela-Martín L, López-Nieva P, Salgado RN, Rodríguez-Perales S,Torres-Ruiz R, López-Lorenzo JL, Cornago J, Llamas P, Fernández-Navarro P,Sánchez-Domínguez R, Segovia JC, Sastre I, Cobos-Fernández MÁ, Menéndez P, Santos J, Fernández-Piqueras J, Villa-Morales M. (2023). Comprehensive characterization of a novel, oncogenic and targetable SEPTIN6::ABL2 fusion in T-ALL. Br J Haematol. 202(3):693-698. doi: 10.1111/bjh.18901.
- Villa-Morales M, Pérez-Gómez L, Pérez-Gómez E, López-Nieva P, Fernández-Navarro P, Santos J. (2023). Identification of NRF2 Activation as a Prognostic Biomarker in T-Cell Acute Lymphoblastic Leukaemia. Int J Mol Sci. 24(12):10350. doi: 10.3390/ijms241210350.



#### **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. María villa-Morales, José luis marín Rubio, José fernández Piqueras. Spain. December 10, 2019. Validity 10 years, extendable.
- SEPT6-ABL2 fusion for use in the diagnosis and/or treatment of cancer. European Patent Application Number EP21382663. Inventors: José Fernández Piqueras, María Villa Morales, Antonio Lahera, Laura Vela-Martín, Pilar López-Nieva, Carmen Ayuso, Pilar Llamas, Jose Luis López Lorenzo, Javier Cornago, Rocio Salgado. PCT/EP2022/065750. Spain. 21/07/2021.

- Lahera A, López-Nieva P, Alarcón H, Marín-Rubio JL, Cobos-Fernández MÁ, Fernández-Navarro P, Fernández AF, Vela-Martín L, Sastre I, Ruiz-García S, Llamas P, López-Lorenzo JL, Cornago J, Santos J, Fernández-Piqueras J, Villa-Morales M. (2023). SOCS3 deregulation contributes to aberrant activation of the JAK/STAT pathway in precursor T-cell neoplasms. Br J Haematol. 201(4):718-724. doi: 10.1111/bjh.18694.
- Tundidor I, Seijo-Vila M, Blasco-Benito S, Rubert-Hernández M, Adámez S, Andradas C, Manzano S, Álvarez-López I, Sarasqueta C, Villa-Morales M, González- Lois C, Ramírez-Medina E, Almoguera B, Sánchez-López AJ, Bindila L, Hamann S, Arnold N, Röcken C, Heras-Murillo I, Sancho D, Moreno-Bueno G, Caffarel MM, Guzmán M, Sánchez C, Pérez-Gómez E. (2023). Identification of fatty acid amide hydrolase as a metastasis suppressor in breast cancer. Nat Commun. 30;14(1):3130. doi: 10.1038/s41467-023-38750-9.



#### Research projects

- IISFJD/UAM-012022. Jiménez Díaz Research Institute (IISFJD). Genomic and transcriptomic analysis in the personalized treatment of T-cell lymphoblastic neoplasms. PI: Javier Santos
- (UAM/CBM/IIS-FJD). 2022-2026. 086812. AP-DRCLAS (Harvard) and Asisa Foundation. Validation of the role of the SEPT6-ABL2 oncogenic fusion. PI: Villa-Morales M (UAM/CBM/IIS-FJD). 2023-2025.
- PMP24/00026. Carlos III Health Institute (ISCIII). SMARTomicS:: prevention of suicidal behavior through therapeutic interventions guided by digital phenotype and pharmacogenetics. Coordinator Enrique Baca García
- (IISFJD). PI (UAM/CBM/IISFJD group): Concepción Vaquero Lorenzo. 2024-2027.
- PI21CIII/00035. ISCIII. Identification of biomarkers of sensitivity to low doses of ionising radiation by studying the expression of microRNAs in T lymphocytes from interventional cardiology specialists. PI: Pablo Fernández-Navarro (ISCIII). 2022-2024.



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

#### **Group members**

**Principal investigator:** Iván Ventoso (PI)

**Staff scientists:** Juan José Berlanga Miguel Angel Rodríguez Margarita Cabrera

Predoctoral fellows: Carla Henares

**Technicians:** José Alcalde

**Undergraduate and Master students:** Jorge Gracía Alonso

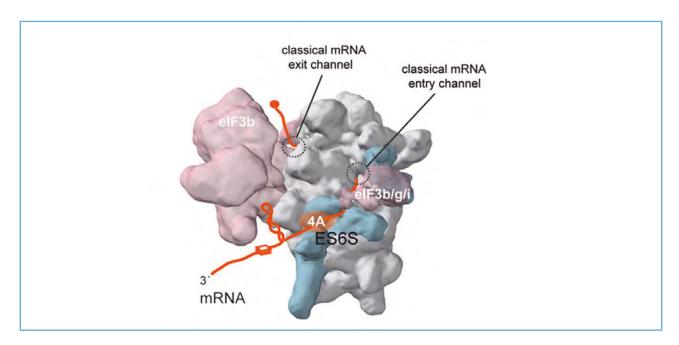




#### Summary

We continue to study mechanistic, functional and physiological aspects of translation regulation in eukaryotes and viruses, by integrating structural analysis of ribosomal initiation complex (PIC), gene targeting in mammalian cells and yeast analysis genome-wide of translation and (polysome profiling). Regarding translation of viral mRNAs, we have described new strategies in alphaviruses and coronavirus to recruit the PIC for viral translation, and new mechanisms to block host translation and interferon secretion. We also continue to study the functional links stress response between translation, proteostasis in mammalian cells and yeast and how these processes can impact ageing and neurodegeneration.





Model of ribosomal preinitiation complex (PIC) with attached mRNA.



#### List of publications

- Martin-Solana E, Diaz-Lopez I, Mohamedi Y, Ventoso I, Fernandez JJ, Fernandez-Fernandez MR (2024) Progressive alterations in polysomal architecture and activation of ribosome stalling relief factors in a mouse model of Huntington's disease. Neurobiol Dis. 195:106488. doi: 10.1016/j.nbd.2024.106488.
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- Ventoso I, Berlanga JJ, Toribio R, Díaz-López I (2024) Translational Control of Alphavirus-Host Interactions: Implications in Viral Evolution, Tropism and Antiviral Response. Viruses. 16(2):205. doi: 10.3390/v16020205.
- Boronat S, Cabrera M, Vega M, Alcalá J, Salas-Pino S, Daga RR, Ayté J, Hidalgo E (2023). Formation of transient protein aggregate-like centers is a general strategy postponing degradation of misfolded intermediates. Int J Mol Sci. 24(13):11202. doi: 10.3390/ijms241311202



#### Research projects

■ PID2021-125844OB-I00. Ministerio de Ciencia e Innovación. Influence of stress-induced translation reprogramming on proteostasis in eukaryotes. From mechanisms to impact on cell survival. PI, Iván Ventoso. From 01/09/2022 to 01/09/2025.



# Genome dynamics and function

## Genome maintenance and instability



### Luis Blanco Dávila

Researchers at the "Genome maintenance and instability" Unit use model systems to decipher mechanisms of genome organization, DNA and chromatin replication and repair. DNA replication itself is an important source of DNA damage, but specialized checkpoints and mechanisms of DNA repair, translesion synthesis, re-priming, histone reloading and telomere maintenance prevent damage accumulation, prolonging organismal fitness while avoiding pathological processes such as cancer.

During this two-year period, the characterization of the mechanism of DNA priming by human PrimPol, that will serve to guide the design of specific PrimPol inhibitors has been completed, together with the characterization of human PrimPol cancer variants with altered primase activity that could have a pronostic value in cancer. Moreover, and continuing a long stand tradition of our research unit, novel DNA polymerases mainly present within enterohemorrhagic, enteropathogenic and enterotoxigenic genomes, were further characterized, and proposed to have a potential role in double strand breaks (DSB) repair. From a biotechnological perspective, novel PrimPols were characterized to improve the PrimPol-based DNA amplification method named TruePrime™, and a novel method of isothermal DNA amplification, based on the combined use of PrimPol and a novel thermostabilized phi29 DNA polymerase variant, was developed and patented to be applied for point of care detection of SARS-CoV2.

The BRCA2 tumor suppressor protein has been further explored as a model to understand the link between genome instability and tumorigenesis. Thus, by using breast cancer variants, the dsDNA binding activity of the N-terminal domain (NTD) of BRCA2 was shown to be essential to prevent and repair ssDNA gaps caused by nucleotide depletion—but not those from PARP inhibition—highlighting distinct gap suppression mechanisms. Besides, the enhanced ssDNA binding of variant R2645G was shown to induce hyper-recombination, causing chromosomal instability, and underscoring the need for tight regulation of BRCA2 DNA binding to maintain genomic stability. As emphasized in a recent review, there are still knowledge gaps in BRCA1/2-related tumorigenesis and still an open debate on haploinsufficiency and its implications for cancer prevention and treatment.



Other important goals of this period were the discovery that telomere fusions are a normal feature of cardiac cell maturation that occur without triggering apoptosis, and that specific telomere fusions which can be detected as circulating DNA in the blood of cancer patients are generated by Alternative Lengthening of Telomeres (ALT), a telomere maintenance mechanism used by some cancers. Besides, a mouse model (generated in collaboration with Vincent Géli's group in France) in which mTert is specifically induced in p21-positive cells, was used to demonstrate that mTert induction in stressed (p21+) cells reverses capillary rarefaction and emphysema, offering a telomerase-based therapeutic strategy.

Moreover, the functional interplay between DNA replication, transcription, and chromatin architecture has been explored, showing that nascent RNAs might facilitate the dynamic repurposing of transcription factories into replication factories during early S-phase, and identifying an unexpected role for the deubiquitinating enzyme ATXN3 in preserving chromatin organization, thereby supporting the coordinated progression of replication and transcription.

Other discoveries highly relevant in the response to replication and cellular stress were: that the AAA ATPase VCP/p97 removes DNA polymerase alpha/ Primase from chromatin in S phase to limit priming during DNA replication of the lagging strand, preventing an excessive activation of the replication stress response; that the E3 ubiquitin ligase and ATPase/helicase Rad5/HLTF, a central protein in DNA damage tolerance, is regulated throughout the cell cycle by the SCF and APC/C complexes; and that Rad5 is regulated by subnuclear relocalization in response to different types of cellular stress, which is required for its function and its own homeostasis.

## Research groups

#### Luis Blanco Dávila

Genome maintenance and variability: enzymology of DNA replication and repair

#### Aura Carreira Moreno

Genome instability and cancer predisposition

#### **Ignacio Flores**

Telomeres in cancer and regeneration

#### María Gómez Vicentefranqueira

Functional organization of the mammalian genome

#### **Emilio Lecona Sagrado**

Chromatin, cancer and the ubiquitin system lab

#### **Jose Antonio Tercero Orduña**

Chromosome replication and genome stability

#### Miguel de Vega José

Maintenance of bacterial genome stability



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

#### **Group members**

**Principal investigator:** Luis Blanco Dávila

**Scientific staff:** María I. Martínezliménez

Predoctoral fellows: Ana Martínez Carrón Cristina Velázquez Ruiz Marcos Jiménez Juliana Nieves Calero Muñoz **Technicians:** Susana Guerra González

**Undergraduate and Master students:**Alejandra Jiménez Mejías

**Visiting scientists:** Paola Libertad García Medel





#### 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 years several labs contributed to demostrate our seminal proposal that the majority of DNA lesions or blocking structures occurring in the template DNA may be "tolerated" during RS, mainly through the generation of PrimPolmediated new replicative starting points (namely primers) in the leading strand, immediately beyond the lesion. Importantly, our discovery of PrimPol as a second primase in human cells, that preferentially makes DNA primers and is specifically involved in alleviating RS both in the nucleus and mitochondria, led 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. Current data strongly suggest that PrimPol is a novel and relevant target in cancer.

In the last two years we characterized human PrimPol cancer variants with altered specific activity and primase deficiency, that could have a pronostic value in cancer, and completed the characterization of the mechanism of DNA priming by human PrimPol, that serves to guide the design of specific PrimPol inhibitors, and to explain the low impact on PrimPol activity of specific nucleotide analogues used as antivirals as remdesivir.

From a biotechnological perspective, we have characterized novel PrimPols in *Thermus thermophilus* and *Enterococcus faecalis*, to improve the PrimPol-based DNA amplification method named TruePrime™, currently exploited by 4BaseBio AG to make GMP synthetic DNA to be used for gene therapy and also for both DNA and RNA vaccines (https://www.4basebio.com).

Moreover, and in close collaboration with Miguel de Vega lab at the CBM, we have developed and patented a novel method of isothermal DNA amplification based on the combination of a PrimPol, as the source of primers, with an engineered version of Phi29 DNA polymerase, still the paradigm of a highly processive and strand-displacement DNA amplification enzyme with a great fidelity. This method has been applied for point of care detection of SARS-CoV2.

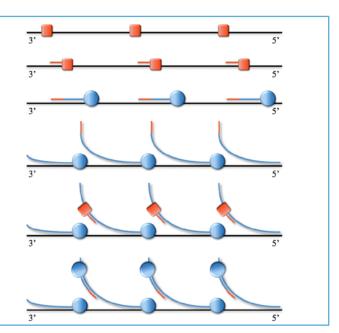


#### **Patents**

- Novel Method for Isothermal amplification of padlock probes for nucleic acid detection and phi29 DNA polymerase variants. Luis Blanco, Miguel de Vega, María Martínez and Alicia del Prado. WO 2024/200376 A1. Date of filing: 24/03/2023. Date of publication: 03/10/2024. Owner: CSIC.
- Methods of amplifying template nucleic acid using a thermostable TthPrimPol. Ana Martínez, Angel Picher and Luis Blanco. EP 4491719A1. Date of filing: 12/07/2023. Date of publication: 15/01/2025. Owners: CSIC and 4basebio S.L.U.



- 1) TthPrimPol binds denatured DNA at multiple sites
- 2) Synthesis of short DNA primers
- 3) Primers are extended by Phi29 DNApol
- Strand displacement by Phi29 DNApol generates new ssDNA
- TthPrimPol synthesizes new primers on the displaced ssDNA
- Phi29 DNApol extends these new DNA primers, resulting in exponential DNA amplification





- Velázquez-Ruiz, C., Blanco, L., Martínez-Jiménez, M. I. (2023) 3´dNTP binding is modulated during primer synthesis and translesion by human PrimPol. Int J Mol Sci. 25(1):51. doi: 10.3390/ijms25010051.
- García-Zarandieta, M., et al. Identification of West Nile virus RNA-dependent RNA polymerase non-nucleoside inhibitors by real-time high throughput fluorescence screening. Antiviral Res. 2023 Apr;212:105568. doi: 10.1016/j.antiviral.2023.105568.
- Alcorlo, M., et al. (2024) Flexible structural arrangement and DNA binding properties of protein p6 from Bacillus subtilis phage Phi29. Nucleic Acids Res. 52, 2045-2065. doi: 10.1093/nar/ gkae041.
- Jiménez-Juliana, M., Martínez-Jiménez, M. I., Blanco, L. (2024) Remdesivir triphosphate is a valid substrate to initiate synthesis of DNA primers by human PrimPol. DNA Repair 143:103772. doi: 10.1016/j.dnarep.2024.103772.



#### Research projects

- Reference: PID2021-125966OB-I00. Funding: MICINN. Title: Optimization of methodologies for isothermal amplification of nucleic acids". PI: Luis Blanco Dávila. Period: 01/09/2022-31/08/2025.
- Reference: SGL2021-03-040 (WP3-IVD Ensayos y prototipos). Funding: Consortium CSIC PTI-SG-DIAGNOSTICO-NextGenerationEU. Title: Methodological variation studies of phi29 polymerase-mediated DNA amplification for SARS Cov2 detection. PIs: Luis Blanco Dávila and Miguel de Vega. Period: 2021-2023.
- Reference: CaixaImpulse Express Call on COVID-19. Funding: "la Caixa" Banking Foundation.Title: "Simple and rapid SARS-COV-2 diagnostic test by phi29 polymerase amplification". PI(s): Luis Blanco Dávila & Miguel de Vega (CBM) and Felipe Cortés Ledesma (CNIO). Period: 13/07/2020-30/06/2023.

Schematic representation of TruePrime™ genomic amplification method. (Adapted from Picher et al. (2016) Nature Commun. 7:13296. DOI: 10.1038/ncomms13296)



#### Other activities

- Luis Blanco and Miguel de Vega organized and directed the Summer Course: "Advances in Molecular Biology and its translation to Biomedicine and Biotechnology", corresponding to the XXI (2023) and XXII (2024) editions of the School of Molecular Biology Eladio Viñuela and Margarita Salas, held at the Menendez Pelayo International University (UIMP), at Santander (Spain).
- 2023. Luis Blanco is elected Vice-President of Margarita Salas Foundation.
- 2023. CONVERGE-EBTON program AWARD to María I. Martínez-Jiménez (Blanco lab) and Alicia del Prado (de Vega lab).
- 2024. Luis Blanco is elected member of Academia Europaea
- 2024. Plaque of Honor of the Spanish Association of Scientists (AEC) to Luis Blanco, in the category of outstanding scientists.
- 2024. ENVALOR program AWARD from Fundación General CSIC-COMTE to María I. Martínez-Jiménez (Blanco lab) and Alicia del Prado (de Vega lab).



#### Doctoral theses

- Ana Martínez Carrón (2023) Optimisation and alternatives to the DNA amplification system TruePrime. University: UAM. Supervisors: Luis Blanco (CBMSO) and Angel Picher (4basebio Plc).
- Cristina Velázquez Ruiz (2023). The 3´dNTP binding of human PrimPol and the impact of cancer variants. University: UAM. Supervisors: Luis Blanco and María I. Martínez Jiménez.



#### GENOME INSTABILITY AND CANCER PREDISPOSITION

#### **Group members**

Principal investigator: Aura Carreira Moreno

**Postdoctoral fellows:** Sree Rama Chaitanya Sridhara Jesus Gomez Escudero

**Predoctoral fellows:** Rady Chaaban Lucía Álvaro Aranda Irene Hernando Herrera

## Technicians:

Juan Carlos Martínez Cañas Natividad Bellido Carreras Carmen Escalona Noguero







#### Summary

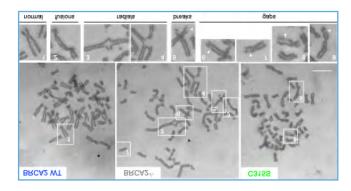
Our lab interrogates the cellular mechanisms deployed to preserve genome integrity using the BRCA2 protein as a model. This serves us to investigate the consequences of BRCA2 mutation in breast cancer predisposition.

During this period, we have focused on two particular topics:

- 1. BRCA2 protein's main functions include DNA repair by homologous recombination (HR) both at induced DNA double-strand breaks (DSB) and spontaneous replicative lesions. At stalled replication forks, BRCA2 protects the DNA from aberrant nucleolytic degradation and is thought to limit the appearance of ssDNA gaps by arresting replication and via post-replicative HR. However, whether and how BRCA2 acts to limit the formation of ssDNA gaps or mediate their repair, remains ill-defined. During this period, we use breast cancer variants affecting different domains of BRCA2 to shed light on this function. We demonstrated that the N-terminal DNA binding domain (NTD), and specifically, its dsDNA binding activity, is required to prevent and repair ssDNA gaps upon nucleotide depletion but not to limit PARPi-induced ssDNA gaps. Thus, these findings suggest that nucleotide depletion and PARPi trigger gaps via distinct mechanisms and that the NTD of BRCA2 prevents nucleotide depletion-induced ssDNA gaps.
- 2. Most pathogenic variants in BRCA2 reside in its canonical DNA binding domain located in the C-terminal region (BRCA2CTD). In this manuscript, we investigated the potential impact of BRCA2 missense variants located in BRCA2CTD on DNA binding and DSS1 interaction to shed light on the role of these activities on the homologous recombination (HR) function of BRCA2 and their pathogenicity. Unexpectedly, we reported that R2645G, a variant recently classified as likely benign and located at the interface with DSS1, increased the ssDNA binding capacity of the BRCA2CTD in vitro resulting in hyperrecombination in cells and prompting chromosomal instability. This instability instigates moderate to high sensitivity to different DNA damage-inducing agents including PARP inhibitors suggesting that BRCA2 DNA binding needs to be tightly regulated to control HR. These findings might have clinical implications for breast cancer.
- 3. Finally, we have published an invited review in the Journal of Molecular Biology in which we highlight the gap of knowledge in the mechanism underlying tumor formation in BRCA1/BRCA2 mutated cancers. We provided an overview of the findings and the ongoing debate regarding BRCA haploinsufficiency. We further put out the challenges in studying this topic and discuss its potential relevance in the prevention and treatment of BRCA-related cancers.

This is a very relevant topic in our lab as we are preparing a manuscript on BRCA2 haploin sufficiency.







- Alvaro-Aranda L, Petitalot A, Djeghmoum Y... Carreira A (2024). The BRCA2 R2645G variant increases DNA binding and induces hyper-recombination. Nucleic Acids Res. 52(12):6964-6976. doi: 10.1093/nar/gkad1222
- Minello A, Carreira A. (2023) BRCA1/2 Haploinsufficiency: Exploring the Impact of Losing one Allele. J Mol Biol. 436(1):168277. doi: 10.1016/j.jmb.2023.168277.
- Vugic D, Dumoulin I, Martin C, Minello A, Alvaro-Aranda L, Gomez-Escudero J, Chaaban R, Lebdy R, von Nicolai C, Boucherit V, Ribeyre C, Constantinou A, Carreira A. (2023) Replication gap suppression depends on the double-strand DNA binding activity of BRCA2. Nat Commun. 14(1):446. doi: 10.1038/s41467-023-36149-0
- Mishra AP, Hartford S, Chittela RK, Sahu S, Kharat SS, Alvaro-Aranda L, Contreras-Perez A, Sullivan T, Martin BK, Albaugh M, Southon E, Burkett S, Karim B, Carreira A, Tessarollo L, Sharan SK. (2023) Characterization of BRCA2 R3052Q variant in mice supports its functional impact as a low-risk variant. Cell Death Dis. 14(11):753. doi: 10.1038/ s41419-023-06289-8.

This image shows a metaphase chromosome spread of BRCA2 deficient cells (BRCA2-/-), BRCA2 normal cells (BRCA2 WT) and cells expressing a variant that accumulates ssDNA gaps (BRCA2 C315S) highlighted by the arrows from Vugic et al., 2023 Nature Communications.



#### Other activities

#### Conferences as invited speaker:

- IRB Barcelona BioMed Conferences: Molecular Determinants of Cancer Therapy Resistance Nov 2024 (Barcelona, SP)
- FASEB Science Research Conference Genetic Recombination and Genome Rearrangements Conference Jul 2024 (Tucson, AZ US)
- I Workshop on Chromatin Alterations in Cancer Development (CBM, Madrid, SP)
- Life Sciences 2 Annual Meeting 2024, Feb 2024 (University of Lausanne, CH)

#### Meeting/course organization:

 Co-organizer of the International course: Genome Instability and Human Disease at Institut Curie, FR (October 2024)

#### Others:

- Thesis doctoral defended at Institut Curie, FR. Anna Minello (2023): Shedding light on the mechanisms of tumor formation linked to BRCA2 using two known pathogenic mutations.
- Member of the Conexion Cancer CSIC hub
- Member of Chromodyst network of excellence



#### Research projects

#### National funders:

- PID2020-115977RB-I00. AEI (Spanish State Research Agency). Role of BRCA2 at challenged replication forks. Aura Carreira (PI). 2021-2024
- PID2023-150953NB-I00. AEI (Spanish State Research Agency). Mechanisms of replication stress-induced ssDNA gaps repair and implications in chemotherapy resistance. Aura Carreira (PI). 2024-2027
- PRYGN234480CARR. AECC. Foundation of the Spanish Association Against Cancer. Development of BRCA2-PARP1 interaction inhibitors (B-Pi) as radiosensitizers for homologous recombination proficient breast tumors. Aura Carreira (PI). 2023-2026

#### **Entidades internacionales:**

- 22-0222. Worldwide Cancer Research (UK). Unveiling mechanisms of tumor formation linked to BRCA2 mutation; Aura Carreira (PI). 2022-2025.
- IRP2021. International Research Project (IRP) CNRS-CSIC. The role of the RNA-binding activity of DNA repair proteins in the dynamics of replication forks: focus on BRCA2. Aura Carreira (co-coordinator) 2022-2027"



#### **Doctoral theses**

Rady Chaaban (2024). Coordination of the chromosome integrity functions of PLK1:BRCA2:PP2A throughout the cell cycle. Genome Dynamics and Function Program CBM/ Genome Integrity, RNA and Cancer Unit, Institut Curie, FR. PSL University. Aura Carreira. N/A.



#### TELOMERES IN CANCER AND REGENERATION

#### **Group members**

**Principal investigator:** Ignacio Flores

**Predoctoral fellows:** Javier Lucas de la Fuente

**Technicians:** Andreea Badarau María José Morillo Chincoa



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#### Summary

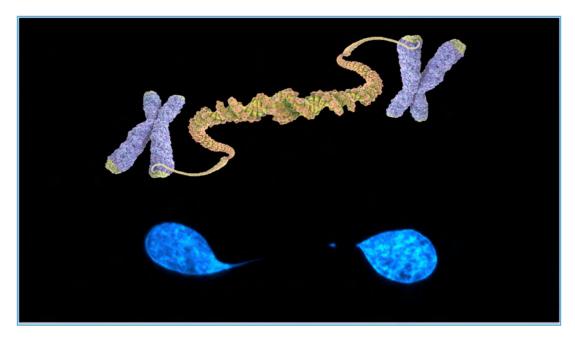
Telomeres are essential components of our genome, protecting it from deterioration and instability. They consist of repetitive DNA sequences and specialized proteins that are located at the ends of our chromosomes. An important aspect of telomere biology is the potential for telomere fusions, which occur when two chromosomes become physically joined at their telomeres. This can trigger a series of genomic rearrangements and oncogenic alterations, ultimately leading to the development of malignant tumors and resistance to chemotherapy. Despite their relevance in tumor evolution, our understanding of the patterns and consequences of telomere fusions in human cancer is still limited. Our group is currently focused on understanding the role of telomeric fusions in cancer and regeneration.

Telomere fusions in cancer: In recent years, we have used whole-genome sequencing data to study the rates and spectrum of somatic telomere fusions (TFs) in over 30 different types of cancer. Our findings indicate that TFs are widespread in human tumors, with rates that vary greatly between and within different types of cancer. In addition to end-to-end fusions, we have identified unique patterns of TFs that are linked to the activity of the alternative lengthening of telomeres (ALT) pathway. We have also detected TFs in the blood of cancer patients, offering a potential method for early cancer detection with high specificity and

sensitivity. Understanding the role of TFs in cancer will be crucial in developing targeted therapies and improving patient outcomes.

Telomere fusions in cardiomyocyte maturation and heart regeneration: Maturation in many mammalian cells, including cardiomyocytes, is accompanied by an increase in the number of chromosomes, or polyploidization. This increase in chromosome sets may contribute to the limited ability of mature cardiomyocytes to divide after a damage, as polyploid cells are often unable to undergo successful cell division. While the importance of cardiomyocyte polyploidy is well-recognized, the mechanism behind it is not understood. In recent years using a high-content imaging approach, we have discovered that approximately half of postnatal mouse cardiomyocytes that enter mitosis have discernible chromosome bridges. Furthermore, we observed that the ploidy of cardiomyocytes is tracked by an increasing number of fusions between chromosome ends, providing evidence of a direct link between TFs and cardiomyocyte polyploidization. Our study suggests that telomere fusions play a role in causing chromosome bridging and polyploidization, and controlling their presence may be crucial in enhancing heart regeneration.





Telomere fusion illustration (top) and image of a chromosome bridge from a U2OS cell (bottom)



- Lipskaia L, Breau M, Cayrou C, Churikov D, Braud L, Jacquet J, Born E, Fouillade C, Curras-Alonso S, Bauwens S, Jourquin F, Fiore F, Castellano R, Josselin E, Sánchez-Ferrer C, Giovinazzo G, Lachaud C, Gilson E, Flores I, Londono-Vallejo A, Adnot S, Géli V.(2024) mTert induction in p21-positive cells counteracts capillary rarefaction and pulmonary emphysema. EMBO Rep. 25(3):1650-1684. doi: 10.1038/s44319-023-00041-1.
- Muyas F, Rodriguez MJG, Cascão R, Afonso A, Sauer CM, Faria CC, Cortés-Ciriano I, Flores I. (2024) The ALT pathway generates telomere fusions that can be detected in the blood of cancer patients. Nat Commun. 15(1):82. doi: 10.1038/s41467-023-44287-8.
- Aix E, Gallinat A, Yago-Díez C, Lucas J, Gómez MJ, Benguría A, Freitag P, Cortez-Toledo E, Fernández de Manuel L, García-Cuasimodo L, Sánchez-Iranzo H, Montoya MC, Dopazo A, Sánchez-Cabo F, Mercader N, López JE, Fleischmann BK, Hesse M and Flores I. (2023) Telomeres Fuse During Cardiomyocyte Maturation. Circulation. 147(21):1634-1636. doi: 10.1161/CIRCULATIONAHA.122.062229.



#### Research projects

REFERENCE: PID2019-110339RB-I00 (2020-2024)
 AGENCY: Ministerio de Ciencia, Innovación y
 Universidades
 TITLE: Role of telomerase and telomeres in polyploid

induction and heart regeneration . Ref:

PI: Dr. Ignacio Flores

DATES FROM: 01/06/2020 TO: 31/05/2024

 REFERENCE: S2022/BMD-7245
 AGENCY: Biomedicine R&D Program. Community of Madrid (CAM)

TITLE: CARDIOBOOST-CM (Bioingeniería de células satélite de músculo esquelético como nueva estrategia de diferenciación a cardiomiocitos)

PI: Dr. Ignacio Flores

DATES FROM: 01/01/2023 TO: 31/12/2027



#### **Doctoral theses**

■ Javier Lucas de la Fuente (2023)

Title: Efectos de la sobrexpresión moderada de la telomerasa en el corazón del ratón neonatal durante homeostasis y tras lesión.

Departamento de Bioquímica. Facultad de Medicina

UAM

Thesis supervisor: Ignacio Flores



#### FUNCTIONAL ORGANIZATION OF THE MAMMALIAN GENOME

#### **Group members**

**Principal investigator:**María Gómez Vicentefranqueira

**Postdoctoral fellows:** Alicia Gallego Jiménez Joana Segura Martínez

Predoctoral fellows: Sara Martín Vírgala Javier Isoler Alcaraz (co-director, César Cobaleda) Sara Tur Gracia (co-director Carlos Estella)

Ran Tong Adrián de Rus

**Undergraduate and Master students:** Miguel La Iglesia

Visiting scientists: Carolos Ordoñez Clemens Blumenthal (Erasmus +)



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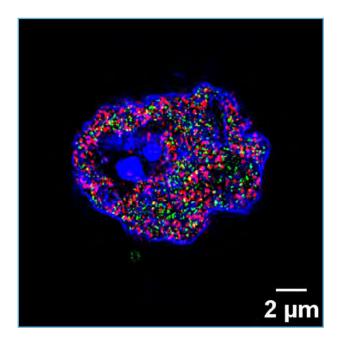


#### Summary

DNA replication and transcription are two essential processes that share the same chromatin template. Evidence accumulated over the past years, including our work, has unveiled the complex relationship between these processes and chromatin structure, which is essential for maintaining genome integrity. To investigate these interdependencies, we work at the interface of DNA replication, transcription, chromatin, and nuclear organization. We employ a multidisciplinary range of state-of-the-art approaches, including multi-omics, super-resolution imaging, chromatin conformation capture, genomic editing, and machine learning. Through this, we aim to gain a molecular understanding of how replication-transcription crosstalk modulates chromatin structure in two key cellular processes: cellular plasticity and stress responses. The knowledge generated may be particularly relevant in the field of cancer, where cellular de-differentiation and replicative stress induced by transcriptional dysregulation are hallmarks.

Over the past two years, we have collaborated with national and international experts and demonstrated: (1) that nascent RNAs can activate replication origins located at the promoter regions of their coding genes by interacting with the human ORC1 subunit of the Origin Recognition Complex, unveiling a non-coding function of RNAs as dynamic components of chromatin; and (2) that the deubiquitinating enzyme Ataxin-3 (ATXN3) regulates the subcellular localization of histone deacetylase 3 (HDAC3), impacting chromatin structure and, subsequently, the coordination between DNA replication and transcription.





Superresolution image of mESC nucleus replicating and transcribing its genome



#### List of publications

- Mas, A.M., Goñi, E., Ruiz de los Mozos, I. R., Arcas, A., Statello, L., González, J., Blázquez, L., Lee, W.T.C., Gupta, D., Sejas, A., Hoshina, S., Armaos, A., Tartaglia, G.G., Waga, S., Ule, J., Rothenberg, E., Gómez, M. and Huarte, M. (2023). ORC1 binds to cis-transcribed RNAs for efficient activation of replication origins. Nat Commun. 14: 4447. doi: 10.1038/s41467-023-40105-3
- Hernández-Carralero, E., Cabrera, E., Rodríguez-Torres, G., Hernández-Reyes, Y., Singh, A.N., Santa-María, C., Fernández-Justel, J. M., Janssens, R.C., Marteijn, J.A., Evert, B.O., Mainland, N., Gómez, M., Ramadan, K., Smits, V.A.J. and Freire, R. (2023). ATXN3 controls DNA replication and transcription by regulating chromatin structure. Nucl Acids Res 11: 5396-5413. doi: 10.1093/nar/gkad212



#### Other activities

- Steering Committee of the CSIC Network "Conexión Genoma" (2024-2026)
- SAB of the Spanish "Excellence Network" Genome Regulation (2024-2025)
- Teacher at the Genetics national Course 2023 (CNG2023): Structure, Function and Evolution of the Genome. 13th-17th March 2023, La Cristalera, Miraflores de la Sierra (Madrid).



#### Research projects

- IGP-SO. Intramural Exploratory Grants Program Severo Ochoa. Integrative approaches to understand cellular fate: NSD2 and hematopoietic lineage specification. PIs: María Gómez Vicentefranqueira and César Cobaleda Hernández. 2024-2026.
- PID2022-141380NB-I00:. Ministry of Science and Innovation | Proyectos Excelencia. Coordination between genomic replication and transcription with cellular metabolism and plasticity. PI: María Gómez Vicentefranqueira. 2023-2026.
- 101072903-RepliFate. European Research Executive Agency. MSCA Doctoral Network | Horizon Europe (HORIZON-MSCA-2021-DN-01). DNA replication as a driver for cell fate decisions. Participant (PI: Emilio Lecona). 2022-2026.
- PID2019-105949GB-I00. Ministry of Science, Innovation and Universities | Proyectos Excelencia. Replication-transcription crosstalk and its impact on genome homeostasis PI: María Gómez Vicentefranqueira. 2020-2023.
- Member of the Spanish "Excellence Network" Chromodyst (2024-2025)"



#### **Doctoral theses**

Cristina Santa María Tobías. 2023. Role of chromatin conformation on transcriptional and post-transcriptional alterations of chromatin-RNAs. Director: María Gómez Vicentefranqueira. Molecular Biosciences Doctorate Program. UAM.



#### CHROMATIN, CANCER AND THE UBIQUITIN SYSTEM

#### **Group members**

**Principal investigator:** Emilio Lecona Sagrado

Scientific staff: Ana Deodora de la Fuente González

**Predoctoral fellows:** Rodrigo Martín Rufo Alicia Gómez Moya Scott B Churcher

**Technicians:** Alba Gil Plaza

Undergraduate and Master students: Miguel Rodríguez Retana Tomás Viuda Moreno Alejandra Perona García Clara Plaza Alonso



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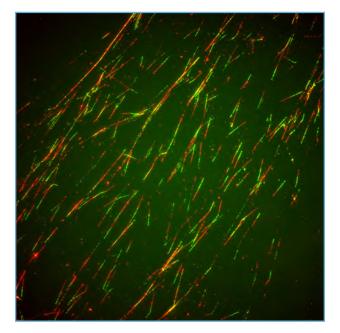
#### **Summary**

DNA replication mediates the copy of the genetic material in a cell and needs to be tightly controlled to ensure genomic stability and to prevent the appearance of mutations and genetic alterations that underlie diseases like cancer. In the Chromatin, cancer and the ubiquitin system lab we study DNA replication not only as the process that mediates the faithful transmission of the genetic information but also as a determinant of cell identity through its influence in chromatin. We focus on the role of the small protein modifiers ubiquitin and SUMO in the control of the DNA replication machinery, known as the replisome. We want to understand how ubiquitin and SUMO drive the presence or removal of DNA replication factors from chromatin to control DNA replication dynamics and cellular identity.

In recent years we have deepened our knowledge on the SUMO/ubiquitin equilibrium in chromatin during DNA replication (Lecona and Fernández Capetillo, Bioessays 2016). We have shown that the SUMO/ubiquitin landscape in active DNA replication forks is sustained by the deubiquitylase USP7 that removes ubiquitin from SUMOylated factors (Lecona et al., NSMB 2016), working together with the AAA ATPase VCP/p97 that removes ubiquitylated and SUMOylated factors from chromatin in Sphase (Franz et al., Cell Rep 2021). In addition, we have shown that USP7 links S phase progression and the activation of the mitotic program through the regulation of

CDK1 (Galarreta et al., EMBO J 2021), what could be relevant for the application of USP7 inhibitors in cancer treatment (Galarreta et al., IJMS 2021). We have proposed that SUMO and ubiquitin modifications time the presence and action of replication factors in S phase (Martín-Rufo et al., Sem Cell Dev Biol 2022). Following this idea, we are now looking for replication factors that are controlled by ubiquitin and SUMO during DNA replication through the analysis of the replication machinery after the inhibition of these pathways. In addition, we are exploring the targets of VCP/p97 in DNA replication. We have observed that VCP/p97 limits the basal activation of the replication stress response (RSR) in an unperturbed S phase by controlling the presence of the DNA polymerase  $\alpha$ /Primase complex in chromatin. We have shown that VCP/p97 regulates DNA replication in the lagging strand (Rodríguez-Acebes, Martín-Rufo et al., bioRxiv 2024) and now we are exploring the mechanisms of control of DNA polymerase  $\alpha$ /Primase extraction from chromatin. Last, we are starting to obtain a collection of replisomes through the systematic analysis of the replication machinery composition in different chromatin environments, during cell differentiation and in the process of malignant transformation. In summary, we explore how the ubiquitin/SUMO pathways shape replisome composition to control DNA replication, cell identity and cancer.





DNA fiber assay for the analysis of DNA replication dynamics.



■ Rodríguez-Acebes S, Martín-Rufo R, Gómez-Moya A, Churcher SB, Fernández-Llorente A, de la Vega-Barranco G, Perona A, Oroz P, Martín-Doncel E, Toledo LI, Méndez J, Lecona E. (2024) "The activation of ATR during unperturbed DNA replication is restricted by VCP/p97 through the extraction of DNA polymerase α/Primase from chromatin" bioRxiv doi: https://doi.org/10.1101/2022.07.25.501345.



#### Other activities

"Chromatin Alterations in Cancer Development" meeting organization, CBM (November 2024)



#### Research projects

- CNS2022-135442. Agencia Estatal de Investigación. "Consolidación Investigadora" Grant. "Shaping specialized replisomes to shape cell identity" Emilio Lecona (PI). July 2023-June 2025
- PID2021-127824OB-I00. Agencia Estatal de Investigación. "Generación de Conocimiento" Grant. "Exploring the ubiquitin/SUMO landscape in DNA replication and cancer" Emilio Lecona (PI). September 2022-August 2025.
- #101072903. HORIZON-MSCA-2021-DN-01, European Commission. "RepliFate Doctoral Network- DNA replication at the heart of cell fate decisions and cancer development" Emilio Lecona (Coordinator). October 2022-September 2026.



#### CHROMOSOME REPLICATION AND GENOME STABILITY

#### **Group members**

**Principal investigator:** Jose Antonio Tercero Orduña

**Postdoctoral fellows:** Carl P. Lehmann

**Predoctoral fellows:** Paula González Fernández Laura de Marcos Maeso

**Undergraduate and Master students:** Alonso Montero Amaya



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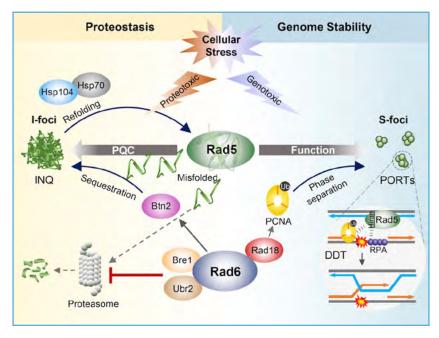
#### **Summary**

The goal of our group is to contribute to the understanding of the mechanisms that prevent genomic instability, a hallmark of cancer cells and a cause of developmental and neurological disorders, premature aging or inflammatory signalling. Our main objective is to provide data to better understand how genome stability is preserved during chromosome replication, especially in the presence of DNA damage or replicative stress. The molecular basis of these processes is evolutionarily conserved, which allows them to be studied in detail using the genetically tractable yeast *Saccharomyces cerevisiae* as a eukaryotic model organism.

During this period, we have investigated different aspects of the regulation and function of the mechanisms of DNA damage tolerance (DDT). These mechanisms help maintain genome stability by overcoming unrepaired DNA lesions that cause replication forks to stall, thus facilitating the completion of genome replication in each cell cycle. We have focused mainly on the study of the central DDT protein Rad5 (human HLTF), an E3 ubiquitin ligase and ATPase/helicase that mediates transient template-switch recombination, an error-free mode of DNA lesion bypass. We have shown that, following cellular stress, Rad5 is regulated by subnuclear relocalization into two types of foci that coexist within the same cell. One type of foci is formed in response to genotoxic stress by liquid-liquid phase

separation, and is associated with Rad5's function in maintaining genome stability. The second type of foci is formed in the presence of proteotoxic stress by chaperone-mediated sequestration at the intranuclear quality control compartment (INQ), and is related to Rad5's own proteostasis. We have found that the relocalization of Rad5 into each type of foci involves different pathways, but in both cases it is driven by the evolutionarily conserved E2 ubiquitin-conjugating enzyme Rad6. This coordinated differential relocalization of Rad5 interconnects DNA damage response and proteostasis networks, highlighting the importance of studying these homeostasis mechanisms in tandem. Spatial regulation of Rad5 under cellular stress conditions thus provides a useful biological model to study cellular homeostasis as a whole. We have also studied the regulation of Rad5 throughout the cell cycle and have found that the levels of this protein oscillate considerably, showing a peak of expression in mid-late S phase, independently of the absence or presence of DNA damage or replicative stress. This is largely due to the degradation of the protein at different times of the cell cycle via the ubiquitin-proteasome system, a process that involves the coordinated participation of the multi-protein E3 ubiquitin ligase complexes SCF (Skp1-Cullin-Fbox) and APC (Anaphase Promoting Complex/Cyclosome).





Model for spatial regulation of DNA damage tolerance protein Rad5 in response to MMS-induced cellular stress



#### List of publications

■ Lehmann, C. P., González-Fernández, P. and Tercero, J. A. (2024) Spatial regulation of DNA damage tolerance protein Rad5 interconnects genome stability maintenance and proteostasis networks. Nucleic Acids Res. 52, 1156-1172. doi: 10.1093/nar/gkad1176



#### Research projects

- PID2020-119708GB-1. Spanish Ministry of Science and Innovation (MICINN). DNA damage tolerance in eukaryotic cells: regulation, mechanisms and importance for genome integrity maintenance. Sep. 2021- Aug. 2024.
- PID2023-148435NB-I00. Spanish Ministry of Science, Innovation and Universities (MICIU). DNA damage tolerance mechanisms and links to nuclear proteostasis networks in the maintenance of cellular homeostasis. Sep. 2024- Aug. 2027.



#### **Doctoral theses**

■ Carl Philip Lehmann (2023). Spatial regulation of the central damage tolerance protein Rad5 in response to cellular stress. Department of Molecular Biology. Universidad Autónoma de Madrid. Supervisor: José Antonio Tercero



#### MAINTENANCE OF BACTERIAL GENOME STABILITY

#### **Group members**

**Principal investigator:** Miguel de Vega José

Postdoctoral fellows: Carlos D. Ordoñez

**Predoctoral fellows:** Silvia Díaz Arco

**Technicians:** Alicia del Prado Díaz Lucía Alba Fernández **Undergraduate and Master students:**Adrián de Rus Moreno

Visiting scientists: James Haslan (Karolinska Intitute)



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#### Summary

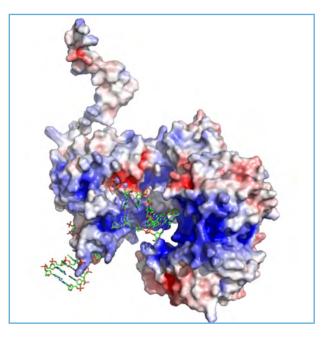
We have continued our investigation on the bacterial IS-excision enhancer (IEE) a protein that promotes the excision of the transposable insertion sequence IS629 that is present in most strains of enterohemorrhagic Escherichia coli O157 and provokes polymorphisms associated with gene inactivation and/or genomic deletions. Our results showed that IEE is endowed with an error-prone DNA polymerase activity in its N-terminal archaeo-eukaryotic primase domain able to promote dislocations of the primer and template strands and bypass DNA lesions. Importantly, IEE has the ability to join DNA termini through a microhomology-mediated end joining reaction, requiring its C-terminal helicase domain that plays a DNA binding role for stabilizing the interaction between the protein and the DNA. It should be note that most bacterial species lack an end-joining mechanism to repair and safeguard the genome following the generation of double stranded DNA breaks (DSBs) by the transposase during the excision of the IS. Our results lead us to propose a functional cooperation between IEE and the transposase encoded by IS629 to mend the resulting DSBs by searching for microhomologies between both ends and further filling of the resulting gaps to allow final sealing of the break.

In closed collaboration with Luis Blanco group (CBM, Madrid) we have developed a novel method of isothermal DNA amplification for detecting ssRNA targets, which does not require addition of DNA primers. In this method, a specific padlock

probe, complementary to a region of the RNA target proximal to the 3´ end, is circularized and amplified by rolling circle amplification (RCA), where the 3' end of the ssRNA target can act as an efficient primer when using a thermostabilized version of phi29 DNA polymerase that we have developed. The padlock-directed concatemeric ssDNA produced by RCA is further amplified by the combination of the polymerase variant and a PrimPol which continuosly synthesizes suitable DNA primers on the RCA product to trigger a hyperbranched RCA, that can be directly analyzed by flourescence, or by colorimetric detection. Both, the amplification method and the thermostabilized phi29 DNA polymerase variant have been patented. Both, the methodology and the new polymerase are currently being implemented for the detection of Mpox Virus.

We have collaborated with Christophe Danelon (Toulouse Biotechnology Institute, France) in a work where the principles of genetic information propagation and evolution within liposomes have been emulated by developing a self-replicator consisting of a linear DNA encoding for the polymerase of phi29 and its initiator protein. It has been shown how the self-replicator survives multiple rounds of replication-coupled transcription-translation reactions, accumulating beneficial mutations. These results form the basis for building genetic complexity in an evolving synthetic cell, and for analizing evolutionary processes in a minimal cell-free system.





Modeling of the interaction of the Insertion Sequence Excision Enhancer protein from E. coli O157:H7 with a primer/template DNA substrate. The predicted structure of IEE was obtained from the Alphafold Database ID A0A0H3JF09, and used under CC BY4.0 License.



- Calvo, P.A., Mateo-Cáceres, V., Díaz-Arco, S., Redrejo-Rodríguez, M. and de Vega, M. (2023) The enterohemorraghic Escherichia coli insertion sequence-excision enhancer protein is a DNA polymerase with microhomology-mediated end-joining activity. Nuc. Acids Res. 51, 1189-1207. doi: 10.1093/nar/gkad017
- Alcorlo, M., Luque-Ortega, J.R., Gago, F., Ortega, A., Castellanos, M., Chacón, P., de Vega, M., Blanco, L., Hermoso, J.M., Serrano, M., Rivas, G. and Hermoso, J.A. (2024) Flexible structural arrangement and DNA-binding properties of protein p6 from Bacillus subtillis phage phi29. Nuc. Acids Res. 52, 2045-2065. doi: 10.1093/nar/gkae041
- Abil, Z., Restrepo-Sierra, A., Stan, A.R., Chane, A., del Prado, A., de Vega, M., Rondelez, Y. and Danelon, C. (2024) Darwinian Evolution of Self-Replicating DNA in a Synthetic Protocell. Nat. Commun. 52:9091. doi: 10.1038/s41467-024-53226-0



#### **Patents**

Novel Method for isothermal amplification of padlock probes for nucleic acid detection and phi29 DNA polymerase variants. Inventors: Luis Blanco, Miguel de Vega, María I. Martínez and Alicia del Prado. PCT WO2024/200376 A1. Priority date 24/03/2023. Owner: CSIC



#### Research projects

- PID2020-115978GB-I00. MICIU/AEI/10.13039/501100011033. New mechanisms of the bacterial enzymes belonging to the Archaea/Eukaryotic Primases superfamily in the repair of DNA breaks and in the excision of insertion elements. Miguel de Vega (PI). 01/09/2021-30/09/2024.
- PID2023-148757NB-I00. MICIU/AEI/10.13039/501100011033 and FEDER, UE. Deciphering the biochemical properties of new bacterial and archaeal DNA polymerases. Miguel de Vega (PI). 01/09/2024-30/08/2027.
- CaixaImpulse Express Call on COVID-19. Funding: "la Caixa" Banking Foundation.Title: "Simple and rapid SARS-COV-2 diagnostic test by phi29 polymerase amplification". PI(s): Luis Blanco Dávila & Miguel de Vega (CBM) and Felipe Cortés Ledesma (CNIO). Period: 13/07/2020-30/06/2023.
- 101195102. Funding: EC Programme: Horizon 22.1-Health. Title: Strengthening epidemiological, genomic and community surveillance of Mpox virus (MPXV) at the Congo River border for DRC and RoC (MPOX-PROBE). Project Partners: Foundation Congolaise pour la Recherche Medicale; Societe Congolaise de Sante Mentale; Universite de Kinshasa; Spanish Research Council (CSIC). CSIC PIs: Antonio Alcamí, Gloria Sánchez, Miguel de Vega and Luis Blanco. Period: 01/10/2024-30/09/2026.
- SGL2021-03-040 (WP3-IVD Ensayos y prototipos). Funding: Consortium CSIC PTI-SG-DIAGNOSTICO-NextGenerationEU. Title: Methodological variation studies of phi29 polymerase-mediated DNA amplification for SARS Cov2 detection. PIs: Luis Blanco Dávila and Miguel de Vega. Period: 2021-2023.

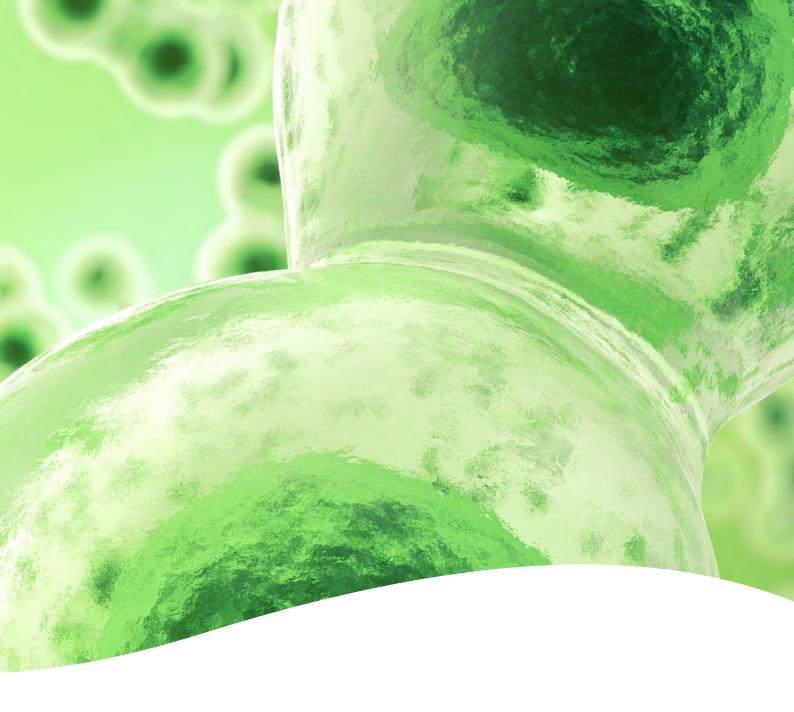


#### Other activities

- Miguel de Vega and Luis Blanco organized and directed the Summer Course: "Advances in Molecular Biology and its translation to Biomedicine and Biotechnology", corresponding to the XXII (2023) and XXIII (2024) editions of the School of Molecular Biology Eladio Viñuela and Margarita Salas, held at the Menendez Pelayo International University (UIMP), at Santander (Spain).
- 2023 CONVERGE-EBTON program AWARD to Alicia del Prado (de Vega lab) and María I. Martínez-Jiménez (Blanco lab). Isothermal nucleic acid amplification for detecting early bacteriophage contaminations in the dairy industry.
- 2024 ENVALOR program AWARD from Fundación General CSIC-COMTE to Alicia del Prado (de Vega lab) and María I. Martínez-Jiménez (Blanco lab). Phage-Phi: Detection of early bacteriophage contaminations in the dairy industry through specific isothermal amplification of their nucleic acids.



# Tissue and organ homeostasis



Cell-cell communication and inflammation unit

Cell architecture and organogenesis Unit



# Tissue and organ homeostasis

## Cell-cell communication and inflammation unit



María Yañez-Mó

Researchers included in the **Cell-Cell Communication and Inflammation Unit**, included in the **Tissue and Organ Homeostasis Program**, focus on understanding how cells interact to ensure proper tissue organization and homeostasis throughout development and healthy life. Disruptions in these cellular interactions are linked to tumor progression, aging, and vascular diseases. The Unit unites several research groups employing diverse cellular and animal models—including mammalian systems and *Drosophila*—to investigate the mechanisms governing cell-cell and cell-matrix interactions.

Together, their work deepened our knowledge of key **biological processes** involved in development or tissue maintenance by functionally deciphering the role of glypicans in the regulation of Hedgehog transport and gradient formation in *Drosophila*, describing how generalized cell death of the wing disc of *Drosophila* triggers the rejuvenation of the entire disc to an earlier developmental stage, or the mechanism by which hypoxia ancillary sequences (HAS) present in several hypoxia target genes contribute to signaling activation and expression of genes related to tissue formation and development. Crucial technical advances were also described, as the development of an *in vivo* model for cellular senescence in the wing imaginal disc of *Drosophila*, or the optimization of chromatographic techniques for the isolation of extracellular vesicles from different biofluids.

This fundamental knowledge is also translated by different groups in the Unit to the understanding of the **etiopathology of different diseases**, like the discovery that tumor cells from B-cell lymphoma/leukemia patients overproduce glutathione, which drives lymphomagenesis in females of a lymphoma mouse model, how the accumulation of the proteoglycan versican in the aortic wall drives aortic aneurysm formation in Marfan syndrome, or how different mutants in position 384 of TIA1, depending on the charge of amino acids, have differential effects in the Welander distal myopathy.



Some of these scientific evidences have a direct translation to the **clinical treatment** of patients, such as the combined treatment targeting the two soluble mediators (IL-1beta and IL-6) that were identified as the main disruptors of the human lung endothelial barrier function during sepsis, the new therapeutic strategy for the treatment of peritoneal metastasis of ovarian cancer based on the use of antibody-drug conjugates targeted directly to carcinoma associated fibroblasts derived from mesothelial to mesenchymal transition, or a novel peritoneal dialysis fluid in which glucose has been substituted by a flavonoid with potent antioxidant activity.

These achievements highlight the Unit's leading role in exploring how cells communicate to achieve tissue homeostasis and how impairment of these mechanisms impacts on disease progression and aging.

# Research groups

# Carlos Anerillas Aljama

Damage response and senescence

# Carlos Cabañas Gutiérrez

Functional interactions between tetraspanin CD9 and cell adhesion molecules

# Miguel R. Campanero

Molecular characterization of vascular pathogenesis and lymphomagenesis

# **Isabel Guerrero Vega**

Mechanisms of cell-cell signaling during development

# José María Izquierdo Juárez

Molecular and cellular basis of the physio(patho) logy associated with the expression of intracellular antigens

# Manuel López Cabrera

Pathophysiology of peritoneal inflammation and fibrosis (PERINFIB)

#### Jaime Millán

Cell biology of inflammation

# María Mittelbrunn Herrero

Immunometabolism and inflammation

# Ginés Morata Pérez / Natalia Azpiazu Torres

Mechanisms of tumorigenesis in *Drosophila* 

# Juan Miguel Redondo Moya

Molecular and translational research in vascular pathologies

# **Fernando Rodriguez Pascual**

Extracellular matrix remodeling in the cardiovascular system

# María Yáñez-Mó

Tetraspanin-enriched membrane microdomains in extracellular vesicles and cell adhesion and migration



# DAMAGE RESPONSE AND SENESCENCE

# **Group members**

**Principal investigator:** Carlos Anerillas Aljama

**Predoctoral fellows:** Aida Perramon Güell

**Undergraduate and Master students:** Paula Sánchez de Rojas de la Osa





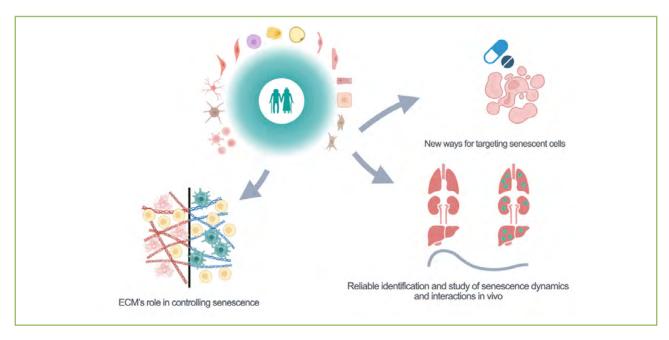
### Summary

Understanding the aging process is crucial as global populations, especially in developed countries, get older. This knowledge can help alleviate healthcare burdens and promote strategies for healthier aging. Research indicates that preventing excessive senescence, which contributes to various pathologies, can enhance tissue function and homeostasis. We have a robust background in studying how senescent cells remain viable despite underlying damage. Our current goal is to connect these findings to defective tissue damage responses, particularly during aging and fibrosis. Our research focuses on:

- Examining the connections between cellular senescence and fibrosis through the extracellular matrix.
- 2. Investigating senescence dynamics in vivo following organ injury.
- 3. Developing therapies targeting senescent cells to improve age-related conditions.

These efforts aim to deepen our understanding of the roles of cellular senescence in multiple contexts, ultimately seeking to mitigate age-dependent functional declines.





Overview of our group's goals



# List of publications

■ Anerillas C\*, Perramon-Güell A, Altés G, Cuesta S, Vaquero M, Olomí A, Rodríguez-Barrueco R, Llobet-Navàs D, Egea J, Dolcet X, Yeramian A, Encinas M\*. Sprouty1 is a broad mediator of cellular senescence. Cell Death Dis. 2024. Apr 26;15(4):296. doi: 10.1038/s41419-024-06689-4. PMID: 38670941.



# Research projects

- LCF/BQ/PI24/12040003. Fundación La Caixa. La Caixa "Postdoctoral Junior Leader Incoming" fellowship. Carlos Anerillas Aljama. 2024-26.
- 101163448. European Commission. ERC Starting Grant for the project ChECMate senescence. Carlos Anerillas Aljama. 2025-30.



#### Other activities

- 2012-13 Final Degree Project award given by the Spanish Government (Beca de Colaboración, MECD)
- 2013-14 Final Master Project award given by the University of Lleida (Spain)
- 2013-18 Assistant Professor of Cell Biology, Lleida School of Medicine, University of Lleida (Spain)
- 2014-19 Ph.D. student, supported by a competitive Ph.D. Fellowship financed by Spanish Government and awarded Cum Laude qualification
- 2016- Member of the Spanish Society for Biochemistry and Molecular Biology (elected full member in 2023)
- 2016 Research Associate, Institute of Genetic Medicine, Newcastle University (UK)
- 2018-19 Award "Ajut Recerca en Salut", providing an extra year for the completion of the Ph.D. thesis
- 2019-23 Postdoctoral (Visiting) Fellowship at the National Institute on Aging, National Institutes of Health (USA)

- 2021- Member of the Association of Spanish Scientists in the USA (ECUSA)
- 2022 NIH's Fellows Award for Research Excellence (FARE) 2022 for the work "A BDNF-TrkB autocrine loop enhances senescent cell viability"
- 2022- Member of the International Cell Senescence Association (ICSA)
- 2023 NIH's Fellows Award for Research Excellence (FARE) 2023 for the work "YAP-TEAD supports senescent cell viability by lowering ER stress"
- 2023- Member of the Spanish Society for Cellular Senescence (SENESCEL)
- 2024 NIH's Fellows Award for Research Excellence (FARE) 2024 for the work "Senescent cells produce and respond to extracellular matrix proteins such as THBS3 to implement a pro-survival EMT-like program"
- 2024 NIH's Matrix Biology Interest Group award for the work "Senescent cells produce and respond to extracellular matrix proteins such as THBS3 to implement a pro-survival EMT-like program"



# FUNCTIONAL INTERACTIONS BETWEEN TETRASPANIN CD9 AND CELL ADHESION MOLECULES

# **Group members**

**Principal investigator:** Carlos Cabañas

**Predoctoral fellows:** Irene Clares Pedrero Almudena Rocha Mulero Lara Álvarez Rodríguez



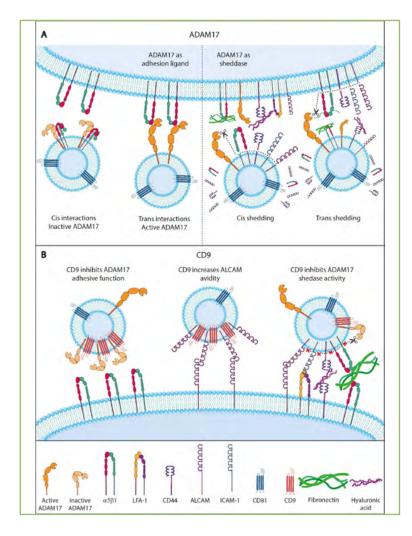


#### Summary

Tetraspanins are highly conserved membrane proteins that function as membrane-organizers and control fundamental cell functions, including adhesion, migration and proliferation. The tetraspanin CD9 specifically associates with different adhesion receptors of the immunoglobulin and integrin families 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 the metalloproteinase ADAM17 on the surface of different types of cells, exerting an inhibitory effect on ADAM17 sheddase activity against a variety of its substrates. Interactions of integrin  $\alpha 5\beta 1$  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  $\alpha 5\beta 1$  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  $\alpha 5\beta 1$ -mediated cell adhesion to its ligands fibronectin and ADAM17-Fc. In situ proximity ligation assays (PLA) and biochemical experiments based on co-immunoprecipitation collectively revealed that the mechanism by which CD9 inhibits  $\alpha 5\beta 1$ -mediated cell adhesion involves the reinforcement of cis interactions between ADAM17 and  $\alpha 5\beta 1$  on the cell surface, which takes place without alteration in  $\alpha 5\beta 1$  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 and metalloproteinases involved in the binding and/ or uptake of exosomes by target cells.





Roles of ADAM17 and tetraspanin CD9 in the targeting and docking of TEVs.



# List of publications

- Pelaez-Prestel HF, Gonzalez-Martin F, Ras-Carmona A, Rocha A, Cabañas C, Lafuente EM, Reche PA. (2024) Oral squamous cell carcinomas drive monocytes into immunosuppressive CD25+CD163+CD206+ macrophages. Oral Oncol. 2024 Dec;159:107078. doi: 10.1016/j. oraloncology.2024.107078.
- Clares-Pedrero I, Rocha-Mulero A, Palma-Cobo M, Cardeñes B, Yáñez-Mó M, Cabañas C. (2024) Molecular Determinants Involved in the Docking and Uptake of Tumor-Derived Extracellular Vesicles: Implications in Cancer. Int J Mol Sci. 2024 Mar 19;25(6):3449. doi: 10.3390/ijms25063449.
- Terri M, Sandoval P, Bontempi G, Montaldo C, Tomero-Sanz H, de Turris V, Trionfetti F, Pascual-Antón L, Clares-Pedrero I, Battistelli C, Valente S, Zwergel C, Mai A, Rosanò L, Del Pozo MÁ, Sánchez-Álvarez M, Cabañas C, Tripodi M, López-Cabrera M, Strippoli R. (2024) HDAC1/2 control mesothelium/ ovarian cancer adhesive interactions impacting on Talin-1-α5β1-integrin-mediated actin cytoskeleton and extracellular matrix protein remodeling. J Exp Clin Cancer Res. 2024 Jan 23;43(1):27. doi: 10.1186/s13046-023-02930-8.



# Research projects

■ PID2021-123199OB-I00. MICINN, State Research Plan, Biomedicine Program. Title: molecular determinants involved in the binding/capture of tumor exosomes by immune and non-immune receptor cells. Principal Investigator: Carlos Cabañas. Dates: 01/09/2022 to 31/08/2025



# **Doctoral theses**

Álvaro Torres Gómez (Ftad. Medicina, UCM). Marzo, 2023

Activación y señalización del receptor de complemento CR3

Departamento de Inmunología, UCM

Carlos Cabañas y M. Esther Lafuente Duarte (Ftad. Medicina, UCM).

Calificación: Sobresaliente cum laude, Tesis con Mención Internacional.



# MOLECULAR CHARACTERIZATION OF VASCULAR PATHOGENESIS AND LYMPHOMAGENESIS

# **Group members**

**Principal investigator:**Miguel R Campanero

Postdoctoral fellows: Cristina Clemente Toribio Alberto Hernández Alcántara Yilin Sun

**Predoctoral fellows:**Carolina Gutiérrez Martínez

**Technicians:** Patricia Martínez Núñez

**Undergraduate and Master students:** Teresa Gil Ruiz



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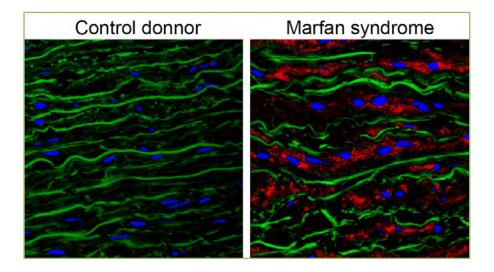


#### **Summary**

This group has investigated, in collaboration with researchers from the USA and Europe, the molecular mechanisms driving lymphomagenesis and some of the most relevant cardiovascular identified diseases. They mediators pathological vascular wall remodeling involved in atherosclerosis, restenosis, intramural hematoma, arterial hypertension, thoracic aortic aneurysm and dissection (TAAD), and abdominal aortic aneurysm. Recently, they discovered novel genes mediating aortic diseases, such as RCAN1, C/EBPβ, ADAMTS1, and NOS2, showing their essential role in maintaining aortic homeostasis. Gene deficiencies were linked to aortic aneurism and dissection (Nat Med 2017; Nat Commun 2018). Their studies unveiled novel pathophysiological mechanisms and therapeutic targets for aortic diseases. Notably, they demonstrated that deficiency of the metalloproteinase Adamts1 triggers aortic pathology in mice via increased Nos2-dependent NO production (Nat Med 2017), reshaping the understanding of TAAD pathophysiology and opening new avenues of research into TAAD pathogenesis that led to the identification of novel targets for pharmacological intervention and potential biomarkers for the follow up of Marfan syndrome (Nat Commun 2021, EMBO Mol Med 2024). The group has also made important contributions to lymphomagenesis and leukemogenesis. They revealed a tumor suppressor role for E2F4 in B-cell

lymphoma as a key transcriptional repressor of the E2F1 oncogene (Leukemia 2012) and identified MAZ as a critical activator of MYB expression, uncovering a novel mechanism to bypass transcriptional repression by E2F-pRB (Nucleic Acids Res 2017). Using gene expression profiling, they identified CDCA7 as a gene overexpressed in lymphoid tumors and critical for lymphoma/leukemia growth in soft gels, tumor formation in immunodeficient mice, and lymphoma migration and invasion via modulation of tubulin and actomyosin cytoskeleton dynamics (Haematologica 2018 & 2020). The selective role of CDCA7 in soft gels is particularly relevant, as tumor initiating cells (TICs), the drivers of tumor resistance and relapse, thrive in these conditions, while differentiated and non-tumor cells do not. In an AECC-funded project, they discovered that culturing lymphoid cells in soft gels exacerbates reactive oxygen species production, killing non-tumor cells while allowing TIC proliferation through increased intracellular glutathione production, while inhibiting glutathione synthesis with buthionine sulphoximine blocked TIC growth in soft gels and hindered tumor growth in a lymphoma mouse model (Cell Death Dis 2024). Overall, this group's pioneering research has reshaped the understanding of vascular and lymphoid malignancies, identifying key genes, mechanisms, and potential therapeutic targets for improved patient outcomes.





Images showing nuclei (blue staning), increased pAKT-S473 staining (red), and disorganized elastic fibers (green) in the aortic wall of a patient with Marfan Syndrome as compared with a control donor.



# List of publications

- A. del Puerto, B. Martí-Prado, A.L. Barrios-Muñoz, C. López-Fonseca, J. Pose-Utrilla, B. Alcover-Sanchez, F. Cesca, G. Schiavo, M.R. Campanero, I. Fariñas, T. Iglesias, and E. Porlan. (2023). Kidins220 sets the threshold for survival of neural stem cells and progenitors to sustain adult neurogenesis. Cell Death Dis 14(8):500. doi: 10.1038/s41419-023-05995-7
- M.J. Ruiz-Rodríguez, J. Oller, M. Toral, S. Martínez-Martínez, A. Colmenar, D. López-Maderuelo, C.B. Kern, J.F. Nistal, A. Evangelista, G. Teixidó-Tura, M.R. Campanero\*, and J.M. Redondo\*. \*Co-senior and corresponding authors. (2024). Versican accumulation drives Nos2 induction and aortic disease in Marfan syndrome via Akt activation. EMBO Mol Med 16(1):132-157. doi: 10.1038/s44321-023-00009-7
- A. H.-Alcántara, O. Kourani, A. Marcos-Jiménez, P. Martínez-Núñez, E. Herranz-Martín, P. Fuentes, M.L. Toribio, C. Muñoz-Calleja, T. Iglesias, and M.R. Campanero. (2024). Glutathione overproduction mediates lymphoma initiating cells survival and has a sex-dependent effect on lymphomagenesis. Cell Death Dis. 15:534 (2024). doi:10.1038/s41419-024-06923-z



# Research projects

- PID2020-115217RB-I00, Spanish Agencia Estatal de Investigación, Roles of smooth muscle and endothelial calcineurin in arterial hypertension and aortic aneurysm. PI: Miguel R. Campanero. 1/9/2021 31/08/2024
- PROYE20060CAMP, Fundación AECC, Identification of efficient therapeutic targets for lymphoid tumors. PI: Miguel R. Campanero. 01/12/2020 30/11/2023
- PID2023-152367OB-I00, Spanish Agencia Estatal de Investigación, Identification and validation of new therapeutic targets for the treatment of hypertension. PI: Miguel R. Campanero. 1/9/2024 31/08/2027
- CIBERCV. Member of the group led by Juan Miguel Redondo.



### **Doctoral theses**

- Andrea de la Fuente-Alonso (2023). Contribution of canonical and non-canonical NO signaling pathways to aortic disease in Marfan syndrome. Departamento de Biología Molecular. Universidad Autónoma de Madrid. Directores: Juan Miguel Redondo y Miguel R. Campanero. Mención internacional
- Yilin Sun (2024). Título. Departamento de Bioquímica. Universidad Autónoma de Madrid. Directores: Miguel R. Campanero y Juan Miguel Redondo
- Alberto Hernández Alcántara (2024). Requerimientos biomecánicos para el crecimiento de los tumores linfoides. Departamento de Bioquímica. Universidad Autónoma de Madrid. Director: Miguel R. Campanero



# MECHANISMS OF CELL-CELL SIGNALING DURING DEVELOPMENT

# **Group members**

**Principal investigator:** Isabel Guerrero Vega

**Scientific staff:** Ana Busturia Jimeno

**Postdoctoral fellows:** Vasiliki Lalioti

Predoctoral fellows: Carlos Jiménez Jiménez Clara Fernández Pardo Víctor Mendoza Grimau

**Technicians:** Lara Maria Rodenstein Undergraduate and Master students: Santiago Lamas de Lazaro Jorge Ruiz Moreno Marina Blanco Rubio

**Visiting scientists:** Pedro Ripoll Quintas



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#### Summary

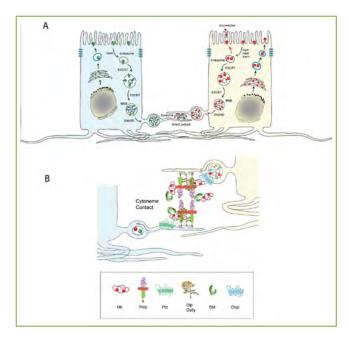
Cell-to-cell communication is a key event during development and its misregulation can cause diseases such as malformations, neurological disorders and cancer. Several signaling molecules (morphogens) act over long distances, forming a gradient distribution within a morphogenetic field. Some morphogens 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. 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 specific objetives are: 1) To study the mechanisms of interaction between cytonemes from Hh-receiving and Hh-sending cells for Hh reception based on the functional interaction of the components of Hh signalling complex on cytonemes (Fig.1). 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 the maintenance of adult homeostasis and tumor progression.

In the same group, the research line "Drosophila Developmental Epigenetics" led by Dr. Ana Busturia. This research aims to understand the epigenetic mechanisms controlling homeostatic development of an organism. This is done by studying the roles played by the the Polycomb (PcG) and trithorax (trxG) group proteins in normal and pathological development. Three research areas are being developed. 1) Organ communication mediated by hemolymph exosomes containing microRNAs that modify PcG/trxG levels in target organs. 2) Effect of the apoptosis mediated by the immune derived Antimicrobial Peptides on the modulation of PcG induced tumor formation. 3) PcG- mediated transgenerational inheritance of the physiological traits observed upon supplementing Drosophila diet with enriched milk fat globule membrane (MFGM).





"Hh signaling conceived as a synapse-like process." A) After an intracellular recycling of Hh and the receptor Ptc, both travel in MVB along cytonemes. B) The Hh co-receptors Ihog, the glypicans (Dally and Dlp) and Shifted (orthologue of vertebrate Wif-1) are recruited to facilitate the reception."



# **Doctoral theses**

■ Carlos Jiménez Jiménez Date: December 4, 2024 Title: Estudio de los procesos de liberación, transporte y recepción de Hedgehog en Drosophila Biociencias Moleculares, Department of Molecular Biology, Universidad Autónoma de Madrid Thesis Advisor: Isabel Guerrero Vega



# List of publications

- LaliotiVS, Gradilla AC, Jiménez-Jiménez C, Fernández-Pardo C, Sánchez-Hernández D, Aguirre-Tamaral A, Sánchez-Platero I, Jordán-Àlvarez S, Wakefield JG, Guerrero I. The Drosophila epidermal growth factor receptor pathway regulates Hedgehog signalling and cytoneme behaviour. Nat Commun. 2025 Feb 26;16(1):1994. doi: 10.1038/s41467-025-57162-5. PMID: 40011425; PMCID: PMC11865286.
- Aguilar G, Bauer M, Vigano MA, Guerrero I, Affolter M. Protocol for generating in-frame seamless knockins in Drosophila using the SEED/Harvest technology. STAR Protoc. 2024 Sep 20;5(3):102932. doi: 10.1016/j. xpro.2024.102932. Epub 2024 Jul 11. PMID: 38996063; PMCID: PMC11296251.
- Aguilar G, Bauer M, Vigano MA, Schnider ST, Brügger L, Jiménez-Jiménez C, Guerrero I, Affolter M. Seamless knockins in Drosophila via CRISPR-triggered singlestrand annealing. Dev Cell. 2024 Oct 7;59(19):2672-2686. e5. doi: 10.1016/j.devcel.2024.06.004. Epub 2024 Jul 5. PMID: 38971155.
- Jiménez-Jiménez C, Grobe K, Guerrero I. Hedgehog on the Move: Glypican-Regulated Transport and Gradient Formation in Drosophila. Cells. 2024 Feb 27;13(5):418. doi: 10.3390/cells13050418. PMID: 38474382; PMCID: PMC10930589.
- Mendoza-Grimau V, Pérez-Gálvez A, Busturia A, Fontecha J. Lipidomic profiling of Drosophila strains Canton-S and white1118 reveals intraspecific lipid variations in basal metabolic rate. Prostaglandins Leukot Essent Fatty Acids. 2024 Feb;201:102618. doi: 10.1016/j.plefa.2024.102618. Epub 2024 May 15. PMID: 38795635.
- Solorzano J, Carrillo-de Santa Pau E, Laguna T, Busturia A. A genome-wide computational approach to define microRNA-Polycomb/trithorax gene regulatory circuits in Drosophila. Dev Biol. 2023 Mar;495:63-75. doi: 10.1016/j. ydbio.2022.12.008. Epub 2022 Dec 31. PMID: 36596335.



# Research projects

Reference: PID2020-114533GB-C21 Funding entity: Ministerio de Ciencia, Innovación y Universidades.

Title: Cellular Communication by Direct Contact: Cytoneme formation in the Hedgehog signaling function during development. Start date: Sept 2021-

End date: July 2025. PI: Isabel Guerrero Vega.

■ Reference: CA19105 https://www.cost.eu/actions/CA19105/ Funding entity: COST- European Cooperation in Science and Technology.

Title: Pan-European Network in Lipidomics and EpiLipidomics EpiLipidNET http://www.epilipid.net Start date - 13/10/2020

End date - 12/10/2024 PI: Ana Busturia

Reference: PRX 2023-00470.

Funding entity: Ministerio de Ciencia, Innovación y

Universidades

Program: ESTANCIA DEL PROGRAMA DE MOVILIDAD EN ENSEÑAÑZA SUPERIOR E INVESTIGACIÓN. Modalidad Senior

Title: Estudio de la función anti-tumoral de los Péptidos Antimicrobianos (AMPs) en Drosophila Host laboratorio: Dr. Bruno Lemaitre en Ecole Polythecnique Federal de Lausanne (EPFL), Lausanne (Switzerland)

Start date: Sept 2024-End date: Feb 2025 PI: Ana Busturia



# MOLECULAR AND CELLULAR BASIS OF THE PHYSIO(PATHO)LOGY ASSOCIATED WITH THE EXPRESSION OF INTRACELLULAR ANTIGENS

# **Group members**

**Principal investigator:** José María Izquierdo Juárez

**Predoctoral fellows:** Rocío Naranjo Sánchez

**Technicians:** José Alcalde García Beatriz Ramos Velasco

**Undergraduate and Master students:** Lorena Torrente Estringana Rodrigo Díaz López



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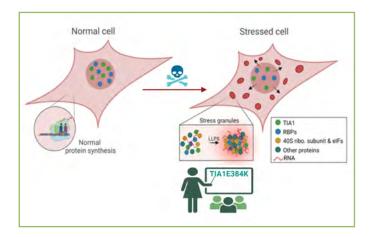


#### Summary

The grammar involves the study of how the letters of the alphabet are assembled into the structure of words and how these words are grouped and interconnected. Each language has its own grammar, complete with its own internal logic. This concept also applies to biological processes, where proper functioning relies on various "grammars" with their own "letters" and "words" that must be accurately constructed, transmitted, and interpreted. For instance, combinations of four letters (A, G, C, and T, as deoxyribonucleotides) encode and store within our DNA the essence of who we are, what we can become, and what we pass on throughout our lives. Similarly, four other letters (A, G, C, and U, as ribonucleotides) form single strands of RNA that temporarily interpret and process the information embedded in our genome. Some of these RNA strands, processed as mRNAs, are read and translated by ribosomes, enabling the sequential and orderly assembly of the protein "bricks" that constitute the tissues/organs of our bodies. In fact, each of the twenty amino acids found in proteins is also represented by a unique letter. Over the past two years, our research group has focused on deciphering the "molecular grammar of amino acids" involved in the formation of transient. non-membrane-bound cellular structures made of RNA and proteins. These structures, known as stress granules (SGs), form in response to cellular stress as a survival mechanism. Specifically, we have studied T-cell intracellular antigen 1 (TIA1)-dependent SGs

(TIA1-SGs) linked to a missense mutation in TIA1 (c.1362 G>A; p.E384K), which is related with Welander distal myopathy (WDM). WDM is an autosomal dominant, late-onset muscular dystrophy that leads to the progressive loss of function in the extensor muscles of the fingers and toes in the upper and lower extremities, between the ages of 40 and 60. This dysfunction is more severe in homozygous patients, who often become wheelchair-bound before the age of 50. WDM has been linked to impaired dynamics of TIA1-SGs. Recently, we have elucidated several molecular and cellular aspects related to the pathophysiology of WDM. Thus, we generated all proteinogenic variants of human TIA1 at position 384 to assess their impact on TIA1-SG formation. We found that positively charged amino acids (K, R, and H), along with C, N, S, and Y residues, enhance the dynamics of TIA1-SG formation through a process known as liquid-liquid phase separation (LLPS), whereas P, V, and W residues do not. Additionally, we demonstrated that the TIA1 paralog, TIAR, is largely unaffected by the most significant mutations, with only R and P altering the dynamics of TIAR-SGs. Understanding the regulatory dynamics tied to these anomalous "grammars" could lay the groundwork for identifying future therapeutic targets. This knowledge is critical for promoting healthy living and enhancing the well-being of individuals at all ages, including patients, their families, and healthcare systems.





Learning the molecular grammar of Welander distal myopathy. The amino acid molecular grammar related to TIA1-dependent stress granules of Welander distal myopathy (c.1362 G>A; p.E384K) plays a key role through the liquid-liquid phase separation phenomenon (LLPS) under oxidative conditions.



# List of publications

- Izquierdo, J. M. (2025) Mitochondria-cGAS-STING axis is a potential therapeutic target for senescence-dependent inflammaging-associated neurodegeneration. Neural Regen. Res. 20, 805-807. doi: 10.4103/NRR.NRR-D-24-00245. Epub 2024 May 17.
- Alcalde-Rey, I., Ramos Velasco B., Alcalde, J., and Izquierdo, J. M. (2024) Decoding the molecular grammar of TIA1-dependent stress granules in proteostasis and Welander distal myopathy under oxidative stress. Cells 13, 1961. doi: 10.3390/cells13231961.
- Izquierdo, J. M. (2024) Pro-inflammatory cytokine 11 plays a pivotal role in inflammaging-associated pathologies. Aging Cell 23, e14360. doi: 10.1111/acel.14360.
- Izquierdo, J. M. (2024) Vitamin D-dependent microbiotaenhancing tumor immunotherapy. Cell. Mol. Immunol. 21; 1083-1086. doi: 10.1038/s41423-024-01184-4.
- Izquierdo, J. M. (2024) Novel insights into the sexual dimorphism-associated immune response. Cell. Mol. Immunol. 21; 804-806. doi: 10.1038/s41423-024-01177-3.
- Ramos Velasco, B., Naranjo, R., and Izquierdo J. M. (2024) Bibliometric overview on T-cell intracellular antigens and their pathological implications. Biology 13, 195. doi: 10.3390/biology13030195.
- Izquierdo, J. M. (2024) Blood platelet factor 4: the elixir of brain rejuvenation. Mol Neurodegener. 19, 3. doi: 10.1186/s13024-023-00681-w.
- Izquierdo, J. M. (2024). Taurine as a possible therapy for immunosenescence and inflammaging. Cell. Mol. Immunol. 21, 3-5. doi: 10.1038/s41423-023-01062-5.



# **Doctoral theses**

- Ms. Lorena Torrente Estringana (University of Burgos, 2024).
- Mr. Rodrigo Díaz López (Autonomous University of Madrid, since November 2024).

- Izquierdo, J. M. (2023) Lost in mTORC1-related translation limits healing, repair and regeneration in mammals. Front. Cell. Dev. Biol. 11, 1294934. doi: 10.3389/fcell.2023.1294934.
- Izquierdo, J. M. (2023) cGAS-STING triggers inflammagingassociated neurodegeneration. Mol Neurodegener. 18, 78. doi: 10.1186/s13024-023-00666-9.
- Izquierdo, J. M. (2023) Chromatin-transcription interface: The secret of eternal youth? Aging Cell 22, e13927. doi: 10.1111/acel.13927.
- Izquierdo, J. M. (2023) Cellular senescence: is the problem a solution for muscle repair? Cell. Mol. Immunol. 20, 429-431. doi: 10.1038/s41423-023-00981-7.



# Research projects

- PID2021-126152OB-I00. Funding entities: MICU-AEI-FEDER-UE. Participating entities: CSIC. Title: "Study of the molecular grammar of TIA1-dependent stress granules in proteostasis and Welander distal myopathy". PI: José María Izquierdo Juárez. Time period: September 2022-February 2026.
- Contract of the Youth Guarantee Program of the Community of Madrid (GARJUVCAM20\_CBM\_TL\_15) for training of technical personnel: Ms. Beatriz Ramos Velasco (March 2021-April 2023).
- Contract of technical personnel associated with PID2021-126152OB-100 project. Ms. Beatriz Ramos Velasco (May 2023-December 2024).
- Contract INVESTIGO of the Community of Madrid (EICS 24211028C098/13-2022-008566). Investigator: Ms. Rocío Naranjo Sánchez (October 2022-September 2023).
- CSIC Rare Diseases Network (RER-CSIC) (established November 2024).



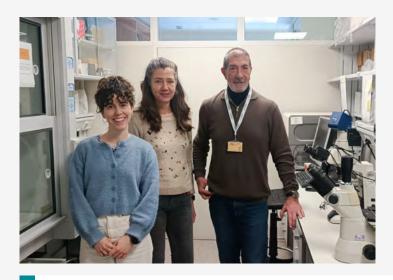
# MOLECULAR PATHOPHYSIOLOGY OF PERITONEAL INFLAMMATION AND FIBROSIS

# **Group members**

**Principal investigator:**Manuel Lopez Cabrera

**Postdoctoral fellows:** Pilar Sandoval Correa

**Predoctoral fellows:** Henar Tomero Sanz







#### Summary

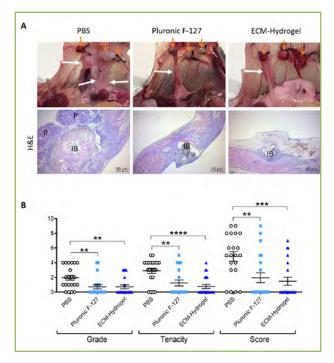
During peritoneal dialysis (PD), the peritoneum is exposed to bio-incompatible solutions that cause inflammation, angiogenesis and fibrosis, resulting in membrane failure. Our group has shown that mesothelial cells undergo an epithelial-mesenchymal transition (EMT) in response to the peritoneal insult. During the last 20 years, we have shown that mesothelial EMT (also termed Mesothelial to Mesenchymal Transition, MMT) is a good marker for membrane failure and a target for preventing PDinduced fibrosis and for the design of biocompatible PD fluids. In this context, we have studied the effect on MMT of a novel PD fluid, patented by our group (PCT/ES2023/070430; WO 2024/008987 A1), in which the glucose has been substituted by Troxerutin, a flavonoid with potent antioxidant activity, as osmotic agent. We could demonstrate that this PD fluid induces less MMT and causes less peritoneal damages and cardiovascular disorders when compared with glucose-based PD fluids in animal models. We have also explored whether MMT plays a role in other 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). We have shown in human peritoneal implant biopsies that a subpopulation of CAFs derives from mesothelial cells through MMT. Our results also suggest that this MMT renders the peritoneum more receptive to implantation of tumor cells, contributes to the

growth and vascularization of secondary tumors and that MMT is a therapeutic target in peritoneal carcinomatosis (Pascual-Antón et al., Adhesions are areas of fibrotic tissue that bind tissues and organs that would normally not be connected, and can be seriously life-threatening. Histological analysis of human post-surgical adhesions has demonstrated that mesothelial cells adjacent to the fibrotic tissue show signs of MMT, suggesting that this could be an initial step in their development. Our results have shown that mechanical injury is the main inducer of MMT during the formation of adhesions. Consequently, molecules involved in mechano-transduction, such as caveolin 1, play a role in the regulation of mechanical MMT and adhesion formation. Recently, we have shown that a extracellular matrix-based hydrogel, which combines physical barrier and MMT-blocking functions, is an effective prophylactic biomaterial to mitigate the formation of peritoneal adhesions in an experimental mouse model (Figure).

# Other activities

- Ayuda del programa CSIC-EBTon (Activa-T, 2024)
- Ayuda del programa de acompañamiento a proyectos innovadores del CSIC INNOVAMAD 2024
- Award from the Universidad de Alcalá to the Best National Patent 2024: "Soluciones para diálisis peritoneal que contienen un flavonoide natural como agente osmótico".







# List of publications

- Tomero-Sanz H, Jiménez-Heffernan JA, Fernández-Chacón MC, Cristóbal-García I, Sainz de la Cuesta R, González-Cortijo L, López-Cabrera M, Sandoval P. Detection of Carcinoma-Associated Fibroblasts Derived from Mesothelial Cells via Mesothelial-to-Mesenchymal Transition in Primary Ovarian Carcinomas. Cancers (Basel). 2024 Jul 29;16(15):2697. doi: 10.3390/cancers16152697
- Terri M, Sandoval P, Bontempi G, Montaldo C, Tomero-Sanz H, de Turris V, Trionfetti F, Pascual-Antón L, Clares-Pedrero I, Battistelli C, Valente S, Zwergel C, Mai A, Rosanò L, Del Pozo MÁ, Sánchez-Álvarez M, Cabañas C, Tripodi M, López-Cabrera M, Strippoli R. HDAC1/2 control mesothelium/ovarian cancer adhesive interactions impacting on Talin-1-α5β1-integrin-mediated actin cytoskeleton and extracellular matrix protein remodeling. J Exp Clin Cancer Res. 2024 Jan 23;43(1):27. doi: 10.1186/s13046-023-02930-8.
- Cetin E, Mazzarino M, González-Mateo GT, Kopytina V, Meran S, Fraser D, López-Cabrera M, Labéta MO, Raby AC. Calprotectin blockade inhibits long-term vascular pathology following peritoneal dialysis-associated bacterial infection. Front Cell Infect Microbiol. 2023 Nov 29;13:1285193. doi: 10.3389/fcimb.2023.1285193.
- Pascual-Antón L, Sandoval P, González-Mateo GT, Kopytina V, Tomero-Sanz H, Arriero-País EM, Jiménez-Heffernan JA, Fabre M, Egaña I, Ferrer C, Simón L, González-Cortijo L, Sainz de la Cuesta R, López-Cabrera M. Targeting carcinoma-associated mesothelial cells with antibody-drug conjugates in ovarian carcinomatosis. J Pathol. 2023 Oct;261(2):238-251. doi: 10.1002/path.6170.
- Trionfetti F, Marchant V, González-Mateo GT, Kawka E, Márquez-Expósito L, Ortiz A, López-Cabrera M, Ruiz-Ortega M, Strippoli R. Novel Aspects of the Immune Response Involved in the Peritoneal Damage in Chronic Kidney Disease Patients under Dialysis. Int J Mol Sci. 2023 Mar 17;24(6):5763. doi: 10.3390/ijms24065763.

ECM-hydrogel treatment ameliorates peritoneal adhesion formation in mice. (A) Representative pictures of adhesions and Haematoxylin & Eosin (H&E) staining. (B) Quantification of adhesions shows significant differences between PBS group with the Pluronic F-127 and ECM-hydrogel groups.



# Research Projects

- Identification and Management of Patients at Risk Outcome and Vascular Events in Peritoneal Dialysis (Acronym: IMPROVE-PD).H2020-MSCA-ITN-2018 (Marie S-Curie Innovative Training Networks. Grant: 287813). 01-01-2019 to 30-06-2023. Coordinator: Manuel López Cabrera.
- Transición Mesotelio-Mesénquimal como diana terapéutica en fibrosis peritonela y en metástasis. Project Ref.: PID2022-142796OB-I00. MINISTERIO DE CIENCIA, INNOVACIÓN Y UNIVERSIDADES. 2023-2026. Principal Investigator: Manuel López Cabrera



# **Doctoral theses**

- Eva María Arriero País. Machine learning and biomarkerbased predictor and diagnosis tool (MAUXI) for the cardiovascular and ultrafiltration failure induced by the mesothelial to mesenchymal transition in peritoneal dialysis. UAM. Supervisors: Manuel López Cabrera
- Valería Kopytina. New horizons in peritoneal dialysis: assessment of novel osmotic agents and evaluation of associated cardiovascular disease. UAM. Supervisors: Manuel López Cabrera and Guadalupe González Mateo
- Lucía Pascual Antón. La Transición mesotelio-mesénquima como diana terapéutica común para el tratamiento de las adhesiones peritoneales postquirúrgicas y la metástasis peritoneal. UAM. Supervisors: Manuel López Cabrera and Pilar Sandoval Correa



#### **Patents**

- Inventors: Manuel López-Cabrera, Guadalupe T. González Mateo, Valeria Kopytina, Juan José Vaquero López, David Sucuza Saenz. Title: Peritoneal dialysis solutions containing a natural flavonoid as an osmotic agent: PCT/ES2023/070430; WO 2024/008987 A1
- Inventors: Eva María Arriero País, Manuel López Cabrera, Pilar Sandoval Correa, Guadalupe González Mateo, María Auxiliadora Bajo Rubio, Gloria del Peso Gilsanz. Title: Software entrenado a base de aprendizaje automático y biomarcadores para la predicción y el diagnóstico de la duración de tratamiento máximo y la causa de fallo de técnica en pacientes de fallo renal en diálisis peritoneal. Registro de invención: SigneBlock certifica que: F1D6E3C5700377F74294090A01FD1D1C455AC70 C7D16f03E54FCf00Af0B295A2 fué grabado en la red Blockchain de SigneBlock.



# CELL BIOLOGY OF INFLAMMATION

# **Group members**

Principal investigator: laime Millán Martínez

**Postdoctoral fellows:** Natalia Colás Algora

Predoctoral fellows: Gema Cerro Tello Carmen López Pujante Pablo Muñoz Pinillos Raúl García Fernández

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

Undergraduate and Master students: Domnica Finis Rubén Perez Llamo



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# Summary

Inflammation is a reparative process triggered by infection, stress, or tissue damage. However, pathological inflammation drives chronic diseases such as atherosclerosis and hepatitis and acute pathologies like sepsis. Inflammatory responses are mediated by soluble factors that disrupt endothelial and epithelial barriers, causing tissue edema and immune cell infiltration. Our objective is to investigate how inflammatory mediators impact cellular barriers and, conversely, how these barriers regulate inflammation.

A cytokine storm, the excessive and pathological release of pro-inflammatory cytokines, disrupts the endothelial barrier, leading to vascular leakage and organ damage. In lung, it causes acute respiratory distress syndrome (ARDS). We have examined the components in the cytokine storm that affect human lung microvascular endothelial cells (HLMVECs). IL- $1\beta$  and IL-6 trans-signaling significantly impaired the endothelial barrier, whereas other cytokines had moderate or negligible effects. The simultaneous inhibition of IL-1 and IL-6 trans-signaling was sufficient to preserve endothelial function in HLMVECs exposed to cytokine storms or septic patient sera, highlighting a combinatorial therapeutic approach to protect pulmonary endothelium (Colas-Algora et al. 2023).

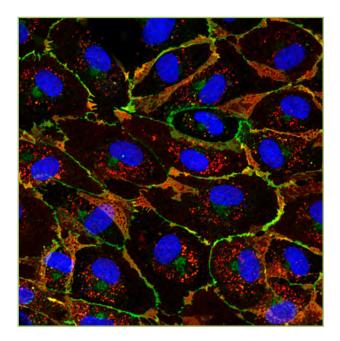
Cytokine storm also drives the progression of COVID19. We translated our studies to murine models of COVID19 and identified IL-6 trans-signaling as a key factor in SARS-CoV-2-induced endotheliopathy. Injection of sgp130Fc, a selective IL-6 trans-signaling

inhibitor, reduced vascular inflammation, endothelial dysfunction, and lung injury (Rodriguez et al. 2024). Collectively, our studies highlight the relevance of IL-6 trans-signaling in pathological endothelial hyperpermeability.

Epithelial polarity is crucial for liver function, particularly in bile canaliculi formation. ICAM-1, an adhesion receptor induced during inflammation, mediates epithelial-immune cell interactions. Our second project revealed that ICAM-1 also regulates apicobasal polarity in hepatic epithelium, independently of its canonical function in leukocyte adhesion. Using ICAM-1 knockout hepatic cells and liver organoids, we demonstrated that ICAM-1 interacts with the adaptor protein EBP50/NHERF1 at the apical membrane, controlling the actomyosin network and bile canalicular dynamics. This indicates that ICAM-1 is critical for maintaining epithelial architecture, a new role that has therapeutic potential for preserving liver function during inflammatory diseases.

Liver organoids are powerful biomedical tools. Cholestasis, impaired bile flow leading to liver damage, is often linked to bile transporter mutations. We have extended our studies with liver organoids by modeling cholestasis. Proteomic analysis from MDR2 knockout mouse organoids revealed upregulation of inflammatory and fibrotic markers, providing a comprehensive molecular profile and potential therapeutic targets for cholestasis (Blazquez et al. 2024).





Human endothelial cells regulate vascular permeability by forming a cellular barrier stabilized by intercellular junctions, primarily composed of VE-cadherin (green) and PECAM-1 (red). Nuclei are labeled in blue.

# Other activities

- Outreach activities:
- TV, "Inflamados". First episode Cazador Cerebros. La 2. Interview. https://www.rtve.es/play/videos/el-cazador-de-cerebros/cazador-cerebros-inflamados/16070239/April2024.
- Press, La Vanguardia. https://www.lavanguardia.com/ vivo/20231025/9308776/como-ganar-batalla-inflamacionciencia.html. Interview. 2023.
- Press. El Pais. https://elpais.com/salud-y-bienestar/2023-05-17/inflamacion-cronica-como-evitar-que-el-cuerpo-viva-en-estado-de-alerta.html#. Interview, 2023



# List of publications

- Colás-Algora N, Muñoz-Pinillos P, Cacho-Navas C, Avendaño-Ortiz J, de Rivas G, Barroso S, López-Collazo E, Millán J. (2023). Simultaneous targeting of IL-1-signaling and IL-6-trans-signaling preserves human pulmonary endothelial barrier function during a cytokine storm. Arterioscler Thromb Vasc Biol. .43:2213-2222. doi: 10.1161/ ATVBAHA.123.319695.
- Cacho-Navas C, López-Pujante C, Reglero-Real N, Colás-Algora N, Cuervo A, Conesa JJ, Barroso S, de Rivas G, Ciordia S, Paradela A, D'Agostino G, Manzo C, Feito J, Andrés G, Molina-Jiménez F, Majano P, Correas I, Carazo JM, Nourshargh S, Huch M, Millán J. (2024). ICAM-1 nanoclusters regulate hepatic epithelial cell polarity by leukocyte adhesion-independent control of apical actomyosin. eLife. https://doi.org/10.7554/eLife.89261
- Rodríguez-Hernández MÁ, Baena-Bustos M, Carneros D, Zurita-Palomo C, Muñoz-Pinillos P, Millán J, Padillo FJ, Smerdou C, von Kobbe C, Rose-John S, Bustos M. (2024). Targeting IL-6 trans-signalling by sgp130Fc attenuates severity in SARS-CoV-2 -infected mice and reduces endotheliopathy. eBiomedicine. 103:105132. doi: 10.1016/j. ebiom.2024.105132.
- Blázquez-García I, Guerrero L, Cacho-Navas C, Djouder N, Millan J, Paradela A, Carmona-Rodríguez L, Corrales FJ. (2024). Molecular Insights of Cholestasis in MDR2 Knockout Murine Liver Organoids. J Proteome Res. 23(4):1433-1442. doi: 10.1021/acs.jproteome.3c00900.



# Research projects

- PID2023-153087OB-I00. AEI. Search for new functions of RhoC in the metabolism and inflammatory response of the vascular endothelium (MIRhoC). PI: Jaime Millán. DURATION: 01/09/2024 31/08/2027.
- P2022/BMD7232. TomoXLiver2. Community of Madrid. PI and vice coordinator: Jaime Millán. Duration: 01/01/2022-31/12/2025.
- PID2020- 119881RB-I00. AEI. Functional characterization of mesoscopic domains of the endothelial surface regulated by the RhoA subfamily of GTPases during the inflammatory response (Endobarrier). PI: Jaime Millán. DURATION: 01/01/2021 31/01/2023. FUNDING: €170,000.



# Doctoral theses

■ Gema Cerro Tello (2024) Organización del citoesqueleto de actina en las barreras celulares de la córnea y su respuesta al estrés. Búsqueda de biomarcadores de queratocono. Universidad Autónoma de Madrid. Sobresaliente Cum Laude



# IMMUNOMETABOLISM AND INFLAMMATION

# **Group members**

**Principal investigator:**Maria Mittelbrunn

Postdoctoral fellows:

Elisa Carrasco Enrique Gabandé Isaac Francos Manuel Montero

#### **Predoctoral fellows:**

Ignacio Escrig Mario Perez Sandra Delgado Marta Villar

**Technicians:** Eva Maria Blanco

**Undergraduate and Master students:**Pablo R Ruiz de Erenchun

Visiting scientists:

Alice Leandri



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#### Summary

Immunosenescence not only leads to increased susceptibility to infections, cancer, autoimmune diseases and reduced vaccine efficacy in the elderly population, but also contribute to inflammaging and increase the risk of many age-associated disorders. Our previous research showed that a mouse model with premature immunosenescence exhibit signs of accelerated aging, highlighting the pivotal role of T cells in maintaining systemic homeostasis. Our results place T cells at the crossroad between inflammation, senescence and aging. We are working to decode the molecular mechanism by old cells contribute to inflammaging and age-related diseases and will discuss novel therapeutic opportunities to promote healthy aging. Furthermore, we are developing strategies to reverse the aged phenotype of T cells in order to reverse immunosenescence. By combining genetic, cellular, pharmacological and nutritional approaches, we aim to mitigate T cell aging in order to restore their function. These strategies are being tested in both murine models and humans, with the ultimate goal of restoring immune competence to foster healthy aging.



### **Doctoral theses**

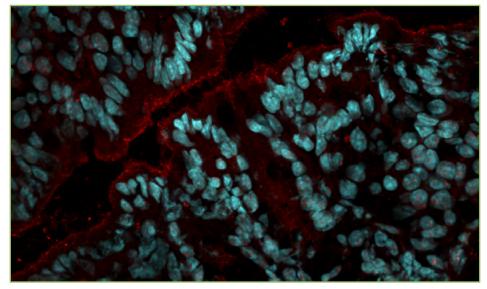
Gonzalo Soto, 2024



# Other activities

- Jaime Serra 2024
- FRACE Real Academia de Ciencias 2023
- Academica Corresponiente Real Academia de Ciencias 2025





Immunofluorescence of an inflamed gut – the gateway to inflammaging, where immunosenescence leaves its mark



# List of publications

- Aranda JF, Ramírez CM, Mittelbrunn M. Inflammaging, a targetable pathway for preventing cardiovascular diseases. Cardiovasc Res. 2024 Nov 12:cvae240. doi: 10.1093/cvr/cvae240. PMID: 39530590
- Enríquez JA, Mittelbrunn M. Warburg Effect Reshapes Tumor Immunogenicity. Cancer Res. 2024 Jul 2;84(13):2043-2045. doi: 10.1158/0008-5472.CAN-24-1304. PMID: 38657107
- Pastor-Fernández A, Gómez de Las Heras MM, Escrig-Larena JI, Barradas M, Pantoja C, Plaza A, Lopez-Aceituno JL, Durán E, Efeyan A, Mittelbrunn M, Martinez L, Fernandez-Marcos PJ. Sexual dimorphism in the antitumor immune responses elicited by the combination of fasting and chemotherapy. Cancer Commun (Lond). 2024 Apr;44(4):508-513. doi: 10.1002/cac2.12535. PMID: 38512765
- Escrig-Larena JI, Delgado-Pulido S, Mittelbrunn M. Mitochondria during T cell aging. Semin Immunol. 2023 Sep;69:101808. doi: 10.1016/j.smim.2023.101808. PMID: 37473558
- Gómez de Las Heras MM, Mittelbrunn M. Old T cells pollute with mito-litter. Nat Aging. 2023 May;3(5):475-476. doi: 10.1038/s43587-023-00412-2. PMID: 37198439
- Soto-Heredero G, Gómez de Las Heras MM, Escrig-Larena JI, Mittelbrunn M. Extremely Differentiated T Cell Subsets Contribute to Tissue Deterioration During Aging. Annu Rev Immunol. 2023 Apr 26;41:181-205. doi: 10.1146/ annurev-immunol-101721-064501. PMID: 37126417
- Gabandé-Rodríguez E, Pfeiffer M, Mittelbrunn M. Immuno(T)herapy for age-related diseases. EMBO Mol Med. 2023 Jan 11;15(1):e16301. doi: 10.15252/ emmm.202216301. PMID: 36373340"



# Research projects

- PID2022 1411690B-100 Gut-To-Brain. State Research Agency. Age-associated T cells in Gut-Brain AxisIP: Maria Mittelbrunn. €455,000. 2024–2026
- ERA4Health CARDINNOV Ener-LIGHT. State Research Agency. Energizing the failing heart. Coordinator: Mahmoud Abdellatif. €1,159,000. 2024–2026.
- ERC CoG 101044248 Let T Be. European Research Council. Letting up senescence and inflammaging through T cells. IP: Maria Mittelbrunn. €1.999.989. 2023–2028
- CAM-Y2020/BIO-6350 NutriSIo. Community of Madrid. Precision nutritional strategies to reactivate the immune system damaged by age, obesity, or chemotherapy. PI: Ana Ramírez, Maria Mittelbrunn, Elisa Carrasco. €616,730. 2022–2024



# MECANISMOS DE TUMORIGÉNESIS EN DROSOPHILA

#### **Group members**

**Principal investigator:** Gines Morata Natalia Azpiazu

Scientific staff: Manuel Calleja Requena

**Postdoctoral fellows:** Noeli Pinal Seoane Mireya Ruiz-Losada

**Predoctoral fellows:** Juan Manuel Garcia Arias

**Technicians:**Angélica Cantarero Mateo
Silvia Vega Esquinas

**Undergraduate and Master students:** 

Carmen Jarabo Caruda Miguel Gascon Ruiz

Leyre Bustamante Ardanaz



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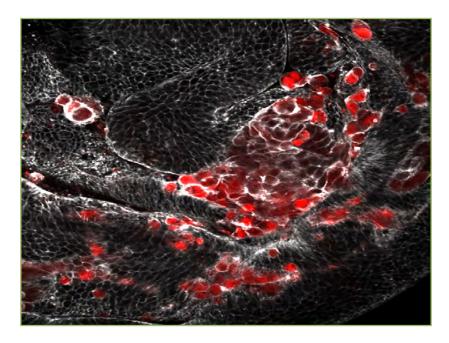
# Summary

Senescent cells represent a class of animal cells that acquire a set unique of features in response to a physiological or chemical stresses. They stop dividing and suffer a number of major metabolic changes that make them different from surrounding cells. They are the study subject of thousands of laboratories in the world, most often utilizing mammalian tissues or cells in culture to investigate their properties. A very relevant feature is the acquisition of the Senescence Associated Secretory Phenotype (SASP): senescent cells secrete diverse types of signals, which are received by neighbor non-senescent cells. Some of the secreted signals behave as growth factors and induce tumorous overgrowths.

In our laboratory we have recently developed a simple and reliable method to generate senescent cells in the fruitfly *Drosophila*. Using this system, we have embarked on a comprehensive project to investigate the properties of *Drosophila* senescent cells and their interactions with non-senescent cells that reside in the same tissue. We are making use of the sophisticated genetic technology of *Drosophila* to study those interactions, and particularly those that cause tumorigenesis. As a model tissue, we use the wing imaginal disc, possibly the animal tissue of the whole of Metazoans in which developmental and genetic parameters are best known.

We have identified the Jun N-Terminal Kinase (JNK) transduction pathway as the driver of cellular senescence in Drosophila and have also demonstrated its role in tumorigenesis by showing that JNK-expressing cells secrete proliferative signals (the ligands Upd, Wg and Dpp) that stimulate the overgrowth of the non-senescent tissue. There are two aspects of the tumorigenic component of cellular senescence that we are currently analyzing, 1) the existence of two types of senescent cells in the wing disc: the tumorigenic ones that appear in the appendage part on the disc, and the nontumorigenic ones, that reside in the thoracic part of the disc and do not produce tumorous overgrowths. We are currently carrying out ATAC-seq screens and RNA sequencing experiments in the wing and thorax to detect and subsequently characterize JNK target genes that are differentially expressed in those regions and may be associated with the tumorigenic processes, 2) the recruitment of new senescent cells by paracrine induction by pre-extant senescent cells. This is an important biological problem and an issue of biomedical interest because in humans the increase of senescent cells has deleterious consequences on health. We are testing the role of some SASP factors (Upd, Wg, Dpp, Notch, dsmitoRNA) for which we have experimental clues they may be involved.





Group of senescent cells in the wing disc. They are labelled red with the TREred construct, indicating high level of activity of the JNK pathway



# List of publications

- Garcia-Arias, JM. Pinal, N. Vargas, SC. Estella, C. And Morata, G. "Lack of apoptosis leads to cellular senescence and tumorigenesis in Drosophila epithelial cells" Cell Death Discovery 10.1038s41420-023-01583-y (2023).
- Pinal N., Ruiz-Losada M. Azpiazu, N. and Morata G. "Size compensation after generalized cell death" Front. Cell Dev. Biol. 11:1301913. doi: 10.3389/fcell.2023.1301913 (2023)
- del Prado, L. et al., (2024). Compensatory activity of the PC-ME1 metabolic axis underlies differential sensitivity to mitocondrial complex I inhibition. Nature Communications. DOI: 10.1038/s41467-024-52968-1
- Ostalé, C.M. et al., (2024). A function of Spalt proteins in heterochromatin organization and maintenance of genomic DNA integrity. bioRxiv. DOI: 10.1101/2024.04.30.591908



# Research projects

■ PID2021-125377NB-100. Ministry of Economy and Innovation. The JNK pathway in tumorigenesis and regeneration processes in Drosophila. IP Ginés Morata. 2021-2025



# **Doctoral theses**

Juan Manuel Garcia-Arias 2024 "Análisis de la función de la vía c-Jun-Terminal quinasa en la inducciín de senescencia celular y la tumorogénesis en Drosophila melanogaster"

Centro de Biología Molecular Universidad Autónoma de Madrid Director: Ginés Morata



# MOLECULAR AND TRANSLATIONAL RESEARCH IN VASCULAR PATHOLOGIES

# **Group members**

Principal investigator: Juan Miguel Redondo Moya (Staff based at CNIC during 2023-2024)

Scientific staff: Sara Martínez Martínez

**Postdoctoral fellows:** Dolores López Maderuelo María Jesús Ruiz Rodríguez

Predoctoral fellows:
Iván Alarcón Ruiz, PhD
Researcher (FPU)
Álvaro Alfayate Hernández,
PhD Researcher
Andrea de la Fuente Alonso,
PhD Researcher (FPU)
Sergio Mateos García, PhD
Researcher (FPI)
Noelia Martín Bermejo, PhD
Researcher (FPI)

**Technicians:** ÁngelColmenar Moreno María José Méndez Olivares

Undergraduate and Master students: MASTER: Noelia Martin Bermejo University: Complutense de Madrid, 2023

Teresa Gil Ruiz (codirector) University: Autónoma de Madrid, 2024

Visiting scientists: Ángel Luis Armesilla Arpa (Professor) Pablo Gómez del Arco (Assitant Professor) Marta Toral Jiménez (Postdoctoral Researcher) Yilin Sun (Postdoctoral Researcher) Fadoua El Abdellaoui Soussi (PhD Researcher (FPI) Paula Sofía Yunes Leites (Postdoctoral Researcher)



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#### Summary

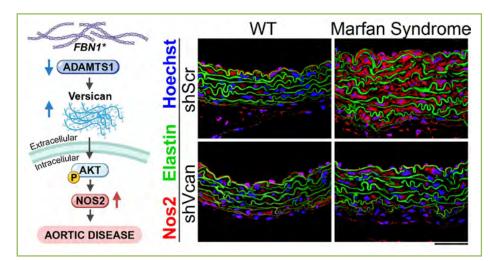
In recent years, our research has focused on the molecular and cellular mechanisms driving vascular remodeling and aortic pathophysiology, particularly in heritable thoracic aortic diseases (HTAD).

Aortic diseases cause 1%-2% of deaths in industrialized countries. Aortic aneurysm (AA) leads to progressive dilation and medial degeneration, often culminating in acute dissection (AD), a life-threatening event. In HTAD such as Marfan syndrome (MFS) and Loeys-Dietz syndrome (LDS), aortic dilation increases AD risk, yet medical treatments and risk predictors remain very limited due to gaps in understanding their molecular mechanisms. Surgery is the only effective intervention, guided by diameter thresholds, but AD can still occur at smaller diameters. There is an urgent need to (i) deepen mechanistic understanding of HTAD, (ii) identify reliable risk markers beyond aortic diameter, and (iii) develop pharmacological strategies.

In collaboration with Miguel Campanero's group, we identified key HTAD mediators, including Adamts1, Nos2, C/EBPβ, Plk1, and Rcan1. Adamts1 deficiency induces aortic disease via NOS2-mediated NO overproduction, and NOS2 inhibitors prevent and reverse pathology in MFS and Adamts1 mouse models. We also found that the NO-sGC-PKG pathway is overactivated in MFS due to extracellular matrix protein accumulation, including Versican (Vcan) and Fibronectin (FN), which activate the AKT-

NOS2 pathway. Genetic Vcan deficiency, lentiviral silencing, or AKT inhibition reversed aortic dilation, highlighting new therapeutic targets. Additionally, FN accumulation promotes MFS aortopathy via integrin signaling, initiating a cascade we have characterized in detail. Mapping these pathways represents a major breakthrough, establishing the most comprehensive signaling network in MFS aortopathy to date and linking extracellular matrix alterations with AKT-NO and integrin-mediated signaling. Our data also suggest that the AKT-NOS2 pathway, previously linked to MFS, also plays a role in LDS, warranting further investigation. Furthermore, in collaboration with Jesús Vázquez's group at CNIC, we conducted proteomic analyses that identified sex-dependent and independent molecular signatures in MFS and LDS, revealing shared patterns absent in controls and distinct clusters differentiating the syndromes. This work has enabled us to identify common mediators, syndrome-specific proteins, and sex-associated markers, all of which require further characterization. Additionally, we identified plasma proteins in MFS patients with high diagnostic accuracy and correlation with clinical severity, supporting their validation as diagnostic and prognostic biomarkers. Overall, our research aims to provide new insights into HTAD pathophysiology and open avenues for therapeutic target discovery and biomarker development in aortic diseases.





Versican (Vcan) accumulates in MFS aortas, activating the AKT/ NOS2 pathway and driving aortic disease. In vivo Vcan silencing or AKT inhibition reverses this pathology. The figure illustrates the Vcan-NOS2 pathway and how Vcan silencing normalizes NOS2 expression and restores vascular wall integrity



# List of publications

- Ruiz-Rodríguez MJ, Oller J, Martínez-Martínez S, Alarcón-Ruiz I, Toral M, Sun Y, Colmenar Á, Méndez-Olivares MJ, López-Maderuelo D, Kern CB, Nistal JF, Evangelista A, Teixido-Tura G, Campanero MR\*, Redondo JM\*. (2024) Versican accumulation drives Nos2 induction and aortic disease in Marfan syndrome via Akt activation. EMBO Mol Med. 16(1):132-157. doi: 10.1038/s44321-023-00009-7. Epub 2024 Jan 2. PMID: 38177536; PMCID: PMC10897446. (affiliation CBM).
- Rodrigues-Diez R, Ballesteros-Martinez C, Moreno-Carriles RM, Nistal F, Díaz Del Campo LS, Cachofeiro V, Dalli J, García-Redondo AB, Redondo JM, Salaices M, Briones AM. (2024) Resolvin D2 prevents vascular remodeling, hypercontractility and endothelial dysfunction in obese hypertensive mice through modulation of vascular and proinflammatory factors. Biomed Pharmacother. 174:116564. doi: 10.1016/j.biopha.2024.116564. Epub 2024 Apr 11. PMID: 38608525. (affiliation CBM).
- Gómez-Del Arco P\*, Isern J, Jimenez-Carretero D, López-Maderuelo D, Piñeiro-Sabarís R, El Abdellaoui-Soussi F, Torroja C, Vera-Pedrosa ML, Grima-Terrén M, Benguria A, Simón-Chica A, Queiro-Palou A, Dopazo A, Sánchez-Cabo F, Jalife J, de la Pompa JL, Filgueiras-Rama D, Muñoz-Cánoves P\*, Redondo JM\*. (2024) The G4 resolvase Dhx36 modulates cardiomyocyte differentiation and ventricular conduction system development. Nat Commun. 4;15(1):8602. doi: 10.1038/s41467-024-52809-1. PMID: 39366945; PMCID: PMC11452623. (affiliation CBM).



#### Other activities

JMR organized the Primera reunión Red temática de Patología Aortica AortinRed, December 13-14, 2024 (Madrid).



# Research projects

- 202334-31. La Marató. Improving Diagnostic Efficiency in AAA Patients. Coordinator: María Sabater Lleal, IP: Juan Miguel Redondo. 01.04.2024 31.03.2027.
- RED2022-134541-T. Ministerio de Ciencia e Innovación. Spanish Network Research in Aortic Pathology (AortinRed). Coordinator: Juan Miguel Redondo. 01.06.2023 31.05.2025. (Research network).
- Our group belongs to the Centro de Investigación Biomédica en Red de Enfermedades Cardiovasculares (CIBERCV):CB16/11/00264 . IP: Juan Miguel Redondo. 01.01.2022 31.12.2024 (annually renewable). (CIBER CV).
- PID2021-122388OB-I00. Ministerio de Ciencia e Innovación. Nitric oxide signaling and mediators in mouse models of Marfan syndrome and in patients with aortic diseases. IP: Juan Miquel Redondo. 01.09.2022 31.12.2025.



# **Doctoral theses**

- Fadoua El Abdellaoui (co-director) (2023). Functional characterization of the development of cardiac arrhythmias and dysfunction: role of Chd4, Znf219 and thPOK interaction. Facultad de ciencias, Universidad Autónoma de Madrid. Pablo Gómez del Arco, Juan Miguel Redondo. International mention.
- Andrea de la Fuente Alonso (2023). Contribution of canonical and non-canonical NO signaling pathways to aortic disease in Marfan syndrome. Facultad de ciencias, Universidad Autónoma de Madrid. Juan Miguel Redondo, Miguel Ramón Campanero. International mention.
- Yilin Sun (co-director) (2024). Contributions of a phosphatase independent activity of smooth muscle cell calcineurin to arterial hypertension. Facultad de ciencias, Universidad Autónoma de Madrid. Miguel Ramón Campanero, Juan Miguel Redondo.



# EXTRACELLULAR MATRIX REMODELING IN THE CARDIOVASCULAR SYSTEM

# **Group members**

**Principal investigator:** Fernando Rodríguez Pascual

**Predoctoral fellows:**Marta Navarro Gutiérrez
Verónica Romero Albillo

**Technicians:** Raquel Jiménez Sánches

**Undergraduate and Master students:** Lucía Rosario Paredes Carbajo



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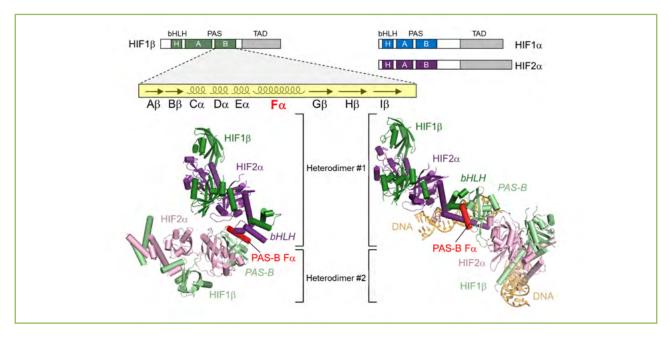


# **Summary**

The extracellular matrix (ECM) constitutes an intricate molecular network that surrounds and integrates cells and tissues in multicellular organisms. Traditionally considered a static mass, today it is widely recognized as a highly dynamic biomaterial that confers mechanical strength to tissues, provides attachment and movement points to cells, and is also essential for the intercellular communication. 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. In the last few years our group has investigated different aspects of this biosynthetic pathway. To this respect, we have focused our work in the study of the biology of lysyl oxidases (LOX), the enzymes responsible for the collagen cross-linking, an essential posttranslational modification that largely determines the biomechanical properties of the collagen.

Another interest of the laboratory has been the regulation by hypoxia of several collagen remodeling enzymes, including lysyl- and prolyl-hydroxylases. Here, we have described the molecular mechanism by which hypoxia through the hypoxia inducible factor (HIF) increases the expression of these matrix biosynthetic enzymes and how this contributes to the deposition of a stiff collagen matrix. Currently, the group explores the functional and molecular links between the stiffness of the matrix, the metabolism and the hypoxia in the development of matrix-related diseases such as the pulmonary fibrosis and other fibrotic disorders.





Model for the interaction between HIF heterodimers. HIF domain organization and schematic of the HIF1 $\beta$  PAS-B showing the position of the the F $\alpha$  helix in red. Crystallographic structures show two HIF2 $\alpha$ /HIF1 $\beta$  heterodimers alone (left) and bound to the HIF element (right) through the F $\alpha$  helix.



# List of publications

- Miguel V, Alcalde-Estévez E, Sirera B, Rodríguez-Pascual F, Lamas S. (2024) Metabolism and bioenergetics in the pathophysiology of organ fibrosis. Free Radical Biology and Medicine, 222: 85–105, DOI: 10.1016/j. freeradbiomed.2024.06.001.
- Rosell-Garcia T, Rivas-Muñoz S, Kin K, Romero-Albillo V, Alcaraz S, Fernandez-Tornero C, Rodriguez-Pascual F. (2023) Multimerization of HIF enhances transcription of target genes containing the hypoxia ancillary sequence. Biochimica et Biophysica Acta Gene Regulatory Mechanisms, 1866 (4), art. no. 194963, DOI: 10.1016/j. bbagrm.2023.194963.



#### Other activities

- Member of the Editorial Board (Molecular Biology Section) of the journal "Scientific Reports", Asia-Pacific Journal of Ophthalmology (Visual Sciences Section), Molecular Biology Reports (Extracellular Matrix Biology Section) and the International Journal of Molecular Sciences.
- Coordinator of the SEBBM group on Extracellular Matrix (2024).



# Research projects

- PID2022-136703OB-I00. Ministry of Science, Innovation, and Universities. "Impact of the extracellular matrix on the cellular biology and metabolic rewiring of organ fibrosis." PI: Fernando Rodríguez Pascual, Santiago Lamas. 09/01/2023-08/31/2027
- CPP2022-009582. Public-Private Partnership Projects of the Ministry of Science, Innovation, and Universities. "Evaluation of M2R therapy in an experimental model of SDRA". Coordinator: Pablo García de la Riva. PI CSIC: Fernando Rodríguez Pascual, Santiago Lamas. 2023-2026
- S2022/BMD-7221 (INNOREN-CM). Biomedical Projects of the Community of Madrid. New diagnostic and therapeutic strategies in chronic kidney disease. PI and coordinator: Fernando Rodríguez Pascual. 01/01/2023-31/12/2026.



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

# **Group Members**

**Principal investigator:** María Yáñez-Mó

**Postdoctoral fellows:** Fernando Gutiérrez del Burgo

Predoctoral fellows: Miguel Palma Cobo Joaquín Morales Beatriz Benayas Víctor Toribio

**Technicians:** Soraya López-Martín

**Visiting scientists:** Celeste Rodríguez



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



#### Summary

Our research team is dedicated to advancing the understanding of tetraspanin-enriched nanodomains, specialized membrane structures crucial in cell adhesion and migration processes. In addition, tetraspanin proteins are highly prevalent on extracellular vesicles (EVs), shuttles for bioactive proteins, lipids, and RNAs, unveiling novel pathways for intercellular communication.

Our investigations aim to unravel the intricate mechanisms underlying EV biogenesis and their subsequent uptake by recipient cells. Within our group we also pioneer innovative approaches for EV isolation, detection and quantification for their use in liquid biopsy in personalized medicine. Moreover, we are at the forefront of engineering synthetic exosome mimetics, harnessing tetraspanin-based tools as therapeutic tools to replicate and enhance natural cellular processes.



# Other activities

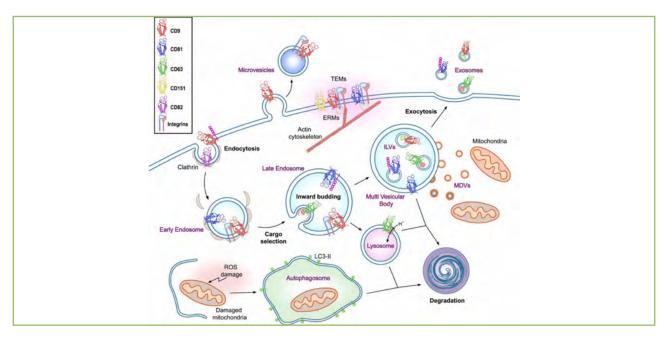
- President of the non-profit association "Grupo español de innovación e investigación en vesículas extracelulares GEIVEX".
- Secretary of the Instituto Universitario de Biología Molecular (IUBM)
- Member of the organizing committee of the GEIVEX workshop "Clinical transfer of EV based therapeutic Products" Badalona June 2023.
- Organizer of the 1st European MOVE meeting on EVs. Malaga October 2023.
- Member of the Scientific committee of the 7th International GEIVEX Symposium 2024 (Palma de Mallorca October 2024)



# **Doctoral theses**

- Víctor Toribio 28/03/2023 "El papel de las tetraspaninas en la relación del aparato endosomal con el metabolismo celular y la identificación de nuevos mecanismos moleculares implicados en la captación de vesículas extracelulares". Biociencias Moleculares. UAM. Directed by María Yáñez-Mo
- Beatriz Benayas 19/04/2024 "Desarrollo y optimización de métodos cromatográficos para el aislamiento de vesículas extracelulares y su uso en biopsia líquida" Biociencias Moleculares. UAM. Directed by María Yáñez-Mo and Pilar Armisén.





Tetraspanins establish microdomains in the membrane, they regulate the endocytosis of many membrane receptors into Early Endosomes that mature into Late Endosomes, and their secretion Microvesicles and Exosomes.



# List of publications

- Benayas B, Morales J, Egea C, Armisén P, Yáñez-Mó M. (2023) Optimization of extracellular vesicle isolation and their separation from lipoproteins by size exclusion chromatography. J Extracell Biol. Jul 8;2(7):e100. doi: 10.1002/jex2.100.
- Benayas B, Morales J, Gori A, Strada A, Gagni P, Frigerio R, Egea C, Armisén P, Cretich M, Yáñez-Mó M. (2023) Proof of concept of using a membrane-sensing peptide for sEVs affinity-based isolation. Front Bioeng Biotechnol. Aug 11;11:1238898. doi: 10.3389/fbioe.2023.1238898.
- Toribio V, Yáñez-Mó M. (2023) Two Complementary Strategies to Quantitate Extracellular Vesicle Uptake Using Bioluminescence and Non-Lipidic Dyes. Methods Mol Biol. 2668:133-144. doi: 10.1007/978-1-0716-3203-1\_11.
- López-Guerrero JA, Valés-Gómez M, Borrás FE, Falcón-Pérez JM, Vicent MJ, Yáñez-Mó M. (2023) Standardising the preanalytical reporting of biospecimens to improve reproducibility in extracellular vesicle research - A GEIVEX study. J Extracell Biol. Mar 31;2(4):e76. doi: 10.1002/jex2.76.
- Welsh JA, et al., (2024) Minimal information for studies of extracellular vesicles (MISEV2023): From basic to advanced approaches. J Extracell Vesicles. 2024 Feb;13(2):e12404. doi: 10.1002/jev2.12404.



# Research projects

- PDC2021-121052-I00, Vaccines based on exosome mimetics. María Yáñez-Mó. 12/01/2021-11/30/2024.
- PID2020-119627GB-I00, Ministry of Science and Innovation. R&D Projects Knowledge Generation. Membrane microdomains, exosomes, viruses, and vaccines. María Yáñez-Mó. 09/01/2021-11/30/2024.
- DTS21/00134, Carlos III Health Institute. Clinical validation of a system for detecting ALK translocations in extracellular vesicles in plasma. María Yáñez-Mó. 01/01/2022-31/12/2024.
- Principal Investigator at Instituto de Investigación Sanitaria la Princesa.
- Group involved in the Spanish Thematic Network on the interaction between cellular metabolism and the microenvironment in homeostasis and diseases. RED2022-134927-T, Ministry of Science, Innovation, and Universities. Coordinator: F Martín-Belmonte. (CSIC). June 1, 2023-May 31, 2025.



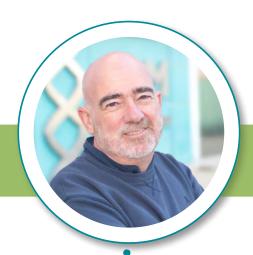
# **Patents**

■ The large extracellular loop (LEL) of CD9 as an immunogenic adjuvant in lipid nanovesicles Soto Alvarez; Yáñez Mó. EP23382365.7. Spain. 19/04/2023. Universidad Autónoma de Madrid.



# Tissue and organ homeostasis

# Cell architecture and organogenesis unit



# Miguel Manzanares

The **Cell Architecture and Organogenesis Unit**, part of the **Tissue and Organ Homeostasis Program**, brings together fourteen research groups dedicated to unravel how cells function as dynamic information hubs. By controlling their shape, polarity, gene expression, and protein activity, cells coordinate to organize themselves in complex tissues and organs through cooperative and highly regulated mechanisms.

Our Unit focuses on understanding how cellular architecture and function are established, maintained, and adapted to sustain tissue homeostasis. At the same time, we investigate how these processes are reprogrammed during development, regeneration or disrupted in pathological contexts. Central to our research is the temporal and spatial orchestration of cell compartmentation, gene expression and protein function.

The Unit integrates diverse expertise across its research groups, combining cutting-edge approaches in **cell biology**, **developmental biology**, and **genomewide studies**. Utilizing model organisms such as flies, worms, zebrafish and mice we explore how cells govern morphology, gene expression and protein networks in specific developmental and physiological contexts. Also, an emerging theme in the Unit is the use of cell-derived tridimensional organoid models, such as brain organoids or gastruloids, which provide powerful platforms to study organogenesis and tissue architecture in vitro.

A highlight of these two-year period has been the incorporation of **three new research groups**: two Junior groups carrying out groundbreaking research on transcriptional regulation in brain cortical organoids, and on stem cells and placenta biology, and a senior group researching novel functions of caspase enzymes.



In this time, the groups of the Unit have achieved important discoveries in diverse areas, such as the biology of formins, immunometabolism of B, or placental development (Álvarez-Sánchez et al, Cell Mol Life Sci 2024). The leading position of the Unit in the field of developmental biology is shown by a string of novel findings on topics such as excretory function, transcriptional regulation and nuclear activity, or cell-to-cell signaling and morphogenesis in Drosophila. Furthermore, unit members have produced insightful reviews on emerging topics in the field such as eye development and its relationship to congenital defects in humans, or the role of chromatin architecture in gene expression. Also, Unit members have been part of major collaborative efforts in different disciplines such as image analysis of cell shape, metabolic control of muscle function, or lineage plasticity of pluripotent stem cells.

These accomplishments underscore the Unit's dynamic role at the forefront of research into how cells shape tissues and organs, balancing fundamentalbiological discovery with translational potential. Looking ahead, the Cell Architecture and Organogenesis Unit remains committed to advancing our understanding of cellular organization principles, organ development, and tissue integrity, while fostering innovation through collaboration and technological integration.

# Research groups

# Miguel Ángel Alonso Isabel Correas Hornero

Cell polarity

#### Luis Alberto Baena

Molecular characterisation of caspase biology

# Leonardo Beccari

Transcriptional regulation of the development of the cerebral cortex

# Paola Bovolenta

Morphogenesis and neurodegeneration of the vertebrate Central Nervous System

# **Carlos Estella**

Antonio Baonza

Cellular response to stress and morphogenesis

#### José Félix de Celis

Cell signalling during imaginal development in *Drosophila* 

# **Miguel Manzanares**

Functional genomics

#### Fernando Martín Belmonte

Intestinal morphogenesis and homeostasis

#### **Nuria Martínez Martín**

Metabolism and B cell function

# **David Míguez**

Biophysics and systems biology

#### Vicente Pérez García

Placenta biology

# Mar Ruiz Gómez Joaquim Culí

Genetic and functional analysis of the renal filtration diaphragm in health and disease

#### **Ernesto Sánchez-Herrero**

Segmental specification and pattern formation in *Drosophila* 

#### **Esther Serrano**

Transcriptional control of sexual differentiation of the nervous system



# **CELL POLARITY**

# **Group members**

**Principal investigator:**Miguel Angel Alonso Lebrero
Isabel Correas Hornero

**Postdoctoral fellows:** Leticia Labat de Hoz

**Technicians:** Laura Fernández Martín





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



Formins are an evolutionarily conserved family of proteins that catalyze the assembly of linear actin filaments. Most formins are maintained in an inactive statethroughanintramolecularinteractionbetweentwo key domains: the diaphanous inhibitory domain (DID) at the N-terminus and the diaphanous autoregulatory domain (DAD) at the C-terminus. Typically, activation requires dissociation of this inhibitory interaction, allowing the formin to catalyze actin assembly. Formins play essential roles in numerous cellular processes, such as mitosis, cytokinesis, cell adhesion and migration, cell polarity, membrane trafficking, and cell and tissue morphogenesis.

INF2 is a formin of particular clinical significance due to its genetic linkage to two degenerative inherited conditions in humans: focal segmental glomerulosclerosis (FSGS), characterized by podocyte loss and impaired renal filtration that may lead to kidney failure, and Charcot-Marie-Tooth disease (CMTD), which affects the peripheral nerves and can result in distal muscle weakness.

Over the past two years, the primary objectives of our laboratory have been:

- 1.To analyze the structural impact of pathogenic mutations on the INF2 DID.
- 2. To elucidate the structure and function of the DAD of INF2.
- 3. To investigate the structure and functional role of the short N-terminal extension preceding the DID in INF2.

4.To determine the cellular mechanisms by which pathogenic INF2 variants cause deleterious effects, particularly in renal cells.

Our findings indicate that the DID of INF2 is particularly prone to destabilization by pathogenic mutations. By using computational tools such as AlphaMissense, we predicted the pathogenicity of these mutations. Additionally, our study of the DAD revealed that specific structural elements within this domain are crucial for actin binding and the normal function of INF2. Notably, we discovered that calmodulin binds to a short N-terminal extension, which is absent in other formins, and that this interaction activates INF2. This activation mechanism is distinct from that of most formins, where activation is mediated by the binding of a specific Rho GTPase to the DID. Finally, we observed that pathogenic INF2 variants lead to deregulated actin polymerization, constitutive activation of the MRTF-SRF transcriptional complex, multipolar spindle formation, and nuclear aberrations. These cellular dysfunctions provide a mechanistic understanding of how these mutations lead to mitotic catastrophe and cell death, contributing disease progression, particularly in FSGS. In conclusion, our research has unveiled critical insights into INF2 regulation, its structural organization, and the pathological consequences of its deregulation. These findings offer crucial insights into the molecular basis of podocyte loss during INF2-linked FSGS progression and suggest potential therapeutic strategies to mitigate its effects.



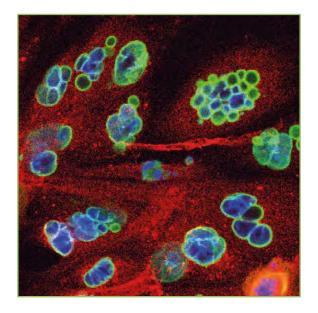


Image of MDCK cells expressing the pathogenic INF2 R218Q variant, showing distinct nuclear aberrations



# List of publications

- Labat-de-Hoz, L., Rubio-Ramos, A., Correas, I. and Alonso, M.A. (2023) The MAL family of proteins: normal function, expression in cancer, and potential use as cancer biomarkers. Cancers 15, 2801. doi: 10.3390/cancers15102801
- Cacho-Navas, C., López-Pujante, C., Reglero-Real, N., Colás-Algora, N., Cuervo, A., Conesa, J. J., Barroso, S., de Rivas, G., Ciordia, S., Paradela, A., D'Agostino, G., Manzo, C., Feito, J., Andrés, G., Molina-Jiménez, F., Majano, P., Correas, I., Carazo, J.M., Nourshargh, S., Huch, M. and Millán, J.(2024) ICAM-1 nanoclusters regulate hepatic epithelial cell polarity by leukocyte adhesion-independent control of apical actomyosin. eLife 12:RP89261. doi: 10.7554/eLife.89261.3
- Labat-de-Hoz, L., Fernández-Martín, L.., Correas, I. and Alonso, M.A. (2024) INF2 formin variants linked to human inherited kidney disease reprogram the transcriptome, causing mitotic chaos and cell death. Cell Mol Life Sci. 25, 279. doi: 10.1007/s00018-024-05323-y
- Labat-de-Hoz, L., Jiménez, M.A, Correas, I. and Alonso, M.A. (2024) Regulation of formin INF2 and its alteration in INF2-linked inherited disorders. Cell Mol Life Sci. 81, 463. doi: 10.1007/s00018-024-05499-3



# Research projects

■ PID2021-123179NB-I00. MCIN/AEI/10.13039/501100011033 and FEDER. Regulation of normal and pathogenic INF2 formin. Miguel A. Alonso. 2022-2025.



# MOLECULAR CHARACTERISATION OF CASPASE BIOLOGY

# **Group members**

**Principal investigator:** Luis Alberto Baena Lopez

**Predoctoral fellows:** Mateo Margareto

**Technicians:**Raquel Marsino

Undergraduate and Master students:

Samuel Navarrete



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



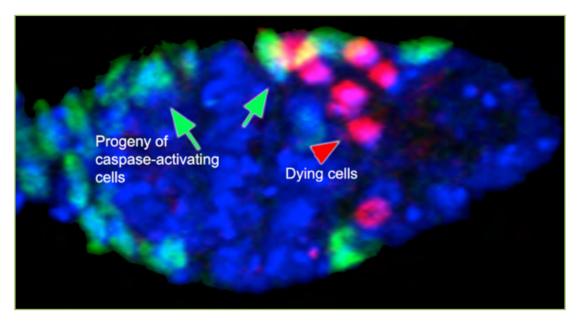
#### Summary

The family of highly conserved enzymes known as caspases has traditionally been studied for their central role in the regulation and execution apoptosis, the programmed cell death process essential for tissue homeostasis and development. However, accumulating evidence over the past decade has dramatically reshaped our understanding of these proteases. Caspases have now been implicated in the regulation of numerous vital cellular processes beyond cell death, including cell proliferation, differentiation, migration, and the maintenance of proteostasis. These non-apoptotic functions are critical for proper cell physiology, tissue integrity, and organismal development. Dysregulation of caspase activity—whether due to defects in apoptotic pathways or in the control of these non-lethal functions—has been increasingly linked to the pathogenesis of a wide array of human diseases, ranging from neurodegenerative disorders and developmental syndromes to cancer and immune system dysfunctions.

Our research group is deeply focused on uncovering the mechanisms that govern caspase activation, substrate specificity, and molecular function in both physiological and pathological settings. To address these questions, we employ a multidisciplinary approach that integrates genetic models in Drosophila and mammalian cells, advanced imaging and microscopy platforms, genome editing technologies such as CRISPR/Cas9, as well as molecular biology and biochemical methods. By combining these powerful tools, we aim to dissect the intricate regulatory networks in which caspases operate, and to define how perturbations in these networks contribute to disease. We anticipate that the insights gained from our work will not only advance fundamental biological knowledge about caspase biology but will also pave the way for novel therapeutic strategies targeting caspasedependent pathways in human disease.

My group was incorporated into the CBM in May 2024, following Luis Alberto Baena López's appointment to a permanent position at the CSIC as Investigador Científico.





Drosophila germarium showing somatic cells and their progeny (green), which, despite transient caspase activation, continue to proliferate and contribute to the tissue. These cells do not colocalize with apoptotic cells labeled by TUNEL (red). Nuclear DNA is counterstained with DAPI (blue).



# List of publications

- The Initiator Caspase Dronc Drives Compensatory Proliferation of Apoptosis-Resistant Cells During Epithelial Tissue Regeneration After Ionizing Radiation. Braun T, Afgin N, Sapozhnikov L, Sivan E, Bergmann A, Baena-Lopez LA, Yacobi-Sharon K, Arama E. bioRxiv [Preprint]. 2024 Sep 2:2024.09.01.610661. doi: 10.1101/2024.09.01.610661. PMID: 39282309.
- Oxidative stress-induced changes in the transcriptomic profile of extracellular vesicles. Dellar ER, Hill C, Carter DRF, Baena-Lopez LA. J Extracell Biol. 2024 Apr 21;3(4):e150. doi: 10.1002/jex2.150. eCollection 2024 Apr. PMID: 38938847
- Cellular stress management by caspases.
   Baena-Lopez LA, Wang L, Wendler F.Curr Opin Cell Biol.
   2024 Feb;86:102314. doi: 10.1016/j.ceb.2023.102314. Epub
   2024 Jan 11.PMID: 38215516
- Kinetic insights into agonist-dependent signalling bias at the pro-inflammatory G-protein coupled receptor GPR84. Luscombe VB, Baena-López LA, Bataille CJR, Russell AJ, Greaves DR.Eur J Pharmacol. 2023 Oct 5;956:175960. doi: 10.1016/j.ejphar.2023.175960. Epub 2023 Aug 3. PMID: 37543157
- Generation of LexA enhancer-trap lines in Drosophila by an international scholastic network.
  Kim ES, et. al. G3 (Bethesda). 2023 Aug 30;13(9):jkad124. doi: 10.1093/g3journal/jkad124.
  PMID: 37279923

- Non-apoptotic caspase activation ensures the homeostasis of ovarian somatic stem cells. Galasso A, Xu DC, Hill C, Iakovleva D, Stefana MI, Baena-Lopez LA. EMBO Rep. 2023 Jun 5;24(6):e51716. doi: 10.15252/embr.202051716. Epub 2023 Apr 11. PMID: 37039000
- Editorial: Regulation and coordination of the different DNA damage responses and their role in tissue homeostasis maintenance.Baena-LópezLA,BaonzaA,EstellaC,HerranzH. Front Cell Dev Biol. 2023 Mar 13;11:1175155. doi: 10.3389/fcell.2023.1175155. eCollection 2023. PMID: 36994105



# Research projects

# The Research of the laboratory has been supported during this time interval by:

- Agencia Estatal de Investigación PID2023-150773NB-I00
- (MCIN/AEI/10.13039/501100011033)
- Cancer Research UK
- Cancer Research UK (C49979/A17516)
- The John Fell Fund (162/001)
- The Edward Penley Abraham Research Funds (RF290, RF286(19))



# Other activities

■ Co-organiser of the 3rd International conference dedicated to discuss the non lethal roles of cell death related proteins. Canada, September 9-12th, 2024. https://www.non-lethal-roles.org/



# TRANSCRIPTIONAL REGULATION OF BRAIN CORTEX DEVELOPMENT

# **Group members**

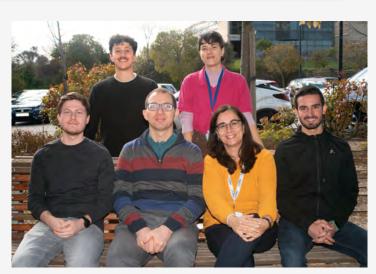
**Principal Investigator:** Leonardo Beccari

Predoctoral fellows: Eva Pajda Szeligowska (co-supervisor IP: Paola Bovolenta) Jorge Mañes García Daniel Felipe Cordero

**Technicians:** Raquel Marco Ferreres

Undergraduate and Master students: Jorge Mañes García Mai Abdelgawad Daniel Felipe Cordero Diego Martin Sanz Matilde Lombardía Ramil Li Ying Chen Chen

Visiting scientists: Alexandra Arcos-Ortiz





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



The cerebral cortex is a highly complex structure responsible for sensory, motor, emotional, and cognitive functions. Its different neuronal types are generated via a process called neurogenesis, controlled by developmental genes expressed dynamically and precisely in time and space.

Our research investigates the transcriptional mechanisms controlling neurodevelopmental gene expression and their role in pathological brain conditions, using human brain organoids derived from iPSCs as model system, thus overcoming the limitations related with the evolutionary divergence of the human non-coding genome with that of classical animal model systems. We currently focus on two main research areas:

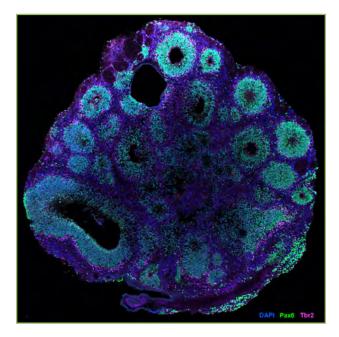
1) Cis-regulatory code and 3D chromatin organization in gene regulation. Gene transcription depends on Cis-Regulatory Elements (CREs) that can act over long distances and fall within Topologically Associated Domains (TADs). Disruptions in CRE activity or TAD organization can severely affect brain development. Over 50.000 CREs are active in the human embryonic cortex and mutations affecting these CREs or altering TAD organization are associated with brain alterations/disorders. For example, the human TBR2 gene encodesa transcription factor playing essential roles in cortical neurogenesis and a large translocation disrupting the TAD organization of its genomic region has been identified in patients with severe brain malformations. We combine the use of human cortical organoids with an enhancer

transgenesis assay methodology that we developed and with CRISPR genome editing and the analysis of chromatin conformational and epigenetic profiles to characterize the *TBR2* cis- regulatory code and understand how structural changes in its regulatory domains impact its expression and cortical neurogenesis.

2) Epigenetic mechanisms in neurodevelopmental gene expression. Epigenetically regulation through histone modifications and nucleosome remodeling controls the access of transcription factor and transcriptional machinery to gene regulatory sequences and allows the inheritance of cell transcriptional states. We investigate the epigenetic mechanisms that contribute to the regulation of human cortical neurogenesis process and how their alteration lead to brain pathological conditions.

We study the CHD2 gene, a chromatin remodeler whose mutations are linked to epilepsy, autism, cognitive impairment, and microcephaly. In fact, CHD2 is the most frequently mutated epigenetic modifier in human epileptic disorders. We combine CRISPR genome editing and human brain organoid cultures with the next-generation sequencing of chromatin epigenetic and transcriptional profiles to study (i) the molecular mechanisms by which CHD2 contributes to the generation of glutamatergic and gabaergic cortical neurons, and (ii) how mutations in this gene impact cortex development leading to the variety of pathological phenotypes observed in patients.





Brain cortical organoid (40 days post aggregation) immunostained for PAX6 and TBR2, marking two main populations of cortical neural progenitors



# List of publications

- Tejedor J.R., Soriano-Sexto A., Beccari L., Castejón-Fernández N.; Correcher P.; Sainz-Ledo L.; Alba-Linares J.J., Urdinguio R.G., Ugarte M; Fernández A.F.; Rodríguez-Pombo P., Fraga M.F., Pérez B.. Integration of multi-omics layers empowers precision diagnosis through unveiling pathogenic mechanisms on Maple Syrup Urine Disease. J Inherit Metab Dis 2025 Jan;48(1):e12829. doi: 10.1002/jimd.12829. Epub 2024 Dec 10.
- Mañes-Garcia, J., Beccari, L Torres, M., Ocaña, O.H. An Efficient Transgenesis Approach for Gene Delivery in the Mouse Embryonic Heart. J Vis Exp. 2024 May 24:(207).doi: 10.3791/66754.
- Mañes-Garcia, J., Marco-Ferreres, R., Beccari, L.# Shaping gene expression and its evolution by chromatin architeture and enhancer activity.. Curr Top Dev Biol. 2024;159:406–437. http://doi: 10.1016/bs.ctdb.2024.01.001.
- Cheron, J., Beccari, L., Hagué, P., Icick, R., Despontin, C., Carusone, T., Defrance, M., Bhogaraju, S., Martin-Garcia, E., Capellan, R., Maldonado, R., Vorspan, F., Bonnefont, J., de Kerchove d'Exaerde, A., 2023. USP7/Maged1-mediated H2A monoubiquitination in the paraventricular thalamus: an epigenetic mechanism involved in cocaine use disorder. Nat Commun 14, 8481. https://doi.org/10.1038/s41467-023-44120-2



# Research projects

- CNS2023-144348. MCIN Grants to Encourage Research Consolidation. Title: Organoid-based analysis of the role of the chromatin remodeler CHD2 in the development of the human cerebral cortex. PI: L. Beccari (2023-2025)
- 20222AT019. MCIN/AEI Talent Attraction Program. Title: Use of brain organoids to study the transcriptional regulation of neurodevelopmental genes. PI: L. Beccari (2022-2025)
- PID2021-127859NA-I00. MCIN Knowledge Generation Project. Title: Use of brain organoids for the analysis of the transcriptional regulation of genes involved in the development of the human embryonic cortex. PI: L. Beccari (2022-2026)



# Other activities

- Several members our group participate in outreach activities, i.e. Semana de la Ciencia 2023-2024, 4°ESO+Empresa 2023-2024, Científic@s en Prácticas 2023-2024.
- Organization of the XII Cajal Conference "Sex and epigenetics perspectives in mental disorders", together with Esther Serrano Sainz and Claudio Toma. Saint Felieu de Guixols (Girona). 22-24 May 2024.



# MORPHOGENESIS AND NEURODEGENERATION OF THE VERTEBRATE CENTRAL NERVOUS SYSTEM

# **Group members**

**Principal investigator:**Paola Bovolenta

Postdoctoral fellows: Marcos Julián Cardozo Adrián Pérez Guadalupe Pereyra

**Predoctoral fellows:** 

Guadalupe Pereyra Pablo Miaja Marcos Martínez Elena Sánchez Eva Pajda

**Technicians:** 

M<sup>a</sup>Jesús Martín Noemí Tabanera Irene Grasa

Carmen Redondo

ozo ows: Undergraduate and Master students: Alejandro Albarrán Mikel Argote Alessandro Battilossi Íñigo Bravo Mónica Gallego Miguel Ángel Garitagoitia Isabel Montejano Jose Manuel Osorio Gabriel Serrano Nuria Soto Irene Cecilia Soto



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



# Summary

Our research focuses on understanding why certain defects occur during brain development and what causes the brain to age or degenerate. Finding answers to these fundamental questions is essential for developing strategies to prevent or treat a wide range of neurological disorders, that span from neurodevelopmental disorders to neurodegenerative conditions such as Alzheimer's disease (AD).

Among the areas we study, we are investigating neurodevelopmental visual disorders arising from defects in embryonic eye formation, which affect both visual and non-visual brain regions. We have examined and reviewed how different vertebrates shape the eye primordium, describing speciesspecific adaptations in the morphogenetic processes underlying optic cup formation and their relationship to the overall developmental timeline of each species. Our work focused on the causes of microphthalmia, anophthalmia, and coloboma (MAC), and we have developed iPSC-based eye organoids to study the roles of various transcriptional regulators in MAC formation.

An unexpected outcome of these studies was the identification of a novel molecular player involved in zebrafish gastrulation. Zebrafish gastrulation begins with the coordinated spreading of blastodermal cells, a process known as epiboly, which is driven by maternal transcripts. We found that maternal vgll4a is a crucial regulator of epiboly. Our research shows that vgll4a controls blastoderm cell adhesion, orchestrating tissue

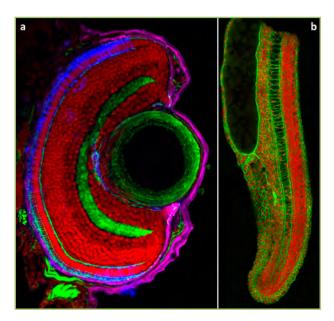
tension and contractility, which ensures proper epiboly progression. The spreading of blastodermal cells shares similarities with the formation of the zebrafish retinal pigment epithelium, raising the question of whether vgll4a plays a similar role in the eye.

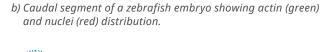
In the context of neurodegeneration, we followed up on our observation that SFRP1, a small secreted protein found in the brain matrisome is involved in AD pathogenesis. Using a transgenic mouse model that overexpresses Sfrp1 in astrocytes, we demonstrated that SFRP1 accumulation causes early dendritic and synaptic defects in adult mice. This is followed by impaired LTP and cognitive decline, which only become apparent as the animals age, mimicking the structuralfunctional temporal distinction observed in AD. This phenotype correlates with proteomic changes, including increased expression of structural synaptic proteins. Thus, excessive SFRP1 seems to impede synaptic protein turnover, reducing synaptic plasticity—a mechanism that may underlie the synaptopathy observed in the brains of prodromal AD patients.

Combined with our earlier studies showing that SFRP1 promotes neuroinflammation and A $\beta$  generation/aggregation, these findings position SFRP1 as a multifaceted contributor to AD pathogenesis and a promising therapeutic target. To advance in this direction, we are optimizing the brain delivery of a monoclonal antibody against SFRP1 to enhance its potential to delay AD progression.

a) Section of a zebrafish embryonic eye showing the distribution of Yap1 activity (magenta); the outer segment of the photoreceptors (blue); actin (green) and nuclei (red)







distribution.

# Other activities

- The PI acted as member of the "Consiglio di Sorveglianza" of the Human Technopole (Milan, Italy), of the EMBO Policy Advisory Board. She also acted as SAB member of the following organizations and institutes: ERA-NET Neuron, Bettencourt-Schueller and Gadea Foundations, Institut de Biologie de Dev de Marseille.
- The PI has been nominated "President elected of FENS" and received the condecoration of "Cavaliere dell' Ordine della Stella d' Italia".
- Several members our group participate in outreach activities, i.e. Semana de la Ciencia 2023-2024, 4°ESO+Empresa 2023-2024, Científic@s en prácticas 2023-2024.



# List of publications

#### Research articles:

- Cardozo MJ, Sanchez-Bustamante E and Bovolenta P (2023) Optic cup morphogenesis across species and related inborn human eye defects. Development, 150, dev200399.
- Pereyra Gand Bovolenta P. (2023) Of dolphins, humans, other long-lived animals and Alzheimer's disease (Commentary on Vacher et al.) Eur J. Neurosci. DOI:10.1111/ejn.15946.
- Camacho-Macorra C, Tabanera N, Sánchez-Bustamante E., Bovolenta P, Cardozo MJ (2024) Maternal vgll4a regulates zebrafish epiboly through Yap1 activity. Front Cell &Dev Biol.
- Pereyra G\*, Mateo MI\*, Martin-Bermejo MJ, Miaja P, Klaassen R, Gruart A, Rueda-Carrasco J, Fernandez-Rodrigo A, Lopez-Merino E, Esteve P, Esteban JA, Smit AB, Delgado-Garcia JM, Bovolenta P. (2024) SFRP1 upregulation causes hippocampal synaptic dysfunction and memory impairment. bioRxiv 2024.04.04.588100; doi: https://doi.org/10.1101/2024.04.04.588100

#### Books:

Marco-Ferreres, R, Padja E, and Bovolenta P. (2024) Morphogenesis and early patterning of the Eye. Encyclopedia of the Eye, Vol 2. Elsevier; p.69-75



#### **Patents**

■ Therapeutic target and monoclonal antibodies against it for the diagnosis and treatment of Alzheimer´s - Disease". Inventores: Bovolenta P, Esteve P, Rueda Carrasco J, Mateo Ruiz MI, Martin Bermejo MJ (CSIC); Dominguez Rodriguez, M; Moreno Iruela I. (ISCIII). Ref. WO2020/165453 A1-



# Research projects

- PID2022-136831OB-I00. AEI. Molecular and cellular mechanisms behind congenital defects and degenerative diseases of the CNS. PI: Paola Bovolenta. 2024-2026.
- Fundacion Tatiana. SFRP1 as a target in the fight against Alzheimer's disease. PI: Paola Bovolenta. 2023-2025.
- Brain4Sight. ERA-NET Neuron . Deconstructing gene regulatorynetworksforimprovingsightandbraindisabilities. Coordinator: Paola Bovolenta (4 grupos).2022-2024.
- RED2022-134100-T. AEI . ReDevNeural 4.0: An integrative approach to understand the logic of neural developmen. Project Coordinator: Paola Bovolenta (15 teams) 2023-2024.
- CIBERER-ACCI. Development of human 3D organoids to understand the differential impact of DYRK1A haploinsufficiency syndrome in the CNS. Mariona Arbones (Paola Bovolenta, PI partner) 2022-2023.
- CSIC Strategic Funds . CSIC Interdisciplinary Thematic Platform. PTI-NEURO-AGING+.PI: Paola Bovolenta. 2022-2023.
- PID2019-104186RB-100. Agencia Estatal de Investigacion. New approaches to understand prevalent and neurodegenerative diseases. PI:Paola Bovolenta. 2020-2023.



# Doctoral theses

Guadalupe Pereyra (2023). Upregulation of Sfrp1 expression causes hippocampal synaptic dysfunction and memory impairment. Facultad de Ciencias, Universidad Autónoma de Madrid.



# CELLULAR RESPONSE TO STRESS AND MORPHOGENESIS

# **Group members**

**Principal investigator:**Carlos Estella
Antonio Baonza

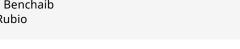
Predoctoral fellows: Gonzalo Garcia Girón César Castellanos Aguilar Inés Kelleher López

Technicians:

Maria Paloma Martín Fernandez

**Undergraduate and Master students:** 

Mario Ortí Muñoz Ibrahim Dris Cadiz Mariam Benchaib Carlos Rubio





https://www.cbm.uam.es/baonza-estella



#### Summary

To preserve tissue homeostasis and organ function, there are mechanisms that coordinate the elimination of defective cells with proliferative processes to replace them. This synchronization is crucial after damage, as it allows cell loss to be compensated by regeneration. Lack of coordination between these processes can lead to the formation of tumors or malformations.

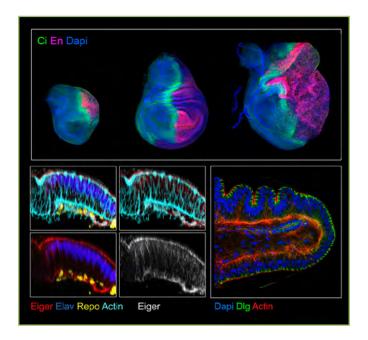
Our research group seeks to understand how cells respond to different types of stress and how the different activated responses are integrated and coordinated. We use Drosophila melanogaster as a model organism due to its remarkable genetic similarity to mammals and the wide availability of genetic tools, which has established it as one of the most powerful and versatile tools for the study of biological processes and human diseases.

# **Objectives and Results**

1. How are appendages formed and shaped? Similar to Origami, the folding of epithelia requires genetic instructions that determine in which specific areas these folds are to be produced. Likewise, it is necessary to generate mechanical compressive forces that assist epithelial folding. To understand how these two processes coordinate to give rise to the characteristic shape of organs we have used leg formation as a model. Our results show that cell proliferation generates compressive forces that contribute to tissue folding, while the Notch signaling pathway plays a critical role in the

- location of these epithelial folds. These results are validated with a mathematical model that predicts epithelial shape and cell behavior.
- 2. Coordination between cell proliferation and apoptosis during tissue homeostasis. When the mechanisms involved in coordinating the elimination of damaged cells and cell proliferation are altered, overgrowths and tumors can occur. We have analyzed the consequences of preventing apoptosis in Drosophila epithelial cells subjected to stress or genetic perturbations that normally induce high cell mortality. We found that in many of these cells the JNK signaling pathway is persistently activated, leading to a senescent state. This results in the induction of tumor overgrowth. In addition, we found that the p53 gene contributes to senescence by enhancing JNK activity.
- 3. Mechanisms of regeneration in the epithelial system. In response to tissue damage, a regenerative process involving increased cell proliferation is induced. The capacity to induce cell proliferation is lost in some tissues as organisms develop or age. Understanding what limits this capacity is critical to the development of new regenerative therapies. Drosophila wing imaginal discs are epithelial structures capable of regenerating after injury, but this ability is lost during development. Our work has revealed that this is because hormones that control the different phases of development can block the induction of proliferation that is activated after tissue damage.





Imaging discs stained with different proteins involved in regeneration, tissue homeostasis and morphogenesis.



#### List of publications

- Ahmed-de-Prado, S., Estella, C. & Baonza, (2024). A. Temporal dynamics of apoptosis-induced proliferation in pupal wing development: implications for regenerative ability. BMC Biol 22, 98. https://doi.org/10.1186/s12915-024-01894-1
- Alonso Rodríguez, David Foronda, Sergio Córdoba, Daniel Felipe-Cordero, Antonio Baonza, David G. Miguez, Carlos Estella. (2024). Cell proliferation and Notch signaling coordinate the formation of epithelial folds in the Drosophila leg. Development 15 April 2024; 151 (8): dev202384. doi: https://doi.org/10.1242/dev.202384
- Garcia-Arias, J.M., Pinal, N., Cristobal-Vargas, S. Estella, Carlos; Morata, Ginés. (2023) Lack of apoptosis leads to cellular senescence and tumorigenesis in Drosophila epithelial cells. Cell Death Discov. 9, 281 (2023). https://doi.org/10.1038/s41420-023-01583-y
- Baena-López, L. A., Baonza, A., Estella, C., & Herranz, H. (2023). Editorial: Regulation and coordination of the different DNA damage responses and their role in tissue homeostasis maintenance. Frontiers in cell and developmental biology, 11, 1175155. https://doi.org/10.3389/fcell.2023.1175155



#### Research projects

■ PID2021-127114NB-I00. Ministry of Science, Innovation, and Universities. Study of the gene regulation network induced by DNA damage and its coordination in different cellular contexts. Principal Investigators: Carlos Estella and Antonio Baonza. From 2022 to 2025.



#### Other activities

#### Carlos Estella:

- Scientific organizer of the Drosophila Spain Meeting. (March 14-16, 2024). Alicante, Spain.
- Moderator of the session "Morphogenesis & Organogenesis" at the 27th European Drosophila Research Conference. October 2023. Lyon, France.
- Member of the Board of Directors and Treasurer of the Spanish Society of Developmental Biology.
- Member of the CSIC Conexión VIDA network and the Conexión GENOMA network.
- Honorary Professor in the Department of Molecular Biology at the Faculty of Sciences of the Autonomous University of Madrid.
- Associate editor in the "Morphogenesis and patterning" and "Cell death and survival" sections of Frontiers Cell and Developmental Biology.

#### Antonio Baonza:

Associate editor of Heliyon magazine, Cell Press.



#### CELL SIGNALLING DURING IMAGINAL DEVELOPMENT IN DROSOPHILA

#### **Group members**

**Principal investigator:** losé Félix de Celis

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

**Predoctoral fellows:** Patricia Vega Cuesta Sandra Herrera Alarcón

**Technicians:** Ana López Varea

**Undergraduate and Master students:** Paula Sanchez de Rojas Raquel Jiménez Bonastre

Carla López Serrano Irene Arias Peralta





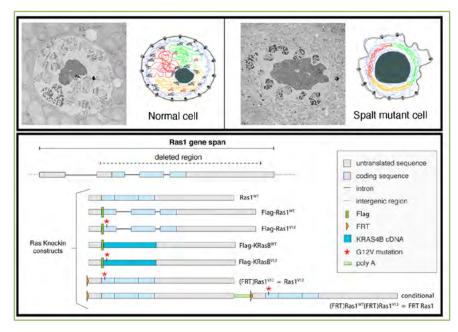


#### Summary

We are analyzing one *Drosophila* protein (Spalt) that has prominent roles during the development of the wing disc epithelium. Spalt is a nuclear protein containing three pairs of Zn fingers, and its human orthologs are involved in Towles-Broke and Okihiro diseases. We identified a DNA response element for Spalt through the analysis of one of its downstream genes (Ostalé et al., 2023). We also identified novel roles of this protein in the development of the prothoracic gland, the endocrine organ responsible for the synthesis of the steroid hormone ecdysone. In this tissue, Spalt is required for chromatin condensation, nuclear membrane stiffness and for the normal operation of the nuclear pore complex. Loss of Spalt prevents nuclear import of phosphorylated ERK, and perturbs the feedback loops that reinforce ERK phosphorylation and trigger the transcription of the enzymes that convert dietary cholesterol into ecdysone (Ostalé et al., 2024a). We also identified a novel requirement for Spalt in the formation/maintenance of heterochromatic regions. We found that faulty heterochromatin formation leads to DNA damage, and trigger DNA damage responses leading to cells stopping in the G2 phase of the cell cycle or entering apoptosis (Ostalé et al., 2024b). All together the analysis of Spalt function suggests that this protein can act both as a canonical transcription factor by direct binding to specific target genes, and as a heterochromatin protein promoting gene silencing by epigenetic

mechanisms. Our second project concerns the Drosophila Ras1 gene. Mutations in human Ras genes (H-Ras, K-Ras and N-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 generated *Drosophila* transgenic lines carrying altered versions of the fly and human Ras genes in the Ras1 locus. We found that expression at normal levels of Ras1V12 or KRasBV12 leads to female sterility, lymphatic tumor formation and a variety of other phenotypes normally caused by increased activity of the RAS/RAF/ERK pathway. We have also found that Ras activating mutations cannot be considered as constitutive. Thus, the function of several Ras upstream elements, such as EGFR or the Guanine nucleotide exchange factor SOS, is required to promote pathway activation in a Ras1V12 background (P. Vega, A. Ruiz and J. F. de Celis in preparation). Finally, we are starting a novel experimental research line to promote the use of Drosophila in cellular toxicology studies. So far, we have been able to show that nano enabled food additives E171 and E551 lead to oxidative stress in Drosophila intestines (Peropadre et al., 2023), and that the fly could be a convenient system to identify and characterize molecules causing endocrine disruption when ingested in the diet (Vega-Cuesta et al., 2024).





Top: Normal and mutant spalt nuclei. Chromosomes (red, green and yellow), nucleolus, nuclear envelope and NPC are indicated. Bottom: Chromosomal region deleted in Ras1 and replaced by activated human KRasB and Drosophila Ras1 (Ras Knockin constructs) in transgenic lines.



#### List of publications

- Ostalé, C. M., Vega-Cuesta, P., González, T., López-Varea, A. and de Celis, J.F.. (2023). RNAi screen in the Drosophila wing of genes encoding proteins related to cytoskeleton organization and cell division. Dev. Biol. 498, 61-76. doi: https://doi.org/10.1016/j.ydbio.2023.03.010
- Peropadre, A., Vega-Cuesta, P., Fernández Freire, P., Pulido, D., Carriere, M. and de Celis, J.F. (2023). Complementary in vitro and in vivo strategies to assess the biological effects of nano enabled food additives E171 and E551. Environmental Science: Nano 10, 1394 1412. DOI: 10.1039/d3en00009e.
- Ostale, C. M., del Prado, A., Esteban, N., Lopez-Varea, A. and de Celis, J.F. (2023). A function of Spalt major as a sequence-specific DNA binding transcription factor mediates repression of knirps in the Drosophila wing imaginal disc. Dev. Biol. 510: 40-49. doi:https://doi.org/10.1016/j.ydbio.2024.03.004

- Cristina M. Ostale, Diego Pulido, Ana Lopez-Varea and Jose F. de Celis (2024a). Developmental analysis of Spalt function in the Drosophila prothorazic gland. Development 151 (16), dev202751. doi: https://doi.org/10.1242/dev.202751
- Ostalé, C. M. Azpiazu, N., Peropadre, A., Martín, M., Ruiz-Losada, M., López-Varea, A., Viales, R. B., Girardot, C., Furlong, E.E.M. and de Celis, J.F. (2024b). A function of Spalt proteins in heterochromatin organization and maintenance of genomic DNA integrity. BioRxiv doi: https://doi.org/10.1101/2024.04.30.591908.
- Vega-Cuesta, P., Pulido, D., Abia, D., Herrera, S., Lopez-Varea, A., Ruiz-Gómez, A., Fernández-Freire, P., Peropadre, A. and de Celis, J.F. (2024). A Drosophila ecdysone-deficient model to identify endocrine disruptors. SSRN. doi: http://dx.doi.org/10.2139/ssrn.4835387



#### Research projects

■ PID2022-141894OB-C21. Ministry of Science and Innovation. Biological tests in *Drosophila* to evaluate the activity of stress response pathways as indicators of toxic effects of food additives and contaminants. Jose F. de Celis. January 2024-December 2026



#### **Doctoral theses**

- Cristina Martínez Ostalé (2023). Regulación transcripcional mediada por los factores de transcripción Spalt.
   Universidad: Departamento de Biología Molecular.
   Universidad Autónoma de Madrid. Jose F. de Celis.
- Patricia Vega Cuesta (2024). Generación y caracterización de alelos mutantes Ras1V12 en locus Ras1 de Drosophila. Departamento de Biología Molecular. Universidad Autónoma de Madrid. Jose F. de Celis and Ana Ruiz Gómez.



#### **FUNCTIONAL GENOMICS**

#### **Group members**

**Principal investigator:** Miguel Manzanares

**Postdoctoral fellows:** Maria Tiana

Elva Martin-Batista

**Predoctoral fellows:** 

Antonio Barral Marta Portela

Aurora Arroyo

**Technicians:** Andrea Maidana

Undergraduate and Master students:

Jazmin Zamora

**Visiting scientists:** 

Camila Quezada



https://www.cbm.uam.es/functional-genomics



#### Summary

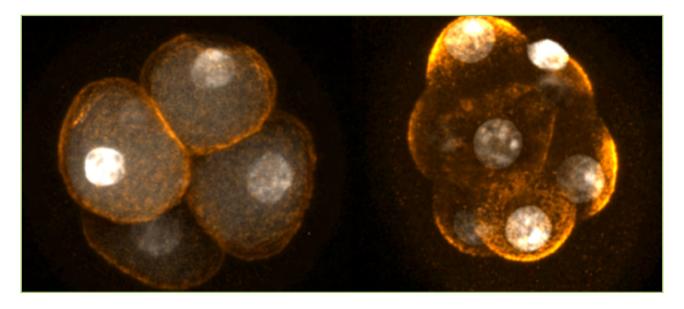
The present and future research objectives of our group are to understand, on the one hand, how genome activity is regulated during the early stages of mammalian development, and on the other, how regulatory and structural variations of the genome can contribute to human disease. To do this, we search for and identify cis regulatory sequences, and we study how they act on their target genes, how they are organized along the 3D structure of chromatin and their role in the gene regulatory networks that underlie a specific biological state. Methodologically, we used a combination of bioinformatics and data analysis tools, structural genomics, genome-wide analysis, gene editing using the CRISPR/Cas9 system, and functional assays in transgenic animals and pluripotent stem cells.

We explore this field using a model in which we have demonstrated our experience and to which we have made relevant contributions: the early stages of embryonic development in mammals. We are experts in the study of the initial phases of mouse development from one cell to the blastocyst of barely a hundred. This is an ideal model, where the combination of genetic, pharmacological, and experimental embryology tools allow analyzing key processes and questions, such as the appearance

of the first differences between cell types that occur in the embryo. In addition, we are pioneers in developing omics approaches where we can interrogate gene expression or chromatin structure in individual mouse embryos of just a dozen cells. We extend these studies in the embryo with the use of pluripotent stem cells, as a complementary system in culture in which we have extensive experience. Also, we will fine-tune the use of gastruloids, embryonic organoids, as an experimental system.

In addition, we want to understand how the progressive loss of differentiation capacity of the embryo cells occurs, from the totipotent state present in the zygote to the gastrulation phases of the embryo. We hypothesize that this knowledge will also allow us to reprogram gene networks in adults in order to initiate regeneration processes in situations of tissue damage. The future of research in our group will be based on the achievements and objectives set out above, with the ultimate goal of answering a basic question: how does the genome act in a coordinated way to give rise to differences between cell types, stages of development, organisms, or disease states.





2-cell (left) and 4-cell (right) mouse embryos, showing the nucleus (white) and Ezrin (orange), a membrane protein, that is enriched in the outside of 4- but not 2-cell embryos.



#### List of publications

- Gimenez-Llorente D, Portela M, Losada A, Manzanares M, Andreu MJ. (2023) A protocol to study three-dimensional genome structure in individual mutant preimplantation mouse embryos. STAR Protoc 4, 102267. doi: 10.1016/j. xpro.2023.102267.
- Ealo T, Sanchez-Gaya V, Respuela P, Muñoz-San Martín M, Martin-Batista E, Haro E, Rada-Iglesias A. (2024) Cooperative insulation of regulatory domains by CTCF-dependent physical insulation and promoter competition. Nat Commun 15, 7258. doi: 10.1038/s41467-024-51602-4.
- Linneberg-Agerholm M, Sell AC, Redó-Riveiro A, Perera M, Proks M, Knudsen TE, Barral A, Manzanares M, Brickman JM. (2024) The primitive endoderm supports lineage plasticity to enable regulative development. Cell 187, 4010-4029. doi: 10.1016/j.cell.2024.05.051.



#### **Doctoral theses**

Antonio Barral (2023)

Control of developmental transitions by OCT4: a new role as a regulator of mTOR activity at gastrulation
Department of Molecular Biology
Universidad Autónoma de Madrid (UAM)
Supervisor: Miguel Manzanares

 Marta Portela (2024)
 Genome replication and early lineage choices during mouse embryonic development
 Department of Molecular Biology
 Universidad Autónoma de Madrid (UAM)
 Supervisor: Miguel Manzanares



#### Research projects

- RED2022-134768-T
   Regulatory Genomics Network
   Agencia Estatal de Investigación (AEI)
   PI: Miguel Manzanares
   June 2023-May 2025
- PID2020-115755GB-I00
   Genome homeostasis and cellular fates in the early mouse embryo
   Agencia Estatal de Investigación (AEI)
   PI: Miguel Manzanares
   September 2021-August 2024
- CA18127
   International Nucleome Consortium.
   European Cooperation in Science & Technology (COST)
   PI: Marc Marti-Renom (CNAG-CRG)
   May 2019-November 2023



#### INTESTINAL MORPHOGENESIS AND HOMEOSTASIS

#### **Group members**

**Principal investigator:** Fernando Martín Belmonte

**Postdoctoral fellows:** Tatiana Alfonso Pérez

Predoctoral fellows: Gabriel Baonza Martínez Gonzalo Herranz Martínez Diego Alonso Larre Laura Akintche Alberto Sánchez de la Cruz Carlos Quintana Quintana **Technicians:** Tamara González Martínez

Undergraduate and Master students: Esther Moreno Salavert





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#### Summary

Our research group is deeply committed to advancing the understanding of intestinal morphogenesis and cell polarity, with a special focus on critical processes such as development, homeostasis, and tissue regeneration. These efforts are crucial for shedding light on diseases like inflammatory bowel disease (IBD) and various forms of cancer.

The intestinal tract plays an essential role in development, regeneration, immunity, and nutrient absorption. However, the underlying mechanisms that drive its formation and function remain largely unexplored. Our work is at the forefront of investigating these fundamental questions, including how neonatal enterocytes mediate metabolic communication with the microbiota and immune cells to establish intestinal homeostasis.

We employ a multidisciplinary approach, combining advanced organotypic cell models, physiologically relevant epithelial tissue models from zebrafish and mice, and cutting-edge stem cell systems using both mouse (mESC) and human (hESC) embryonic stem cells. These tools enable us to study epithelial formation, asymmetric cell division, and the dynamics of epithelialization during intestinal development with unprecedented depth.

In addition, we are pioneering the integration of organ-on-chip intestinal models with in vivo systems to revolutionize our understanding of intestinal morphogenesis and its connections to disease,

including cancer. This holistic strategy allows us to unravel the molecular networks governing tissue patterning, metabolic regulation, and homeostasis, with the ultimate goal of identifying novel biomarkers and therapeutic targets.

Our main research objectives include:

Deciphering the role of cyclin B1 in mitotic spindle orientation and its impact on carcinoma progression in intestinal tissues.

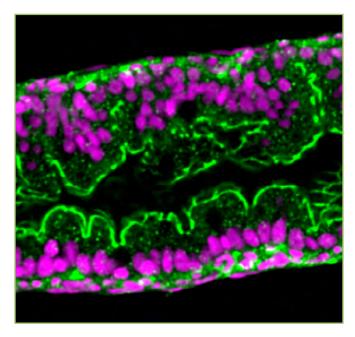
Investigating the function of lysosome-rich neonatal enterocytes (NEs) during early stages of intestinal development and metabolism.

Unraveling the metabolic interplay between the microbiota, epithelial cells, and immune cells in maintaining intestinal balance.

Exploring epithelialization processes in stem cells and their implications for lumen formation during intestinal and embryonic development, both in health and disease contexts.

Finally, we are committed to translating our findings from the bench to the bedside. Through collaboration with the Ramón y Cajal Institute for Health Research (IRYCIS-Madrid), we are analyzing the expression of these key proteins in human epithelial tumors, paving the way for potential clinical studies on tumor progression.





Confocal image of an Intestinal epithelial cell (IEC) in neonatal gut (P7). Confocal image of a seven-day mice gut showing the enrichment of lysosomal vesicles in Lysosome-rich-enterocytes (LREs). Stained for Actin (green) and Nuclei (purple).



#### List of publications

- Marta Iborra-Pernichi, Jonathan Ruiz García, María Velasco de la Esperanza, Belén Seco Estrada, Cristina Prieto Carro, Tamara González Martínez, José García-Consuegra, Fernanda Rey-Stolle, Francisco Javier Rupérez, Milagros Guerra Rodriguez, Rafael Argüello, Sara Cogliati, Martín-Belmonte F, Nuria Martínez-Martín. (2024) Defective mitochondria remodelling in B cells leads to an aged immune response. Nature Comm 15, 2569 (2024). https://doi.org/10.1038/s41467-024-46763-1
- Andrés-San Román JA, Gordillo-Vázquez C, Franco-Barranco D, Morato L, Fernández-Espartero CH, Baonza G, Tagua A, Vicente-Munuera P, Palacios AM, Gavilán MP, Martín-Belmonte F, Annese V, Gómez-Gálvez P, Arganda-Carreras I, Escudero LM. (2023) CartoCell, a high-content pipeline for 3D image analysis, unveils cell morphology patterns in epithelia. Cell Rep Methods. 2023 Oct 23;3(10):100597. doi: 10.1016/j.crmeth.2023.100597



#### **Doctoral theses**

■ Titulo: Caracterización de las propiedades fisiológicas y metabólicas de los enterocitos durente la etapa neonatal. PhD estudiante: Gonzalo Herranz Gómez. Universidad: Universidad Autónoma de Madrid. Facultad: CBMSO. Date: 07/2024.



#### Other activities

2020–2023 Scientific board of French Agency of Research (ANR) (Cell, evolution, DevBiol)



#### Research projects

- PID2023-151844OB-I00. Ministry of Science and Innovation. Neonatal Intestinal Microenvironment: The Role of Enterocytes in Homeostasis. PI: Fernando Martin-Belmonte. €310,000 09/2024-08/2027. +FPI contract 4 years.
- Horizon-MSCA-2022-DN -101119504. EU. Epithelial Exchange Surfaces From organizing principles to ex vivo disease models of the gatekeepers of the body (SurfEx)
   PI: Fernando Martin-Belmonte. Coordinator: Michel Boxem (Utrecht University). €450,000 12/2023-12/2027.
- AECC Researcher. Mitotic spindle regulation by kinases during cell division in breast cancer. Tatiana Alfonso (PI) Fernando Martin-Belmonte (Coordinator). €200,000 12/2023 -11/2027.
- RED2022-134927-T. MICINN. Spanish Thematic Network on the Interaction between Cellular Metabolism and the Microenvironment in Homeostasis and Diseases. Coordinator: Fernando Martín Belmonte. €20,000 9/2023-8/2025.
- H2020-MSCAIF-2019-897948. Understanding the role of CyclinB1-NuMA interaction in spindle orientation and epithelial morphogenesis (NuMA\_CyclinB1\_EM). Fernando Martin-Belmonte. €347,000. 09/2022 -08/2024.
- PID2020-120367GB-I00. Ministry of Science and Innovation. Molecular mechanisms of formation, differentiation, and homeostasis of intestinal tubules and their relationship to human pathologies. PI: Fernando Martin-Belmonte. €320,000 09/2021 -08/2024. +FPI contract 4 years.



#### METABOLISM AND B CELL FUNCTION

#### **Group members**

**Principal investigator:**Nuria Martínez Martín

**Postdoctoral fellows:** Elena Giulia Rodríguez Bovolenta

Predoctoral fellows: Marta Iborra Pernichi Jonathan Ruiz García Belén Seco Estrada Ana María Prieto

**Technicians:** María Velasco de la Esperanza

**Undergraduate and Master students:**Virgilia Olivé Rodriguez
Carlos Quintana Quintana



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



#### Summary

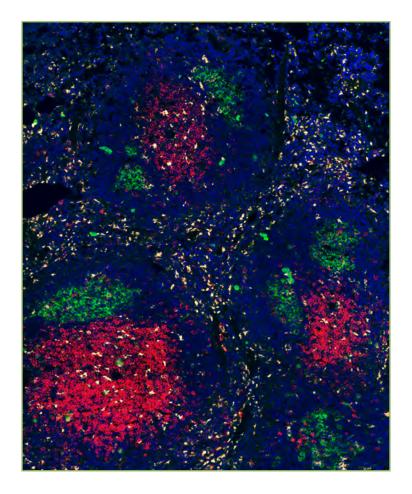
In our organism, infection leads to the orchestration of a finetuned immune response. This response relies on the activation of several immune cells; among them, activation of B lymphocytes is crucial, giving place to the production of specific high-affinity antibody-producing cells (plasma cells, PC) and memory B cells (MBC). Activated B cells proliferate and differentiate, originating a macroscopic structure within the follicles of secondary lymphoid organs known as Germinal Centers (GC). In GCs, B cells proliferate and differentiate into PC and MBC.

Deciphering molecular mechanisms controlling GC initiation and how different pathogens trigger this initiation have attracted the attention of immunologists. In this regard, GC initiation entails critical metabolic changes in the immune cells. Thus, the characterisation of the metabolic profile of each immune cell and understanding how metabolism instructs intrinsic cell activation, proliferation, and differentiation in the GC has become an essential field of study. However, GC is a double-edged sword: while a deficient GC initiation can lead to immunodeficiencies, an overactivation of GC can lead to GC-derived lymphomas or autoimmune diseases. Therefore, in addition to GC initiation, the processes leading to the GC reaction and termination of the cellular expansion are also of high scientific and clinical interest.

We have generated data demonstrating the role of mitochondria during the GC reaction, shaping intrinsic B cell function. Moreover, we have evidence suggesting that the metabolic activity of B lymphocytes impacts B cells themselves and instructs the metabolic rewiring and fate differentiation of neighbouring T lymphocytes. We have defined this process as "metabolic communication". We believe this process should be fundamental during the GC reaction, shaping initiation and shutdown of the immune response. Importantly, our work has taken us to develop stateof-the-art techniques based on flow cytometry to evaluate B cell metabolism during the immune response, such as a technique to quantify the relative amount of ATP in live cells, and metabolic profile of specific GC subsets.

Our laboratory aims to apply multi-omics and functional approaches in genetically modified mice with a dysfunctional metabolism to characterise the "metabolic communication" and identify all the molecular entities that mediate it. Moreover, we will evaluate its role in GC initiation, depending on the pathogen's nature and the GC shutdown. The obtained information will be evaluated in terms of its therapeutic potential in B cell lymphomas and autoimmune diseases and to improve, in the future, vaccination strategies.





Germinal centre structures in a spleen from immunized mouse (CD4; red; GL7: gren; DAPI: blue)



#### List of publications

- Cosgrove, J., Marçais, A., Hartmann, F. J., et. al. Bossche, J. V. den. (2024). A call for accessible tools to unlock single-cell immunometabolism research. Nat. Metab. 6, 779–782.
- Marta Iborra-Pernichi, Jonathan Ruiz García, María Velasco de la Esperanza, et al. Nuria Martínez-Martín. (2024). Defective mitochondria remodelling in B cells leads to an aged immune response. Nat. Commun. 15, 2569 doi: 10.1038/s41467-024-46763-1.
- Martinez-Martin, N. & Alarcon, B. (2023) Physiological and therapeutic relevance of T cell receptor-mediated antigen trogocytosis. Biomed. J. 100630. doi:10.1016/j. bj.2023.100630.
- Pernichi, M. I., García, J. R. & Martínez-Martín, N. (2023) Metabolic Reprogramming, Methods and Protocols. 109–115. doi:10.1007/978-1-0716-3247-5\_9.



#### Research projects

- FBBVA. Becas Leonardo a Investigadores y Creadores Culturales. "Decoding mitochondria entry into B cells: recognition unveiled". PI Nuria Martínez-Martín. 2024-2025.
- CNS2022-135636. AEI. Consolidación. "Exploration of Polyamine Metabolism as a Driver of Diffuse B-cell Lymphoma". PI Nuria Martínez-Martín. 2023-2025.
- HR22-00447. "La Caixa" Foundation. "Metabolic communication between immune cells orchestrates the response to pathogens". PI Nuria Martínez-Martín. 2023-2025
- PID2021-126298OB-100. AEI. "Generación de conocimiento 2021". "Deciphering how B cell metabolism shapes germinal centre shutdown and its relation with immune system disorders". 2022-2025.



#### **BIOPHYSICS AND SYSTEMS BIOLOGY**

#### **Group members**

**Principal investigator:**David Míguez Gómez

**Postdoctoral fellows:** Mario Ledewsma Terrón

**Predoctoral fellows:** Diego Pérez Dones Ahmed Naser Fayad Diego Mazo Durán

**Undergraduate and Master students:** Gemma Navarro





#### Summary

Our lab focuses on the study of the dynamics of developmental systems, combining experimental, theoretical, and computational tools. Our main interests are:

## 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 trigger 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

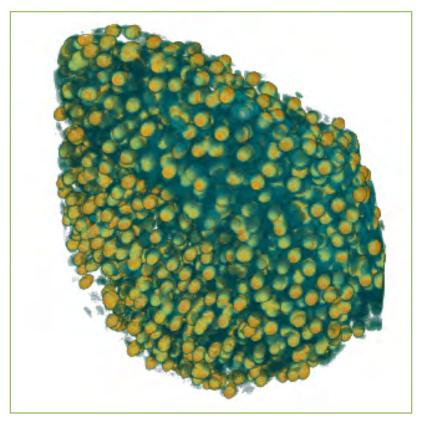
Small-molecule inhibitors display significant potential as a treatment for diseases involving deregulation of signal transduction pathways. These inhibitors are developed based on their target specificity and binding affinity. We focus on the fact that the

numerous signaling proteins and feedback loops in signaling pathways strongly influence the efficiency of pharmacological treatment. Several regulatory positive and negative feedback loops create 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 the effect of perturbations in biological networks

Biological networks control cellular behaviour both at the intracellular and at the intercellular level. These highly interconnected networks need to perform in a 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 experimentally and computationally the dynamics of fluctuations in minimal network motifs.





Digital reconstruction of a developing zebrafish retina imaged in 3D



#### List of publications

- A.N. Fayad, D. Mazo-Durán, D.G. Míguez, (2024). Cellular Compartmentalization as a Physical Regulatory Mechanism of Signaling Pathways. Biophysica 4, 634-650
- A. Rodríguez et al, (2024). Cell proliferation and Notch signaling coordinate the formation of epithelial folds in the Drosophila leg, Development 151 (8): dev202384
- J.C. Herrero Martín et al. (2024). An ETFDH-driven metabolon supports OXPHOS efficiency in skeletal muscle by regulating coenzyme Q homeostasis. Nat Metab. 6, 209–225



#### Research projects

- PDC2022-133147-I00, I+D+i Proof of Concept Projects: "OSCAR: an Object Segmentation Counter And Analysis Resource" (David Míguez) 2022-2025
- PID2022-140421NB-I00. National I+D+i Plan Ministry of Science of Spain "Interaction between physical and molecular mechanisms in the regulation of vertebrate retina formation" (David Míguez) 2023-2026



#### PLACENTA BIOLOGY

#### **Group members**

**Principal investigator:** Vicente Pérez García

**Postdoctoral fellows:** Sergio Navarro Serna Johanna Grinat

Predoctoral fellows: Paula Doria Borrell Eva González Cantó Ana Ferrero Micó Yurani Nathaly Hernández Díaz





#### Summary

The placenta is a transient organ that connects the embryo to the mother during pregnancy and mediates nutrient and oxygen supply to sustain normal growth. Hence, the placenta orchestrates developmental outcomes and fetal growth. Functionality of the placenta depends on the earliest steps during placentation when trophoblast cells, the building block of the placenta, invade into the endometrium to establish the definitive maternal-fetal interface. Several pregnancy complications such as miscarriage and preeclampsia are caused by defects in the process of trophoblast invasion.

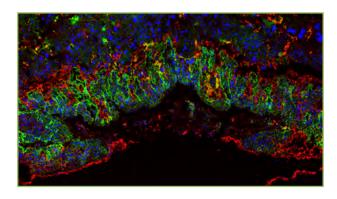
Intriguingly, trophoblast cells share some key similarities with carcinomas. These similarities include the ability to invade healthy tissues, the formation of new vessels and the promotion of an immunotolerant environment. A key biological question remains: do tumour cells repurpose the same genes and mechanisms that are critical for trophoblast invasion?

By using CRISPR/Cas9 genome editing systems, we will genetically manipulate trophoblast stem cells and organoids to:

- 1) Identify the molecular signatures characteristics of invasive placental cells.
- 2) Unravel the common molecular pathways between trophoblast invasion and cancer metastasis.

Our results will provide fundamental insights into the cellular invasive mechanisms that coordinate placentation and the potential implication of these same mechanisms in tumour metastasis.





Development of the placental labyrinth in a mouse. Image showing how the labyrinth structure forms in the mouse placenta during development. This region is essential for the exchange of nutrients and oxygen between the mother and the fetus.



#### **Articles**

- González-Cantó, E., Monteiro, M., Aghababyan, C., Ferrero-Micó, A., Navarro-Serna, S., Mellado-López, M., Tomás-Pérez, S., Sandoval, J., Llueca, A., Herreros-Pomares, A., Gilabert-Estellés, J., Pérez-García, V.\* and Marí-Alexandre, J.\* (2024) Reduced levels of miR-145-3p drive cell cycle progression in advanced high-grade serous ovarian cancer. Cells 13, 1904. doi: 10.3390/cells13221904. \*Equal contribution.
- Doria-Borrell, P. and Pérez-García, V. (2024) Understanding the intersection between placental development and cancer: Lessons from the tumor suppressor BAP1. Commun. Biol. 7, 1053. doi: 10.1038/s42003-024-06689-2.
- Walewska, E., Makowczenko, K. G., Witek, K., Laniecka, E., Molcan, T., Alvarez-Sanchez, A., Kelsey, G., Pérez-García, V.\* and Galvão, A. M.\* (2024) Fetal growth restriction and placental defects in obese mice are associated with impaired decidualisation: the role of increased leptin signalling modulators SOCS3 and PTPN2. Cell. Mol. Life Sci. 81, 329. doi: 10.1007/s00018-024-05336-7. \*Equal Contribution.
- Álvarez-Sánchez, A., Grinat, J., Doria-Borrell, P., Mellado-López, M., Pedrera-Alcócer, É., Malenchini, M., Meseguer, S., Hemberger, M. and Pérez-García, V. (2024) The GPI-anchor biosynthesis pathway is critical for syncytiotrophoblast differentiation and placental development. Cell. Mol. Life Sci. 81, 246. doi: 10.1007/s00018-024-05284-2.

#### **Book Chapters**

- Pérez-García, I. and Pérez-García, V. (2024) CRISPR activation in mouse trophoblast stem cells. In: Turco, M. Y. and Hemberger, M. (eds) Methods in Molecular Biology, vol. 2781. Springer, New York, NY, pp. 93–103. doi: 10.1007/978-1-0716-3746-3\_9.
- Doria-Borrell, P., Moya-Navamuel, M., Hemberger, M. and Pérez-García, V. (2024) Generation of knockout mouse trophoblast stem cells by CRISPR/Cas9. In: Turco, M. Y. and Hemberger, M. (eds) Methods in Molecular Biology, vol. 2781. Springer, New York, NY, pp. 81–91. doi: 10.1007/978-1-0716-3746-3\_8.



- Reference: PID2020-114459RA-I00
  Funding entity: Ministry of Science and Innovation
  Title: Deciphering the Molecular Mechanisms Regulated by
  the BAP1 PR-DUB Complex in Cell Invasion Shared between
  Human Trophoblast and Cancer Cells
  Principal Investigator (PI): Vicente Pérez García
  Dates: 01/09/2021 31/08/2024
- Reference: PLEC2022-009246
   Funding entity: Ministry of Science, Innovation and
   Universities Strategic Lines Projects Call, within the State
   Plan for Scientific, Technical and Innovation Research 2021–2023, under the Recovery, Transformation and Resilience
   Plan (PRTR)

Title: Replacement of the Mouse Embryo Assay with the Bovine Embryo Assay (or How to Prevent the Sacrifice of One Million Mice per Year in Europe). MEA-for-BEA Co-Principal Investigator (Co-PI): Vicente Pérez García Dates: 01/11/2022 – 31/10/2025
EU Funding: Project funded by the European Union – NextGenerationEU, under the PRTR

Reference: ARECES
 Funding entity: Ramón Areces Foundation
 Title: Trophoblast Invasion: New Insights into Cancer
 Metastasis Using Human Placenta (INVADE)
 Principal Investigator (PI): Vicente Pérez García
 Dates: 14/04/2023 - 14/04/2026

Reference: CNS2022-135933 (PRTR)

- Funding entity: Ministry of Science, Innovation and Universities

  Title: Mirrors of Invasion: The Placenta–Cancer Connection Principal Investigator (PI): Vicente Pérez García Dates: 01/07/2023 31/12/2025

  EU Funding: Funded by the European Union through the Recovery, Transformation and Resilience Plan (PRTR),
- NextGenerationEU funds

  Reference: PID2023-148535OB-I00
  Funding entity: Ministry of Science, Innovation and Universities
  Title: CRISPR Activation in Trophoblast Invasion: New Insights into Cancer Metastasis Using Human Placenta Principal Investigator (PI): Vicente Pérez García Dates: 01/09/2024 31/12/2027
  EU Funding: Project funded by the European Union (FEDER) Reference: 2024ICT291
  Funding entity: Spanish National Research Council (CSIC)
  Title: Extraordinary Grants for the Recruitment of Tenured Scientists, OEP 2020–2021
  Principal Investigator (PI): Vicente Pérez García Dates: 28/06/2024 30/05/2025
- Reference: Grant Agreement ID 101120126 (AFRODITA)
   Funding entity: European Commission Horizon Europe,
   Marie Skłodowska-Curie Actions (Doctoral Networks)
   Title: Advancing Fertility and Reproduction through
   Dedicated and Innovative Technological Applications (AFRODITA)

Partner – Co-PI, Agencia Estatal CSIC participates as a named Partner in this doctoral network Dates: 01/03/2024 – 29/02/2028



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

#### **Group members**

**Principal investigator:**Mar Ruiz Gómez (PI)
Joaquim Culi Espigul (co-PI)

#### Scientific staff:

Juan Modolell Mainou (Ad Honorem Professor) Sonsoles Campuzano Corrales

#### Postdoctoral fellows:

Marta Carrasco Rando

#### **Technicians:**

Raúl Somavilla Cabrero



htpps://www.cbm.uam.es/m\_ruiz



#### Summary

Chronic kidney disease (CKD) is a prevalent pathology whose incidence is increasing partly due to the scarcity of reliable markers for early diagnosis of kidney damage. The slit diaphragm (SD), a modified cell junction present in vertebrate podocytes, is a main target of injury leading to CKD. Our lab's pioneering work established that *Drosophila* nephrocytes, the cells involved in ultrafiltration, have SDs homologous to those of mammalian podocytes, and promoted the use of *Drosophila* to model nephropathies. Since then, further studies from many groups confirmed *Drosophila* suitability as a model organism to advance the understanding of the molecular mechanisms involved in SD assembly and stability.

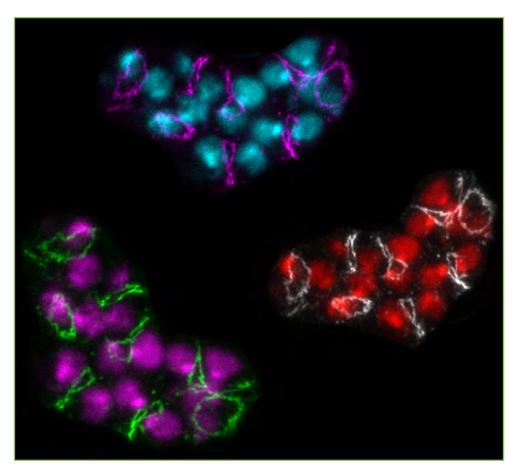
Our group exploits *Drosophila*'s genetic versatility and the efficiency and speed in generating transgenic flies to identify novel SD components and regulators. Next, we validate these discoveries in vertebrates using the zebrafish model, establishing the evolutionary conservation and relevance of these genes and mechanisms.

Our studies of the early stages of SD formation during *Drosophila* embryogenesis, revealed that SD assembly requires the creation of cell membrane asymmetries enriched in phosphatidylinositol 4,5-bisphosphate (PIP2). These PIP2-rich domains provide an optimal microenvironment for the

stability of SD proteins, ultimately leading to SD assembly. During podocyte development it has been proposed that apical proteins, which partially co-localise with SD components, may facilitate their eventual basal localization as tight junctions are replaced by SDs. However, our research in nephrocytes showed that, despite co-localization of apical and SD proteins at PIP2-rich microdomains, apical proteins are not required for the recruitment of SD components.

A key finding from our research was the identification of the phospholipid scramblase Scramb1 as a novel component of the nephrocyte SD. We discovered that Scramb1 specifically localizes to SDs and plays a crucial role in their assembly, particularly in recruiting the key SD protein Dumbfounded (Duf)/Neph1. This process likely involves Scramb1's interaction with Polychaetoid (Pyd)/ZO-1, potentially influencing Pyd-Duf association. Furthermore, our analysis of Scramb1's interactome revealed its association with membrane remodelling proteins, suggesting its active role in SD membrane dynamics. Notably, we found that calcium-binding sites on Scramb1 are critical for its function, suggesting that calcium signalling, known to be crucial in podocytes, regulates SD assembly via modulation of Scramb1 activity.





Collage of stage 14 Drosophila nephrocytes. The slit diaphragm component Duf (green, magenta and white) accumulates at the equatorial cell cortex that separates the sibling nuclei (magenta, cyan and red) of the binucleated nephrocytes. For further details, see article by Carrasco-Rando et al. 2023.



#### List of publications

- Castillo Mancho, V., Atienza-Manuel, A., Sarmiento-Jiménez, J., Ruiz-Gómez, M.\* and Culi, J.\* (2024) Phospholipid scramblase 1: an essential component of the nephrocyte slit diaphragm. Cell Mol Life Sci 81, 261. doi: https://doi.org/10.1007/s00018-024-05287-z.
- Carrasco-Rando, M., Culi, J., Campuzano, S. and Ruiz-Gómez, M.\* (2023) An acytokinetic cell division creates PIP2-enriched membrane asymmetries leading to slit diaphragm assembly in Drosophila nephrocytes. Development 150, dev201708. doi:10.1242/dev.201708.



#### Research projects

- PID2019-105492GB-I00. MICINN. Building a renal slit diaphragm: a developmental and proteomic approach. PIs: M. Ruiz-Gómez and J. Culi. 2020-2023.
- PID2022-140267NB-I00. MCIN. Assembly and dynamics of the kidney slit diaphragm junction. PIs: M. Ruiz-Gómez and J. Culi. 2023-2026.



#### Other activities

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" (2023, 2024)



## SEGMENTAL SPECIFICATION AND PATTERN FORMATION IN DROSOPHILA

#### **Group members**

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

**Postdoctoral fellows:** David Foronda Alvaro

**Technicians:** 

Paloma Martín Fernández Daniel Tovar Manzano

#### **Undergraduate and Master students:**

Blanca López Arrabal

Sergio Palomino Gálvez

Laura Utiel Ortega

Daniel Sánchez Calera

Manuel Agundez Montero





https://www.cbm.uam.es/ernesto\_sanchez-herrero



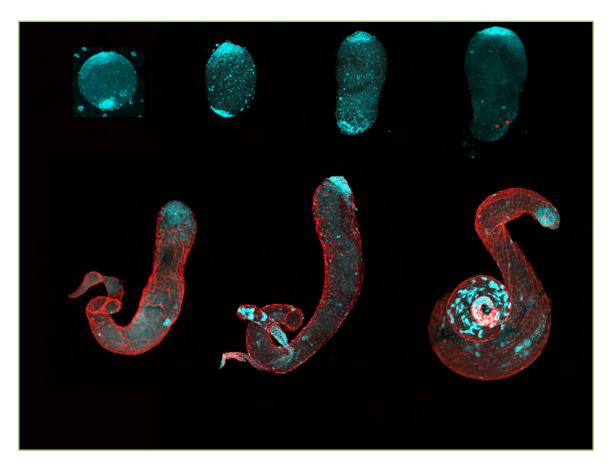
#### Summary

The Hox genes are a group of genes conserved in evolution that specify the anteroposterior axis in bilaterians. They code for proteins with a DNAbinding domain, the homeodomain, and regulate downstream genes acting a transcription factors. We are concentrating in the analysis of Hox gene function during Drosophila development. More specifically, we have studied two functions of the Drosophila Hox gene Abdominal-B (Abd-B): the development of the posterior abdomen and the formation of testes. In males, the seventh abdominal segment develops during larva but is eliminated by cell extrusion during pupa, and this suppression depends on Abd-B activity. We have been investigating the role of the Hox cofactors Extradenticle (Exd) and Homothorax (Hth), together with Abd-B, in this process. We have found that high levels of Abd-B down-regulate exd and hth, that hth is needed for Abd-B expression and that Exd and Hth proteins antagonize Abd-B activity. The Exd protein normally interacts with Hox proteins through a Hexapeptide domain, N-terminal to the Hox homeodomain, which included a tryptophane, conserved in all *Drosophila* and mouse Hox proteins. Exd and Abd-B proteins physically interact in the posterior abdomen, but we have found that this tryptophane is not needed for this interaction of for a correct activity of Abd-B, although is essential for its activity in nervous system development. Our results support an ambivalent role of Abd-B with respect to

Exd and Hth, whereby these proteins can cooperate or be antagonistic depending of the tissue context and levels of expression. Our analysis also reveals intrinsic protein requirements of different Abd-B domains, which are also organ-specific.

During pupal development the male genital disc everts and contact with the testes; muscle cells migrate from the genital disc, surround the gonads and, subsequently, the testes elongate and coil dextrally. We have found that the Hox gene Abd-B is required in the muscles for the correct development of the testes. Elongation is also prevented if muscle cells are mutant for the Notch pathway or change the sex from male to female. The testes are surrounded by a basal membrane, between muscle cells and cells making spermatogenesis, and in this space there are proteins of the extracellular matrix. If metalloproteases are inhibited in muscle cells the testes do not elongate. As to the dextral coiling, this depends on the activity of the myosin ID, and we have found this is required in the muscles that surround the testes. We have used explants testes to confirm Myosin ID role and also to show that If Abd-B is mutant in muscle cells we have found the rotation can be normal, inverted (sinistral) or testes do not rotate and grow straight. Our results identify the genes and signals required for the complex morphogenesis of these organs.





Successive stages of testis development (from left to right, from third instar larva to adult). Topro, marking nuclei, is in cyan and the muscle tissue is marked by Tropomyosin (in red).



#### List of publications

■ Rodríguez, A., Foronda, D., Córdoba, S., Felipe-Cordero, D., Baonza, A,. Miguez, D.G. and Estella, C. (2024) Cell proliferation and Notch signaling coordinate the formation of epithelial folds in the Drosophila leg. Development 151(8):dev202384. doi: 10.1242/dev.202384



#### Research projects

■ PID2020-113318GB-I00. Agencia Estatal de Investigación-Ministerio de Ciencia, Innovación y Universidades. "Study of the activity of Drosophila melanogaster Hox genes in organ development". Ernesto Sánchez-Herrero Arbide / September 2021 - September 2024



# TRANSCRIPTIONAL CONTROL OF SEXUAL DIFFERENTIATION OF THE NERVOUS SYSTEM

#### **Group members**

**Principal investigator:** Esther Serrano Saiz

Predoctoral fellows: Ana Bermejo Santos Rafael Casado Navarro Rodrigo Torrillas de la Cal Andrea Arroyo

**Undergraduate and Master students:** 

Virgilia Olive Miguel Rubio

Emilio Martina Pérez



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



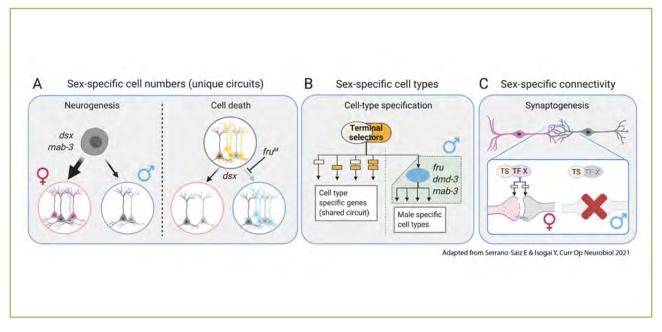
#### **Summary**

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 different at the molecular and structural levels. 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 dependent 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 ancient family of DMRT genes encode conserved transcription factors involved in the regulation of sexual features in every animal species so far studied. However, the role of DMRTs in the vertebrate nervous system has not been profusely investigated. We propose that DMRTs control sex-specific neuron identities and numbers, connectivity and constriction of sexual transcriptional differences in cooperation with sexlinked factors and sex-specific splicing mechanisms.

We are approaching this question using genetic and genomicapproachesinmiceincluding RNA-Seq, ATAC-Seq and single cell RNA-Seq. In order to translate our findings to humans, we are establishing 3D human midbrain and cortical organoids from XX and XY iPCS.

We have presented compelling evidence that Dmrt2, a member of the conserved family of doublesex and mab-3-related transcription factors (Dmrts), is a key orchestrator of proliferation and neurogenesis in the mouse cingulate cortex. Intriguingly, Dmrt2 levels are higher in males before gonadal hormone secretion, suggesting a novel, hormone-independent mechanism of early brain sexual differentiation. Our research provided the first evidence of a Dmrt gene intersecting with sex-specific development in the vertebrate nervous system. Our findings reveal novel principles: unlike invertebrates, we observe quantitative rather than binary, dimorphic expression patterns. These subtle differences nevertheless significantly impact developmental progression. Misregulation of DMRTs might lead to sex-specific susceptibility to neurodevelopmental and other psychiatric disorders.





Conserved logic in sexual circuit formation by DMRTs



#### Research projects

- Fundación Alicia Koplowitz. Combined genetic and functional studies to establish the role of DMRTA1 and DMRTA2 in susceptibility to attention deficit-hyperactivity disorder (ADHD) and its sexual skewing. Esther Serrano (IP), Claudio Toma (co-IP). 2022-2024
- PID2021-127235NB-I00. MCIN. Transcriptional control of sexual differentiation across the mouse nervous system by Dmrt2, Dmrt3 and Dmrt5. Esther Serrano Saiz (IP).2022-2025
- CNS2022-135227. MCIN. Dmrt5, a novel strategy to prevent sexual differences in the brain. Esther Serrano Saiz (IP)
- ReDevNeural 4.0 "Una aproximación integrativa hacia el entendimiento de la lógica del desarrollo neuronal" RED2022-134100-T (AEI). Coordinator: Paola Bovolenta. 2022-2024

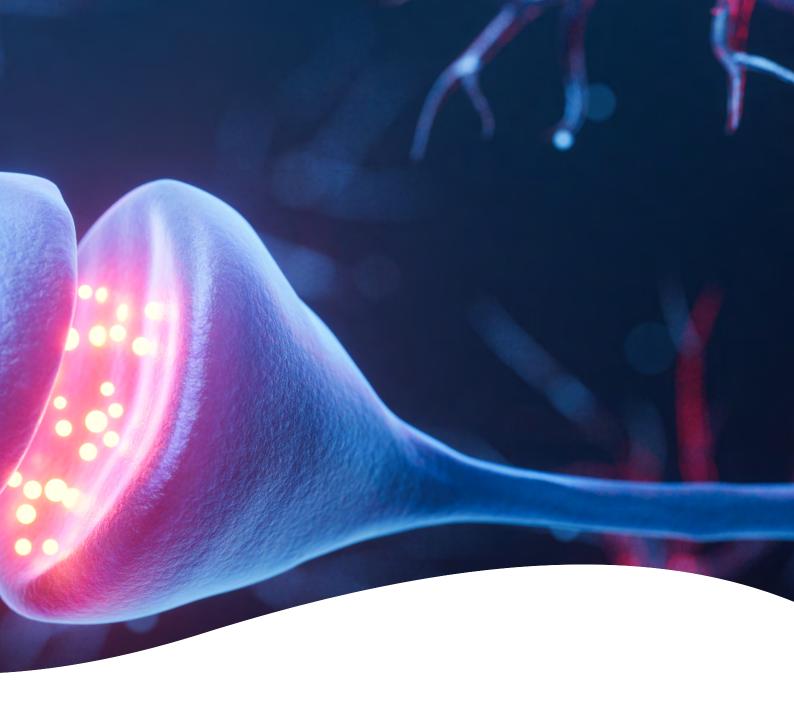


#### Other activities

Organisation of the XII Cajal Conference 2024 (SENC)
 SENC-Day Symposium (2023)



Physiological and pathological processes



Molecular neuropathology unit

Metabolic and signaling networks in disease unit



# Physiological and pathological processes

Molecular neuropathology



### José Javier Lucas Lozano

Between 2023 and 2024, the Molecular Neuropathology Unit at the CBM has made significant contributions to the understanding of key molecular mechanisms involved in brain function, adult neurogenesis, and neurodegeneration. Our research has shed light on how the regulation of stem cell survival, neuronal integration, and synaptic plasticity underpin both physiological processes and pathological conditions in the brain.

One of the most notable advances was the identification of Kidins220 as a critical regulator of neural stem cell and progenitor survival. By setting the threshold for their viability, this protein ensures the maintenance of adult neurogenesis, a process essential for cognitive flexibility and brain repair. Complementing this, it was found that GSK3 $\beta$  orchestrates the inhibitory innervation of adult-born granule cells in the dentate gyrus, fine-tuning their functional integration into hippocampal circuits. Further exploring the modulation of neurogenesis under stress conditions, we found that azithromycin, a widely used antibiotic, preserves hippocampal neurogenesis and mitigates behavioral alterations in a mouse model of sepsis, suggesting a novel neuroprotective role. Additionally, our work revealed that cyclic and neuron-restricted overexpression of Yamanaka factors can reverse age-associated neural decline and enhance memory, pointing to promising rejuvenation strategies through epigenetic reprogramming.

Through human in vivo imaging, an association between herpes simplex virus and increased cerebral amyloid-beta deposition was demonstrated in cognitively normal elderly individuals, reinforcing the relevance of viral-host interactions in Alzheimer's disease. In parallel, sphingomyelin 16:0 was identified as a pathogenic lipid species and a therapeutic target in acid sphingomyelinase deficiency, a rare but fatal neurodegenerative disorder. These findings open new therapeutic avenues targeting lipid metabolism and neuroinflammation.

On the front of gene regulation, we uncovered disease-related alterations in RNA splicing. In schizophrenia, alike what we had previously reported regarding autism, we identified pathogenic mis-splicing of CPEB4, a key regulator of translation of genes involved synaptic plasticity, supporting a transcriptomic contribution to the disease. We also revealed that intron retention in the MAPT gene can produce functional RNA species, challenging conventional views and offering insights into tau-related neurodegeneration mechanisms.



## Research groups

#### Jesús Ávila Félix Hernández

Tau function and dysfunction in Alzheimer disease

María Jesús Bullido

Pathogenic mechanisms of Alzheimer's disease

Beatriz Cubelos

Molecular mechanisms of oligodendrocyteneuron interaction & pathologies associated with myelin

Javier Díaz Nido

Physiopathology and Therapy of Neurodegeneration: Friedreich's Ataxia

Francisco Javier Díez Guerra

Molecular basis of neuronal plasticity

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**Carlos Dotti** 

Survival and plasticity in the aging brain

José Antonio Esteban

Mechanisms of synaptic plasticity, and contribution to cognitive function

•••••

María Dolores Ledesma

Lipids in neuronal physiology and pathology

María Llorens-Martín

Adult neurogenesis and neurodegenerative diseases

Beatriz López-Corcuera Francisco Zafra

Physiopathological aspects of glycine transporters in glycinergic neurotransmission: hyperekplexia and pain

José Javier Lucas Lozano

Molecular basis of Huntington's disease and other central nervous system disorders

••••••

**Ernest Palomer** 

Neuroepigenetics of brain physiology and pathology

Marta Pérez Pereira

Biology of human stem cells in translational neuroscience

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**Eva Porlan** 

Neural Stem Cells in the Adult Brain

•••••

**Claudio Toma** 

Genomic approaches to identify genes implicated across major psychiatric disorders

Francisco Wandosell

Molecular mechanisms of neurodegeneration

Finally, we made progress in understanding the signaling pathways that support synaptic plasticity and memory formation. Our findings showed that PI3K, via its regulatory subunit p85 $\alpha$ , couples' long-term synaptic potentiation with actin cytoskeleton remodeling in dendritic spines, a fundamental mechanism for learning and memory consolidation.

Altogether, the work carried out by the Molecular Neuropathology Unit during this period reflects our commitment to unraveling the complex molecular landscape of the brain in health and disease. These advances lay the groundwork for new diagnostic and therapeutic approaches aimed at enhancing brain resilience and function throughout life.



#### TAU FUNCTION AND DYSFUNCTION IN ALZHEIMER DISEASE

#### **Group members**

**Principal investigator:** Jesús Ávila Félix Hernández

**Scientific staff:** Vega García-Escudero Alejandro Antón

**Postdoctoral fellows:** Laura Vallés

Predoctoral fellows: Daniel Gabarre-Ruiz Indalo Domene Francisco Vallejo **Technicians:**Raquel Cuadros
Rocio Peinado
Nuria de la Torre

Undergraduate and Master students: Anastasia Stoliarov Marta Roldán Alvaro Ruiz Aaron Abdelkader Ana Mariscal Margarita Sánchez-Pece



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



#### Summary

Tau is a neuronal protein expressed in multiple isoforms, some of which arise from alternative splicing and exclude certain exons of the full Tau protein. Recently, in addition to these known isoforms, we identified a novel variant generated by intron retention in exon 12. In the 2023-2024 period, we have further characterized two additional isoforms—one resulting from intron retention in exon 3 and another from the simultaneous retention of introns 12 and 3. We analyzed the three-dimensional structures of these isoforms using AlphaFold.

Since aging is the primary risk factor for Alzheimer's disease, we also investigated:

a) Tau's role in pathological aging – Tau proteins in the nucleus can interact with pericentromeric heterochromatin, leading to its condensation. Heterochromatin loss is associated physiological aging, but nuclear Tau may accelerate this process. This loss can activate previously silenced genetic regions, including long and short repeated sequences, which, after transcription and reverse transcription, may integrate as transposons in other genomic regions, potentially harming aged cells. We found that treatment with lamivudine, a reverse transcriptase inhibitor, prevents these pathological changes.

b) Neuronal rejuvenation strategies – We explored methods to rejuvenate brain cells in aged mice and identified a key role of folate receptor alpha (FR $\alpha$ ) in partially rejuvenating dentate gyrus cells. This rejuvenation was linked to cognitive improvements in old mice. Notably, FR $\alpha$  activation induces the expression of Yamanaka factors (YF), previously hypothesized to be involved in neuronal rejuvenation. Our findings confirmed this hypothesis, showing that restricting YF overexpression to neurons enhances memory performance in aged mice.



#### **Patents**

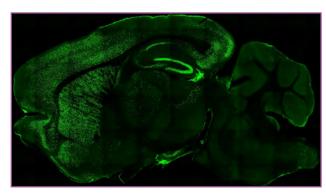
■ Peptide for cognitive improvement, Jesus Avila, Alejandro Antón, Félix Hernández, EP23382113.1, 08-02-2023.



#### **Doctoral theses**

Daniel Ruiz-Gabarre (2023). ¿Tine el gen MAPT algo nuevo que decir? Descubrimiento y caracterización de nuevas isoformas de la prteína tau y su relevancia en la enfermedad de Alzheimer. Anatomia, Histología y Neurociencia. UAM. Jesús Avila & Vega García-Escudero.





Expression of the Yamanaka factor Klf4 in neurons of the mouse central nervous system



#### List of publications

- Avila, J., Perry G. (2023) Memory, Sleep, and Tau Function. J Alzheimers Dis 94, 491-495. doi: 10.3233/JAD-230230
- Domene-Serrano, I., Cuadros R., Hernandez F., Avila J., Santa-Maria I. (2023) Tridimensional Structural Analysis of Tau Isoforms Generated by Intronic Retention. J Alzheimers Dis Rep 7, 1259-1265. doi: 10.3233/ADR-230074
- Alfaro-Ruiz, R., Aguado C., Martin-Belmonte A., Moreno-Martinez A. E., Merchan-Rubira J., Hernandez F., Avila J., Fukazawa Y., Lujan R. (2023) Different modes of synaptic and extrasynaptic NMDA receptor alteration in the hippocampus of P301S tau transgenic mice. Brain Pathol 33, e13115. doi: 10.1111/bpa.13115
- Valles-Saiz, L., Avila J., Hernandez F. (2023) Lamivudine (3TC), a Nucleoside Reverse Transcriptase Inhibitor, Prevents the Neuropathological Alterations Present in Mutant Tau Transgenic Mice. Int J Mol Sci 24, doi: 10.3390/ijms241311144
- Anton-Fernandez, A., Valles-Saiz L., Avila J., Hernandez F. (2023) Neuronal nuclear tau and neurodegeneration. Neuroscience 518, 178-184. doi: 10.1016/j. neuroscience.2022.07.015
- Avila, J., Santa-Maria I., Sotiropulos I. (2023) Editorial Special Issue Neuroscience "Tauopathies". Neuroscience 518, 1-3. doi: 10.1016/j.neuroscience.2023.03.021
- Hernandez, F., Ferrer I., Perez M., Zabala J. C., Del Rio J. A., Avila J. (2023) Tau Aggregation. Neuroscience 518, 64-69. doi: 10.1016/j.neuroscience.2022.04.024
- Molinero, N., Anton-Fernandez A., Hernandez F., Avila J., Bartolome B., Moreno-Arribas M. V. (2023) Gut Microbiota, an Additional Hallmark of Human Aging and Neurodegeneration. Neuroscience 518, 141-161. doi: 10.1016/j.neuroscience.2023.02.014

- Avila, J. (2024) Delaying Brain Aging or Decreasing Tau Levels as Strategies to Prevent Alzheimer's Disease: In Memoriam of Mark A. Smith. J Alzheimers Dis 100, S265-S270. doi: 10.3233/JAD-240500
- Avila, J., Marco J., Plascencia-Villa G., Bajic V. P., Perry G. (2024) Could there be an experimental way to link consciousness and quantum computations of brain microtubules? Front Neurosci 18, 1430432. doi: 10.3389/fnins.2024.1430432
- Moreira, P. I., Avila J., Galimberti D., Pappolla M. A., Plascencia-Villa G., Sorensen A. A., Zhu X., Perry G. (2024) Therapeutic Trials in Alzheimer's Disease: Where Are We Now? J Alzheimers Dis 101, S1-S2. doi: 10.3233/JAD-249016
- Ruiz-Gabarre, D., Valles-Saiz L., Carnero-Espejo A., Ferrer I., Hernandez F., Garcia-Escudero R., Avila J., Garcia-Escudero V. (2024) Intron retention as a productive mechanism in human MAPT: RNA species generated by retention of intron 3. EBioMedicine 100, 104953. doi: 10.1016/j.ebiom.2023.104953
- Anton-Fernandez, A., Cuadros R., Peinado-Cahuchola R., Hernandez F., Avila J. (2024) Role of folate receptor alpha in the partial rejuvenation of dentate gyrus cells: Improvement of cognitive function in 21-month-old aged mice. Sci Rep 14, 6915. doi: 10.1038/s41598-024-57095-x
- Anton-Fernandez, A., Roldan-Lazaro M., Valles-Saiz L., Avila J., Hernandez F. (2024) In vivo cyclic overexpression of Yamanaka factors restricted to neurons reverses ageassociated phenotypes and enhances memory performance. Commun Biol 7, 631. doi: 10.1038/s42003-024-06328-w
- Sala-Jarque, J., Gil V., Andres-Benito P., Martinez-Soria I., Picon-Pages P., Hernandez F., Avila J., Lanciego J. L., Nuvolone M., Aguzzi A.et al. (2024) The cellular prion protein does not affect tau seeding and spreading of sarkosyl-insoluble fractions from Alzheimer's disease. Sci Rep 14, 21622. doi: 10.1038/s41598-024-72232-2



#### Research projects

- PID2021-123859OB-I00. MCIU. Functions of Tau Isoforms. Jesús Avila. 01/09/22-31/08/25
- 201920E104. CSIC. Neuronal rejuvenation through metabolite-mediated reprogramming. Jesús Avila. 01/10/2019-30/09/2023
- PID2020-113204GB-I00. MCIU. Neuroregeneration in Alzheimer's disease through the expression of pluripotency factors in vivo. Félix Hernández. 01/09/21-31/05/25
- PID2023-149460NB-I00. MCIU. Activation of transposons in animal models of Alzheimer's disease: implication of the tau protein. Félix Hernández. 01/09/24-31/08/27
- ION-ARPA. Ionis Pharmaceuticals Inc. 3' replacement of TaumRNAs in Alzheimer's disease by a replicative transsplicing ribozyme-ion-arpa. Vega García-Escudero. 01-07-2023-30/6/2025
- Part of the Networking Research Center on Neurodegenerative Diseases (CIBERNED).



#### PATHOGENIC MECHANISMS OF ALZHEIMER'S DISEASE

#### **Group members**

**Principal investigator:**María Jesús Bullido Gómez-Heras

**Scientific staff:** Jesús Aldudo Soto María Recuero Vicente

**Predoctoral fellows:**Blanca Salgado Fuentes

**Technicians:** Isabel Sastre Merlín María Martín Rico

**Undergraduate and Master students:** Alba Romero Solares Inés Beamonte Palomero Silvia Socas Delgado



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#### Summary

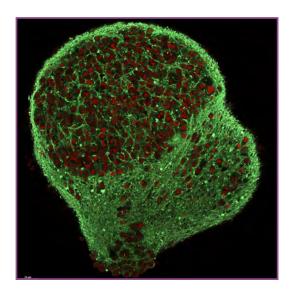
Our team is devoted to the study of functional connections between Alzheimer's disease (AD)-like neurodegeneration and herpes simplex I (HSV-1) infection. We have developed several models of HSV-1 infection in neuroblastoma cells, in which we have described an AD-like phenotype characterized by the intracellular accumulation of A $\beta$  and hyperphosphorylated tau and by autophagic alterations. Functional transcriptomic analyses of those models pointed to the lysosomal pathway and cholesterol metabolism as the most prominent processes bridging the infection with neurodegeneration.

To validate these pathways, we study biomarkers related to HSV 1 infection, cholesterol and the lysosome pathway in patients. We have found a decrease in cholesterol content of peripheral blood cells in patients with mild cognitive impairment, supporting the notion that cholesterol metabolism is altered early in the pathogenesis of AD, both in the CNS and in the periphery. Additionally, the decrease in CD8+ and increase in CD16+ cells support the existence of alterations in the adaptive and innate immune system of the patients. We also looked for correlations between HSV-1 and AB accumulation, and we found that serum anti-HSV IgG levels positively correlate with AB load in the brain of aged cognitively normal subjects. This suggests that infection is selectively related to cerebral Aβ deposition in aging, and supports the involvement of HSV-1 in preclinical phases of the degenerative process.

To gain insight into these processes in a scenario closer to the actual disease than neuroblastoma models provide, we are currently developing new cell models of human neural cells differentiated from neural progenitors in 2D and 3D platforms.

In LUHMES cells, a human embryonic neural precursor cell line that differentiates into mature neurons, we have confirmed the ability of HSV-1 to induce the accumulation of AB peptide and hyperphosphorylated tau and to alter the autophagic pathway in differentiated cells. Preliminary results suggest that HSV-1 establishes a latent infection in these cells. The LUHMES 3D platforms are therefore promising for further research into the role of HSV-1 primary infection, latency and reactivation in neurodegeneration. ReNcell VM is a human neural progenitor cell line that differentiate into neuronal and glial cells, thus providing the possibility to study the interplay between different cell lineages. We have optimized a 2D ReNcell VM model of infection that reproduce an AD-like neurodegenerative phenotype in the differentiated cells. In addition, we have developed two complementary 3D platforms, based on Matrigel embedded cells and in matrix-free, neurospheroid-like structures, respectively. In both platforms, HSV-1 spreads throughout the structures and induce neurodegeneration markers, making ReNcell VM a valuable platform for future studies on the role of neuroimmflamation in HSV-1 induced neurodegeneration.





A neurospheroid of differentiated LUHMES cells visualised by confocal microscopy. Nuclei are labelled with DAPI (red) and neurofilaments with an antibody specific for intermediate filaments of the cytoskeleton (green).



#### List of publications

- Le Borgne J, Gomez L, Heikkinen S, et al. X-chromosomewide association study for Alzheimer's disease. Mol Psychiatry. Published online December 4, 2024. doi:10.1038/ s41380-024-02838-5
- Puerta R, de Rojas I, García-González P, et al. Connecting genomic and proteomic signatures of amyloid burden in the brain. Preprint. medRxiv. 2024;2024.09.06.24313124. Published 2024 Sep 6. doi:10.1101/2024.09.06.24313124
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- European Alzheimer's & Dementia Biobank Mendelian Randomization (EADB-MR) Collaboration, LuoJ, Thomassen JQ, et al. Genetic Associations Between Modifiable Risk Factors and Alzheimer Disease [published correction appears in JAMA Netw Open. 2023 Jun 1;6(6):e2321189. doi: 10.1001/jamanetworkopen.2023.21189.]. JAMA Netw Open. 2023;6(5):e2313734. Published 2023 May 1. doi:10.1001/jamanetworkopen.2023.13734
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- García-GonzálezP,deRojasI,Moreno-GrauS,etal.Mendelian Randomisation Confirms the Role of Y-Chromosome Loss in Alzheimer's Disease Aetiopathogenesis in Men. Int J Mol Sci. 2023;24(2):898. Published 2023 Jan 4. doi:10.3390/ ijms24020898
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#### Research projects

- CIBERNED-ISCIII. Role of HSV-1 infection in Alzheimer's disease: effects on myelin damage and therapeutic approaches. Coordinator: Eva Carro; Participating group PI: MJBullido; Duration: June 2024-May 2026.
- PID2020-113921RB-I00. MICINN. Cholesterol homeostasis and lysosomal pathway in HSV-1-induced neurodegeneration and Alzheimer's disease: pathogenic mechanisms and biomarkers; PI: MJ Bullido. Duration: September 2021-May 2025.
- CIBERNED-ISCIII. Progressive supranuclear palsy: identification of susceptibility loci, cellularity involved, and molecular pathways for drug development: PSP/DEGESCO Program. Coordinator: Agustín Ruiz; PI of the participating group: MJBullido; Duration: 2021-23
- CIBER-NED. Group CB06/05/0007; PI: MJBullido
- IdiPAZ. Group "Neurology and cerebrovascular diseases." PI: E Díez-Tejedor.



# MOLECULAR MECHANISMS OF OLIGODENDROCYTE-NEURON INTERACTION & PATHOLOGIES ASSOCIATED WITH MYELIN

#### **Group members**

**Principal investigator:**Beatriz Cubelos

Scientific staff: Gema María Esteban Ortega Gonzalo García Martín María Sáez Santacruz

**Postdoctoral fellows:** Gema María Esteban Ortega

**Predoctoral fellows:** Gonzalo García Martín

**Undergraduate and Master students:**María Sáez Santacruz



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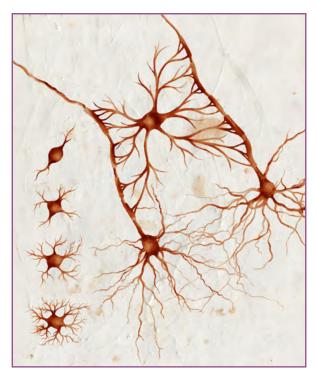
#### Summary

In our laboratory, we focus on studying the neurological component of demyelinating pathologies, with a specific emphasis investigating the molecular mechanisms responsible for myelination processes in the Central Nervous System (CNS). We know that proper myelination is crucial for the correct transmission of nerve impulses. In the CNS, oligodendrocytes play a fundamental role in myelinating neuronal axons, a complex process involving multiple cellular interactions. Diseases resulting from improper myelination, such as Multiple Sclerosis or leukodystrophies, currently lack effective treatments.

Therefore, understanding the molecular mechanisms responsible for oligodendrocyte maturation and how oligodendrocyte-neuron interaction is established is crucial for advancing the study of these myelin diseases. Our group has demonstrated the importance of R-Ras1 and R-Ras2 GTPases, essential proteins in oligodendrocyte differentiation and survival, as well as in maintaining energy homeostasis.

Our experimental models, lacking R-Ras1 and/ or R-Ras2, faithfully reproduce the symptomatic characteristics of myelin diseases, suggesting that they could be used as models for the development of new treatments based on the neurological component. Overall, our work seeks to shed light on the molecular processes underlying demyelinating pathologies and open new avenues for the development of effective therapies.





Myelination. Cajal style



#### List of publications

#### **Artículos:**

Alcover-Sanchez B, Garcia-Martin G, Paleo-García V, Quintas A, Dopazo A, Gruart A, Delgado-García JM, de la Villa P, Wandosell F, Pereira MP, Cubelos B. (2024) R-Ras1 and R-Ras2 regulate mature oligodendrocyte subpopulations. Glia. 2024 Nov doi: 10.1002/glia.24643.



#### Research projects

- PID2021-123269OB-I00. Spanish Ministry of Science and Innovation. Role of R-Ras1 and R-Ras2 in oligodendrocyte differentiation and specification. Beatriz Cubelos Álvarez. January 1, 2021 December 31, 2024.
- 101057609. CIBERNED, Spanish Ministry of Science and Innovation. Alzheimer's disease: effects on myelin damage and therapeutic approaches Eva Ma Carro Diaz. 06/30/2024 06/30/2026.



#### **Patents**

■ Transgenic Mouse Model of Myelin Pathologies, Beatriz Cubelos Alvarez. & Berta Alcover-Sanchez, N.º EP3977848, Europe and extension, October 2024. UAM



#### **Doctoral theses**

Berta Alcover Sanchez (2024). Papel de R-Ras1 y R-Ras2 en la diferenciación y maduración oligodendrocitaria. Biociencias moleculares. Universidad Autónoma de Madrid. Sobresaliente Cum laude. Directora: Beatriz Cubelos Alvarez



# PHYSIOPATHOLOGY AND THERAPY OF NEURODEGENERATION: FRIEDREICH'S ATAXIA

#### **Group members**

**Principal investigator:** Javier Díaz Nido

**Scientific staff:** Saíl Herranz Martín

Predoctoral fellows: Andrés Vicente Acosta

**Undergraduate and Master students:** Jorge Galán-Cruz

Visiting scientists: Frida Loría-Salinas





#### Summary

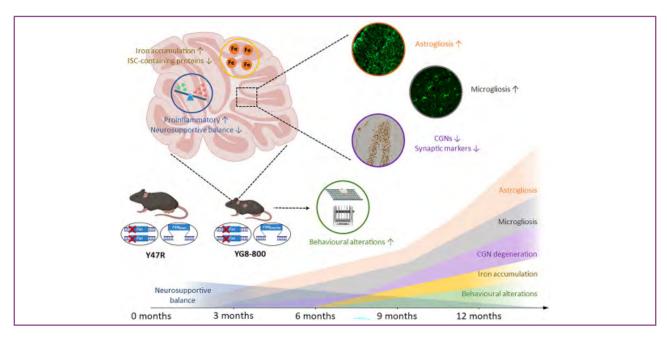
Our research group is interested in the study of Friedreich's ataxia, which is the most common hereditary ataxia in the Spanish population. We try to elucidate the molecular basis of this disease and develop novel therapies.

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 have described that an agonist of the Sonic Hedgehog signalling pathway SAG reduces mitochondrial dysfunction and neurotoxicity of frataxin-deficient astrocytes.

Currently we are characterizing a novel mouse model for Friedreich's ataxia in which we have observed an increase in glial reactivity followed by neurodegeneration in the cerebellum. 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.





Chronology of changes observed in the cerebellum of frataxin-deficient mice



#### List of publications

■ Vicente-Acosta A, Herranz-Martín S, Pazos MR, Galán-Cruz J, Amores M, Loria F, Díaz-Nido J. (2024) Glial cell activation precedes neurodegeneration in the cerebellar cortex of the YG8-800 murine model of Friedreich ataxia. J. Neurobiol Dis. 200:106631. doi: 10.1016/j.nbd.2024.106631. Epub 2024 Aug 5.PMID: 39111701



#### **Doctoral theses**

Andres Vicente Acosta (2023) Efectos de la deficiencia de frataxina en astrocitos y su modulación por el agonista de Sonic hedgehog SAG.Departamento de Biología Molecular. Universidad Autónoma de Madrid. Directores: Javier Díaz-Nido y Frida Loría Salinas. Mención Internacional.



#### Other activities

■ 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.



#### Research projects

- PID2022-143030OB-I00. Ministry of Science and Innovation. Title: Neurodegeneration in the cerebellum of Friedreich's ataxia models: Molecular bases and therapeutic approaches. Duration: 01/06/2023 31/05/2026. Funding (in euros): 141,250
- PID2019-111338RB-I00. Ministry of Science and Innovation. Title: Regulation of energetic metabolism in the brain: implications for neurodegeneration in Friedreich's ataxia. Duration: 01/06/2020 31/05/2023. Funding (in euros): 108,900
- Our research group also belongs to the Puerta de Hierro Majadahonda Hospital Health Research Institute.



#### MOLECULAR BASES OF NEURONAL PLASTICITY

#### **Group members**

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

**Postdoctoral fellows:** Elena Martínez Blanco

**Technicians:** Lucía Baratas Álvarez Ainhoa Rodríguez Moya

**Undergraduate and Master students:** Ricardo Pérez Velasco Pedro Cadenato Martínez de Salinas

Visiting scientists: Kiran Anushka Dogra



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



#### Summary

Higher cognitive functions differentiate humans from other animal species and rely on the activity of complex neural networks in the forebrain. As we age or suffer neurological or psychiatric disorders, these networks lose functionality due to synapse dysfunction and loss, eventually leading to neurodegeneration. In the development of the mammalian central nervous system, there are critical periods of synaptic plasticity which exhibit highly increased synaptogenesis and synaptic remodeling. Those are driven by sensory stimulation and are essential for establishing the proper configuration and functionality of adult neural networks. Understanding how these periods of heightened synaptic plasticity work -and how to stimulate them-could be key to preventing, alleviating, or reversing the cognitive decline associated with aging and neurological disorders.

In our lab, we have replicated in vitro several features of these high-plasticity periods using primary cultures of dissociated embryonic neurons. Our findings emphasize the critical role of neuronal activity, and we are working to uncover the molecular and cellular mechanisms behind it. Specifically, we investigate how synaptic activity, and in particular local intracellular calcium (Ca<sup>2+</sup>) oscillations, modulate signaling pathways that regulate synapse formation and rejuvenation.

A central player in this process is calmodulin (CaM), a calcium-binding protein that translates Ca<sup>2+</sup> oscillations into intracellular signals, leading to both short-term

and long-term structural and functional changes, including modulation of gene expression. The activity of CaM is regulated by proteins like Neurogranin (Ng), a prominent CaM-sequestering protein located in the postsynaptic compartment of principal forebrain neurons. Interestingly, the levels of Ng in the brain are closely linked to cognitive performance in humans.

Our research focuses on understanding how Ng expression is regulated and what is its functional role in the postsynaptic environment. To achieve this, we employ a range of techniques, including biochemistry, cellular and molecular biology, transcriptomics, proteomics, advanced light microscopy and image analysis. Additionally, we develop genetically encoded biosensors to identify key molecules and interactions involved in synaptic generation and remodeling.

Based on our findings, we propose that Neurogranin (Ng) is a promising target for strategies aimed at preventing, alleviating, or even reversing cognitive impairment. Since Ng expression is confined to forebrain regions and late developmental stages, interventions designed to promote Ng expression are unlikely to produce unwanted collateral effects.

A better understanding of the role of CaMsequestering proteins in synaptic plasticity could lead to therapies that enhance cognitive function and improve the quality of life for aging individuals and those with early-stage neurodegenerative diseases.



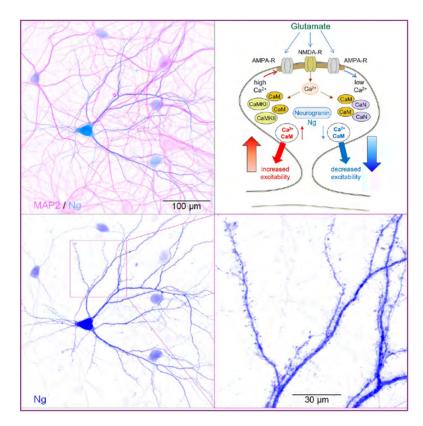


Figure 307-2.- Calmodulin sequestering protein Neurogranin modulates synaptic plasticity and enhances cognitive functions. Neurogranin present in dendrites and spines controls post-synaptic excitability by regulating Calmodulin (CaM) availability in mature hippocampal neurons in culture (DIV18).



#### List of publications

De Andrés R, Martínez-Blanco E, Díez-Guerra FJ. (2024) HDAC4 Inhibits NMDA Receptor-mediated Stimulation of Neurogranin Expression. Mol Neurobiol. 25 November 2024. https://doi.org/10.1007/s12035-024-04598-3



#### Research projects

■ Intramural Project UAM/194 "Molecular Basis of Neuronal Plasticity." Principal Investigator: Francisco Javier Díez Guerra. September 2023–September 2027.



#### **Doctoral theses**

- Raquel de Andrés Hernaiz (2023) "Estudio de la Regulación de la Expresión de Neurogranina por Actividad Sináptica". Departamento de Biología Molecular, Universidad Autónoma de Madrid. Director: Fco. Javier Díez Guerra
- Elena Martínez Blanco (2024) "Papel de Neurogranina en la Regulación de la Señalización de Calcio/ Calmodulina y su Impacto en la Plasticidad Neuronal". Departamento de Biología Molecular, Universidad Autónoma de Madrid. Director: Fco. Javier Díez Guerra"



#### SURVIVAL AND PLASTICITY IN THE AGING BRAIN

#### **Group members**

**Principal investigator:**Carlos Dotti

**Postdoctoral fellows:** Inés Berenguer López Marta Carús Cadavieco

**Predoctoral fellows:** Raquel García Rodríguez Lucía Martín Rodríguez

**Technicians:** Mercedes Hernández del Cerro

Undergraduate and Master students:
Maite Jimémez Lozano
Eva Díaz Cava
José Luis Mazo Varas
Laura Rosado Florido
Susana Mendaza Ruiz

**Visiting scientists:** Sahba Mobini



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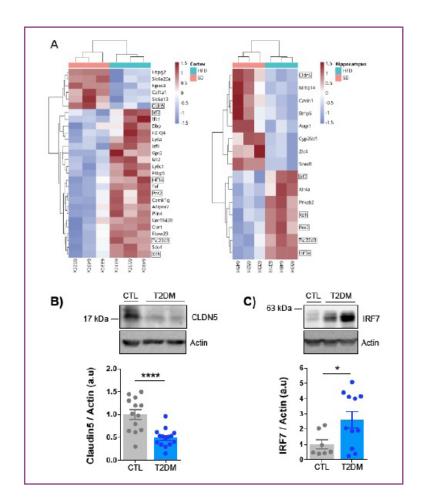
#### Summary

Our research focuses on understanding the mechanisms that maintain the balance between synaptic plasticity and neuronal survival during aging, as well as the conditions that disrupt this balance and lead to pathological cognitive disorders. Notably, we have demonstrated that aging impacts the lipid composition of neuronal plasma membranes. This alteration, due to the allosteric function of lipids, results in the tonic activation of various receptors, leading to their desensitization. Our findings indicate that this desensitization affects receptors for trophic factors, such as BDNF and insulin, as well as the excitatory neurotransmitter glutamate. Although desensitization manifests as a "loss-of-function" effect on synaptic plasticity observable in biochemical, gene expression, and functional terms—it concurrently produces a "gainof-function" effect on cell survival, suggesting that the functional decline associated with aging is a trade-off for neuronal longevity.

In addition to examining the balance between plasticity and survival in normal aging, we have made significant progress in understanding the mechanisms that lead to pathological cognitive changes. Our investigations, particularly using a type 2 diabetes mellitus (T2DM) model, indicate that for T2DM to induce significant cognitive changes similar to Alzheimer's disease, the affected individual must possess an underlying subclinical neurological pathology.

Currently, we are investigating the changes in gene expression in brain cells induced by long-term diabetes mellitus and how these changes may contribute to the development of cognitive deficits in older adults.





Gene expression analysis of the cerebral cortex and hippocampus in aged diabetic mice, at the onset of cognitive deficits: up-regulation of Interferon Regulatory Factor 7 (Irf7) and downregulation of Claudin 5 (Cldn5) (A). These changes were confirmed at the protein (B and C).



#### List of publications

- Martín MG, Dotti CG. (2023). Game-changer in the fight against Huntington's disease: Cholesterol nanoparticles restore brain function in mice. Pharmacol Res. 194:106879. doi: 10.1016/j.phrs.2023.106879. E
- Casadomé-Perales Á, Naya S, Fernández-Martínez E, Mille BG, Guerrero-Valero M, Peinado H, Guix FX, Dotti CG, Palomer E. (2023) Neuronal Prosurvival Role of Ceramide Synthase 2 by Olidogendrocyte-to-Neuron Extracellular Vesicle Transfer. Int J Mol Sci. 24(6):5986. doi: 10.3390/ ijms24065986.
- Carús-Cadavieco M, Berenguer López I, Montoro Canelo A, Serrano-Lope MA, González-de la Fuente S, Aguado B, Fernández-Rodrigo A, Saido TC, Frank García A, Venero C, Esteban JA, Guix F, Dotti CG. (2023) Cognitive decline in diabetic mice predisposed to Alzheimer's disease is greater than in wild type. Life Sci Alliance. 6(6):e202201789. doi: 10.26508/lsa.202201789. Print 2023 Jun.
- Carús-Cadavieco M, González de la Fuente S, Berenguer López I, Serrano-Lope MA, Aguado B, Guix F, Palomer E, Dotti CG. (2024) Loss of Cldn5 -and increase in Irf7-in the hippocampus and cerebral cortex of diabetic mice at the early symptomatic stage. Nutr Diabetes. 14(1):64. doi: 10.1038/s41387-024-00325-y.



#### Research projects

PID2022-138334OB-I00. AEI. On the mechanisms of cognitive defects induced by type 2 diabetes in mice predisposed to alzheimer's disease. PI: Carlos Dotti. 2022-2025



# MECHANISMS OF SYNAPTIC PLASTICITY, AND CONTRIBUTION TO COGNITIVE FUNCTION

#### **Group members**

**Principal investigator:** losé A. Esteban

**Postdoctoral fellows:** Irene B. Maroto Martínez

Predoctoral fellows: Eneko Merino Casamayor Alba Fernández Rodrigo Esperanza López Merino Celia García Vilela

**Technicians:** Silvia Gutiérrez Eisman Undergraduate and Master students: Izascun Los Arcos López de Pariza Pablo Bueno Calvo Christina Reyburn Valés Marta Piñeiro Pérez Aitana Jiménez Marín



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



#### 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.

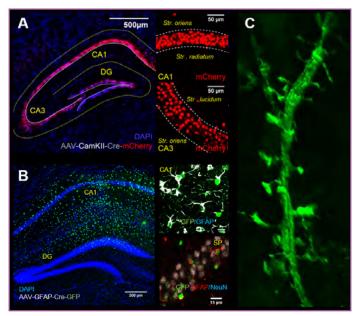
In recent years, our research is concentrated on two intersecting directions. On the one hand, we continue uncovering the molecular components and signaling cascades that control the remodeling of synapses during plasticity. We are dedicating particular effort to the signaling pathway controlled by phosphoinositide 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. Indeed, we are now finding that different PI3K and Ras isoforms contribute to specialized functions in neurons and glial cells, coupling with different presynaptic and postsynaptic components during synaptic plasticity. We are also investigating how these mechanisms impact on cognitive function and behavioral aspects such as anxiety and sociability.

On the other hand, we are investigating how cellular metabolism, both in neurons and in astrocytes, impacts on synaptic function and plasticity. In fact, the PI3K pathway is a well-known signaling hub for the regulation of metabolic fluxes. Using cell type-specific genetically modified mice, we are now finding that astrocytic PI3K controls synaptic plasticity in an indirect manner, by providing important metabolites for neurons to undergo synaptic plasticity. In addition, we are investigating how the energy balance required to sustain synaptic function is shared between neurons and astrocytes.

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





A, B. Analysis of neuronal function by genetic deletion using in vivo injection of adenoassociated viral vectors expressing the cre recombinase under neuronal (A) or astrocytic (B) promoters. C. Fluorescence image of a CA1 pyramidal neuron expressing GFP-HRas obtained with STED microscopy.



- López-García, S., López-Merino, E., Fernández-Rodrigo, A., Zamorano-González, P., Gutiérrez-Eisman, S., Jiménez-Sánchez, R. and Esteban, J. A. (2024) PI3K couples long-term synaptic potentiation with cofilin recruitment and actin polymerization in dendritic spines via its regulatory subunit p85α. Cell Mol Life Sci 81, 358. doi: 10.1007/s00018-024-05394-x.
- Toledano-Zaragoza, A., Enriquez-Zarralanga, V., Naya-Forcano, S., Briz, V., Alfaro-Ruíz, R., Parra-Martínez, M., Mitroi, D. N., Luján, R., Esteban, J. A. and Ledesma, M. D. (2024) Enhanced mGluR5 intracellular activity causes psychiatric alterations in Niemann Pick type C disease. Cell Death Dis 15, 771. doi: 10.1038/s41419-024-07158-8.
- del Prado, L., Jaraíz-Rodríguez, M., Agro, M., Zamora-Dorta, M., Azpiazu, N., Calleja, M., López-Manzaneda, M., de Juan-Sanz, J., Fernández-Rodrigo, A., Esteban, J. A., Girona, M., Quintana, A. and Balsa, E. (2024) Compensatory activity of the PC-ME1 metabolic axis underlies differential sensitivity to mitochondrial complex I inhibition. Nat Commun 15, 8682. doi: 10.1038/s41467-024-52968-1.

- López-Merino, E., Cuartero, M. I., Esteban, J. A.\* and Briz, V.\* (2023) Perinatal exposure to pesticides alters synaptic plasticity signaling and induces behavioral deficits associated with neurodevelopmental disorders. Cell Biol Toxicol 39, 2089-2111. \*Co-corresponding authors. doi: 10.1007/s10565-022-09697-2.
- Pedraza, N., Monserrat, M. V., Ferrezuelo, F., Torres-Rosell, J., Colomina, N., Miguez-Cabello, F., Párraga, J. P., Soto, D., López-Merino, E., García-Vilela, C., Esteban, J. A., Egea, J., Garí, E. (2023) Cyclin D1-Cdk4 regulates neuronal activity through phosphorylation of GABAA receptors. Cell Mol Life Sci 80, 280. doi: 10.1007/s00018-023-04920-7.
- Carús-Cadavieco, M., Berenguer-López, I., Montoro-Canelo, A., Serrano-Lope, M. A., González-de la Fuente, S., Aguado, B., Fernández-Rodrigo, A., Saido, T. C., Frank-García, A., Venero, C., Esteban, J. A., Guix, F., Dotti, C. G. (2023) Cognitive decline in diabetic mice predisposed to Alzheimer's disease is greater than in wild type. Life Sci Alliance 6, e202201789. doi: 10.26508/lsa.202201789.



# Research projects

- PID2023-149056OB-I00. Ministerio de Ciencia, Innovación y Universidades. "Metabolic regulation subserving synaptic plasticity". PI: José A. Esteban. 2024-2027.
- PDC2021-120815-I00. Ministerio de Ciencia e Innovación/ NextGenerationEU. "GSK3alpha, an unsuspected therapeutic target for Alzheimers disease". PI: José A. Esteban. 2022-2024.
- PID2020-117651RB. Ministerio de Ciencia e Innovación. "Interplay between synaptic and metabolic plasticity. Relevance for mental disease". PI: José A. Esteban. 2021-2024.



# Doctoral theses

- Alba Fernández Rodrigo (2024). "Role of PI3K isoforms in linking synaptic plasticity and metabolism in neurons and astrocytes". Departamento de Biología Molecular. Universidad Autónoma de Madrid. Directores: José A. Esteban y María Isabel Cuartero. Mención internacional.
- Sergio López García (2023). "Role of PI3-kinase regulatory subunit (p85) isoforms in synaptic plasticity". Departamento de Biología Molecular. Universidad Autónoma de Madrid. Director: José A. Esteban.



# LIPIDS IN NEURONAL PHYSIOLOGY AND PATHOLOGY

# **Group members**

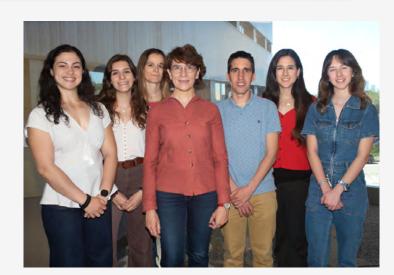
**Principal investigator:**María Dolores Ledesma Muñoz

**Postdoctoral fellows:** Marta Guerrero Valero Ángel Gaudioso Guirado

Predoctoral fellows: Ana Toledano Zaragoza Sara Naya Forcano Elena Melgarejo De la Peña Violeta Enriquez Zarralanga

**Undergraduate and Master students:**Pilar Moreno Huguet
Ana Villa María

**Visiting scientists:** Ameer Taha



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



# **Summary**

During the period 2023-24 our laboratory has focused on understanding the role in neurons of two abundant lipids in these cells, sphingomyelin (SM) and cholesterol. Using mouse models for two rare diseases in which these lipids accumulate, the acid sphingomyelinase deficiency (ASMD) and Niemann Pick type C (NPC), respectively, we have also unveiled pathological consequences of their alterations and assessed therapeutic strategies for these fatal disorders. We have shown that, among the different SM species, SM16:0 is the most accumulating and toxic for neurons lacking the acid sphingomyelinase (ASMko). Genetic silencing of the SM16:0 anabolic ceramide synthase 5, ameliorated behavioral alterations and brain pathology in ASMko mice, which mimic ASMD. Moreover, SM16:0 might be a good biomarker for brain pathology in this disease. We have also assessed the efficacy of different diets to diminish lipid overload in ASMD. While a diet lacking the SM precursor Choline did not show any benefit, oral intake of the polyphenol ellagic acid reduced SM levels and pathological hallmarks in the ASMko mouse brain by enhancing secretion of extracellular vesicles. Regarding cholesterol, we have demonstrated that accumulation of this lipid in lysosomes of NPC neurons leads to increased levels of the metabotropic glutamate receptor type 5 (mGluR5) in these organelles. Lysosomal mGluR5 remains active leading to the abnormal increase of mGluR5 dependent long-term depression (mGluR5-

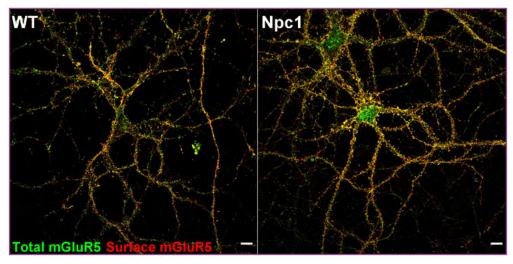
LTD). Pharmacological inhibition of intracellular mGluR5 in NPC neurons normalized mGluR5-LTD and ameliorated psychiatric alterations in the NPC mouse model. These results provide knowledge on the role of SM and cholesterol in synaptic plasticity and neuronal survival and open perspectives for the treatment of ASMD and NPC. Besides neuronal dysfunction and degeneration, these lysosomal storage disorders, show chronic neuroinflammation that worsens brain disease symptoms including motor, cognitive and psychiatric alterations. In collaboration with the visiting scientist Prof. Ameer Taha (University of California Davis, US) and Prof. Coral Barbas (CEMBIO, Madrid), we have set new methodology to quantify oxylipins, which are oxidized fatty acids involved in the resolution of inflammation. We propose that the altered oxylipin profile observed in the ASMko mouse brain contributes to neuroinflammation. We found low levels of pro-resolving oxylipins in the active freeform, while most oxylipins were found inactivated by esterification to other lipids. These results prompt us to target oxylipin metabolic pathways to fight neuroinflammation in ASMD and other neurological lysosomal storage disorders.



# Other activities

 MD Ledesma was appointed member of the Executive Committee of the Federation of European Neuroscience Societies (FENS) (June 2024)





Immunofluorescences against total mGluR5 (green) and surface mGluR5 (red) in cultured primary cortical neurons derived from wild type mice or mice deficient in the cholesterol transport protein Npc1. Scale bar= 10 micrometers



# List of publications

- Toledano-Zaragoza, A., Enriquez-Zarralanga, V., Naya-Forcano, S., Briz, V., Alfaro-Ruíz, R., Parra-Martínez, M., Mitroi D.N., Luján, R., Esteban, J.A., Ledesma M.D. (2024) Enhanced mGluR5 intracellular activity causes psychiatric alterations in Niemann Pick type C disease. Cell Death Dis. 15:771. doi: 10.1038/s41419-024-07158-8.
- Taha, A.Y., Gaudioso, Á., Moran-Garrido, M., Camunas-Alberca, S.M., Bachiller-Hernández, J, Sáiz, J., Ledesma, M.D., Barbas, C. (2024) Neurons regulate the esterification of bioactivelipid mediators in the brain of acid sphingomyelinase deficient mice. Prog Neuropsychopharmacol Biol Psychiatry. 129:110896. doi: 10.1016/j.pnpbp.2023.110896.
- Gaudioso, Á., Moreno-Huguet, P., Casas, J., Schuchman, E.H., Ledesma, M.D. (2023) Modulation of Dietary Choline Uptake in a Mouse Model of Acid Sphingomyelinase Deficiency. Int J Mol Sci. 24:9756. doi: 10.3390/ijms24119756.
- Soto-Huelin, B., Babiy, B., Pastor, O., Díaz-García, M., Toledano-Zaragoza, A., Frutos, M.D., Espín, J.C., Tomás-Barberán, F.A., Busto, R., Ledesma, M.D. (2023) Ellagic acid and its metabolites urolithins A/B ameliorate most common disease phenotypes in cellular and mouse models for lysosomal storage disorders by enhancing extracellular vesicle secretion. Neurobiol Dis. 182:106141. doi: 10.1016/j.nbd.2023.106141.
- Frutos, M.F., Pardo-Marqués, V., Torrecilla-Parra, M., Rada, P., Pérez-García, A., Martín-Martín, Y., de la Peña, G., Gómez, A, Toledano-Zaragoza, A., Gómez-Coronado, D., Casarejos, M.J., Solís, J.M., Rotllan, N., Pastor, Ó., Ledesma, M.D, Valverde, Á.M, Busto, R., Ramírez, C.M. (2023) MiR-7 controls cholesterol biosynthesis through posttranscriptional regulation of DHCR24 expression. Biochim Biophys Acta Gene Regul Mech. 1866:194938. doi: 10.1016/j.bbagrm.2023.194938.
- Gaudioso, Á., Jiang, X., Casas, J., Schuchman, E.H., Ledesma, M.D. (2023) Sphingomyelin 16:0 is a therapeutic target for neuronal death in acid sphingomyelinase deficiency. Cell Death Dis. 14:248. doi: 10.1038/s41419-023-05784-2.

■ Kalinichenko, L.S, Mühle, C., Jia, T., Anderheiden, F., Datz, M., Eberle, A.L, Eulenburg, V., Granzow, J., Hofer, M., Hohenschild, J., Huber, S.E., Kämpf, S., Kogias, G., Lacatusu, L., Lugmair, C., Taku, S.M., Meixner, D., Sembritzki, N.K., Praetner, M., Rhein, C., Sauer, C., Scholz, J., Ulrich, F., Valenta, F., Weigand, E., Werner, M., Tay, N., Mc Veigh, C.J., Haase, J., Wang, A.L., Abdel-Hafiz, L., Huston, J.P., Smaga, I., Frankowska, M., Filip, M., Lourdusamy, A., Kirchner, P., Ekici, A.B., Marx, L.M, Suresh, N.P., Frischknecht, R., Fejtova, A., Saied, E.M., Arenz, C., Bozec, A., Wank, I., Kreitz, S., Hess, A., Bäuerle, T., Ledesma, M.D., Mitroi, D.N., Miranda, A.M., Oliveira, T.G., Lenz, B., Schumann, G., Kornhuber, J., Müller, C.P. (2023) Adult alcohol drinking and emotional tone are mediated by neutral sphingomyelinase during development in males. Cereb Cortex. 33:844-864. doi: 10.1093/cercor/bhac106.



# Research projects

- PID2023-149557OB-I00. Plan Nacional I+D Ministerio Español de Ciencia e Innovación. Characterization and prevention of sphingomyelin-induced alterations in oligodendrocytes as a cause for neurodegeneration in lysosomal storage disorders. PI: MD Ledesma. (01/09/24-31/08/27)
- MDBR grant programme. University of Pennsylvania (US). Targeting the endocannabinoid system to treat epilepsy in Niemann Pick disease type C. PI: MD Ledesma. (01/02/23-31/01/24)
- Wylder Nation Foundation (US). Understanding and treating brain pathology in the acid sphingomyelinase deficiency. PI: MD Ledesma. (01/09/22-31/08/25)
- PID2020-112830RB-I00. Plan Nacional I+D, Ministerio Español de Ciencia e Innovación. Non-conventional approaches to understand and treat Niemann Pick diseases. PI: MD Ledesma. (01/09/21-31/08/24)



# ADULT NEUROGENESIS AND NEURODEGENERATIVE DISEASES

# **Group members**

**Principal investigator:**María Llorens-Martín

Scientific staff: Alba Ruiz Vinuesa

Postdoctoral fellows: Berenice Márquez-Valadez Julia Terreros Roncal Elena Moreno Jiménez

**Predoctoral fellows:** 

Julia Terreros Roncal Elena Moreno Jiménez Miguel de la Flor García Marta Gallardo Caballero Marta Alonso Moreno Ana V. Prádanos Senén Javier Molina Hernández **Technicians:** Carla Rodríguez Moreno

**Undergraduate and Master students:**Javier Molina Hernández
Evgenia Kokosali



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



#### **Summary**

Certain regions of the mammalian brain continue growing new neurons throughout life. This process, named adult neurogenesis, confers an extraordinary degree of plasticity to the central nervous system. The LlorensLab is focused on understanding the regulation of adult neurogenesis in distinct mammalian species. Our final goal is to exploit the potential of adult neurogenesis to counteract the consequences of physiological and pathological aging.

Our research group investigates the mechanisms that control adult hippocampal neurogenesis, both under physiological and pathological conditions. We perform in vivo and in vitro studies animal models of different diseases, and also use human brain samples. In particular, we are interested in determining the therapeutic potential of increasing adult hippocampal neurogenesis for the treatment of neurodegenerative diseases such as Alzheimer's disease (AD), Huntington's disease, Parkinson's disease, or ALS, and psychiatric conditions. The hippocampus plays a pivotal role in learning and memory. Furthermore, a unique feature of this structure is the existence of adult neurogenesis. The process of adult hippocampal neurogenesis encompasses the birth and functional integration of newborn neurons throughout lifetime. Adult hippocampal circuit confers and extraordinary degree of plasticity to the circuit. Thus, we work to develop strategies capable of increasing the functionality of newborn neurons.



# Other activities

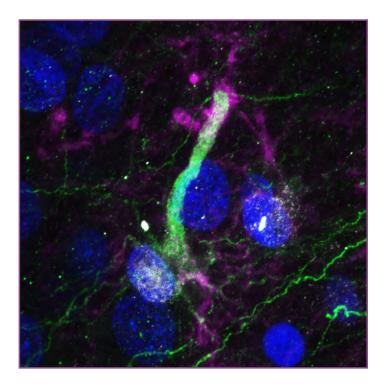
#### 2023:

- Cátedra VIU-NED en Neurociencia Global y Cambio Social, Best doctoral thesis award Julia Terreros-Roncal.
- Best Doctoral Thesis Award "Premio Fundación Lilly a la Mejor Tesis Doctoral en Bioquímica y Biología Molecular". Julia Terreros-Roncal.
- Mención Honorífica de Doctorado (Premio Extraordinario de Doctorado) curso 2022/2023. Julia Terreros-Roncal.
- Mención Honorífica de Doctorado (Premio Extraordinario de Doctorado) curso 2022/2023. Miguel de la Flor García.
- Premio Jóvenes Investigadores en Neurociencias -Instituto de Neurociencias "Federico Olóriz" de Granada. Elena Moreno-liménez

#### 2024:

- Boehringer-Ingelheim/FENS Research Award, Boehringer-Ingelheim Foundation and FENS. María Llorens-Martín
- Premio Carmen y Severo Ochoa de Investigación en Biología Molecular, Fundación Carmen y Severo Ochoa. María Llorens-Martín.
- Premio Real Academia de Doctores de España en Ciencias de la Vida y de la Salud.
- Premio PINP "Premio a la mejor Tesis Doctoral" Centro de Biología Molecular "Severo Ochoa" (CBMSO-CSIC)
- Primer Premio de Investigación Científica y Tecnológica Margarita Salas, Categoría Ciencias de la Vida -Ayuntamiento de Madrid y Fundación Margarita Salas
- Áccesit Premio Fundación Lilly a la Mejor Tesis Doctoral en Bioquímica y Biología Molecular - Sociedad Española de Bioquímica y Biología Molecular (SEBBM)
- Best Poster Award at 5th Eurogenesis Meeting Brain Organoid & Systems Neuroscience Journal.





Human hippocampal neural stem cell



- Rodríguez-Moreno CB, Cañeque-Rufo H, Flor-García M, Terreros-Roncal J, Moreno-Jiménez EP, Pallas-Bazarra N, Bressa C, Larrosa M, Cafini F, Llorens-Martín M (2024). Azithromycin preserves adult hippocampal neurogenesis and behavior in a mouse model of sepsis. Brain Behav Immun. Mar;117:135-148. doi: 10.1016/j.bbi.2024.01.005.
- Gallardo-Caballero M, Rodríguez-Moreno CB, Álvarez-Méndez L, Terreros-Roncal J, Flor-García M, Moreno-Jiménez EP, Rábano A, Llorens-Martín M. Prolonged fixation and post-mortem delay impede the study of adult neurogenesis in mice (2023). Commun Biol. Sep 23;6(1):978. doi: 10.1038/s42003-023-05367-z.
- Moreno-Jiménez EP, Flor-García M, Hernández-Vivanco A, Terreros-Roncal J, Rodríguez-Moreno CB, Toni N, Méndez P, Llorens-Martín M. GSK-3β orchestrates the inhibitory innervation of adult-born dentate granule cells in vivo (2023). Cell Mol Life Sci. Jul 23;80(8):225. doi: 10.1007/ s00018-023-04874-w.
- Llorens-Martín M. (New neurons) singing in the avian brain (2023). Nat Rev Neurosci. Jun;24(6):333. doi: 10.1038/s41583-023-00695-9.
- Terreros-Roncal J, Flor-García M, Moreno-Jiménez EP, Rodríguez-Moreno CB, Márquez-Valadez B, Gallardo-Caballero M, Rábano A, Llorens-Martín M (2023). Methods to study adult hippocampal neurogenesis in humans and across the phylogeny. Hippocampus. Apr;33(4):271-306. doi: 10.1002/hipo.23474.



# Research projects

- PID2023-1465720B-I00. Spanish Ministry of Science and Innovation: Proyectos de Generación del conocimiento. PI: María Llorens-Martín. 09/01/2024 08/31/2027.
- A2024021S. Alzheimer's disease Research Grant (BrightFocus Foundation, USA), PI: María Llorens-Martín. 07/01/2024 – 06/30/2027.
- ERC-CoG-2020-101001916. ERC Consolidator Grant 2020 (European Commission), PI: María Llorens-Martín. 10/01/2021 09/30/2026.
- PID2020-113007RB-I00. Spanish Ministry of Science and Innovation: Programa Estatal I+D+i orientada a los retos de la sociedad. PI: María Llorens-Martín. 09/01/2021 08/31/2024.



#### **Doctoral theses**

- Miguel de la Flor García (2023). Neurogénesis hipocampal adulta en seres humanos. Efectos del envejecimiento y las enfermedades neurodegenerativas. Departamento de Biología Molecular, Universidad Autónoma de Madrid. Directora: María Llorens-Martín.
- Elena Moreno Jiménez (2023). Proceso de neurogénesis hipocampal adulta en la especie humana. Alteración de la integración de las nuevas neuronas granulares en condiciones patológicas. Departamento de Biología Molecular, Universidad Autónoma de Madrid. Directora: María Llorens-Martín.



# PHYSIOPATHOLOGICAL ASPECTS OF GLYCINE TRANSPORTERS IN GLYCINERGIC NEUROTRANSMISSION: HYPEREKPLEXIA AND PAIN

# **Group members**

**Principal investigator:**Beatriz López-Corcuera (PI)
Francisco Zafra (co-PI)

Predoctoral fellows: Raquel Felipe Mendía Chiara D'Agostino Sara Orugo Medina Jorge Sarmiento-Jiménez

**Undergraduate and Master students:** Araceli Vázquez Medel Beatriz Morales González



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



#### Summary

Our group studies the physiological and pathological aspects of glycinergic neurotransmission including hyperekplexia (HPX) and pain. HPX (OMIM 149400) is a rare neurological syndrome of perinatal clinical importance caused by deficient glycinergic inhibition. It is caused by mutations in crucial proteins for glycinergic neurotransmission, such as the neuronal glycine transporter GlyT2 (SLC6A5 gene). Our work aims to understand the effects of GlyT2 mutations in HPX patients. Bioinformatics tools will guide intervention strategies on the protein (folding, traffic or activity), aiming to rescue mutant phenotypes or produce modulatory ligands with diverse applications, including analgesia. Our experimental systems involve cell lines, neuronal cultures from wild-type and mutant mice, Xenopus laevis oocytes, and Zebrafish. The research delves into the 3D structure, intracellular trafficking, regulation and developmental consequences of the GlyT2 variants. We also wish to unravel new transporter interactome as it influences transporter trafficking. For this we are optimizing new evolutions of the proximity labelling technique suitable for its use in native tissue coupled to a proteomic analysis. By using biochemical and proteomics techniques, we have identified during this period a new GlyT2 variant found in a child patient with HPX and we have characterized it as a loss-of-function variant with respect to transporter activity, but a gain-offunction variant that alters cellular proteostasis,

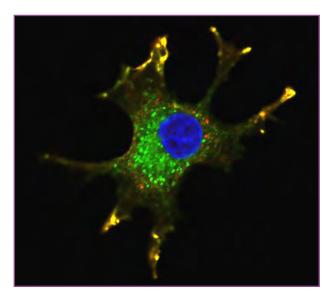
activating the unfolded protein response (UPR) and interfering with processes occurring in lipid rafts. Another aspect of our research aims to understand the severe phenotype of HPX in the neonatal period and its posterior partial relief. We have characterized the role of GlyT2 in perinatal development by showing that stable expression of GlyT2 in PC12 cells leads to the activation of the ERK and CREB pathways involved in neuronal differentiation, elevation of intracellular calcium concentration, and increased number and length of neurites. Our results clearly implicate GlyT2 in growth cone progression and differentiation of PC12 cells into neurons. Finally, we have demonstrated the regulation of GlyT2 by the Hedgehog pathway can be emulated by D2 dopamine receptor agonists, what indicates glycinergic interneurons containing GlyT2 receive dopaminergic inputs in response to the Hedgehog pathway activation. The biological significance of this regulation in the control of peripherally acting neuronal nuclei is being further investigated.



#### Doctoral theses

Raquel Felipe Mendía. 26 octubre 2023. Regulación y modificación del transportador neuronal de glicina GlyT2. Departamento de Biología Molecular. Universidad Autónoma de Madrid. Supervisors: Beatriz López Corcuera. Emilio Camafeita Fernández.







- Piniella D, Canseco A, Vidal S, Xiol C, Díaz de Bustamante A, Martí-Carrera I, Armstrong J, Bastolla U, Zafra F. (2023) Experimental and Bioinformatic Insights into the Effects of Epileptogenic Variants on the Function and Trafficking of the GABA Transporter GAT-1. Int J Mol Sci. 24(2):955. doi: 10.3390/ijms24020955.
- Piniella D, Zafra F. (2023) Functional crosstalk of the glycine transporter GlyT1 and NMDA receptors. Neuropharmacology. 232:109514. doi: 10.1016/j.neuropharm.2023.109514.
- Arribas-Carreira L, Castro M, García F, Navarrete R, Bravo-Alonso I, Zafra F, Ugarte M, Richard E, Pérez B, Rodríguez-Pombo P. (2024) Metabolic Rewiring and Altered Glial Differentiation in an iPSC-Derived Astrocyte Model Derived from a Nonketotic Hyperglycinemia Patient. Int J Mol Sci. 25(5):2814. doi: 10.3390/ijms25052814.
- Felipe R, Sarmiento-Jiménez J, Camafeita E, Vázquez J, López-Corcuera B. (2024) Role of palmitoylation on the neuronal glycine transporter GlyT2. J Neurochem.168(9):2056-2072. doi: 10.1111/jnc.16181.



# Research projects

- PID2023-150608OB-I00. Pathologies of the neuronal glycine transporter GlyT2: hyperekplexia and pain. implications on development. Ministry of Science and Innovation. PI: B. López Corcuera/F. Zafra. 1/9/2024-1/9/2027. 180.000,00 €.
- PID2020-119399RB-I00. The neuronal glycine transporter GlyT2 in pain and in hyperekplexia: pathological implications in development. Ministerio de Ciencia e Innovación. PI: B. López Corcuera. 2021-2024. 145.000,00 €.
- CIVP20A6612, Fundación Ramón Areces. El transportador neuronal de glicina GlyT2 en hiperplexia: una patología glicinérgica del desarrollo. PI: B. López Corcuera. 01/05/2021 to 01/05/2024. 78.800,00 €.

PC12 cells stably expressing GlyT2 differentiated with NGF. GlyT2, green. GAP43, red. DAPI, blue.



# Other activities

■ The group is member of the Research Group "Involvement of Glycinergic and Glutamatergic Systems in Pathologies of the Central Nervous System" of the IdiPAZ Biosanitary Research Institute.

#### Beatriz López Corcuera:

- Deputy Director of the Severo Ochoa Molecular Biology Center since March 27, 2023.
- Coordinator of the Final Degree Project for the Bachelor's Degree in Biochemistry (2013-2023). 80 students per academic year.
- Teaching: University Professor since October 2021 with teching duties since 1993. Theoretical and practical courses in 23-24 in the degrees in Biochemistry and Food Science and Technology. This includes formal lectures, seminars, laboratory instruction and individual supervision of students.
- Two Final Degree Projects supervised in 23-24.

#### Francisco Zafra:

- Member of the Advisory Committee of SLC6A1-Spain (Spanish association of relatives and people affected by epileptic and developmental encephalopathy in the SLC6A1 gene).
- Teaching: University Professor since January 2008 has held various academic positions in the organizational chart of the Department of Molecular Biology, teaching the degrees in Biology and Biochemistry, as well as the Master's degree in Biomolecules and Cell Dynamics. Until October 2023 he was coordinator of the PhD program in Molecular Biosciences.



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

# **Group members**

**Principal investigator:** José Javier Lucas Lozano

**Postdoctoral fellows:** Julia Pose Utrilla

Predoctoral fellows: Ivana Ollà Claudia Rodríguez López David Lozano Muñoz Alejandra Arroyo García

**Technicians:**Miriam Lucas Santamaría
Irene Ruiz Blas
Sandra García Pereira

**Undergraduate and Master students:** Marta Coiduras del Olmo Avetik Arsen Khachatryan



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



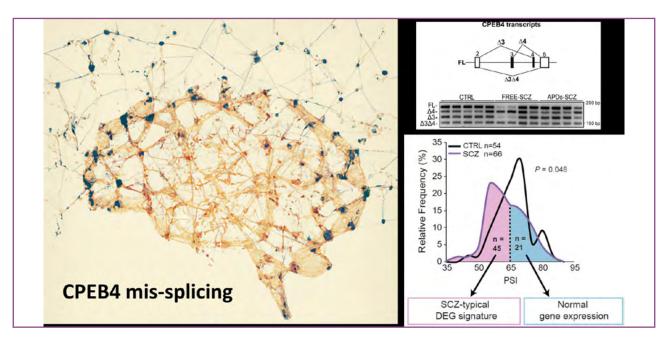
#### Summary

In our laboratory, we investigate the molecular bases of brain diseases by generating and characterizing genetically modified mouse models that recapitulate pathogenesis, as well as neuropathology and symptoms (construct and face validity), and often allow the characterization of existing therapies (predictive validity) and the testing of new ones.

Recently, we have focused on abnormal RNA processing. We found alterations in splicing factors and aberrant alternative splicing events in Huntington's disease (HD) (Fernández-Nogales et al., Nat Med, 2014; Cabrera JR & Lucas, Brain Pathol., 2017; Hernández et al., Brain, 2020) and Alzheimer's disease (AD) (García-Escudero et al., Acta Neuropathol., 2021). The relevance of the human/mouse intersection RNAseq method we developed to establish the global mis-splicing signature in postmortem HD brain (Elorza et al., Brain, 2021) was highlighted in the review by Xing et al. in Trends Neurosci., 2021.

We also observed alterations in microexon splicing and regulated transcriptome polyadenylation in autism (Parras et al., Nature, 2018; García-Cabau et al., Nature, 2025), schizophrenia (Ollà et al., Biol Psychiatry, 2023), epilepsy (Parras et al., Brain, 2020; de Diego-García et al., Epilepsia, 2023), and HD (Picó S, et al., Sci Transl Med., 2021). The latter evidenced a cerebral thiamine deficiency in HD, leading to a patent and the ongoing clinical trial HUNTIAM (NCT04478734). Recently, we discovered a TAF1 alteration in HD that revealed a key role for this protein in multiple sclerosis.





Reduced inclusion of the CPEB4 microexon in schizophrenia (SCZ) brains correlates with lower CPEB4-target SCZ risk gene expression, particularly in individuals not on antipsychotics. In SCZ cases with PSI<65, differentially expressed gene signatures align with known SCZ profiles



- Li, X., Hernandez I., Koyuncu S., Kis B., Haggblad M., Lidemalm L., Abbas A. A., Bendeguz S., Goblos A., Brautigam L.et al. (2024) The anti-leprosy drug clofazimine reduces polyQ toxicity through activation of PPARgamma. EBioMedicine 103, 105124. doi: 10.1016/j. ebiom.2024.105124
- Martinez-Pizarro, A., Pico S., Lopez-Marquez A., Rodriguez-Lopez C., Montalvo E., Alvarez M., Castro M., Ramon-Maiques S., Perez B., Lucas J. J.et al. (2024) PAH deficient pathology in humanized c.1066-11G>A phenylketonuria mice. Hum Mol Genet 33, 1074-1089. doi: 10.1093/hmg/ddae051
- Olla, I., Pardinas A. F., Parras A., Hernandez I. H., Santos-Galindo M., Pico S., Callado L. F., Elorza A., Rodriguez-Lopez C., Fernandez-Miranda G.et al. (2023) Pathogenic Mis-splicing of CPEB4 in Schizophrenia. Biol Psychiatry 94, 341-351. doi: 10.1016/j.biopsych.2023.03.010
- de Diego-Garcia, L., Brennan G. P., Auer T., Menendez-Mendez A., Parras A., Martin-Gil A., Mitra M., Olla I., Villalba-Benito L., Gil B.et al. (2023) CPEB4-CLOCK crosstalk during temporal lobe epilepsy. Epilepsia 64, 2827-2840. doi: 10.1111/epi.17736



# Research projects

■ PID2021-1231410B-I00, Altered RNA processing at the convergence of Huntington's disease and other neural diseases. Ministerio de Ciencia e Innovación. NED2022/03, Analysis of thiamine deficiency in Huntington's for disease progression biomarker and assessment of therapeutic response. CIBERNED Part of the Networking Research Center on Neurodegenerative Diseases (CIBERNED) (https://www.ciberned.es/qrupos/qrupo-de-investigacion?id=28582)



# **Doctoral theses**

Papel de las CPEBs y de la poliadenilación anómala en la esquizofrenia. Ivana Ollá. Universidad Autónoma de Madrid. January 2023.



#### **Patents**

Jose J. Lucas; Claudia Rodriguez Lopez; Ivo H. Hernandez. Method for the diagnosis of multiple esclerosis España. Aplication N: EP24382907.4. Tittle holder: CSIC. Priority Country: Spain. Priority date: 14 August 2024.



# NEUROEPIGENETICS OF BRAIN PHYSIOLOGY AND PATHOLOGY

# **Group members**

**Principal investigator:** Ernest Palomer Vila

**Predoctoral fellows:** Lucía Cañizares Moscato

**Undergraduate and Master students:** 

Alejandro Novo Merlo Inés Cabrera Martorell Jose Ruiz Iglesias



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



# **Summary**

We launched the lab in January 2023 with the goal of understanding how different brain cell types alter their expression patterns through epigenetic mechanisms during Alzheimer's disease (AD) progression. By deciphering these changes, we aim to develop strategies to reverse aberrant epigenetic modifications, restore cellular function, and mitigate memory loss. Ultimately, our mission is to identify new molecular targets to halt the progression of this devastating disease.

To achieve this, we developed EpiFlow: a cuttingedge multiplexed flow cytometry approach that enables the simultaneous analysis of multiple epigenetic marks alongside the identification of major brain cell types at the single-cell level. Our initial EpiFlow findings demonstrated significant promise, prompting us to expand its application across eukaryotic systems, including various mouse tissues, and to explore its potential in epigenetic drug discovery. Beyond its research applications, EpiFlow holds great promise for clinical translation, which could be used as a biomarker or prognostic tool and may play a pivotal role in identifying novel epigenetic treatments.



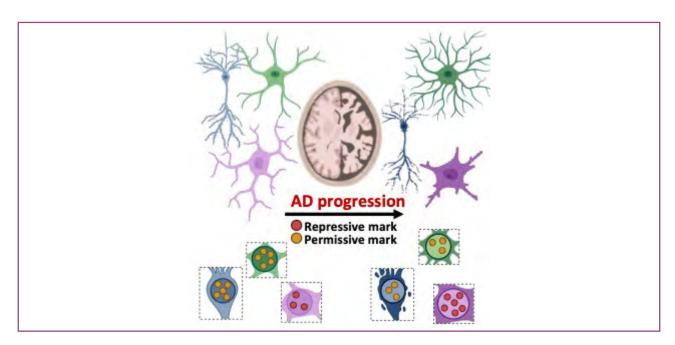


Illustration of our research goals where epigenetic changes favour Alzheimer's disease-associated aberrant cellular phenotypes during AD progression.



# List of publications

- Carús-Cadavieco, M., González de la Fuente, S., Berenguer López, I., Serrano-Lope, M.A., Aguado, B., Guix, F.X., Palomer, E., Dotti, C.G. (2024). Loss of Cldn5 -and increase in Irf7-in the hippocampus and cerebral cortex of diabetic mice at the early symptomatic stage. Nutr. Diabetes. doi: 10.1038/s41387-024-00325-y
- Casadomé-Perales, Á., Naya, S., Fernández-Martínez, E., Mille, B.G., Guerrero-Valero, M., Peinado, H., Guix, F.X., Dotti, C.G., Palomer, E. (2023) Neuronal Prosurvival Role of Ceramide Synthase 2 by Olidogendrocyte-to-Neuron Extracellular Vesicle Transfer. Int. J. Mol. Sci. doi: 10.3390/ ijms24065986



# Research projects

- EUR2024-153539. Agencia Estatal de Investigación. Identificación de metilación de histonas específicas de tipo celular en la enfermedad de Alzheimer: nuevas dianas para la intervención terapéutica. Ernest Palomer. 01/12/2024-30/11/2026.
- PID2022-137384OA-I00. Agencia Estatal de Investigación. Studying the cell-type role of KMT5s in Alzheimer's disease: new therapeutical targets. Ernest Palomer. 01/09/2023-31/08/2026.
- RYC2021-031713-I. Agencia Estatal de Investigación. Studying the role of H4K20 methylation and methyltransferases in AD; new targets for therapeutic Intervention. Ernest Palomer. 01/01/2023-31/12/2028.



# BIOLOGY OF HUMAN STEM CELLS IN TRANSLATIONAL NEUROSCIENCE

# **Group members**

**Principal investigator:** Marta P. Pereira

**Postdoctoral fellows:** Cristina Ulecia Morón

Predoctoral fellows: Marina Rodriguez Rubio Brina Stancic Miguel Esteban Lucía Rafael Romero Pérez

**Technicians:** Paula Paredes Mayoral

**Undergraduate and Master students:** Ines Anguiano

Visiting scientists: Andrea Corsi



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



# Summary

Research in animal models of neurodegenerative diseases has shown that stem cells exert beneficial effects mainly through brain plasticity rather than direct cell replacement. However, response variability across species highlights the need for human-specific studies due to the unique characteristics of the human brain.

Our research investigates the biology of stem cells as a source of human neurons and their therapeutic applications in neurological disorders, particularly Parkinson's disease. A key aspect is generating A9 dopaminergic neurons, significantly impacted in Parkinson's. To optimize neuronal cultures, we analyzed various surfaces, identifying the critical roles of material composition and topography at nano- and microscale levels in developing innovative culture systems.

Stem cells are essential for developing research models that improve understanding of cell biology, disease processes, and advanced therapies. This is particularly relevant for the human central nervous system, where accessibility is limited. Given their ability to self-renew and differentiate, stem cells require precise regulation of growth and development mechanisms. These processes are influenced by biochemical and physical factors, leading to techniques using specialized materials and micro/nanostructured surfaces. Building on these insights, we developed human

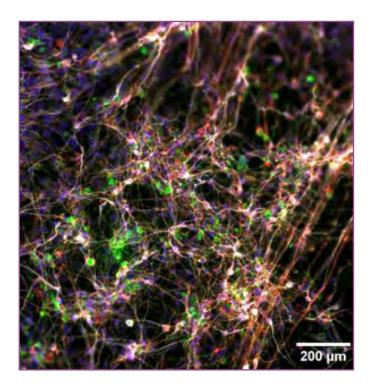
brain organoids to improve knowledge of neuronal circuits and diverse neuron types. This aligns with global efforts to reduce reliance on animal models while advancing neural tissue research. By refining cerebral organoids with improved structural features, we enhanced their utility in preclinical studies.

Current projects optimize scaffold designs by incorporating materials and textures validated in 2D cultures, fostering neuronal growth and supporting electrical conductivity. Advances in micro- and nanoscale fabrication enable precise substrate modifications, influencing how cells interact with their environment. In neural stem cells, surface patterns affect their ability to grow, divide, and develop into specialized types.

Additionally, topographical modifications help guide neuronal organization, mimicking spatial arrangements in the human brain. Unlike traditional cultures, where neurons grow randomly, structured surfaces promote orderly growth, crucial for neural network development.

Our research aimed to advance stem cell therapies by improving understanding of human neuron responses. The ultimate goal remains to develop brain organoid models that replicate human brain complexity, providing valuable platforms for studying neurological disorders and exploring new treatments.





Neuronas dopaminérgicas derivadas de células madre humanas



- Nelke A, García-López S, Caso JR, Pereira MP. The therapeutic use of clonal neural stem cells in experimental Parkinson's disease. Stem Cell Res Ther. 2024 Oct 9;15(1):356. doi: 10.1186/s13287-024-03965-0
- Alcover-Sanchez, B., et al., R-Ras1 and R-Ras2 regulate mature oligodendrocyte subpopulations.Glia, Epub 2024 Nov 19 doi: 10.1002/glia.24643.
- Pereira MP, García-Bueno B, Caso JR. Editorial: Neuroinflammatory and oxidative/nitrosative pathways in neuropsychiatric and neurological diseases and their possible neuropharmacological regulation, Volume II. Front Pharmacol. 2023 Mar 31;14:1191907. doi: 10.3389/ fphar.2023.1191907.
- Vasudevan, S., et al., Omnidirectional leaky optoelectrical fiber for optogenetic control of neurons in cell replacement therapy. Bioelectrochemistry, 2023. 149: p. 108306



#### **Doctoral theses**

- Marina Rodríguez Rubio (2023) Bioingeniería de organoides cerebrales. Dept. Biología Molecular, UAM. Director: Marta Pérez Pereira. Mención internacional.
- Miguel Esteban Lucía (2024) Micro/nanoscale topographical cues in stem cell differentiation and alignment. Dept. Biología Molecular, UAM. Directoras: Marta Pérez Pereira (UAM), Isabel Rodríguez Fernández (IMDEA-Nanociencia).



# Research projects

- GA-101047177, HORIZON-EIC-2021-PATHFINDEROPEN-01, Opto-Electronic Neural Connectoid Model Implemented for Neurodegenerative Disease (OpenMIND). Coordinator: Jenny Emnéus. PI Node UAM Marta P Pereira. 01/11/2022-30/10/2025.
- PID2020-118189RB-I00, AEI, Ministry of Science and Innovation. Brain organoids: development and complexity.PI Marta P Pereira 01/09/2021- 28/02/2025. Projects as collaborator
- PID2022-137932NB-I00, AEI, Ministry of Science and Innovation. Barrier permeability and neuroinflammation in the context of neuropsychiatric diseases. A preclinical and clinical approach. PI: Juan C Leza (UCM). 01/09/2023-31/08/2027.
- PID2020-113103RB-I00, AEI, Ministry of Science and Innovation, Mitofagia, innate immunity, and oxidative stress in psychotic illness and major depression: characterization of their role and possible pharmacological and epigenetic modulation. PI: Javier R Caso (UCM). 01/09/2021-31/12/2024.



# NEURAL STEM CELLS IN THE ADULT BRAIN

# **Group members**

**Principal investigator:**Eva Porlan

**Predoctoral fellows:** Coral López Fonseca

**Technicians:** Beatriz Ortigosa

**Undergraduate and Master students:** 

Laura Isabel Aguilar

Marta Chico

Raquel Sáez



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

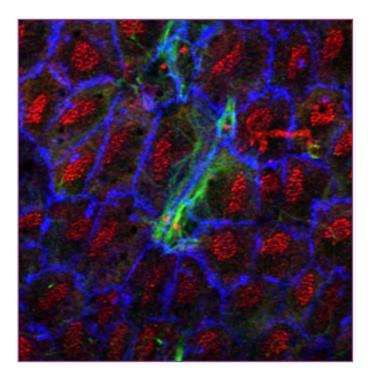


#### Summary

In adult vertebrates, somatic stem cells (SC) are selfrenewing and multipotent undifferentiated cells, that maintain the integrity of the host tissue and offer a potential source of cells for regeneration after injury at young ages. SC are defined by their ability to self-perpetuate whilst being the source of tissue-specific differentiated progeny. Impairing the homeostatic balance between SC self-renewal and differentiation paves the way to either tissue functional and structural impairment and may lead to tumorigenesis. SC have been found in the mammalian central nervous system, where they contribute to the homeostatic balance by addition of new neurons and glial cells to the brain once development has concluded. Within specialized niches, the microenvironments where they dwell, neural stem cells (NSC), derived progenitors and differentiated progeny are stratified within a highly regulated hierarchy and coordinated to maintain the necessary cellular production to uphold adult tissue renewal. Notwithstanding NSC contribution to homeostasis, their regenerative capacity is limited and unable to replace lost neural populations to induce a real functional recovery in situations of brain damage. However, since they can activate in reaction to some types of lesions, the population of NSC is regarded as a potential cellular target for regenerative medicine.

In our group, we focus on the molecular characterization of the pathways involved in mammalian NSC self-renewal and differentiation to neuronal and glial populations. To this end, we use tissue specific conditional and inducible loss- and gain-of-function mouse models. We are mostly interested in pinpointing molecular targets to effectively manipulate these processes on demand. This strategy is a very promising venue for the future of cell replacement therapies, since it holds the potential for exploiting NSC and their progeny for replacement/regenerative therapeutic interventions, in physiological ageing or pathological situations stemmed from neurodegeneration or demyelination.





Neural stem cells in the mouse lateral ventricle (green) amidst a field of multiciliated ependymal cells (blue)



# List of publications

- Del Puerto A, Lopez-Fonseca C, Simon-Garcia A, Marti-Prado B, Barrios-Munoz AL, Pose-Utrilla J, Lopez-Menendez C, Alcover-Sanchez B, Cesca F, Schiavo G, Campanero MR, Farinas I, Iglesias T\*, Porlan E\*. Kidins220 sets the threshold for survival of neural stem cells and progenitors to sustain adult neurogenesis. Cell Death & Disease 14, no. 8 (Aug 4, 2023): 500. DOI: 10.1038/s41419-023-05995-7
- Domingo-Muelas A, Morante-Redolat JM, Moncho-Amor V, Jordan-Pla A, Perez-Villalba A, Carrillo-Barbera P, Belenguer G, Porlan E, Kirstein M, Bachs O, Ferron SR, Lovell-Badge R, Farinas I. The rates of adult neurogenesis and oligodendrogenesis are linked to cell cycle regulation through p27-dependent gene repression of SOX2. Cell Mol Life Sci. 2023;80(1):36. DOI: 10.1007/s00018-022-04676-6
- Sirerol-Piquer MS, Morante-Redolat JM, Porlan E. Editorial: "Unravelling neural stem cell biology: players and strategies". Front Cell Dev Biol. 2023;11:1206339. DOI: 10.3389/fcell.2023.1206339



# Research projects

- PID2023-146945NB-I00. AEI and ERDF (EU). Pharmacological targets in neural stem cell fate decisions: implications for regeneration. PI: Eva Porlan. 01/09/2024- 31/08/2027
- PID2019-104763RB-I00. AEI. Novel druggable regulators of adult neurogenesis and direct lineage reprogramming: implications for regeneration. PI: Eva Porlan. 06/01/2020-05/31/2023.
- Collaborating group CIBERNED-CB06/05/1122 IdiPaz



#### **Doctoral theses**

Lopez Fonseca, Coral (2024). Efectos de la quinasa Plk1 en la regulación de factores de transcripción proneurales. Departamento de Biología Molecular, UAM. Directora: Porlan Alonso, Eva



# THE GENETICS OF PSYCHIATRIC DISEASES

# **Group members**

**Principal investigator:** Claudio Toma

**Predoctoral fellows:**Miriam Martínez Jiménez
Inés Garcia Ortiz

**Technicians:** Sergio Espartero Boza Raúl Somavilla Cabrero

Undergraduate and Master students: Miriam Martínez Jiménez Inés García Ortiz Jose Ignacio Gómez Blanco (supervisor; co-supervisor: Esther Serrano-Saiz) Ángela Calzado González (co-supervisor; supervisor: Javier Gilabert) Andrea Cendón Durán Cristina Rodríguez-Osorio Puigdueta Susana Inés Rodríguez Sanz Alessia Ruggiero

**Visiting scientists:** Blanca Sánchez Moreno (from Dr. Javier Gilabert's lab)



http://www.cbm.uam.es/claudio.toma



#### Summary

Our research group is dedicated to advancing psychiatric genetics by identifying susceptibility genes involved in bipolar disorder (BD), schizophrenia (SCZ), and suicide (SUI), while developing novel translational tools. We employ cutting-edge genomic approaches such as whole-exome sequencing, whole-genome sequencing, RNA sequencing, and genome-wide association studies (GWAS) to uncover both common and rare variants associated with psychiatric conditions. By integrating genetic risk profiling, digital phenotyping, and machine learning, we aim to enhance precision medicine in psychiatry.

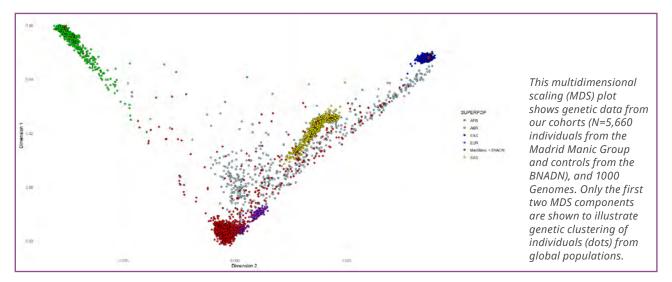
In 2022, we established the Madrid Manic Group (MadManic), a collaborative Spanish node in psychiatric genetics that brings together geneticists, bioinformaticians, psychiatrists, and clinicians. The translational medicine aims of our studies are based on a cohort, comprising over 3,000 individuals and a collection of >9,000 biospecimens for high-throughput arrays from psychiatric patients and controls. Currently, the genomic data available from our MadManic cohort includes genome-wide SNP data for 5,700 individuals, which includes the largest sample for BD and suicide in Southern Europe, and RNA sequencing of >700 subjects of BD patients and controls. The genomic data are combined with

extensive phenotyping for translational aims, using the MeMind platform, which records over 2,000 clinical variables for bipolar patients. Additionally, the SMARTcrisis 3.0 clinical trial through smartphone apps (MeMind and eB2) continuously track suicidality monitoring sleep, mood fluctuations, and other behavioral markers.

Through this initiative, we drive innovative medical intervention strategies to tackle key clinical challenges: i) Develop methods for identifying psychiatric patients at high risk of suicidal behavior through machine learning approaches, mobile device data, and individual genetic profiles; ii) Reduce the time required to identify the most effective pharmacological treatment for BD patients, preventing symptom worsening and reducing suicide risk; iii) Expand collaborative networks to advance genetic research in BD and suicide, lithium pharmacogenomics, and clinical studies of BD.

Our goal is to develop innovative strategies for identifying high-risk individuals, predicting treatment response, and improving medical interventions for major psychiatric disorders.







- O'Connell KS, et al., (2023) Genetic diversity enhances gene discovery for bipolar disorder. doi. org/10.1101/2023.10.07.23296687
- Ollà I., et al., (2023) Pathogenic mis-splicing of CPEB4 in schizophrenia. Biological Psychiatry 94 (4), 341-351 doi. org/10.1016/j.biopsych.2023.03.010
- Zwicker A., et al. (2023) Polygenic scores and onset of major mood or psychotic disorders among offspring of affected parents. American Journal of Psychiatry 180 (4), 285-293. doi.org/10.1176/appi.ajp.20220476
- Koromina M., et al., (2024) Fine-mapping genomic loci refines bipolar risk genes. MedRxiv doi.org/10.1101/2024 .02.12.24302716.
- Rodríguez-López C., et al. (2024) TAF1-dependent transcriptional dysregulation underlies multiple sclerosis. doi.org/10.1101/2024.08.23.609325



#### Other activities

XII Cajal Conference 22-24 May 2024 (Society of Spanish Neuroscience, SENC) (co-organized with Serrano-Saiz E and Beccari L)



# Research projects

- PID2023-149154OB-I00. Ministerio de Ciencia e Innovación (Proyectos I+D+i): RNA sequencing (RNAseq) for personalized medicine in bipolar disorder: gene expression analyses and pharmacotranscriptomics applied to treatment response of lithium. PI (N: 1): Toma C. 2024-27
- PMP24/00026. Instituto de Salud Carlos III (Proyectos de Investigación Orientados a la Implantación de la Medicina Personalizada de Precisión): SMARTomicS: prevention of the suicide behaviour through guided therapeutic interventions via digital phenotyping and pharmacogenomics. Coordinator: Baca-Garcia E. 2024-2026
- LINK24026. I-LINK program CSIC: Clinical, genetic and pharmacogenomics analyses to advance in the understanding of bipolar disorder. PIs (N: 5): Toma C, Fullerton J, Papiol Miró S, Paracchini S, Schofield P. 2024-26.
- CNS2022-135318. Ministerio de Ciencia e Innovación (Proyectos Consolidación): Pioneering approaches in personalized medicine applied to bipolar disorder using genomic profiles, transcriptomics and telemedicine. PI (N: 1): Toma C; 2023-25.

- Fundación Alicia Koplowitz: Combined genetic and functional studies to establish the role of DMRTA1 and DMRTA2 in susceptibility to attention deficit-hyperactivity disorder (ADHD) and its sexual skewing. PIs (N: 2): Serrano E, Toma C; 2023-24.
- PID2020-114996RB-I00. Ministerio de Ciencia e Innovación (Proyectos I+D+i): Advances in understanding bipolar disorder through the combination of digital phenotyping and genome-wide approaches. PIs (N: 1): Toma C; 2021-24.
- 2020/MRF1200428. NHMRC & Medical Research Futures Fund Grant (Australia): A multifaceted approach to the pharmacogenomic signatures of bipolar disorder for improving treatment outcomes phenotyping and genome-wide approaches. PIs (N: 4): Fullerton J, Green M, Scholfied P, Toma C; 2021-25.

#### Networks:

- Psychiatric Genomics Consortium Bipolar Disorder (PGC-BD)
- Psychiatric Genomics Consortium Suicide (PGC-SUI)
- Global Bipolar Cohort (GBC)
- Consortium on Lithium Genetics (ConLiGen) (http://www.conligen.org/mem-es.html)
- WeSEQ (Sequencing of patients with severe forms of mental conditions)



# MOLECULAR MECHANISMS OF NEURODEGENERATION

# **Group members**

**Principal investigator:** Francisco Wandosell

Scientific staff: Lara Ordoñez Gutierrez

Postdoctoral fellows: Sergio Rivas Muñoz

**Predoctoral fellows:**Alba Orantes
Jorge de los Santos Galan

**Undergraduate and Master students:** Violeta Sebastián Serrano Ana Isabel Zabik



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



# Summary

Our group, "Molecular mechanisms of Neuro-degeneration", is a stablish line in the CBM over several years. 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. We are interested in describe common molecular mechanisms underlying the age-dependency of these brain disorders. Data from different animal models strongly support that 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 supports the hypothesis that proteostasis is altered in AD. We analyse the role of mTORC1 and AMPK activities in the generation of amyloid in AD transgenic models. Both protein complex controls important aspects of protein synthesis and macroautophagy. Our data indicated mTORC1 inhibition reduced that beta amyloid accumulation in AD mouse model brains, ex vivo and in vivo. In contrast, AMPK activation did not support a similar effect on either in macroautophagy or amyloid accumulation effect.

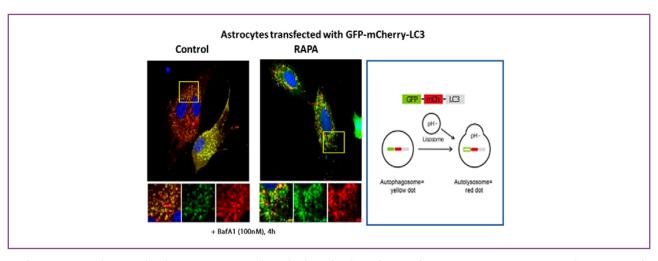
Our second goal is the analysis of a new oncogenic pathway that we recently described in glioma: Akt/WIP/YAP/TAZ. Our data indicated that Akt and WIP are responsible of the cell division of cancerstem cells and the maintenance of its stem-like phenotype. Our current work is about defining the elements 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)].



#### Other activities

Máster en Biomedicina Molecular. Departamento de Biología Molecular y de Bioquímica (UAM). 32817- Avances en la Investigación sobre Enfermedades del Sistema Nervioso (BMM5): Coordinadores: Javier Díaz Nido, Francisco Wandosell e Isabel Lastres Becker (2021-2024)





- Máster en Biomedicina Molecular. Departamento de Biología Molecular y de Bioquímica (UAM). 32817- Avances en la Investigación sobre Enfermedades del Sistema Nervioso (BMM5): Coordinadores: Javier Díaz Nido, Francisco Wandosell e Isabel Lastres Becker (2021-2024)



# List of publications

- L. Carrero, D. Antequera, I. Alcalde, D. Megias, L. Ordoñez-Gutierrez, C. Gutierrez, J. Merayo-Lloves, F. Wandosell, C. Municio, E. Carro (2023). "Altered clock gene expression in female APP/PS1 mice and Aquaporin-dependent amyloid accumulation in the retina". Inter.J.Mol. Science- 24:21-doi: 10.3390/ijms242115679.
- M. Garcia-Juan, L. Ordoñez-Gutierrez,and F. Wandosell, (2023). "Clearance of β-amyloid mediated by autophagy is enhanced by MTORC1 inhibition but not AMPK activation in APP/PSEN1 astrocytes".Glia 72 (3) pp.588-606-doi: 10.1002/GLIA.24492.
- M. Villa-González, M. Rubio, G. Martín-López, P. R Mallavibarrena, L. Vallés-Saiz, D. Vivien, F. Wandosell \* and M.José Pérez-Álvarez\* (2023). "Pharmacological inhibition of mTORC1 reduces neural death and damage volume after MCAO by modulating microglial reactivity". Biol Direct 19, 26 (2024) doi.org/10.1186/s13062-024-00470-5--bioRxiv preprint, doi: https://doi.org/10.1101/2023.12.31.571469.
- B. Alcover-Sanchez, G. Garcia-Martin1, M. Sanz-Rodriguez, A. Gruart, J. M. Delgado-García, P. de la Villa-Polo, F. Wandosell1, M. P. Pereira and B. Cubelos (2024). "R-Ras1 and R-Ras2 regulate mature oligodendrocyte subpopulations". Glia, doi: 10.1002/glia.24643.



#### **Doctoral theses**

- Lcdo. Mario Villa Gonzalez. "Papel de mTORC en la fisiología y patología cerebral (19 /01/ 2023)" Universidad: Unv. Autónoma de Madrid. Directores: Dra Ma Jose Perez & Dr. Francisco Wandosell.
- Lcda. Marta García Juan. "Análisis de los mecanismos que regulan la autofagia en astrocitos . y su papel en la eliminación del péptido beta-amiloide". (21 /02/ 2024). Unv. Autónoma de Madrid. Directores: Dr. Francisco Wandosell y Drª Lara Ordoñez.

- M. J. Benitez\*, D. Retana1\*, L. Ordoñez-Gutiérrez\*, I. Colmena,M. J. Goméz, R. Álvarez, M. Ciorraga1, A. Dopazo, F. Wandosell, J. J. Garrido. (2024) "Transcriptomic alterations in APP/PS1 mice astrocytes lead to early postnatal axon initial segment structural changes". Cell. Mol. Life Science, 81:1, doi:.10.1007/s00018-024-05485-9.
- García-Juan M, Villa M, Benito-Cuesta I, Ordóñez-Gutiérrez L. and Wandosell F. (2024). "Reassessing the balance AMPK-MTORC1 in autophagy in CNS" Neural Regeneration Research. doi: 10.4103/NRR.NRR-D-24-00733.
- Villa-González, M., García-Juan, M., Ordóñez-Gutiérrez, L., Pérez-Álvarez M. J. and Wandosell, F. (2024) "
- HIF-1α downregulates the APP protein after oxygen and glucose deprivation in the APPswe/PSEN1 mouse model of Alzheimer's disease". Journal Neurochemestry . https://doi.org/10.1016/j.neuint.2024.105923



#### Research projects

- ISCIII-CIBERNED n°: CB06/05/0067 "Mecanismos Moleculares de Neurodegeneración" Grupo del CBM--"Financiación anual competitiva" IP: Dr. F. Wandosell (CIBERNED group -PI- F. Wandosell: http://ciberned.es/grupo-wandosell.htlm,)
- Grant: I+D+i-RETOS-PID2021-124801NB-I00. (2022-2025). "Nuevos Componentes de la ruta WIP, YAP/TAZ en Sistema Nervioso Central: Proliferación versus Diferenciación" (01/09/ 2022.- 31/08/25). IP1- Dr. F. Wandosell; IP2-Drª I. M. Antón Gutiérrez
- Grant: Early Stage Plus (2023-2024)- "Amyloid-β burden and neuroinflammation dual targeted nanomedicines for Alzheimer's disease" (AβNANO). Coord: Dr. Santiago Grijalbo (IQAC-CSIC); IP CBM Dr. F. Wandosell.



# Physiological and pathological processes

Metabolic and signaling networks in disease



# Laura Formentini

The research conducted by the "Metabolic and signaling networks in disease" Unit aims to understand the complex molecular mechanisms that govern cellular function, with a focus on the intersections of cell metabolism, signaling, immune regulation and disease development. Leveraging a diverse range of expertise and cutting-edge technologies, our Unit is dedicated to pioneering approaches that explore the physiopathological roles of various molecular pathways in health and disease. The research within our Unit spans several domains, including metabolic diseases, ageing, cancer, inflammation, and neurological conditions, with a particular emphasis on identifying novel therapeutic targets for prevalent and often underexplored conditions.

One of the central research areas focuses on understanding the role of mitochondria in cellular and tissue homeostasis. Mitochondria are essential organelles responsible for energy production and for integrating bioenergetics with cellular processes such as signalling, metabolism, cell death, and immunity. Research in this area investigates how mitochondrial dysfunction contributes to disease setting and progression, particularly in the context of cancer, immune response, and metabolic disorders. The research delves into various mitochondrial pathways, genetically modified animal models of dysfunction, tissue-specific nutrient preferences, and lipid or calcium-dependent carriers and shuttles that are critical for mitochondrial health, with the aim of developing targeted therapies for diseases linked to mitochondrial dysfunction. Another important focus is the exploration of how metabolism influences tissue fibrosis, particularly in organs such as the liver and kidneys, and assess the impact of diet and environmental factors. Additionally, the Unit investigates how biological sex influences metabolism, with a particular emphasis on the role of sex hormones in modulating mitochondrial function and disease susceptibility.

Cellular signaling also plays a crucial role in disease, and the Unit investigates the complexity of signaling networks, particularly those regulated by G protein-coupled receptor kinase 2 (GRK2). This kinase acts as a key hub that integrates signals from various pathways, influencing processes such as inflammation, metabolism, and tumor progression. Researchers focus on understanding how GRK2 networks are engaged in diseases like obesity, skin-disorders, cancer, and inflammatory conditions, with the goal of identifying novel therapeutic targets for these diseases.



# Research groups

#### **Eduardo Balsa**

Mitochondrial dysfunction in metabolic diseases

# Pedro Bonay

Functional glycogenomics

# Susana Cadenas

Mitochondrial pathophysiology

# ••••••

# Sara Cogliati

Molecular mechanisms of sex-differences in metabolism physiology and disease

# Laura Formentini

Translational energy metabolism

# Santiago Lamas

Molecular pathophysiology of fibrosis

# Federico Mayor

# Cristina Murga

Patho-physiological implications of G proteincoupled receptors signaling networks

# Petronila Penela

Cellular signaling networks in cancer

#### **Belén Pérez**

#### Pilar Rodríguez Pombo

Translational medicine in inborn errors of metabolism and other rare genetic diseases

# Natalia Reglero

Vascular inflammation and autophagy

# Catalina Ribas

Cell communication in homeostasis and disease through new Gq-GPCR signaling nodes

# Lourdes Ruiz Desviat

# Eva María Richard

Physiopathology studies and therapeutical approaches in animal and cellular models of neurometabolic diseases

# Beatriz Pardo

# Araceli del Arco

Calcium signaling in mitochondria: metabolic control and mitochondria physiopathology

#### ••••••

#### **Javier Traba**

Mitochondrial biology in immune modulation

The Unit is also dedicated to advancing research into inherited metabolic disorders (IEMs), with a focus on understanding the genetic and molecular mechanisms underlying conditions such as metabolic acidemias. These disorders are characterized by defective metabolic pathways, leading to a variety of complications, including neurological and cardiac abnormalities. By utilizing patient-derived iPSCs and genetically modified animal models, researchers aim to uncover new therapeutic strategies for IEMs. This includes using CRISPR/Cas9 technology to correct genetic defects and developing novel disease models that more accurately replicate human conditions. Bridging functional genomics with clinical applications, the Unit aims at providing valuable insights into disease mechanisms, paving the way for innovative therapies for these complex and challenging disorders.

The Unit also conducts research on how glycosylation, a key post-translational modification, influences protein function and disease progression.

In addition to metabolic dysfunction, the Unit places a strong emphasis on understanding immune cell function, particularly how immune cells interact with the vasculature and influence inflammatory responses. The research aims to explore how endothelial autophagy, a process by which cells degrade and recycle damaged components, regulates immune responses and prevents tissue damage.

The collective aim of the "Metabolic and Signaling Networks in Disease" Unit is to bridge the gaps between basic molecular biology, disease mechanisms, and therapeutic applications. By investigating how metabolic and signaling pathways regulate cellular processes in health and disease, the research provides new insights into disease etiology and uncovers novel targets for drug development. Ultimately, the research aims to deepen our understanding of complex diseases and pave the way for more effective and personalized therapeutic strategies, advancing the diagnosis, prognosis, and treatment of pathologies.



# MITOCHONDRIAL DYSFUNCTION IN METABOLIC DISEASES

# **Group members**

**Principal investigator:** Eduardo Balsa

**Postdoctoral fellows:** Alba Roca Portoles Sara Laine Menendez

Predoctoral fellows: Lucia del Prado Montero Marcos Javier Zamora Dorta Pablo Castillo Serrulla Víctor Manuel Cruz Vilchez

**Technicians:**Natalia del Pozo Ramos
Raquel Losada de Paz

**Undergraduate and Master students:** Natalia Brun Rubio

Visiting scientists
Sophie Coaker



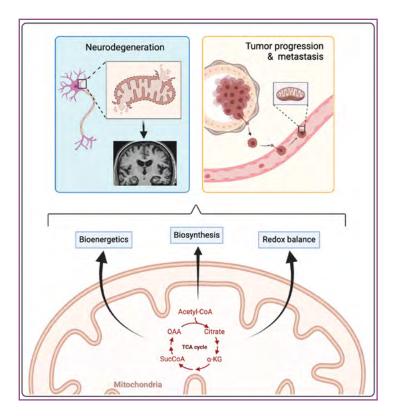
https://www.cbm.uam.es/balsalab



# Summary

Our laboratory is dedicated to unraveling the fundamental molecular components governing mitochondrial function and their implications in human physiology and disease. We focus on two core areas: Firstly, First, we aim to elucidate how mitochondrial dysfunction compromises cellular and organ fitness. We have identified novel genes that regulate mammalian oxidative phosphorylation (OXPHOS) activity, with significant implications for human disease (PMID: 40301572). In parallel, we discovered a previously unrecognized mechanism that accounts for the differential sensitivity of brain cell types to mitochondrial dysfunction, offering critical insights for the development of targeted therapies for mitochondrial disorders (PMID: 39375345). Building upon these findings, we have generated new mouse models to investigate the metabolic compatibility of brain cells, with particular astrocyte-neuron communication. focus on Preliminary data suggest that mitochondrial dysfunction in astrocytes severely impairs brain activity and reduces lifespan. Mechanistically, we are dissecting how mitochondrial activity in astrocytes maintains the homeostasis of specific neurotransmitters, and how its disruption triggers a range of severe neurological phenotypes, including epilepsy, ataxia, weight loss, and ultimately death. Secondly, we investigate the nutrient and metabolic dependencies of metastasizing cancer cells. Supported by CRIS cancer foundations, our ambitious project explores metabolic heterogeneity within tumors as a key determinant of metastatic outcomes. We've identified metabolic adaptations enabling cancer cells to navigate and thrive in the metastatic cascade, facilitating colonization of distant organs such as the liver and lungs. Additionally, we have developed and implemented an innovative strategy that combines cellular barcoding with single-cell clonal tracking to reconstruct the nutrient landscapes of the tumor microenvironment. This approach allows us to link specific metabolic conditions to metastatic outcomes and to identify distinct genetic programs that drive these aggressive phenotypes. Overall, our research highlights the central role of metabolism in both neurodegeneration and cancer progression. By uncovering how mitochondrial dysfunction drives organ failure and how metabolic adaptation supports metastasis, we provide key insights into disease mechanisms. Our models and approaches offer new avenues for targeting metabolic vulnerabilities, with the potential to guide innovative therapies for mitochondrial disorders and metastatic cancer.





The Balsa laboratory focus on two central areas; First, we aim to elucidate the molecular mechanisms whereby mitochondrial dysfunction compromises cellular and organ fitness. Second, we focus on understanding the nutrient and metabolic dependencies of metastasizing cancer cells.



# List of publications

#### Research articles:

- Lucia Del Prado, Myriam Jaraíz-Rodríguez, Mauro Agro, Marcos Zamora-Dorta, Natalia Azpiazu, Manuel Calleja, Mario Lopez-Manzaneda, Jaime de Juan-Sanz, Alba Fernández-Rodrigo, José A Esteban, Mònica Girona, Albert Quintana, Eduardo Balsa. Compensatory activity of the PC-ME1 metabolic axis underlies differential sensitivity to mitochondrial complex I inhibition. Nat Communications. 2024 DOI: 10.1038/s41467-024-52968
- Liliana M Sanmarco, Joseph M Rone, Carolina M Polonio, Gonzalo Fernandez Lahore, Federico Giovannoni, Kylynne Ferrara, Cristina Gutierrez-Vazquez, Ning Li, Anna Sokolovska, Agustin Plasencia, Camilo Faust Akl, Payal Nanda, Evelin S Heck, Zhaorong Li, Hong-Gyun Lee, Chun-Cheih Chao, Claudia M Rejano-Gordillo, Pedro H Fonseca-Castro, Tomer Illouz, Mathias Linnerbauer, Jessica E Kenison, Rocky M Barilla, Daniel Farrenkopf, Nikolas A Stevens, Gavin Piester, Elizabeth N Chung, Lucas Dailey, Vijay K Kuchroo, David Hava, Michael A Wheeler, Clary Clish, Roni Nowarski, Eduardo Balsa, Jose M Lora, Francisco J Quintana. Lactate limits CNS autoimmunity by stabilizing HIF-1α in dendritic cells. Nature. 2023. DOI: 10.1038/s41586-023-06409-6

# Review:

Myriam Jaraíz-Rodríguez, Lucia Del Prado, Eduardo Balsa. Metabolic remodeling in astrocytes: Paving the path to brain tumor development. Neurobiology of Disease. 2023. DOI: 10.1016/j.nbd.2023.106327



# Research projects

- PR\_EX\_2022-01. CRIS Cancer Foundation. Metabolic heterogeneity as a critical determinant of melanoma metastasis. Eduardo Balsa Martinez. 01/03/2023 -31/03/2028
- PID2022-137404OB-I00. State Research Agency. Deciphering mitochondrial metabolism as a diary for tumor progression and metastasis. Eduardo Balsa Martinez. 01/09/2023-31/08/2026.



#### **Patents**

■ TREATMENT, DIAGNOSIS AND/OR PROGNOSIS OF MITOCHONDRIAL DISEASES, Eduardo Balsa Martinez. EP24382547.8. Spain. 23/05/2024. Universidad Autónoma de Madrid.



# FUNCTIONAL GLYCOGENOMICS

# **Group members**

Principal investigator: Pedro Bonay Miarons

**Undergraduate and Master students:** 

Ivan Sanchez Mellado

Norma Ines Sandoval





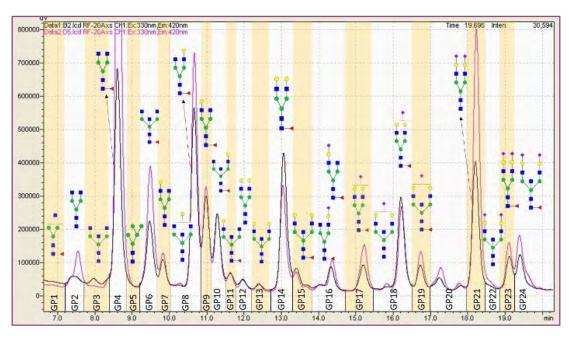
#### Summary

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 glycomic 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.





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



# Research projects

European Project HORIZON-EIC-2023-PATHFINDERCHALLENGES, Linking Intestinal Bacteria and Host Metabolism to Tackle Type 2 Diabetes with Novel Food. DiBaN, November 2024-2027.



# Other activities

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). Ratification in 2025. 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 highschool, undergraduate and graduate education



# MITOCHONDRIAL PATHOPHYSIOLOGY

# **Group members**

**Principal investigator:** Susana Cadenas Álvarez

**Postdoctoral fellows:** Alina Luchkova

Predoctoral fellows: Ana Mata Villanueva

**Undergraduate and Master students:** Daniel Santa Cruz Serra María González Noé

**Visiting scientists:**Thilo Hagen (National University of Singapore, Singapore)



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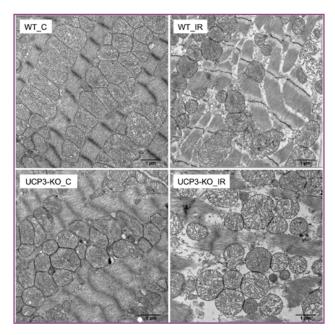


#### Summary

Uncoupling protein UCP3 belongs to the mitochondrial carrier family (solute carrier family 25, SLC25) and presents a high amino acid sequence homology with UCP1, the first uncoupling protein identified, and with UCP2. UCP3 is abundantly expressed in skeletal muscle, and it is also present in brown adipose tissue and heart. Unlike UCP1, the function of which is adaptive thermogenesis in brown adipose tissue, the physiological function of UCP3 is unclear. Although a role similar to UCP1 in proton transport from the intermembrane space into the mitochondrial matrix, thereby uncoupling oxidative phosphorylation, was initially proposed, this does not appear to be its primary function. Other proposed functions are the prevention of excessive production of mitochondrial reactive oxygen species (ROS), and its participation in the transport or oxidation of fatty acids. Our recent work, using mice lacking this protein (UCP3-KO mice), supports its importance in limiting the production of mitochondrial ROS, and also reveals a protective role of UCP3 against myocardial damage due to ischemia-reperfusion (IR). Thus, we found that infarct size in isolated perfused hearts subjected to IR ex vivo was larger in adult and old UCP3-KO mice than in equivalent wild-type mice, and was accompanied by higher levels of creatine kinase in the effluent. UCP3-KO hearts also showed more pronounced mitochondrial structural changes after IR as determined by transmission electron

microscopy. The greater myocardial damage in UCP3-KO hearts was confirmed in vivo after coronary artery occlusion followed by reperfusion. Addition of S1QEL, a suppressor of superoxide generation from site IQ in complex I, at the time of reperfusion, limited infarct size in UCP3-KO hearts, pointing to exacerbated superoxide production as a possible cause of the damage. Metabolomics analysis of isolated perfused hearts confirmed the reported accumulation of succinate, xanthine and hypoxanthine during ischemia, and a shift to anaerobic glucose utilization, which all recovered upon reoxygenation. The metabolic response to ischemia and IR was similar in UCP3-KO and wildtype hearts, being lipid and energy metabolism the most affected pathways. A bioenergetics analysis of the hearts showed that fatty acid oxidation and complex I (but not complex II) activity were equally impaired after IR. Overall, our results indicate that UCP3 deficiency promotes enhanced superoxide generation and mitochondrial structural changes that increase the vulnerability of the myocardium to IR injury.





Transmission electron micrographs of wild-type (WT) and UCP3-KO hearts before (left) and after (right) IR. Mitochondria appear well-preserved and organized in healthy hearts, whereas IR causes disorganization and disruption of mitochondrial ultrastructure, especially in hearts lacking UCP3.



# List of publications

- Luchkova, A., Mata, A., Cadenas, S. (2024) Nrf2 as a regulator of energy metabolism and mitocondrial function. FEBS Lett. 598, 2092-2105. doi: 10.1002/1873-3468.14993
- Sánchez-Pérez, P., Mata, A., Torp, M.K., López-Bernardo, E., Heiestad, C.M., Aronsen, J.M., Molina-Iracheta, A., Jiménez-Borreguero, L.J., García-Roves, P., Costa, A.S.H., Frezza, C., Murphy, M.P., Stensløkken, K.O., Cadenas, S. (2023) Energy substrate metabolism, mitochondrial structure and oxidative stress after cardiac ischemia-reperfusion in mice lacking UCP3. Free Radic. Biol. Med. 205, 244-261. doi: 10.1016/j.freeradbiomed.2023.05.014



# Research projects

- PI23/01846. Instituto de Salud Carlos III, Spanish Ministry of Health. Mitochondrial energy metabolism and redox signaling in myocardial ischemia-reperfusion injury. Susana Cadenas. 2024-2026.
- PI19/01030. Instituto de Salud Carlos III, Spanish Ministry of Health. Redox-sensitive mechanisms for cardioprotection in ischemia-reperfusion injury. Susana Cadenas. 2020-2023.
- CA20121. COST (European Cooperation on Science and Technology) Action. Bench to Bedside transition for Pharmacological regulation of NRF2 in non communicable diseases (BenBedPhar). Antonio Cuadrado. 2021-2025.



# Other activities

- Paper of the Year Award 2023 in Free Radical Biology and Medicine. Awarded jointly by the Society for Free Radical Research-Europe (SFRR-E) and the Society for Redox Biology and Medicine (SfRBM).
- Coordinator of the "Free Radical and Oxidative Stress" Group Meeting of the Spanish Society of Biochemistry and Molecular Biology (SEBBM) (2020-2023).



# MOLECULAR MECHANISMS OF SEX-DIFFERENCES IN METABOLISM PHYSIOLOGY AND DISEASE

# **Group members**

**Principal investigator:** Sara Cogliati

**Predoctoral fellows:**José García-Consuegra Martínez FPI
Ariadna Qin Gil FPI
Tahmineh Ebrahimi

**Technicians:** Ana Sagrera Aparisi

**Undergraduate and Master students:** Cristina Peralbo Aviles Vecchione Stefano

**Visiting scientists**Beatriz Linillos Pradillo



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#### Summary

Metabolism exhibits significant differences between the sexes, which can lead to sex-dependent disease manifestations. Women generally have greater longevity than men but are more frequently affected by Alzheimer's disease, osteoporosis, and autoimmune disorders. Conversely, men have a higher risk of developing cardiovascular disease, cancer, Type-2 diabetes (T2D), Parkinson's disease, and stroke. However, after menopause, women experience a poorer prognosis following their first myocardial infarction, and their incidence of T2D becomes comparable to that of men.

Given this context, it is crucial to characterize biological sex differences as part of the advancement toward personalized medicine.

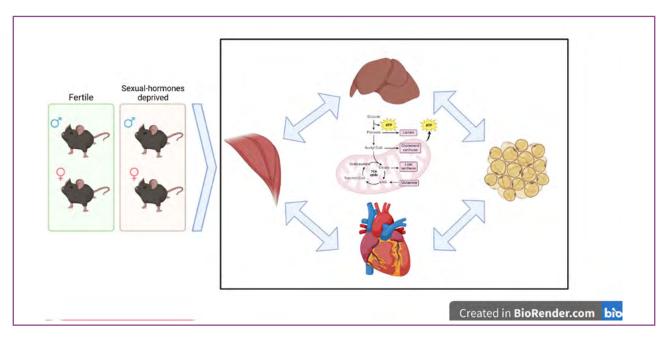
Our laboratory aims to understand the molecular mechanisms underlying metabolic sex differences in health and disease, with a particular focus on the role of mitochondria. We are conducting an extensive analysis of mitochondrial function and morphology across various tissues (liver, muscle, heart, and adipose tissue), comparing males and females. Additionally, we are investigating the influence of sex hormones using surgical and pharmacological models of gonadal deprivation to mimic postmenopause, andropause, and aging.

Our findings indicate that mitochondrial morphology and function are significantly affected by sex hormone deprivation, influencing inter-organ communication and contributing to pathologies such as muscle atrophy, fat accumulation, glucose metabolism dysregulation, and cardiac insufficiency. Through gene expression analysis and metabolomics approaches, we are identifying differentially regulated pathways to uncover specific molecular targets modulated by sex hormones.

Males and females adapt to their specific metabolic needs through distinct mechanisms in response to environmental conditions. To further explore these molecular mechanisms, we collaborate with Prof. Casuso and Prof. Huertas (University of Granada) to study how men and women assemble respiratory chain supercomplexes in response to high-intensity exercise. Additionally, we have shown that muscle and heart tissues assemble supercomplexes differently under moderate natural hypoxia (J Physiol. Apr 17. doi: 10.1113/JP285516).

Overall, our research demonstrates that mitochondrial function responds differently to sex hormones and environmental cues, playing a crucial role in modulating and adapting the body's metabolic needs.





Comparison of fertile and hormone-deprived male and female mice to investigate metabolic sex differences. Using a combination of biochemical and omics analyses, we elucidate the molecular pathways underlying these differences.



# List of publications

- Iborra-Pernichi M, Ruiz García J, Velasco de la Esperanza M, Estrada BS, Bovolenta ER, Cifuentes C, Prieto Carro C, González Martínez T, García-Consuegra J, Rey-Stolle MF, Rupérez FJ, Guerra Rodriguez M, Argüello RJ, Cogliati S, Martín-Belmonte F, Martínez-Martín N. (2024). Defective mitochondria remodelling in B cells leads to an aged immune response. Nat Commun. 2024 Mar 22;15(1):2569. doi: 10.1038/s41467-024-46763-1.
- Jerónimo Aragón-Vela, Rafael A Casuso, Ana Sagrera Aparisi, Julio Plaza-Díaz, Ascensión Rueda-Robles, Agustín Hidalgo-Gutiérrez, Luis Carlos López, Andrea Rodríguez-Carrillo, José Antonio Enriquez, Sara Cogliati\*, Jesús R Huertas\* (\*co-correspondent authors). (2024). Early heart and skeletal muscle mitochondrial response to a moderate hypobaric hypoxia environment. J Physiol. Apr 17. doi: 10.1113/JP285516.
- Sevilla-Lorente R, Marmol-Perez A, Gonzalez-Garcia P, Rodríguez-Miranda N, Riquelme-Gallego B, Aragon-Vela J, Martinez-Gálvez JM, Molina-Garcia P, Alcantara JMA, Garcia-Consuegra J, Cogliati S, Salmeron LM, Huertas JR, Lopez LC, Ruiz JR, Amaro-Gahete FJ. (2024). Sexual dimorphism on the acute effect of exercise in the morning vs. evening: A randomized crossover study. J Sport Health Sci Dec 22:101021. doi:10.1016/j.jshs.2024.101021. Online ahead of print.



# Research projects

- CNS2023-143646. State research agency. Call for proposals 2023 - "Incentives for research consolidation". Discovery of mitochondrial pathways related to cardiac protection from estradiol as a potential treatment for heart failure during menopause (ESTHER). Principal investigator (PI). Sara Cogliati Dates. 06/01/2024-06/01/2026
- Intramural Exploratory Grants Program Severo Ochoa (IGP-SO) 2024. Unlocking Therapeutic Avenues: Targeting Purine and Pyrimidine Synthesis Pathways to tackle Double-Hit Lymphoma. Principal investigator (PI). Sara Cogliati and Ana Ortega Molina Dates: 01/06/2024-31/05/2026
- PID2023-148516OB-I00. State Research Agency, National Plan 2023, Knowledge Generation Projects 2023. EstradioL and fatty acid oxidation: deciphering the liaison to protect post-menopausal females' hearts (LYDIA). Principal investigator (PI). Sara Cogliati Dates. 01/09/2024-31/08/2027
- Research Network RED2022-134927-T. Spanish thematic research network on the interaction between cellular metabolism and the microenvironment in homeostasis and diseases.



# TRANSLATIONAL ENERGY METABOLISM

# **Group members**

**Principal investigator:** Laura Formentini

Postdoctoral fellows: Pablo Sanchez Aguilera

**Predoctoral fellows:** Beñat Salegi Ansa José María Lanza Arnaiz

**Technicians:** Tzoliné Topdjian Panosián

Undergraduate and Master students: Francisco Javier Arenas Cerro Laura Naharro Naharro

Visiting scientists Linda Civettini



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



# Summary

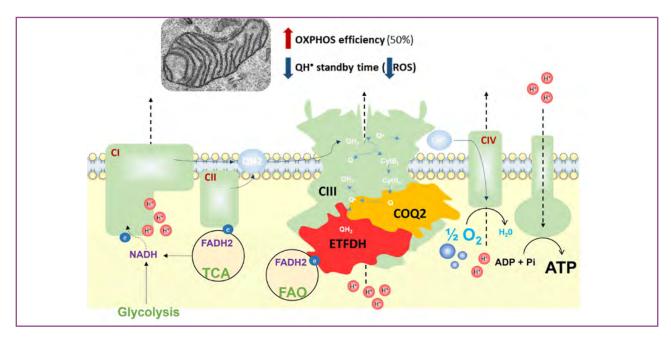
Mitochondria play essential roles in cellular metabolism, cell death regulation, and intracellular signaling. During the last decade, our research has focused on unraveling how mitochondrial energy metabolism integrates cellular functions, contributing to the onset and progression of age-related pathologies, including metabolic disorders and cancer. Beyond ATP production, mitochondrial metabolism is tightly regulated to meet cellular demands: we have previously demonstrated that oxidative phosphorylation (OXPHOS) plays a critical role in modulating immune responses and inflammation (Cell Reports 2017, PMID: 28494869) and in regulating both intra- and intercellular oncogenic signaling (Nature Communications 2020, PMID: 32681016). Additionally, mitochondrial dysfunction profoundly impacts adipose tissue and skeletal muscle lipid metabolism, altering lipid species composition (Diabetologia 2017, PMID: 28770317; EMBO J. 2020, PMID: 32488939). These metabolic disturbances disrupt ROS and calcium signaling, leading to significant structural changes in muscle tissue (Cell Death and Disease 2022, PMID: 32488939), which have emerged as key hallmarks of myopathies.

Very recently, we identified a metabolon in skeletal muscle that integrates nutrient catabolism with mitochondrial efficiency. To investigate its role, we developed the first conditional and tissue-specific mouse model targeting the lipid-catabolism Q-reductase ETFDH. These "LowFAO" mice exhibit significantly reduced fatty acid  $\beta$ -oxidation (FAO),

providing a unique tool to explore mitochondrial dysfunction. Using this model, we demonstrated that the OXPHOS is not a rigid system composed of 5 complexes but instead relies on specific interactors to optimize electron-proton flux, prevent electron leakage to oxygen, minimize ROS production, and enhance nutrient-dependent bioenergetic efficiency (Nat Metabolism 2024, PMID: 38243131). Understanding the mechanisms governing the metabolon assembly and disruption opens new avenues for identifying therapeutic targets in mitochondrial-related diseases.

Current Aims. Our current research, supported by national and EU funding, as well as the Fundación Ramón Areces, focuses on advancing our understanding of mitochondrial metabolism in pathophysiology. We specialize in developing and characterizing animal models with impaired mitochondrial activity, enabling us to investigate how mitochondrial dysfunctions, environmental factors, and diets shape metabolism at the cellular, tissue, and systemic levels. Our objective is to identify the key mitochondrial functions required to maintain cellular homeostasis and determine which metabolic products are essential for overall organismal health. Additionally, we aim to elucidate how cells acquire or transform these metabolites within physiological and pathological tissue environments. This knowledge is crucial for refining mitochondrial medicine and developing targeted therapeutic strategies.





The new OXPHOS scheme, as in Herrero Martín et al., Nat Metab 2024.



- Herrero Martín JC, Salegi Ansa B, Álvarez-Rivera G, Domínguez-Zorita S, Rodríguez-Pombo P, Pérez B, Calvo E, Paradela A, Miguez DG, Cifuentes A, Cuezva JM, Formentini L. (2024) An ETFDH-driven metabolon supports OXPHOS efficiency in skeletal muscle by regulating coenzyme Q homeostasis. Nat Metab. Feb;6(2):209-225. doi: 10.1038/s42255-023-00956-y. PMID: 38243131.
- Albericio G, Higuera M, Araque P, Sánchez C, Herrero D, García-Brenes MA, Formentini L, Torán JL, Mora C, Bernad A. (2024) Development of a Bmi1+ Cardiac Mouse Progenitor Immortalized Model to Unravel the Relationship with Its Protective Vascular Endothelial Niche. Int J Mol Sci. Aug 13;25(16):8815. doi: 10.3390/ijms25168815. PMID: 39201501.



# Research projects

- HORIZON-MSCA-2023-DN-01-01. Grant number: 101167421 UNION (Understanding frailty towards a future of healthy ageing (UNION). Coordinator: Massimiliano Rusica, University of Milan (Italy) PI: Laura Formentini. €3,423,000. 2024-2028
- PID2022-136738OB-I00. Ministry of Science and Innovation. PI: Laura Formentini. €343,750. 2023-2026
- Ramon Areces Foundation. PI: Laura Formentini. €120,000. 2023-2026
- PID2019-104241RB-I00-Ministry of Science and Innovation. PI: Laura Formentini. €160,000. 2020-2023
- The TEM Group is part of the IIS: Instituto de Investigación Hospital 12 de octubre (i+12)



# MOLECULAR PATHOPHYSIOLOGY OF FIBROSIS

# **Group members**

**Principal investigator:** Santiago Lamas

**Postdoctoral fellows:** Elena Alcalde Estévez Celia Pinto Díez

**Predoctoral fellows:** Belén Sirera Conca

**Technicians:** Jessica Tituaña Fajardo

**Undergraduate and Master students:**María Madejón Sánchez
Sadaf Barakzoy
Jorge Santiago Yebra



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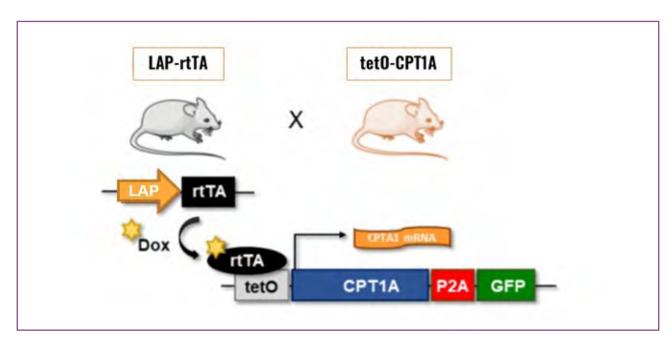


#### Summary

Fibrosis results from an unbalanced cellular response to inflammation and wound healing leading to the activation of specific subpopulations of resident mesenchymal cells promoting their transition towards myofibroblasts. These cells synthesize extracellular matrix components, such as collagen, that ultimately replace the cellular living tissue and establish fibrosis. While major advances regarding the mechanistic knowledge on the underlying cell biology alterations in fibrosis have helped to characterize the main phases and mediators involved, this knowledge has not yielded significant progress in treatment. This is due in part to a very incomplete understanding of the cellular types involved in the fibrotic response, the metabolic features associated to each of them, their mutual influence and the changes ultimately related to the metabolic shift or reprogramming occurring in the transition from physiological to pathological conditions. Metabolic derangement is now identified as a key culprit in the pathophysiology of fibrogenesis.

During the past years we have studied the role of metabolism in the genesis of renal injury focusing on: a) the importance of fatty acid oxidation (FAO) and b) the crosstalk between fibrosis and circadian regulation. To this end we use animal models with specific gain-of-function for critical enzymes involved in fatty acid oxidation, such as Cpt1a, as well as animals with a disruption of the circadian rhythm. These studies have been complemented by cellular models and biochemical approaches directed towards the study of mitochondrial biogenesis and function. We have found that overexpression of the enzyme carnitine palmitoyl transferase 1A (CPT1A) in kidney tubules promotes enhanced FAO, restores mitochondrial homeostasis and protects from fibrosis. By dissecting the role of specific components of the circadian clock through the employment of genetically modified mouse models, we demonstrated the cross-regulation between the circadian rhythm and kidney inflammation and fibrosis, with a particular emphasis on the metabolic component. We are currently studying the importance of FAO in liver fibrosis by using a model of CPT1A conditional expression in the liver/kidney and the differential impact of FAO in hepatocytes and stellate cells. We believe these studies will be relevant for establishing pharmacological targets for liver fibrosis in the context of metabolic-dysfunction associated steatohepatitis (MASH).





Generation of a conditional mouse model for CPT1A over-expression in liver and kidney



# List of publications

- Miguel V, Alcalde-Estévez E, Sirera B, Rodríguez-Pascual F, Lamas S. Metabolism and bioenergetics in the pathophysiology of organ fibrosis. Free Radic Biol Med. 2024 Sep;222:85-105. doi: 10.1016/j.freeradbiomed.2024.06.001.
- Miguel V, Rey-Serra C, Tituaña J, Sirera B, Alcalde-Estévez E, Herrero JI, Ranz I, Fernández L, Castillo C, Sevilla L, Nagai J, Reimer KC, Jansen J, Kramann R, Costa IG, Castro A, Sancho D, Rodríguez González-Moro JM, Lamas S. Enhanced fatty acid oxidation through metformin and baicalin as therapy for COVID-19 and associated inflammatory states in lung and kidney. Redox Biol. 2023 Dec;68:102957. doi: 10.1016/j. redox.2023.102957. Epub 2023 Nov 3.
- Wculek SK, Heras-Murillo I, Mastrangelo A, Mañanes D, Galán M, Miguel V, Curtabbi A, Barbas C, Chandel NS, EnríquezJA, Lamas S, Sancho D. Oxidative phosphorylation selectively orchestrates tissue macrophage homeostasis. Immunity, 2023 Mar 14;56(3):516-530.e9. doi: 10.1016/j. immuni.2023.01.011.
- Rey-Serra C, Tituaña J, Lin T, Herrero JI, Miguel V, Barbas C, Meseguer A, Ramos R, Chaix A, Panda S, Lamas S. Reciprocal regulation between the molecular clock and kidneyinjury. Life Sci Alliance. 2023 Jul 24;6(10):e202201886. doi: 10.26508/lsa.202201886.
- Cuevas-Delgado P, Miguel V, Rupérez FJ, Lamas S, Barbas C. Impact of renal tubular Cpt1a overexpression on the kidney metabolome in the folic acid-induced fibrosis mouse model. Front Mol Biosci. 2023 Jun 12;10:1161036. doi: 10.3389/fmolb.2023.1161036.



# Research projects

- P2022/BMD-7221. INNOREN-CM. New diagnostic and therapeutic strategies in chronic kidney disease. 2023-2026.
- PID2022-136703OB-I00. Impact of the extracellular matrix on cell biology and metabolic reprogramming of fibrosis. 2023-2027.
- PID2019-104233RB-I00: Metabolic reprogramming to combat renal fibrosis. June 1, 2020, to May 31, 2023.
- CPP2022-009582. Evaluation of M2R therapy in an experimental model of ARDS. 2023-2026.



#### Doctoral theses

■ Gaurangkumar Patel (2023). Role of Antipsychotics-□ Induced Mitochondrial Dysfunction in Increased Cardiovascular Risk. Departamento de Bioquímica, UAM. Directores: María Monsalve y Santiago Lamas



#### Other activities

- Doctor Honoris Causa Lecture, Universidad de la República, Uruguay. March 2023
- UNIA International Meeting, Metabolic basis of organ fibrosis, Baeza, October 2023.



# PATHO-PHYSIOLOGICAL IMPLICATIONS OF G PROTEIN-COUPLED RECEPTORS SIGNALING NETWORKS

# **Group members**

Principal investigator: PI: Federico Mayor Jr co-PI: Cristina Murga

**Scientific staff:** Irene García Higuera

Postdoctoral fellows: Carmen Vida Rueda Yadileini Portilla Tundidor **Predoctoral fellows:** Rocío Santos Clemente Alejandro Asensio López

Viviana Marolda Ángela Albitre Raquel Huertas Lárez Ana Romo Gallo

# Technicians:

Cristina Delgado Arévalo Estefanía Vázquez de Oro

**Undergraduate and Master students:**Daniel Oña Sánchez
Sergio Carretero



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Summary

The intricate intercommunication among cell types in their specific tissue environment or via organ crosstalk, often involving cells of the immune system, is essential for homeostasis. Physiologically, cells integrate multiple signals emanating systemically or from the microenvironment via highly interconnected signaling networks, while alterations in such homeostatic interplays lead to pathological situations.

Therefore, a better understanding of the complexity of cellular signalling networks and of their maladaptive rewiring in pathological conditions is key to unveiling the molecular basis of disease. The role of regulatory nodes is especially important in such pathological reshaping, given their ability to integrate multiple upstream modulatory inputs and their downstream cell-type specific interactomes (i.e., cellular partners with different functions).

Our group has pioneered research on the G proteincoupled receptor kinase 2 (GRK2) node in cellular communication and put forward its participation in the onset or progression of certain metabolic, cardiovascular, tumoral or inflammatory conditions.

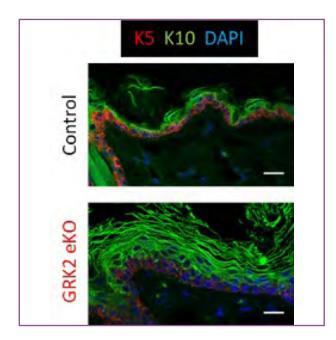
The main aim of our group is to further elucidate how GRK2 interaction networks are engaged and integrated in a cell type- or context-specific way and their potential contribution to the progression of pathological situations in which GRK2 expression or functionality is altered, such as breast and skin cancer, skin inflammation or obesity-related diseases. By analysing the phenotype of cellular and animal models with altered GRK2 functionality

using molecular, omics and cell biology approaches, we seek to gain a deeper understanding of the role of such GRK2 interactomes in these contexts and to identify new targets for combined therapies in certain prevalent and relevant diseases.

# Main lines of research:

- Role of signalling pathways governed by myeloid GRK2 in the maladaptive reshaping of metabolic and inflammatory networks and in the early stages of response to obesogenic diets: i) GRK2 interactome involved in the polarization and proinflammatory characteristics of macrophages in response to metabolic or inflammatory stress signals; ii) Role of GRK2 in the modulation of neutrophil trafficking and functionality and in the early alterations of the myeloid cell landscapes triggered by diet in tissues.
- GRK2 interactome as an integrator of signals acting in the breast cancer tumor microenvironment.
   Involvement in invasion, reprogramming and metabolism (in collaboration with Petronila Penela's group).
- Participation of GRK2 and Gq in the interconnection between different cell types in the HNSCC tumor microenvironment (in collaboration with Catalina Ribas and Inmaculada Navarro-Lérida).
- Role of GRK2 in epidermal homeostasis and in keratinocyte-immune system communication. Implication in the susceptibility to the development of inflammatory skin diseases or squamous cell skin tumors (in collaboration with Catalina Ribas).







- Rivas, V., González-Muñoz, T., Albitre, A., Lafarga, V., Delgado-Arévalo, C., Mayor, F. Jr. and Penela, P. (2024) GRK2-mediated AKT activation controls cell cycle progression and G2 checkpoint in a p53-dependent manner. Cell Death Discov. 10(1):385. doi: 10.1038/s41420-024-02143-8
- Vida, C., Portilla, Y., and Murga, C. (2024) Adrenergic modulation of neutrophil and macrophage functions: pathophysiological cues. Current Opinion in Physiology. 41:100780. doi:10.1016/j.cophys.2024.100780
- Asensio, A., Sanz-Flores, M., Liakath-Ali, K., Palacios-García, J., Paramio, J.M., García-Escudero, R., Mayor jr, F., and Ribas, C. (2024) The G Protein-Coupled Receptor Kinase 2 Orchestrates Hair Follicle Homeostasis. bioXriv. doi: 10.1101/2024.04.11.589052
- Mayor, F Jr. and Murga, C. (2023) G Protein-Coupled Receptor Kinases Take Central Stage. Cells 12(1), 23. doi:10.3390/cells12010023



#### Other activities

■ Federico Mayor: Director, Institute of Molecular Biology, Universidad Autónoma, Madrid. Director, Physiological and pathological processes Program CBM. Member of Scientific advisory boards of the Lilly Foundation Spain, IDIBAPS-Clinic (Barcelona, Spain) and Instituto de Investigación Sanitaria "Fundación Jiménez Díaz" (Madrid, Spain). Member of the Editorial Board of Revista SEBBM (scientific dissemination and policy magazine). Honorary Member of the Spanish Society for Biochemistry and Molecular Biology-SEBBM (2023). Cristina Murga: Member of the Rector Board of the Instituto de Investigación Sanitaria "La Princesa" (Madrid, Spain). President, Senior University Program of the UAM.

Fluorescence image showing the dermis and epidermis of control mice and of an animal in which the kinase GRK2 has been genetically depleted



#### **Doctoral theses**

- Viviana Marolda (2024). GRK2-Associated Protein Networks in Breast Cancer Cell Interplay with the Tumour Microenvironment. Departamento de Biología Molecular. Universidad Autónoma de Madrid. Supervisors: Petronila Penela and Federico Mayor.
- Alejandro Asensio (2024). Role of GRK2 in epidermal homeostasis. Departamento de Biología Molecular. Universidad Autónoma de Madrid. Supervisors: Catalina Ribas and Federico Mayor. Internacional Mention.
- Ángela Albitre (2023).GRK2 networks in mammary gland development and tumorigenesis. Departamento de Biología Molecular. Universidad Autónoma de Madrid. Supervisors: Petronila Penela and Federico Mayor. Internacional Mention.



# Research projects

- PID2023-1467350B-I00. Agencia Estatal de Investigación (AEI), Spain.GRK2 interactomes in cellular signalling and disease (GRK2INTER). Principal Investigator (PI): Federico Mayor Jr. and Cristina Murga (co-PI).2024 -2027.
- INTEGRAMUNE-CM/ P2022/BMD-7209. Madrid Regional Excellence Networks in Biomedicine. Integrated cellular and molecular systems in immune-inflammatory pathophysiology. Coordinator PI: Federico Mayor (4 research groups). 2023-2026
- Grant agreement 860229. European Union:H2020-MSCA Programme, ONCORNET2.0 (ONCOgenic Receptor Network of Excellence and Training). Coordinator: Martine Smit, Amsterdam, Federico Mayor Jr., Network group PI. 2020-2024.
- PID2020-117218RB-I00. Agencia Estatal de Investigación (AEI), Spain. Integrated GRK2 signaling networks and molecular mechanisms of disease. Principal Investigator (PI): Federico Mayor Jr. and Cristina Murga (co-PI). 2021-2024.
- Group CB16/11/00278. Cardiovascular Research Consortium (CIBER CARDIOVASCULAR) Instituto de Salud Carlos III. Group PI: Federico Mayor Jr. 2017-2023.
- Affiliation to the Health Research Institute (IIS) La Princesa



# CELLULAR SIGNALING NETWORKS IN CANCER

# **Group members**

**Principal investigator:** Petronila Penela Márquez

Scientific staff: Laura Nogués Vera

**Postdoctoral fellows:** Teresa González Muñoz

Predoctoral fellows: Clara Moyano Jimeno Viviana Marolda Ángela Albitre Belén Ortiz del Castillo Technicians: Cristina Delgado Arévalo Carolina Maroto González

Undergraduate and Master students: Clara Rodriguez Blanco Javier Arroba Gallego Paula Cassasano Mateos



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#### Summary

Intratumor cell heterogeneity and the intrinsic adaptability of tumour cells are fundamental features of cancer, with significant consequences for tumour evolution and the emergence of resistances. Cellular heterogeneity and the dynamic rewiring of signaling networks are key contributors to tumor adaptability and progression. Moreover, lifestyle factors can influence tumor heterogeneity, drastically affecting treatment efficacy.

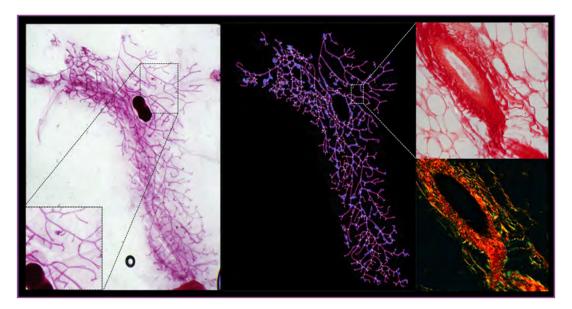
Understanding the mechanisms underlying the interplay between environmental factors and malignant cells is crucial to identifying molecular dependencies that could serve as the foundation for new therapeutic strategies. The dynamic reconfiguration of cellular signaling in response to tumor risk factors may contribute to increased cellular instability and alterations in the vascular stroma, thereby promoting tumor progression. In this context, the dysregulation of proteins that function as signaling nodes and mediate post-translational modifications may play a critical role in various aspects of tumor cell behavior, including motility, cell cycle control, and stress responses.

Our research focussed on the role of kinases and other posttranslational modifiers in the dynamic rewiring of cell signaling networks, which may represent potential therapeutic targets.

We have demonstrated that G protein-coupled receptor kinase2 (GRK2) is a versatile molecular hub, modulating signalling mediated by many GPCRs and an expanding array of cellular partners. GRK2 is emerging as a relevant onco-modulator in breast cancer through complex regulatory loops affecting stress-related RNA binding proteins (HuR) in the angiogenic response to hypoxic and adrenergic stresses, E3 ubiquitin ligases (MDM2) on centrosome dynamics and cytosolic protein deacetylases (HDAC6) to foster EGF signalling and motility. The concurrent up-regulation of GRK2 and these factors constitutes a functional signature across various breast cancer types, extending beyond hormone-dependent tumors.

Our research aims are to identify a) GRK2-governed signalling circuits involved in breast cancer progression and resistance, deciphering key targets modified by phosphorylation, acetylation, ubiquitination; b) consequences of GRK2-based signalosomes on cell cycle dynamics under stress conditions, and their role in cell cycle decision-making to differentiation, proliferation or senescence; c) influence of hormonal (adrenergic, estrogenic), metabolic stresses and micro-environmental conditions on GRK2 interactions with relevant partners in genomic stability an stroma remodelling, analysing their impact in mammary gland morphology, epithelial organization, angiogenesis and tissue fibrosis that facilitate tumour growth and dissemination.







#### List of publications

- Rivas V, González-Muñoz T., Albitre A., Lafarga V., Mayor Jr F., Penela P. (2024) GRK2-mediated AKT activation controls cell cycle progression and G2-checkpoint in a p53-dependent manner. Cell Death Discov. 10, 385. doi:10.1038/s41420-024-02143-8
- Diez-Alonso L., Arroyo-Ródenas J., Romencín P.A., Martínez A., Gómez-Rosel M., Blanco B., Jiménez-Reinoso A., Mayado A., Pérez-Pons A., Aguilar-Sopeña Ó., Ramírez-Fernández Á., Segura-Tudela A., Perez-Amill L., Tapia-Galisteo A., Domínguez-Alonso C., Rubio-Pérez L., Jara M., Solé F., Hangiu O., Almagro L., Albitre Á., Penela P., Sanz L., Anguita E., Valeri A., García-Ortiz A., Río P., Juan M., Martínez-López J., Roda-Navarro P., Martín-Antonio B., Orfao A., Menéndez P., Bueno C., Álvarez-Vallina L. (2024) T lymphocytes secreting anti-BCMA T cell engagers demonstrate superior antitumor activity than anti-BCMA CAR-T cells. Sci. Transl. Med. 16, eadg7962. doi: 10.1126/scitranslmed.adg7962
- Albitre Á, Reglero C, González-Muñoz T, Penela P. (2023) The Stress Connection in Cancer: the Adrenergic Fuelling of Breast Tumors. Curr. Opin. Physiol. 36, 100720. doi:10.1016/j.cophys.2023.100720



#### **Doctoral theses**

- Viviana Marolda (2024) CXCR4/ACKR3/GRK2-governed networks in cancer cell migration and metastasis. Departamento de Biología Molecular, UAM. Supervisors, Petronila Penela and Federico Mayor
- Angela Albitre Sanz (2023) GRK2 networks in mammary gland development and tumorigenesis. Departamento de Biología Molecular, UAM. European Mention. Supervisors, Petronila Penela and Federico Mayor
- Belen Ortiz del Castillo (2023) The role of GRK2 in orienting cell division and cell cycle decision-making to proliferation. Departamento de Biología Molecular, UAM. Supervisor: Petronila Penela

Imaging analysis of mouse mammary glands. Carmine-stained whole-mount histology (left panel), skeletonized image (middle panel) and picrosirius red staining (left panels) of mammary glands for gross morphological analysis of ducts, branching complexity and tissue stroma



- CIVP20A6618. Fundation Ramón Areces "Exploring posttranslational regulation of angiogenic and inflammatoryrelated processes during colorectal cancer progression and differential recurrence" P. Penela. 2021-2024.
- PI21/01834. Carlos III Institute of Health (FIS) "Exploring the role of GRK2 in BRCA1 dysfuntion and mechanisms of PARP-inhibitor resistance in breast tumor models beyond BRCA status". P. Penela. 2022-2024.
- INTEGRAMUNE-CM / P2022/BMD-7209. COM. MADRID-Programa de Actividades I+D en BIOMEDICINA/ Coordinator: F. Mayor, UAM. P. Penela, 2023-2026
- CIBER CARDIOVASCULAR, Instituto de Salud Carlos III, Group CB16/11/00278 (PI, F Mayor), network's researcher, P. Penela 2017-2023
- European Union:H2020-MSCA Programme, Grant agreement 860229 ONCORNET2.0 (ONCOgenic Receptor Network of Excellence and Training) -Coordinator: Martine Smit, Amsterdam. UAM hub: F. Mayor and P. Penela, Ethical Board and trainee supervisor, 2020-2024.
- Instituto de Investigación Sanitaria Hospital La Princesa. Area 1. Group 13 (PI: P. Penela)



#### TRANSLATIONAL MEDICINE IN INBORN ERRORS OF METABOLISM AND OTHER RARE GENETIC DISEASES

#### **Group members**

Principal investigator: Belén Pérez Pilar Rodriguez-Pombo

Scientific staff: Alejandra Gámez

**Postdoctoral fellows:** Rafael Hencke Tresbach

**Predoctoral fellows:** Laura Arribas Diana Gallego Obdulia Sánchez Lijarcio Cristina Segovia Falquina Diana Gallego Alejandro Soriano Sexto Alicia Vilas Lagoa

**Technicians:** Blanca Rodríguez Fátima Leal Rosa Navarrete

Undergraduate and Master students: Alexia Pérez Pestourie Vera Ruiz Montés

Visiting scientists: Martina Randazzo





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



#### Summary

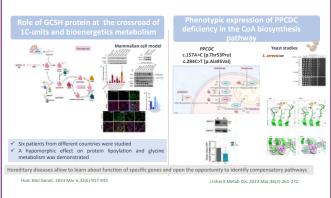
Regarding gene discovery, we uncovered a previously unrecognized link between CoA synthesis and severe cardiac pathology. Our findings identified pathogenic variants in an enzyme involved in CoA biosynthesis, and functional studies in fibroblasts and yeast confirmed their role in disease development. We also examined the phenotypic variability in patients with mutations in the GCSH gene, which encodes protein H, important for lipoylation and one-carbon metabolism. Functional analyses in fibroblasts, COS cells, and yeast clarified the clinical differences among these patients. This work highlights the necessity of functional assays for understanding disease mechanisms and informing personalized treatments. Additionally, we expanded the mutational spectrum of inborn errors of metabolism (IEMs) by utilizing transcriptomics and functional genomics to identify hard-to-detect promoter variants, deep intronic mutations, and splicing defects that standard exome sequencing often misses.

On the therapeutic front, we developed a CRISPR-Cas9 PMM2 knockout model in the HepG2 hepatoma cell line, which accurately replicates the PMM2-CDG phenotype. This model exhibits complete loss of PMM2 protein and mRNA, a 90% reduction in enzymatic activity, and altered glycoprotein patterns (ICAM-1, LAMP1, and A1AT). This system serves as a powerful tool for characterizing diseasecausing variants and evaluating potential therapies. Additionally, an RNA-seq-based transcriptomic study in patient-derived fibroblasts provided deeper insight into the pathophysiological mechanisms

underlying PMM2-CDG. By applying systems biology methods, we identified key dysregulated pathways, including senescence, bone regulation, extracellular matrix (ECM) composition, cell adhesion, and cytokine response, all of which may contribute to disease progression. Functional validation assays in fibroblasts confirmed defects in cell proliferation, cell cycle regulation, and migration, suggesting a possible role for inflammation in disease pathology. Notably, treatment with a previously described pharmacological chaperone successfully reversed the differential expression of several dysregulated genes, highlighting its potential as a therapeutic candidate. These findings establish a valuable platform for drug discovery and therapy evaluation, supporting efforts to repurpose existing drugs. We have also described how GLDC gene variants, responsible for nonketotic hyperglycinemia (NKH), affect the differentiation of induced pluripotent stem cells (iPSCs) into astrocytes, revealing a metabolic shift in the serine-glycine-onecarbon metabolism and a coordinated cell growth response, offering new insights into the disease's pathology and potential therapeutic options.

Overall, our work advances genomic diagnosis and therapeutic development for IEMs, bridging the gap between functional genomics and clinical application. By combining cutting-edge sequencing technologies, functional assays, and innovative disease models, we aim to improve diagnostic accuracy, uncover new therapeutic targets, and pave the way for precision medicine strategies in inherited metabolic disorders.





Insights into the Role of GCSH in Bioenergetics and the Impact of PPCDC Deficiency on CoA Biosynthesis



- J Alicia Vilas, et al. (2024) HepG2 PMM2-CDG knockout model: A versatile platform for variant and therapeutic evaluation. Mol Genet Metab 143(1-2): 108538. doi: 10.1016/j.ymgme.2024.108538.
- Nastassja Himmelreich, et al. (2024) Significance of utilizing in silico structural analysis and phenotypic data to characterize phenylalanine hydroxylase variants: A PAH landscape. Mol Genet Metab;142(3):108514. doi: 10.1016/j. ymgme.2024.108514. Online ahead of print.
- Houge G, et al. (2024) Comparison of the ABC and ACMG systems for variant classification. Eur J Hum Genet . doi: 10.1038/s41431-024-01617-8. Online ahead of print.
- Diana Gallego, et al. (2024) Transcriptomic analysis identifies dysregulated pathways and therapeutic targets in PMM2-CDG. Biochim Biophys Acta Mol Basis Dis. 2024 Jun;1870(5):167163. doi:10.1016 / j.bbadis. 2024. 167163.
- Laura Arribas-Carreira, et al. (2024) Metabolic Rewiring and Altered Glial Differentiation in an iPSC-Derived Astrocyte Model Derived from a Nonketotic Hyperglycinemia Patient. Int J Mol Sci ;25(5):2814. doi: 10.3390/ijms25052814.
- Jose Córdoba-Caballero, et al. (2024) Exploring miRNA-target gene pair detection in disease with coRmiT. Brief Bioinform;25(2):bbae060. doi: 10.1093/bib/bbae060.
- Ainhoa Martínez-Pizarro, et al. (2024) PAH deficient pathology in humanized c.1066-11G>A phenylketonuria mice. Hum Mol Genet: ddae051. doi: 10.1093/hmg/ddae051. Online ahead of print.
- Juan Cruz Herrero Martín, et al. (2024) An ETFDH-driven metabolon supports OXPHOS efficiency in skeletal muscle by regulating coenzyme Q homeostasis. Nat Metab.;6(2):209-225. doi: 10.1038/s42255-023-00956

# Other activities

Belén Pérez: Member of the Organizing Committee of the AEGH Interdisciplinary Congress.

Belén Pérez: Advisor to the Ministry of Health for the SNS Service Portfolio.

Belén Pérez: Vice President of AEGH. Belén Pérez: Deputy Director of CEDEM.

Alejandra Gámez: President of the Gender Equality

Program at CBMSO.

Pilar Rodríguez-Pombo: Erasmus Coordinator.



#### Research projects

- Fundación Ramón Areces. Nanoformulaciones biocompatibles para la entrega de fármacos: Un nuevo enfoque para el tratamiento enfermedades metabolicas hereditarias. Belén Pérez. 2025-27.
- Instituto de Salud Carlos III. Avanzando hacia la medicina de precisión en enfermedades raras y tumores de baja frecuencia. Belén Pérez. Enero 2025-diciembre 2026.
- EU. HORIZON-WIDERA (Number 101160079). BRIDGing the research and INnovation Gap for Rare Diseases in Europe by upgrading the excellence of IMGGE (BRIDGING-RD). Belén Pérez. September 2024-september 2027.
- CIBERER, ISCIII. Posicionamiento y posibilidades de la secuenciación de cuarta generación en el diagnóstico de enfermedades raras (ER24P7AC7651). Belén Pérez.
- CIBERER, ISCIII. Nanomedicina para la administración de fármacos en errores congénitos del metabolismo: prueba de concepto en PMM2-CDG. Belén Pérez. Enero 2024-marzo 2025.
- Instituto de Salud Carlos III. Increasing diagnosis rates and developing therapeutic strategies for inborn errors of metabolism via the genomic, transcriptomic, epigenomic and functional analysis of patients and models. Belén Pérez. 01/01/2023-31/12/2025.



#### **Doctoral theses**

- Cristina Segovia Falquina (2023) Generation of a functional variant analysis platform for PMM2 with diagnostic and therapeutic purposes. Department of Molecular Biology. UAM PhD Supervisors: Belén Pérez and Alejandra Gámez
- Laura Arribas Carreira (2023). Implications of glycine metabolism in human health and disease: Non-ketotic hyperglycinemia, a disease with a neurological clinical phenotype. Department of Molecular Biology. UAM . PhD Supervisor: Pilar Rodríguez Pombo
- Alicia Vilas Lagoa (2023) Development of diagnostic and therapeutic models for phosphomannomutase 2 deficiency. Department of Molecular Biology. UAM . PhD Supervisor: Belén Pérez. International Distinction
- Diana Gallego Martínez (2024) Functional genomics applied to the identification of therapeutic targets in inherited metabolic diseases. Department of Molecular Biology. UAM PhD Supervisor: Belén Pérez. International Distinction
- Obdulia Sánchez Lijarcio (2024). Multi-omic and functional genomics strategy for improving the diagnosis of neurometabolic diseases. Department of Molecular Biology. UAM. PhD Supervisor: Belén Pérez"



#### VASCULAR INFLAMMATION AND AUTOPHAGY

#### **Group members**

**Principal investigator:** Natalia Reglero

Scientific staff: Irene Carriles Moar

**Postdoctoral fellows:** Dafne García Mateos

**Predoctoral fellows:**Guillermo Marcos Zapatero

Undergraduate and Master students:

Edurne Armesto Longueira



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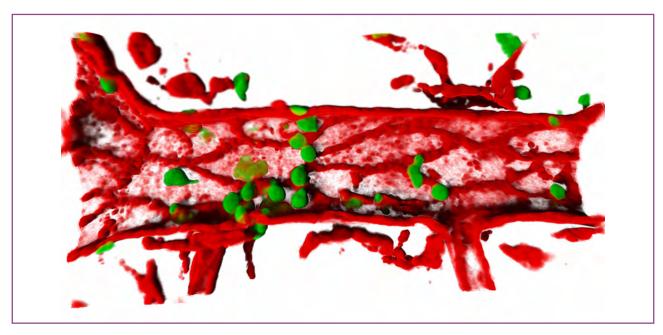
#### Summary

Our laboratory investigates the dynamic interplay between immune cells and the vasculature during physiological and pathological inflammation with the aim of identifying novel therapeutic targets to modulate inflammatory diseases. Although inflammation is essential for host defense and tissue repair, this response must be tightly regulated to avoid excessive tissue damage and chronic disease. Dysregulated immune cell recruitment and vascular dysfunction contribute to the pathogenesis of numerous conditions, including cardiovascular, metabolic, and infectious diseases, as well as cancer progression. Understanding how the vasculature dysfunctional becomes during pathological inflammation is therefore a critical area of research.

Autophagy has emerged as a fundamental regulator of immune responses, with extensive evidence demonstrating its role in immune cell-intrinsic processes such as survival, differentiation, and inflammatory mediator production. Autophagy is a tightly regulated cellular degradation and recycling process that maintains homeostasis under stress conditions; its impairment is a hallmark of ageing and is linked to chronic inflammatory and metabolic diseases. However, the contribution of autophagy beyond immune cells—particularly within the vascular endothelium—remains poorly understood. Endothelial cells serve as key regulators of leukocyte and macromolecule trafficking into tissues, and our

group has demonstrated that endothelial autophagy is essential for limiting neutrophil recruitment and associated tissue damage during acute inflammation (Reglero-Real et al. Immunity, 2021). Our work, supported by advanced imaging strategies such as 4D intravital confocal microscopy, has revealed that endothelial autophagy critically modulates the ability of microvessels to guide immune cell migration, thereby promoting effective resolution of inflammation. These findings suggest novel links between vascular autophagy, immune function, and age-related inflammatory pathologies. Yet, the molecular mechanisms governing this response remain unknown. To address this, our laboratory employs a multidisciplinary approach, integrating high-resolution microscopy, spectral flow cytometry, and in vivo models of inflammation to dissect the pathways through which endothelial autophagy shapes immune responses. By defining the initiating factors, molecular regulators, and pathophysiological relevance of endothelial autophagy during acute and chronic inflammation, as well as ageing-associated pathologies, we aim to advance our understanding of vascular-immune crosstalk and identify new strategies for targeting inflammatory disorders.





Inflamed cremasteric postcapillary venule illustrating the interaction of neutrophils (eGFP; green) with endothelial cell junctions (PECAM-1; red).



#### List of publications

#### **CBM** affiliation:

- Reglero-Real N\*, Pérez-Gutiérrez L, Saleeb RS, Nourshargh S, Collinson L\*, Yoshimura A\*. (2024). Volume correlative light X-ray and electron microscopy (CLXEM) of endothelial cells in mouse tissue. Star Protocols Sep 20;5(3):103257 DOI: https://doi.org/10.1016/j.xpro.2024.103257
- Cacho-Navas C, López-Pujante C, Reglero-Real N, Colás-Algora N, Cuervo A, Conesa JJ, Barroso S, de Rivas G, Ciordia S, Paradela A, D'Agostino G, Manzo C, Feito J, Andrés G, Molina-Jiménez F, Majano P, Correas I, Carazo JM, Nourshargh S, Huch M, Millán J. ICAM-1 nanoclusters regulate hepatic epithelial cell polarity by leukocyte adhesion-independent control of apical actomyosin. Elife. 2024 Apr 10;12:RP89261. DOI: 10.7554/eLife.89261.



#### Research projects

- CBM Intramural Project / "Mención de Excelencia Severo Ochoa" CBM, Spain / "Investigating the immune and vascular metabolic signature in sepsis" / Principal Investigators: Natalia Reglero Real, Nuria Martínez-Martín and Jaime Millán./ 01/06/2024 01/06/2026.
- PID2022-137552OA-I00 / Ministry of Science and Innovation, Spain / "Unmasking novel roles for endothelial autophagy pathways during inflammation" / Principal Investigator: Natalia Reglero Real / 01/09/2023-01/09/2026
- RYC2021-031221-I / Ministry of Science and Innovation,
   Spain / " Imaging the Molecular Processes of
   Inflammation: Novel roles for Autophagy Pathways" /
   Principal Investigator: Natalia Reglero Real / 01/01/2023-31/12/2027



#### Other activities

Organisation of the "2<sup>nd</sup> Endothelial Workshop: From Physiology to Disease". CIMA, Santiago de Compostela, Spain. 09/06/2023.



# CELL COMMUNICATION IN HOMEOSTASIS AND DISEASE THROUGH NEW GQ-GPCR SIGNALING NODES

#### **Group members**

**Principal investigator:** Catalina Ribas Núñez

**Scientific staff:** Inmaculada Navarro Lérida

**Postdoctoral fellows:** Dafne García Mateos Alejandro Asensio López

**Predoctoral fellows:** Alejandro Asensio López Raquel Huertas Lárez

**Technicians:** Isabel Jiménez López

Undergraduate and Master students: Raúl García Fernández Edurne Armesto Longueira Ana Romo Gallo

Visiting scientists: Ellen Richard



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#### Summary

Cell-cell communication and the interactions that occur between them are key aspects for cellular homeostasis, regulating individual cellular processes and intercellular relationships. The microenvironment of a cell is an organized combination of extracellular matrix, stromal tissue (blood vessels, immune cells, fibroblasts and signaling molecules) that influences the cellular phenotype through physical, mechanical and biochemical mechanisms.

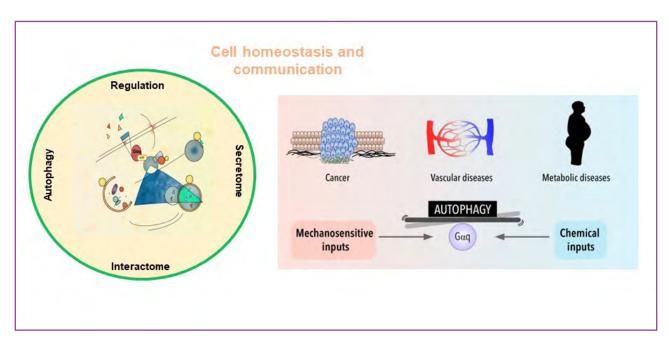
Our main objective is to understand how cells communicate to each other and their surroundings under physiological and pathological conditions, focusing on two GPCR signaling nodes, G $\alpha$ q and GRK2: (1) Interaction between tumor cells and their surrounding stroma (2) Epidermal homeostasis and keratinocyte-immune cells crosstalk.

(1) The Gaq interactome has expanded considerably and our group has contributed to this, through the identification of a new interaction region in Gaq. This non-canonical Gaq signaling is relevant for the maintenance of cell homeostasis, revealing a novel role of Gaq as a central modulator of mTORC1 and the autophagic process. Considering the importance of GPCR in cancer and the novel role of the Gaq as regulator of autophagy, **one of our goals** is to investigate the involvement of Gaq as a link between both processes in the context of oral cancer. We are focused on assessing the importance of the GPCR-Gaq pathway in the regulation of tumor-associated stromal architecture by CAFs (cancer-associated

fibroblasts) and its role as a regulator in the interplay between exosome secretion and autophagic flux, as well as in the modulation of vascular tumor flushing and tumor immune responses. (2) Proper functional interactions between epidermal, dermal, and immune cell infiltration are necessary for correct homeostasis in stratified epithelia (such as skin or oral tissues). We have reported that GRK2 downregulation correlates with tumor malignancy and increases invasion in oral carcinomas. Considering that GRK2 loss induces alterations in epidermal homeostasis, our second qoal will be to analyze how is the dialogue between keratinocytes and their cellular microenvironment fine-tuned to facilitate coordinated responses to maintain homeostasis against inflammatory diseases and cancer. We are focused in assessing the impact of GRK2 deletion in keratinocytes in the skin immune cell landscape, barrier function, skin-microbiome interaction and in the susceptibility to inflammatory diseases and squamous cell carcinomas (in collaboration with F.Mayor).

By using biochemical, cell biology, different "omic" methodologies, high-resolution microscopy, flow cytometry, and 2D and 3D migration/invasion systems, combined with animal models with altered Gaq or GRK2 expression, we will contribute to the understanding of the molecular mechanisms preserving epithelial homeostasis and lead to the discovery of novel targets and treatments associated with the development of inflammatory diseases and carcinomas.





Gaq signaling and autophagy at the crossroads of a balance between mechanical and chemical cues and their impact in cancer, metabolic and cardiovascular pathologies



#### List of publications

- Asensio, A., Sanz-Flores, Liajath-Ali, K., Palacios-García, J., M., Paramio, J.M., García-Escudero, R., Mayor jr, F.\* and Ribas, C.\* (2024) The G Protein-Coupled Receptor Kinase 2 (GRK2) Orchestrates Hair Follicle Homeostasis. bioRxiv 2024.04.11.58905. https://doi.org/10.1101/2024.04.11.589052. \*Co-corresponding authors. In final revision in Journal of investigative Dermatology
- Colás-Algora, N., Muñoz-Pinillos, P., Barroso, S., Cacho-Navas, C., Caballero, A., Cerro-Tello, G., de Rivas, G., González-Fernández, M., Jiménez-Alfaro, I., Fresno, M., Ribas, C., Paradela, A., López-Collazo, E., Fernández, J.J. and Millán, J. (2024) Harnessing homeostatically active RhoC at cell junctions preserves human endothelial barrier function during inflammation. bioRxiv. 2024.05.17.594667. https://doi.org/10.1101/2024.05.17.594667



#### **Doctoral theses**

Alejandro Asensio López (2024). "Role of GRK2 in epidermal homeostasis". Departamento de Biología Molecular. Universidad Autónoma de Madrid. Supervisors: Catalina Ribas and Federico Mayor. Internacional Mention.



#### Research projects

- CIVP21S13241. Ramon Areces Foundation. "Gαq interactome in cell homeostasis and communication: impact on tissue architecture remodeling and endothelial dysfunction" IP: Catalina Ribas Núñez. Fechas: 01/01/2023-31/12/2025.
- PI22/00966. Instituto de Salud Carlos III. "Gαq signaling networks in cellular homeostasis and communication: impact on endothelial dysfunction and inflammation". IP: Catalina Ribas Núñez. Fechas: 01/01/2023-31/12/2025.
- INTEGRAMUNE-CM/P2022/BMD-7209, Madrid Regional Excellence Networks in Biomedicine. "Integrated cellular and molecular systems in immune-inflammatory pathophysiology". Team member (IP: Federico Mayor). Fechas: 01/01/2023-31/12/2026.
- Grupo CIBERCV CB16/11/00278. Cardiovascular Research Consortium (CIBER CARDIOVASCULAR). Instituto de Salud Carlos III. Team member. Fechas: 2017-2023



#### Other activities

Catalina Ribas: Member of TRAIN@CBM and Help&Hand committes at CBM. Partipation in the Innovation Project "SWI+Microworld (Small World Initiative)", Guest speaker in "Commemorative event for the feast of San Alberto Magno" (2023) Faculty of Science (UAM)



# PHYSIOPATHOLOGY STUDIES AND THERAPEUTICAL APPROACHES IN ANIMAL AND CELLULAR MODELS OF NEUROMETABOLIC DISEASES

#### **Group members**

Principal investigator: Lourdes Ruiz Desviat (PI) Eva Richard (co-PI)

**Postdoctoral fellows:** Ainhoa Martínez-Pizarro

**Predoctoral fellows:** Emilio Martínez García-Tenorio

**Technicians:** Mar Álvarez Elena Montalvo Gonzalo Villena Undergraduate and Master students: Mónica Gallego Bonhomme Beatriz Alvera Irene González Garnacho Alvaro Arévalo Nuevo José Vicente del Alamo

**Visiting scientists:** Selma Elabed



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#### Summary

The group belongs to CIBERER and to Health Research Institute Hospital La Paz (IdiPAZ) and actively collaborates with CEDEM (Science Faculty, UAM). Our research focuses on neurometabolic diseases, propionic acidemia (PA) and hyperphenylalaninemias among others, enzymatic deficiencies of autosomal recessive inheritance, characterized by the toxic accumulation of precursors and lack of downstream metabolites.

One line of research involves the analysis of the physiopathological mechanisms underlying the multiorgan complications associated to PA, one of the most frequent organic acidemias. Chronic kidney disease (CKD) is a known long-term complication. We have investigated the renal phenotype of a hypomorphic murine PA model (Pcca-/-(A138T)) in which we have shown altered mitochondrial networks and a marked reduction of mitophagy in kidney tissue, along with a steep reduction of PGC-1- $\alpha$ . The results indicate that impairment of mitochondrial homeostasis and quality control are involved in CKD development in PA. We have also generated iPSCs from PA patients' fibroblasts and differentiated them to neuronal precursors, astrocytes and cardiomyocytes, relevant cell lineages for this disease. PA cardiomyocytes exhibit greatly depressed cell excitability and an increased risk of arrythmias. In iPSCs-derived PA astrocytes we observe altered mitochondrial function, miRNAs deregulation and astrogliosis. Overall, our results provide a basis for identifying new therapeutic targets for intervention strategies for the disease.

We have also used CRISPR/Cas9 technology to generate novel cellular and animal models of disease. We have generated hepatoma cellular models of phenylketonuria (PKU), knock-in with different patient specific mutations in exon 11 of the PAH gene, all of them causing splicing defects. We have identified splice modulating antisense oligonucleotides that correct the exon skipping defect of several of these PAH variants and confirmed the functional rescue of the hepatic enzyme phenylalanine hydroxylase in the gene edited hepatoma cell lines. We have also generated a novel PKU mouse model, carrying the highly prevalent PAH splicing variant c.1066-11G>A. Homozygous mice, with a partially humanized intron 10 sequence with the variant, accurately recapitulate the splicing defect and present almost undetectable hepatic PAH activity and exhibit the biochemical and clinical phenotype of the most severe form of the disease. They exhibit fur hypopigmentation, lower brain and body weight, reduced survival, and decreased monoamine neurotransmitter levels. They present behavioral deficits, locomotor deficiencies, correlating with changes in the morphology of glial cells and decreased myelinization. This PKU mouse model with a prevalent human variant represents a useful tool for pathophysiology research and for novel therapies development.





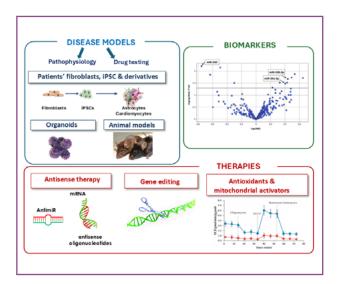
#### List of publications

- Schumann A, Martinez-Pizarro A, Richard E, Schell C, Kössinger AL, Zeyer KA, Tholen S, Schilling O, Barry M, Neubauer B, Köttgen M, Hannibal L, Desviat LR, Spiekerkötter U. (2024) Renal phenotyping in a hypomorphic murine model of propionic aciduria reveals common pathomechanisms in organic acidurias. Sci. Rep. 14(1):30478. doi: 10.1038/s41598-024-79572-z.
- Richard E, Martínez-Pizarro A, Desviat LR. (2024) Exploring RNA therapeutics for urea cycle disorders. J. Inherit. Metab. Dis. 47(6):1269-1277. doi: 10.1002/jimd.12807.
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#### Other activities

Lourdes Ruiz Desviat: Premio EPF (Enfermedades Poco Frecuentes) Martínez-Pardo de Enfermedades Metabólicas 2024.



Research tools and methods used to understand the pathophysiology of neurometabolic diseases and for the development of novel therapies.



#### Research projects

- PID2022-137238OB-I00 MCINN y FEDER. Rare neurometabolic diseases: from research in novel disease models to targeted therapies. Lourdes R Desviat & Eva Richard (2023-2026).
- PAF113. Propionic acidemia Foundation. Elucidation of cardiac electrophysiological alterations in propionic acidemia: Towards the identification of targets for therapeutics. Eva Richard (2023-2024).
- Fundación Ramón Areces XX Concurso Nacional. Acidemia propiónica: impacto en el epigenoma y el proteoma en relación con el fenotipo cardiaco y neurológico. Eva Richard (2021-2024).
- EU COST Action CA21113. Gene Editing for the treatment of Human Disease (GenE-HumDi). Lourdes R Desviat (participant) (2022-26).

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- CIBER DE ENFERMEDADES RARAS (CIBERER). ISCIII CB06/07/0017.
- Instituto de Investigación Sanitaria Hospital La Paz (IdiPaz).



#### **Patents**

■ Splice shifting oligonucleotides for use in the treatment of diseases characterized by altered inclusion of microexons. Lourdes Ruiz-Desviat, Ainhoa Martínez-Pizarro, Sara Picó, José J Lucas, Brage S Andresen. Ref. WO/2023/057575; EP4163373, published 13th April 2023.Title holders: UAM/CSIC/University of Southern Denmark.



# CALCIUM SIGNALING IN MITOCHONDRIA: METABOLIC CONTROL AND MITOCHONDRIA PHYSIOPATHOLOGY

#### **Group members**

**Principal investigator:**Beatriz Pardo Merino
Araceli del Arco Martínez

#### Scientific staff:

Laura Contreras Balsa José María Carrascosa Baeza Jorgina Satrústegui Gil-Delgado Cayetano von Kobbe Alonso

#### Predoctoral fellows:

Luis González Moreno Eduardo Herrada Soler Ismael García Lobo Andrea Santamaría Cano Miguel Angel Serrano Lope Teresa Mingo Morcillo

#### **Technicians:**Bárbara Sesé Cobos

Undergraduate and Master students: Ismael García Lobo Raúl García Martínez Daniel Marcos Ríos Mikel Freire Bastardo Patricia Picallo Ramos Alba Cantalejo Rodrigo Sara Ferrero



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



#### Summary

Our interests are understanding calcium regulation of mitochondrial function by way of the calcium-dependent mitochondrial carriers of aspartate-glutamate/AGCs (Aralar/AGC1 and citrin/AGC2), components of the malate aspartate shuttle (MAS), or ATP-Mg2+/Pi carriers (SCaMCs). These carriers have Ca2+-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.

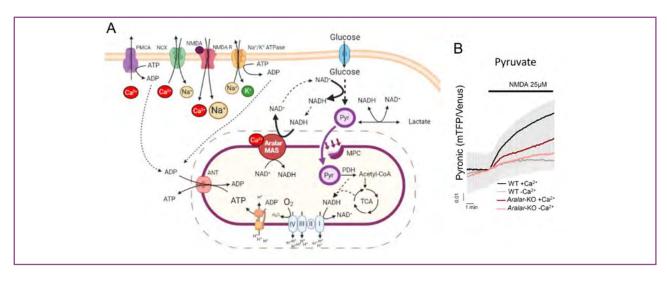
In humans, Aralar/AGC1 deficiency is a rare disease presenting neurological and muscular affectation. Postnatal hypomyelination, epilepsy, hypotonia and delayed neurodevelopment are the main traits in

patients. Global Aralar-KO mice have a short lifespan and well recapitulates the human phenotype. Our main interest is focused on deciphering the physiopathological role of Aralar/AGC1 in specific brain cell types and in muscle. To face these issues, we have generated oligodendroglial- and neuron-specific Aralar-KO mice. To answer relevant questions as: (a) the role of neuronal Aralar in the severe phenotype of the global Aralar-KO mice (b) the involvement of muscular- and neuronal-Aralar in motor coordination deficits and hypotonia, or (c) the contribution of oligodendroglial and neuronal Aralar to postnatal myelination, and demyelination-remyelination processes.

Citrin deficiency is a urea cycle disorder with different manifestations. Citrin/AGC2 is mainly expressed in liver and main clinical symptoms are hypoglycemia, hyperammonemia and dyslipidemia. In the frame of the Citrin Foundation, we are exploring i) whether the exogenous expression of Aralar, which has low expression in human liver, as possible therapy for Citrin deficiency and ii) the role of citrin/AGC2 in liver in the mitochondrial response to Ca2+ mobilizing agonists and its impact in liver metabolism. For that purpose, we will generate cellular and transgenic mouse models to better recapitulate the human disease.

As part of a COVID-19 project (CvK), a therapy targeting senescent cells, was shown to reduce mortality and morbidity induced by SARS CoV 2 in the transgenic animal models used.





A Ca2+-dependent mechanism boosting pyruvate production, glycolysis and OXPHOS by activating Aralar/malate-aspartate shuttle, upon neuronal stimulation. (A) Cytosolic and mitochondrial processes. (B) Cytosolic pyruvate. In Aralar-KO neurons, NMDA fails to activate glycolysis, and increase pyruvate.



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#### MITOCHONDRIAL BIOLOGY IN IMMUNE MODULATION

#### **Group members**

**Principal investigator:** Javier Traba

Predoctoral fellows: Aurea Oliva Herrero Carolina Meroño Ortega Yara Cuesta Valero

**Undergraduate and Master students:** 

Rubén Rincón Álvarez Andrea Alonso Gómez Marta Pascual Fernández Natalia Garcia Acosta







#### Summary

The activation and differentiation of immune cells require intricate metabolic reprogramming, with mitochondria playing a central role. These organelles are essential not only for energy production but also as sources of metabolic intermediates and signaling molecules that shape immune responses. Our laboratory is dedicated to investigating the interplay between mitochondrial function and the regulation of both innate and adaptive immunity. Specifically, we focus on the roles of key mitochondrial proteins in maintaining homeostasis and modulating immune cell function:

 Posttranslational Modifications (PTMs) and Macrophage Function:

We are particularly interested in how PTMs of mitochondrial proteins—such as lysine acetylation and succinylation—regulate macrophage activation and polarization. Our research focuses on mitochondrial nicotinamide adenine dinucleotide (NAD+)-dependent enzymes, including deacetylases (e.g., Sirtuin 3, SIRT3), desuccinylases (e.g., Sirtuin 5, SIRT5), and deacylases targeting more complex modifications (e.g., SIRT4-mediated hydroxymethylglutarylation of lysines). Notably, our recent findings reveal that SIRT3 plays a key role in controlling the secretion of type I interferons, which are critical antiviral molecules.

- Mitochondrial Metabolites in Immune Cell Activation:

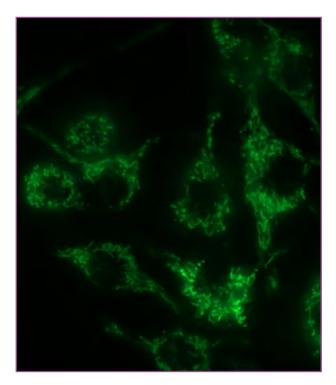
Our lab also explores the role of mitochondrial metabolites-including adenine nucleotides (AMP, ADP, ATP) and NAD\*-in the activation and differentiation of macrophages and T lymphocytes. As metabolite transport across the inner mitochondrial membrane is mediated by Mitochondrial Carrier Family proteins, we are investigating the impact of altering the expression of specific mitochondrial carriers, such as SLC25A51/MCART1, the recently identified NAD\* transporter, in immune cells.

- Targeting Metabolism to Modulate Inflammation:

We are exploring strategies to mitigate inflammation in a psoriatic mouse model by modulating mitochondrial function, fidelity, and quality control pathways.

Our research aims to uncover fundamental mechanisms by which mitochondria shape immune responses, providing new insights into the metabolic regulation of immunity. Moving forward, we will continue to investigate novel therapeutic strategies targeting mitochondrial function in inflammatory and autoimmune diseases, with the goal of translating our findings into innovative interventions for immune-related disorders.







#### List of publications

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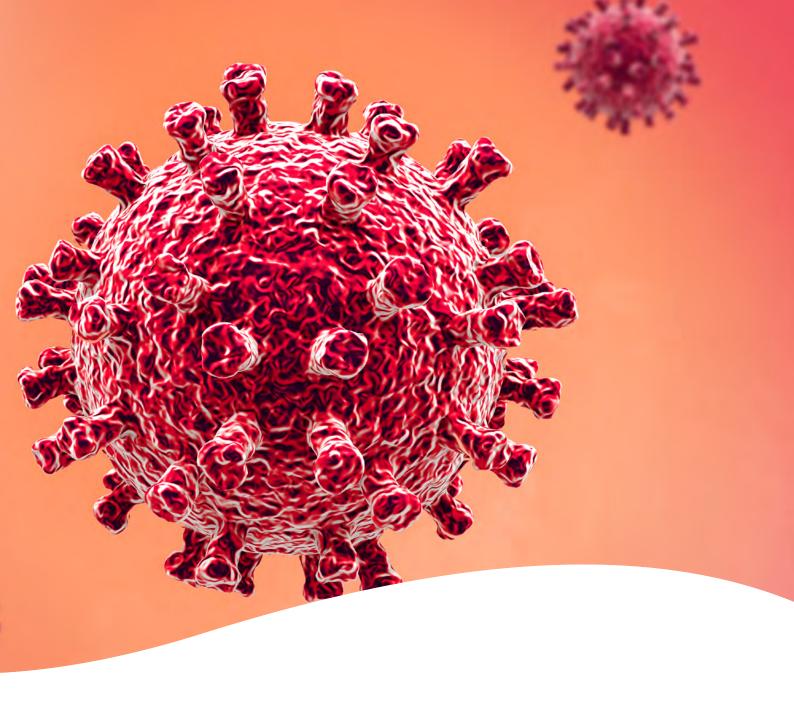
RAW 264.7 mouse macrophages expressing a fluorescent mitochondrial biosensor for nicotinamide adenine dinucleotide (NAD+)



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- PID2019-105665RA-I00. State Research Agency. Immunometabolic regulation through the mitochondrial protein Sirtuin 3. Javier Traba. 01/06/2020-31/03/2024.
- SBPLY/21/180501/000161. Regional Government of Castilla-La Mancha. Study of the molecular basis of Fontaine-Petty progeroid syndrome. Araceli del Arco Martínez. 01/09/2022-31/03/2025.
- Membership of regional or national networks of excellence: RED2022-134927-T. Spanish thematic network on the interaction between cellular metabolism and the microenvironment in homeostasis and diseases. Coordinator: Fernando Martín Belmonte (CBM). 06/01/2023-05/31/2025.



# Interactions with the environment



Immune system development and function unit

Microbes in health and welfare unit



# Interactions with the environment

#### Immune system development and function unit



#### César Cobaleda

Over the past two years, our "Immune" unit has made significant contributions across diverse fields of immunology, inflammation, cancer biology, cellular signaling, and host-pathogen interactions. The diverse research lines represented in our groups share a common commitment to understanding and therapeutically targeting the mechanisms that govern immune responses, tissue homeostasis, and disease pathogenesis.

Key advances were made in uncovering immune responses to parasitic and viral infections, such as *Trypanosoma cruzi*, the agent of Chagas disease; microRNA and metabolomic profiling identified biomarkers predictive of disease progression and treatment response, and markers regulating reactive species in infected macrophages revealed novel immunomodulatory mechanisms. A strong translational component also emerged from the exploration of viral immune evasion and environmental virology; work on poxviruses dissected how viral proteins interfere with host immune sensing and cytokine signaling, with implications for anti-inflammatory drug development.

At the intersection of immunity and cancer, other groups examined how immune pathways are altered in malignancies. In aggressive double-hit B cell lymphomas, transcriptomic and metabolic profiling uncovered vulnerabilities involving the unfolded protein response and epigenetic control, suggesting strategies to resensitize immunologically "cold" tumors. R-RAS2 was also shown to drive leukemia and breast cancer in mouse models, linking it to lymphocyte signaling and transformation.

Cell signaling in autoimmunity and inflammation remains a central focus. Autoimmune signaling studies revealed that IL-23 governs T cell pathogenicity by modulating migration, metabolism, and protein synthesis, offering targets for IL-23/IL-17-driven diseases. Complementary work showed that nitric oxide and electrophilic lipids shape T cell activity via redox-sensitive transcriptional regulation.

Research into T cell receptor (TCR) signaling advanced our understanding of TCR nanoclusters and on the conformational changes that enhance antigen sensitivity. Inhibitors blocking adaptor recruitment to the TCR showed promise for modulating autoimmunity while preserving host defense. Synthetic biology strategies enabled the design of logic-gated CAR-T cells and miniTCRs for targeted cancer therapy.



### Research groups

# Balbino Alarcón Signal transduction by the T-cell antigen receptor Antonio Alcamí Immunity & Viromics César Cobaleda Cellular plasticity in development and cancer Margarita del Val Viral immunology Manuel Fresno Regulation and function of proinflammatory mediators and their involvement in immune and inflammatory mediated diseases Núria Gironés Immunoregulatory mechanisms in the development

of Chagas disease: translational applications

#### Miguel Angel Íñiguez Juan Manuel Serrador

Nitric oxide and bioactive lipids signalling in the immune response

#### María N. Navarro

Intracellular signalling in inflammatory processes

#### Ana Ortega Molina

Metabolism in cancer and aging

#### Felipe X. Pimentel-Muiños

Unconventional autophagy in health and disease

#### 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

Non-conventional autophagy emerged as a key regulator in immune signaling and stress responses. Studies on ATG16L1 highlighted its role in cytokine receptor trafficking, immunogenic cell death, and antibacterial defense, suggesting therapeutic targets for inflammatory diseases like Crohn's.

In hematopoietic malignancies, the unit studied how transcriptional and epigenetic regulators shape cell fate. Infection-induced stress was found to trigger B-ALL in predisposed individuals, linking environmental and genetic factors in leukemia initiation. Further insight came from TCFL5 isoforms, which modulate leukemogenesis by interacting with signaling pathways that control survival and cell cycle progression.

Progress was also made in the translation of knowledge from developmental immunology to antitumoral immunotherapy; the identification of the pre-TCR as a target in T-ALL enabled antibody–drug conjugates and CAR-T cells with improved specificity. Mapping thymus aging and regeneration using spatial biology aimed to reverse immunosenescence and restore immune function.

In viral ecology, the unit identified novel viruses in polar and alpine environments and detected the first avian influenza cases in Antarctica. These efforts were complemented by studies on airborne transmission and emerging zoonoses, reinforcing our role in global health surveillance.

The period has also seen a solid output of doctoral training, with fourteen doctoral theses defended across multiple thematic areas. Members of the unit have received notable recognition, including regional and national awards for young investigators, and invitations to present at leading international conferences.

Taken together, the research conducted over the past two years exemplifies the strength and cohesion of the unit in tackling complex biomedical challenges. Through collaborative efforts and a strong foundation in molecular and cellular biology, the unit remains at the forefront of discovery, training, and innovation in immunology and related disciplines.



#### SIGNAL TRANSDUCTION BY THE T-CELL ANTIGEN RECEPTOR

#### **Group members**

**Principal investigator:**Balbino Alarcón

Scientific staff: Aldo Borroto

Postdoctoral fellows: Alejandro Hortal Claudia Cifuentes Ana Villanueva

**Predoctoral fellows:**Rut Tercero
Alice Lombard

**Technicians:** Tania Gómez Aranda Irene Arellano Undergraduate and Master students: Alexandra Shirikova Marta Lacuna Pilar Grosso

Visiting scientists: Claudia Juraske Anastasia Kremser Lena Grubisic



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

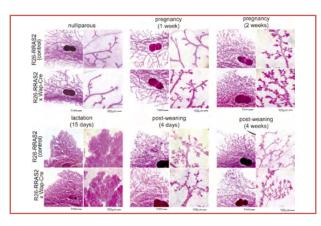


#### Summary

T-cell activation requires the recognition of antigenic peptides presented on major histocompatibility complexes (pMHC) by the T-cell antigen receptor (TCR). The TCR interacts with the antigen extracellularly through its variable  $\alpha$  and  $\beta$  chains, which have very short cytoplasmic tails. Consequently, signal initiation is relayed to the CD3 subunits, which contain longer cytoplasmic tails capable of engaging intracellular effectors. We have discovered that, in the plasma membrane of resting T cells, the TCR is organized into oligomers of up to 20 receptors, which we refer to as TCR nanoclusters. We propose that nanoclusters contribute to the remarkable sensitivity of T cells to antigens, despite the low affinity of individual TCRs for pMHC. Indeed, our recent findings suggest that TCR nanoclustering enables cooperative interactions between pMHC-engaged and non-engaged TCRs. Furthermore, we have proposed that conformational changes mediate the conversion of pMHC-TCR interactions into CD3-driven intracellular signals, thereby triggering TCR activation. One consequence of these conformational changes is the exposure of a proline-rich sequence (PRS) in CD3E, which becomes accessible for binding to the adaptor protein Nck. We have developed small-molecule inhibitors that block Nck recruitment to the TCR as potential immunomodulatory agents. These inhibitors are orally bioavailable and demonstrate potent prophylactic and therapeutic effects in various autoimmune disease models while preserving T cell responses to

pathogens. Another direct effector of the TCR is the small GTPase R-RAS2 (also known as TC21), which constitutively binds to the non-phosphorylated TCR and plays a crucial role in homeostatic signaling via PI3K. R-RAS2 is also a direct effector of the B cell antigen receptor (BCR). We are currently investigating the role of R-RAS2 in key physiological processes of T and B lymphocytes, including homeostatic population control, immunological synapse formation, thymic selection, and germinal center formation. Additionally, we are studying its involvement in pathological conditions, such as the development of T and B cell lymphomas and leukemias. Our most exciting recent findings suggest that wild-type R-RAS2 acts as an oncogenic driver when over expressed. Over expression of R-RAS2 in genetically modified mice leads to the development of chronic lymphocytic leukemia (CLL), a B cell leukemia, as well as the emergence of triplenegative breast cancers. Interestingly, breast cancer development occurs exclusively in parous female mice and not in nulliparous ones. This observation aligns with the high RRAS2 expression found in human triplenegative breast cancers associated with parity. We are currently working to elucidate the mechanisms by which R-RAS2 overexpression drives transformation in both CLL and breast cancer. Additionally, we aim to identify RRAS2-associated markers that could predict the onset and prognosis of these cancers.





Carmine alum stainings of mammary glands of control Rosa26-RRAS2fl/fl and Rosa26-RRAS2fl/fl x Wap-Cre females taken at different stages: 8-week old nulliparous mice, at 1 week and 2 weeks of pregnancy, at day 4 after ceasing lactation (postweaning), and at 4 weeks post-weaning.



#### Research projects

- PDC2021-121170-I00. State Research Agency. Mouse model of chronic lymphocytic leukemia for the validation of new therapeutic interventions. Balbino Alarcón (PI). 2022-2023.
- P2022/BMD-7209. Community of Madrid. Integrated cellular and molecular systems in immune-inflammatory pathophysiology. (INTEGRAMUNE 2023-2026).
- PID2022-136745OB-I00. State Research Agency. TCR signaling mechanisms and T-B interactions in germinal centers for the generation of antibodies of clinical and commercial interest. Balbino Alarcón (PI). 2023-2026.



#### List of publications

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#### **Book chapters:**

■ Van Santen HM and Alarcón B. (2024). TCR signaling: Proximal signaling. In: Ratcliffe, MJH (ed) Encyclopedia of Immunobiology 2nd edition. Elsevier Ltd, pp. 1-11. doi: 10.1016/B978-0-128-24465-4.00097-1



#### **Doctoral theses**

- Ana Villanueva García (2023). Validation and characterization of new family of inhibitors for RAS-driven tumors. Departamento de Biología Molecular. Universidad Autónoma de Madrid. Director: Balbino Alarcón.
- Alejandro Miguel Hortal Borowski (2023). Overexpression of wild type RRAS2, without oncogenic mutations, drives chronic lymphocytic leukemia. Departamento de Biología Molecular. Universidad Autónoma de Madrid. Director: Balbino Alarcón.
- Lydia B. Hörndler Gil (2024). Development and optimization of a combinatorial CAR-T cell therapy for AML to overcome on-target off-tumor responses. Departamento de Biología Molecular. Universidad Autónoma de Madrid. Directores: Hisse M van Santen and Balbino Alarcón.
- Claudia Cifuentes Caballero (2024). Role of RRAS2 overexpression behind breast cancer development. Departamento de Biología Molecular. Universidad Autónoma de Madrid. Director: Balbino Alarcón.



#### **IMMUNITY & VIROMICS**

#### **Group members**

**Principal investigator:** Antonio Alcamí

**Scientific staff:** Bruno Hernáez

Postdoctoral fellows: Angela Vázquez Calvo Rafael González Serrano Pilar Delgado Ana Moraga Quintanilla

Predoctoral fellows: Sergio Sánchez Carrillo Javier Alvarez de Miranda Rodríguez Isabel Alonso Sánchez

Alazne Rubio Unanue

**Technicians:** 

Rocío Martín Hernández María del Carmen Fernández Moyano Carolina Sánchez Fernández María Teresa García Castey

Undergraduate and Master students: Samuel Donaire Daniela Casamayor



www.cbm.uam.es/antonio.alcami



#### Summary

We are investigating the evasion strategies used by poxviruses to evade the host immune response. We are characterizing soluble decoy receptors for cytokines, such as type I interferon, tumor necrosis factor and chemokines, that are secreted from infected cells and modulate cytokine activity. We also work on poxin/schlafen, a viral protein that interferes with DNA sensing and prevents activation of type I interferon. The contribution of these viral proteins to pathogenesis is tested in a mouse model of infection (mousepox). Based on viral immune modulatory activities, we are developing new anti-inflammatory therapeutic approaches to block human inflammatory diseases. Since the 2022 mpox virus international outbreak, we are characterizing mpox virus proteins that may contribute to virus adaptation to human replication and transmission. We have applied new nanofiber filters to capture viruses to characterize the potential airborne transmission of mpox virus. We were also involved in the identification of mpox virus in wastewater to evaluate the presence of mpox virus in the population.

In this time, Bruno Hernaez was awarded a grant from AEI 2022 to characterize mpox virus mechanisms that modulate chemokine activity.

We are also interested in environmental viral metagenomics for the identification of new viruses in Antarctic Dry Valleys, Antarctic and Arctic lake sediments, and water samples from Alpine ecosystems in the Ordesa and Monte Perdido National Park in the Pyrenees. These studies will identify common properties of viruses present in similar extreme ecosystem separated geographically.

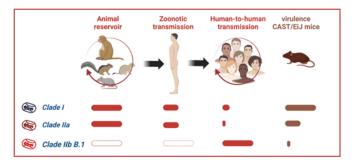
We work on airborne virus transmission and, in collaboration with other CSIC groups, the development of methods for the inactivation of viruses present in air and surfaces.

We participated in the Spanish Antarctic Campaign 2023-2024 and the International HPAI Australis Expedition 2024 aboard the sailboat Australis, and identified the first cases of avian influenza virus in Antarctica.



- Advisor to the World Health Organization Advisory Committee on Variola Virus Research.
- Member of the Scientific Advisory Board of CSIC





Transmission properties of different clades of MPXV and their virulence in humans and mice. From Alcami, A. 2023 PNAS USA 120(13):e2301662120.



#### List of publications

- Aguado, B., et al. (2024) Searching for high pathogenicity avian influenza virus in Antarctica. Nat. Microbiol. 9(12), 3081-3083. doi:10.1038/s41564-024-01868-7.
- Aguado, B., et al. (2024) Detection of highly pathogenic avian influenza virus in Antarctica during the International HPAI Australis Expedition 2024. DIGITAL.CSIC https://doi.org/10.20350/digitalCSIC/16596.
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- Hernaez, B., et al. (2023) Monitoring monkeypoxvirus in saliva and air samples in Spain: a cross-sectional study. Lancet Microbe 4(1):e21-e28. doi: 10.1016/S2666-5247(22)00291-9.
- Hernaez, B. and Alcamí, A. (2023) Detection of Chemokine Binding Proteins Association to Cell Surface Glycosaminoglycans by Flow Cell Cytometry and Indirect Immunofluorescence. Methods Mol. Biol. 2597:121-129. doi:10.1007/978-1-0716-2835-5\_10.
- Alejo, A., et al. (2023) African swine fever virus transmembrane protein pEP84R guidescore assembly. Plos Pathogens 9(1):e1011136. DOI: 10.1371/journal.ppat.1011136.



- Ref. 101195102. EU Horizon EDCTP Program. Strengthening epidemiological, genomic and community surveillance of Mpox virus at the Congo Reiver border for DRC and RoC (MPOX-PROBE) Coordinator: Dario Scaramuzzi; PI: A. Alcamí. 10/24-09/26
- Ref. 101121308. Horizon EU. SysTem for SAFE Biological chEmical Radiological and Nuclear Assessment, Rescue and Decontamination (STBERNARD). Coordinator: JL Pérez-Díaz. PI: A. Alcamí.09/24-10/26.
- Ref. HR23-00058. Fundación La Caixa. Monkeypox virus cytokine decoy receptors: adaptation to human immune evasion and transmission (MPOX\_ADAPT). PI: A. Alcamí. 01/24-21/26.
- Ref. 202320E224. Proyecto Intramural CSIC. Monitoring of avian influenza H5N1 in penguin colonies in Antarctica (PENGUIN\_FLU). PI: A. Alcamí. 11/23-11/24.
- Ref. HR22-00813. Fundación La Caixa. High-efficient technology for clean and safe air: development of advanced air purifiers to inactivate pathogens in aerosols (SafeAir). Coordinator: M.A.Bañares. PI: A. Alcamí.12/22-11/25.

- Ref. 101084097. Horizon EU. Healthy environmental-friendly and resilient farm to work (HE-FARM). Coordinator: JL Pérez-Díaz. PI: A. Alcamí.11/22-10/25.
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- Ref. 505820, Contract No. DE-AC02- 05CH11231. Joint Genome Institute, U.S. Department of Energy. Diversity of DNA and RNA viruses in Antarctica. PIs: A. Alcamí and C. Cary 01/20-12/24.
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#### CELLULAR PLASTICITY IN DEVELOPMENT AND CANCER

#### **Group members**

**Principal investigator:** César Cobaleda Hernandez

Postdoctoral fellows: Jorge Martínez Cano

Predoctoral fellows: lorge Martínez Cano

Javier Isoler Alcaraz (co-IP: María Gómez)

Rodrigo Bailey Maldonado



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



#### **Summary**

How is the identity of blood cells determined? When their identity is disrupted, cells can become tumoral (leukemia) or non-functional (immunodeficiencies), so understanding cell plasticity is essential for the development of therapies. We study how hematopoiesis is altered in disease using samples from human patients, and mice modified to recapitulate human hematologic diseases.

#### **Cellular Plasticity**

Cellular plasticity is the ability of cells to change identity, adopting new gene expression profiles and functions. This process, controlled by transcription factors and epigenetic regulators, enables cells to activate or suppress developmental programs. Dysregulation can lead to abnormal identities, as seen in cancer and genetic syndromes. Our group investigates how lymphocyte identity is determined and disrupted in conditions like leukemia and immunodeficiencies, using genetically engineered mouse models to modify the expression of normal and oncogenic transcription factors and epigenetic regulators.

#### Cellular Plasticity in Leukemia

B-cell acute lymphoblastic leukemia (B-ALL) is the most common childhood cancer. Nearly 5% of newborns have a genetic predisposition, yet less than 1% develop B-ALL, requiring a secondary hit. The triggers remain unclear, but rising B-ALL incidence correlates with modern lifestyles. Immune stress—potentially from infections, antibiotics, diet, or microbiota alterations—has been proposed as a key factor. We developed mouse models expressing oncogenic

lesions linked to B-ALL and demonstrated that common infections are major triggers. Furthermore, preventive treatment with oncogenic pathway inhibitors protected predisposed mice, suggesting B-ALL may be preventable. Given that premalignant cells exist in many cancers, our findings could inform broader cancer prevention strategies.

#### **Cellular Plasticity in Immunodeficiencies**

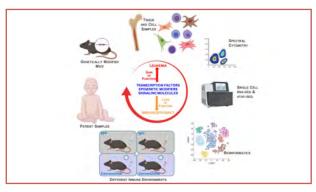
Wolf Hirschhorn Syndrome (WHS) is a rare disorder caused by deletions on chromosome 4, leading to severe immunodeficiencies. NSD2, an epigenetic regulator affected in WHS, is also mutated in B-ALL and Multiple Myeloma.

We use genetically engineered mouse models with altered Nsd2 function to study WHS-related hematopoietic defects. Our research shows that Whsc1 loss is crucial for normal B-cell differentiation and function, linking it to WHS immunodeficiency. This model serves as a preclinical tool for therapy development.

#### Cellular Plasticity in Multiple Myeloma

Multiple myeloma is a cancer of plasma cells, the cells responsible for producing antibodies. It is a severe disease that leads to abnormal cell proliferation in the bone marrow, weakening bones, impairing immune function, and causing organ damage. We contributed to developing NSD2-based multiple myeloma mouse models that replicate human disease. These models establish correlations between tumor genetics, immune characteristics, and responses to preclinical therapies, aiding prediction of treatment outcomes for next-generation immunotherapies.





We use genetically modified mice modeling human hematopoietic diseases. Using spectral cytometry, single-cell transcriptomics, and bioinformatics, we study genetic and environmental interactions in disease development. Findings are validated in human samples to explore new therapies.



#### **Patents**

■ Title: "Animal Model of MMSET-associated Multiple Myeloma Associated Identified as Rosa26-hMMSET-IIStop-Floxed Mouse Strain". Inventors: Cobaleda, César; Campos-Sanchez, Elena; Martínez-Cano, Jorge. Licensing Contract signed by CSIC and the spin-off company MIMO Biosciences.



#### **Doctoral theses**

Jorge Martínez Cano (2023). Regulación del metabolismo mitocondrial y la diferenciación normal y patológica de los linfocitos B por Wolf-Hirschhorn Syndrome Candidate 1. Departamento de Biología Molecular. Universidad Autónoma de Madrid.

Director: César Cobaleda.



#### List of publications

- Larrayoz M, Garcia-Barchino MJ, Celay J, Etxebeste A, Jimenez M, Perez C, Ordoñez R, Cobaleda C, Botta C, Fresquet V, Roa S, Goicoechea I, Maia C, Lasaga M, Chesi M, Bergsagel PL, Larrayoz MJ, Calasanz MJ, Campos-Sanchez E, Martinez- Cano J, Panizo C, Rodriguez-Otero P, Vicent S, Roncador G, Gonzalez P, Takahashi S, Katz SG, Walensky LD, Ruppert SM, Lasater EA, Amann M, Lozano T, Llopiz D, Sarobe P, Lasarte JJ, Planell N, Gomez-Cabrero D, Kudryashova O, Kurilovich A, Revuelta MV, Cerchietti L, Agirre X, San Miguel J, Paiva B, Prosper F, Martinez- Climent JA. (2023) Preclinical models for prediction of immunotherapy outcomes and immune evasion mechanisms in genetically heterogeneous multiple myeloma. Nat. Med. 29, 632-645. doi: 10.1038/s41591-022-02178-3.
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#### Research projects

- CUNINA 2. Fundación UNOENTRECIENMIL. Validating novel molecular determinants of predisposition to Childhood Acute Lymphoblastic Leukemia. March-2024/ February-2027
- 100B1. Intramural Exploratory Grants Program-Severo Ochoa. Integrative approaches to understand cellular fate: NSD2 and hematopoietic lineage specification. June-2024/May-2026
- PRYCO211305SANC. Asociación Española Contra el Cáncer (AECC). Infectious triggers and novel therapeutic opportunities in childhood B cell leukemia (PREVENT). October-2021/September-2026
- PID2021-122787OB-I00. Agencia Estatal de Investigación. Alterations of the Epigenetic Control of Development in Rare Diseases and Leukemia (AERIAL). September-2022/August-2025



#### Other activities

- 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)].
- Member of the Animal Experimentation Ethics Committee of the Severo Ochoa Center for Molecular Biology (CEEA/ CBMSO)

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#### VIRAL IMMUNOLOGY

#### **Group members**

**Principal investigator:** Margarita del Val Latorre

Scientific staff: Luis C. Antón Canto Manuel Ramos Álvarez-Buylla

**Postdoctoral fellows:** Elena Campos Sánchez Cristina Rodríguez Rojas

Predoctoral fellows: Cristina Rodríguez Rojas Víctor Muñoz Abad Andrés Soto Zaragoza Laura Luna Gutiérrez Oliva **Technicians:**Beatriz Mena Romero
Carolina Rubio Cachazo

Undergraduate and Master students: María Fernández Martínez



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



#### Summary

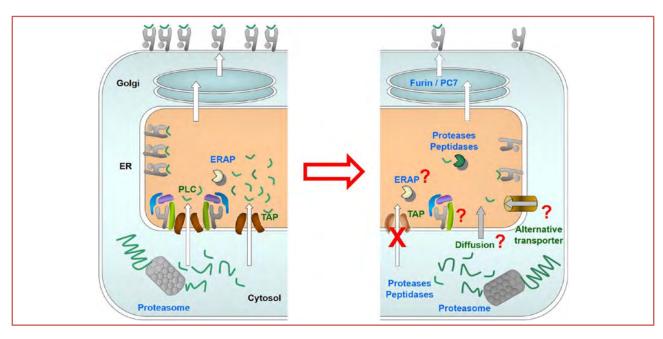
Our field of interest aims improving the control of chronic and opportunistic infections by the immune system, for which the cellular immune response, as opposed to antibodies alone, plays a leading role. Our long-term goal is contributing to an improved design of new vaccines. Therefore, our main goal is developing approaches to induce a potent, longlasting T-lymphocyte immune response. Our work has also focused on vulnerable populations, such as immunocompromised individuals as well as the elderly, trying to address approaches improving vaccine efficiency as well as measuring the strength and durability of their immune response to vaccination, aiming at revealing critical parameters of vaccine-mediated protection from disease in these populations.

We study the immune response to infection in immunocompromised individuals using a mouse model 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. TAP is frequently deleted in metastases and is also targeted by viruses, resulting in both instances in evasion from the detection, and elimination, by these T lymphocytes. The viral infection models we are using are vaccinia virus (VACV) and murine cytomegalovirus (MCMV). VACV is the viral vaccine vector used for the complete eradication of variola

virus, the agent causing smallpox and of the family of the poxvirus causing monkeypox. We have observed that VACV infection is able to overcome the deficiency of TAP for effective MHC-I antigen presentation. By using constructs with the virus proteins and minigenes containing the specific epitopes, we are studying how to promote this independent presentation of TAP. MCMV, as a model for human cytomegalovirus, a key factor in transplant viability, remains latent and causes increased mortality in frail population. We are also analysing the TAP independent presentation from MCMV. Both models of infection are being evaluated in ICARO project, funded by the European Innovation Council, that aims the use of intracellular carriers against resistant microorganisms (https://icaro-project.eu/).

We are also focusing on the antiviral immunity in the elderly, a highly vulnerable segment of the population. In the wake of the pandemic caused by SARS-CoV-2, the group refocused part of its research and know-how to address the immune response to infection by, and vaccination against, SARS-CoV-2. We are carrying out a global analysis of the adaptive and innate immune response, establishing the presence of neutralizing antibody and T-lymphocyte anti-SARS-CoV-2 responses. We are also pursuing to extend this knowledge to new emerging diseases, by focusing on the *mpox* outbreaks, analyzing the protective immune response in the elderly from smallpox vaccinations performed more than fifty years earlier.





MHC class I viral antigen processing and presentation pathway. TAP transporter plays a central role (left) and its inactivation has a large impact (right). Potential alternative routes that may mediate the low-level presentation of MHC class I viral antigens in TAP absence are indicated (right).



#### List of publications

- Perdiguero B, Marcos-Villar L, López-Bravo M, Sánchez-Cordón PJ, Zamora C, Valverde JR, Sorzano COS, Sin L, Álvarez E, Ramos M, del Val M, Esteban M, Gómez CE. (2023) Immunogenicity and efficacy of a novel multi-patch SARS-CoV-2/COVID-19 vaccine candidate. Front Immunol. 14: 1160065. doi: 10.3389/fimmu.2023.1160065.
- Sanchiz A, Martín R, del Val M, Corell A, Alcamí A. (2023) MPXV and SARS-CoV-2 in the air of nightclubs in Spain. Lancet microbe 4: e389. doi: 10.1016/S2666-5247(23)00104-0
- Jiménez-Arroyo C, Molinero N, Sabater C, Margolles A, Terrón-Camero LC, Andrés-León E, Ramos M, del Val M, Moreno-Arribas MV. (2024) Gut microbiome and clinical and lifestyle host factors associated with recurrent positive RT-PCR for SARS-CoV-2. Front. cell. infect. Microbiol. 14: 1494193. doi: 10.3389/fcimb.2024.1494193.



#### Other activities

■ 16 awards to Margarita del Val, including those awarded by AlumniUAM, the Transforma España Foundation, and the International University of La Rioja, including seven as a female scientist, six in the field of education, five in the media, and two in the field of healthcare.



#### Research projects

- PID2023-153045OB-I00, MCIU. Novel strategies to potentiate antigen presentation for cellular immunity to infections and vaccination in vulnerable populations. PI: Margarita Del Val Latorre. 2024-2027.
- #101046927, European Commission. Intracellular Carrier Against Resistant microOrganisms (ICARO). Program: PATHFINDER-OPEN-2021/ HORIZON-EIC. IPs: Coordinator Arrays-for-cell Nanodevices; IP Subproject: Margarita Del Val. 2022-2026.
- RED2022-134831-T, MCIU. Proyecto de investigación Red de Inmunoterapia del Cáncer. REINCA. Call MCI-AEI REDES. Network coordinator: Juan J. Lasarte. 2022-2025
- PID2019-110407RB-I00, MCIU. Strengthening of immune memory and antigen presentation as tools to overcome immunodeficiencies in CD8+ T cell responses (IMPRIMEM). PI: Margarita Del Val Latorre. 2020-2024.



#### Doctoral theses

- Víctor Muñoz Abad (2024) Identification of translocation pathways alternative to the transporter TAP involved in antigen presentation to CD8<sup>+</sup> T lymphocytes. Dept. of Molecular Biology, UAM. Dirs.: Luis C. Antón and Margarita Del Val Latorre.
- Cristina Rodríguez Rojas (2024) Role of TAP and Proteasomes in Antigen Presentation during Cytomegalovirus Infection. Dept. of Molecular Biology, UAM. Dir.: Margarita Del Val Latorre.



# REGULATION AND FUNCTION OF PROINFLAMMATORY MEDIATORS AND THEIR INVOLVEMENT IN IMMUNE AND INFLAMMATORY MEDIATED DISEASES

#### **Group members**

**Principal investigator:**Manuel Fresno Escudero

**Scientific staff:**Konstantinos Stamatakis
Andriani

Postdoctoral fellows: Antonio Lahera Alonso Angela Rodríguez García-Rendueles

Predoctoral fellows: Teresa Garcia Prieto Patricia Torres Gérica Javier Merino Valverde Alfonso Herreros Cabello Sergio Polo Nicoli Technicians: Carolina González Maroto Maria Del Carmen Maza

Undergraduate and Master students: Victoria Raquel Pérez Fernández Paula Martínez Cenalmol Natalia Olivares Vaquerizo

**Visiting scientists:** Hector Omar Rodríguez



http://www.cbm.uam.es/manuel.fresno



#### Summary

The biological activity of LPS is more complex than generally thought. we found that the "so called" atypical LPS interact with Toll like receptors (TLR) very differently from classical LPS. The interaction of lipid A with TLR4, is affected by the number and length of acyl chains and subtle differences in LPS core structure affect TLR4/MD2 interaction, and homo and heterodimerization with TLR2. Those atypical LPS have peculiar immunological properties making them suitable for their use in inflammatory pathologies. Congenital diaphragmatic hernia (CDH), a rare disease affecting diaphragm and lung development. Interestingly, atypical LPS influence anti-inflammatory macrophage activation that is effective in curing CDH in genetic and mutagenic animal models. The immune system of the fetus is responsible for repairing the damage and closure of the hernia in the diaphragm and enhanced proper lung development after treatment. Those TLR ligands are a promising solution for CDH that led to their designation as orphan drug by EMA.

TCFL5 is a bHLH transcription factor with two major isoforms (TCFL5\_E8 and CHA) that showed a reciprocal regulation. We found an opposing role of TCFL5\_E8 vs CHA in the oncogenic development of B-and T-acute lymphoblastic leukemia (ALL) and B- cell lymphoma cell lines, being Cha prooncogenic. In T-ALL TCFL5\_E8/CHA physically interact with NOTCH1, binding together to the promoters of prooncogenic genes as antiapoptotic BCL2 or HES-1 repressing/activating them respectively. In B cell lymphomas, TCFL5 is induced by CD40 or BCR signaling depending on LYN. In those

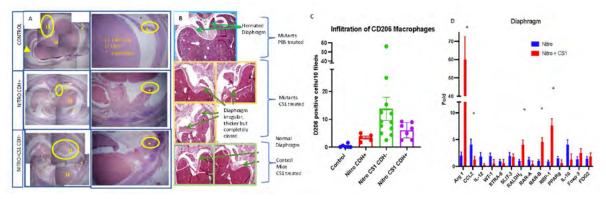
cells, CHA interacts with MYC. In TCFL5 KO lymphomas BCR or CD40 signaling is dramatically reduced. The lack of TCFL5 caused a radical decrease in BCR signaling molecules as SYK, CD19 and BLNK, resulting in an inability to respond to stimuli, an increase in cell death and an abnormal cell cycle. Besides, TCFL5\_E8 regulates non canonical NF-kB2 activation. TCFL5\_E8 and CHA differently bind to SYK promoter. Interestingly, down regulation Tcfl5 expression seems to lead to more active mitochondria.

Using Tcfl5 deficient mice, we found that TCFL5 is involved in the formation of germinal centers and in pro-B to pre-B cells differentiation, two processes that are MYC dependent. They also have a defect in DN3 to DN4 thymocyte transition consistent with NOTCH1 regulation.

In an effort to understand the COVID19 pandemic, we analyzed the immunological responses of infected patients, including long COVID- Serum ACE2 levels 2 adiscriminate different type of symptoms. Besides, children develop a different immune response than adults. They can be protected by natural antibodies to previous CoV infection and develop a B-1 IgM-dependent innate immune response to SARS-Cov2.

We are also working on the protozoan parasite *T. cruzi* causative agent of Chagas' disease analyzing its genetic variability, clinical outcome and immunopathology in close collaboration with Nuria Gironès.





TLR ligand CS1 cures CDH. A) Nitrofen fetus treated with CS1 had a complete diaphragm. B CS1-treated G2-GATA4Cre;Wt1fl/fl pregnant mice. C) M2 macrophage diaphragm infiltration. D) CS1 treatment significantly increase M2 marker Arg1, and retinol pathway genes Aldh1a2, Rbp1 and Rarb in diaphragm.



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- Horndler L, et al. (2023). Decreased breadth of the antibody response to the spike protein of SARS-CoV-2 after repeated vaccination. Front Immunol.Apr 3;14:1157263. doi: 10.3389/fimmu.2023.1157263.
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#### **Doctoral theses**

- Patricia Torres Gérica. 2023. Papel de la proteína Galectina-1 en el desarrollo del tejido adiposo y el metabolismo de los adipocitos. UAM. Director: Manuel Fresno Escudero.
- Teresa García Prieto. 2023. Las isoformas de Akt y TLR5 en la función de la barrera intestinal y la enfermedad inflamatoria intestinal. UAM. Director: Manuel Fresno Escudero.
- Javier Merino Valverde. 2024. Lipopolisacáridos atípicos: estructura, actividad biológica y posibles aplicaciones clínicas. UAM. Director: Manuel Fresno Escudero.
- Alfonso Herreros Cabello. 2024. Aproximaciones ómicas para la biología y genética de *Trypanosoma cruzi* y la definición de biomarcadores en la enfermedad de Chagas. UAM. Directores. Manuel Fresno Escudero y Núria Gironés Pujol

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- Piredda R, et al. (2024). Assessment of molecular modulation by multifrequency electromagnetic pulses to preferably eradicate tumorigenic cells. Sci Rep.2024 Dec 3;14(1):30150.
- Villar SR, et al. (2024). Discovery of circulating miRNAs as biomarkers of chronic Chagas heart disease via a small RNA-Seq approach. Sci Rep. Aug9;14(1):18514. doi: 10.1038/s41598-024-69310-w.
- Fresno, M., et al. (2024). Identification of Chagas disease biomarkers using untargeted metabolomics. Scientific Reports, 14, 187668.doi: 10.1038/s41598-024-69205-w.
- Herreros-Cabello A, et al. (2024).Quantitative Proteomic Analysis of Macrophages Infected with Trypanosoma cruzi Reveals Different Responses Dependent on the SLAMF1 Receptor and the Parasite Strain. Int J Mol Sci. Jul 8;25(13):7493. doi: 10.3390/ijms25137493.
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- Validation of neuroinflammatory pharmacological targets for the treatment of chronic pain. Manuel Fresno. 20.9.2023-30.6.2024.
- PID2022-137487OB-I00. Role of TCFL5 in processes of differentiation, immunosenescence, inflammation, and disorders associated with aging. Manuel Fresno. September 1, 2023–August 31, 2026.
- Head of Group 12. Princesa Health Research Institute. Manuel Fresno Escudero.



# IMMUNOREGULATORY MECHANISMS IN THE DEVELOPMENT OF CHAGAS DISEASE: TRANSLATIONAL APPLICATIONS

#### **Group members**

**Principal investigator:** Nuria Gironés

**Predoctoral fellows:** 

Alfonso Herreros Cabello (Co-director)

**Technicians:** 

Diana Karolina Santos Peñaloza

**Undergraduate and Master students:** 

Andrea Rodrigo Castro Mario Gómez Montes José Francisco Mares

Visiting scientists:

Héctor Omar Rodríguez Angulo

Esteban Lozano



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



#### Summary

Chagas disease, caused by *Trypanosoma cruzi*, is a neglected tropical disease with no reliable biomarkers for disease progression or treatment efficacy. During this period, in collaboration with physicians from several hospitals, we identified a circulating microRNA signature specific to chronic Chagas patients, which will be further investigated in individuals with chronic Chagas cardiomyopathy. Additionally, our metabolomic study identified several circulating biomarkers in chronic Chagas patients that may help predict disease progression and assess treatment efficacy.

We also studied the role of the SLAMF1 receptor in modulating macrophage responses to *T. cruzi* infection. Our findings indicate that SLAMF1 inhibits the production of reactive species in macrophages infected by a lethal *T. cruzi* strain. Through a proteomic analysis of wild-type and SLAMF1-deficient macrophages, we identified significant differences in their responses to parasite strains with varying genomic and virulence profiles. These findings may help explain the diverse clinical outcomes observed in patients and experimental infections.

In collaboration with an Argentinean research group, we demonstrated that Sec22b-dependent antigen cross-presentation plays a crucial role in T cell priming during *T. cruzi* infection.

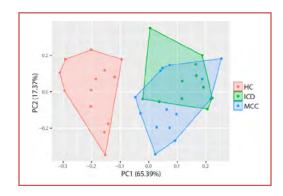
Furthermore, we contributed to the Chagas Disease Working Group of Madrid, participating in a study on the prevalence of Chagas disease among Latin American pregnant women in Madrid, Spain (2011–2016).

Beyond Chagas disease research, we collaborated with two groups on the experimental model of SARS-CoV-2 infection for validating products aimed at controlling viral infection.



 Alfonso Herreros Cabello (2024)
 Omics approaches for the biology and genetics of Trypanosoma cruzi and the definition of biomarkers in Chagas disease Molecular Biology Autonomous University of Madrid International Mention





Principal Component Analysis of circulating microRNA in Chagas disease patients. HC: healthy controls; ICD: Indeterminate Chagas patients; MCC: Mild-moderate Chagas Cardiomyophaty patients.



#### List of publications

- Herreros-Cabello A, Bosch-Nicolau P, Pérez-Molina JA, Salvador F, Monge-Maillo B, Rodriguez-Palomares JF, Ribeiro ALP, Sánchez-Montalvá A, Sabino EC, Norman FF, Fresno M, Gironès N, Molina I. Herreros-Cabello A, et al. Among Identification of Chagas disease biomarkers using untargeted metabolomics.Sci Rep. 2024 Aug 13;14(1):18768. doi: 10.1038/s41598-024-69205-w.
- Herreros-Cabello A, Del Moral-Salmoral J, Morato E, Marina A, Barrocal B, Fresno M, Gironès N. Quantitative Proteomic Analysis of Macrophages Infected with Trypanosoma cruzi Reveals Different Responses Dependent on the SLAMF1 Receptor and the Parasite Strain. Int J Mol Sci. 2024 Jul 8;25(13):7493. doi: 10.3390/ijms25137493.
- Villar SR, Herreros-Cabello A, Callejas-Hernández F, Maza MC, Del Moral-Salmoral J, Gómez-Montes M, Rodríguez-Angulo HO, Carrillo I, Górgolas M, Bosch-Nicolau P, Molina I, Pérez-Molina JA, Monge-Maillo B, Bottasso OA, Beloscar J, Pérez AR, Fresno M, Gironès N. Discovery of circulating miRNAs as biomarkers of chronic Chagas heart disease via a small RNA-Seq approach. Sci Rep. 2024 Jan 12;14(1):1187. doi: 10.1038/s41598-024-51487-9.
- Herrero-Martínez JM, Trigo E, Navarro M, García-Alcázar D, Carrillo I, Fernández-López T, Calderón-Moreno M, Millán-Pérez R, Cuadros J, Velasco M, García-Bujalance S, Lizasoain M, Martín-Rabadán P, Pérez-Ayala A, Flores-Chávez M; Working Group on Chagas Disease of Madrid. Herrero-Martínez JM, et al. Prevalence of Chagas disease in Latin American pregnant women in Madrid, Spain: A multicentre cross-sectional study from 2011 to 2016. Trop Med Int Health. 2023 Dec;28(12):912-922. doi: 10.1111/tmi.13942.
- Andreu S, von Kobbe C, Delgado P, Ripa I, Buzón MJ, Genescà M, Gironès N, Del Moral-Salmoral J, Ramírez GA, Zúñiga S, Enjuanes L, López-Guerrero JA, Bello-Morales R. Andreu S, et al. Dextran sulfate from Leuconostoc mesenteroides B512F exerts potent antiviral activity against SARS-CoV-2 in vitro and in vivo. Front Microbiol. 2023 May 3;14:1185504. doi: 10.3389/fmicb.2023.1185504.

- Biscari L, Maza MC, Farré C, Kaufman CD, Amigorena S, Fresno M, Gironès N, Alloatti A. Biscari L, et al. Sec22b-dependent antigen cross-presentation is a significant contributor of T cell priming during infection with the parasite Trypanosoma cruzi. Front Cell Dev Biol. 2023 Mar 1;11:1138571. doi: 10.3389/fcell.2023.1138571.
- Pastor-Fernández A, Bertos AR, Sierra-Ramírez A, Del Moral-Salmoral J, Merino J, de Ávila AI, Olagüe C, Villares R, González-Aseguinolaza G, Rodríguez MÁ, Fresno M, Gironés N, Bustos M, Smerdou C, Fernandez-Marcos PJ, von Kobbe C. Pastor-Fernández A, et al. Treatment with the senolytics dasatinib/quercetin reduces SARS-CoV-2-related mortality in mice.Aging Cell. 2023 Mar;22(3):e13771. doi: 10.1111/acel.13771.



- PID2021-123389OB-I00. MICINN/FEDER. Role of the Trypanosoma cruzi SLAMF1 receptor ligand and microRNAs during infection: applications in diagnosis and therapy (SLAMIRNA). Principal Investigator: Nuria Girones Pujol From: Sep 1st 2023 to: Sep 30th 2025.
- Comunidad de Madrid / REACT UE/ FEDER. Preclinical platforms and models for a multidisciplinary approach to COVID-19 and in response to future pandemics (COVTRAVI-19-CM). From: Jan 1 2020 to: Dec 31t 2022. Extended to June 2023. Coordinator: Manuel Fresno Escudero
- Co-coordinators of the Platform for preclinical models of infection and pathogenesis: Núria Gironès Pujol and Ivan Ventoso.



# NITRIC OXIDE AND BIOACTIVE LIPIDS SIGNALLING IN THE IMMUNE RESPONSE

#### **Group members**

**Principal investigators:** Juan Manuel Serrador Peiró Miguel Ángel Íñiquez Peña

**Predoctoral fellows:** Leticia Villadangos Reyes

#### **Technicians:**

Ana Renshaw Calderón Yaiza Gutiérrez de la Torre Alicia Rodríguez Cárdenas

#### **Undergraduate and Master students:**

Leticia Villadangos Reyes Sergio de la Torre Rodríguez Roberto Rosado Quirós



https://www.cbm.uam.es/immune\_NO\_ bioactive\_lipids



#### Summary

Nitric oxide (NO) and bioactive lipids as nitro-fatty acids (NO<sub>2</sub>-FA) or prostaglandins (PGs), are key mediators in a variety of physiological and pathological processes, with an essential role in inflammation. Recent studies indicate that both mediators can play an important role in the modulation of the immune response. Our research is dedicated to study the role played by NO, NO<sub>3</sub>-FA and PGs in inflammation and in the activation and differentiation of T lymphocytes, analysing their involvement in the regulation of gene expression and activation of transcription factors. We are also interested in the analysis of other parameters of activation such as chemotaxis, intercellular adhesion and the organization of adhesion and signalling receptors at the immune synapse. The analysis of the actions exerted by these agents in the activation and function of human T lymphocytes will allow us to determine their role in the modulation of the immune response.

#### 1. Nitric oxide and adaptive immunity

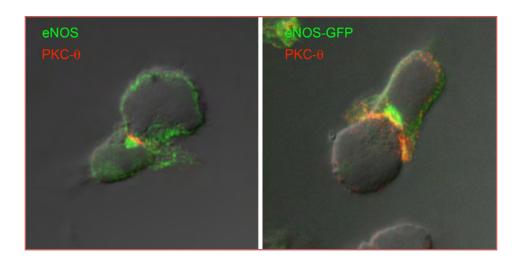
NO is a key messenger in the pathogenesis of inflammation. In the immune system, NO has been considered to be a cytotoxic molecule associated with the response of phagocytic cells to pathogens as part of the first line of host defence against infection. However, NO can also regulate the adaptive immune response, linking innate and adaptive immunity. By targeting signalling molecules, NO influences 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, nitration of fatty acids and nitroalkylation of proteins by NO<sub>2</sub>-FA, as important post-translational modifications by which NO can act as a signalling molecule during T cell-mediated immunity.

## 2. Actions of bioactive lipids in inflammatory processes

Fatty acid oxidative modifications result in the production of bioactive lipids, which display a variety of actions in pathophysiological processes. These compounds include PGs and NO<sub>2</sub>-FA, important signalling molecules that can modulate the inflammatory process and the immune response. To this end, we analyse 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 in the immune response, will contribute to the understanding of the potential therapeutic benefits of these compounds.





Endothelial Nitric Oxide Synthase (green) on the Golgi and plasma membrane controls the coalescence and activation of Protein Kinase C-theta (red) at the central Supramolecular Activation Cluster (c-SMAC) of the T Cell Immune Synapse (IS).



#### List of publications

- Guerra-Espinosa, C., Jiménez-Fernández, M., Sánchez-Madrid F. and Serrador, J:M. (2024). ICAMs in Immunity, Intercellular Adhesion and Communication. Cells, 13, 339. doi: 10.3390/cells13040339
- Villadangos, L. and Serrador J.M. (2024). Subcellular Localization Guides eNOS Function. Int. J. Mol. Sci., 25, 13402; doi: 10.3390/ijms252413402
- Bago, A. Cayuela, M.L., Gil A. Calvo E., Vázquez, J., Queiro A., Schopfer, F.J., Radi, R. Serrador J.M. and Íñiguez, M.A. (2023). Nitro-oleic acid regulates T cell activation through post-translational modification of calcineurin. Proc. Natl. Acad. Sci. U.S.A. 120 (4) e2208924120, doi: 10.1073/pnas.2208924120.



- PID2022-140155OB-I00. MICIU. Actions of Nitric Oxide and Nitro Fatty acids in the regulation of T cell function. Juan M. Serrador Peiró and Miguel A. Íñiguez Peña. September 2023 to 2027.
- IIS Hospital Universitario de la Princesa Group 18: Actions of bioactive lipids in the immune and inflammatory response.



#### INTRACELLULAR SIGNALLING IN INFLAMMATORY PROCESSES

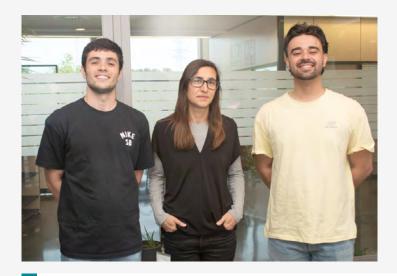
#### **Group members**

**Principal investigator:** María N. Navarro

**Predoctoral fellows:** Jorge Santiago Yebra

**Technicians:** María Jesús Valle Pastor

Undergraduate and Master students: Master student: Ignacio Cayuela Gracia. Undergraduate student: Rebeca Fraile Rodríguez



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



#### Summary

Autoimmunity represent a major health and scientific challenge, and demands the development of effective and specific therapies. We study the function, signalling cascade and metabolic remodelling of interleukin 23 (IL-23), as an strategy to develop new therapies for IL-23/IL-17related autoimmune diseases such as psoriasis, inflammatory bowel diseases or rheumatoid arthritis. The chronic inflammatory diseases are initiated by uncontrolled immune responses that trigger the hyper-activation of signalling cascades, the accumulation of pro-inflammatory mediators and finally, the manifestation of clinical symptoms. The axis formed by interleukin 23 and interleukin 17 (IL-23/IL-17) has emerged as a key signalling hub in autoimmune pathologies 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 interleukin 17 and 22 (IL-17/IL-22). IL-23 pathogenic actions are mostly restricted to distinct subpopulations of T lymphocytes: the CD4 helper subset Th17 and the TCRy $\delta$  subpopulation Tyδ17. 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.

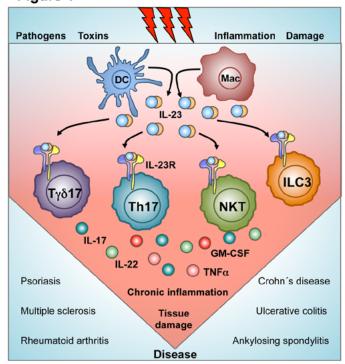
Our lab is interested in the characterisation of the signalling network triggered by IL-23 and other pro-inflammatory cytokines, as a strategy to uncover novel mediators of cytokine signalling for the development of therapeutic tools based on the interference with intracellular signalling pathways. Recently, the lab has discovered how interleukin 23 promotes pathogenic T cell migration to the inflamed site, and that interleukin-23 controls protein synthesis and cellular metabolism to induce pathogenic functions in T cells. 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.



Decoding IL-23 Signaling Cascade for New Therapeutic Opportunities. Cells. 2020

Pastor-Fernández G, Mariblanca IR, Navarro MN

#### Figure 1



Role of IL-23 in autoimmune diseases



#### Research projects

- Project. ref. CEX2021-001154-S. Centro de Biología Molecular Severo Ochoa. Targeting immunometabolism to restrain autoimmunity. Maria N Navarro. 03/06/2024-31/05/2026.
- Project. Ref. PID2022-141729OB-I00. Ministerio de Ciencia, Innovación y Universidades. Mitochondrial and metabolic interference in interleukin 23-related autoimmune pathologies (MITMEET23). María N. Navarro. 01/11/2023-31/10/2026.
- Project. Ref. PID2019-110511RB-I00. Ministerio de Ciencia e Innovación. Intracellular Signalling Blockage for inflammatory diseases (iSigB). María N. Navarro. (. 01/08/2020-31/07/2023.



#### **Doctoral theses**

Gloria Pastor Fernández (2024). "Papel de las quinasas mTORC1 y AMPK en la cascada de señalización de Interleuquina 23". Interacciones con el ambiente. Universidad Autónoma de Madrid. María N. Navarro.



#### METABOLISM IN CANCER AND AGING

#### **Group members**

**Principal investigator:** Ana Ortega Molina

**Postdoctoral fellows:** Rebeca Gema Jimeno Lumeras

Predoctoral fellows: Beatriz Lopez Martin-Lunas Sara Salazar Ortego Marta Gonzalez Perez

**Technicians:** Cristina Prieto Carro

**Undergraduate and Master students:** Sara Salazar Ortego Marta González Pérez



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



#### Summary

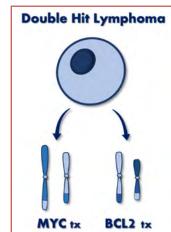
Since 2021, our lab at CBM has focused on understanding the biology and identifying new therapeuticstrategiesforhigh-gradeB-celllymphomas (HGBL) with MYC and BCL2 rearrangements, known as "double-hit" lymphoma (DHL). These tumors, comprising ~10% of diffuse large B-cell lymphomas (DLBCL), are among the most aggressive and chemoresistant subtypes, with minimal treatment options and poor outcomes.

Our research lines include:

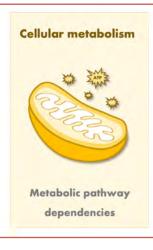
- Metabolic Characterization & Targeted Therapies: We analyze the metabolic features of DHL using transcriptomic and metabolomic tools in preclinical models and patient samples. Our goal is to identify metabolic vulnerabilities that could serve as novel therapeutic targets, addressing the poor response of these lymphomas to standard treatments.
- Unfolded Protein Response (UPR) & Tumor Microenvironment (TME): Transcriptomic analysis of DHL patients reveals increased ATF4 expression, a key transcription factor regulating autophagy, redox homeostasis, and the unfolded protein response (UPR). As a pro-survival pathway activated under endoplasmic reticulum (ER) stress, UPR's role in DH-HGBL remains unclear but may expose targetable vulnerabilities. We aim to decipher this pathway and explore its effects on the tumor microenvironment.

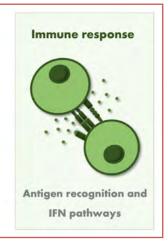
• Immune Landscape & Epigenetic Reprogramming: DH-HGBL tumors exhibit reduced expression of immune and inflammatory signatures, including interferon responses, suggesting a low dependence on the microenvironment and classifying them as "non-inflamed" lymphomas. We seek to characterize their TME and investigate whether epigenetic reprogramming could enhance sensitivity to immunotherapeutic agents. Our work aims to uncover novel treatment avenues for these aggressive lymphomas with extremely poor prognoses.











ER stress, metabolic pathways, and the composition of the tumor microenvironment are significantly altered in HGBL-DH-BCL2 and may contribute to tumor progression, therapy resistance, and disease aggressiveness. Understanding these changes is crucial for identifying potential therapeutic targets.



#### List of publications

Ortega-Molina A. (AC); Lebrero-Fernandez C.; Sanz A; et al; Efeyan A (AC). (1/20). (2024). A mild increase in nutrient signaling to mTORC1 in mice leads to parenchymal damage, myeloid inflammation and shortened lifespan. Nature Aging. 4-6. DOI: 10.1038/s43587-024-00635-x



#### Other activities

#### Invited oral presentations at national and international conferences:

- EHA Research Conference 2024. 18-21 March 2024. Borovets, Bulgaria
- Cellular Aging and Metabolism Symposium. 22-24 July 2024. A Coruña. Spain.

#### Worshop organizer:

October 2024: I Worshop on Chromatin Alterations in Cancer Development Symposium. CBM. 100 attendees.

#### Prizes:

- January 2023. "Margarita Salas" Award for young scientists under 40 from Regional Government of Madrid.
- July 2024. Elected as a new member of the Young Academy of Spain"



- CMBSO IGP-SO 2024. Intramural Grant Programme-SO 2024. Unlocking Therapeutic Avenues: Targeting Purine and Pyrimidine Synthesis Pathways to tackle Double-Hit Lymphoma. Principal Investigators: Ana Ortega-Molina and Sara Cogliati. 01/06/24-31/05/26.
- LABAE235101ORTE. AECC Lab 2023. Fundación Científica Asociación Española Contra el Cáncer. Decoding new strategies to reduce immune evasion in "double hit"-high grade B cell lymphomas. Principal Investigator: Ana Ortega-Molina. 01/12/23-30/11/26.
- LEO22-2-2003. Beca Leonardo 2022. Fundación BBVA. Estudio de posibles alternativas para superar la resistencia a inmunoterapia de los linfomas B agresivos. Principal Investigator: Ana Ortega-Molina. 01/11/22-30/04/24.
- PID2021-124601OB-I00. Agencia Estatal de Investigación (AEI). Ministerio de Ciencia, Innovación y Universidades (MICIU). Deciphering metabolic dependencies as targetable vulnerabilities in double-hit lymphomas. Principal Investigator: Ana Ortega-Molina. 01/09/22-31/08/25.
- BFERO2021.05. I FERO-BMS Fellowship. Fundación FERO. Targeting unfolding protein response in high-grade B cell lymphomas. Principal Investigator: Ana Ortega-Molina. 01/12/21-30/11/23.
- 20212AT020. Proyecto Intramural Especial CSIC. Targeting metabolic dependencies in high-grade B cell lymphomas. Principal Investigator: Ana Ortega-Molina. 01/09/21-31/08/24.
- RYC2019-027280-I. Programa Ramón y Cajal. Agencia Estatal de Investigación (AEI). Ministerio de Ciencia, Innovación y Universidades (MICIU). Functional genomics and new therapeutic interventions in Follicular Lymphoma. Principal Investigator: Ana Ortega-Molina. 01/09/21-31/08/26.



#### UNCONVENTIONAL AUTOPHAGY IN HEALTH AND DISEASE

#### **Group members**

**Principal investigator:** Felipe X. Pimentel Muiños

**Predoctoral fellows:** Elena Terraza Sivestre Julia Bandera Linero Daniel Oña Sánchez

**Undergraduate and Master students:** 

Clara Moyano Jimeno

Blanca Vioque Hacha



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



#### Summary

Autophagy is a degradative process that eliminates cytoplasmic components and is critical for cellular homeostasis. Judging from the pathological phenotypes observed in mice lacking members of the autophagic machinery (ATGs), autophagy protects against different pathologies, including cancer, inflammatory diseases or neurodegeneration. This protective capacity suggests that manipulation of autophagy could hold therapeutic value. However, it is now clear that the different ATGs also have alternative functions not strictly related to the canonical autophagic pathway. The contribution of these alternative activities to preventing the pathological phenotypes caused by ATG elimination is currently unclear. Therefore, characterization of these activities is important because it could help the way ATG activity should be manipulated with therapeutic purposes.

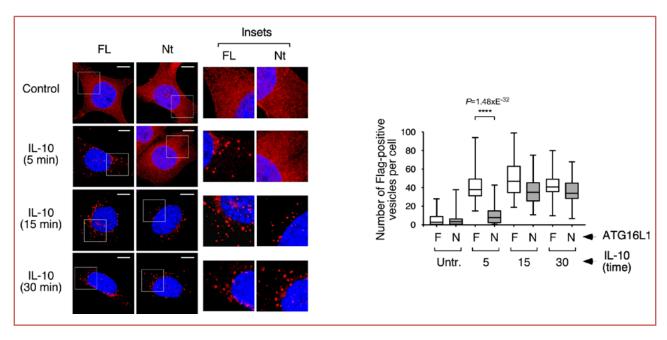
With this goal in mind, the lab focuses on ATG16L1 as a paradigm to discover new unconventional activities of the autophagic machinery. In its most canonical function, ATG16L1 is critical for proper formation of autophagosomes, double-membrane vesicles that sequester the cellular material that will eventually be degraded in the lysosome. However, ATG16L1 includes a C-terminal domain that is irrelevant for this activity and seems to play unconventional roles in a wide variety of cellular processes. Projects currently being developed in

the lab intend to discover and characterize in detail novel unconventional functions of ATG16L1 carried out through this domain, and explore how they interact with the conventional pathway. Areas of interest in the lab in the 2023-24 period have focused on the role of unconventional autophagy in:

- 1) The regulation of endocytosis, trafficking and signaling output of cytokine receptors
- 3) The regulation of immunogenic cell death and its impact in cancer biology
- 4) The innate immune response against intracellular bacterial infections

To identify and characterize these processes we use genetically manipulated cellular systems, proteomics and bioinformatics approaches. We are trying to characterize the underlying mechanisms and explore how they may be influenced by a coding allele of ATG16L1 (T300A) whose presence increases the risk of suffering Crohn's disease, a serious intestinal inflammatory pathology. Exploring and establishing ways to manipulate atypical autophagy with therapeutic intentions is also a long-term goal of our group that we intend to develop over the next few years.





Defective IL10-induced IL10R (red, anti-flag) endocytosis in cells lacking the WD40 domain of ATG16L1 (Nt)



■ Terraza-Silvestre, E; Villamuera, R; Bandera-Linero, J; Letek, M; Oña-Sánchez, D; Ramón-Barros, C; Moyano-Jimeno, C; Pimentel-Muiños, FX. (2023) An unconventional autophagic pathway that inhibits ATP secretion during apoptotic cell death. Under revision. Published in BiorXiv; link: https://www.biorxiv.org/content/10.1101/2024.01.21.576513v1



#### **Doctoral theses**

- Alvaro Fernandez Cabrera (2023). "Unraveling the unconventional autophagic activities of ATG16L1 and their links with the transmembrane molecule TMEM59". Univ. Salamanca. Director: Felipe X. Pimentel Muiños. (Sobresaliente cum laude).
- Elena Terraza Silvestre (2024). "Identification of an autophagic pathway that inhibits ATP release during apoptotic cell death". Univ. Autonoma de Madrid. Director: Felipe X. Pimentel Muiños. (Sobresaliente cum laude).



- PID2023-148747OB-100. Ministerio de Ciencia e Innovación. Expanding the characterization of novel unconventional functions of autophagy and their relevance in disease prevention. Principal investigator: Felipe X. Pimentel Muiños. 01/09/2024 31/08/2027
- Intramural exploratory grants program Severo Ochoa. Role of unconventional forms of autophagy in the infection of Citrobacter rodentium. Principal investigators: Felipe X. Pimentel Muiños and David Ruano Gallego. 01/06/2024 – 31/05/2026



## DEVELOPMENT OF THE HUMAN LYMPHOHEMATOPOIETIC SYSTEM

#### **Group members**

**Principal investigator:** María Luisa Toribio García

**Scientific staff:** Marina García Peydró

**Postdoctoral fellows:** Marina García Peydró Patricia Fuentes Villarejo

Predoctoral fellows: Fátima Bayón Calderón Carmela Cela Rodríguez Raquel Victoria Pérez Fernández Ángela Aparicio Valencia

**Technicians:**Juan Alcain Sánchez
Eloísa Castillo Gutiérrez

Undergraduate and Master students: Sandra Lucía López García Ángela Aparicio Valencia

Visiting scientists:
Sara González García.
Universidad Complutense
de Madrid.
Apostol Apostolov. Josep
Carreras Leukaemia
Research Institute.
Barcelona.
Ilaria Sergio. Sapienza
University of Rome



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



#### Summary

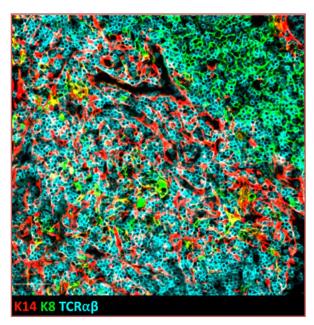
Our group studies the molecular pathways that control T lymphocyte development in the human thymus, with the aim of deciphering how dysregulation of these pathways results in T-cell acute lymphoblastic leukemia (T-ALL), an aggressive tumor of developing T cells with high rates associated with poor outcome of refractory/relapsed cases (r/rT-ALL). Our final goal is to translate mechanistic insights into antigen-targeted immunotherapies that could improve r/rT-ALL patient survival. This is a challenging yet unmet aim in the field, because shared antigen expression between leukemic and normal T cells has prevented the identification of T-ALL-specific targets. Focusing on a novel model of de novo generation of human T-ALL, we identified the pre-TCR, a surface receptor of T-cell progenitors absent on mature T cells, as a leukemia-initiating cell (LIC) biomarker expressed in 55% of T-ALL cases. Pre-TCR targeting with antibody drug conjugates (ADCs) derived from a proprietary pre-TCR-specific antibody impaired LIC activity and tumor progression of pre-TCR+ T-ALL patient xenografts in preclinical mouse models. These results point to the pre-TCR as a promising T-ALLspecific target for immunotherapy, providing proof of concept for implementation of novel immunotherapy strategies overcoming lack of T-ALL antigen specificity.

We also focused on T cells armed with chimeric antigen receptors (CAR-T) as an additional pre-TCR-targeting immunotherapy. This strategy, however, still faces important limitations resulting from signaling

through non-physiological receptors like CARs. In addition, CAR-T cell therapy is typically restricted to the use of autologous T cells from patients to avoid graft-versus-host disease (GvHD), this imposing numerical and functional limitations of healthy T cells from T-ALL patients. By combining complementary innovative strategies, our current aim is to overcome limitations associated to both target recognition by CARs and effector function of autologous T cells armed with them. Particularly, we are generating: 1) endogenously-regulated TCR-fused anti-pre-TCR CAR constructs (TRuCs) and 2) a platform of universal CAR-T cells (uCAR-T) to express them, consisting on either  $\alpha\beta$  T cells devoid of allorecognition by TRAC locus gene editing, or VΔ1+ ΓΔT cells differentiated de novo from cord blood hematopoietic progenitors. These strategies are expected to provide the foundations conductive to an efficient and safe, universal, allogeneic, off-the-shelf anti-pre-TCR CAR-T cell immunotherapy for r/r T-ALL.

Also, we have investigated the molecular pathways underlying age-dependent thymus involution. By implementing an ultrahigh-plex spatial biology platform (CODEX®, Co-Detection by Indexing) that enables comprehensive spatial phenotyping of thymic architecture and identification of structural and cellular changes associated to thymus aging, our goal is to develop novel strategies for thymus regeneration and functional recovery from immunosenescence.







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- Sánchez-Cerrillo I, et al. (2024). Combined Dendritic Cell And Anti-TIGIT Immunotherapy Potentiate Trail+ Memory NK Cells Against HIV-1 Infected Cells bioRxiv 2024.04.09.587160; doi: https://doi.org/10.1101/2024.04.09.587160
- Tirado N, et al (2024). CAR-T cells targeting CCR9 and CD1a for the treatment of T cell acute lymphoblastic leukemia 2024.09.02.610843; doi: https://doi.org/10.1101/2024.09.02.610843
- H-Alcántara A, et al. (2024). Glutathione overproduction mediates lymphoma initiating cells survival and has a sex-dependent effect on lymphomagenesis. Cell Death Dis.15, 534-545. doi: 10.1038/s41419-024-06923-z.
- Materna M, et al. (2024). The immunopathological landscape of human pre-TCRα deficiency: From rare to common variants. Science 383(6686):eadh4059. doi: 10.1126/science.adh4059.
- Toribio ML, González-García S. (2023). Notch Partners in the Long Journey of T-ALL Pathogenesis. Int J Mol Sci. 24,1383-1414. doi: 10.3390/ijms24021383.



### **Patents**

María Luisa Toribio García, Patricia Fuentes Villarejo, Sara González Carcía, Fátima Bayón Calderón, Carmela Cela Rodríguez, Juan Alcain Sánchez. Title: Antibody-Drug Conjugates or CAR-T cells for the treatment and/or prevention of Leukemia.

Inventors:

N° of application: EP23382727 Date of register: 2023

Figure 1. Representative image of an 8 wks-old mouse thymus microenvironment analysed by CODEX highly multiplex immunofluorescence imaging. K14-red (medullary Thymic Epithelial Cells -TECs-), K8-green (cortical TECs), TCRAβ-blue (mature T cells).



#### **Doctoral theses**

Fátima Bayón Calderón (2023)
 Title: Pre-TCR specific CAR-T cells as a novel immunotherapy for T-ALL
 Department of Molecular Biology. Facultad de Ciencias.
 Universidad Autónoma de Madrid.
 Supervisors: María Luisa Toribio García and Sara González García.



- HR23-00608. La Caixa Foundation Social Work.
   Deciphering the Impact of Leukaemia on Immunological Fitness Beyond Disease. PI: María Luisa Toribio (2023 -2026)
- X UNOENTRECIENMIL RESEARCH GRANT. Unoentrecienmil Foundation. Universal CAR-NK cells specific for pre-TCR for immunotherapy against acute T-cell lymphoblastic leukaemia (T-ALL) PI: María Luisa Toribio (2023 - 2025)
- PID2022-138880OB-I00. State Research Agency.
  Overcoming the current challenges of CAR-T
  immunotherapy against ALL-T leukemia: optimization
  of CARs (TRuCs) and generation of universal allogeneic
  effector T cells. PI: María Luisa Toribio (2023 2025)
- REINCA: State Research Agency. Cancer Immunotherapy Network PI: María Luisa Toribio (2023 - 2025)
- Grants for research projects related to childhood cancer. Inocente Inocente Foundation. Improving the quality of life of pediatric patients with T-ALL through universal CAR-T immunotherapy. PI: María Luisa Toribio (2022 -2025)
- PLEC2022-009312. State Research Agency. Treg-less: Addressing CCR7-mediated homeostasis of regulatory T cells to break immune tolerance in solid tumors PI: María Luisa Toribio (2022 - 2025)
- PDC2021-121238-I00. State Research Agency. Towards a new immunotherapy for T-cell acute lymphoblastic leukemia (T-ALL) based on a unique anti-preTCR antibody conjugated to drugs. PI: María Luisa Toribio (2021 2024)



# TCR DOMAINS IN T CELL DIFFERENTIATION AND PATHOPHYSIOLOGICAL AND THERAPEUTIC RESPONSES

#### **Group members**

**Principal investigator:** Hisse Martien van Santen

Predoctoral fellows: Ivaylo Balabanov Lydia Horndler Gil Irene Serrano Pérez Lucía Domínguez Esteban

**Undergraduate and Master students:** Alba Rodríguez Macía María Rodríguez Moraga María Gutíerrez Pliego



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



#### Summary

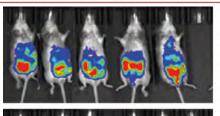
In recent years both antibody-based and cellular cancer immunotherapies have established themselves as successful, and sometimes even curative, treatments for cancer, complementing the traditional surgical, radiotherapy and chemotherapy approaches.

The clinically most established type of cellular immunotherapy is Chimeric Antigen Receptor (CAR)-T cell therapy, with treatments approved for various types of B cell leukemia. Wider implantation of this approach is hampered by the 'on target off tumor' response of CAR-T cells: recognition of the correct antigen on non-cancerous cells, due to the fact that most CAR-T cell targeted antigens are tumor-associated and not tumor-specific. In the case of Acute Myeloid Leukemia, a cancer with very limited treatment options, co-expression of CAR-T cell targeted antigens by healthy myeloid precursors gives rise to unacceptable toxicities. Taking into account that tumor cells often lose expression of specific MHC alleles, and that R/R AML patients when possible can undergo haplo-identical hematopietic stem cell transplants, we developed a logical gate formed by an activating CAR recognizing an antigen expressed by most AMLs but also precursors and an inhibitory, NK cell-derived immune receptor that recognizes defined MHC alleles. We obtained evidence with AML patient samples and in preclinical models of CAR-T cell cancer immunotherapy that logically gated CAR-T cells can discriminate between

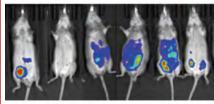
CAR target-expressing cells based on presence or absence of the ligand for the inhibitory receptor. These findings support the development of logic gate-based CAR-T cell therapy against AML and other types of cancer where 'on target – off tumor' toxicity by conventional CAR-T cell therapy prohibits clinical application.

We also study tumor-specific miniTCRs that combine antigen recognition domains of the TCR with CAR-like signaling domains. We have found that the identity and topology of the signaling domains of these miniTCRs determines their efficiency with respect to activating the T cells. This has a direct implication for improving CAR-based cellular cancer immunotherapy. They also provide new insights into the mechanisms that underlie full TCR function, which we are currently following-up upon. In addition, we are developing miniTCR variants that can provide antigen-regulated switches for precision control of gene expression of, for example, factors with proven ability to strengthen anti-tumor responses but whose systemic delivery is too toxic.

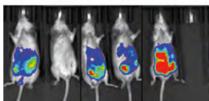




Non-transduced T cells



CAR-transduced T cells



CAR + inhibitortransduced T cells

Leukemia growth in mice inoculated with human AML cells and control human T cells, human T cells transduced only with the AML-specific CAR or with the CAR and the AML MHC-recognizing inhibitory receptor. Overlaid color scale (blue to red) quantifies the number of AML cells present in each mouse.



#### List of publications

- Menon AP, Villanueva H, Meraviglia-Crivelli D, van Santen HM, Hellmeier J, Zheleva A, Nonateli F, Peters T, Wachsmann TLA, Hernandez-Rueda M, Huppa JB, Schütz GJ, Sevcsik E, Moreno B, Pastor F (2024). CD3 aptamers promote expansion and persistence of tumor-reactive T cells for adoptive T cell therapy in cancer. Mol Ther Nucleic Acids 35(2):102198; doi: 10.1016/j.omtn.2024.102198
- Menon AP, Moreno B, Meraviglia-Crivelli D, Nonatelli F, Villanueva H, Barainka M, Zheleva A, van Santen HM, Pastor F (2023). Modulating T Cell Responses by Targeting CD3. Cancers 15(4), 1189; doi: 10.3390/cancers15041189
- Horndler L, Delgado P, Romero-Pinedo S, Quesada M, Balabanov I, Laguna-Goya R, Almendro-Vázquez P, Llamas MA, Fresno M, Paz-Artal E, van Santen HM, Álvarez-Fernández S, Olmo A, Alarcón B (2023). Decreased breadth of the antibody response to the spike protein of SARS-CoV-2 after repeated vaccination. Front. Immunol. 14, 1157263; doi: 10.3389/fimmu.2023.11572
- van Santen, HM and Alarcón, B (2024) TCR Signaling: Proximal Signaling. In: Encyclopedia of Immunobiology, 2nd edition doi:10.1016/B978-0-128-24465-4.00097-1



#### Other activities

Scientific collaboration with Vivia Biotech, Tres Cantos, Madrid, Spain; project: Multiparameter analysis of CAR-T cell-mediated killing (2024).



## Research projects

- PID2022-139934OB-I00; Ministerio de Ciencia e Innovación, proyectos i+D+i; Recombining and combining receptors of the immune system to improve specificity of cellular cancer immunotherapy (RECOMUNE); PI: HM van Santen; 2023 2026
- Fundación LAIR; Preclinical evaluation of CAR-T cells with KIR-based logic gates for selectively targeting myeloid leukemias; PI: HM van Santen; 2022 2024
- PID2019-104703GB-I00; Ministerio de Ciencia e Innovación, proyectos i+D+i; Identity, topology and geometry of TCR signaling domains in protective, therapeutic and autoimmune responses (TCRmap); PI: HM van Santen; 2020 2023



#### **Doctoral theses**

- Ivaylo Balabanov (2023); Recombinant T cell receptors for cancer immunotherapy as a model for study of full TCR function; Faculty of Sciences, Dept. of Molecular Biology, Universidad Autónoma de Madrid; Director: HM van Santen
- Lydia Horndler Gil (2024); Development and optimization of a combinatorial CAR-T cell therapy to overcome on target off tumor responses; Faculty of Sciences, Dept. of Molecular Biology, Universidad Autónoma de Madrid; Director: HM van Santen



## Interactions with the environment

## Microbes in health and welfare



## Margarita Sáiz

The overall scientific objective of the Microorganisms in Health and Welfare Unit is the study of both basic and applied aspects of the interactions of microorganisms with their hosts and the environment, including cellular and acellular entities. Microorganisms are an ideal model for the study of cellular biology, evolution, and the pathological processes and immune responses associated with infection. They are also unique tools for the generation of biotechnological and biomedical products that provide benefits to health and the economy.

The contributions in the last two years of the virology groups include the study of DNA and RNA viruses, which are relevant human and animal pathogens, within the one-health concept. Both fundamental research and the generation of new antiviral strategies have been addressed. Among them, the role of an RNA dodecahedral cage in human rhinovirus (HRV) assembly and uncoating has been identified together with the RNA release process from viral particles and its modulation by antiviral drugs. Also, several studies on human immunodeficiency virus 1 (HIV-1) have revealed a mechanism needed for the correct assembly of the HIV-1 mature capsid and new methods have been developed to determine error frequencies in complementary DNAs synthesized by HIV-1 reverse transcriptases. The role of magnesium concentration and temperature on the accuracy of the retroviral polymerase has been shown.

Some studies have established that African swine fever virus (ASFV) virulence relies on the control of type I IFN, by the action of specific viral genes. New and innovative vaccine approaches against this virus including live attenuated vaccines or partially replicative vaccines have been developed. In addition, self-organizing neural maps have been derived to identify relationships among minority haplotypes of SARS-CoV-2 quasispecies. Remarkably, in infected patients a rapid functional diversification of the virus was found, as well as the presence of minority sequences that anticipate those that will dominate at later phases of the pandemic. Also, new information on tropism determinants and the oncolytic capacity of Minute Virus of Mice (MVM) has been generated. The ability of the proteases encoded by foot-and-mouth disease virus (FMDV) to subvert the antiviral response triggered by the cGAS/STING pathway has been documented.

New antiviral strategies against coronaviruses have also been explored as i) the synergistic lethal mutagenesis, shown to be effective in extinguishing SARS-CoV-2, ii) the combination of host-targeted and direct-acting antivirals proven effective against infection by human coronaviruses or iii) the use of non-coding RNAs derived from the FMDV genome able to trigger broad antiviral activity against coronaviruses.



## Research groups

#### José María Almendral

ssDNA virus evolution, pathogenesis and anti-cancer potential

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#### **Ricardo Amils**

Molecular ecology of extreme environments

• • • • • • • • • • • • •

#### **Esteban Domingo**

Genetic variability of RNA viruses

### María Fernandez Lobato

Yeast enzymes bioengineering to generate bioactive compounds

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#### Mauricio García Mateu

Virus engineering and nanobiotechnology

#### Paulino Gómez-Puertas

Molecular modelling

•••••

#### **Aurelio Hidalgo**

Ultrahigh-throughput discovery and engineering of enzymes for biotechnological applications

••••••

## Wilfried J. Meijer

Conjugation in Gram-positive bacteria

Mario Mencía

Biotechnology and genetics of extreme

thermophiles

Luis Menéndez-Arias

Human immunodeficiency virus reverse transcriptase and antiretroviral therapy

••••••

**Manuel Pazos** 

Bacterial cell envelope during preseptal growth

• • • • • • • • • • • • •

María Graciela Pucciarelli

RNA-based control of Listeria adaptation to stress

and virulence

Yolanda Revilla

Virus-cell interaction. The ASFV model

**David Ruano** 

Biotechnology of probiotics and intestinal

pathogens

•••••

Margarita Sáiz

Modulation of antiviral immunity by viral

proteases and noncoding RNAs

Francisco Sobrino

New strategies for prevention and control of viral diseases: foot-and-mouth disease virus as a model

The Molecular Modeling Group has developed novel "in silico" drug design systems like MiniACE2 proteins to block SARS-CoV2 infection and generated new quantitative methods for computational biology.

The Unit's groups focused on prokaryotic microorganisms have made significant contributions in both fundamental and applied research. A novel two-component processive transcription antitermination system in many G+ conjugative plasmids has been discovered, providing insights into conjugation regulation and offering potential strategies to disrupt the process.

The production of honey oligosaccharides showing prebiotic properties in packed-bed reactors with immobilized enzymes and chitinous material bioconversion by three new chitinases from non-conventional yeast have been achieved. New ultra-high-throughput screening methods based on microfluidics to identify genes and enzymes of interest in natural or artificial diversity have been developed. Studies on molecular ecology of extreme environments have uncovered the coupled C, H, N, S and Fe biogeochemical cycles operating in the continental deep subsurface of the Iberian Pyrite Belt. New information on the recombinational repair pathways operating in extreme thermophiles to cope with DNA damage caused by high temperatures has been generated.



# ssDNA VIRUS EVOLUTION, PATHOGENESIS AND ANTI-CANCER POTENTIAL

#### **Group members**

**Principal investigator:** losé M. Almendral del Río

**Scientific staff:** Alberto López-Bueno

Postdoctoral fellows: Tania Calvo López Cecilia Lotufo

Predoctoral fellows:
Pedro Arroyo Gil
María Gutiérrez Fombona
Jorge Martínez-Ortega
Alejandro Fernández-Llorente

**Undergraduate and Master students:** Gonzalo Fechoso Fernández Ana Ruíz Medina



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



### **Summary**

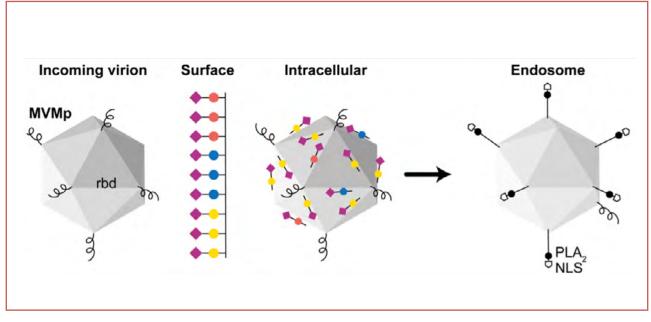
Our research is aimed at understanding the molecular basis of the oncolytic capacity of the Minute Virus of Mice (MVM), a member of the Parvoviridae, toward its development as a novel anticancer biological agent. Main previous results showed the capacity of MVM to infect eliminate cancer stem cells obtained from glioblastoma patients implanted in the brain of rodents. The virus disrupted brain tumours developed from stem cells carrying deregulated p53 responses, raising hopes for a new personalized and biosafe medicine based on this virus.

Our recent efforts are focused on enhancing MVM anti-cancer properties by re-targeting the virus to the neovascularization process required for tumor growth. We are engineering different domains of MVM capsid with heterologous peptides able to block vascular endothelial growth factor (VEGF) functions, to either induce antibodies that may reduce tumor growth, or to drive the tropism of the virus specifically to VEGF-R expressing vascular cells supporting the tumor vascularization. Some of the VEGF-chimeric viruses engineered in the tropism determinant of the capsid localized in a depression at the 2-fold axis did show an increased tropism towards human glioblastoma cells, by the differential recognition of sialic acids receptors on the cancer cell surface (Figure).

Other approaches to increase MVM oncotropism are exploring experimental evolutionary strategies based on the selection of (i) MVM variants naturally evolved in mice with distinct tropism and pathogenicity, (ii) chimeric viruses spontaneously emerging after coinfection with two MVM strains, and (iii) MVM mutants affected at the capsid domain recognizing sialic acid receptors obtained from directed evolution strategies.

We are aware that multi-functional non-structural proteins may also critically contribute to viral tropism, and therefore other ongoing research concern the mechanisms mediated by the NS1 and NS2 non-structural proteins in their roles to regulate the differential toxicity of the MVM infection in normal versus human transformed cells.





MVM capsid-sialic acid receptors interaction on parvovirus tropism. Virus binding to pro-traffic and inhibitory sialic acid receptors on the cell surface impacts the traffic to the endosome and exposure of the VP1-Nter sequence. (from Calvo-López et al., 2023).



#### List of publications

- Calvo-López, T., Grueso E., Sánchez-Martínez, C., and J. M. Almendral (2023). Intracellular virion trafficto the endosome driven by cell type specific sialic acid receptors determines parvovirus tropism. Frontiers in Microbiology, 13:1063706. doi: 10.3389/fmicb.2022.1063706.
- Mietzsch M, Qiu J, Almendral JM and Söderlund-Venermo M (2023) Editorial: Parvoviruses: from basic research to biomedical and biotechnological applications. Front. Microbiol. 14:1194926. doi: 10.3389/fmicb.2023.1194926.
- Piredda, R., Rodríguez, L.G., Stakamakis K., Martinez-Ortega J., López A., Almendral J.M., and Revilla, Y. (2024). Assessment of molecular modulation by multifrequency electromagnetic pulses to preferably eradicate tumorigenic cells. Scientific Reports, 14, 30150. https://doi.org/10.1038/s41598-024-81171-x.
- Sánchez-Martínez, C., Grueso, E., Calvo-López, T., Martinez-Ortega, J., Ana Ruiz, A., and José M. Almendral. (2024) VEGF-Virus interactions: pathogenic mechanisms and therapeutic applications. Cells, 13(21), 1815; https://doi.org/10.3390/cells13211815.

#### **Book Chapters**

Lopez-Bueno, A., Gil-Ranedo, J., and Almendral, J.M. (2024) Assembly of structurally simple icosahedral viruses. In: Mateu M.G. (ed) Structure and physics of viruses. Springer. Subcellular Biochemistry vol. 105, pag. 403-430.



#### Research projects

- Ref: PID2019-111146RB-100. Spanish Ministerio de Ciencia Innovación. "Directed evolution of Parvoviruses for human cancer therapy". PI, A. López-Bueno and J.M. Almendral. June 2020-June 2023.
- Ref: PID2022-141799OB-I00. Spanish Ministerio de Ciencia Innovación. "Retargeting parvovirus infection to cellular processes determining human cancer". PI, J.M. Almendral. Sept 2023- Oct 2026.
- Paso Alto S.L. "Uso de radiación no ionizante (modulación) para potenciar el tratamiento del cáncer: Efectos sobre viabilidad celular y rutas de señalización en modelos tumorales", PI, JM Almendral and Y. Revilla. November 2022-November 2023.



#### Other activities

- Participation as UAM in S4i Science for industry. Madrid 2024.
- Organization (J.M.-O.) II International Congress Synbio. Barcelona 2023.



## MOLECULAR ECOLOGY OF EXTREME ENVIRONMENTS

#### **Group members**

**Principal investigator:** Ricardo Amils Pibernat

**Scientific staff:** Jose. Manuel Martínez Vicenta de la Fuente

Postdoctoral fellows: Zamira Elena Soto

Predoctoral fellows: Adrián Martínez Bonilla Guillermo Mateos Budiño Esther Velasco Enrique Ignacio Carrasco Cai Qian **Technicians:** Nuría Rodríguez González

Undergraduate and Master students: Andrea Vidal Esteban Sabroso Violeta Gallego

Visiting scientists:
David Fernández Remolar
(Macau University, China)
Erik Zettler (NIOZ,
Nederland)
Linda Amaral (NIOZ,
Nederland)
Cristina Escudero
(Tübingen University,
Germany)



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#### Summary

This area of research has the following objectives:

Geomicrobiology of the Iberian Pyrite Belt (IPB): characterization of the underground bioreactor responsible of the origin of the extreme conditions detected in the Río Tinto basin. This objective is developed in collaboration with Professor J.L. Sanz from the Department of Molecular Biology (UAM). This objective aims to identify the microorganisms involved in the coupled operation of the C, H, N, S and Fe biogeochemical cycles in the deep subsurface of the IPB in the absence of light, their isolation, phenotypic and genotypic characterization, and their involvement in the oxidation of metal sulfides, mainly pyrite, in strict anaerobic conditions.

Acidophiles: conventional microbial ecology, molecular ecvology, molecular biology and biotechnology of extreme acidic environments. This objective is mainly devoted to the exploration of the biotechnological applications (biomining, bioremediation, biomineralization and phytoremediation) of acidophilic organisms inhabiting the Tinto basin.



## Other activities

■ Encyclopedia of Astrobiology, editor

Characterization of extreme environments of astrobiological interest: Río Tinto and Iberian Pyrite Belt, Uyuni Salt Lake (Bolivia), Dallol in the Danakil depression (Ethiopia). This objective aims to characterize different extreme environments to evaluate the limits of life and the habitability in different planets and moons of the Solar System and from exoplanets.

**Microbial ecology of heavy water:** this objective is devoted to the characterization of microorganisms able to develop in pure D2O, an extremely toxic compund.

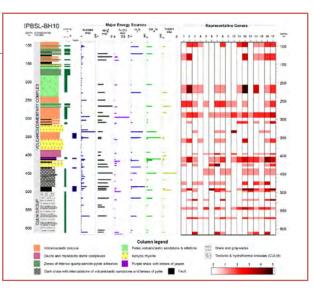


- PID2022-13660NB-I00. MICIU. Operation of the deep subsurface bioreactor that originate the extreme conditions of Río Tinto and biotechnological application of the Iberian Pyrite Belt biodiversity. R.Amils and I. Marín (IPs). 2023-2026.
- TED2021-129563B-I00. MICINN. Decifering the metabolism of Fe(II) Oxidation associated to the Reduction of Nitratte and its utilization for the bioremediation of nitrate contaminated waters R. Amils and J.L. Sanz (IPs) 2023-2025.
- Consolidator Grant 81602. ERC. The physicochemicakl nature ofwateronearlyMars.A.G. FairénandJ.L. Sanz (IPs).. 2019-20234 Red Nacional de Microorganismos Extremófilos (ReDex). 2023-2025.





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- Fairén, A.G., Sanz, J.L., Rodríguez, N., Sánchez-García, L., Rojas, P., Uceda, E.R., Carrizo, D., Amils, R. (2023) Ecological successions throughout the dessication of the hypersaline Tirez lagoon (Spain) as an astrobiological time-analog for the wet-to-dry transition on early Mars. Sci. Rep., 13: 1423 doi: 10.1038/s41598-023-28327-3
- Hallsworth, J.E., Udaondo, Z., Pedrós-Alió, C., Höfer, J., Benison, K.C., Lloid, K.G., Cordero, R.J.B., de Campos, C.B.L., Yakimov, M.M., Amils, R. (2023) Scientific novelty beyond the experiment. Microbiol. Biotech., 2023;161131-1173 doi: 10.1111/1751-7915-14222.
- Sánchez-León, E., Huang-Lin, E., Amils, R., Abrusci, C. (2023) Production and characterization of an exopolysaccharide by Bacillus amyloliquefaciens: Biotechnological applications. Polymers2023,15,1550 doi:10.3390/polym15061550
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- Escudero, S., Amils, R. (2023) Hard Rock Dark Biosphere and Habitability. Front. Astron. Space Sci. 10:1203845 doi: 10.3389/fspas.2023.1203845.
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Mineralogy, physicochemical variables and microbial diversity detected in the deep subsurface of the Iberian Pyrite Belt

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## GENETIC VARIABILITY OF RNA VIRUSES

#### **Group members**

**Principal investigator:** Esteban Domingo Solans

Scientific staff: Celia Perales Viejo

**Postdoctoral fellows:** Pilar Somovilla Crespo

Predoctoral fellows: Carlos García Crespo

**Technicians:** Ana Isabel de Ávila Lucas María Eugenia Soria Benito Undergraduate and Master students: Antoni Durán i Pastor Esther Uribe-Echeverría Carmen Dominguez Navarro Mario Rucio Guallar

Visiting scientists: Sergio Ortega Josep Sardanyes Tomás Lázaro Oriol Llopis



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



### Summary

#### Functional diversification of SARS-CoV-2

Through a collaborative interdisciplinary project, we have analyzed the quantitative and biological extent of viral sequence diversity in nasopharyngeal isolates of SARS-CoV-2, the causative agent of COVID-19. Self-Organising Maps (SOM, an unsupervised neural network) have allowed clustering different haplotypes from individual patients, depicting their location in sequence space and quantifying their frequency (see figure). Several virus isolates that reached Madrid during the first wave of COVID-19 share minority haplotypes (present at low frequency) indicating amino acid substitutions in the virus polymerase, which affect viral RNA synthesis. The effect was quantified by an in vitro primer extension assay using the corresponding minimal replication complex (nsp12-nsp8-nsp7 mutant proteins). These results have documented for the first time that the incipient diversification that SARS-CoV-2 undergoes in infected individuals is functionally relevant. In addition, mapping of amino acid changes in the three-dimensional structure of the replication complex identified a contact surface between nsp12 and nsp8 which is critical for enzymatic activity, and constitutes a new potential target for therapeutic interventions.

Possible predictive value of mutant spectra Mutant spectra of individual SARS-CoV-2 isolates from different waves (belonging to different phylogenetic groups or clades) have revealed a new facet of viral quasispecies: the presence in mutant spectra of clusters of mutations and deletions that are not typical for the isolate being analyzed, but served to define clades that appeared months or years later during the pandemic spread of the virus. We call these clade-discordant sequences, and their presence suggests the possibility that mutant spectra may have predictive value for genomic sequences of future virus isolates, an aspect we are currently investigating.

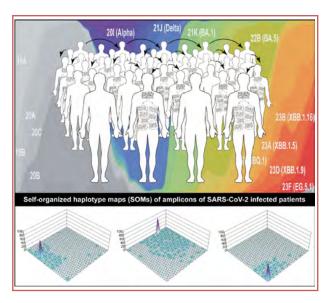
#### New therapeutic options

Lethal mutagenesis, or viral extinction by antiviral agents that induce an increase in the number of mutations in viral genomes, is an antiviral strategy in whose development our group has played a direct role. In this context, we have documented that remdesivir and ribavirin exert a synergistic lethal mutagenesis against SARS-CoV-2, leading to the extinction of the virus in a cell culture model of infection. This suggests a new avenue of therapeutic intervention for people with severe COVID-19, another aspect of our current work. All our studies have been carried out in collaboration with other groups, in particular Celia Perales' group at the CNB-CSIC and her associates at the Fundación Jiménez Díaz hospital group. The different groups that have collaborated in our research, other issues addressed during the last two years, as well as review articles in which we have participated, can be identified by consulting the list of publications.





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- Ariza-Mateos A., et al. (2023). Natural languages and RNA virus evolution. J Physiol. 602(11):2565-2580.
- Domingo E., et al. (2023). Puzzles, challenges, and information reservoir of SARS-CoV-2 quasispecies. J. Virol. 97(12):e0151123.
- Somovilla, P., et al. (2023). Atypical mutational spectrum of SARS-CoV-2 replicating in the presence of ribavirin. Antimicrob. Agents and Chemother. 67(1):e0131522.
- García-Crespo, C., et al. (2023). Hepatitis C virus fitness can influence the extent of infection mediated epigenetic modifications in the host cells. Front. Cell. Infect. Microbiol. 13:1057082.
- Domingo, E., et al. (2023). Viral Fitness, Population Complexity, Host Interactions, and Resistance to Antiviral Agents. Curr Top Microbiol Immunol. 439:197-235.
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- Martínez-González, B. et al. (2023). Fitness-Dependent, Mild Mutagenic Activity of Sofosbuvir for Hepatitis C Virus. Antimicrobial Agents and Chemotherapy. 67(7):e0039423.
- Delgado, S. et al. (2024). Incipient functional SARS-CoV-2 diversification identified through neural network haplotype maps. Proceedings of the National Academy of Sciences. USA. 121(10):e2317851121.
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- Martínez-González, B. et al. (2024). SARS-CoV-2 mutant spectra as variant of concern nurseries: endless variation? Frontiers in Microbiology. 15:1358258. 14. Conde, A. et al. (2024). Fluoride anodic films on stainless steel fomites to reduce transmission infections. Applied and Environmental Microbiology. 90(2):e0189223.
- Domingo, E. et al. (2024). Reply to Qu et al., "Quasispecies are constantly selected through virus encoded intracellular reproductive population bottlenecking". Journal of Virology. 98(4):e0004624.
- Berkhout, B. et al. (2024). Virus Research: 40 years and still going strong. Virus Research, 350, 199493. 17. Ferrer-Orta, C. et al. (2024) Point mutations at specific sites of the nsp12-nsp8 interface dramatically affect the RNA polymerization activity of SARS CoV-2. Proceedings of the National Academy of Sciences. 121(29): e2317977121.
- Landa, CR. et al. (2024). Adapting the rhizome concept to an extended definition of viral quasispecies and the implications for molecular evolution. Scientific Reports. 14(1) pp. 17914.
- Domingo, E,; Witzany, G. (2024). Quasispecies productivity.
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- Sardanyes, J. et al. (2024) Quasispecies theory and emerging viruses: challenges and applications. npj Viruses 2. Article number: 54.



The pandemic spread of SARS-CoV-2 (upper part) has been analyzed by ultra-deep sequencing of nasopharyngeal isolates, and self-organized neural maps to display different haplotypes within isolates (bottom part)



#### Other activities

- 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.



- PID2020-113888RB-I00. Ministry of Science and Innovation Molecular disorganization during lethal mutagénesis of SARS-CoV-2: involvement of structured and unstructured RNA regions. PIs: Jordi Gómez and Esteban Domingo. (2021-2024)
- S2018/BAA-4370. Community of Madrid/ERDF. Platform for the development of animal health control strategies. PLATESA2. Coordinator: Noemí Sevilla. (2019-2023). VIRNA group PI: E. Domingo.
- PTI+ Global Health, within WP9 Antivirals, CSIC. Synergistic lethal mutagenesis for SARS-CoV-2 with virus samples from patients. (2022-2023). PIs: Nuria Verdaguer, Esteban Domingo, and Celia Perales.
- 525/C/2021. Fundació La Marató TV3. Towards understanding the molecular mechanisms of lethal mutagenesis in SARS-CoV-2. PI: Nuria Verdaguer and Celia Perales. Collaborator: Esteban Domingo. (2021-2024)
- PID2023-146622OB-I00. Ministry of Science, Innovation, and Universities. Composition analysis and dynamics of SARS-CoV-2 mutant spectra, and implications for improved tests of antiviral efficacy. PIs: Celia Perales and Esteban Domingo (2024-2027).
- Member of the Biomedical Research Network for Liver and Digestive Diseases (CIBERehd). Group CB06/04/0086.
- Member of the Health Research Institute (Jiménez Díaz Foundation).



# YEAST ENZYMES BIOENGINEERING TO GENERATE BIOACTIVE COMPOUNDS

#### **Group members**

**Principal investigator:** María Fernandez Lobato

Scientific staff: Miguel Remacha Moreno

Postdoctoral fellows: Noa Minguet

Predoctoral fellows: David Piedrabuena Estrada Marina Minguet Lobato Egle Narmontaite Maria Martinez Ranz

**Technicians:** 

Laura Barahona Pérez Pilar Sánchez Pozo Beatriz Gracia Gómez Undergraduate and Master students: Gloria Gómez Montesino Marcos Fuentes de Frutos David Blázquez García Beatriz Gracia Gómez

Visiting scientists: Fadia V. Cervantes (ICP, Madrid)



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



#### Summary

We work with microorganisms producing bioactive compounds and try to connect the generation of knowledge to the development of new biocatalysts for biotechnological applications. We focus on characterization of enzymes producing new compounds, analysis of their structural-functional determinants, their operational improvement, the purification, and characterization of the molecules they produce as well as the evaluation of their potential biological activity.

During the last years we have continued with the characterization and study of proteins from nonconventional yeast showing glycosyltransferase production applicable in the activity, heterooligosaccharides and glycoconjugate derivatives with putative prebiotic, antibiotic, antiviral or antioxidant properties, among others. We have also characterized new fungal and yeast chitinases, chitosanases and chitin deacetylases that can hydrolyze or modified different chitinolytic materials, general waste from industrial activity, to generate different types of chitooligosaccharides with varied acetylation patterns and promising bioactive properties. In general, most of the characterized proteins are glycosyl hydrolases (GH) structurally included in families GH32, 31, 13, 18 or 84. Interestingly, some of the characterized enzymes can also glycosylate compounds including aromatic rings such as hydroxytyrosol, epicatechin gallate or

phloretin, which confers the new products formed a special biotechnological interest. We have obtained numerous variants of biocatalysts that increase or alter the pattern of the biosynthetic products formed. The biological activities of these new molecules are currently being analyzed. Objectives included in those of the Glicoenz consortium (http://www.glicoenz.org/p/glicoenz.html).



#### **Doctoral theses**

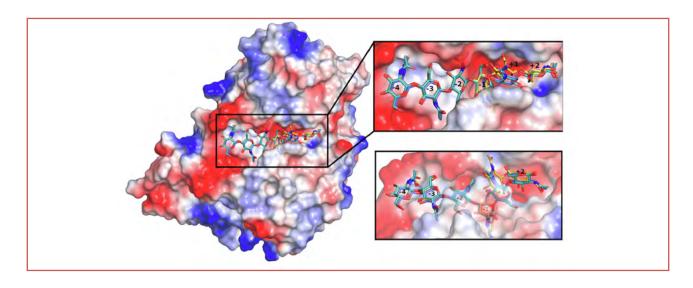
David Piedrabuena Estrada (2023). "Nuevo protocolo para la obtención de oligosacáridos prebióticos mediante el empleo de actividades glicosilhidrolíticas ofrecidas por la levadura no convencional Schwanniomyces occidentalis". Department of Molecular Biology. University Autónoma from Madrid. Directors: María Fernández Lobato and Ma José Hernaiz.



## Other activities

 M. Fernández Lobato: Director of the Molecular Biology Department, University Autónoma from Madrid.
 M. Remacha Moreno: Director of the Doctoral School, University Autónoma from Madrid.





Structural traits of complexes give insights into the chitinase Chit42 machinery. Superposition of Chit42 D169A/E171A complexed with NAG6 (teal) and NAG4/NAG2 (gold/slate; left-hand). Electron density map of complexes with NAG6 (top right), NAG4/NAG2 cocrys (botton right).



## List of publications

- Garcia-Gonzalez, M., Narmontaite, E., Cervantes, F.V., Plou, F.J., Betancor, L., Fernández-Lobato, M. (2023) Continuous production of honey oligosaccharides in packed-bed reactors with immobilized α-glucosidase from Metschnikowia reukaufii. Biocatalysis and Agricultural Biotechnology 47, 102590 (pp 1-13). doi: 10.1016/j. bcab.2022.102590
- Minguet-Lobato, M., Cervantes, F.V., Míguez, N., Plou, F.J. and Fernández-Lobato, M. (2024) Chitinous material bioconversion by three new chitinases from the yeast Mestchnikowia pulcherrima. Microb. Cell Fact. 23, 31 (pp 1-15). doi: 10.1186/s12934-024-02300-9
- Merdzo, Z., Narmontaite, E., Gonzalez-Alfonso, J.L., Poveda, A., Jimenez-Barbero, J., Plou, F.J., Fernández-Lobato, M. (2024) Insights into the transglucosylation activity of α-glucosidase from Schwanniomyces occidentalis. Appl. Microbiol. Biotechnol. 108, 443 (pp 1-13). doi: 10.1007/ s00253-024-13262-8
- Rodriguez-Garcia, D., Uceda, C., Barahona, L., Ruiz-Nuñez, M., Ballesteros, A.O., Desmet, T., Sanz-Aparicio, J., Fernandez-Lobato, M., Gonzalez-Alfonso, J.A. and Plou, F.J. (2024) Enzymatic modification of dihydromyricetin by glucosylation and acylation, and its effect on the solubility and antioxidant activity. Org. Biomol. Chem. 23, 1136-1145. Published on line 2024 Dec 10. doiI: 10.1039/d4ob01682c
- González-Alfonso, J.L., Rodríguez-García, D., Moreno-García, M., Uceda, C., Ubiparip, Z., Barahona, L., Ballesteros, A.O., Desmet, T., Fernández-Lobato, M., Plou, F.J. (2024) Enzymatic modification of polyphenols derived from plant-based residues. In: Romero García, JM, Morán Alarcón, LC, Padilla Rascón, C, Castro Galiano, E (eds.) Iberoamerican Congress on Biorefineries (pp. 564) Jaen University Publications. ISBN 978-84-9159-626-4



- Fundación Ramón Areces. XIX Concurso Nacional-Ciencias de la Vida y la Materia. Production of second-generation prebiotics and polyphenol glycosides. Validation of its bioactive properties for use in functional food. PI: M. Fernández Lobato. 04/2019-04/2023.
- PID2019-105838RB-C32. MCI-Programa Estatal de Investigación, I+D+i-Retos. Prospecting and development of microbial enzymes to obtain new glycosylated compounds of pharmacological interest. PI: M. Fernández Lobato. 01/06/2020-31/05/2024.
- TED2021-129288B-C22. AEI- Projects oriented towards the ecological transition and the digital transition. Simplification of the use of chitin-enriched waste for the enzymatic production of bioactive chitooligosaccharides. PI: M. Fernández Lobato. 01/12/2022-30/09/2025.
- PDC2022-133134-C22.EAI-Proof of Concept Projects. Scaling up the production of glycosidases to obtain modified flavonoids and their evaluation in biomedical applications. PI: M. Fernández Lobato. 01/12/2022-30/09/2025.
- PID2022-136367OB-C32. MCI/AEI-Programa Estatal de Investigación Científica, Técnica y de Innovación. Production and functional modification of glycoenzymes for the sustainable obtaining of glycoderivatives by transglycosylation. PI: M. Fernández Lobato. 01/09/2023-31/08/2026.



## VIRUS ENGINEERING AND NANOBIOTECHNOLOGY

#### **Group members**

Principal investigator: Mauricio García Mateu

**Scientific staff:** Alejandro Valbuena Jiménez

Postdoctoral fellows: Juan Carlos Gil Redondo

**Predoctoral fellows:**Luis Valiente Martínez-Sicluna
Judith Escrig Traver
Valentín Riomoros Barahona





#### Summary

We use protein engineering and biochemical, biophysical and biological analyses to study virus assembly, conformational stability and dynamics, and physical properties, and their biological relevance. We aim at providing further insights into viral infection, and guidelines for the application of this knowledge for the design of vaccines, antiviral drugs, and novel bio/nanomaterials.

Some specific subjects that are currently being researched in our laboratory include: i) the relationship between the mechanics and dynamics of a virus capsid and virus assembly or genome uncoating; ii) the structural determinants of the mechanical properties of viruses; iii) the biological relevance of the mechanical properties of viruses; iv) the development of new antiviral drugs that modify the mechanical properties of viruses; v) the development of mechanically resistant virus- or protein-based nanostructured materials.

Scientific relevance and implications. Some major scientific contributions in the last two years include: i) further experimental evidence on the biological relevance of mechanical properties of viruses; ii) new insights into the intimate relationship between virus mechanical elasticity and conformational dynamics at equilibrium; iii) Physical and chemical conditions that control virus capsid assembly pathways and the architecture of the human immunodeficiency

virus capsid particles assembled in vitro; iv) singleparticle analysis of the kinetics of viral genome uncoating; v) the discovery of the possibility to develop new antiviral drugs that interfere with the mechanical elasticity of viral particles required for genome uncoating; v) the discovery of the biological relevance of a viral RNA dodecahedral cage in human rhinovirus that promotes virion assembly and modulates viral genome uncoating.



#### Research projects

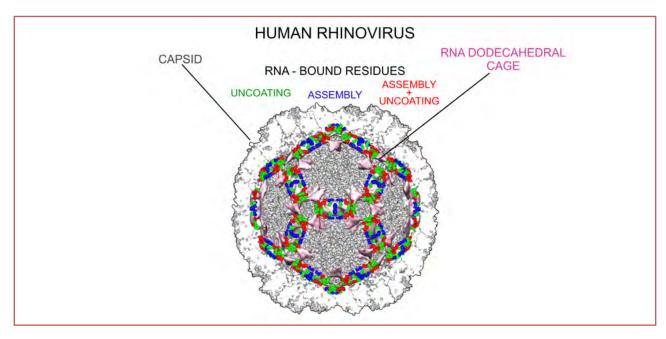
- PID2021-126973OB-I00. Ministerio de Ciencia e Innovación. Biomechanics and dynamics of human viruses for the development of antiviral drugs and engineered protein nanomaterials for biomedical or nanotechnological uses- Biomecánica y dinámica de virus humanos para el desarrollo de fármacos antivirales y materiales modificados por ingeniería de proteínas para usos biomédicos o nanotecnológicos. P.I. Prof. Mauricio G. Mateu. 2022-2026.
- Network on Physical Virology- Red Temática en Física Virológica. Ministerio de Ciencia e Innovación. P.I.: Prof. David Reguera. 2023-2025



#### Other activities

- M.G. Mateu: Edition of the book Structure and Physics of Viruses 2nd edition. Springer Nature
- M.G. Mateu: Member of the Editorial Board of Virus Research





The interaction in human rhinovirus between a dodecahedral cage made of RNA and the viral capsid modulates virus assembly and viral genome uncoating



- Strobl, K., Mateu, M.G., de Pablo. P.J. (2023). Exploring nucleic acid condensation and release from individual parvovirus particles with different physicochemical cues. Virology 581, 1-7.
- Luque, D., Ortega, A., Valbuena, A., Vilas J.L., Rodríguez-Huete, A., Mateu, M.G., Castón, J.R. (2023). Equilibrium dynamics of a biomolecular complex analyzed at singleamino acid resolution by cryo-electron microscopy. J. Mol. Biol. 435, 168024.
- Fuertes, M.A., López, D., Valiente, L., Rodríguez-Huete, A., Valbuena, A., Mateu, M.G. (2023) Electrostatic screening, acidic pH and macromolecular crowding increase the self-assembly efficiency of the minute virus of mice capsid in vitro. Viruses 15, 1054.
- Reguera, D., de Pablo, P.J., Abrescia, N.A., Mateu, M.G., Hernández-Rojas, J., Castón, J.R., San Martín, C. (2023). Physical Virology in Spain. Biophysica 3, 598-619.
- Valbuena, A., Strobl, K., Gil-Redondo, J.C., Valiente, L., de Pablo, P.J., Mateu, M.G. (2024). Single-molecule analysis of genome uncoating from individual human rhinovirus particles, and modulation by antiviral drugs. Small 20, e2304722.
- Escrig, J., Marcos-Alcalde, I., Domínguez-Zotes, S., Abia, D., Gómez-Puertas, P., Valbuena, A., Mateu, M.G. (2024). Structural basis for self-assembly pathways leading to different human immunodeficiency virus capsid-like nanoparticles ACS Nano 18, 27465-27478.

- Gil-Cantero D., Luque, D., Mata, C.P., Valiente, L., Rodriguez-Huete, A., Valbuena, A., Mateu, M.G., Castón, J.R. (2024). Cryo-EM of human rhinovirus reveals capsid-RNA duplex interactions that provide insights into virus assembly and genome uncoating. Commun. Biol. 7, 1501.
- Valiente, L., Riomoros-Barahona, V., Gil-Redondo, J.C., Valbuena, A., Mateu, M.G. (2024). A capsid-bound RNA dodecahedral cage determines human rhinovirus infectivity and biological fitness by modulating virion assembly and genome uncoating. J. Mol. Biol. 437, 168922.

#### Capítulos de libros:

- Mateu, M.G. (2023). Virus mechanics: a structure-based biological perspective. En "Physical Virology" (Comas-Garcia, M. & Rosales-Mendoza, S., eds.), pp. 237-282. Springer Nature, Singapore.
- Mateu, M.G. (2024) Introduction: The structural basis of virus function. En "Structure and Physics of Viruses" 2nd edition (Mateu M.G., ed.). Springer Nature, Switzerland. Subcell. Biochem. 105, 3-53.
- de Pablo, P.J., Mateu, M.G. (2024) Mechanical properties of viruses. En "Structure and Physics of Viruses" 2nd edition (Mateu M.G., ed.). Springer Nature, Switzerland. Subcell. Biochem. 105, 629-691.
- Mateu, M.G., Valbuena, A. (2024). Bio/nanotechnological applications of virus particles. En "Structure and Physics of Viruses" 2nd edition (Mateu M.G., ed.). Springer Nature, Switzerland. Subcell. Biochem 105, 823-878.



## MOLECULAR MODELING

#### **Group members**

**Principal investigator:** Paulino Gómez-Puertas

Scientific staff: Íñigo Marcos-Alcalde

**Postdoctoral fellows:** David Ros-Pardo



http://bioweb.cbm.uam.es/



#### 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. Carbapenemases: Dynamic simulation of the interaction of bacterial carbapenemases (VIM-2, KPC-2, OXA-48) with substrates and known inhibitors.

#### **Doctoral theses**

■ David Ros-Pardo (2024). Homology modeling and molecular dynamics techniques in the study of the functionality of GSDMB and the cohesin complex. Universidad Autónoma de Madrid. Supervisors: Íñigo Marcos-Alcalde and Paulino Gómez-Puertas.

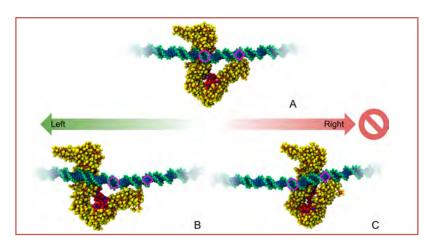
- B. Development of a new and efficient drug design system based on computational dynamic simulation of macromolecular structures. Based on the analysis of enzyme active centers, the method developed by our group consists of simulating these protein structures through molecular dynamics for several hundred nanoseconds, selecting different representative structures and filtering a database of 3D compounds for each one of them. Used for the design of new carbapenemases inhibitors and the design of specific inhibitors as antitumor drugs.
- C. In silico design of peptides as new antivirals against SARS-CoV-2 infection.
- D. Development of efficient methods for calculating paths of minimum or maximum parameter values (i.e. minimum energy paths) through surfaces of any number of dimensions.



#### **Patents**

MiniACE2 proteins to block SARS-CoV2 infection". 2022-2024. Patent no: NC2022/0005322. Patent in collaboration with the Instituto Distrital de Ciencia, Biotecnología e Innovación en Salud (IDCBIS) of Bogotá, Colombia. [WO2023/209619A1]





STAG2-RAD21 complex: A unidirectional DNA ratchet mechanism in loop extrusion.



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## Other activities

- Dr. Gomez-Puertas, associated researcher to the Institute
  - since 2012. ■ Molecular Modeling Group, member of the "Cancer Hub" network of the Spanish National Research Council - CSIC since 2022.

for Health Research of La Paz University Hospital (IdiPAZ)

- Molecular Modeling Group, member "Computational Biology and Bioinformatics" network of the Spanish National Research Council - CSIC since 2023
- Molecular Modeling Group, member of the "Rare Diseases Hub" network of the Spanish National Research Council -CSIC since 2024.



# ULTRAHIGH-THROUGHPUT DISCOVERY AND ENGINEERING OF ENZYMES FOR BIOTECHNOLOGICAL APPLICATIONS

#### **Group members**

**Principal investigator:** Aurelio Hidalgo

Scientific staff: Patricia Pérez Arnaiz

Postdoctoral fellows: Davide Agostino Cecchini Álvaro Lorente Arévalo María Gimeno Pérez

**Predoctoral fellows:** Laura Blas Muñoz John Martínez Salvador Laura Pilar Saiz Álvarez **Technicians:** Carmen Ortega Plaza Sara Aparicio Gutierrez

Undergraduate and Master students: Aitor Palacios Azcona Martina Escorial García Carlos Huertas Díaz

**Visiting scientists:** Theofania Andreadaki Diletta Arceri





www.cbm.uam.es/ahidalgo



#### Summary

Microbial diversity is a vast reservoir of genetic information that can be valorized to provide sustainable solutions to urgent societal needs, such as the drive for a greener industry, accurate and low-volume diagnostics or biodiversity monitoring and conservation. It can also be used to unravel fundamental knowledge about sequence-structurefunction relationships of proteins or the composition of functions of microbiomes. While in silico methods are fast and widely used for biodiscovery, their very same nature precludes the discovery of true novelty in sequence space. Functional screening, on the other hand, is unbiased by query motifs but is time, reagent and resource intensive. Consequently, our objective is to leverage the synergy between biology and and microtechnologies to find novel and industrially relevant genes, enzymes and compounds faster, more efficiently and sustainably than the state of the art, particularly enabling academic labs to undertake biodiscovery campaigns until now costly and limited to large enterprises.

In the HT Discovery Lab, we carry out discovery and engineering campaigns both in the natural or artificial genetic diversity. One of the tools to discover new or improved enzymes are biological selections: inexpensive methods to find enzymes that couple the improved fitness of a protein to the survival of a biological host under selective pressure. In our group, we develop and apply biological selections to enhance the activity of enzymes with "unnatural"

substrates relevant for the pharma and fine chemical industries and 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 selective and stable transferases in the frame of regional and national research grants. Subsequently, 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".

However, the complexity of cellular metabolism limits the applicability of biological selections. For this reason, we also work on screening methods, which involve individually assaying in vivo or vitro each bacterium, gene or protein for a desired property. 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 assay volume, use of reagents, cost and environmental footprint. Moreover, microfluidics enables the conversion of general lab operations (additions, aliquoting, detection of a given property) into a particular device design. Using ultrahigh-throughput screening, we have discovered novel esterases, KREDs, lyases, and other enzymes in cooperation with several industrial stakeholders during the EU-funded projects MetaFluidics, RadicalZ, CC-TOP and BlueTools.



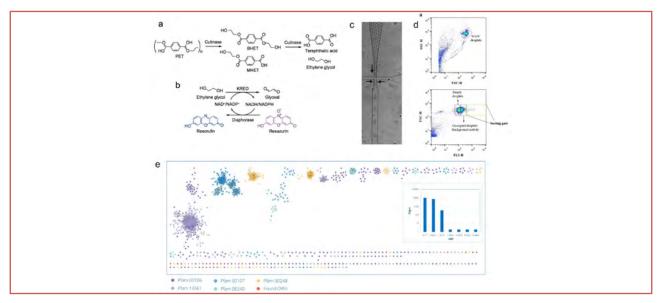


Figure 1: From biodiscovery to insight. The metagenomic diversity is screened for industrially relevant enzymes (A,B) in microfluidic droplets (C), which are then sorted by FACS (D) and the resulting sequences characterized, e.g with sequence similarity networks (E).



#### Research articles:

- Sánchez-Costa, M., Gola, S., Rodríguez-Sáiz, M., Barredo, J., Hidalgo, A. & Berenguer, J. (2024). From accurate genome sequence to biotechnological application: The thermophile Mycolicibacterium hassiacum as experimental model. Microb. Biotechnol. 17, e14290. DOI: 10.1111/1751-7915.14290
- Ribeiro, A. L. J. L., Pérez-Arnaiz, P., Sánchez-Costa, M., Pérez, L., Almendros, M., Vliet, L. van, Gielen, F., Lim, J., Charnock, S., Hollfelder, F., González-Pastor, J. E., Berenguer, J. & Hidalgo, A. (2024) Thermostable *in vitro* transcription-translation compatible with microfluidic droplets. Microb. Cell Factories 23, 169. DOI: 10.1186/s12934-024-02440-y

#### **Book chapters:**

Ribeiro, A.-L., Sánchez, M., Bosch, S., Berenguer, J. & Hidalgo, A. Stabilization of Enzymes by Using Thermophiles. (2023). In: Methods Mol. Biol. (Clifton, NJ) 2704, 313–328. DOI: 10.1007/978-1-0716-3385-4\_19



#### Other activities

- Best poster communication. Laura Blas. CMIBM, Madrid, June 2024
- Best oral communication. Laura Blas. Jornadas Españolas de Biocatálisis. Donostia-San Sebastián, September 2024
- Participation in the European Night of Researchers. CaixaForum Madrid, September 2023.
- Licensing agreement for 7 ketoreductases to Prozomix, Ltd. October 2023.



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- H2020-SC2, GA 10100560. European Commission. Rapid discovery and development of enzymes for novel and greener consumer products (RadicalZ). Aurelio Hidalgo (PI). 01/06/2021- 31/05/2025. Role: coordinator
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## CONJUGATION IN GRAM-POSITIVE BACTERIA

#### **Group members**

**Principal investigator:** Wilfried Meijer

**Postdoctoral fellows:** Andrés Miguel Arribas

**Predoctoral fellows:** Fernando Freire Gómez

**Technicians:** 

Daniel González Álvarez, Laura Blanca Pariente

**Undergraduate and Master students:** 

Aroa Vaillo González Laura Navarro

María Victoria del Castillo Sarduy

Sara Broekman

Andrés Francesch Vázquez



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



#### Summary

Bacterial conjugation is the process in which a conjugative DNA element transfers from a donor to a recipient bacterial cell via a specialized channel. Often found on conjugative plasmids, these elements carry genes essential for conjugation and frequently harbour antibiotic resistance (AR) genes. The horizontal transfer of AR is a major global health concern, as it contributes to antibiotic-resistant infections that are difficult to treat.

Despite its significance, much remains unknown about conjugation in Gram-positive (G+) bacteria. While it facilitates AR spread, conjugative plasmids can also serve as valuable tools for genetic modification, aiding biotechnology, clinical research, and industrial applications. Our lab focuses on two main goals: developing genetic tools for G+ bacteria and deepening our understanding of conjugation at genetic, functional, and molecular levels. By dissecting conjugation mechanics, we aim to improve bacterial genetic manipulation and explore therapeutic strategies to inhibit conjugation, reducing AR transmission.

Amultidisciplinary approach is central to our work, with bioinformatics playing a crucial role. Computational analysis has helped us classify the conjugative plasmid pLS20 into four distinct clades, allowing us to identify conserved genes, protein domains, and motifs across different plasmids. This knowledge enhances our understanding of conjugation's genetic architecture.

One of our major achievements is developing a pLS20-based system that enables the exchange

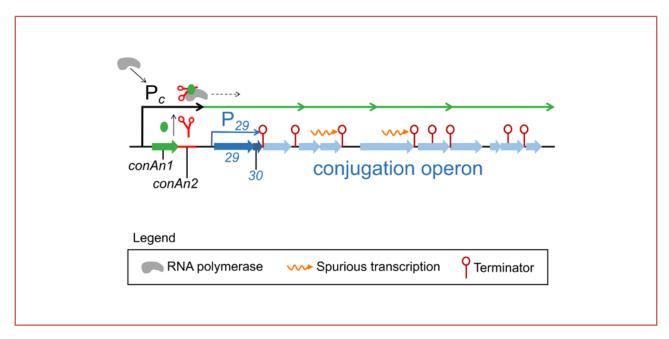
of chromosomal regions exceeding 100 kilobases between bacterial strains. This advancement holds promise for synthetic biology, metabolic engineering, and therapeutic development, as large-scale genetic exchanges allow for more complex and precise modifications.

Beyond tool development, we investigate the genetic and molecular stages of conjugation, seeking potential intervention targets to prevent AR gene transfer. A key discovery from our lab is a novel two-component processive antitermination system in many G+conjugative plasmids. This system enables continuous transcription across long distances by bypassing multiple terminators, ensuring proper expression of conjugation operon. The presence of a unique "antitermination-resistant terminator signal" at the operon's end prevents transcription read through in downstream DNA regions.

Our results showed that this antitermination mechanism is widespread in G+ bacteria, providing insights into conjugation regulation and offering potential strategies to disrupt the process. Targeting this system could hinder AR gene transfer, offering new approaches to combat antibiotic resistance.

Through genetic tool development, bioinformatics, and molecular research, our work advances bacterial conjugation studies with both theoretical and practical applications. By pushing the boundaries of understanding and innovation, we aim to contribute meaningful solutions to the global antibiotic resistance crisis.





Molecular basis of the two-component processive antitermination system present at the start of the conjugation operon. Association of the ConAn1 (protein) and conAn2 (RNA) component with the transcribing RNA polymerase alter the characteristics allowing it to bypass transcriptional terminators



## List of publications

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- PID2019-108778GB-C21P. Ministerio de ciencia y innovación. Estudios de proteínas involucrados en diferentes aspectos del proceso de la conjugación del plásmido pLS20 de Bacillus subtilis. W.J.J. Meijer. 01/07/2020 30/06/2023
- 2023AEP024, : Consejo Superior de Investigaciones Científicas. Cnjugacion en bacterias Gram positivas. W.J.J. Meijer. 01/07/2023 – 31/08/2023
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- BCV-2025-1-0026, Red Española de supercomputacion. Next level research in antibiotic resistance by predicting interactions between proteins encoded by a conjugative element and those encoded by the donor cell. W.J.J. Meijer, D. Abia. 01/03/2025 31/05/2025



## BIOTECHNOLOGY AND GENETICS OF EXTREME THERMOPHILES

#### **Group members**

Principal investigator: Mario Mencía Caballero

Postdoctoral fellows: Bruna Fernanda Silva de Sousa

**Technicians:** Samuel Jimena López Noemí López Rubio

**Undergraduate and Master students:** 

Sara Quiles Hernández

Hannah Imbusch



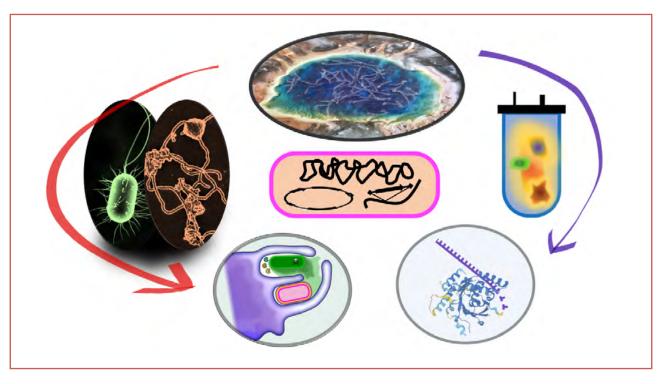


#### Summary

As a way to harness the evolution of a workhorse organism in biotechnology, such as *Thermus* thermophilus, we want to understand the processes that contribute to the plasticity of this organism's genome. Some of the most important of these processes are, the mechanisms of horizontal gene transfer and the corresponding defense systems against them, as well as the genome repair pathways and their mutual interactions. The main defense systems we are currently studying are the programmable nuclease Argonaute, the DNA primase-polymerase Primpol, and the AddAB complex (equivalent to RecBCD). The recombinational repair pathways we are studying are RecFOR, RecJ, and HerA-NurA. Analysis of these pathways will also reveal the strategies used by an extreme thermophile to cope with DNA damage caused by high temperatures. Next, we are developing a new system for continuous cultivation, the generation of induced diversity, and the selection of improved variants in an iterative, automated process. The new systems we are developing will be used for the discovery of thermostable proteins and the isolation of thermostable enzyme variants that could better meet the requirements of industrial biocatalysts or in other applications in the field of molecular biology, such as gene editing. Along the same lines, we are exploring the potential of this organism in the degradation and metabolism of plastic waste (polyethylene terephthalate and polystyrene) to try to generate a possible solution to the problem of plastic waste accumulation worldwide.

Also sharing the theme of evolution, I am interested in the early evolutionary events that led to the split of Bacteria and Archaea in the first place, and the emergence of Eukarya in a second stage. Regarding the Bacteria-Archaea split, I postulate a divergence based on the proton gradient and the critical, evolutionarily unchanged differences between the membranes of archaea and bacteria. These differences eventually carried over from bacteria to mitochondria and eukarya. Regarding the emergence of eukarya, the fundamental event would be the association of a protophagocytic organism (derived from Asgard archaea) with an alpha-proteobacterium that allowed the process of acid digestion to appear, and this would be critical for the emergence of phagocytosis, mitochondria, and eventually the rest of the eukaryotic characteristics.





We investigate three converging themes: i) generating new ways to evolve proteins for biotechnology using thermophilic bacteria, ii) understanding the mechanisms of genome plasticity of the genus Thermus, and iii) shedding light on early events in the evolution of Bacteria, Archaea and Eukarya



## List of publications

Gómez-Campo, C. L., Abdelmoteleb, A., Pulido, V., Gost, M., Sánchez-Hevia, D. L., Berenguer, J., and Mencía, M. (2024). Differential requirement for RECFOR pathway components in Thermus thermophilus. Environ. Microbiol. Rep. 16, e13269. DOI: 10.1111/1758-2229.13269



# HUMAN IMMUNODEFICIENCY VIRUS REVERSE TRANSCRIPTASE AND ANTIRETROVIRAL THERAPY

#### **Group members**

**Principal investigator:** Luis Menéndez Arias

**Predoctoral fellows:** Javier Martínez del Río

**Technicians:** Nerea López Carrobles

Undergraduate and Master students: Gabriel Pino Peco Nuria Rello García Rubén del Caz Calvo Rebeca Estévez Barcia Alicia Vallejo Tomás **Visiting scientists:**María de las Mercedes
Alcón Calderón
Yesit Bello Lemus



www.cbm.uam.es\lmenendezariaslab



#### Summary

Infections caused by human immunodeficiency viruses (HIV) constitute a major burden to human health worldwide. Despite significant advances in antiretroviral therapy, more than 600,000 people die each year from HIV-related causes, while a cure is yet to be found. 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 antiretroviral therapies.

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.

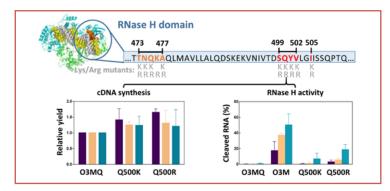
The accuracy of RTs is a crucial property in biotechnological applications. We have recently developed a novel NGS-based assay for measuring RT error rates using a relatively small number of reads. This method was validated by showing that the accuracy of HIV-1 RT in RNA-dependent DNA polymerization reactions was higher at lower concentrations of Mg2+ (i.e. 0.5 mM). In fact, the HIV-1<sub>BH10</sub> RT error rate obtained under these conditions was 9.0 x 10-6, only modestly higher than eukaryotic transcription error rates calculated for human cells. In these studies, we also found similar RT error rates when the template used was *in vitro*-transcribed viral RNA than in experiments where the RNA was obtained from HIV-infected cells. However,

mutational patterns were somewhat different depending on the template.

Despite remarkable advances in antiretroviral therapy, prevalence of drug-resistant HIV strains is increasing around the World, particularly in less-developed countries. The search for unexploited targets of antiretroviral intervention is still important for the future clinical management of the disease. One of these targets is the viral ribonuclease H (RNase H). However, RNase H inhibitors have not been approved for clinical use. Recent research in our laboratory highlighted the role of the HIV-1 RT's RNase H domain in nucleic acid binding affinity. We have also identified amino acid substitutions away from the active site that inactivate the RNase H while maintaining the nucleic acid binding affinity and DNA polymerization efficiency of HIV-1 RT.

In collaboration with medicinal chemists from Shandong University (Jinan, China), we have identified and characterized HIV-1 RNase H inhibitors with antiviral activity in cell culture assays. These drugs, specific for HIV-1 and HIV-2, could also be developed into broader spectrum inhibitors blocking the replication of other viruses targeting nucleases that share the same mechanism of action and have significant structural similarity. Examples are the hepatitis B virus RNase H, retroviral integrases or nucleases found in the terminase complex of herpesviruses.





Charge engineering of the nucleic acid binding cleft of a thermostable HIV-1 RT. Substituting Arg or Lys for GIn500 inactivates RNase H activity while maintaining cDNA synthesis efficiency. For details, see Martínez del Río et al. (2023) J. Mol. Biol. 435, 168219.



#### **Doctoral theses**

Moisés A. Árquez Mendoza (2024). Interactions of the nucleocapsid and reverse transcriptase of human immunodeficiency virus type-1 and their implications for drug resistance. Faculty of Health Sciences, Universidad del Valle, Santiago de Cali, Colombia. Directors: Antonio Acosta Hoyos and Luis Menéndez Arias.



#### Other activities

Luis Menéndez Arias is Academic Editor of PLoS ONE, Associate Editor of Frontiers in Virology, and member of the Editorial Boards of Antimicrobial Agents and Chemotherapy, Antiviral Research, Antiviral Therapy, Frontiers in Drug Discovery, Journal of Biological Chemistry, Pharmaceutical Fronts, Viruses and Virus Research. Member of the Global Virus Network (www.gvn.org).



## List of publications

- Martínez del Río, J., López-Carrobles, N., Mendieta-Moreno, J. I., Herrera-Chacón, O., Sánchez-Ibáñez, A., Mendieta, J. and Menéndez-Arias, L. (2023) Charge engineering of the nucleic acid binding cleft of a thermostable HIV-1 reverse transcriptase reveals key interactions and a novel mechanism of RNase H inactivation. J. Mol. Biol. 435, 168219; doi:10.1016/j.jmb.2023.168219
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- PID2019-104176RB-I00: Novel HIV reverse transcriptases with improved biochemical properties for biotechnological applications (2020-2023), Spanish Ministry of Science and Innovation.
- PID2022-136725OB-I00: Novel antiviral candidates targeting the ribonuclease H fold in HIV and other viruses and reverse transcriptase engineering for biotechnology (2023-2026), Spanish Ministry of Science, Innovation and Universities.



## BACTERIAL CELL ENVELOPE DURING PRESEPTAL GROWTH

#### **Group members**

**Principal investigator:**Manuel Pazos Don Pedro

**Predoctoral fellows:** Aitana Belloso Casuso Daniel Ballesteros San José

**Undergraduate and Master students:**Paula de la Fuente de Domingo
Luis Fernando Bermejo López



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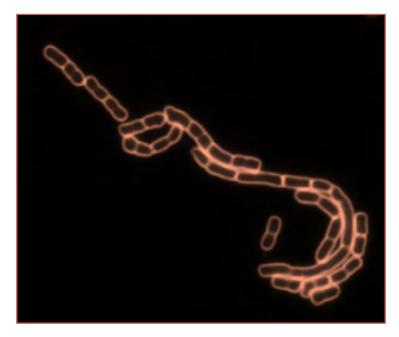


#### Summary

A major and essential structural component of the cell envelope of most bacteria is the peptidoglycan sacculus, and its synthesis is by far the most antibiotic-targeted process. In Gram-negative bacteria, the thin and net-like peptidoglycan layer is surrounded by an asymmetric and hydrophobic lipid bilayer (the outer membrane), which acts as a permeability barrier against external agents like many clinically used antibiotics. During the bacterial cell cycle both peptidoglycan and membrane biogenesis machineries are coordinated and regulated to ensure the robust growth of the cell envelope and the viability of the cell. Contrary to cell elongation and cell division, the transition between both stages called preseptal growth - synthesis of cell envelope at the division site before septation remains poorly characterized.

The research in the laboratory focuses on understanding the molecular mechanisms regulating the biogenesis of the bacterial cell envelope of Gram-negative bacteria, using gastrointestinal pathogenic organisms. Using a multidisciplinary approach combining genetics, biochemistry, cell biology and different microscopy techniques we aim to identify and characterize the protein interactions and their impact on the peptidoglycan enzymatic activities, the mechanical and structural properties of the cell envelope, and the impact on virulence.





We integrate molecular biology and biochemistry to investigate the molecular mechanisms driving bacterial cell envelope biogenesis, particularly at the preseptal stage. Our research aims to uncover how envelope architecture and integrity contribute to the pathogenicity of Gram-negative bacteria.



- PID2022-140818OA-I0. Spanish Ministry of Science and Innovation. Molecular basis of the cell division arrest mediated by SPOR proteins. 01/09/2023-30/08/2026. PI: Manuel Pazos. Role: Principal Investigator.
- 2020-T1/BMD-19970. Comunidad de Madrid. Molecular and therapeutic basis of preseptal growth in gastrointestinal infections. 01/01/2022 31/12/2026. PI: Manuel Pazos. Role: Principal Investigator.



## RNA-BASED CONTROL OF LISTERIA ADAPTATION TO STRESS AND VIRULENCE

#### **Group members**

**Principal investigator:**María Graciela Pucciarelli Morrone

**Predoctoral fellows:**Marcos Peñalver Medina



https://www.cbm.uam.es/mg.pucciarelli



#### Summary

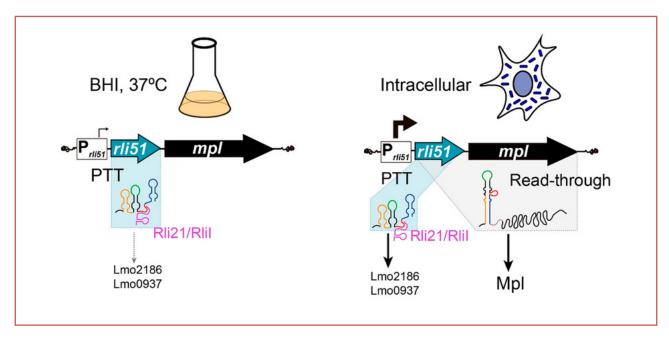
Listeria monocytogenes is an intracellular bacterial pathogen that causes food-borne diseases collectively known as listeriosis, which are severe and mostly affect pregnant women, elderly, and immunocompromised individuals as well as livestock. Our group aims to understand how *L. monocytogenes* tolerates diverse stresses, mainly the adaptation to low temperatures and the crosstalk between the response to stress and virulence. The importance of these studies is denoted by the extraordinary capacity of *L. monocytogenes* to proliferate at refrigeration temperatures (0-4°C) despite being a mesophilic bacterium. This property makes *L. monocytogenes* one of the most difficult pathogens to control by the food industry.

In the last two years, the group has focused on deciphering the mode of action of a small RNA of *L. monocytogenes*, named Rli51, which our previous transcriptomic analyses uncovered as up-regulated when this pathogen grows in a nutrient-rich medium at 4°C. Interestingly, Rli51 maps in the 5'-untranslated region (UTR) of a gene coding for a virulence protein, the metalloprotease Mpl. Our study in Rli51 has revealed that this specific small RNA acts as regulatory element both in cis and trans. Thus, when acting in cis it controls expression of the full mlp transcript folding as transcriptional terminator when it pairs with the Rli21 small RNA. In addition, Rli51 can operate in trans by modulating

expression of iron-scavenger (heme-binding) receptors. Importantly, Rli51 is up-regulated during the intracellular infection, exceeding the levels required to titrate Rli21 and allowing in this manner transcription of the full mlp transcript.

The unique response of Rli51 to low temperature and the cues from the intracellular environment of the host cell prompts us to study in the future more examples of regulatory elements exhibiting similar features. The elucidation of the molecular mechanisms by which these small RNAs control transcription and virulence is therefore one of our major goals for the next future.





Model of the mechanism of regulation mediated by the Listeria monocytogenes sRNA Rli51



- Morón Á, Ortiz-Miravalles L, Peñalver M, García-Del Portillo F, Pucciarelli MG, Ortega AD. (2024) Rli51 Attenuates Transcription of the Listeria Pathogenicity Island 1 Gene mpl and Functions as a trans-Acting sRNA in Intracellular Bacteria. Int J Mol Sci. 25(17):9380. doi: 10.3390/ijms25179380.
- Peñalver M, Paradela A, Palacios-Cuéllar C, Pucciarelli MG, García-Del Portillo F. (2024) Experimental evidence of D-glutamate racemase activity in the uncultivated bacterium Candidatus Saccharimonas aalborgensis. Environ. Microbiol. 26(4):e16621. doi: 10.1111/1462-2920.16621.



## **Doctoral theses**

David López Escarpa (2023). Title: Regulación de proteínas morfogenéticas de Salmonella enterica que unen penicilina y su contribución a la vida intracelular. Departamento Biología Molecular. Universidad Autonoma de Madrid (UAM). Directors: M. Graciela Pucciarelli & Francisco García del Portillo



## VIRUS-HOST INTERACTIONS: THE ASFV MODEL

#### **Group members**

Principal investigator: Yolanda Revilla Novella

Scientific staff: Daniel Pérez Núñez

Postdoctoral fellows: Roberta Piredda Raquel García Belmonte Elena Riera Laguna

Predoctoral fellows: Raquel García Belmonte Elena Riera Laguna Gonzalo Vigara Astillero Julia Gata de Benito

**Technicians:**Carmen García Valdepeñas
Cecilia Flores



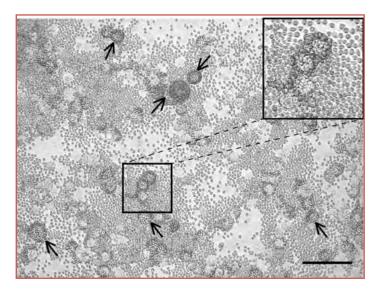


#### Summary

The laboratory is led by Dr Yolanda Revilla and studies the viral and cellular mechanisms occurring during ASFV infection, along with the interaction of the virus and the host immune system, in a further attempt to develop safe and effective vaccines. ASFV infects domestic pigs and wild boar and is currently the great animal pandemic worldwide, being the biggest problem in the global pig industry as well as responsible for a major ecological disaster. The natural target cell of this virus is exclusively porcine monocytes and macrophages, consequently the viral and cellular factors that determine ASFV tropism are a focus of attention in our laboratory. Another relevant, basic objective, is the molecular mechanisms involved in ASFV virulence, directly related to the control that the virus exerts on different branches of the immune response, in particular, on the innate response by regulating type I IFN, as we have firstly described in the group. To this end, we study virus-host interaction using cell biology, biochemical, microscopy and cytometry techniques, together with the generation of recombinant viruses using CRISPR-Cas9 technology as well as their genetic characterization using NGS techniques such as Illumina and Nanopore.

Last but not least, the group is generating vaccine candidates against ASFV, based on (i) fully safe live attenuated vaccines (LAV) and (ii) vaccines prototypes only partially replicative in the vaccinated animal, which would be a world first in the field and would represent a breakthrough in terms of safety and commercialization of our prototypes. To achieve this, our group collaborates, under the umbrella of several European and international projects with other leading groups in the field, and with the specialised commercial sector.





ASFV-infected cells surrounded by erythrocytes forming "rosettes" (indicated by arrows, magnification in inset). This phenomenon is known as "hamadsorption" and is linked to virulence in natural ASFV strains.



### List of publications

- Roberta Piredda 1 , Luis G Rodríguez Martínez 2 , Konstantinos Stamatakis 1 3 , Jorge Martinez-Ortega 1 , Alejandro López Ferráz 4 , José M Almendral 5 , Yolanda Revilla. Assessment of molecular modulation by multifrequency electromagnetic pulses to preferably eradicate tumorigenic cells. Sci Rep. 2024 Dec 3;14(1):30150. doi: 10.1038/s41598-024-81171-x.
- Sunwoo, Sun-Young; Garcia-Belmonte, Raquel; Walczak, Marek; et al; Perez-Nunez, Daniel. 2024. Deletion of MGF505-2R Gene Activates the cGAS-STING Pathway Leading to Attenuation and Protection against Virulent African Swine Fever Virus. VACCINES. MDPI. 12. WOS (3) https://doi.org/10.3390/vaccines12040407
- Perez-Nunez, Daniel; Madden, Daniel W.; Vigara-Astillero, Gonzalo; et al; Richt, Juergen A.2024. Generation and Genetic Stability of a PolX and 5' MGF-Deficient African Swine Fever Virus Mutant for Vaccine Development. VACCINES. MDPI. 12. WOS (0) https://doi.org/10.3390/vaccines12101125
- Daniel Pérez Núñez; Raquel García Belmonte; Elena Riera; Marta Hernadez Fernadez-Sesma; Gonzalo Vigara Astillero; Yolanda Revilla. 2023. Signal peptide and N-glycosylation of N-terminal-CD2v determine the hemadsorption of African swine fever virus. Journal of Virology. ASM Journal. pp.1-35.
- Elena Riera Laguna; Raquel García Belmonte; Ricardo Madrid; et al; Yolanda Revilla Novella. 2023. African swine fever virus ubiquitin-conjugating enzyme pI215L inhibits IFN-I signaling pathway through STAT2 degradation. Frontiers Microbiology. PMID: 36713190. Jan 13;-1081035, pp.doi:10.3389/fmicb.2022.1081035.



#### **Patents**

Attenuated African swine fever virus and uses thereof. Yolanda Revilla and Daniel Pérez Núñez. PCT/ES2022/070527.. Spain. 5/08/2022-2025. CSIC / Careside (South Korea).



## Research projects

- PID2020-117300RB-I00. AEI. Modulation of the innate response by the african swine fever virus (ASFV) in relation to vaccine development. PI: Yolanda Revilla Novella. 2020-2023.
- PCI2020-120684-2. International coordination of research on infectious animal diseases ERANET (ICRAD)/ (PCI). "Characterization of virus and host-specific modulation of type I IFN in African Swine Fever Virus virulence or attenuation. IFNASF. Yolanda Revilla Novella PI, Coordinator. 2021-2024.
- 3- PID2023-1515910B-I00, AEI. Molecular markers of ASFV virulence with a view to vaccine development. PI: Yolanda Revilla. 2024-2027.
- HORIZON-CL6-2023-FARM2FORK-01. Commission of the European Union. Topic: HORIZON-CL6-2023-FARM2FORK-01-5 Type of Action: HORIZON-RIA Proposal number: 101136439. Proposal acronym: VAX4ASF Tittle: "New technologies for African swine fever vaccines". 1-APRIL 2024-30 DECEMBER 2027. Yolanda Revilla, IP of CSIC Team and Co-Coordinator.
- CPP2022-009531. Ministerio de Ciencia e Innovación. Agencia Estatal de Investigación. New strategies to improve vaccines against VPPA. PI: Yolanda Revilla. April 2023-April 2026.



#### **Doctoral theses**

Elena Riera Laguna, (2023) Tittle: Implicación de los genes virales I215L, A238L y EP402R del Virus de la Peste Porcina Africana en la modulación de la respuesta inmune innata. Programa de Doctorado en Biociencias Moleculares por la Universidad Autónoma de Madrid (UAM). Directores de la Tesis: Yolanda Revilla Novella y Ricardo Madrid González



## BIOTECHNOLOGY OF PROBIOTICS AND INTESTINAL PATHOGENS

#### **Group members**

**Principal investigator:**David Ruano Gallego

**Technicians:** Laura Gómez Ruiz

Undergraduate and Master students: Sol Gabriel y Galán Maroto Inés Ochoa Arizu Marina Bueno Alonso





#### Summary

The intestine plays a crucial role in our body, not only in absorbing nutrients but also in hosting a complex network of interactions involving intestinal enterocytes, microbiome bacteria, and the immune system. These elements work in a delicate balance, ensuring both proper digestion and protection against harmful pathogens. However, any disruption in this balance due to external factors such as infections, diet, or genetic predispositions can cause diseases such as inflammatory bowel disease (IBD), infections, or even colorectal cancer.

In our research laboratory, we focus on studying how pathogenic bacteria utilize a specialized mechanism called the Type 3 Secretion System (T3SS) to infect the intestine and evade the immune system's defenses. It is a sophisticated molecular syringe that injects specialized proteins, known as effectors, directly into host cells. The effectors play a crucial role in manipulating host cellular processes, allowing bacteria to establish infection, subvert immune responses, and exploit host metabolism for their survival. The network of interactions established by the effectors is critical for the infection process, as they enable bacteria to outcompete beneficial microbes and gain a foothold in the intestinal environment.

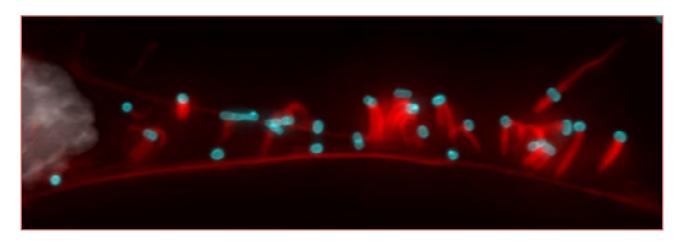
Our goal is to unravel this network of protein interactions to gain a better understanding of how infections occur and how the immune system responds to different infection strategies. To

achieve this, we employ a combination of genetic, biochemical, and imaging techniques. We create mutant strains of these pathogenic bacteria, each lacking specific effector proteins, to determine their role in infection. By using these mutants to infect mice with different genetic backgrounds and observing their effects on the intestine, we can gain valuable insights into the mechanisms underlying infection and immunity, shedding light on how effectors influence disease progression.

Additionally, we aim to harness the power of probiotics for biotechnological applications in the field of biomedicine. Probiotics, which are beneficial bacteria that support gut health, have shown immense promise in preventing and treating intestinal diseases. Using advanced techniques from synthetic biology, we intend to develop methods for engineering bacterial strains to improve resistance intestinal pathogens, disrupt their colonization and enhance immune responses. This could pave the way for the development of nextgeneration probiotics that act as living therapeutics, capable of sensing and responding to disease signals in real-time.

We are particularly interested in E. coli Nissle 1917, a strain that is amenable for genetic manipulation and considered safe. Endowing this strain with new capabilities to better compete with pathogens and other harmful bacteria will contribute to generating alternative treatments to antibiotics.





Effect of the infection of Citrobacter rodentium on actin cytoskeleton



Mustieles-Del-Ser P, Ruano-Gallego D, Parro V. (2024) Immunoanalytical Detection of Conserved Peptides: Refining the Universe of Biomarker Targets in Planetary Exploration. Anal Chem. 96(12):4764-4773. doi: 10.1021/ acs.analchem.3c04165



- Intramural project. Centro de Biología Molecular Severo Ochoa. Role of unconventional forms of autophagy in the infection of Citrobacter rodentium. David Ruano Gallego. 2024-2026.
- PID2022-138782OA-I00. Ministerio de Ciencia e Innovación. Deciphering Type 3 Secretion System Effector Networks (DEFFNET). David Ruano Gallego. 2023-2026.
- RYC2021-031342-I. Ministerio de Ciencia e Innovación. Type III Secretion System effector networks: artificial intelligence applied to study intestinal bacterial infections. David Ruano Gallego. 2023-2028.



# MODULATION OF ANTIVIRAL IMMUNITY BY VIRAL PROTEASES AND NONCODING RNAS

#### **Group members**

**Principal investigator:** Margarita Sáiz Zalabardo

Postdoctoral fellows: Miguel Rodríguez Pulido Catalina Grabowski Miguel Ángel Sanz Hernández

**Predoctoral fellows:** Miryam Polo Hernández

**Undergraduate and Master students:** Laura Priego González



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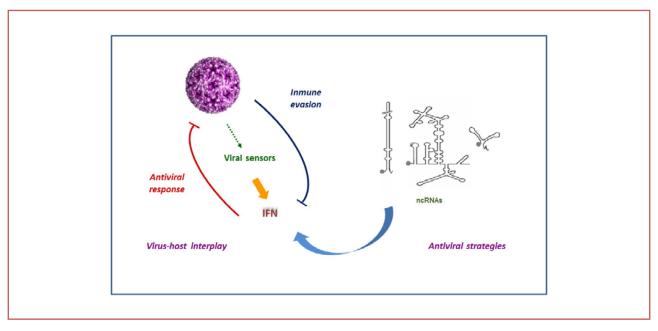


#### Summary

Our research interest is focused on the study of the interactions between RNA viruses and host cells involving the non-coding regions (NCR) of the viral genomes and the catalytic activity of the viral proteases. Our studies include the identification of proteins sensing infection and viral RNA genomes, as well as the characterization of the immune evasion molecular 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. We use foot-and-mouth disease virus (FMDV), a highly infectious and worldwide distributed pathogen, as a model and we are actively working on the identification of host innate effector proteins which are targets for the proteolytic activity of the two virally encoded proteases (Lpro and 3Cpro) for interference with different signaling routes known to respond to infection by RNA viruses. We have reported the redundant degradation of the DNA immune sensor cGAS by 3Cpro and Lpro adding a new viral target to the host proteins involved in antiviral immunity degraded during infection. We had previously shown the Lpro- dependent cleavage of the RNA immune sensors LGP2 and MDA5. These interactions have a negative impact on antiviral immunity promoting, in turn, viral infection. We also work in the characterization of

the structural domains in the FMDV 5' and 3'NCR of the FMDV genome, which are involved in the viral cycle and in the immune response elicited. Our previous studies on the FMDV NCRs have led us to test the biotherapeutic applications of synthetic non-coding RNAs derived from the FMDV 5'and 3'NCR (ncRNAs) and we have shown their ability to induce a broad spectrum antiviral activity against infection by pathogens of different viral families including relevant zoonotic viruses (FMDV, West Nile virus, Rift Valley fever virus, African swine fever virus). The pan-coronaviruses antiviral activity of these ncRNAs has been recently addressed showing the intrinsic prophylactic activity of naked RNA against SARS-CoV-2 in a COVID-19 mouse model. We are also working on the development of new nanotechnology-based formulations for the ncRNAs delivery. 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 current and newly emerging viruses. 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 against type I interferonopathies.





Schematic representation of virus host-interplay and biotherapeutic approach based on the FMDV noncoding RNAs



#### List of publications

- Sanz, M.Á, Polo, M, Rodríguez-Pulido, M., Huildore Bommanna, R. and Sáiz, M. (2024) The antiviral response triggered by the cGAS/STING pathway is subverted by the foot-and-mouth disease virus proteases. Cell. Mol. Life Sci. 81(1):148. doi: 10.1007/s00018-024-05190-7
- de León, P., Cañas-Arranz, R., Bustos, M.J., Sáiz, M. and Sobrino, F. (2023) Inhibition of Human Coronaviruses by Combinations of Host-Targeted and Direct-Acting Antivirals. Antimicrob. Agents Chemother. 67(4):e0170322. doi: 10.1128/aac.01703-22
- Rodríguez-Pulido, M., Calvo-Pinilla, E., Polo, M., Saiz, J.C., Fernández-González, R., Pericuesta, E., Gutiérrez-Adán, A., Sobrino, F., Martín-Acebes, M.A. and Saiz, M. (2023) Non-coding RNAs derived from the foot-and-mouth disease virus genome trigger broad antiviral activity against coronaviruses. Front. Immunol. 11:718. doi: 10.1038/s41419-020-02931-x



#### Research projects

- PID2020-113184RB-C21 (MICINN) (2021-2024) Peptide vaccines, antiviral strategies and viral immune evasion mechanisms. Biotherapeutic applications. PIs: F. Sobrino and M. Sáiz.
- SGL2103051 (PTI+, CSIC) (2022-2023). Subgroup "Target-based biological models". Protection range against SARS-CoV-2 of different antiviral molecules. Analysis in mice and against new variants. PIs: M Sáiz and F. Sobrino.
- PID2023-148273NB-I00. (2024-26) Functional study of virus-targeted RBPs involved in translation control, antiviral immunity and disease. New antiviral developments PIs: M Sáiz and E. Martínez-Salas.
- P2018/BAA437 (CAM) (2019-2023). Platform for development of control strategies in animal health. Partners: INIA (N Sevilla, PI), CBMSO (F Sobrino, PI), UAM, UCM, UAH.



#### Other activities

Associate editor of Frontiers in Molecular and Infection Microbiology-Virus and Host section (since 2018). Participation as proffesor in the Virology Master (Universidad Complutense de Madrid) subject "Applications of Virology" academic years 2022-2023 and 2023-2024.



## NEW STRATEGIES FOR PREVENTION AND CONTROL OF VIRAL DISEASES: FOOT-AND-MOUTH DISEASE VIRUS AS A MODEL

#### **Group members**

**Principal investigator:** Francisco Sobrino Castelló

**Postdoctoral fellows:** Patricia de León Valdés

**Technicians:** Maria José Bustos

**Visiting scientists:** Rebecca Hernández Antolín (Laboratorio Subterráneo de Canfranc)



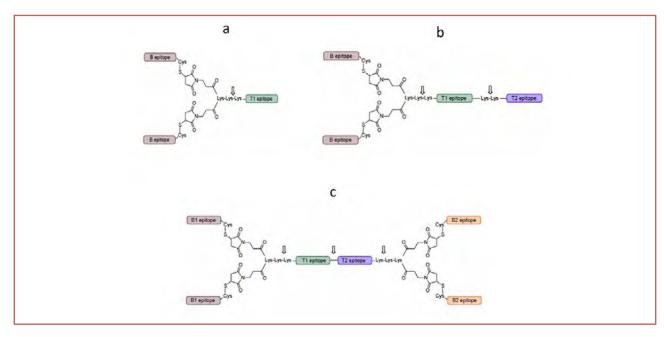
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#### **Summary**

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 with clear connections with the "One Health" approach. 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 different arrangements of FMDV Band T- cell epitopes to elicit efficient T-cell specific antibody responses as to improve feasible FMD field vaccines. This vaccine approach can be extended to other pathogens provided continuous B- cell epitopes and efficient T-cell epitopes are identified. As part of the analysis of the functional role of FMDV proteins on the viral cycle and the mechanisms mediating the pathogenesis of FMDV and other related viruses causing vesicular diseases -- such as swine vesicular disease virus (SVDV), vesicular stomatitis virus (VSV)--, we have characterized the inhibitory effect of antivirals targeting cellular metabolism such as valproic acid (VPA), lauryl gallate (LG) and cerulenin (CRL) on the multiplication of FMDV, SVDV, as well as different enveloped viruses, like African swine fever virus (ASFV), VSV, type I herpesvirus and human coronaviruses, including SARS-CoV-2. The expertise of our group is being applied to address the antiviral synergy against different coronaviruses, and eventually other emerging viruses such as monkeypox virus, of combinations of broad antiviral spectrum host-targeted compounds such as VPA or LG and direct acting antivirals (i.e. remdesivir). In parallel, we collaborate with R. Amils (CBMSO) and other groups in the characterization of the sources of carbon and energy used by bacteria found to be metabolically active and in highly pure heavy water, and learning how these microorganisms can adapt to this extremely detrimental environment.





Dendrimer peptide vaccines: a modular approach. Bivalent B-cell epitope immunogens (B2T) conjugated to one (a) or two (b) T-cell epitopes. (c) Tail-to-tail fusion of two B2T molecules (Click chemistry), leading to a B2T-TB2 multivalent platform.



#### List of publications

- de León, P., Cañas-Arranz, R., Bustos, M.J. Sáiz, M. and Sobrino, F. Inhibition of human coronaviruses by combinations of host-targeted and direct-acting antivirals. Antimicrob. Agents Chemother. 8;67(4):e0170322. doi. org/10.1128/aac.01703-22 (2023).
- Rodríguez-Pulido, M., Calvo-Pinilla, E., Polo, M., Saiz, J.C., Gutiérrez-Adán, A., Sobrino, F., Martín-Acebes, M.A. and Sáiz, M. Non-coding RNAs derived from a viral genome trigger broad antiviral activity against coronaviruses. Front. Immunol. 14:1166725. doi: 10.3389/fimmu.2023.1166725 (2023).
- Ariza-Mateos, A., Briones, C., Perales, C., Sobrino, F., Domingo, E. and Gómez, J. Archaeological approach to virus evolution. J. Phys. 602.11 2469–2478 (2024).
- Ariza-Mateos, A., Briones, C., Perales, C., Sobrino, F., Domingo, E. and Gómez, J. Natural languages, molecular biology and RNA virus evolution. J. Phys. 602.11 2565–2580 (2024).



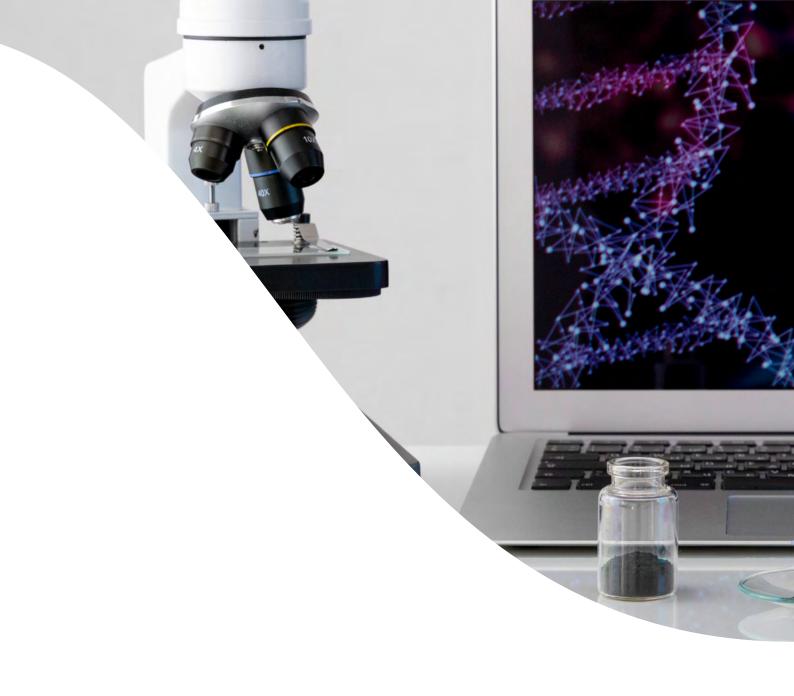
#### **Patents**

Lauryl gallate for use as antiviral agent. de León, A. L. Carrascosa, M.J. Bustos, F. Sobrino. E. Torres, M. Sáiz, R. Cañas EP21382535.9. Date of presentation: 17-06-2021. Abandoned: 8-2023.



#### Research projects

- PID2020-113184RB-C2 (AEI-MCI) 1. Project coordinated with Pompeu Fabra University (D. Andreu). Peptide vaccines, antiviral strategies, and immune evasion mechanisms. Biotherapeutic applications. F. Sobrino Coordinator (2021-23).
- CAM S2018/BAA-4370. Platform for the development of animal health control strategies (PLATESA2-CM) Participating entities: INIA, CSIC (CBM), UCM, UAM. Principal investigator for the NESTRANVIR subproject (CBM): F. Sobrino. (2019-2022). 2019-2022. (CBM subproject). (2019-23).
- SGL2103051. PTI+, CSIC. Group funded within the subproject "Target-based biological models based on targets". Spectrum of protection against SARS-CoV-2 of different antiviral molecules. Analysis in mice and against new variants. M Sáiz and F. Sobrino. 2022-23.
- EOI-38-2022. Potential energy sources used by viable bacteria in pure D2O. Canfranc Underground Laboratory (LSC). F. Sobrino Coordinator (2022-25).
- Exchange grant within PIE 202120E047- Conexiones-Life or LifeHUB.CSIC. LifeHUB3\_06. F. Sobrino Coordinator (2024).
- PID2023-148273NB-I00. (2024-26) Functional studies of RBPs involved in translational control, antiviral immunity and pathology. New antiviral developments. PIs: M Sáiz and E. Martínez-Salas.



# Scientific facilities





#### ADVANCED LIGHT MICROSCOPY FACILITY

#### **Group Members**

**Technical director:** Álvaro Sahún Español

**Scientific supervisor:** Francisco Javier Díez Guerra

Staff:

Carmen Sánchez Jiménez Elena Calvo Cazalilla Carlos Gallego García Maite Villalba Villacorta





#### Description

The Advanced Light Microscopy Facility (SMOA) is a core research support platform at the Centro de Biolog'iaMolecular Severo Ochoa, offering access to advanced optical microscopy technologies. Its instrumentation includes four widefield microscopes, seven laser scanning confocal microscopes—one featuring STED super-resolution and FLIM capabilities—and a spinning disk fluorescence microscope equipped with dual cameras and a high-resolution SoRa disk. The facility is also equipped for sample preparation, with two stereomicroscopes and a vibratome, and provides a wide range of microscopy-related reagents, such as primary and secondary antibodies, fluorescent dyes, mounting media, and imaging chambers. Highperformance computing systems are available for image processing and analysis.

SMOA's technical staff is responsible for the maintenance and operation of all microscopy and computational resources. They offer comprehensive support to researchers, including expert guidance, troubleshooting, and training through theoretical and practical courses, workshops, and seminars on microscopy techniques.

Recognized as a reference center by Evident/ Olympus, SMOA is integrated into national networks such as the Spanish Network for Advanced Optical Microscopy (REMOA) and the Red de Laboratorios e Infraestructuras (RedLab). The facility also holds ISO 9001:2015 (AENOR) certification, ensuring compliance with international standards in quality management.



#### CBM courses:

■ Good practices in optical microscopy

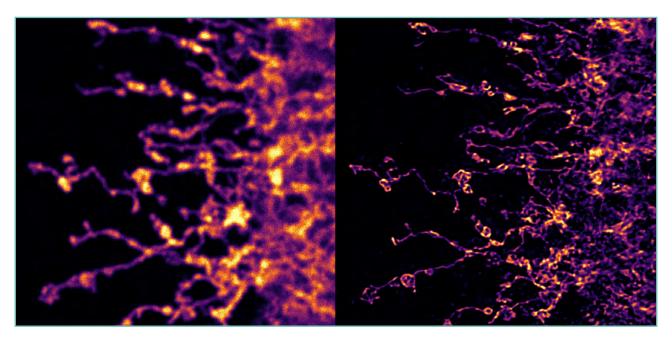
#### Visits:

- Spanish Biology Olympiad
- Latin-American Biology Olympiad
- 4°ESO+Empresa programme
- Cientific@s en prácticas programme
- Monthly scholar visits

#### **Organiced conferences:**

- Spanish & Portuguese Advanced Optical Microscopy Meeting 2024 (SPAOM24) Toledo (Spain), 20-22 November 2024 (Organiser: Alvaro Sahún).
- 1st Junior Group Workshop for the European Society for Virology (ESV): Imaging Viruses Madrid (Spain), 13-14. February 2025 (Organiser: Carlos Gallego).





Cytonemes in the imaginal wing disc of Drosophila melanogaster are emitted from Hedgehog-producing cells to receptor cells. Cytonemes are labelled through Ihog protein fused with RFP fluorescent protein. On the left, confocal microscopy; on the right, STED super-resolution microscopy.

Sample preparation by Clara Fernandez (Isabel Guerrero's Lab). Image acquisition by Carmen Sánchez (SMOA).



- Preventive and corrective maintenance of equipment to ensure optimal performance.
- Scientific and **technical assistance** (experimental design, sample preparation, image acquisition, image visualization and analysis and results publication).
- Training.
- Organization of seminars and tutorials, as well as the development of theoretical and practical guides.
- Storage and distribution of common reagents and consumables.
- Control of access to the service and the use of its resources.
- Management of billing and reservations.
- Regular maintenance and updates of the website.
- Assess, evaluate, and discuss researchers' needs and advancements in optical microscopy to ensure cuttingedge scientific capabilities.
- Collaborate and negotiate with commercial companies to explore their offerings and maximize the benefits of their systems, capabilities and expertise.
- Secure funding to facilitate necessary and timely upgrades.



#### **ANIMAL FACILITY**

#### **Group Members**

**Technical director:**Blanca Estévez Santiago

**Scientific supervisor:** María Mittelbrum Herrero

#### Staff:

Miguel A. Bordallo Martín-Fontecha Alfonso Gutiérrez García Marta González Mella José María Sedano Torres Laura López Martínez Beatriz García Martínez Virginia González de la Torre José Ignacio Herrero Lahuerta Isidro García Gómez María Soledad López Martín Haisul CY Chang Hsiao





#### Description

This is a modern facility, inaugurated in January 2011, covering approximately 1,500 m². It was designed and equipped with the latest advances in laboratory animal housing and care. The facility breeds and maintains mice (mostly transgenic), frogs, and fish.

The animal facility is divided into two main areas:

- An SPF (Specific Pathogen Free) zone dedicated to the breeding and maintenance of mouse lines, where new lines are introduced exclusively via embryo rederivation, thanks to the Reproductive Biotechnology Service.
- An experimental zone that includes rooms classified as BSL1, BSL2, and BSL3 (Biosafety Levels 1, 2, and 3).

There are dedicated rooms for behavioral studies both in the SPF area and in BSL1 and BSL2.

The facility works in close collaboration with the Preclinical Biomedical Services—housed within the animal facility—as well as with the Biosafety Service, which enables the irradiation of mice.

All mice are housed in individually ventilated cages (IVCs) placed on ventilated racks, and are handled inside dedicated Biological Safety Cabinets using forceps to prevent contamination. Fish (zebrafish—many of them genetically modified—and medaka fish) and frogs (Xenopus laevis) are kept in dedicated aquatic systems specifically designed for their maintenance.

To support the maintenance and handling of mice, the facility is equipped with more than 50 ventilated racks, 18 Biological Safety Cabinets, 5 changing stations, 2 gas exposure cabinets, 6 anesthesia units, microsurgical magnifiers, and other standard laboratory equipment.

The designated veterinarian has a dedicated pharmacy for administering drugs necessary to support research activities, promote successful experimental outcomes, and ensure animal welfare at all times.

For facility maintenance, there is a dedicated technical area housing HVAC systems and other equipment required to maintain stable environmental conditions.





Culture of Care: commitment to improving animal welfare, scientific quality, care of the staff and transparency for the stakeholders.

In the washing area—where all materials are cleaned and processed—the facility is equipped with a rack washer, a tunnel washer, a bedding aspiration system, a bottle washer and filler, and a water treatment unit. Two double-door autoclaves and a hydrogen peroxide pass-through chamber (SAS) are available for material entry into the SPF zone. The BSL3 area of the facility has an additional 2,000-liter double-door autoclave and two Security Airlocks (SAS). One of these connects directly to the BSL3 Cell Culture facility, a rare and highly valuable feature for conducting certain types of studies.

Access to the facility and its various rooms is controlled via magnetic key cards, ensuring that users can only enter authorized zones. Access to biocontainment areas also requires approval from the Center's Biosafety Service. The center is fully authorized to work with genetically modified organisms at Biosafety Levels I and II.



#### Services

#### Internal Services Provided by the Animal Facility:

- Breeding and maintenance of mice under SPF conditions, conventional housing, and Biosafety Levels 2 and 3.
- Management of transgenic mouse colonies.
- Breeding and maintenance of zebrafish and medaka fish.
- Technical support for researchers in the execution of various experimental procedures.
- Technical and ethical advisory services.
- Coordination of national and international animal shipments and arrivals.
- Veterinary supervision and oversight.
- Health monitoring of housed animals, conducted through accredited external services.

- Reproductive biotechnology services.
- Transgenesis service in collaboration with the CNB (National Center for Biotechnology).

All activities are carried out in strict compliance with current regulations, with particular attention to animal health and welfare, as well as the training and qualification of personnel involved in animal care and handling.

#### Courses

- 4°ESO+Empresa programme
- Científic@s en practicas programme
- Spanish Biology Olympiad



#### BIOCOMPUTATIONAL ANALYSIS FACILITY

#### **Group Members**

**Technical director:** Sandra González de la Fuente

Scientific supervisor: Begoña Aguado (until november 2023 in the Genomics and NGS Core Facility and later in the Biocomputational Analysis Core Facility).

#### Staff:

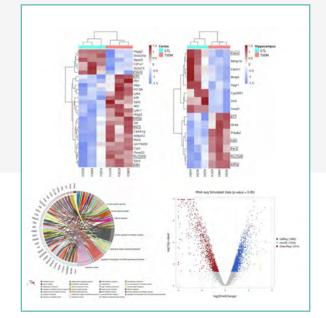
Sandra González de la Fuente (until november 2023, as technical supervisor of the NGS section of the Genomics and NGS Core Facility, and since then, of the Biocomputational Analysis Core Facility) Paula Martínez García (until november 2023 in the Genomics and **NGS Core Facility** and since then in the Biocomputational Analysis Core Facility)

Description

Iván Lorca Alonso (until november 2023 in the Genomics and NGS Core Facility and since then in the Biocomputational Analysis Facility) Maria Santos Galindo (until november 2023 in the Genomics and NGS Core Facility and since then in the **Biocomputational Analysis** Facility) Maria Santos Romero (since 20/11/24) Laura Tabera Moreno (until november 2023, as technical supervisor of the Genomics section of the Genomics and NGS Core Facility) Maria Gabriela Atencia Cibreiro (until november 2023 as part of the Genomics and NGS Core



CBM 2023-2024



Example of personalized graphics generated in the Biocomputational Analysis Core Facility

#### In November 2023, the Genomics and NGS Core Facility was divided into two independent facilities: The Genomics Core Facility and the Biocomputational Analysis Core Facility.

Facility)

The Biocomputational Analysis Service (SABio) is specialized in providing state-of-the-art solutions for computational analysis of massive data, covering a wide range of technologies and applications. From meticulous selection of preprocessing and analysis methods to custom software development, our expert team is qualified to handle and integrate all types of multi-omics data. We offer:

- Technical scientific advice, experimental design, training to research groups with data analysis needs.
- Multiomics data analysis: massive sequencing (NGS), proteomics, metabolomics, etc.; omics integration, specific software development and biostatistical data analysis.
- Secure and confidential management of raw data acting as collaborators ("brokers") of the European ENA repository (EBI-EMBL).
- Support to users in accessing CSIC scientific computing resources (NodoBio CCC, DRAGO).

Depending on the objectives, the technology used for data generation, types of samples and sample size and study organizations among others, we design the best method of data processing.



CBM 2023-2024 Scientific facilities





#### List of publications

- Aguado, B., Begeman, L., Günther, A., Iervolino, M., Soto, F., Vanstreels, R. E. T., Reade, A., Coerper, A., Wallis, B., Alcamí, A., & Dewar, M. (2024). Searching for high pathogenicity avian influenza virus in Antarctica. Nature microbiology, 9(12), 3081–3083. https://doi.org/10.1038/s41564-024-01868-7
- Adán-Jiménez, J., Sánchez-Salvador, A., Morato, E., Solana, J. C., Aguado, B., & Requena, J. M. (2024). A Proteogenomic Approach to Unravel New Proteins Encoded in the Leishmania donovani (HU3) Genome. Genes, 15(6), 775. https://doi.org/10.3390/genes15060775
- Torres-Sánchez, E., Lorca-Alonso, I., González-de la Fuente, S., Hernández-Ledesma, B., & Gutiérrez, L. F. (2024). Antioxidant Peptides from Sacha Inchi Meal: An In Vitro, Ex Vivo, and In Silico Approach. Foods (Basel, Switzerland), 13(23), 3924. https://doi.org/10.3390/foods13233924
- Oggerin, M., Del Moral, C., Rodriguez, N., Fernandez-Gonzalez, N., Martínez, J. M., Lorca, I., & Amils, R. (2024). Metal tolerance of Río Tinto fungi. Frontiers in fungal biology, 5, 1446674. https://doi.org/10.3389/ffunb.2024.1446674
- Carús-Cadavieco, M., González de la Fuente, S., Berenguer López, I., Serrano-Lope, M. A., Aguado, B., Guix, F., Palomer, E., & Dotti, C. G. (2024). Loss of Cldn5 -and increase in Irf7-in the hippocampus and cerebral cortex of diabetic mice at the early symptomatic stage. Nutrition & diabetes, 14(1), 64. https://doi.org/10.1038/s41387-024-00325-y
- Cacheiro-Llaguno, C., Mösges, R., Calzada, D., Gonzálezde la Fuente, S., Quintero, E., & Carnés, J. (2024). Polysensitisation is associated with more severe symptoms: The reality of patients with allergy. Clinical and experimental allergy: journal of the British Society for Allergy and Clinical Immunology, 54(8), 607–620. https://doi.org/10.1111/cea.14486
- González-de la Fuente, S., Requena, J. M., & Aguado, B. (2023). Datasets of Iso-Seq transcripts for decoding transcriptome complexity in four Leishmania species. Data in brief, 52, 109838. https://doi.org/10.1016/j.dib.2023.109838
- Sánchez-Salvador, A., González-de la Fuente, S., Aguado, B., Yates, P. A., & Requena, J. M. (2023). Refinement of Leishmania donovani Genome Annotations in the Light of Ribosome-Protected mRNAs Fragments (Ribo-Seq Data). Genes, 14(8), 1637. https://doi.org/10.3390/genes14081637
- Carús-Cadavieco, M., Berenguer López, I., Montoro Canelo, A., Serrano-Lope, M. A., González-de la Fuente, S., Aguado, B., Fernández-Rodrigo, A., Saido, T. C., Frank García, A., Venero, C., Esteban, J. A., Guix, F., & Dotti, C. G. (2023). Cognitive decline in diabetic mice predisposed to Alzheimer's disease is greater than in wild type. Life science alliance, 6(6), e202201789. https://doi.org/10.26508/lsa.202201789
- Camacho, E., González-de la Fuente, S., Solana, J. C., Tabera, L., Carrasco-Ramiro, F., Aguado, B., & Requena, J. M. (2023). Leishmania infantum (JPCM5) Transcriptome, Gene Models and Resources for an Active Curation of Gene Annotations. Genes, 14(4), 866. https://doi.org/10.3390/genes14040866



- Selection of Preprocessing and Analysis Methods: We identify the tools and methods of preprocessing and data analysis suitable for the type of sequencing.
- Quality Control Planning: We define strategies for quality control of sequencing data, such as removal of low quality sequences, artifact detection and coverage assessment.
- Computational analysis of massive sequencing (NGS) data: re-sequencing (DNA-seq), de novo assembly, RNA-seq, ChIP-seq, ATAC-seq, CLIP-seq, Exome-seq, Ribo-seq, IsoSeq, Single-cell, metabolomics, 16S, 18S, ITS and other amplicon and shotgun metagenomics. Pathway enrichment analysis, search for germline or somatic variants, SNP/InDel, CNV and structural variants, microarrays, etc. Graphical representation of results in a customized way according to user requirements.
- Computational analysis of proteomics and metabolomics data: Multivariate statistical analysis of proteomics and metabolomics data, principal component analysis (PCA) or linear discrimination analysis (LDA) for clustering of samples depending on the expression of certain proteins/ metabolites. Mapping of proteins/metabolites to known metabolic pathways to analyze differential profiles. Graphical representation of results in a customized way according to user requirements.
- Multiomics data integration analysis: Combination of NGS omics data such as genomics, transcriptomics, etc. with metabolomics and proteomics data for complete understanding of biological processes. Machine learning analysis to build predictive models. Graphical representation of results in a customized way according to user requirements.
- Development, implementation and creation of customized software for data analysis: Programming in different languages according to the needs of the Service (Phyton, R, Bash, Tcsh, etc.) to develop ad hoc scripts.
- Statistical analysis of experimental data: exploratory analysis, hypothesis testing with parametric and non-parametric methods, robust statistical methods, ANOVA, principal component analysis (PCA), data mining (machine learning), ROC curves, etc. Graphical representation of results in a customized way according to user requirements.
- Sending sequences to European repositories, specifically to the European Nucleotide Archive (ENA) and European Genome-phenome Archive (EGA) belonging to the European Bioinformatics Institute (EBI). It is a secure and confidential way of maintaining the data, in addition to being an essential requirement when publishing the results in scientific journals. As collaborating users of ENA (Brokers), we take care of the maintenance of the data, as well as renewing the privacy of the data.



- Specialized courses tailored to the needs of the users.
- Gabinete de formación del CSIC: Análisis de datos de NGS 2023
- Gabinete de formación del CSIC: Análisis de datos de NGS 2024 (First edition)
- Gabinete de formación del CSIC: Análisis de datos de NGS 2024 (Second edition)



#### **BIOINFORMATICS FACILITY**

#### **Group Members**

**Technical director:** David Abia Holgado

Scientific supervisor: Ugo Bastolla Bufalini

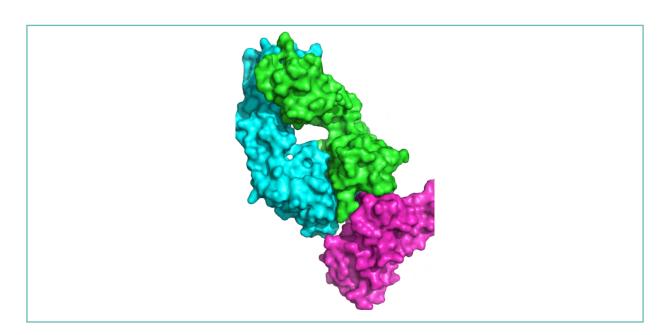




#### Description

The mission of the Bioinformatics Facility is to support research groups of the CBMSO (and occasionally other centres) in the bioinformatics analysis of their data, to orient their research through computational predictions and to advice on the computational aspects of their projects. The personnel in the Facility teach at master's courses and participate in bioinformatics training of master students. Nowadays, bioinformatics analyses and predictions must complement experiments in order to rationalize their results and to maximize the knowledge that they provide. This is particularly true for high throughput experiments, whose bioinformatics analysis is necessary for extracting the information of interest. In addition, in silico predictions may help optimizing the design of the experiments. We perform these analyses and predictions through computer programs that are publicly available or we develop specific programs to tackle specific problems, and we run the computations in the computing infrastructure of the Bioinformatics Facility, which consists of a high performance computing cluster, with infiniband connection, a shared parallel file system and computational nodes with up to 512GB of memory and 60 CPU cores.







#### List of publications

The bioinformatics service staff has participated in 12 scientific publications between 2021-2022. Below are some of the most relevant ones.

- Bastolla, Ugo; Chambers, Patrick; Abia, David; Garcia-Bermejo, Maria-Laura; Fresno, Manuel; (2022) Is Covid-19 severity associated with ACE2 degradation? Frontiers in Drug Discovery Vol 1, page 789710
- Horndler, Lydia; Delgado, Pilar; Abia, David; Balabanov, Ivaylo; Martínez-Fleta, Pedro; Cornish, Georgina; Llamas, Miguel A; Serrano-Villar, Sergio; Sánchez-Madrid, Francisco; Fresno, Manuel; (2021) Flow cytometry multiplexed method for the detection of neutralizing human antibodies to the native SARS-CoV-2 spike protein. EMBO molecular medicine Vol 13, Num 3, page e13549
- Erkelens, Amanda M; Qin, Liang; van Erp, Bert; Miguel-Arribas, Andrés; Abia, David; Keek, Helena GJ; Markus, Dorijn; Cajili, Marc KM; Schwab, Samuel; Meijer, Wilfried JJ; (2022) The B. subtilis Rok protein is an atypical H-NS-like protein irresponsive to physico-chemical cues. Nucleic Acids Research. Vol 50, Num 21, pages 12166-12185
- Hortal, Alejandro M; Oeste, Clara L; Cifuentes, Claudia; Alcoceba, Miguel; Fernández-Pisonero, Isabel; Clavaín, Laura; Tercero, Rut; Mendoza, Pilar; Domínguez, Verónica; García-Flores, Marta; (2022) Overexpression of wild type RRAS2, without oncogenic mutations, drives chronic lymphocytic leukemia. Molecular Cancer. Vol 21, Num 1, page ene-24
- Bovolenta, Elena R; García-Cuesta, Eva M; Horndler, Lydia; Ponomarenko, Julia; Schamel, Wolfgang W; Mellado, Mario; Castro, Mario; Abia, David; van Santen, Hisse M; (2022) A set point in the selection of the αβTCR T cell repertoire imposed by pre-TCR signaling strength. Proceedings of the National Academy of Sciences Vol 119, Num 22, page e2201907119



#### Services

■ The main functions of the Facility are the analysis of proteins at the level of sequence (homologous search, family characterization, prediction of properties) and structure (modeling of non-crystallized or mutant proteins and characterization of protein interactions using molecular dynamics).

#### Summary list of services offered

Among the services provided during these years, we would highlight the research projects that concerned the Covid-19 pandemics. The first one, 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, investigated 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 transgene 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.



#### DROSOPHILA TRANSGENESIS FACILITY

#### **Group Members**

**Technical director:** Mar Casado García Nuria Esteban Delgado

Scientific supervisor:

Carlos Estella

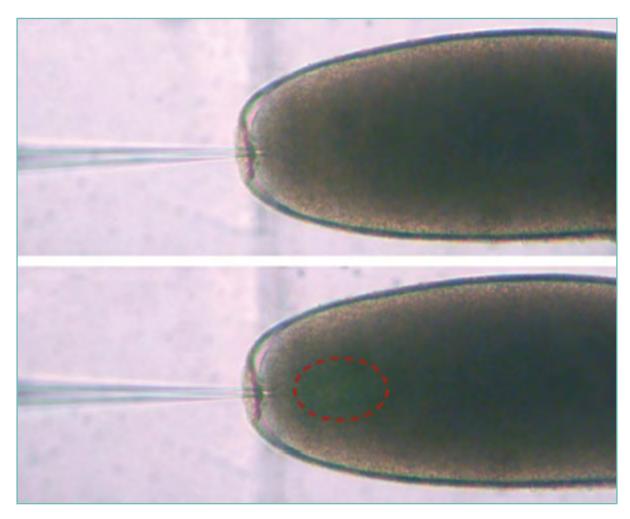




#### Description

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.





Drosophila embryos injection



- Generation of transgenic strains mediated by the transposon P (random insertion into yw or w strains) Helper DNA is provided by the facility, that also prepares the injection mixture.
- Targeted insertion of transgenes mediated by the PhiC31 integrase into the following acceptor strains: ZH-attP22A, ZH-attP-51D, ZH-attP-68E, ZH-attP-86Fb.
- Transgenesis with BAC vectors [P(acman) collection] » Injection of DNA cocktails for gene editing using CRISPR/ Cas technology in the strains: 25C, 68A, Nos-cas9, Vasacas9 and Vasa-cas9 RFP-.
- Insertion of DNA into specific strains provided by the costumer.

- Shipment of injected embryos or generation and shipment of transgenic lines according to the requested service.
- Shipment of Drosophila stocks from the collection.
- Plating and shipment of cDNA plasmids.



#### **ELECTRON MICROSCOPY FACILITY**

#### **Group Members**

**Technical director:** María Teresa Rejas

Staff:

Milagros Guerra Rodríguez Tania Matamoros Grande María Josefa Rodríguez Gómez Martin Christoph Sachse



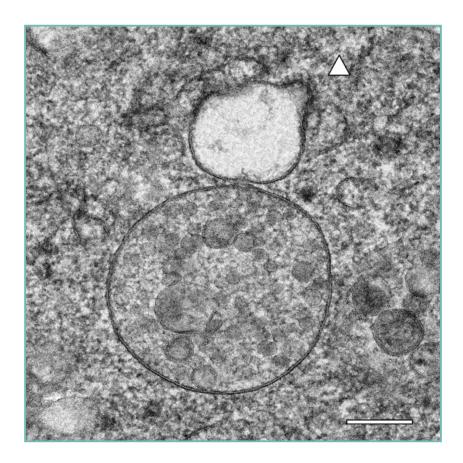


#### Description

The Electron microscopy service (SME) of the CBM provides help to scientists of the CBM and from outside the institute to address their morphological questions within their research. For this, the SME is specialized in conventional and cryo-sample preparation for transmission electron microscopy of cell biological samples, such as bacteria, fungi, cultured cells, and tissues. In addition, bacteria, purified viruses or proteins can be analysed by negative staining, as well as nanoparticles for material science. The service has a 100 kV and a 120 kV TEM, both equipped with a 4x4 K CMOS camera.

We offer a full service for sample preparation for morphological and immunohistochemical studies. The analysis at the microscope can be done by the scientists themselves after a training, as well as by the service. The members of the SME can provide help with the analysis and interpretation of the results.





In 2023 a high pressure freezing machine was installed at the SME. The image shows a late endosome of a high pressure frozen Vero cell. Next to it is an electron lucent vacuole with a clathrin coated bud (white arrowhead) at the limiting membrane. Scale bar 200 nm.



#### List of publications

- Gurriaran-Rodriguez U, Kodippili K, Datzkiw D, Javandoost E, Xiao F, Rejas MT, Rudnicki MA. (2024) Wnt7a is required for regeneration of dystrophic skeletal muscle. Skelet Muscle. 14(1):34. doi: 10.1186/s13395-024-00367-x.
- Iborra-Pernichi M, Ruiz García J, Velasco de la Esperanza M, Estrada BS, Bovolenta ER, Cifuentes C, Prieto Carro C, González Martínez T, García-Consuegra J, Rey-Stolle MF, Rupérez FJ, Guerra Rodriguez M, Argüello RJ, Cogliati S, Martín-Belmonte F, Martínez-Martín N. (2024). Defective mitochondria remodelling in B cells leads to an aged immune response. Nat Commun. 15:2569. doi: 10.1038/s41467-024-46763-1.
- Martin V, Guerra B, Hernaez B, Kappler-Gratias S, Gallardo F, Guerra M, Andres G, Alejo A. (2024). A novel live DNA tagging system for African swine fever virus shows that bisbenzimide Hoechst 33342 can effectively block its replication. Antiviral Res. 230:105973. doi: 10.1016/j. antiviral.2024.105973.
- Alejo A, García-Castey M, Guerra M, Hernáez B, Martín V, Matamoros T, Andrés G. (2023). African swine fever virus transmembrane protein pEP84R guides core assembly. PLoS Pathog. 19:e1011136. doi: 10.1371/journal. ppat.1011136.



#### Services

- Technical and scientific supervision on experimental design and data analysis.
- User assistance and training.
- Negative staining of macromolecular complexes, extracellular vesicles, viruses and nanoparticles.
- Chemical fixation and resin-embedding of biological specimens.
- Cryofixation, freeze-substitution and low temperature embedding.
- Ultramicrotomy of resin embedded samples.
- Tokuyasu cryosectioning.
- Immunolectron microscopy with gold conjugates.
- Correlative light-electron microscopy.
- Freeze-fracture, freeze-etching and Pt-C replication.
- Electron microscopy of nucleic acids.



#### FLOW CYTOMETRY FACILITY

#### **Group Members**

**Technical director:** Berta Raposo

**Scientific supervisor:** Maria Luisa Toribio

Staff:

Silvia Andrade

Raquel Nieto





#### Description

The flow cytometry facility provides access to state-of-the-art multiparametric flow cytometry, spectral flow cytometry and cell sorting. Available flow cytometry techniques range from classic cell immunophenotype, cell cycle and apoptosis determinations, 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. Spectral flow cytometry maximizes sensitivity, decreases background, and allows investigation of an increasing number of molecules of interest. Cell sorting provides high purity cell separations, single-cell sorting, and particle enrichment. Determinations of proteinprotein interactions based on FRET are also provided. Highly experienced and dedicated personnel offers training and advice on flow cytometry principles and applications, cell-sorting operation and equipment calibration and maintenance.

Equipment includes one analogical cytometer 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. Spectral flow cytometry

equipment includes two Cytek cytometer (AURORA) equipped with 4 lasers (V/B/YG/R) and 5 lasers (UV/V/B/YG/R), 4 analysis stations equipped with a Spectroflo and Flowjo licenses, and a nextgeneration software (OMIQ) containing the most commonly used algorithms for multidimensional data analysis. The facility also includes a cell sorter, FACS Aria Fusion integrated inside a Class II biosafety cabinet, equipped with four lasers and sixteen fluorescence detectors. For cell metabolism analysis, the facility is equipped with a Seahorse metabolic analyzer, that enables the detection of discrete changes in cellular bioenergetics in realtime using living cells in a 96-well platform, and with a multiwell motorized microscope (Cytation) that allows normalization of seahorse assays and other assays.



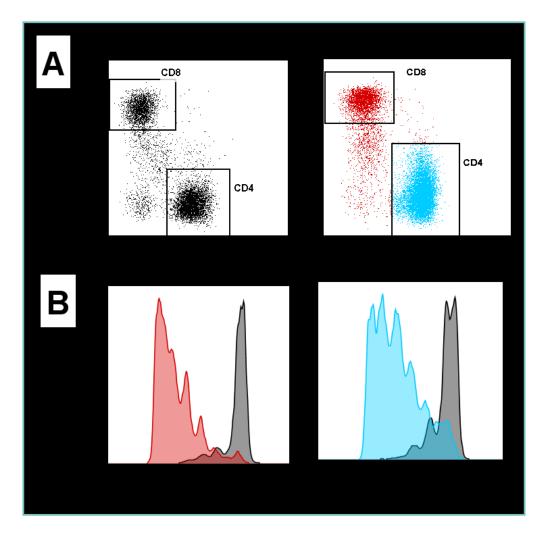


FIGURE 1. Tracking human T cell division. Human peripheral blood lymphocytes, either non-stimulated or stimulated with anti-human CD3 antibody were labeled with anti-CD3-PE, anti-CD4-FITC and anti-CD8-PECy7 antibodies and Cell Trace Violet.



- Training and advice on instrument operation.
- Advice on new experimental designs and adaptive setups.
- Training on post-acquisition data analysis using FlowJo, FACSDiva, Spectroflo and OMIQ softwares.
- Equipment operation and maintenance.
- Availability to commonly used reagents.



- III CURSO ANÁLISIS AVANZADO de datos en citometría de flujo (co-organized by CBM, CIEMAT and PALEX)
- Gabinete de Formación del CSIC (2024): Introducción a la citometría de flujo (co-organized by CBM, CNB and CIB)
- Gabinete de Formación del CSIC (2024): Análisis de datos de citometria de flujo: DIVA y FlowJo
- Gabinete de Formación del CSIC (2024): Aplicaciones y uso de la citometría de flujo en investigación (co-organized by CNB, CBM and CIB)



#### **GENOMICS FACILITY**

#### **Group Members**

**Technical director:** Laura Tabera Moreno

#### Scientific supervisor:

Begoña Aguado Orea (until november 2023 in the Genomics and NGS Core Facility) Leonardo Beccari (since november 2023 in the Genomics Core Facility)

#### Staff:

Maria Gabriela Atencia Cibreiro (until november 2023 as part of the Genomics and NGS Core Facility and since then in the Genomics Core facility)
Sandra González de la Fuente (until november 2023 as technical supervisor of the NGS section of the Genomics and NGS Core Facility)
Paula Martínez García (until november 2023 in the

Genomics and NGS Core Facility)

Iván Lorca Alonso (until november 2023 in the Genomics and NGS CoreFacility)

María Santos Galindo (until november 2023 in the

Genomics and NGS Core Facility)





#### Description

In november 2023, the Genomics and NGS Core Facility was divided into two independent facilities: the Genomics Core Facility and the Biocomputacional Core Facility.

The Genomics Core Facility provides a comprehensive range of molecular biology and -omics services, technical advice, and experimental design support to research teams at CBMSO and external users. Additionally, it offers specialized training courses.

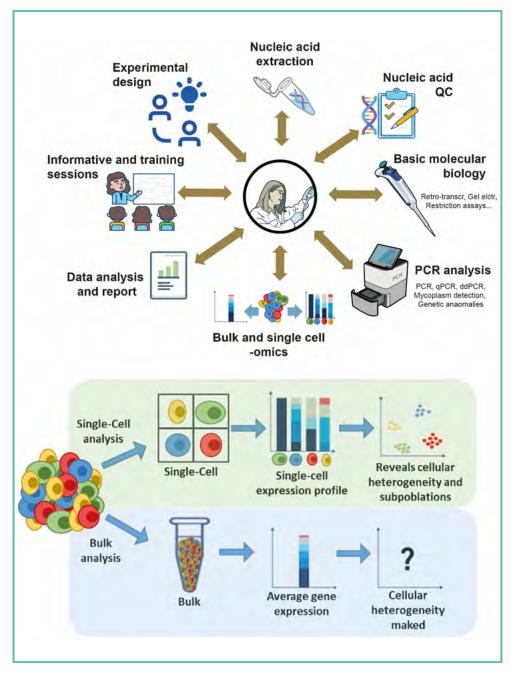
With extensive experience in PCR, qPCR, RT-qPCR, and digital droplet PCR (ddPCR), the facility also supports nucleic acid extraction and their quantification, and quality control. In the past two years, its capabilities have significantly expanded to include genomic abnormality analysis, mycoplasma detection in cell cultures, and NGS library preparation for bulk and single-cell experiments using diverse technologies.

Notably, we have integrated various scRNA-seq methodologies, including microfluidic-based (10x Genomics), combinatorial cell barcoding, and gelseparation techniques, enabling adaptation to different research needs.

Keeping pace with rapid advancements in -omics technologies, the facility is committed to incorporating new technologies (long-read sequencing, scATAC-seq, CITE-seq, spatial transcriptomics), when required to respond user's needs. Furthermore, it actively informs CBMSO users about the latest technological developments through seminars and periodic reports.

The Genomics Core Facility also offers users the option to independently access its equipment via an electronic reservation system, allowing flexibility in their level of involvement based on specific research requirements.





Overview of the services offered by the Genomics core facility and differences between bulk and single cell RNA seq experiments



#### Services

- Experimental design, performance and data analysis.
- Training courses.
- Primer design
- Nucleic acids extraction
- Nucleic acids quantification (using spectrophotometry or fluorimetry)
- RNA, DNA and NGS libraries quality control
- Reverse transcription (RT)
- PCR, RT-PCR, qPCR, RT-qPCR
- Droplet Digital PCR
- Single cell experiments



#### MOUSE GENE EDITING FACILITY

#### **Group Members**

**Technical director:**Ma Belén Pintado Sanjuanbenito

Staff:

Verónica Domínguez Plaza Coral Pedrero García





#### Description

The Service is shared between two CSIC centres: CBM and CNB. Our mission is to generate new genetically altered mouse models supporting the activity of research groups. We implement traditional technologies like additive transgenesis or ES cell mediated gene targeting, with genome edition with CRISPR/Cas9. We perform the technical generation of models through microinjection, electroporation or ES cell handling. We also support researchers on the design of the most appropriate mouse model for the specific purpose of their project: transgenic, KO, KI or conditional. The Service can design and test guides for genome edition and for additive transgenesis and ES-based gene targeting. We support research groups on the design of constructs, vectors, and genotyping if requested.

We also advise in breeding strategies of genetically altered mouse colonies, and facilitate the location and the incorporation of existing mouse lines from different sources. We can generate new mouse ES cell lines or collect embryos at different developmental stages. We perform cell line gene edition on request, designing guides and electroporating cells provided by the different research groups. All our services are open to any researcher who demand our help, except those under patent restriction, that are only provided to internal users from CSIC or UAM.

As part of our supporting activities for the research community, we organize two courses included in the annual CSIC catalogue, one focused on advanced handling of complex genotypes of genetically altered mouse models: conditional and inducible and a second one to help researchers to write the official request for authorization of experiments with animals according to Directive 2010/63/EU.

The service occupies physically three rooms at CNB, plus specifically assigned space in animal facilities of CNB and CBM. We count with the following equipment: P2 laboratory of ES cells with with 2 CO<sub>2</sub> incubators for cell culture, Class II Biological Safety cabinet, Laminar flow hood, stereomicroscope, inverted microscope for tissue culture with camera, Electroporator, Cell electrofuser, Refrigerated centrifuge, Microinjection laboratory with two microinjection settings, consisting of inverted microscope with micromanipulator system, piezo drill, stereomicroscopes, pipette puller, Nepa 21 electroporator for embryos, CO<sub>2</sub> incubator for embryo culture and a molecular biology laboratory with: PCRThermocycler, basic laboratory equipment.





Chimeric mice generated by microinjecting embryonic stem (ES) cells into blastocysts



#### List of publications

- Martinez-Campanario,M.C., Cortés,M., Moreno-Lanceta, Han, L., Ninfali, C., Domínguez, V., Andrés-Manzano M.J., Farràs, M., Esteve-Codina, A., Enrich, C., Díaz-Crespo,F.J., Pintado, B., Escolà-Gil,J.C. García de Frutos, P. Andrés, V., Melgar-Lesmes, P. and Postigo, A.(2023) Atherosclerotic plaque development in mice is enhanced by myeloid ZEB1 downregulation. Nat Commun. 2023; 14: 8316. doi: 10.1038/s41467-023-43896-7 PMCID: PMC10721632
- Cortés, M., Agnese Brischetto, A., Martinez-Campanario, M.C., Ninfali, C. Domínguez, V., Fernández, S., Celis, R., Esteve-Codina, A., Lozano, J.J., Sidorova, J., Garrabou, G., Siegert, A.M., Enrich, C., Pintado, B., Morales-Ruiz, M., Castro, P., Cañete, J.D. and Postigo, A (2023). Inflammatory macrophages reprogram to immunosuppression by reducing mitochondrial translation. Nat Commun. 2023; 14:7471.doi:10.1038/s41467-023-42277-4 PMCID: PMC10656499
- Ninfali,C., Cortés,M., Martínez-Campanario, M.C., Domínguez, V., Han,L., Tobías,E., Esteve-Codina,A., Enrich,C., Pintado,B., Garrabou, G. and Postigo, A. (2023). The adaptive antioxidant response during fasting-induced muscle atrophy is oppositely regulated by ZEB1 and ZEB2. Proc Natl Acad Sci U S A. 120(46): e2301120120. doi: 10.1073/pnas.2301120120



#### Courses

- Maintenance and management of conditional and inducible mouse lines
- Drafting and monitoring of projects for the authorization of the use of experimental animals within the framework of Royal Decree 53/2013



#### Services

- Survey in international repositories and web resources to locate existing mice lines with the desired genotype
- Advise and technical support in the design of targeting vectors or constructs for microinjection and guides for genome editing with CRISPR/Cas9 technology
- Collection and culture of mouse embryos from 1 cell to blastocyst
- Pronuclear microinjection of DNA
- Vector electroporation in ES cell lines
- CRISPR/ Cas9 based genome edition of mouse embryo including design of guides
- CRISPR/Cas9 edition of cell lines, including design of quides
- International consortia ES cells handling and injection to generate chimeras
- Derivation of murine ES cell lines
- Genotyping by PCR, LR-PCR and sequencing
- Reproductive biotechnology to solve breeding problems of genetically modified mice
- DNA construct design and cloning for genome modification on demand.
- Support in establishment and management of genetically altered mouse lines
- Specific training in collection, handling and culture of mouse embryos in preimplantational stages
- Advise in creation and establishment of genetically altered mouse models according to Directive 63/2010/EU, including preliminary welfare assessment



#### PRECLINICAL BIOMEDICINE FACILITY

**Group Members** 

**Technical director:** Haisul CY Chang Hsiao

Scientific supervisor:

Konstantinos Stamatakis Andriani





#### Description

**Preclinical Biomedicine** is a scientific facility inaugurated in 2024, located in the CBM's animal facility. It provides scientific-technical guidance and support for experimental design and the use of specialized equipment with mice.

Our goal is to offer reliable, high-quality services that meet the needs and expectations of researchers, while complying with ethical and regulatory standards in animal research. The service is available to all institute researchers as well as external collaborators interested in using the equipment.

Training and guidance are offered on equipment usage, along with support for data analysis and interpretation. The personnel have expertise and qualifications in preclinical biomedicine and can assist researchers in designing and conducting their experiments.

Haisul CY Chang Hsiao, our experimental microsurgeon with experience more than 20 years in the field of animal experimental surgery, provides creative and practical advises for the experimental surgical settings and, if some are not skilled enough to maximize the animal wellness and etical issues to obtend the samples for our services, Haisul also helps our investigators in small animal manipulations. In the near future, the first IVIM Intravital Microscope in europe is incorporated in our unit in CBMSO. Until then,

Haisul takes charge of the training of the IVIM Intravital Microscope operations to access observation windows for the IVIM Intravital Microscope in brain, lung, skin, liver, small intestines, spleen, kidney, mammary glands, adipose tissue, lymph nodes, y peritoneum. By using IVESTA 3 Greenough Microscope, sophisticated microsurgeries can be done like heterotopic heart transplantation or orthotopic kidney transplantation in mice by Haisul.

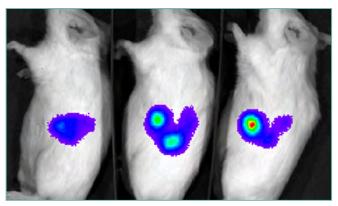
Konstantinos Stamatakis Andriani, our experienced expert without doults in IVIS imaging experiment settings, gives full usefulness of our two IVIS imaging sistems in two animal rooms with different sanitary status: the Room P2 (where virus vectors and mouse tumor can be employed) and the Room Preclinical Biomedicine (convencial space free of potencial pathogenic agents).

BELOW IS A DESCRIPTION OF THE EQUIPMENT AVAILABLE:

#### **Metabolic Cages for Mice**

PhenoMaster (TSE Systems) and CLAMS (Comprehensive Lab Animal Monitoring System): These systems allow for the measurement of various metabolic parameters in mice, including food and water intake, energy expenditure, activity, temperature, bodyweight, and oxygen consumption. Capacity for housing 12 mice individually.





Provide scientific-technical advice and support for experimental design and the use of equipment for research with mice ("mus musculus") in the field of biomedicine

## Services

- Training and guidance on how to use the available equipment.
- Advice on data analysis and interpretation.
- The service staff have experience and qualifications in the field of preclinical biomedicine and can assist researchers with their experimental design and execution.

#### **Stereoscopic Magnifier**

**IVESTA 3 Greenough Microscope**: Unlike conventional stereoscopic microscopes, the Ivesta 3 incorporates **FusionOptics** technology, combining high resolution with a large depth of field simultaneously. Includes downloadable proprietary software for streamlined image use on laptops and smartphones.

#### **Mouse Endoscope**

**COLOVIEW Mainz by Karl Storz**: Enables visualization of the colon, fluid injection, and color video recording in anesthetized mice.

#### **In Vivo Imaging Systems**

**IVIS Lumina II and IVIS Spectrum**: Provide non-invasive imaging of live animals using optical, fluorescence, and bioluminescence modalities.

#### **Element HT5 Hemocytometer**

A multi-channel analyzer using laser flow cytometry, impedance technology, and colorimetric detection for rapid and accurate hematological profiling across 20 parameters in under a minute using only 15  $\mu$ L of whole blood. Also supports safe analysis of non-blood cavity fluids, delivering immediate cellular content information. Results are displayed numerically and as WBC scatter plots and WBC/PLT histograms.

#### **Biochemical Analyzer**

**VetScan VS2:** A compact system for chemical, gasometric, and electrolyte blood analysis completed in 12 minutes with just 100 μL of blood. Includes an intelligent quality control system (**iQC**®) for reliability. Each disposable rotor contains all necessary reagents and diluent for multivariate analysis. Available rotor profiles:

Rotor	Description	Parameters
Comprehensive Diagnostic Profile	Full chemical and electrolyte analysis	ALB, ALP, ALT, AMY, BUN, Ca, CRE, GLOB, GLU, K+, Na+, PHOS, TBIL, TP
Preventive Care Profile Plus	Comprehensive health screening	ALB, ALP, ALT, AST, BUN, Ca, Cl-, CRE, GLOB, GLU, K+, Na+, TBIL, tCO2, TP
Electrolytes Plus	Acid-base and osmotic balance	Cl–, K+, Na+, tCO2
Prep Profile II	Basic health assessment	ALP, ALT, BUN, CRE, GLU, TP
Critical Care Plus	Serial testing and reassessments	ALT, BUN, CI–, CRE, GLU, K+, Na+, tCO2
Kidney Profile Plus	Renal monitoring and evaluation	ALB, BUN, Ca, CI-, CRE, GLU, K+, Na+, PHOS, tCO2
Mammalian Liver Profile	Liver function baseline	ALB, ALP, ALT, BA, BUN, CHOL, GGT, TBIL
Phenobarbital Profile	Drug-level monitoring + liver markers	ALB, ALP, ALT, AST, BUN, GGT, PHB, TBIL
T4/Cholesterol Profile	Thyroid screening	T4, CHOL



#### PROTEIN BIOTECHNOLOGY FACILITY

#### **Group Members**

**Technical director:** 

Dionisio Ureña Rodríguez (until May 2024) María Gimeno Pérez (since Jan 2024)

**Scientific supervisor:** 

José Berenguer Carlos (until Jun 2023) Aurelio Hidalgo Huertas (since Jan 2024)

Staff:

Isabel Carrascal Blanco





#### Description

The Biotechnology Protein Facility, formerly known as the Fermentation Facility, provides scientific and technical support to research groups at CBM and other public or private institutions, offering guidance on the best strategies for biomass and protein production. We advise users on selecting appropriate bacterial strains, plasmid expression systems, and conditions for the overproduction of recombinant proteins. The facility also enables scaling up production processes and cultivating a wide range of non-recombinant microorganisms in large volumes, utilizing the fermenters available and monitoring key parameters—such as temperature, stirring, pH, oxygen concentration, foam, and biomass—in compliance with cGMP (current Good Manufacturing Practice) regulations. To evaluate protein production, we offer cell culture disruption services and SDS-PAGE analysis.

Additionally, the Biotechnology Protein Facility provides ready-to-use competent preparations of various *E. coli* strains suitable for gene cloning or recombinant protein expression. It also produces DNA size markers for internal use by most research groups at CBMSO.





Prueba de expresión de proteínas fluorescentes en matraz



#### Services

- Bioprocesses for microbial culture production at laboratory and pilot scale.
- Optimization of batch, feed-batch and continuous cultures.
- Heterologous protein expression in prokaryotes and veasts.
- Ready-to-use *Escherichia coli* competent cells.

- DNA molecular weight markers.
- Disruption of cell cultures.
- SDS-PAGE electrophoresis and gel staining.



#### PROTEOMICS FACILITY

#### **Group Members**

**Technical director:** Carlos García García Esperanza Morato López

**Scientific supervisor:** José Manuel Cuezva Marcos María Yáñez Mo

Staff:

Tamara Rosell García Alberto Rodríguez Izquierdo





#### Description

The Proteomics Facility offers advance proteomics analytical services to the CBM scientific community, and also to any researcher from other public research centers, universities, hospitals and companies. As a support service for research activity, we provide personalized scientific/technical support to each project, which may include the stages before and after analysis: experimental design, projects review, data analysis and advice/support on the biological interpretation of generated data.

Since 2005, our laboratory has been part of ProteoRed, a proteomics platform whose main objective is to integrate and coordinate the activities of the Spanish Proteomics Services as well as to evaluate the quality of the services offered (participating in the human proteome sequencing project (SpHP)). We are also members of the Spanish Proteomics Society (SEProt) and the European Proteomics Association (EuPA). Besides, we are the Proteomics Service of the IIS- Princesa.



#### Services

#### Protein QC (Quality Control):

- Single-protein analysis
- Confirmation of protein overexpression in complex samples
- Protein identification in a gel spot/band

#### Protein-protein interactions:

- Characterization of protein-protein interactions: IPs, Pull-down, TAP system, BioID,
- Avidin/Biotin....
- Interactomics ID

### Proteins identification/characterization by LC-MS/MS and bioinformatic analysis:

- High-throughput protein identification
- PTMs (Post-translational modifications) identification and characterization
- Discovery/validation of proteins of interest/biomarkers
- Targeted Proteomics (Peptide selection by Single Mass Ion Monitoring (SMIM))
- Characterization of molecular mechanisms

#### **Quantitative Proteomics:**

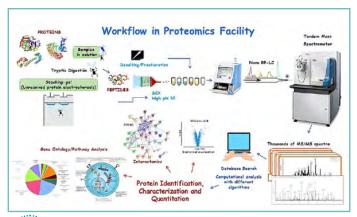
- Label-free protein quantitation in complex samples
- Protein quantitation in complex samples by iTRAQ/TMT
- Differential expression analysis in two or more sample groups
- Quantitation of post-translational modifications (PTMs)
- Proposals/Budget review for projects calls
- Collaboration in research projects
- Training in proteomics and bioinformatics tools



Training in proteomics and bioinformatics tools

CBM 2023-2024 Scientific facilities





Workflow in Proteomics Facility



#### List of publications

- Rosell-Garcia, T., Rivas-Muñoz, S., Kin, K., Romero-Albillo, V., Alcaraz, S., Fernandez-Tornero, C., & Rodriguez-Pascual, F. (2023). Multimerization of HIF enhances transcription of target genes containing the hypoxia ancillary sequence. Biochimica et biophysica acta. Gene regulatory mechanisms, 1866(4), 194963. https://doi.org/10.1016/j. bbagrm.2023.194963
- Franca-Oliveira, G., Martinez-Rodriguez, A. J., Morato, E., & Hernández-Ledesma, B. (2023). Contribution of Proteins and Peptides to the Impact of a Soy Protein Isolate on Oxidative Stress and Inflammation-Associated Biomarkers in an Innate Immune Cell Model. Plants, 12(10), 2011. https://doi.org/10.3390/plants12102011
- Pablo Martí-Andrés, Isabela, Salvador Pérez, Sergio Rius-Pérez, Isabel Torres-Cuevas, Hildegard Colino-Lage, David Guerrero-Gómez, Esperanza Morato, Anabel Marina, Patrycja Michalska, Rafael León, Qing Cheng, Antonio Miranda-Vizuete, Edward E. Schmidt, Antonio Martínez-Ruiz, Elias S.J. Arnér, Juan Sastre. (2023). TRP14 is the cellular cystine reductase and also reduces cysteinylated proteins. Free Radical Biology and Medicine, Volume 201, Supplement 1, Page 15 https://doi.org/10.1016/j. freeradbiomed.2023.03.078.
- Romina Medeiros Figueredo, Elizabeth López-Achigar, Jorge Cantero Piñanez, Margot Paulino Zunini, Esperanza Morato López, Carlos García García, Antonio Martinez Ruiz (2023). Identification of tyrosine nitration as a nitroxidative postranslational modification in fibrinogen. Free Radical Biology and Medicine. Volume 201, Supplement 1, Pages 63-64. https://doi.org/10.1016/j.freeradbiomed.2023.03.258.
- Pablo Martí-Andrés, Isabela Finamor, Isabel Torres, Salvador Pérez, Sergio Rius-Pérez, Hildegard Colino-Lage, David Guerrero-Gómez, Esperanza Morato, Anabel Marina, Patrycja Michalska, Rafael León, Qing Cheng, Eszter Petra Jurányi, Klaudia Borbényi-Galambos, Iván Millán, Péter Nagy, Antonio Miranda-Vizuete, Edward E. Schmidt, Antonio Martínez-Ruiz, Elias S.J. Arnér, Juan Sastre. (2024). Beyond reduction: unveiling TRP14's dual role in cysteine homeostasis and redox dynamics. Free Radical Biology and Medicine, Volume 218, Supplement 1, Page 8. https://doi.org/10.1016/j.freeradbiomed.2024.04.034.
- Romina Medeiros Figueredo, Elizabeth López-Achigar, Esperanza Morato López, Carlos García, García, Antonio Martinez Ruiz. (2024). Identification of nitrated proteins in human thrombi from ischemic cerebrovascular events. Free Radical Biology and Medicine, Volume 218, Supplement 1, Page 48. https://doi.org/10.1016/j.freeradbiomed.2024.04.171.

- Benito-Vázquez, I., Muñoz-Labrador, A., Garrido-Romero, M., Hontoria-Caballo, G., García-García, C., Diez-Municio, M., & Moreno, F. J. (2024). New Pipeline for Analysing Fruit Proteolytic Products Used as Digestive Health Nutraceuticals. International journal of molecular sciences, 25(19), 10315. https://doi.org/10.3390/ijms251910315
- Benito-Vázquez, I., Garrido-Romero, M., Hontoria-Caballo, G., García-García, C., Díez-Municio, M., & Moreno, F. J. (2024). Carob (Ceratonia siliqua) Flour as Source of Bioactive Compounds: Production, Characterization and Nutraceutical Value. Foods (Basel, Switzerland), 13(19), 3024. https://doi.org/10.3390/foods13193024
- Martí-Andrés P, Finamor I, Torres-Cuevas I, Pérez S, Rius-Pérez S, Colino-Lage H, Guerrero-Gómez D, Morato E, Marina A, Michalska P, León R, Cheng Q, Jurányi EP, Borbényi-Galambos K, Millán I, Nagy P, Miranda-Vizuete A, Schmidt EE, Martínez-Ruiz A, Arnér ES, Sastre J. (2024). TRP14 is the rate-limiting enzyme for intracellular cystine reduction and regulates proteome cysteinylation. EMBO J. 43(13):2789-2812. doi: 10.1038/s44318-024-00117-1.
- Adán-Jiménez, J., Sánchez-Salvador, A., Morato, E., Solana, J. C., Aguado, B., & Requena, J. M. (2024). A Proteogenomic Approach to Unravel New Proteins Encoded in the Leishmania donovani (HU3) Genome. Genes, 15(6), 775. https://doi.org/10.3390/genes15060775
- Torres, A., Bernardo, L., Sánchez, C. et al. (2024). Comparing the Proteomic Profiles of Extracellular Vesicles Isolated using Different Methods from Long-term Stored Plasma Samples. Biol Proced Online 26, 18. https://doi.org/10.1186/s12575-024-00243-4
- Herreros-Cabello, A., del Moral-Salmoral, J., Morato, E., Marina, A., Barrocal, B., Fresno, M., & Gironès, N. (2024). Quantitative Proteomic Analysis of Macrophages Infected with Trypanosoma cruzi Reveals Different Responses Dependent on the SLAMF1 Receptor and the Parasite Strain. International Journal of Molecular Sciences, 25(13), 7493. https://doi.org/10.3390/ijms25137493
- Torres-Sánchez, E., Morato, E., Hernández-Ledesma, B., & Gutiérrez, L.-F. (2024). Proteomic Analysis of the Major Alkali-Soluble Inca Peanut (Plukenetia volubilis) Proteins. Foods, 13(20), 3275. https://doi.org/10.3390/foods13203275
- Paterson, S., Alonso-Pintre, L., Morato-López, E., González de la Fuente, S., Gómez-Cortés, P., & Hernández-Ledesma, B. (2025). Microalga Nannochloropsis gaditana as a Sustainable Source of Bioactive Peptides: A Proteomic and In Silico Approach. Foods, 14(2), 252. https://doi.org/10.3390/foods14020252



Technical services and operations teams



#### **TECHNICAL SERVICES**



#### Cell culture, glass wash and media preparation

- Mercedes Dávila Cerrato
- Antonia Cerrato Gómez
- Anunciación Gaceo Esteban
- Mª Angeles Blanco Ferreras
- María Cazorla Plaza
- Marta Fierro Fernández
- Anunciación Gaceo Esteban
- Miriam García Carrascal

- Lorena García Murillo
- María Teresa Gómez Buendía
- María Nieves Martín Bermejo
- Francisco Borja Mirasol Burgos
- Ana MªPérez Colmenar
- Juan Antonio Rebelles Vicente

#### **Facilities Maintenance**

- César Martos Valladares
- Adrián Carrasco Alonso
- Pedro Pablo Cordón Polanco
- Jorge Ignacio Ocampo Pérez
- Miguel Angel Ollero Serrano
- José María Sanz Mejías

#### **Technical Maintenance**

- Francisco Gutiérrez de la Cruz
- Juan Antonio Delgado Rodríguez
- Fernando Muñoz Maqueda

#### Photography

Jose Antonio Perez Gracia

## Information Technology and Services (IT&S)

- Pedro Pemau Alonso
- Eugenio Barrientos Ruiz
- Vicente Monreal López
- Diego Díaz Rodilla
- María Peña Pérez García
- Óscar Santos de Miguel

## Occupational Health and Safety (OHS)

Fernando García Muñoz

## Biosafety and radioprotection

- Gema Caparrós de la Jara
- Iris Esparza Collado
- María Letre Nieto

#### **OPERATIONS TEAMS**



#### Finance and grants

- Montserrat Barbero Maturana
- David Arjona Lara
- Jaime García Martín-Delgado
- Sonia Rubio Lago
- Gregorio Javier Sánchez García
- Mª Belén Villar Pérez

#### **Human resources**

- Lucía Horrillo Muñoz
- Cristina González Espinosa
- Mario Marcos Sánchez
- Jesica Martínez Santos
- María Paz Yubero Martín

## Communications & Scientific Outreach

- Maria José Martín Pereira
- Maria Josefa Rodriguez Gómez
- Teresa de los Reyes Corrales
- Iole Ferrara Romeo

- Francisco Jiménez Condes
- Miguel Pérez Pulido
- Lidia Barbero Jiménez
- Montserrat de la Fuente Chivato
- Marta García Martínez
- María Rosa Sánchez Caballero

#### **Executive assistant**

Antonia Condés Cano

#### **Grant support**

Carmen Arroyo Martín

## National and international programs

■ Volga del Castillo Domingo

#### Quality management

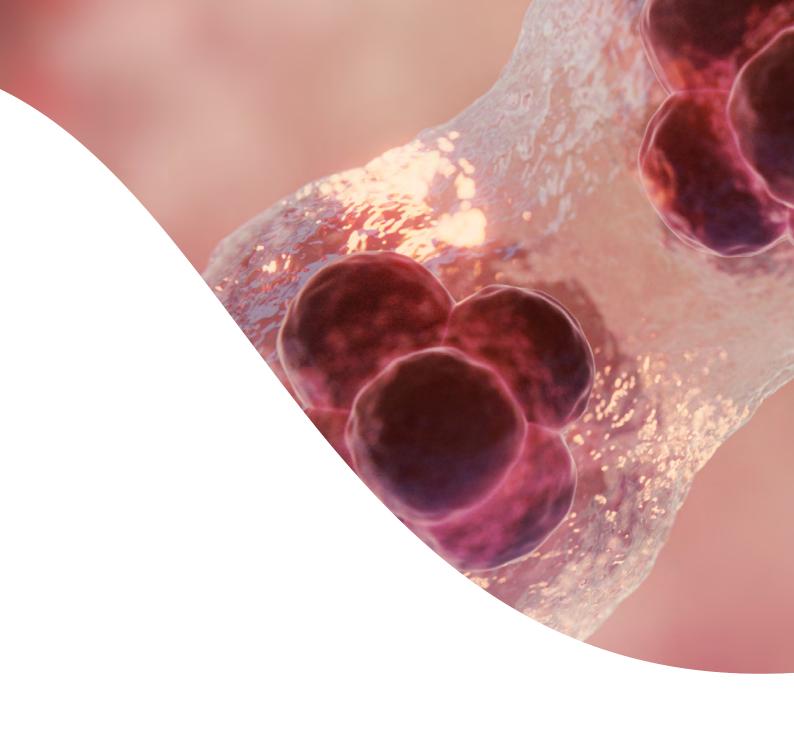
■ Maria del Carmen López Vara

#### **Procurement**

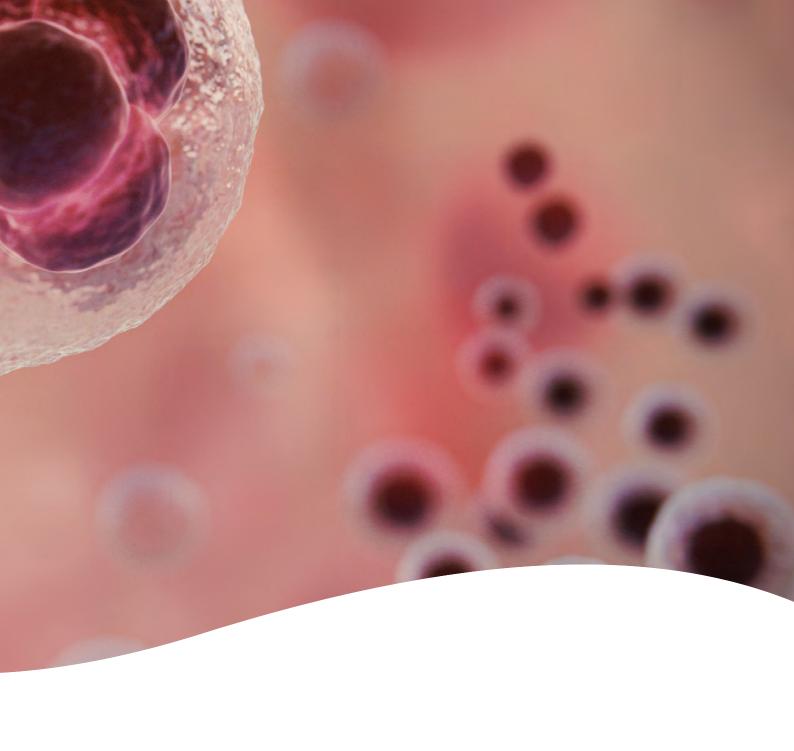
- Leyre Campos Talaverón
- Jose Miguel Celestén Martín
- Mª José Fernández Martín
- Carmen González Martínez
- Jesús Miguel Hernández Largo
- Antonio Hernán-Pérez Fernández
- Mercedes Marín Atienza
- Rosa María Pastor Rodríguez
- Teodoro Pedraza Caro
- Adriana Veronica Susperreguy Orefice

#### Institutional relations

- Almudena Hernando
- Carmen Hermoso Crispín



## Committees





#### TRAIN@CBM COMMITTEE

#### **Group Members**

Coordinators: Miguel Manzanares Laura Formentini

Members:
Carlos Estella
Enrique Gabandé
Maria Gómez Vicentefranqueira
María José Martín Pereira
Marta Pérez Pereira
Catalina Ribas
Valentín Riomoros Barahona
Álvaro Sahún
Claudio Toma



#### Description

The TRAIN@CBM program is designed to support CBM early career researchers (ECRs), including PhD students and postdoctoral researchers, by providing them with the tools, skills, and opportunities necessary to advance their professional development. Our mission is to equip researchers for diverse career paths, foster a collaborative scientific community, and create a positive and healthy environment that supports the CBM's development as a leading research institution.

Our program is built around five key objectives:

- a) TRAINING ECRs in healthy research habits.
- **b)** REPARING ECRs for the job market in academia, industry, and beyond.
- **c)** CREATING an integrated and collaborative research community within CBM.
- d) ATTRACTING international talent.
- e) PROMOTING ethics and equality in science.

Our TRAIN@CBM Committee consists of two PIs from each CBM Scientific Program, along with representatives from the predoctoral and postdoctoral community and scientific services. Together, they are responsible for achieving the program's objectives and ensuring its successful implementation.



#### Other activities

■ The TRAIN@CBM committee have met every two months to engage in detailed discussions about the ongoing development and implementation of training initiatives for ECRs. These meetings provide a platform for evaluating the progress of various programs, identifying emerging needs, and refining strategies to enhance the professional growth of PhD students, postdoctoral researchers, and other members of the scientific community. During these sessions, the committee members collaborate to review current activities, propose new initiatives, and ensure that all training efforts are aligned with the overarching goals of fostering a highly skilled, interdisciplinary, and globally competitive research community.

CBM 2023-2024 Committees





Members of the TRAIN@CBM committee



#### **Activities**

Over the past two years, we have successfully implemented several key initiatives aimed at fostering the professional development of our researchers:

- PhD Thesis Advisory Committee (PhDAC): This initiative ensures that every PhD researcher of the CBM receives comprehensive and continuous guidance throughout their doctoral studies. The PhDAC is composed of the PhD supervisor, one internal advisor, and one external advisor with demonstrated expertise in the research field, providing students with a diverse range of perspectives and specialized knowledge. The committee meets annually to review the student's goals and experimental plans, track progress, and address any obstacles—whether experimental, conceptual, or practical—that arise. As a result of this initiative, 46 ECRs from the CBM have voluntarily joined the program, benefiting from this tailored support system.
- CBM Academy: Building upon the previous initiative, we have established a comprehensive training program for CBM ECRs consisting of courses, talks, and workshops held throughout the four years of PhD studies. Topics range from research ethics and integrity to scientific writing and the translation of research findings. The program is led by experts from our community, along with external researchers and collaborators, offering a well-rounded and practical approach to scientific development.

- Scientific Integrity and Ethics School: As part of the CBM Training Program, the Ethics School is a one-day event organized in collaboration with the CSIC Ethics Committee, UAM Animal Research Ethics Committee, ISCIII Clinical Research Ethics Committees, and the ISCIII Commission for the Use of Human Cells and Tissues. This program provides researchers with essential knowledge on ethical standards and best practices in scientific research.
- Off-Academia Seminar Series: to help ECRs explore nonacademic career options, we have been offering bimonthly seminars since early 2024. These sessions provide insights into various professional sectors, broadening the career horizons of our researchers and supporting their transition beyond academia. To date, we have conducted five seminars, covering a range of topics such as science policy, scientific editorials, science communication, and private sector research and development.
- Postdoctoral Call: We have launched an international call to recruit talented researchers from around the world in any field covered by CBM research. This initiative has led to the successful recruitment of 8 postdoctoral researchers, who have joined both UAM and CSIC research lines, contributing their expertise and advancing ongoing investigations across a wide range of scientific areas.



## **EQUALITY COMMITTEE**

#### **Group Members**

#### 2023

**President:**Sonsoles Campuzano
Corrales

**Vice president :** Jose María Carrascosa Baeza

**Members:** 

Beatriz López Corcuera Alejandra Gámez Abascal Jaime Millán Martínez Esther Serrano Saiz 2024

**President:**Alejandra Gámez Abascal

**Vice president:** Esther Serrano Saiz

Members:

José Mª Carrascosa Baeza Beatriz López-Corcuera Álvaro Sahún Elisa Carrasco Cerro María José Martín Pereira





### Description

Established in 2021, the Equality Committee of the CBM was created following the Equality policies of the State General Administration and the CSIC Equality Plan. The inception of the committee was not only approved but also continues to receive strong support from the CBM director, underscoring the institution's commitment to fostering an inclusive and equitable workplace.

The primary role of the Equality Committee, although not executive, is pivotal in maintaining and promoting equality within the CBM. It serves as a vital conduit, relaying concerns and issues related to equality directly to the Board of Directors of the CBM, ensuring high-level attention and action. Aligned with the Equality Plans of both the CSIC and the Universidad Autónoma de Madrid, the committee is dedicated to creating an environment where all members feel respected and valued, regardless of their gender, race, ideology, age, affective-sexual orientation, or religion.

Key objectives of the Equality Committee include the dissemination and promotion of the CSIC and UAM's equality policies. By making these policies accessible and understood within the CBM, the committee ensures that all staff are well-informed about their rights and the support mechanisms in place. Additionally, the committee acts as a vital channel through which staff can express proposals or suggestions related to equality, fostering a culture of open communication and participatory policy development.

The committee also provides confidential support to any member of the CBM experiencing harassment, discrimination, or any other type of inappropriate behavior, ensuring a safe and trusting environment. It champions policies that facilitate the reconciliation of work, personal, and family life for all employees, promoting a balanced approach to professional and personal responsibilities.

Through a detailed assessment of the current equality situation at the CBM, based on available data, the committee identifies and implements specific measures to address any areas of improvement. This proactive stance is supported by initiatives aimed at training all CBM employees on issues of gender equality and diversity, specifically focusing on managing sexual and gender-based harassment within the framework of the CSIC Training Plan. Furthermore, the committee is committed to promoting equality within the functional and organizational structures of the CBM. It advocates for the inclusion of sex/gender analysis in research and innovation, enhancing the scientific rigor and relevance of research outcomes. The committee also works diligently to increase the visibility of women's roles within the CBM and the broader scientific community, setting a powerful example for inclusivity and equality in science, and inspiring current and future generations.







## Participation in projects

Teaching Innovation Project INNOVA "Coeducating in Equality to Teaching and Research Staff of UAM".

Funded by: Universidad Autónoma de Madrid

IP: Equality Unit and IUEM (2023-2024)

Alejandra Gamez is part of the research team

Teaching Innovation Project IMPLANTA: "Gender and Intersectionality in Teaching Practice".

Funded by: Universidad Autónoma de Madrid

IP: Equality Unit and IUEM (2024-2025)

Alejandra Gamez is part of the research team



#### Other activities

 ACCESSIT ACCREDITATION IN GENDER EQUALITY 2024 Centro de Biología Molecular Severo Ochoa (CBM).

Driving Inclusion: The CBM Equality Committee's activities Towards Diversity and Equality



#### **Activities**

#### **SEMINARS**

- Dr. Silvia Gallego, ICMM-CSIC, "Equality Networks. My Experience at CEI UAM+CSIC" March 21, 2024, commemorating International Women's Day.
- Dr. Paola Boloventa, Director of CBM and CSIC Research Professor, "Breaking the Glass Ceiling without Quotas" June 10, 2024.
- Professor José Ignacio Pichardo, tenured at UCM, "LGBTIQ+ Diversity at in university and work environments". Pride Month. June 2024.
- Dr. Aitor Villafranca, CSIC Research Professor and Director of Education at the PRISMA association, "LGBTIQ+ Diversity in Science: Current Situation and Actions for Equality. July 2024

#### **WORKSHOPS**

- Lola Ledesma "Do you want to be a (Neuro)Scientist?" IES Palomeras-Vallecas 2024
- Esther Serrano-Saiz "When I grow up, I want to be... a scientist". Webinar for ESO students as part of the seminar series "Women and Science at CBM" (2024).
- Educational workshop by María José Martin Pereira in collaboration with the Spanish Association Against Cancer at Arcángel Rafael International School. 2024
- Meeting with students, featuring Margarita del Val (organized by IRNASA – CSIC) 2024
- Talk in the "Women Scientists Cycle" by Dr. Natalia Azpiazu at IES Francisco Nieva de Valdepeñas for 8M International Women's Day. 2024
- Educational workshop by Alejandra Gámez Abascal at Greenwich School, February 11th 2024.
- School workshop at CBM to award a 1st-grade student who won the "Draw a Scientist" prize from SEBMM. 2024
- "Microbiology is Written with an M for Woman" event organized by the Microbiology Area - E.S. of Experimental Sciences and Technology at Rey Juan Carlos University by Dr. Bruna Fernanda de Sousa. 2024.
- Reading of names of murdered women and a family picture with signs against gender violence. November 25, 2024.

#### **DIGITAL CONTENT**

- Creation of a commemorative video for March 8, 2024: International Women's Day.
- Creation of a video and poster for the IV Equality Committees Encounter at CSIC.

#### PROJECTS: VISIBILITY AND CONCILIATION

- Setup and inauguration of a lactation room at CBM (January 2024)
- Violet space in CBM hall (March 8, 2024)

#### DISSEMINATION

Monthly newsletter from the CBM Equality Commission to disseminate activities related to Equality, Diversity, and Inclusion. Distributed at IUEM, Inter-center CSIC Commission, CBM, IU UAM.

## CONTRIBUTION TO THE ESCAPE ROAD OF THE INTERCENTERS CSIC-UAM NETWORK

Creation of Gerty Cori and Christiane Nüsslein-Volhard posters that are part of the escape road "In Search of Nobel and Non-Nobel Women"

### **MULTICULTURAL DAY**

■ A interactive world map celebration the cultural diversity day (21st May) in the CBM hall where individuals can pin their country of origin to celebrate the worldwide diversity of the CBM community

#### **GAY PRIDE MONTH**

- Seminars by Aitor Villafranca and José Ignacio Pichardo
- Stand with diverse information about the LGTBIQA community with posters of 8 key LGTBIQA+ scientists and lists of movies and books related to the community.
- Game to visualize affective-sexual diversity at our center with 6 flasks: Heterosexual, Bisexual, Gay, Lesbian, Asexual, and Others to put a small colored ball inside the flask that most represents you



## PREDOC & POSTDOC COMMITTEE

#### **Group Members**

President::

Elena Rodríguez Bovolenta

**Vice president (or Secretary):** Nerea Murugarren Garrido

Members:
Celia García Vilela
Valentín Riomoros Barahona
Miguel Palma Cobo
Marta Alonso Moreno
Miryam Polo Hernández
Joana Segura Martínez
Alba Roca Portoles



## Description

The CBM Predoc and Postdoc (P&P) Committee aims to:

- Represent: To be the voice of the P&P community before CBM governance bodies and other relevant bodies.
- Identify and Act: To know and address the needs and problems that affect the P&P community of the center.
- Create Community: Foster the consolidation of a social and academic community among the P&P members of the CBM.
- **Promote:** Organize events of interest to the P&P community, promoting collaboration and knowledge sharing.
- **Welcome:** Facilitate the integration of new P&P members, providing support and orientation.

- Connect: Serve as a liaison between the CBM P&P community and other internal groups or services, as well as establish connections with external organizations.
- Influence: Encourage and influence the CBM's P&P training program, ensuring a training environment that is responsive to our needs and aspirations.

Our aim is to ensure that every young researcher feels supported, whether through academic initiatives, training programs, or social activities that help create a strong and inclusive network.

CBM 2023-2024 Committees





Members of the Predoc & Postdoc committee at the CBM.



#### **Activities**

- Periodic meetings (twice per month) to share suggested ideas and concerns of the P&P community to contribute to the functioning and objectives of the CBM.
- Organising Scientific events and meetings, such as:
  - Student and Postdoc symposium: This symposium is organised to give the opportunity to youth researchers to present their work and increase their networking. We secured funding and sponsorships to extend the Symposium to two days, provide attendees with some merchandising, and end the event with a small party.
  - Molecular mix: where pre- and postdocs can present their research in a more informal setting without PIs.

We have also contributed to the organization of special CBM seminars, workshops, and training sessions. Beyond academic activities, we are also involved in organizing social events to strengthen the bonds between researchers. For instance:

- Welcome Meeting: introductory presentation where we explain the organisation of the CBM for new beginners.
- Biomingle: networking events are organised once per month, and we contribute by providing a social activity to engage participation.
- Collaboration with other committees such as the Help&Hand committee, and TRAIN@CBM committee.
- Increase the awareness and attendance of program seminars.
- Help and suggest ideas for invited speakers for the special seminars.
- Contribute to outreach activities: guided tour of the CBM.



## HELP@HAND

#### **Group Members**

**Presidents:**Catalina Ribas
Claudio Toma

Members:

Elisa Carrasco (representative of the Equality Committee)

Joana Segura Martinez (representative of the Pre&Postdoc committee)

Juan Antonio Delgado Rodríguez

Silvia Gutierrez Eisman

Andrés Soto Zaragoza (representative of the Equality committee) Miguel Palma Cobo (representative of the Pre&Postdoc committee)

Maria José Martin Pereira



#### Description

The Help@Hand committee at CBM is an initiative aiming to support doctoral and postdoctoral researchers by fostering a positive and constructive work environment. The Help@Hand provides confidential assistance in resolving potential workplace conflicts and concerns.

#### **Objectives:**

- a) PROVIDING confidential, neutral, and informal assistance to CBM personnel facing workplace concerns.
- b) PREVENTING conflicts and promoting a peaceful work environment through mediation and support.
- c) ADVOCATING for fair and equitable application of workplace procedures.
- d) ENSURING strict confidentiality, offering a safe space for discussion without formal documentation.
- e) IDENTIFYING trends to contribute to a more respectful and harmonious workplace.

#### Help@Hand Team:

The Help@Hand committee is led by Claudio Toma and Catalina Ribas, CBM PIs and members of the TRAIN@CBM committee. Their role is to offer an external and neutral perspective on conflicts or difficult situations encountered by doctoral and postdoctoral researchers and other staff. While the team does not take formal action, it facilitates mediation and, in severe cases, guides individuals to the appropriate offices responsible for handling potential misconduct.





Members of the Help@Hand committee

#### **Activities:**

- Impartial and active listening: Providing a space where researchers can voice concerns without fear of repercussions.
- Assessment of available options: Helping individuals understand possible solutions and approaches to their situation.
- **Providing information and referrals:** Guiding researchers to the relevant institutional support when necessary.
- **Informal fact-finding:** Clarifying issues without initiating formal investigations.
- Conflict management and mediation: Facilitating resolution through discussion and diplomatic efforts.
- **Confidentiality:** Ensuring that no information is shared without explicit consent.
- Feedback to CBM authorities: Compiling anonymoustrendanalysesandrecommendations in an annual report.

#### **Scope and Limitations:**

- The Help@Hand board does **not** act as a formal complaints office or investigative body.
- It does **not** replace official institutional channels for conflict resolution.
- No records are kept, and no formal statements are made in official proceedings.
- The team does **not** have management authority or policy-making capabilities but seeks to contribute to a better workplace culture.

By providing informal conflict resolution and support, Help@Hand complements the broader TRAIN@CBM initiative, ensuring that researchers at CBM can thrive in a collaborative, fair, and respectful environment.



#### **Activities**

- Periodic meetings to share ideas in order to decide on the functioning and objectives of the committee.
- An anonymous questionnaire has been created to assess the general level of workplace conditions, including concerns related to interpersonal dynamics, research environment, and overall well-being. The collected data will help identify trends, highlight systemic issues, and inform future actions. Conversations and support supply for those CBM members who have contacted the Help & Hand committee.



## SUSTAINABILITY COMMITTEE

#### **Group Members**

**President:** 

**Raquel Marco Ferreres** 

**Vice President:** 

Carmela Cela Rodríguez

**Members:** 

César Martos Valladares Mercedes Dávila Cerrato

Gema Caparrós de la Jara

Elena Calvo Cazalilla

Isidro García Gómez

Hisse M. van Santen

Leyre Campos Talaverón (since November 2024)

María José Martín Pereira

Carolina Sánchez Fernández

Mª Carmen López Vara Pedro Pemau Alonso Jessica Martínez Santos (until December 2024) Antonio Hernán-Pérez (until October 2024) Beatriz Barrocal López (until January 2024)

**Colaborators:** 

Álvaro Sahún Español Francisco Gutiérrez de la Cruz Andrés Soto Zaragoza Fernando García Muñoz David Foronda Alfaro (until December 2024)



#### Description

The CBM Sustainability Committee aims to promote and implement sustainable practices across all areas of activity within the center, ensuring a firm commitment to reducing our environmental impact and carbon footprint.

#### **Objectives**

In response to the unprecedented climate crisis, the committee works to minimize the environmental impact of CBM's research activities by:

- Encouraging energy and water efficiency in laboratories.
- Reducing plastic waste.
- Implementing sustainable initiatives that are simple yet effective in lowering the center's carbon footprint.

## **Implications**

Sustainability in research centers is both a responsibility and a necessity. By adopting ecofriendly measures, CBM not only complies with environmental regulations but also sets an example within the scientific community. Sustainable practices improve efficiency, reduce costs, and create a healthier work environment.

The committee works closely with researchers, technical staff, and administration to implement feasible solutions, from reducing energy consumption in ultra-low temperature freezers to optimizing waste management and promoting sustainable mobility.

#### Relevance

Scientific research plays a crucial role in developing solutions for global environmental challenges. However, it is essential to ensure that the research process itself is sustainable. By fostering a culture of environmental responsibility, the CBM Sustainability Committee helps create a positive and lasting impact both within the center and beyond.





## Other activities

- Waste Management in Cell Culture Rooms: We evaluated plastic waste recycling options, promoted reduction and reuse, and installed recycling bins with instructions on each floor.
- Sustainability Best Practices Guide: A guide with recommendations for sustainable practices was created and distributed to all CBM members.
- Sustainability Representatives: We requested each lab to have a Sustainability Representative to communicate with the committee and ensure sustainable practices.
- Monthly Sustainability Actions: We launched a series of monthly sustainability campaigns in order to promote sustainability actions at the CBM. They were promoted through posters and internal communications. Some examples of this campaign are: reduction of plastic use, reduction of paper use, switching off equipment that is not being used, having a right maintenance of the -80°C ultrafreezers.
- Sustainability Surveys: We conducted surveys to gather ideas, assess interest in sustainability challenges, and evaluate the impact of monthly actions.
- Collaboration with the Festivities Committee: We promoted actions to make the Spring Celebration more sustainable, such as reusable cups to refill with drinks.

- Mobility Survey: We assessed staff commuting habits to explore sustainable transport options.
- Seminars: Recycling training sessions organized by FCC Medioambiente and the Ayuntamiento de Madrid. A series of three sessions that took place on February 21 and 28, and April 10, 2024. The talks were given by several environmental educators coordinated by Francisco J Cerrillo López.



Measures to enhance energy and water efficiency at the CBM



# Scientific outreach



Scientific outreach CBM 2023-2024

## SCIENTIFIC OUTREACH

Science and technology are part of our cultural heritage. Activities related to science communication and scientific dissemination are increasingly important for researchers and society, as they:



Allow society to learn more about the work of researchers and the impact that science has on all areas of our daily lives,



Increase the interest and support of governments and entities that provide financial support to scientific work, and



Promote scientific vocations in young people.

# The **CBM Communications and Science Outreach department** has the following objectives and tasks:

- Promote the national and international relevance of CBM.
- Increase the visibility of our scientific achievements through the dissemination of articles, grants, scholarships, conferences, seminars, theses, etc.
- Coordinate and scale the centre's communication channels: social networks, website, etc.
- Promote the relationship with the press and other media, including through the CSIC and UAM communications departments
- Organise outreach and educational events and activities at the CBM and beyond, and inform the public about them and other news and initiatives of interest
- Facilitate and encourage the participation of our scientists in outreach events, led by the CBM and in collaboration with the CSIC, the UAM and other national and international organisations.
- Promote the attraction of talent to the CBM
- Collaborate with other CSIC centres on the UAM campus and outside it, to develop activities and events
- Coordinate internal communications for CBM staff

CBM 2023-2024 Scientific outreach

## SCIENTIFIC OUTREACH ACTIVITIES

Our public engagement programmes involve many activities throughout the year, described below. Furthermore, due to the excellence of our research and the scientific dissemination capacity of our members, the CBM has a long tradition of collaboration and participation in countless press, radio, television and digital media formats, which grows every year.









## Ciencia Contigo

Ciencia Contigo is a science outreach event for all audiences organized annually by the Fundación Severo Ochoa (FSO), with the support of the Community of Madrid and the CBM. The FSO Board of Trustees includes CBM researchers such as Federico Mayor Menéndez and Jesús Ávila, among others, and its secretary is Paola Bovolenta, director of the CBM.

In 2023 and 2024, we celebrated the second and third editions of the Ciencia Contigo outreach event. The first and second editions were held at the Residencia de Estudiantes, with a great reception by the Madrid public. These two events had very complete programs with presentations by leading speakers in research and outreach in the areas covered: general topics in the first edition, a focus on neuroscience on the occasion of the Cajal Year in the second edition.

In 2023, the Ciencia Contigo event was accompanied by a science+art exhibition consisting of pairs of images, one a selected Cajal drawing, and the other an experimental image related to the drawing. The experimental images were submitted by scientists from all over Spain and Europe.

In 2024 the theme of the event was 'The MicroLife' and the presentations focused on the importance of microorganisms (bacteria, viruses, etc.) and their impact on various areas of our daily life, society and technology. The event included several speakers in different formats: short talks and round tables. Ricardo Moure was the master of ceremonies for the entire event, which took pkace at the CaixaForum Madrid, and was attended by more than 300 people.

The speakers were experts in topics as diverse and interesting for the public as the antimicrobial resistance crisis, emerging viruses, the functioning of our immune system, the effects of our microbiome on mental health, the possible characteristics of life outside the Earth or the use of microorganisms in biotechnology. These experts came from various centers of the CSIC and other research centers in Spain.

Scientific outreach CBM 2023-2024

## Guided tours for schools

The guided visits to the CBM offer a unique opportunity to get to know closely one of the main scientific research institutes in Spain, founded in 1975 by Nobel prize winner Severo Ochoa on the campus of the Autonomous University of Madrid. We have more than 90 leading research groups in various fields of biomedicine, supported by 20 high-quality scientific-technical services.

We offer these guided tours completely free of charge, at least once a month during the academic year. During the guided visit, students will be able to tour the centre's modern facilities, including state-of-the-art laboratories, scientific services, specialised research areas and collaborative workspaces. Scientists from the centre will explain their most outstanding projects in areas such as genetics, microbiology, and neuroscience, showing first-hand how their research contributes to the advancement of scientific knowledge and also how they have come to work in a centre like the CBM. During the visits, students learn about and see our labs and scientific facilities.

### Science Week

Our outreach activities in the framework of Science Week include face-to-face and online talks, interactive workshops and an open day at the CBM for Schools and Educational Centers.









CBM 2023-2024 Scientific outreach

## European Researchers' Night

The CBM participates in the European Researchers' Night as a key member of the CSIC-UAM network of research centres. With the support of European funding (Horizon Europe, Marie Skłodowska-Curie actions, Grant Agreement No. 101.162.110), this network of 10 institutes runs its own node of the Night. In 2023, the node was located at the 12 de Octubre hospital, and in 2024, the activities took place at the Fundación Telefónica building in the Gran Vía, the very heart of Madrid's city centre.

The full-day event included a morning science fair for school groups (Primary, Secondary, and High School), featuring activities such as the "Science Cart," workshops on superconductivity, catalysts, antioxidants, and the Escape Road scavenger hunt focused on female Nobel prize awardees in science.

In the afternoon, the fair opened to the general public with interactive stands, workshops (DNA extraction, plant biotechnology, algae as alternative food, X-ray vision, etc.), and, as a new feature, a science stand-up comedy contest where researchers competed through humorous monologues. All activities were free to attend and more than 1000 people enjoyed them.









Scientific outreach CBM 2023-2024

## Madrid es Ciencia science fair

The CBM actively participated in the 2023 and 2024 editions of the "Madrid es Ciencia" fair, held at IFEMA and organized by the Community of Madrid. As part of the CSIC institutional stand, the center offered demonstrations aimed at students and the general public to bring biomedical research closer to society.

The activities prepared by our early career researchers included topics in neuroscience, such as how neurons and the brain work; microbiology, with the observation of microorganisms and their role in health; astrobiology, exploring the search for life on other planets and the role of extremophilic bacteria; and biotechnology, with simple experiments showing how antibiotics are produced.







CBM 2023-2024 Scientific outreach

## 4°ESO+Empresa Program

The CBM participated in the 4°ESO+Empresa program organized by the Community of Madrid during the 2022–23 and 2023-24 academic years. A total of 14 students per year completed 4-day internships within the CBM program, and 6 additional students completed their internships in CBM laboratories. The internships included hands-on activities in protein biotechnology, animal facility, cell culture, genomics, bioinformatics analysis, proteomics, optical and electron microscopy, and flow cytometry.







## Spanish Biology Olympics

CBM collaborates with the Spanish Biology Olympics, annually hosting national finalists. After the regional phases, the selected 6 students carry out a 5-day internship in our laboratories, engaging in scientific activities related to molecular biology and biotechnology. This initiative reflects our strong commitment to science outreach and support for young scientific talent.



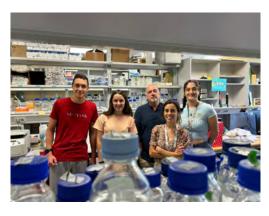


Scientific outreach CBM 2023-2024

## Científic@s en prácticas Program

The Científic@s en prácticas program is a CSIC initiative offering one-week research internships to 3rd-year ESO and 2nd-year PMAR students from disadvantaged backgrounds who show strong academic interest and effort. In 2023 and 2024, the CBM hosted several students in the research groups of María Gómez Vicentefranqueira and Miguel Manzanares. The program also includes school talks, research center visits, and the production of outreach videos in collaboration with trainees and the Complutense University of Madrid (UCM).









## **Europe Day**

We participate in this event organized by the network of CSIC centers on the UAM campus. In 2024 and again in 2025, our researchers with european granst and fellowships gave talks and collaborated with the Mujeres Nobel Escape Road scavenger hunt at the Eugenio Trías Library in Retiro.

CBM 2023-2024 Scientific outreach

## Researchers at Schools

As part of this project carried out in collaboration with the CSIC-UAM campus network of research centers, CBM researchers gave talks at selected schools chosen by the network, and we organized guided visits and workshops at our facilities for these same schools.

## Severo Ochoa Week

During the 1st Severo Ochoa Week, organized by the Royal Academy of Medicine of Spain in collaboration with the Carmen and Severo Ochoa Foundation, Nebrija University, and SEBBM, CBM researcher María Llorens received the Foundation's annual award. The ceremony featured a lecture by María Blasco, CBM alumna and former award recipient. The Week included a presentation on the CBM as an awardee of the Severo Ochoa Excellence certificate, and our strategy and research areas were outlined by our director, Paola Bovolenta, and three researchers (Ana Ortega, Miguel Mananares and José Antonio Esteban). Our early career researchers also participated in a reading session of Severo Ochoa's works. The CBM will continue to collaborate with this yearly initiative celebrating the life and legacy of our founder, Severo Ochoa.

## Science with Chocolate

An informative programme of talks, originally accompanied by chocolate, and now accompanied by a drink with friends at the Moe Club. The monthly talks are prganised by Enrique de la Rosa and CBM researcher Margarita del Val, and supported by the Centro de Biología Molecular Severo Ochoa, the Centro de Investigaciones Biológicas Margarita Salas, the Spanish Association of Scientists, the DRO Foundation, the Spanish Society of Immunology and the Royal Spanish Society of Chemistry.

### Pint of Science

Pint of Science is the largest free science outreach festival worldwide. In Madrid, over 80 talks took place in about a dozen bars, organized into six thematic areas ranging from neuroscience to law and art. Prominent CBM researchers participated in the festival in 2023 and 2024. Pint of Science Spain, active since 2015, has gathered over 110,000 attendees and continues to grow.

Scientific outreach CBM 2023-2024



# The following Facilities & Services, research groups and people have volunteered in our outreach activities during 2023-204.

RES	SEARCH GROUPS (PI)
Ana Ortega	Jose Felix de Celis
Antonio Alcamí	Jose María Requena y Begoña Aguado
Aura Carreira	Leonardo Beccari
Aurelio Hidalgo	Lola Ledesma
Belén Pérez	Manuel Fresno
Carlos Anerillas	Mar Ruiz y Joaquim Culí
Carlos Cabañas	Margarita del Val
Carlos Estella y Antonio Baonza	Margarita Sáiz Zalabardo
Claudio Toma	María Fernandez Lobato
Crisanto Gutiérrez	María Gómez Vicentefranqueira
David Ruano	María Jesús Bullido
Emilio Lecona Sagrado	María Llorens
Ernesto Sánchez Herrero	María Mittelbrunn
Esther Serrano	Mauricio García Mateu
Ginés Morata y Natalia Azpiazu	Miguel Manzanares
Isabel Guerrero	Nuria Gironès
Jaime Millán	Nuria Martínez Martín
Jesús Ávila de Grado	Paola Bovolenta
Jose Antonio Esteban	Yolanda Revilla

CBM 2023-2024 Scientific outreach



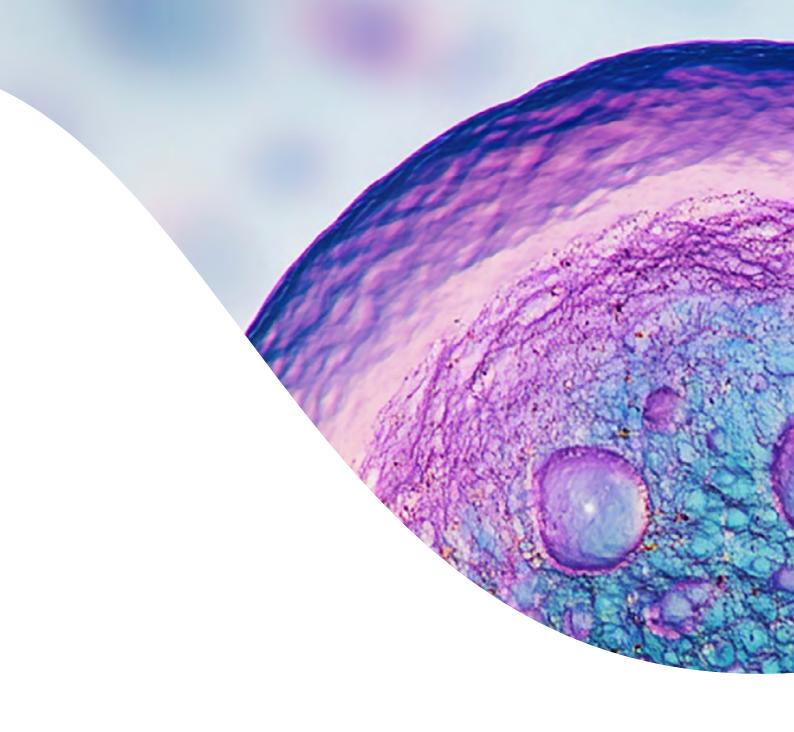
	FACILITIES AND SERVICES
Advanced Light Microscopy	
Animal facility	
Biocomputational Analysis	
Bioinformatics	
Biosafety and radioprotection	
Cell and Tissue Culture	
Drosophila transgenesis	
Electron Microscopy	
Flow Cytometry	
Genomics	
Proteomics	
Protein Biotechnology	



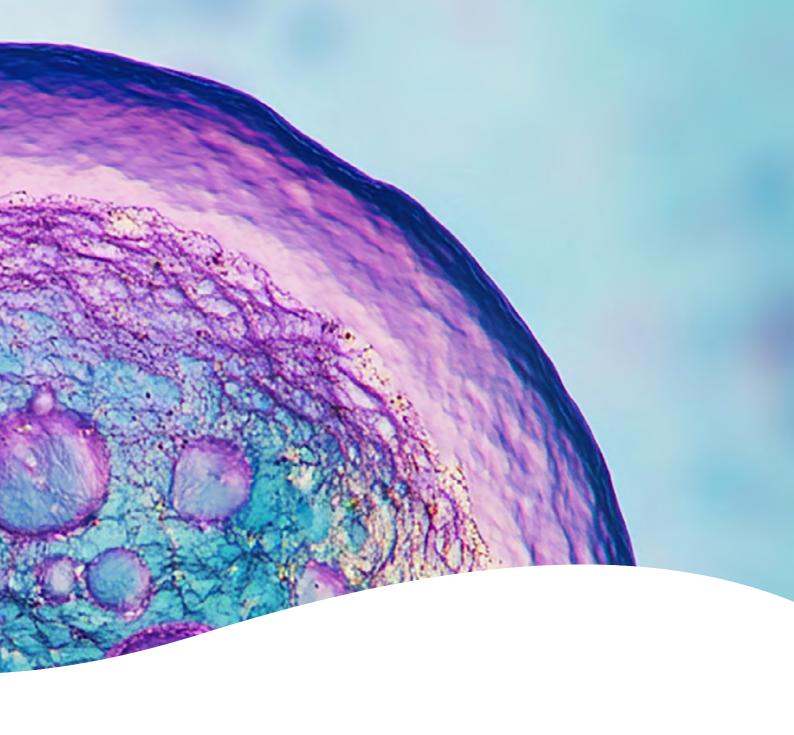
VOLUNTEERS		
Adrián Martínez Bonilla	Jorge Mañes García	
Adrián Pérez Ramos	Jose Antonio López Guerrero	
África Sanchiz	Juan Manuel García Arias	
Ainhoa Martínez Pizarro	Julia Emiliani	
Alberto Hernández	Julia Gata de Benito	
Alicia Gomez Moya	Lara Alvarez Rodriguez	
Alicia Vilas Lagoa	Laura Barahona Pérez	
Almudena Hernando	Laura Luna Gutiérrez Oliva	
Álvaro Arévalo	Laura Tabera	
Álvaro Sahun Español	Lucía Alvaro Aranda	
Ana Isabel de Ávila	Maite Rejas	
Ana Victoria Prádanos	Mar Casado García	
Andrés Soto Zaragoza	Mar Ruiz Gómez	
Antonio Alcamí	Marcos Martínez Baños	
Antonio Baonza Cuenca	Margarita del Val	
Arturo Martín Martínez	María Gimeno	
Aurelio Hidalgo	María Gómez Vicentefranqueira	
Beatriz Barrocal	Maria Isabel Carrascal Blanco	
Begoña Aguado	María Martín Rico	
Berta Raposo	Maria Martinez Ranz	
Blanca Estévez Santiago	María Santos Romero	
Blanca Rodríguez Mercader	María Sol Gómez	
Carlos Gallego García	María Tiana	
Carlos García	Marta Carrasco Rando	

CBM 2023-2024 Scientific outreach

VOL	LUNTEERS
Carmen María Sánchez-Valdepeñas Villegas	Marta Fierro
Carmen Sanchez Jimenez	Mercedes Dávila Cerrato
Celia García Vilela	Miguel Manzanares
César Castellanos Aguilar	Milagros Guerra
Clara Fernández Pardo	Milagros Guerra Rodríguez
David Abia	Mireya Ruiz Losada
David Foronda Álvaro	Miriam Martínez Jiménez
David Ruano Gallego	Miryam Polo Hernandez
Diana Karolina Santos Peñaloza	Mónica Gallego Bonhomme
Diego Martín Sanz	Natalia Azpiazu
Dionisio Ureña	Noemi Tabanera Anguita
Elena Calvo Cazalilla	Nuria Esteban
Elena Campos Sánchez	Patricia Vega Cuesta
Elena Rodríguez Bovolenta	Paula García
Elena Sánchez Bustamante	Paula Sánchez de Rojas de la Osa
Elisa Carrasco	Rafael Casado Navarro
Emilio Lecona Sagrado	Raquel Marco Ferreres
Esperanza Morato	Raquel Nieto Pintado
Esther Serrano	Rebeca Jimeno
Eva Padja Szeligowska	Rodrigo Martin
Eva Sacristán	Ruben Minguez
Fernando Carrasco	Samuel Donaire
Gabriela Atencia	Sandra González de la Fuente
Gema Caparrós de la Jara	Sara Tur Gracia
Iole Ferrara Romeo	Silvia Andrade
Jaime Millán	Silvia Andrade Calvo
Javier Álvarez de Miranda	Tamara Rosell
Javier Molina Hernández	Tania Matamoros Grande
Jesús Ávila de Grado	Teresa Villalba
Jesús Gómez Escudero	Valentín Riomoros Barahona
loana Segura Martinez	Violeta Enríquez Zarralanga



# Training activities



DATE	DOCTORAL STUDENT	PROGRAM	DIRECTOR	TITLE
24/01/2023	Lucía Pascual Antón	TISSUE AND ORGAN HOEMOSTASIS	Manuel L. Cabrera / Pilar Sandoval	La transición mesotelio-mesénquima como diana terapéutica común para el tratamiento de las adhesiones peritoneales postquirúrgicas y la metástasis peritoneal
21/02/2023	Daniel Ruiz Gabarre	PHISIOLOGICAL AND PATHOLOGICAL PROCESSES	Jesús Ávila	¿Tiene el gen MAPT algo nuevo que decir? Descubrimiento y caracterización de nuevas isoformas de la proteína tau y su relevancia en la enfermedad de Alzheimer
24/02/2023	Sergio López García	PHISIOLOGICAL AND PATHOLOGICAL PROCESSES	José A. Esteban	Role of P13-kinase regulatory subunit (p85) isoforms in synaptic plasticity
03/03/2023	Ivana Ollà	PHISIOLOGICAL AND PATHOLOGICAL PROCESSES	José J. Lucas	Alteración patológica del splicing de CPEB4 en esquizofrenia
09/03/2023	Belén Ortiz del Castillo	PHISIOLOGICAL AND PATHOLOGICAL PROCESSES	Petronila Penela	Role of GRK2 in orienting cell division and cell cycle decision making to proliferation
10/03/2023	Carlos Verdú Cano	INTERACTIONS WITH THE ENVIROMENT	José Berenguer	New insights on mechanisms and barriers against horizontal gene transfer in Thermus thermophilus
17/03/2023	Guadalupe Pereyra Gómez	TISSUE AND ORGAN HOEMOSTASIS	Paola Bovolenta	Upregulation of Sfrp1 expression causes hippocampal synaptic dysfunction and memory impairment
28/03/2023	Víctor Toribio Serrano	TISSUE AND ORGAN HOEMOSTASIS	María Yáñez-Mó	El papel de las tetraspaninas en la relación del aparato endosomal con el metabolismo celular y la identificación de nuevos mecanismos moleculares implicados en la captación de vesículas
30/03/2023	Carl Philip Lehmann	GENOME DYNAMICS AND FUNCTION	José A. Tercero	Spatial regulation of the central DNA damage tolerance protein Rad5 in response to cellular stress
14/04/2023	Alicia Vilas Lagoa	PHISIOLOGICAL AND PATHOLOGICAL PROCESSES	Belén Pérez	Desarrollo de modelos con fines diagnósticos y terapéuticos para la deficiencia de fosfomanomutasa 2
18/04/2023	Cristina Santa María Tobías	GENOME DYNAMICS AND FUNCTION	María Gómez	Role of chromatin conformation on transcriptional and post-transcriptional alterations of chromatin-RNAs
27/04/2023	Ana Villanueva García	INTERACTIONS WITH THE ENVIROMENT	Balbino Alarcón	Validation and characterization of new family of inhibitors for RAS-driven tumors
04/05/2023	Ivaylo Evgueniev Balabanov	INTERACTIONS WITH THE ENVIROMENT	Hisse van Santen	Recombinant T-cell receptors for cancer immunotherapy as a model for study of full TCR functions

DATE	DOCTORAL STUDENT	PROGRAM	DIRECTOR	TITLE
26/05/2023	Valeria Kopytina	TISSUE AND ORGAN HOEMOSTASIS	Manuel López Cabrera	New horizons in peritoneal dialysis: assessment of novel osmotic agents and evaluation of associated cardiovascular disease
30/05/2023	Elena Riera Laguna	INTERACTIONS WITH THE ENVIROMENT	Yolanda Revilla / Ricardo Madrid	Implicación de los genes virales I215L, A238L y EP402R del Virus de la Peste Porcina Africana en la modulación de la respuesta inmune innata
02/06/2023	Antonio Barral Gil	TISSUE AND ORGAN HOEMOSTASIS	Miguel Manzanares	Control of developmental transitions by OCT4: A new role as a regulator of mTOR activity at gastrulation
19/06/2023	Laura Arribas Carreira	PHISIOLOGICAL AND PATHOLOGICAL PROCESSES	Pilar Rodríguez Pombo	Implicaciones del Metabolismo de la Glicina en la Salud y la Enfermedad Humana: Hiperglicinemia no Cetósica, una Enfermedad con Fenotipo Clínico Neurológico
21/06/2023	Raquel de Andrés Hernaiz	PHISIOLOGICAL AND PATHOLOGICAL PROCESSES	Javier Díez Guerra	Estudio de la regulación de la expresión de Neurogranina por actividad sináptica
23/06/2023	Andrés Vicente Acosta	PHISIOLOGICAL AND PATHOLOGICAL PROCESSES	Javier Díaz Nido / Frida Loria Salinas	Efectos de la deficiencia de frataxina en astrocitos y su modulación por el agonista de Sonic hedgehog SAG
13/07/2023	Ana Martínez Carrón	GENOME DYNAMICS AND FUNCTION	Luis Blanco / Ángel Picher	Optimisation and alternatives to the DNA amplification system TruePrime®
14/07/2023	Miguel de la Flor García	PHISIOLOGICAL AND PATHOLOGICAL PROCESSES	María Llorens	Neurogénesis hipocampal adulta en seres humanos. Efectos del envejecimiento y las enfermedades neurodegenerativas
04/09/2023	Cristina Segovia Falquina	PHISIOLOGICAL AND PATHOLOGICAL PROCESSES	Belén Pérez / Alejandra Gámez	Generación de una plataforma de análisis funcional de variantes en PMM2 con fines diagnósticos y terapéuticos
22/09/2023	Patricia Torres Gérica	INTERACTIONS WITH THE ENVIROMENT	Manuel Fresno	Role of galectin-1 in adipose tissue development and adipocyte metabolism
28/09/2023	Alejandro Miguel Hortal Borowski	INTERACTIONS WITH THE ENVIROMENT	Balbino Alarcón / Clara Oeste	Overexpression of wild type RRAS2, without oncogenic mutations, drives chronic lymphocytic leukemia
06/10/2023	Teresa García Prieto	INTERACTIONS WITH THE ENVIROMENT	Manuel Fresno	Akt isoforms and TLR5 in intestinal barrier function and inflammatory bowel disease / Las isoformas de Akt y TLR5 en la función de la barrer intestinal y la enfermedad inflamatoria intestinal

DATE	DOCTORAL STUDENT	PROGRAM	DIRECTOR	TITLE
10/10/2023	David Piedrabuena Estrada	INTERACTIONS WITH THE ENVIROMENT	María Fernández Lobato / Josefa Hernáiz	Producción sostenible de nuevos glicoconjugados con potenciales propiedades prebióticas mediante la β-fructofuranosidasa Ffase de Schwanniomyces occidentalis
16/10/2023	Marina Rodríguez Rubio	PHISIOLOGICAL AND PATHOLOGICAL PROCESSES	Marta Pérez Pereira	Bioengineering of brain organoids
26/10/2023	Raquel Felipe Mendía	PHISIOLOGICAL AND PATHOLOGICAL PROCESSES	Beatriz López Corcuera	Regulación y modificación del transportador neuronal de glicina GlyT2
27/10/2023	Inés Romero Carramiñana	PHISIOLOGICAL AND PATHOLOGICAL PROCESSES	José Manuel Cuezva	IF1 como regulador de la función mitocondrial y su implicación en el sistema inmune
27/10/2023	Vicente Castillo Mancho	TISSUE AND ORGAN HOEMOSTASIS	Mar Ruiz / Joaquín Culí	Caracterización del interactoma de Scramb1, un organizador del ensamblaje del diafragma de filtración en zonas de membrana de tipo balsa lipídica
02/11/2023	Cristina Martínez Ostalé	TISSUE AND ORGAN HOEMOSTASIS	José F. de Celis	Regulación de la transcripción mediada por los factores de transcripción Spalt en Drosophila melanogaster"
03/11/2023	Cristina Velázquez Ruiz	GENOME DYNAMICS AND FUNCTION	Luis Blanco / María Martínez	he 3´dNTP binding of human PrimPol and the impact of cancer variants
10/11/2023	Fátima Bayón Calderón	INTERACTIONS WITH THE ENVIROMENT	M. Luisa Toribio / Sara González	Células CAR-T pre-TCR específicas como nueva estrategia inmunoterapéutica frente a la leucemia linfoblástica aguda de células T
16/11/2023	Jorge Martínez Cano	INTERACTIONS WITH THE ENVIROMENT	César Cobaleda	Regulación del metabolismo mitocondrial y la diferenciación normal y patológica de los linfocitos B por Wolf- Hirschhorn Syndrome Candidate 1
21/11/2023	Javier Lucas de la Fuente	GENOME DYNAMICS AND FUNCTION	Ignacio Flores	Efectos de la sobrexpresión moderada de la telomerasa en el corazón del ratón neonatal durante homeostasis y tras lesión
24/11/2023	Elena Moreno Jiménez	PHISIOLOGICAL AND PATHOLOGICAL PROCESSES	María Llorens	Proceso de neurogénesis hipocampal adulta en la especie humana. Alteración de la integración de las nuevas neuronas granulares en condiciones patológicas
15/12/2023	Ángela Albitre Sanz	PHISIOLOGICAL AND PATHOLOGICAL PROCESSES	Petronila Penela / Federico Mayor	GRK2 networks in mammary gland development and tumorigenesis

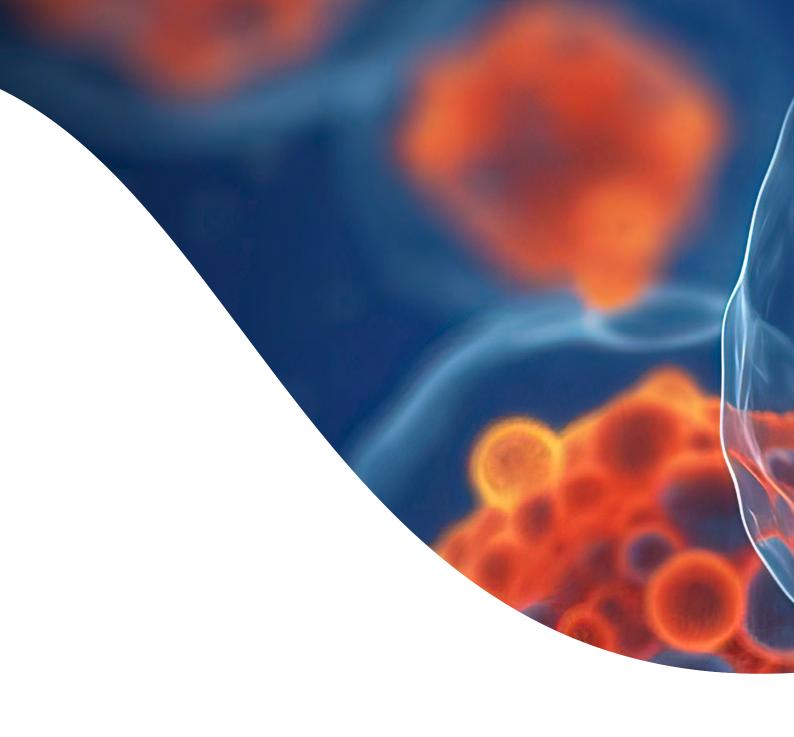
DATE	DOCTORAL STUDENT	PROGRAM	DIRECTOR	TITLE
10/01/2024	Eva Mª Arriero	TISSUE AND ORGAN HOEMOSTASIS	Manuel López Cabrera	Machine–Learning– and biomarker– based predictor and diagnosis tool (MAUXI) for the Cardiovascular Outcome and Ultrafiltration failure induced by the Mesothelial–to– Mesenchymal transition in Peritoneal Dialysis
02/02/2024	Lydia B. Hörndler Gil	IINTERACTIONS WITH THE ENVIROMENT	Hisse van Santen	Development and optimization of a combinatorial CAR-T cell therapy for AML to overcome on-target-off tumor responses
09/02/2024	Gonzalo Soto Heredero	TISSUE AND ORGAN HOEMOSTASIS	María Mittelbrunn	Caracterización de los linfocitos T asociados a la edad y su contribución al envejecimiento
15/02/2024	Marta García Juan	PHISIOLOGICAL AND PATHOLOGICAL PROCESSES	Francisco Wandosell / Lara Ordóñez	Análisis de los mecanismos que regulan la autofagia en astrocitos y su papel en la eliminación del péptido beta-amiloide
07/03/2024	Yilin Sun	TISSUE AND ORGAN HOEMOSTASIS	Miguel R. Campanero / Juan M. Redondo	Contribución de la actividad independiente de fosfatasa de la calcineurina de las células del músculo liso a la hipertensión arterial
08/03/2024	Gema Cerro Tello	TISSUE AND ORGAN HOEMOSTASIS	Jaime Millán / Jade Louber	Organización del citoesqueleto de actina en las barreras celulares de la córnea y su respuesta al estrés. Búsqueda de biomarcadores de queratocono
05/04/2024	Claudia Cifuentes Caballero	IINTERACTIONS WITH THE ENVIROMENT	Balbino Alarcón	Role of <i>RRAS2</i> overexpression behind breast cancer development
19/04/2024	Coral López Fonseca	PHISIOLOGICAL AND PATHOLOGICAL PROCESSES	Eva Porlan	Efectos de la quinasa Plk1 en la regulación de factores de transcripción proneurales
19/04/2024	Beatriz Benayas López	TISSUE AND ORGAN HOEMOSTASIS	María Yáñez-Mó / Pilar Armisén	Desarrollo y optimización de métodos cromatográficos para el aislamiento de vesículas extracelulares y su uso en biopsia líquida
26/04/2024	Víctor Muñoz Abad	IINTERACTIONS WITH THE ENVIROMENT	Luis Antón / Margarita del Val	Identification of translocation pathways alternative to the transporter TAP involved in antigen presentation to CD8+T lymphocytes
10/05/2024	Cristina Rodríguez Rojas	IINTERACTIONS WITH THE ENVIROMENT	Margarita del Val	Role of TAP and proteasomes in antigen presentation during cytomegalovirus infection
17/05/2024	Alberto Hernández Alcántara	TISSUE AND ORGAN HOEMOSTASIS	Miguel R. Campanero	Requerimientos biomecánicos para el crecimiento de los tumores linfoides

DATE	DOCTORAL STUDENT	PROGRAM	DIRECTOR	TITLE
23/05/2024	Diana Gallego Martínez	PHISIOLOGICAL AND PATHOLOGICAL PROCESSES	Belén Pérez	Genómica funcional aplicada a la identificación de dianas terapéuticas en enfermedades metabólicas hereditarias
04/06/2024	Alejandro Asensio López	PHISIOLOGICAL AND PATHOLOGICAL PROCESSES	Federico Mayor / Catalina Ribas	Role of GRK2 in epidermal homeostasis
05/07/2024	Elena Martínez Blanco	PHISIOLOGICAL AND PATHOLOGICAL PROCESSES	F. Javier Díez Guerra	Papel de Neurogranina en la regulación de la señalización de Calcio/Calmodulina y su impacto en la plasticidad neuronal
09/07/2024	Marta Portela Martínez	TISSUE AND ORGAN HOEMOSTASIS	Miguel Manzanares	Genome replication and early lineage choices during mouse embryonic development
12/07/2024	Elena Terraza Silvestre	IINTERACTIONS WITH THE ENVIROMENT	Felipe Pimentel	Identificación de una ruta autofágica no convencional que reprime la liberación de atp durante la muerte celular apoptótica
30/07/2024	Alba Fernández	PHISIOLOGICAL AND PATHOLOGICAL PROCESSES	José A. Esteban / M. Isabel Cuartero	Role of PI3K isoforms in linking synaptic plasticity and metabolism in neurons and astrocytes
11/10/2024	Laura Vela Martín	GENOME DYNAMICS AND FUNCTION	María Villa Morales / José L. Marín Rubio	Estudio de las bases moleculares subyacentes a las neoplasias de células T precursoras para aplicar la medicina personalizada: caracterización de la fusión SEPTIN6::ABL2 como nueva diana terapéutica e identificación del gen AHR como biomarcador de respuesta al tratamiento
14/10/2024	Alfonso Herreros Cabello	IINTERACTIONS WITH THE ENVIROMENT	Manuel Fresno / Núria Gironés	Omics approaches for the biology and genetics of <i>Trypanosoma</i> cruzi and the definition of biomarkers in Chagas disease
18/10/2024	Javier Merino Valverde	IINTERACTIONS WITH THE ENVIROMENT	Manuel Fresno	Atypical lipopolysaccharides: Structure, Biological Activity and Potential Clinical Applications
23/10/2024	Viviana Marolda	PHISIOLOGICAL AND PATHOLOGICAL PROCESSES	Federico Mayor	GRK2-Associated Protein Networks in Breast Cancer Cell Interplay with the Tumour Microenvironment
25/10/2024	Berta Alcover Sánchez	PHISIOLOGICAL AND PATHOLOGICAL PROCESSES	Beatriz Cubelos	Papel de R-Ras1 y R-Ras2 en la diferenciación y maduración oligodendrocitaria
31/10/2024	Juan Manuel García	TISSUE AND ORGAN HOEMOSTASIS	Ginés Morata	Análisis de la función de la vía c-Jun N-terminal quinasa en la inducción de senescencia celular y la tumorogénesis en <i>Drosophila melanogaster</i>

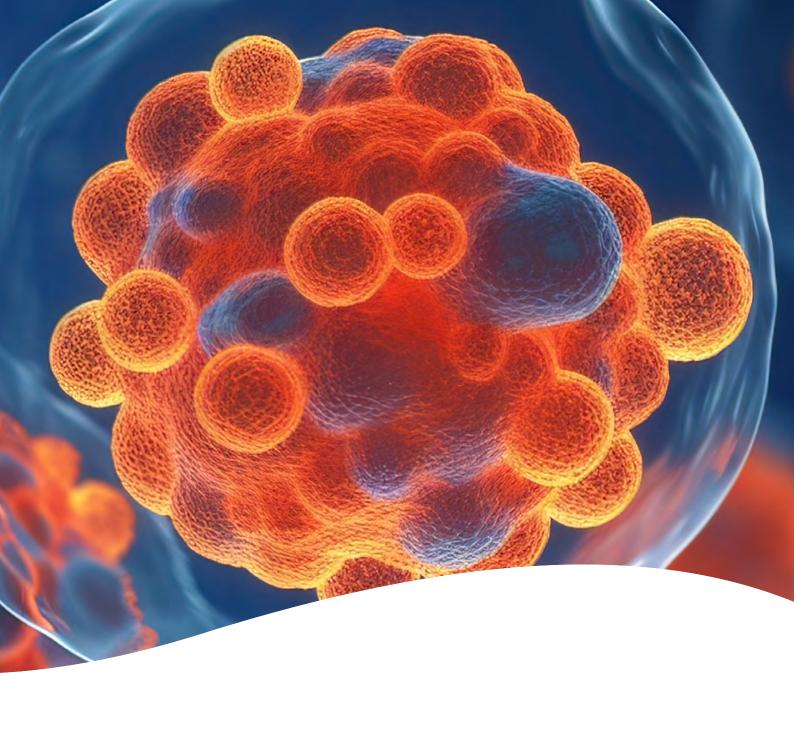
Training activities

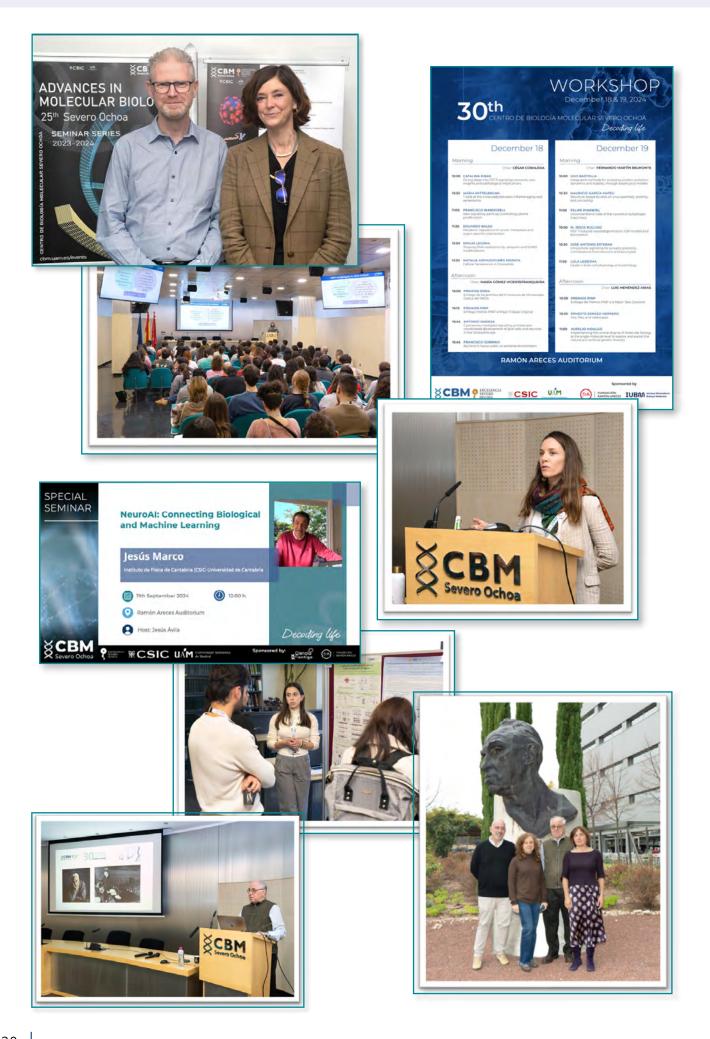
## DOCTORAL THESES

DATE	DOCTORAL STUDENT	PROGRAM	DIRECTOR	TITLE
19/11/2024	David Ros Pardo	IINTERACTIONS WITH THE ENVIROMENT	Paulino G. Puertas	Homology modeling and molecular dynamics techniques in the study of the functionality of GSDMB and the cohesin complex
22/11/2024	Patricia Vega Cuesta	TISSUE AND ORGAN HOEMOSTASIS	José F. de Celis	Generación y caracterización de alelos mutantes Ras1 <sup>v12</sup> en el locus Ras1 de <i>Drosophila</i>
22/11/2024	Gloria Pastor Fernández	IINTERACTIONS WITH THE ENVIROMENT	María N. Navarro Lobato	Papel de las quinasas mTORC1 y AMPK en la cascada de señalización de Interleuquina 23
05/12/2024	Carlos Jiménez Jiménez	TISSUE AND ORGAN HOEMOSTASIS	Isabel Guerrero	Estudio de los procesos de liberación, transporte y recepción de Hedgehog en <i>Drosophila</i>
10/12/2024	Rady Chaaban	GENOME DYNAMICS AND FUNCTION	Aura Carreira	Coordination of the chromosome integrity functions of PLK1:BRCA2:PP2A throughout the cell cycle
13/13/24	Gonzalo Herranz	TISSUE AND ORGAN HOEMOSTASIS	Fernando Martín Belmonte	Caracterización de las propiedades fisiológicas y metabólicas de los enterocitos durante la etapa neonatal



# Seminars and lectures





Seminars and lectures



# **SEVERO OCHOA SEMINAR SERIES 2023 & 2024**

DATE	NAME	CENTER	TITLE
03/02/2023	Tristán Rodríguez	Faculty of Medicine, National Heart & Lung Institute. Imperial College London. London, UK	Cell competition and how cells deal with their stressed neighbours
10/02/2023	David Rubinsztein	Cambridge Institute of Medical Research, Cambridge, UK	Autophagy and neurodegeneration
24/02/2023	Adriana Maggi	Univeristy of Milan, Italy	Metabolism:the issue of gender
14/04/2023	Lluís Ribas	Institut de Recerca Biomèdica, IRB Barcelona	The Genetic Code: origin, functional limits, and biotechnology
21/04/2023	Oliver Hobert	Department of Biological Sciences. Columbia University of New York. NY. USA	Organizational principles of nervous system specification in the nematode C. elegans
27/06/2023	David Sancho	Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madri	Connecting myeloid cell metabolism and function
14/09/2023	Haruhiko Bito	Department of Neurochemistry, University of Tokio, Tokio, Japan	Capturing and measuring neuronal circuit dynamics in active ensembles
06/10/2023	Carola Vinuesa	The Crick Institute, London, UK	How genomic medicine freed Kathleen Folbigg after 20 years in prison
27/10/2023	Sjoerd Wanrooij	Umeå University, Umeå, Sweden	The Role of G4 Structures in human Mitochondrial DNA instability
03/11/2023	Bruno Silva-Santos	Instituto de Medicina Molecular (IMM), University of Lisbon, Portugal	Molecular mechanisms of differentiation and activation of effector gamma-delta T cell subsets
17/11/2023	Irene Miguel Aliaga	MRC Laboratory of Medical Sciencies, Imperial College London, London, UK	The sex and geometry of organs

# SEVERO OCHOA SEMINAR SERIES 2023 & 2024

DATE	NAME	CENTER	TITLE
26/01/2024	Álvaro Rada Iglesias	Instituto de Biomedicina y Biotecnología de Cantabria, Santander	Synergistic insulation of regulatory domains by developmental genes and clusters of CTCF sites
02/02/2024	María de la Fuente Freire	nstituto de Investigación Sanitaria de Santiago de Compostela (IDIS), La Coruña, Spain	From lab to market: Nanomedicine transforming healthcare by addressing unmet clinical needs
23/02/2024	Sebastian Jessberger	Brain Research Institute, University of Zürich, Zürich, Switzerland	Molecular and celular principles of hipocampal neurogénesis across the adult mouse lifespan"
01/03/2024	Burkhard Becher	Institute of Experimental Immunology, University of Zurich, Switzerland	IL-12 and IL-23: paradoxes in inflammation
08/03/2024	Michela Matteoli	Humanitas University, Milan, Italy	How the immune system shapes synapses
25/04/2024	Sarah-Maria Fendt	VIB KU Leuven Center for Cancer Biology. Leuven, Belgium	Metabolic Rewiring Driving Metastasis Formation
07/06/2024	Madalena Tarsunas	Department of Oncology, University of Oxford, Oxford, UK	TOP1-induced DNA Damage Underlies PARP Inhibitor Sensitivity in BRCA1-deficient Cells
25/10/2024	Marcin Nowotny	International Institute of Molecular of Cell Biology (IIMCB) Warsaw, Poland	Mechanisms of bacterial DNA repair
29/11/2024	Johannes Meiser	Luxembourg Institute of Health, Luxembourg	Functions of Formate in Cancer

DATE	NAME	CENTER	TITLE
10/01/2023	Vicente Pérez García	Centro de Investigación Príncipe Felipe, Valencia	Understanding the links between placenta development and cancer
17/01/2023	Daniel Abankwa	Cancer Cell Biology at the University of Luxembourg	Novel PDE6D inhibitors block K-Ras trafficking to stemness defining organelles
15/02/2023	César Augusto Ramírez Segura	Unidad de Ingeniería Celular y Molecular del IDCBIS, Bogotá	Diseño de una proteína para bloquear la infección por SARS- CoV2
21/02/2023	Isabel Fabregat	Bellvitge Biomedical Research Institute (IDIBELL). CIBER de Enfermedades Hepáticas y Digestivas (CIBEREHD)	TGF-beta and NADPH oxidases: a perverse association in liver fibrosis and cancer
02/03/2023	Tatiana Hurtado de Mendoza	University of California San Diego	Targeting Neoantigens to solid tumors to enable immunotherapy
16/03/2023	Ana Ruiz Sáenz	Department of Cell Biology. Erasmus MC. The Netherlands)	Overcoming cancer resistance to targeting therapies
21/03/2023	Luis Alberto Baena- López	Medical Sciences Division. Oxford University. Oxford, UK	From "killing me softly" to "I will survive": Caspase activation sustains basic cellular functions
17/04/2023	Aleix Bayona Feliú	Institute for Research in Biomedicine (IRB BARCELONA), Barcelona (Spain)	New roles of the chromatin network in the maintenance of genome stability
19/04/2023	Nicolás Gutiérrez	Champalimaud Foundation, Lisboa, Portugal	A hypothalamic node for the cyclical control of female sexual rejection
19/05/2023	Marie-Noëlle Prioleau	Institut Jacques Monod, Paris, France	Spatiotemporal control of DNA replication in vertebrates
22/06/2023	Mateo Sánchez	King's College London. London, UK)	Directed evolution of molecular tools for cell biology and neuroscience
28/06/2023	Manuel Daza Martín	The Institute of Cancer Research. London, UK	When DNA becomes its own enemy
11/09/2023	Alon Monsonego	Ben-Gurion University of the Negev. Beer Sheva, Israel	T cells in the biology of aging and Alzheimer's disease

DATE	NAME	CENTER	TITLE
26/09/2023	Marco Milán	Institute for Research in Biomedicine (IRB Barcelona)	Tissue Biology of Chromosomal Instability: insights from Drosophila
02/10/2023	Jordi Casanova	Institut de Biologia Molecular de Barcelona; IRB Barcelona)	A story of life and death: cell plasticity, autophagy and stress protection
24/10/2023	Álvaro Galvic Maurer	Universidad de Chile)	Epigenetic effects of low- nutrient diet in the adult brain of Drosophila
12/12/2023	Manuel Irimia	Centre for Genomic Regulation (CRG) and Universitat Pompeu Fabra (UPF), Barcelona)	Evolution of tissue-specific transcriptomes across bilaterians
14/12/2023	Carlos Matute	Achucarro Basque Center for Neuroscience, University of Basque Country (UPV/EHU) and CIBERNED	Is brain myelin an energy source/ store?

DATE	NAME	CENTER	TITLE
06/02/2024	Antonio Giraldez	Department of Genetic, University of Yale, USA	From development to therapeutic mRNAs: Combining genomics and expansion microscopy to understand the basic mechanisms of transcription and translation
08/02/2024	Alessio Delogu	Department of Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology and Neuroscience, King's College London, UK	Seeding the thalamus with inhibition: a developmental perspective of GABA interneurons in the rodent thalamus
13/02/2024	Iván Plaza	Spanish National Cancer Research Centre (CNIO). Madrid, Spain	Structure, function and pharmacology of (oncogenic) protein kinases
15/02/2024	Josefa González	Instituto de Biología Evolutiva, Barcelona, Spain	Transposable elements drive regulatory, coding, and epigenetic variation in Drosophila natural populations
16/02/2024	Tomohiro Kurosaki	University of Osaka, Japan	Regulation of humoral memory compartments
24/04/2024	Sonia Ferández Veledo	Institut d'Investigació Sanitària Pere Virgili (IISPV) Hospital Universitari Joan XXIII, Tarragona CIBERDEM - Universitat Rovira Virgili (URV)	Beyond Expectations: Unlocking New Physiological Functions of the succinate G-Protein-Coupled Receptor SUCNR1
07/05/2024	Javier S. Burgos	Unidad Predepartamental de Medicina. Facultad de Ciencias de la Salud. Universitat Jaume I	aRukon, una nueva solución tecnológica para la reutilización racional y valorización de muestras de experimentación animal
04/06/2024	Alberto Bartolomé	Instituto de Investigaciones Biomédicas Sols-Morreale (IIBM) CSIC-UAM	$\beta$ cell mass and determinants of $\beta$ cell dysfunction: a history of mice and men
13/05/2024	Paula Jáuregui	Senior Editor, Nature Immunology and Nature Microbiology)	Publishing at Nature Research Journals
18/06/2024	Pablo Guasp	Memorial Sloan Kettering Cancer Center, NY.	Personalized mRNA vaccines induce long-lived functional CD8+ T cells in pancreatic cancer
26/06/2024	Carlos Chillón- Marinas	Department of Cellular and Molecular Medicine, University of California at San Diego, La Jolla, CA, USA	Special Spatial Transcriptomics to understand Huntington's Disease and generation of new neurons in the adult brain

DATE	NAME	CENTER	TITLE
11/09/2024	Jesús Marco	Instituto de Física de Cantabria	NeuroAI: Connecting Biological and Machine Learning
13/09/2024	Moisés Mayo	Instituto Gulbenkian Ciência, Oieras, Portugal	Legs or genitals? Ask Tgfbr1
17/09/2024	Elena Bossi	Department of Biotechnology and Life Sciences and Biotechnology, University of Insubria, Italy	Unraveling the Relevance of Heterologous Expression in Xenopus Oocytes in the Modern Era of Membrane Transporter Studies: Investigating GABA and Betaine Transport by SLC6A1
17/10/2024	Cristina González Aguilera	Centro Andaluz de Biología Molecular & Medicina Regenerativa (CABIMER), Sevilla, Spain	Chromatin replication as a source of gene expression variability
22/10/2024	Alicia González Martín	Instituto Investigaciones Biomédicas Sols-Morreale (CSIC- UAM, Madrid, Spain	Deciphering microRNA control of B cell tolerance
29/10/2024	Vanesa Lafarga	Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid, Spain	Exploring the rfole of nucleolar stress in ageing and neurodegeneration
31/10/2024	Mario Corrado	CECAD Research Center, University of Cologne, Germany	Trick or Treat - Twisting immunity with metabolism
12/11/2024	Miguel Bernabé Rubio	Centre for Gene Therapy and Regenerative Medicine, King's College London. London, UK	Mechanisms of dedifferentiation: Linking injury-induced plasticity to cancer initiation
14/11/2024	Mario Enrique Zurita Ortega	Instituto de Biotecnología. Universidad Nacional Autónoma de México. Cuernavaca, México	The Response to Transcriptional Stress and Long Non-Coding RNAs
20/11/2024	Antonio Alcamí	Centro de Biología Molecular Severo Ochoa, Madrid, Spain	Expedición Científica a la Antártida en busca de gripe aviar
03/12/2024	Miguel Vicente- Manzanares	Instituto de Biología Molecular y Celular del Cáncer/CSIC, Salamanca, Spain	Novel modes of funcional regulation of non-muscle myosin Il by point mutations, anti-mitotic chemotherapy and during T cell activation
11/12/2024	Daniele Fachinetti	Institut Curie, Paris, France	Causes and consequences of centromeric instability

# JOB SEMINARS 2023 & 2024

DATE	NAME	CENTER	TITLE
19/09/2023	Carlos Pardo Pastor	Randall Centre for Cell & Molecular Biophysics, King's College London	Tales from the bending machine: Piezo1 and clathrin-mediated endocytosis
27/11/2023	Hernán López Schier	New York University Abu Dhabi, Abu Dhavi, UAE	Long-term morphostasis in a regenerating organ
		• 2024 •	
10/01/2024	Carolina Villarroya Beltri	Spanish National Cancer Research Centre (CNIO). Madrid, Spain	Targeting aneuploidy to beat cancer
29/04/2024	María Fernández Rubín de Celis	Beth Israel Deaconess Medical Center / Harvard Medical School	Modulation of cellular senescence as a new strategy to treat metabolism-related diseases
09/07/2024	Juan A. Torreño-Piña	Centre for Genomic Regulation (CRG), Barcelona	Early steps of individual multi- receptor viral interactions dissected at the single virus level
17/09/2024	Sergio Juárez-Carreño	Institute for Research in Biomedicine (IRB), Barcelona	Macrophages as Master Regulators of Inter-Organ Communication in Physiology and Disease
24/09/2024	Katernina Karcali	Centre for Genomic Regulation (CRG), Barcelona	Structural integration in Drosophila CNS Development and Homeostasis

# **MEMORIAL LECTURES 2023 & 2024**

NAME	CENTER	TITLE
III MARGARITA SALAS MEMORIAL LECTURE	Stephen Kowalczykowski (Department of Molecular and Cellular Biology University of California, Davis, CA, USA)	Watching DNA Recombination and Replication, One Molecule at a Time: It's not the way textbooks show
XXIX SEVERO OCHOA MEMORIAL LECTURE	Christoph Hess (Cambridge University, Cambridge, UK)	Epstein Barr Virus - searching for a metabolic Achilles' heel
	• 2024 •	
IV MARGARITA SALAS MEMORIAL LECTURE	Andrew Jackson (MRC Human Genetics Unit Institute of Genetics and Cancer, University of Edinburth, UK	Insights from the smallest people in the world: AI, DNA replication, epigenetics and stem cells
XXX SEVERO OCHOA MEMORIAL LECTURE	Alfonso Martínez Arias (Universitat Pompeu Fabra, Barcelona, Spain)	Mammalian gastrulation: a pluripotent stem cell perspective
	III MARGARITA SALAS MEMORIAL LECTURE  XXIX SEVERO OCHOA MEMORIAL LECTURE  IV MARGARITA SALAS MEMORIAL LECTURE  XXX SEVERO OCHOA	III MARGARITA SALAS MEMORIAL LECTURE Cellular Biology University of California, Davis, CA, USA)  XXIX SEVERO OCHOA MEMORIAL LECTURE  IV MARGARITA SALAS MEMORIAL LECTURE  Andrew Jackson (MRC Human Genetics Unit Institute of Genetics and Cancer, University of Edinburth, UK  XXX SEVERO OCHOA MEMORIAL LECTURE  Alfonso Martínez Arias (Universitat Pompeu Fabra,



























## CENTRO DE BIOLOGÍA MOLECULAR SEVERO OCHOA

C/ Nicolás Cabrera, 1 28049 Madrid