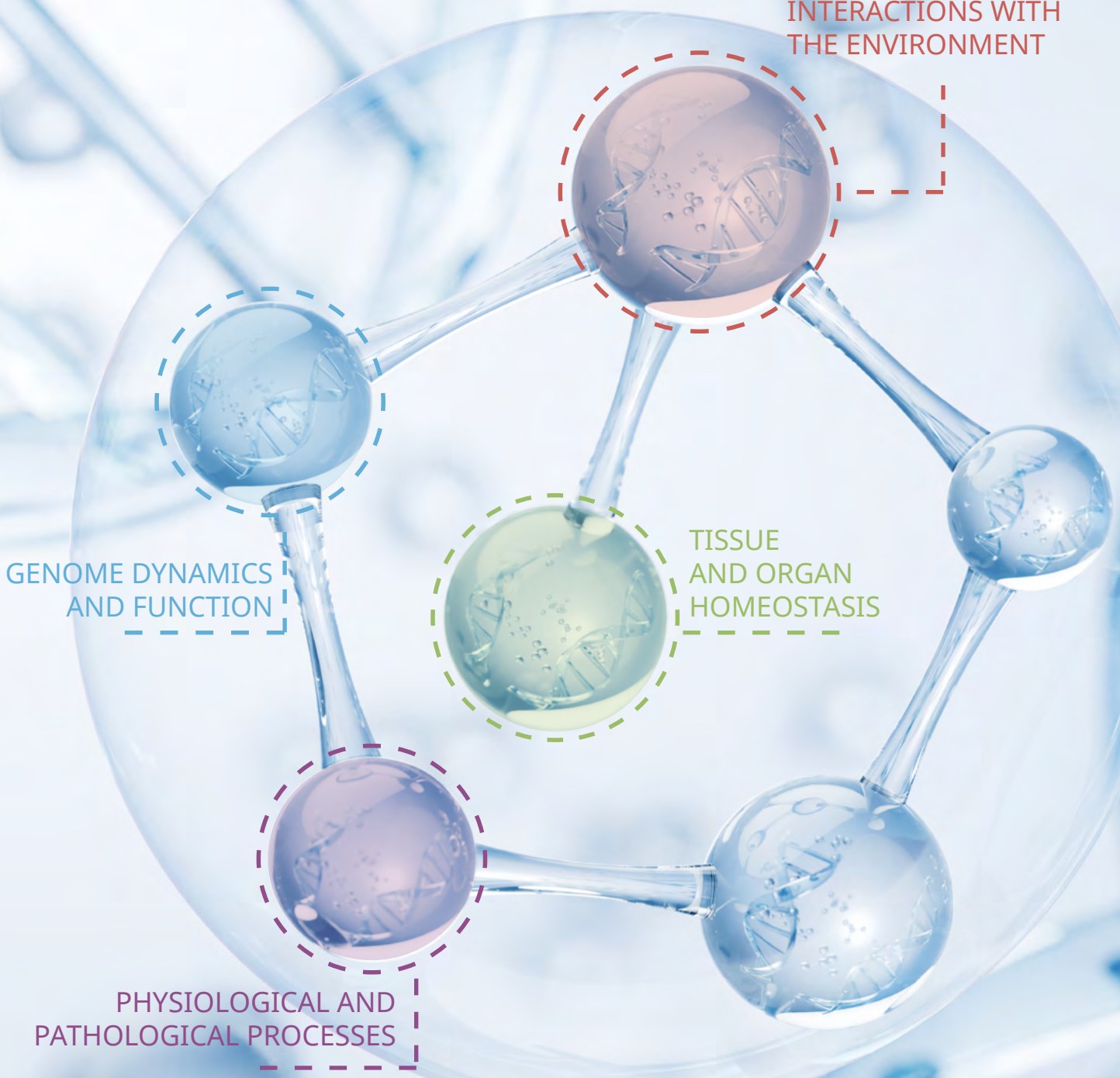
SCIENTIFIC REPORT
2021-2022

CBM Severo Ochoa
CENTRO DE BIOLOGÍA MOLECULAR
CSIC UAM

CENTRO DE BIOLOGÍA MOLECULAR
SEVERO OCHOA



2021-2022

SCIENTIFIC REPORT 2021 - 2022

INDEX

DIRECTOR REPORT

Lourdes Ruiz
CBM Director

Dear colleagues and friends,

I am pleased to present the scientific report 2021-22 of the Centro de Biología Molecular Severo Ochoa (CBM), giving an overview of the events and scientific and strategic activities during this period. This will be my last contribution that I write as Director, as on March 2023 Paola Bovolenta has been designated CBM Director, after being elected by the CBM General Board (Claustro Cientifico) and proposed by the Governing Board (Junta de Gobierno).

In 2021-2022 we have started to see the fruits of the work carried out in the past few years, thanks to the changes in the scientific organization and the recruitment of promising scientists, young PIs as well as senior consolidated groups, adding their expertise in state-of-the-art technological and scientific approaches. We have seen a significant improvement in research outputs in terms of high-quality publications as well as in fund-raising, with several relevant EU-funded and national and regional grants obtained. Scientific projects focused in the COVID19 pandemics have continued during these two years, adding to the knowledge of the biology of the virus, its airborne detection, immune response generated and the development of diagnostic and potential treatment strategies. One of our researchers coordinates the CSIC Interdisciplinary Thematic Platform known as Global Health Platform against COVID-19 pandemic. The Genomics facility has been performing diagnostic PCRs for COVID19 for CSIC personnel, in collaboration with CSIC Occupational Health Service.

Of note, and closely related to the COVID19 pandemics and the development of new vaccines, several CBM researchers have been very active in science dissemination and communication to the lay public in mass media and have received prestigious awards for this activity. Also, in 2022, we launched a specific science communication event with the support of Comunidad de Madrid, "Ciencia Contigo", carried out at the Residencia de Estudiantes in Madrid, that was highly successful in terms of the number of attendees and the feedback and comments in social media.

We have continued with our strong commitment to training, exemplified by the more than 48 predoctoral students who defended their PhD Thesis with the highest marks in 2021-22, 65 Master Thesis and more than 100 Final Degree Projects carried out supervised by CBM researchers. A total of 147 seminars and conferences by prestigious national and international researchers have taken place in these two years.

In 2021 the CBM Equality Working Group was established and, working in close contact with CSIC and UAM Equality Committees, carried out specific dissemination and other activities to ensure and foster inclusiveness and equal opportunities for all in CBM activities. As a result of this, CBM received the CSIC Equality Distinction Award 2021.

I would also like to mention that CBM researchers have received different awards in this period, underlying those granted to young researchers, as is the case of the National Research Award "Gabriela Morreale", in Medicine and Health Sciences granted to María Llorens and the "Margarita Salas Award" from Comunidad de Madrid granted to Ana Ortega.

Finally, thanks to the excellent research outputs, and a very well-defined strategic plan for the next four years, focused on the Interplay among Immunity, Inflammation and Metabolism (I3M), CBM obtained the recognition as Severo Ochoa Centre of Excellence in the 2021 Call, with Paola Bovolenta as Scientific Director. This represents an excellent opportunity for the CBM to consolidate a leading position in the Spanish research landscape, which I am confident will be achieved. All the proposed actions and the commitment and excellent work of researchers and personnel in scientific and technical services undoubtedly ensure success in this aim.

I have had the privilege of serving the CBM as Director for the past four years. I had never anticipated that I would one day be Director when I completed my PhD research in this centre (a too long time ago!). I have always been aware of the scientific excellence of the CBM, but during these years I have had the opportunity to realize the complexity and the impressive efficiency of the scientific, technical, administrative and management staff in carrying out "housekeeping" activities, that allow investigators to perform their research and attain their scientific objectives.

I would like to thank all the many people who have helped me along, each in their own corresponding sphere of activity and responsibility, with special thanks to the Vice-director Jaime Millán. I am grateful to have had this time serving as Director and I am excited with the prospect of continuing to be part of the CBM community going forward.

Lourdes Ruiz
CBM Director

IN MEMORIAM

PROF. JUAN MODOLELL (1937-2023)

On February 28th, 2023, Juan Modolell, ad honorem Professor of Research of the Spanish National Research Council (CSIC), passed away in Madrid at age 85. Juan was born in Barcelona in 1937. He obtained two bachelor's degrees: in Biological Sciences (Universidad de Barcelona, 1959) and in Biochemistry (Universidad de La Laguna, 1962). He attained a PhD in Biochemistry from Ohio State University in 1966. Afterwards, he made a successful post-doc at Prof. Bernard Davis´ lab (Harvard Medical School), studying the inhibition of protein biosynthesis by the antibiotic streptomycin. Juan came back to Spain in 1970 to join the laboratory of Prof. David Vazquez, at the "Centro de Investigaciones Biológicas", obtained a second PhD in Chemical Sciences (Universidad Complutense de Madrid, 1971) and soon after, became a staff member of the CSIC. In 1977, he moved to the recently founded "Centro de Biología Molecular Severo Ochoa" (CBMSO) where he has been an active member for the rest of his life.

Juan made landmark discoveries in two unrelated fields of work, *Escherichia coli* protein biosynthesis and *Drosophila melanogaster* Developmental Biology. His early work described the mechanisms of interaction of aminoacyl-tRNA and the elongation factors EF-G and EFTu with the ribosome. He also determined the stoichiometry of ribosomal translocation and revealed the mode of action of several antibiotics that specifically inhibit bacterial protein synthesis.

Juan was an established researcher in the biosynthesis of proteins field when, it 1980, he made a risky transition to start working in *Drosophila* Developmental Biology. After a fruitful conversation with Antonio García-Bellido, Juan embarked on the analysis of pattern formation, namely, to discover the molecular and cellular mechanisms underlying the formation of the pattern of bristles that cover the thorax of the adult *Drosophila*. Juan and his group pioneered the use of Molecular Biology techniques to clone and functionally characterize the genes of the achaete-scute complex, whose expression confer to epidermal cells the ability to become neural precursors and, accordingly, coined for them the term "proneural genes". The key role of the achaete/scute genes in the patterning of the thoracic bristles relies in their spatially and temporally restricted pattern of expression in the wing imaginal disc, since bristle precursors are selected from among the clusters of cells that express achaete/scute. Furthermore, Juan´s team demonstrated that the expression of these genes depends on the activity of enhancer elements, which drive their transcription at precise positions of the wing disc and reinforce it in the neural precursors. Remarkably, these were ones of the firsts position specific enhancers characterized in the *Drosophila* genome.

Later, Juan´s group succeeded in the characterization of genes that control achaete-scute expression acting through those enhancers (the prepattern genes), or regulate the activity of the proneural proteins. These discoveries were paradigmatic since they stablished how hierarchical gene activity leads to the generation of morphological patterns and pave the way to the identification of proneural genes in other organisms, thus demonstrating the universality of the proneural function. After this pioneering work on pattern formation, he continued making important contributions to our understating of other developmental processes in *Drosophila* and Xenopus and participated in international initiatives such as the sequencing of the *Drosophila* genome.

Juan played an important role as mentor of several generations of researchers, which he considered his second family, and was always eager to receive visiting scientists that benefited from the know-how and the excellent scientific environment of Juan´s lab at the CBMSO. He was also a great listener to all who approaches him seeking for advice and guidance. The scientific community has lost an extraordinary colleague, brilliant, generous, and forward-thinking. We will never forget him.

Sonsoles Campuzano

BOARD OF DIRECTORS AND MANAGER

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CBM Director

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CBM Vice-Director

Germán Lerma Rodrigo
CBM Manager

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Representative of CSIC at the CBM Scientific Committee

Federico Mayor Jr
Director of the Instituto Universitario de Biología Molecular (UAM)

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Paola Bovolenta Nicolao
Tissue and Organ Homeostasis

Maria Luisa Toribio García
Interactions with the Environment

Carlos Dotti
Physiological and Pathological Processes

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Director of the CBM Equality Committee

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Basel

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Genomics at the VU University
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Amsterdam, The Netherlands

Dr. Giampietro Schiavo
UCL Institute of Neurology
Discoveries Centre for Regenerative
and Precision Medicine, UCL Campus
University College London.Queen
Square. London WC1N 3BG, UK

PROGRAMS

GENOME DYNAMICS AND FUNCTION

14 PIs

TISSUE AND ORGAN HOMEOSTASIS

22 PIs

PHYSIOLOGICAL AND PATHOLOGICAL PROCESSES

29 PIs

INTERACTIONS WITH THE ENVIRONMENT

27 PIs

UNITS

GENOME DECODING

GENOME MAINTENANCE AND INSTABILITY

CELL-CELL COMMUNICATION AND INFLAMMATION

CELL ARCHITECTURE AND ORGANOGENESIS

MOLECULAR NEUROPATHOLOGY

METABOLIC AND SIGNALING NETWORKS IN DISEASE

IMMUNE SYSTEM DEVELOPMENT AND FUNCTION

MICROBES IN HEALTH AND WELFARE

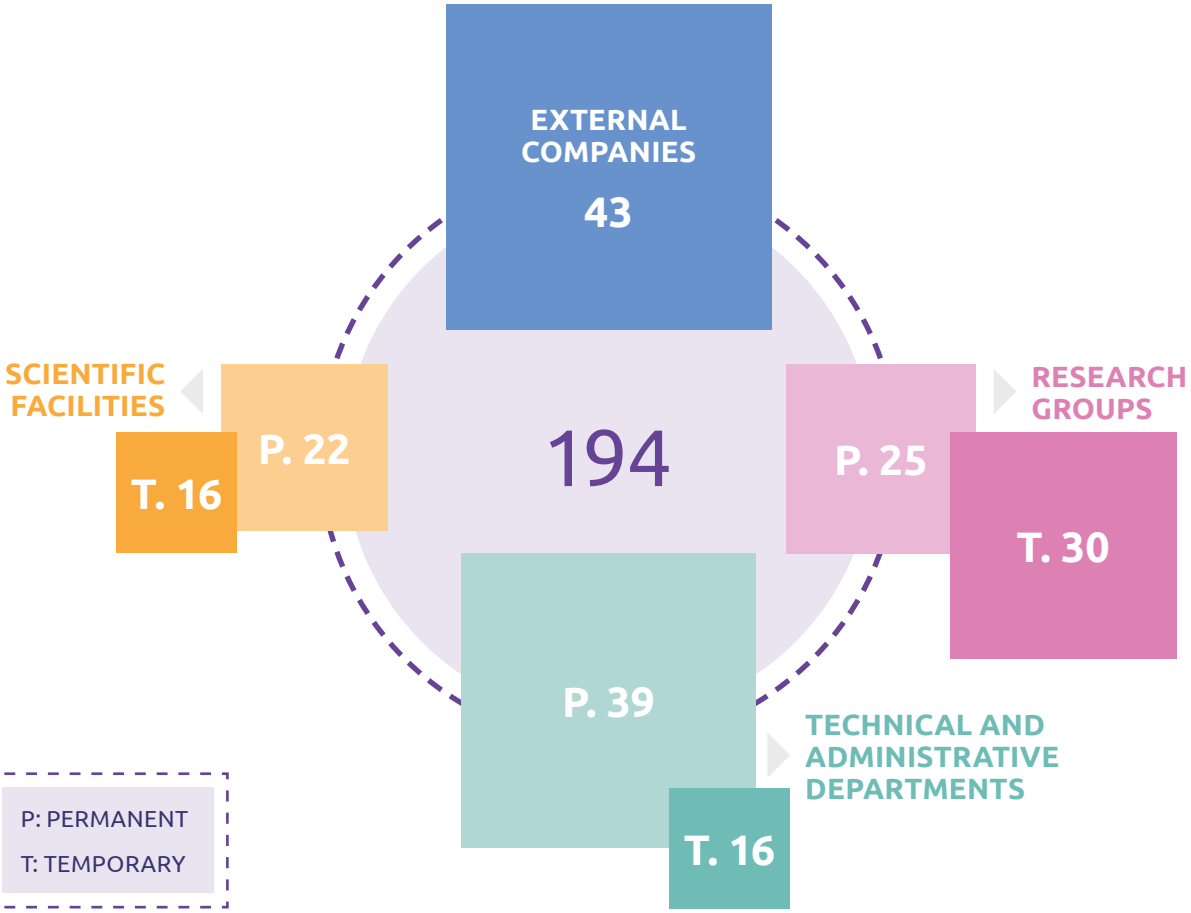
SCIENTIFIC STAFF

SCIENTIFIC PERSONNEL (CSIC-UAM) **494**

- RESEARCH PROFESSORS UNIVERSITY PROFESSORS — 38
- RESEARCH INVESTIGATORS RESEARCH SCIENTISTS ASSOCIATE PROFESSORS — 64
- TENURE TRACK RESEARCHERS TENURE TRACK PROFESSORS — 27
- "AD HONOREM" — 6

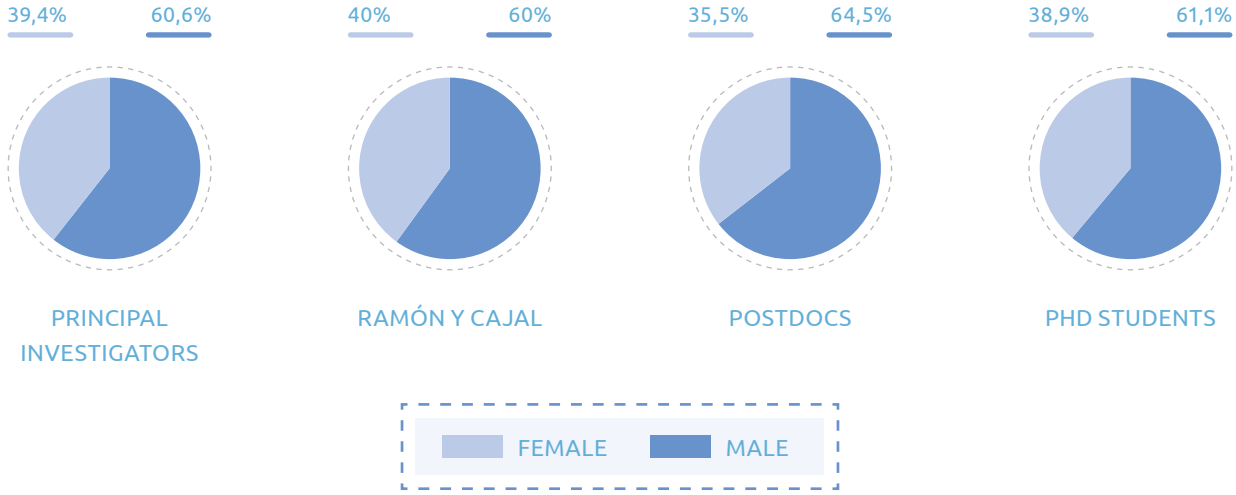
- STUDENTS — 178
- RESEARCHERS — 181

RESEARCH SUPPORT STAFF

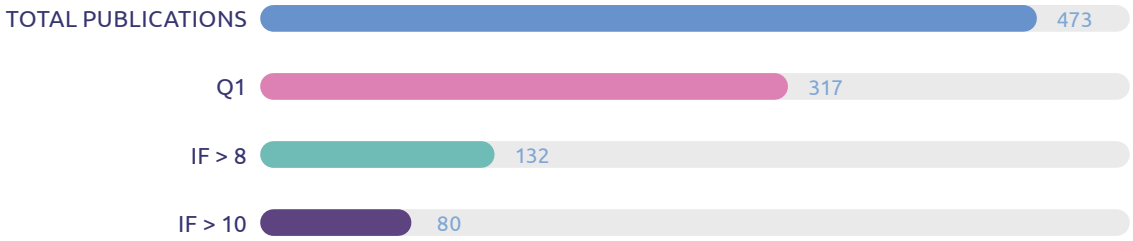


GENDER EQUALITY

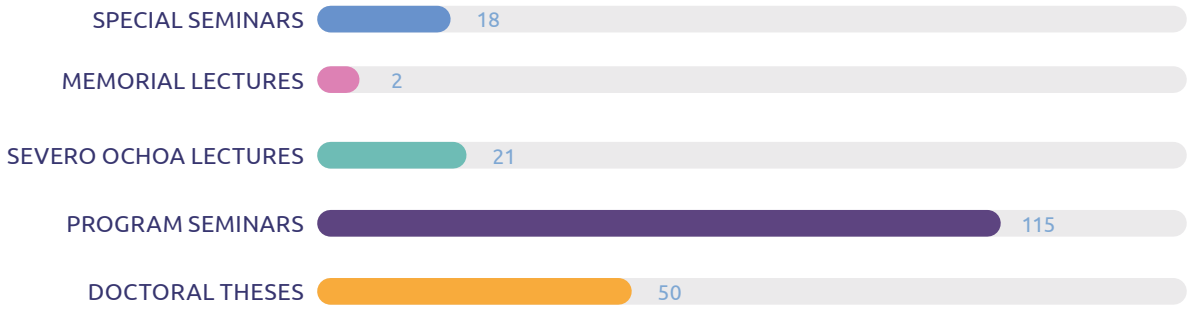
Gender Equality



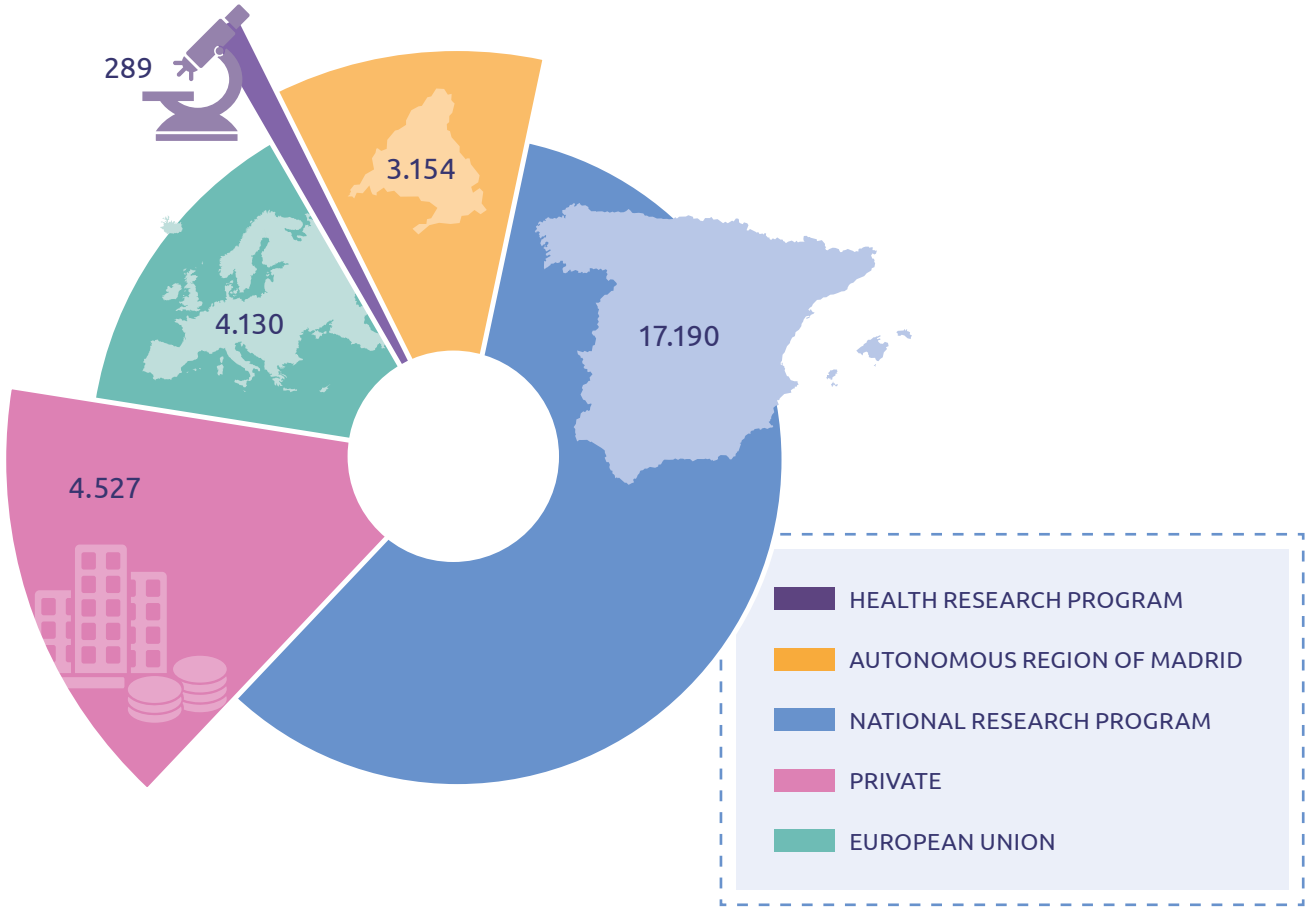
PUBLICATIONS



SEMINARS, LECTURES AND THESES



PROJECTS (*)



* FIGURE IN THOUSAND OF EUROS

PATENTS

45 PATENTS APPLIED FOR

LICENSED PATENTS

» JURKAT-S(ALPHA), JURKAT-S(DELTA) Y JURKAT-S(KAPPA), Hisse Martien van Santen, Maria del Pilar Delgado Cañaveras, Lydia Begoña Horndler Gil, Balbino Jose Alarcón Sánchez; application number: 271/2021. 29/10/2021. CSIC.

» JURKAT-SGFP, Hisse Martien van Santen, María del Pilar Delgado Cañaveras, Balbino José Alarcón Sánchez, Lydia Begoña Horndler Gil; application number: 81/2021. 02/01/2021. CSIC.

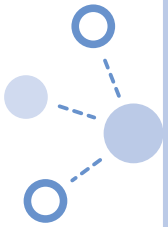
» FLOW CYTOMETRY MULTIPLEXED METHOD FOR THE DETECTION OF SARS-COV-2 ANTIBODIES, Lydia Begoña Horndler Gil, María del Pilar Delgado Cañaveras, Hisse Martien van Santen, Balbino José Alarcón Sánchez; application number: 20382667. 24/07/2020. CSIC.

» METHOD OF PRODUCING VDELTA1+ T CELLS, Juan Alcaín Sánchez, Patricia Fuentes Villarejo, María Jesús García León, M. Luisa Toribio García; application number: 22382013. 01/12/2022. CSIC/ONECHAIN IMMUNOTHERAPEUTICS.

» PRIMER-INDEPENDENT DNA POLYMERASES AND THEIR USE FOR DNA SYNTHESIS, Modesto Redrejo Rodríguez; application number: 201731236. 10/20/2017. CSIC/INSTITUT PASTEUR.

SERVICES

SCIENTIFIC FACILITIES



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

TECHNICAL SERVICES

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

ADMINISTRATIVE SERVICES

- ADMINISTRATION
- HUMAN RESOURCES
- NATIONAL AND INTERNATIONAL PROGRAMS
- INSTITUTIONAL RELATIONS
- OTHER ADMINISTRATIVE SERVICES
- PURCHASING AND STOCKROOM
- QUALITY

AWARDS AND HONORS 2021

Margarita del Val

PREMIO COMUNICACIÓN CIENTÍFICA CSIC FUNDACION BBVA

MEDALLA DE HONOR UIMP A LOS VALORES SOCIALES

José Antonio López Guerrero

PREMIO COMUNICACIÓN CIENTIFICA CSIC FUNDACION BBVA

PREMIO XV TERTULIA EN INVESTIGACIÓN

Encarnación Martínez-Salas

MIEMBRO DE LA ACADEMIA EUROPEA

Veronica Miguel Herranz

PREMIO INVESTIGACIÓN BÁSICA FUND. RENAL IÑIGO ALVAREZ DE TOLEDO

Santiago Lamas

DOCTOR HONORIS CAUSA UNIVERSIDAD REPUBLICA URUGAY

María Llorens-Martín

MIEMBRO ELECTO DE LA ACADEMIA JOVEN DE ESPAÑA

AWARDS AND HONORS 2022

María Llorens-Martín

PREMIO DE INNOVACION JÓVENES INVESTIGADORES PFIZER.

PREMIO NACIONAL DE INVESTIGACIÓN GABRIELA MORREALE.

MIEMBRO ACADEMIAS: JOVEN DE ESPAÑA; EUROPEA; ACADEMIANET

PREMIO DE INVESTIGACIÓN MÉDICA TRANSLACIONAL DE LA REAL ACADEMIA DE MEDICINA

Ana Ortega

PREMIO DE INVESTIGACIÓN DE LA CM "MARGARITA SALAS"

Santiago Lamas

BASIC SCIENCE AWARD DE LA EUROPEAN SOCIETY FOR FREE RADICAL RECHEARCH

Julia Terreros Roncal

PREMIO JOVEN INVESTIGADOR CIBERNED

Carmela Cela Rodríguez

I PREMIO DIVULGACIÓN ORO YO INVESTIGO YO SOY CSIC

Jesús Ávila

ACADÉMICO REAL ACADEMIA NACIONAL DE MEDICINA

ERCs 2021 AND 2022

MITTELBRUNN, MARIA

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

ENDOLYSOSOMAL-MITOCHONDRIA CROSSTALK IN CELL AND ORGANISM HOMEOSTASIS

Fecha Inicio y Fecha Fin	Organismo	Máx. contribución UE
01/03/2017 • 28/02/2023	U.A.M.	1.199.875,00 €

GUTIERREZ ARMENTA, CRISANTO

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

EXPLOITING GENOME REPLICATION TO DESIGN IMPROVED PLANT GROWTH STRATEGIES

Fecha Inicio y Fecha Fin	Organismo	Máx. contribución UE
01/06/2019 • 31/05/2024	C.S.I.C.	2 497 800,00 €

BALSA MARTINEZ, EDUARDO

Ref.:948478 -MitoCure-2020 ERC-Stg

MOLECULAR AND METABOLIC MECHANISMS UNDERLYING MITOCHONDRIAL DYSFUNCTION

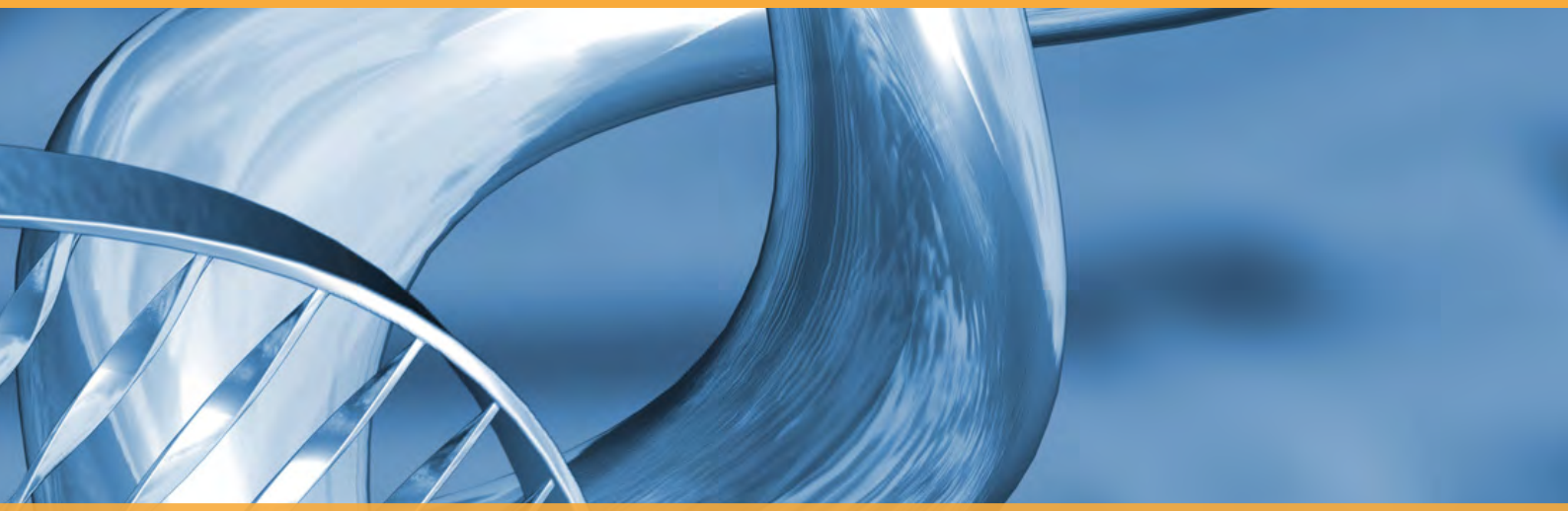
Fecha Inicio y Fecha Fin	Organismo	Máx. contribución UE
01/01/2021 • 31/12/2025	U.A.M.	1.463.410,00 €

LLORENS-MARTÍN, MARÍA

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

INTERROGATING NEURAL ADULT HIPPOCAMPAL NEUROGENESIS

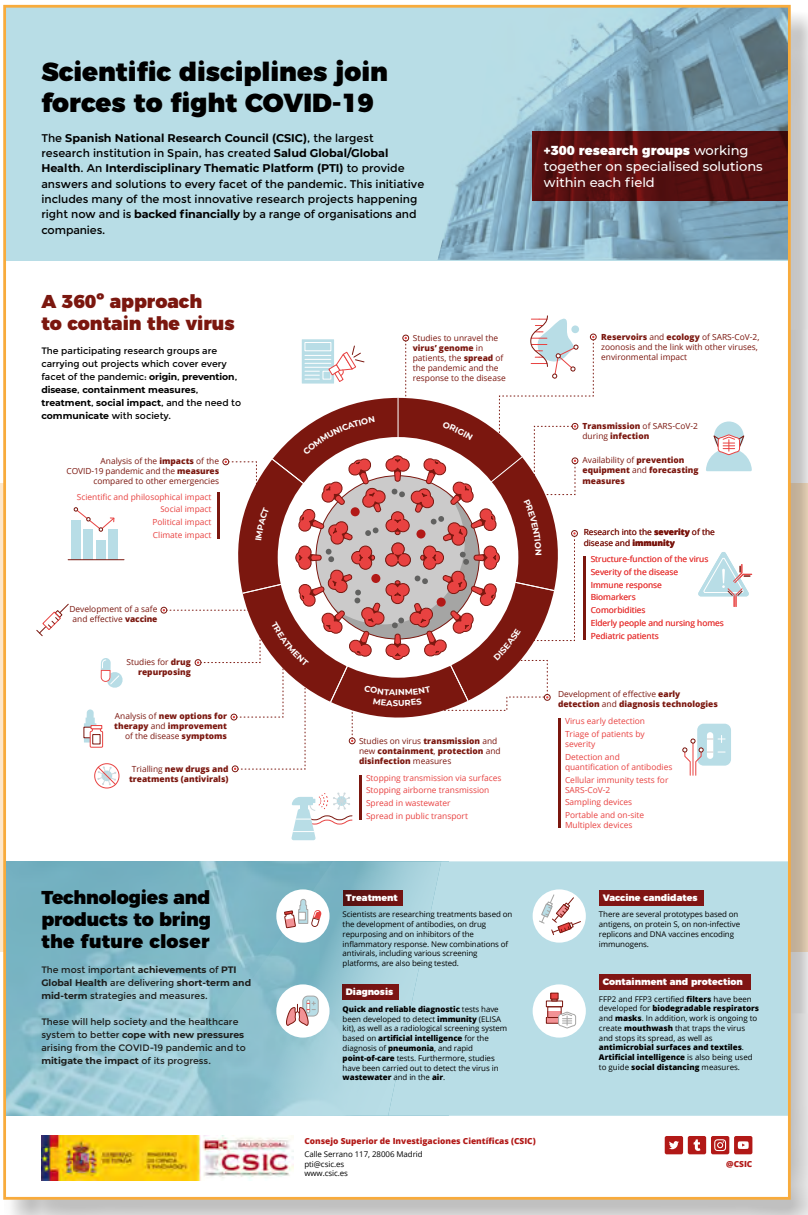
Fecha Inicio y Fecha Fin	Organismo	Máx. contribución UE
01/09/2021 • 31/08/2026	C.S.I.C.	1.994375,00 €



Platforms and singular projects

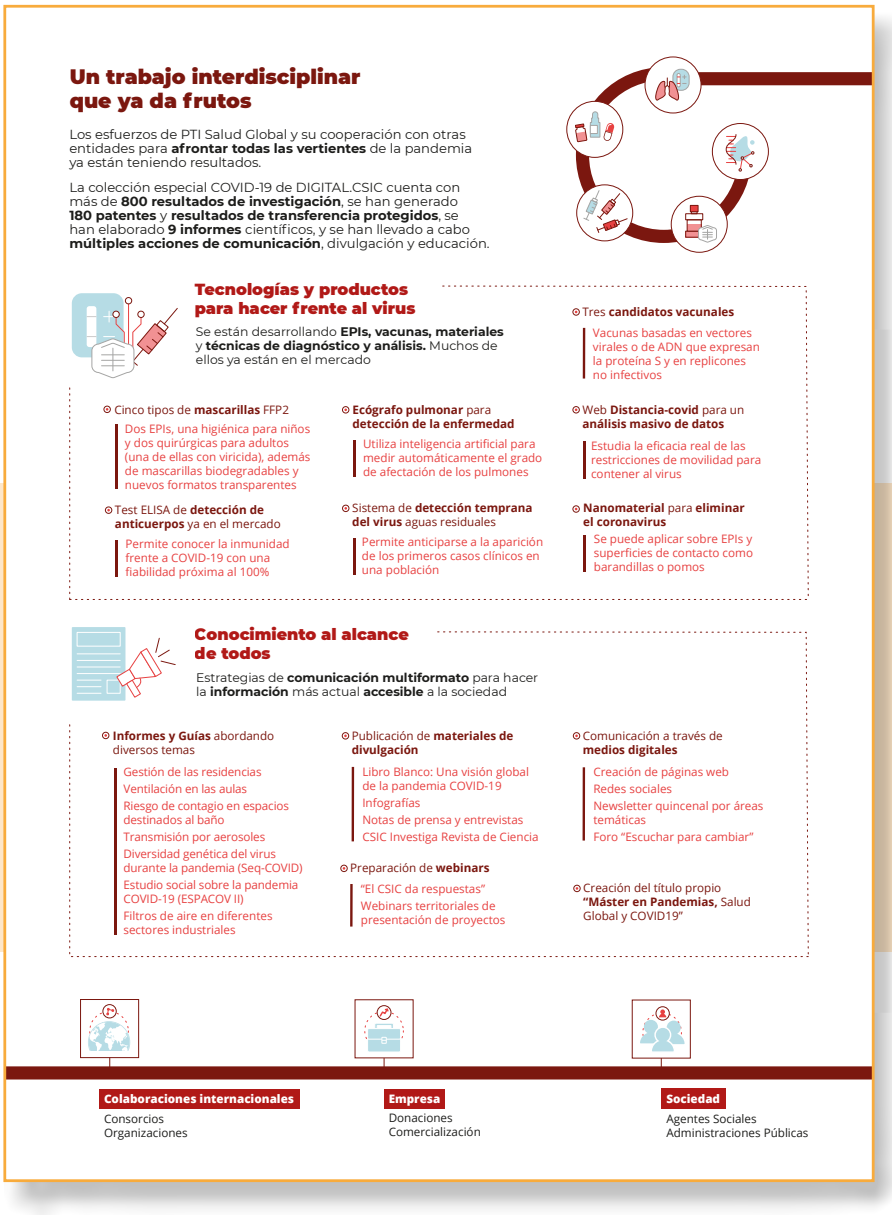
PLATFORM FOR INTERDISCIPLINARY RESEARCH IN GLOBAL HEALTH (PTI SALUD GLOBAL) OF THE SPANISH NATIONAL RESEARCH COUNCIL (CSIC), OUTSTATION AT CBMSO

The Platform for Interdisciplinary Research in Global Health (PTI Salud Global) of the Spanish National Research Council (CSIC) emerged in March 2020 to initially deal with the SARS coronavirus 2 pandemic and is now conducting research on emerging challenges, outbreaks and epidemics by potentially disruptive infectious agents. These include the West Nile virus in Sevilla since 2020 and *mpox* in 2022. Avian flu and Marburg virus are on watch. This national cluster is a virtual institution that proposes advanced solutions in the short, medium and long term. Margarita Del Val (CBMSO, CSIC-UAM) and Iñaki Comas (IBV, CSIC) are the scientific Coordinators. The Platform comprises more than 400 researchers from 144 research groups from different disciplines, representing the wide range of fields of knowledge at CSIC. Areas covered include prevention, diagnostic, disease, containment, immunity, antiviral therapies and vaccines, social impact and communication, connecting human health with animal and environmental health. Focus is strongly oriented towards transferring knowledge, products, procedures and information to society and industry.



At CBMSO, seven research groups joined the Platform in 2020. M Del Val coordinates the Platform and leads work on immunity to infection and vaccination, especially in the very elderly population, to both coronavirus and recently *mpox*. U Bastolla and M Fresno proposed and provided data for a mechanistic association between the severity of SARS-CoV-2 infection and the differential expression of its receptor protein ACE2 and showed that its expression influences the outcome of infections, especially in children. J Millan focuses on inflammation-mediated endothelium damage by SARS-CoV-2. A Alcamí leads studies on improved detection in aerosols of both coronavirus and recently *mpox*, especially in education and transportation, and also develops technologies for their inactivation. L Blanco and M de Vega develop a novel diagnostic method for SARS-CoV-2 infection, based on the isothermal amplification of specific padlock probes, under protection by a CSIC patent. E Domingo and C Perales investigate novel antiviral strategies based on synergic lethal mutagenesis and study virus variability, fitness and quasispecies dynamics. Finally, F Sobrino and M Saiz assay the anti-coronavirus activity of cell-targeted drugs as well as synthetic noncoding RNAs in cultured cells and in a COVID-19 mouse model.

Acknowledgements: The Platform responded to the pressing demand during the coronavirus pandemic, supported and sponsored since March 2020 by donor private Spanish companies. Since January 2021, the European Union Recovery and Resilience Mechanism, NextGenerationEU, established by Regulation (EU) 2020/2094, is the main financing body.



PLATFORM AND PRECLINICAL MODELS FOR A MULTIDISCIPLINARY APPROACH IN COVID19 AND IN RESPONSES TO FUTURE PANDEMICS. (COVTRAVI-19 CM)

Group Members

Principal Investigators (PI, co-PI)
Manuel Fresno Escudero

Scientific Staff
José Mª Almendral del Rio
Juan José Berlanga Chiquero
Sara Cogliati
José M. Cuezva Marcos
María Fernández Lobato
Laura Formentini
Núria Gironés Pujol
Aurelio Hidalgo Huertas
José Antonio López Guerrero
Alberto López-Bueno
Federico Mayor Menéndez
Petronila Penela Márquez
Alberto Rastrojo
Catalina Ribas Núñez
Miguel Angel Rodríguez Gabriel
Konstantinos Stamatakis Andriani
Javier Traba Dominguez
Iván Ventoso Bande
María Yáñez-Mo

Hired Postdoctoral fellows
Mª del Pilar Ubeda Cantera
Dafne García Mateos
Dania Matamoros Grande
Vicente Carpio Ruiz
Davide Cecchini
Xavier Cabodevilla Bravo
Cecilia Maricel Lotufo
Jorge Martínez Ortega

Project Manager
Marta García Sanchez

Hired Technicians
Carolina Maroto González
Eugenio Barrientos Ruiz
Javier del Moral Samoral
Diana Karolina Santos
Cristina Sánchez Blanco
Rubén Chaboy Cansado
Paula Cobeta Martínez
Rocío Moreno Palomares
Javier Merino Valverde
Miguel Alejandro Hortal Borowski
Peter Elías Kidibule
Laura de la Bastida Casero
María Arribas Barrios

Summary

This project was awarded with resilience funds focused on advances in the Knowledge, Treatment and Surveillance of SARS-CoV-2 infection.. Structured in 3 research lines: 1) Pathogenic mechanisms and complications derived from SARS-CoV-2 infection, especially inflammatory, metabolic, cardiovascular and reproductive in acute or persistent COVID-19; 2) New antiviral strategies for SARS-CoV-2 and future pandemics, with new broad spectrum compounds and novel screening strategies; 3) Metagenomic surveillance, both in birds and mammals from diverse ecosystems, as well as in human samples. This project included many independent groups as well as the development of new platforms with newly acquired state of the art technologies and equipment.

Among the results obtained, we have described: a) the molecular and metabolic alterations derived from SARS-CoV-2-mitochondria interaction in vitro and the contribution of mitochondria to SARS-CoV-2 infection, pathogenesis of COVID-19 in affected organs, cytokine profile and immune system activation in murine models of infection. b) Molecular basis of endothelial dysfunction and inflammatory response in the heart caused by SARS-CoV-2 which is essential for the development of new therapeutic strategies. c) Alterations in fertility derived from SARS-CoV-2 infection. d) immunomodulatory capacity of several partial agonists of TLRs receptors that have demonstrated therapeutic capacity in preclinical models of sepsis with the K18-hACE2 transgenic mouse model. e) Design, production and identification of antiviral phytochemical compound; Development of broad-spectrum antivirals based on ribosome blockade; anionic polymers as antivirals

and development of broad-spectrum antivirals based on NDTs in microfluidic platforms with fluorescence sorting capability (on-chip sorting). f) Prevention and metagenomic surveillance. The microbial diversity associated with animals carrying potential emerging human pathogens and the diversity of viruses. Genetic analysis of emerging viruses associated with human hematopoietic pathologies.

Among the new equipment acquired and incorporated in to platforms are:

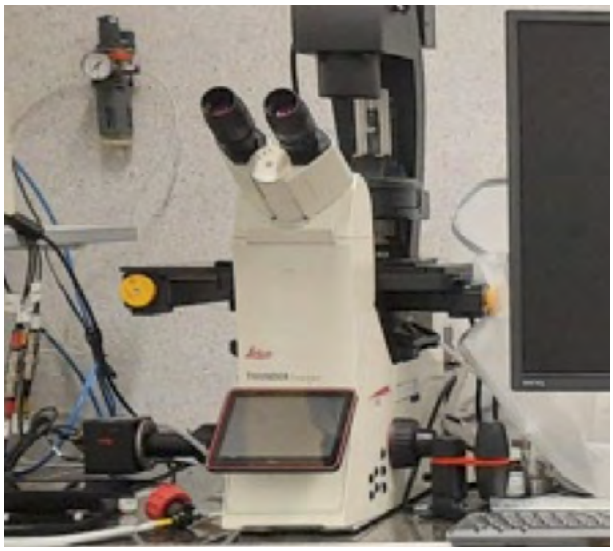
Antiviral In Vitro Screening Platform

» Integrated high throughput and highresolution Microscopy and Cytometry System

Cross-sectional platform for preclinical models of infection and pathogenesis

» Optical Imaging Systems (fluorescence and bioluminescence) for small animals

» CLAMS system of modular metabolic cages



Optical Imaging Systems (fluorescence and bioluminescence) for small animals. B) Integrated high throughput and high resolution Microscopy and Cytometry System

List of publications

» Horndler L, Delgado P, Abia D, Balabanov I, Martínez-Fleta P, Cornish G, Llamas MA, Serrano-Villar S, Sánchez-Madrid F, Fresno M, van Santen HM, Alarcón B. (2021). Flow cytometry multiplexed method for the detection of neutralizing human antibodies to the native SARS-CoV-2 spike protein. EMBO Mol Med. Mar 5;13(3):e13549. doi: 10.15252/emmm.202013549.

» Maza MDC, Úbeda M, Delgado P, Horndler L, Llamas MA, van Santen HM, Alarcón B, Abia D, García-Bermejo L, Serrano-Villar S, Bastolla U, Fresno M. (2022) ACE2 Serum Levels as Predictor of Infectability and Outcome in COVID-19. Front Immunol Mar 23;13:836516. doi: 10.3389/fimmu.2022.836516.

» Bastolla U, Chambers P, Abia D, Garcia-Bermejo M-L, Fresno M (2022) Is Covid-19 Severity Associated With ACE2 Degradation? Frontiers in Drug Discovery 1DOI: 10.3389/fddsv.2021.789710

» Bello-Morales R, Andreu S, Ruiz-Carpio V, Ripa I, López-Guerrero JA. 2022. Extracellular Polymeric Substances: Still Promising Antivirals. 2022 Viruses. Jun 19;14(6):1337. doi: 10.3390/v14061337. Review.

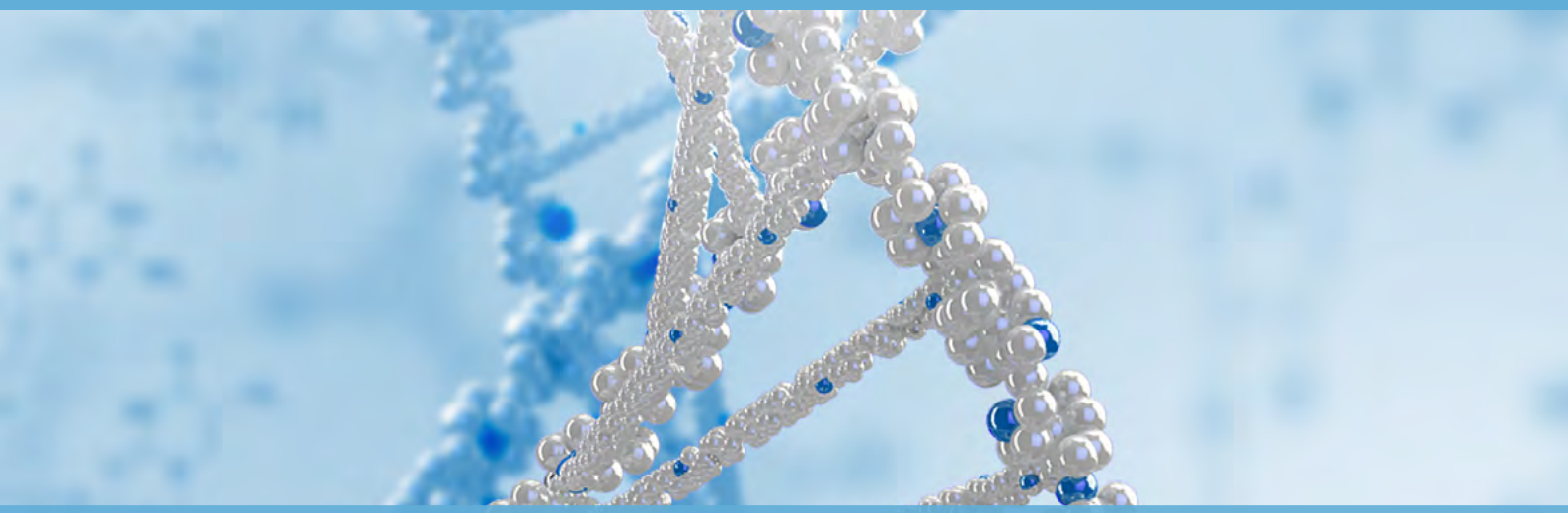
» Praena B, Mascaraque M, Andreu S, Bello-Morales R, Abarca-Lachen E, Rapozzi V, Gilaberte Y, González S, López-Guerrero JA*, Juarranz Á. 2022. Potent Virucidal Activity In Vitro of Photodynamic Therapy with Hypericum Extract as Photosensitizer and White Light against Human Coronavirus HCoV-229E. Pharmaceutics. Nov 2;14(11):2364. doi: 10.3390/pharmaceutics14112364.

» Andreu, S., von Kobbe, C., Delgado, P., Ripa, I., Buzón, M.J., Genescà, M., Gironès, N., del Moral-Salmoral, J., Ramírez, G.A., Zúñiga, S., Enjuanes, L., López-Guerrero, J.A., Bello-Morales, R. 2022. Dextran sulfate from Leuconostoc mesenteroides B512F exerts potent antiviral activity against SARS-CoV-2 in vitro and *in vivo* (2023. in press Antiviral Research.

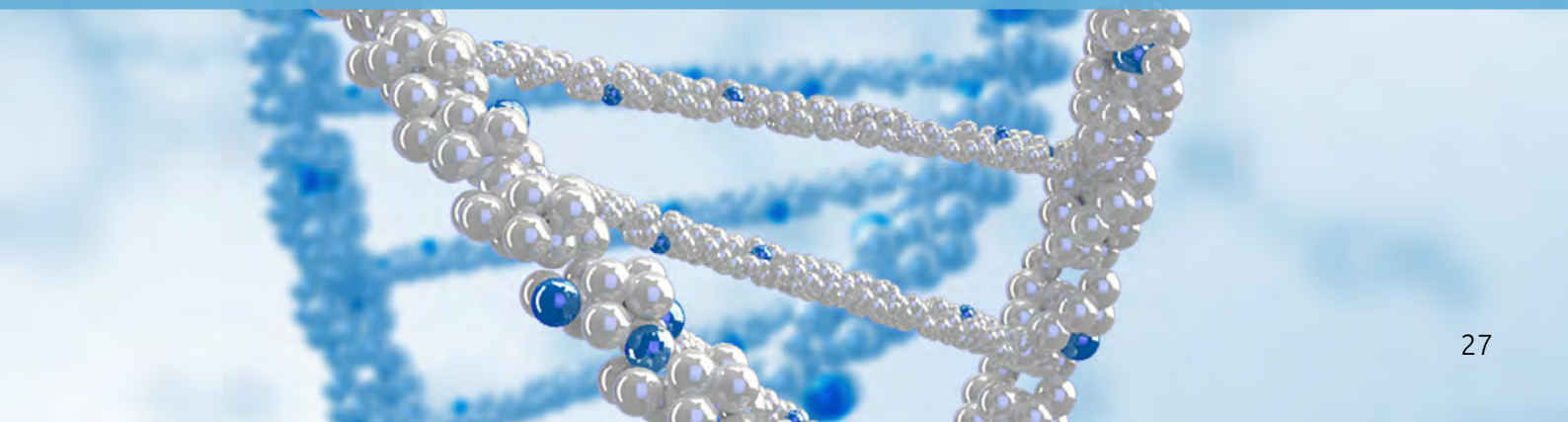
» Cecchini DA, Sánchez-Costa M, Orrego AH, Fernández-Lucas J, Hidalgo A. 2022. Ultrahigh-Throughput Screening of Metagenomic Libraries Using Droplet Microfluidics. Methods Mol Biol., 2397:19-32. doi: 10.1007/978-1-0716-1826-4_2. PMID: 34813057.

» Amorim et al. (2022) Engineering *Saccharomyces cerevisiae* for the one-step production of a functional sweetening mixture towards food applications. Food Bioprod. Process. 135, 123-134. doi:10.1016/j.fbp.2022.07.006

PROGRAM



Genome Dynamics and Function



Genome Dynamics and Function

Genome Decoding Unit

Encarnación Martínez-Salas

Research of the Genome Decoding UNIT is centered to investigate fundamental processes governing genome regulation in cells and model organisms. Complementary and collaborative activities within the Unit are deciphering the molecular basis of essential cellular processes to better understand how genomic information is interpreted, and to provide the basis for new diagnostic tools and therapeutic strategies. Crisanto Gutiérrez (CG) is interested in understanding how complex gene regulatory networks impinge on cell cycle progression and DNA replication and how epigenetics mechanisms affect these processes in the model plant Arabidopsis thaliana. Carlos Perea (CP) aims to understand how transcription rewires as cells transit through mitosis, leading to gene expression reprogramming and change of cell fate. José María Requena (JMR) is engaged in generating improved genome assemblies, transcriptome and gene annotations for pathogenic *Leishmania* species using massive sequencing and proteogenomic approaches. Ugo Bastolla (UB) researches in computational biology, inferring structural and physico-chemical properties of proteins. Javier Santos (JS) researches precursor T-cell lymphoblastic neoplasms; they found circRNAs discriminating between different stages of thymocyte differentiation and a new method for lncRNAs quantification. Encarna Martínez-Salas (EMS) research is aimed at understanding the principles guiding alternative mechanisms of translation initiation through the functional and structural characterization of RNA structural elements and RNA-binding proteins. Iván Ventoso/Juan José Berlanga/Miguel Angel Rodríguez (IV/JJB/MAR) is focused to study the mechanics and regulation of mRNA translation in eukaryotes and viruses, and their implications for stress response and aging.

Highlights of the unit

» Coordination of cell division, growth and fate as well as cell cycle dynamics during organogenesis using a multiple fluorescent sensor to identify cell cycle phases. Identification of the role of ORC1 proteins in DNA replication and heterochromatin dynamics (CG)

» The CP group began on May 2022 supported by the *Talento* program. Implementation of the SNAP technology for *in vivo* labelling and purification of proteins and degron approaches, for the functional characterization of factors.

» The *Leishmania infantum* genome/proteome has been incorporated as reference in the specialized database TriTryDB (https://tritrypdb.org). Genome and protein annotations for L. braziliensis, L. donovani, L. major and L. infantum resources are available in NCBI, ENA, and UniProt (JMR).

» Phylogenetic inference model considering selection for protein folding stability, native dynamics, and structural response to mutations. Hypothesis testing the role of the cellular receptor ACE2 on the response to SARS-COV-2 infection. Studies of GGN motifs and nucleosome organization as determinants of eukaryotic genome replication origins (UB).

» In-vitro method for quantifying the cellular content of specific RNAs. European patent. EP21382615.9. SEPT6-ABL2 fusion for use in the diagnosis and/or treatment of cancer. European patent. PCT/EP2022/065750 (JS).

» Loss of function of biallelic pathogenic Gemin5 variants, oligomerization and RNA-binding defects of Gemin5 clinical variants. Homodecamer assembly of Gemin5 determines binding to cognate RNAs and translation. Gemin5 regulation of selective association to polysomes of mRNAs controlling cell proliferation (EMS)

» Effects of pathogenic arginine-rich peptides on gene expression (IV/Fernández-Capetillo´s group (CNIO). Translation control by eIF2 phosphorylation is regulating proteostasis and lifespan extension in *Schizosaccharomyces pombe* (JJB/MAR)

Ugo Bastolla Bufalini
COMPUTATIONAL BIOLOGY AND BIOINFORMATICS

Crisanto Gutierrez
CELL DIVISION, GENOME REPLICATION AND CHROMATIN

Encarnación Martínez-Salas
INTERNAL INITIATION OF TRANSLATION IN EUKARYOTIC mRNAs

Carlos Perea-Resa
DYNAMIC AND RECYCLING OF THE TRANSCRIPTIONAL MACHINERY ACROSS MITOSIS

José Fernández Piqueras
GENETICS AND CELL BIOLOGY OF CANCER: T-CELL LYMPHOBLASTIC NEOPLASMS

Jose María Requena Rolanía
REGULATION OF GENE EXPRESSION IN LEISHMANIA

Iván Ventoso / Juan José Berlanga / Miguel Ángel Rodríguez / Margarita Cabrera
REGULATION OF mRNA TRANSLATION IN EUKARYOTES AND ITS IMPLICATIONS FOR ORGANISMAL LIFE

COMPUTATIONAL BIOLOGY AND BIOINFORMATICS

Group Members

Principal Investigator:
Ugo Bastolla Bufalini

Postdoctoral fellow:
Yves Dehouck (until April 2022)

Master student
Ivan Lorca

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Summary

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

Along line (1), we studied how non-harmonic motions affect the normal modes that ground our prediction of protein native dynamics. 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 sampling protein loops and a new method for estimating the change of stability due to mutations (collaborations with Pablo Chacón's group, IQFR-CSIC). Along line (2), we developed the structure and stability-constrained substitution model of protein evolution, based on our new predictions of the structural effect of mutations. We showed that it greatly improves evolutionary inference with respect to our previous model that only takes into account protein folding stability (https://www.biorxiv.org/content/10.1101/2023.01.22.525075v1). 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 folding does not particularly limit recombination. We developed hybrid protein alignments based on sequence and structure similarity, which improve the alignment quality and the estimated divergence time with respect to methods that take into account only one type of information (https://www.biorxiv.org/content/10.1101/2023.01.22.525078v1).

Along line (3), in collaboration with Crisanto Gutierrez's and María Gómez's groups (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 DNA secondary structure). 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-CSIC), 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 (https://www.biorxiv.org/content/10.1101/2022.09.11.507163v1).

Finally, we proposed a mechanistic association between the severity of SARS-CoV-2 infection and the differential expression of its receptor protein ACE2, which downregulates the inflammatory peptides of the Angiotensin-Bradykinin system, whose upregulation in aging, chronic inflammation and SARS-CoV-2 infection may play a key role in infection severity. In collaboration with Manuel Fresno's group (CBMSO), we showed that the expression of ACE2 influences the probability and the outcome of infections.

List of publications

» Del Amparo, R., González-Vázquez, L.D., Rodríguez-Moure, L., Bastolla, U. and Arenas, M. (2023) Consequences of Genetic Recombination on Protein Folding Stability. J Mol Evol. 91, 33-45. doi: 10.1007/s00239-022-10080-2.

» López-Blanco, J.R., Dehouck, Y., Bastolla, U. and Chacón, P. (2022) Local Normal Mode Analysis for Fast Loop Conformational Sampling. J. Chem. Inf. Model. 62, 4561-4568. doi: 10.1021/acs.jcim.2c00870.

» Maza, M.D.C., Úbeda, M., Delgado, P., Horndler, L., Llamas, M.A., van Santen, H.M., Alarcón, B., Abia, D., García-Bermejo, L., Serrano-Villar, S., Bastolla, U. and Fresno, M. (2022) ACE2 serum levels as predictor of infectability and outcome in COVID-19. Front Immunol. 13, 836516. doi: 10.3389/fimmu.2022.836516.

» Bastolla, U., Chambers, P., Abia, D., Garcia-Bermejo, M.L. and Fresno, M. (2022) Is Covid-19 Severity Associated With ACE2 Degradation? Front. Drug Discov. 1. doi: 10.3389/fddsv.2021.789710.

» Dehouck, Y. and Bastolla, U. (2021) Why are large conformational changes well described by harmonic normal modes? Biophys J. 120, 5343-5354. doi: 10.1016/j.bpj.2021.10.027.

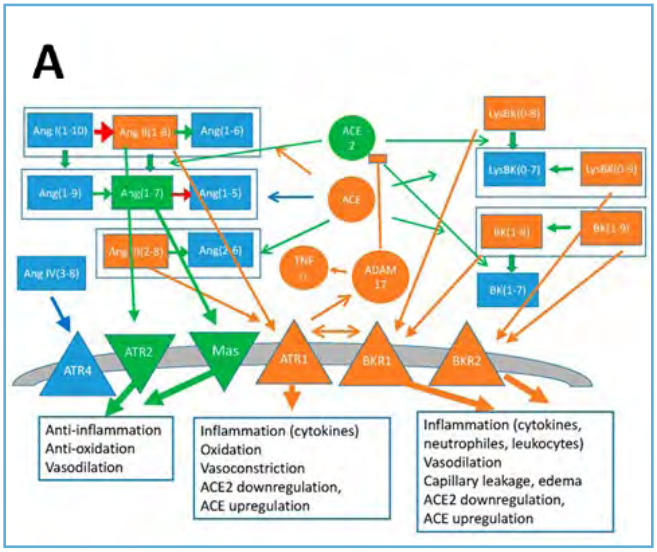
» Bastolla, U. (2021) Mathematical Model of SARS-Cov-2 Propagation Versus ACE2 Fits COVID-19 Lethality Across Age and Sex and Predicts That of SARS. Front Mol Biosci. 8, 706122. doi: 10.3389/fmolb.2021.706122.

Research projects

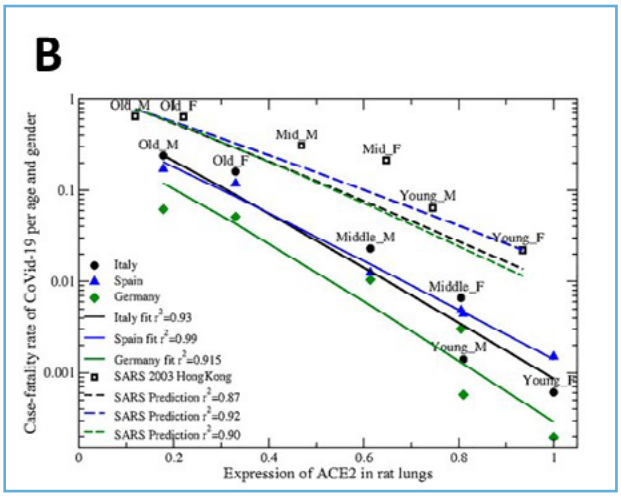
» Theoretical and computational investigation of tuberculosis antimicrobial resistance development based on extensive experimental library of mycobacterium strains. H2020-MSCA-RISE-2018-823922. European Commission. 11 teams coordinated by Aston University (UK).

» Angiotensin converting enzyme serum levels as predictor of infection and clinical outcome in COVID-19. CSIC-COV19-108. PIs: Ugo Bastolla and Manuel Fresno. From 05/2020.

» 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.



A) Schematic representation of the RAS (left) and bradykinin (right) inflammatory system downregulated by the SARS-COV-2 receptor ACE2. Signaling peptides are represented as rectangles, peptidases (ACE, ACE2), proteases (ADAM17) and cytokines (TNFα) as circles and membrane receptors as triangles.



B) Expression of the ACE2 protein in rat lung (horizontal axis) vs. case fatality rate of SARS-CoV-2 and SARS for humans of same sex and age class (vertical axis). Lines depict predictions of the mathematical model.

CELL DIVISION, GENOME REPLICATION AND CHROMATIN

Group Members

Principal Investigator:
Crisanto Gutierrez

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Bénédicte Desvoyes

Postdoctoral fellows:
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Jorge Fung Uceda
María Sol Gómez
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(until September 2022)
Diego Gomez Martinez
Ivan del Olmo Montoro

Predoctoral fellows:
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(until July 2022)
Rocío Núñez Vázquez

Technicians:
Elisa Alonso Pérez
Carla Alonso Rodriguez
(until August 2022)

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

A major advance has been the generation of Arabidopsis lines (PlaCCI) expressing three fluorescent markers (CDT1a-CFP, H3.1-mCherry and CYCB1;1-YFP) that allow the identification of each cell cycle phase by unique combinations of colors. We are using these tools to decipher the gene networks and molecular basis controlling stem cell biology, proliferation of their derivatives and their differentiation to form a root tip (Fig. 1). We have been expanding these studies to other organs and how dividing cells communicate with each other.

Expanding this project we are interested in learning new pathways controlling the response to different types of stress in three different avenues. One is the identification of molecular determinants of DNA replication origin (ORI) function. Based on previous genome-wide maps of ORIs and their association with epigenetic marks in 9 chromatin states we are exploring the role of individual modifications in ORI function using CRISPR-dCas9 tools. Another is the link of DNA replication factors in the deposition and maintenance of epigenetic marks and how they affect the reprogramming of gene expression in growth under normal and stress conditions. Finally, we are 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 salt and water deficit stress.

Our studies are ultimately aimed at designing strategies to improve plant growth and performance using pathways not explored so far.

List of publications

» Gutierrez, C. (2021) Chromatin, DNA replication and transcription: closing the triangle. Trends Plant Sci. 26, 10-12 doi: 10.1016/j.tplants.2020.10.010

» Elena-Real, C., Gonzalez-Arzola, K., Diaz-Quintana, A., Velazquez-Campoy, A., Devoyes, B., Gutierrez, C., de la Rosa, M., Diaz-Moreno, I. (2021) Proposed mechanism for regulation of H 2 O 2 -induced programmed cell death in plants by binding of cytochrome c to 14-3-3 proteins. Plant J. 106, 74-85. doi: 10.1111/tpj.15146

» Echevarria, C., Gutierrez, C., Desvoyes, B. (2021) Tools for assessing cell cycle progression in plants. Plant Cell Physiol. 62, 1231-1238. doi: 10.1093/pcp/pcab066

» D'Ario, M., Tavares, R., Schiessl, K., Desvoyes, B., Gutierrez, C., Howard, M., Sablowski, R. (2021) Cell size controlled in plants using DNA content as an internal scale. Science 372, 1176-1181. doi: 10.1126/science.abb4348

» Simonini, S., Bemer, M., Bencivenga, S., Gagliardini, V., Pires, N., Desvoyes, B., van der Graaff, E., Gutierrez, C., Grossniklaus, U. (2021) The Polycomb group protein MEDEA controls cell proliferation and embryonic patterning in *Arabidopsis*. Dev Cell 56, 1945-1960. doi: 10.1016/j.devcel.2021.06.004

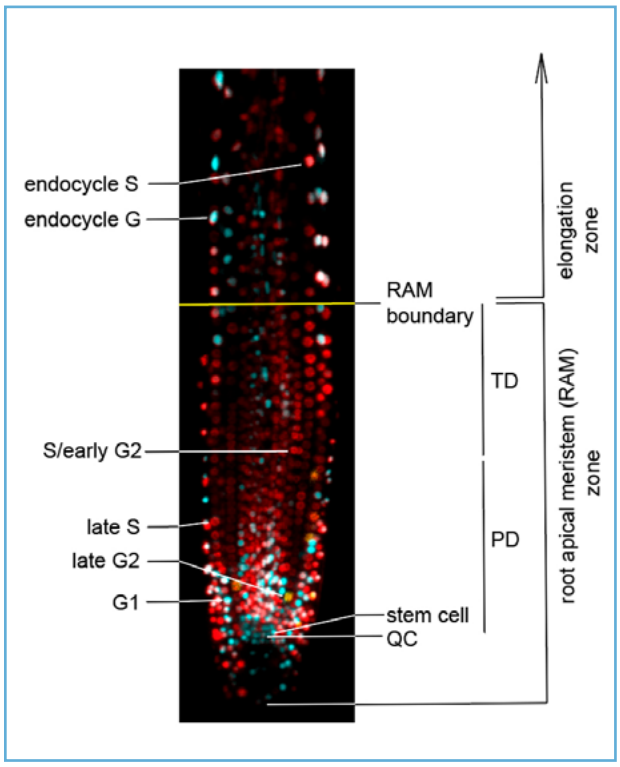
» Desvoyes, B., Echevarria, C., Gutierrez, C. (2021) Cell proliferation in the root apical meristem. J. Exp. Bot 72, 6708-6715. doi: 10.1093/jxb/erab303

» Sablowski, R., Gutierrez, C. (2022) Cycling in a crowd: coordination of plant cell division, growth and cell fate. Plant Cell 34, 193–208. doi: 10.1093/plcell/koab222

» Gutierrez, C. (2022) A journey to the core of the plant cell cycle. Int. J. Mol. Sci. 23, 8154. doi: 10.3390/ijms23158154

» Han, S-K., Herrmann, A., Yang, J., Iwasaki, R., Sakamoto, T., Desvoyes, B., Kimura, S., Gutierrez, C., Kim, E-D., Torii, K. (2022) Deceleration of cell cycle underpins a switch from proliferative to terminal division in plant stomatal lineage. Dev. Cell 57, 569-582.e6. doi: 10.1016/j.devcel.2022.01.014

» Núñez-Vázquez, R., Desvoyes, B., Gutierrez, C. (2022) Histone variants and modifications during abiotic stress response. Frontiers Plant Sci. 13, 984702 (2022). doi: 10.3389/fpls.2022.984702



Cell cycle, endocycle and cell differentiation dynamics in a developing Arabidopsis root.

Participation in projects

» Chromatin dynamics and cell proliferation control during organogenesis in Arabidopsis. Implications on the abiotic stress response (CHROMALINKS), RTI2018-094793-B-I00, Crisanto Gutierrez (PI), 2019-2022.

» Exploiting genome replication to design improved plant growth strategies (PLANTGROWTH), ERC-2018-AdG_833617, Crisanto Gutierrez (PI), 2019-2024.

» Integration of cell cycle control and chromatin dynamics: the Arabidopsis model (ChromCC), PID2021-123319NB-I00, Crisanto Gutierrez P), 2022-2025.

Doctoral theses

» Clara Echevarria Zomeño (2022) Coordination of cell proliferation with developmental programs in Arabidopsis. Universidad Autónoma de Madrid. Directors: Crisanto Gutierrez and Bénédicte Desvoyes. Cum laude, European Mention.

Other activities

» Crisanto Gutiérrez: Chair of the Cell and Developmental Biology Section of Academia Europaea (since 2021). Member of the EMBO Council (since 2020).Member of the Scientific Advisory Board of Fundación Gadea – Ciencia. Member of Editorial Board of EMBO J. and Eur. J. Cell Biol., and Editor of Plant J. Medal Margarita Salas to Mentoring activities by CSIC (2022)

INTERNAL INITIATION OF TRANSLATION IN EUKARYOTIC mRNAs

Group Members

Group leader:
Encarnación Martínez-Salas

Postdoctoral fellows:
Rosario Francisco-Velilla
Azman Embarc-Buh (January 2021-April 2022)

Predoctoral fellows:
Azadeh Nahavandi Araghi (2021-)
Salvador Abellán Pérez (2022-)

Technicians:
Jorge Ramajo

Master students:
Salvador Abellán Pérez (2021)

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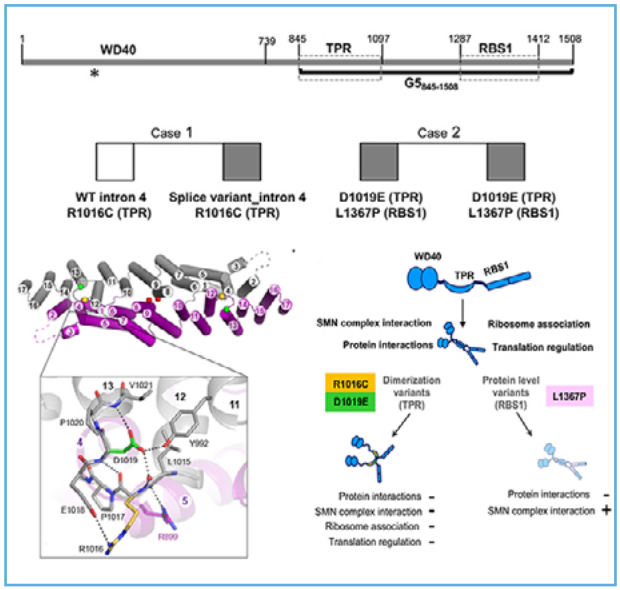
Summary

Our aims are focused to understand the principles guiding translation regulation through the characterization of RNA-binding proteins (RBPs). Gemin5 is a member of the survival of the motor neurons (SMN) complex and a translation reprogramming factor. Thus, this protein is acting on fundamental steps of the mRNA life cycle, splicing and translation. Gemin5 is organized in functional domains with a distinctive structural organization. The C-terminal region adopts a homodecamer architecture comprised of a dimer of pentamers, critical to bind cognate RNA ligands and to regulate translation. However, only a fraction of the RNA targets was enriched in polysomes. Two subsets of these mRNAs carry unique cis-acting regulatory elements, the 5' terminal oligopyrimidine tracts or the histone stem-loop structure at the 3' end, respectively. In agreement with this, Gemin5 stimulates translation of mRNAs encoding ribosomal proteins and histones. Dysregulation of RBPs produce widespread effects, challenging the identification of the mechanism contributing to disease. Biallelic variants in Gemin5 gene found in patients with neurodevelopmental disorders affect key domains of the protein, the dimerization module (TPR) and the noncanonical RNA-binding site (RBS1) (Figure 1). Whereas the RBS1 variant confers protein instability, the TPR variants disrupt protein dimerization and fail to associate with ribosomes, hampering its role in translation control. All mutants are defective in the interaction with protein networks involved in translation and RNA-driven pathways. Thus, in-depth studies of Gemin5 variants provided a molecular basis of disease associated with malfunction of this protein, opening new avenues of research for this multifunctional protein.



Summary of Gemin5 biallelic variants found in patients developing neurodevelopmental disorders.

List of publications

» Francisco-Velilla, R., Embarc-Buh, A., Del Caño-Ochoa, F., Abellan, S., Vilar, M., Alvarez, S., Fernandez-Jaen, A., Kour, S., Rajan, D.S., Pandey, U.B., Ramón-Maiques, S., and Martinez-Salas, E. (2022) Functional and structural deficiencies of Gemin5 variants associated with neurological disorders. Life Sci. Alliance. 5(7), e202201403. doi: 10.26508/lsa.202201403.

» Rajan, D.S., Kour, S., Fortuna, T.R., Cousin, M.A., Barnett, S.S., Niu, Z., Babovic-Vuksanovic, D., Klee, E.W., Kirmse, B., Innes, M., Rydning, S.L., Selmer, K.K., Vigeland, M.D., Erichsen, A.K., Nemeth, A.H., Millan, F., DeVile, C., Fawcett, K., Legendre, A., Sims, D., Schnekenberg, R.P., Burglen, L., Mercier, S., Bakhtiari, S., Martinez-Salas, E., Wigby, K., Lenberg, J., Friedman, J.R., Kruer, M.C., and Pandey, U.B. (2022) Autosomal Recessive Cerebellar Atrophy and Spastic Ataxia in Patients With Pathogenic Biallelic Variants in GEMIN5. Front. Cell Dev. Biol. 10, 783762. doi: 10.3389/fcell.2022.783762.

» Guo, Q., Zhao, S., Francisco-Velilla, R., Zhang, J., Embarc-Buh, A., Abellan, S., Lv, M., Tang, P., Gong, Q., Shen, H., Sun, L., Yao, X., Min, J., Shi, Y., Martínez-Salas, E., Zhang, K., and Xu, C. (2022) Structural basis for Gemin5 decamer-mediated mRNA binding. Nat. Commun. 13(1), 5166. doi: 10.1038/s41467-022-32883-z.

» Embarc-Buh, A., Francisco-Velilla, R., Garcia-Martin, J.A., Abellan, S., Ramajo, R., and Martinez-Salas, E. (2022) Gemin5-dependent RNA association with polysomes enables selective translation of ribosomal and histone mRNAs. Cell. Mol. Life Sci. 79(9), 490. doi: 10.1007/s00018-022-04519-4.

» Francisco-Velilla, R., Embarc-Buh, A., Abellan, S., del Caño-Ochoa, F., Ramón-Maiques, S., and Martinez-Salas, E. (2022) Phosphorylation of T897 in the dimerization domain of Gemin5 modulates protein interactions and translation regulation. Comput. Struct. Biotechnol. J. 20, 6182-6191. doi: 10.1016/j.csbj.2022.11.018.

» Francisco-Velilla, R., Embarc-Buh, A., Abellan, S., and Martinez-Salas, E. (2022) Picornavirus translation strategies. FEBS Open Bio. 12(6), 1125-1141. doi: 10.1002/2211-5463.13400.

» Escos, A., Martín-Gómez, J., González-Romero, D., Díaz-Mora, E., Francisco-Velilla, R., Santiago, C., Cuezva, J.M., Domínguez-Zorita, S., Martínez-Salas, E., Sonenberg, N., Sanz-Ezquerro, J.J., Mehdi Jafarnejad, S., and Cuenda, A. (2022) TPL2 kinase expression is regulated by p38γ/p38δ-dependent association of Aconitase-1 with TPL2 mRNA. Proc. Nat. Acad. Sci. U. S. A. 119(35), e2204752119. doi: 10.1073/pnas.2204752119.

» Embarc-Buh, A., Francisco-Velilla, R., Camero, S., Perez-Cañadillas, J.M., and Martínez-Salas, E. (2021) The RBS1 domain of Gemin5 is intrinsically unstructured and interacts with RNA through conserved Arg and aromatic residues. RNA Biology. 18(sup1), 496-506. doi: 10.1080/15476286.2021.1962666.

» Saiz, M., and Martinez-Salas, E. (2021) Uncovering targets of the Leader protease: Linking RNA-mediated pathways and antiviral defense. Wiley Interdiscip. Rev. RNA. 12(4), e1645. doi: 10.1002/wrna.1645.

» Fernandez-Chamorro, J., Francisco-Velilla, R., Embarck-Buk, A., and Martínez-Salas, E. (2021) Identification of novel RNA-binding proteins recognizing RNA structural elements. Meth. Mol. Biol. 2323, 109-119. doi: 10.1007/978-1-0716-1499-0_9.

» Embarc-Buh, A., Francisco-Velilla, R., and Martínez-Salas, E. (2021) RNA-binding proteins at the host-pathogen interface targeting viral regulatory elements. Viruses. 13(6), 952 doi: 10.3390/v13060952.

» Rangel-Guerrero, S., Franco, P., Martínez-Salas, E, and Alvarez Salas, L. (2021) Structural insights of the pre-let7-interaction with LIN28B. Nuc. Nucl. Nucleic Acids. 40(2), 194-211. doi: 10.1080/15257770.2020.1859116.

Participation in projects

» PID2020-115096RB-100 MCIN (2021-2024) Gemin5 function in RNA-driven processes: impact on neuromuscular diseases

» B2017/BMD-3770, RyPSE-CM, CM (2018-2022) RNA and RNA-binding proteins: impact in health and disease

» NATIONAL EXCELLENCE NETWORK RNAlife-2 RED2018-102467-T

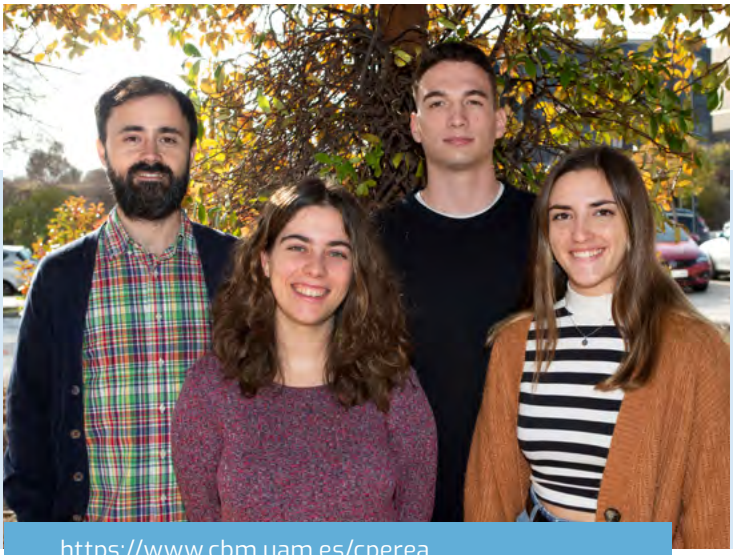
» EU-COST OC-2021-1-25480 (2022-2025) TRANSLACORE. Translational control in Cancer European network

Other activities

» SAB committee IBMC-CNRS, Strasbourg, France, 2021. Elected member of Academia Europea, 2021. Member of the Editorial Board: Virology, Virus Res, Front Microbiol.

» MASTER Supervisor: Salvador Abellan Perez (2021) Impact of Gemin5 Ser and Thr residues on protein stability. UAM.

DYNAMIC AND RECYCLING OF THE TRANSCRIPTIONAL MACHINERY ACROSS MITOSIS



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

Group Members

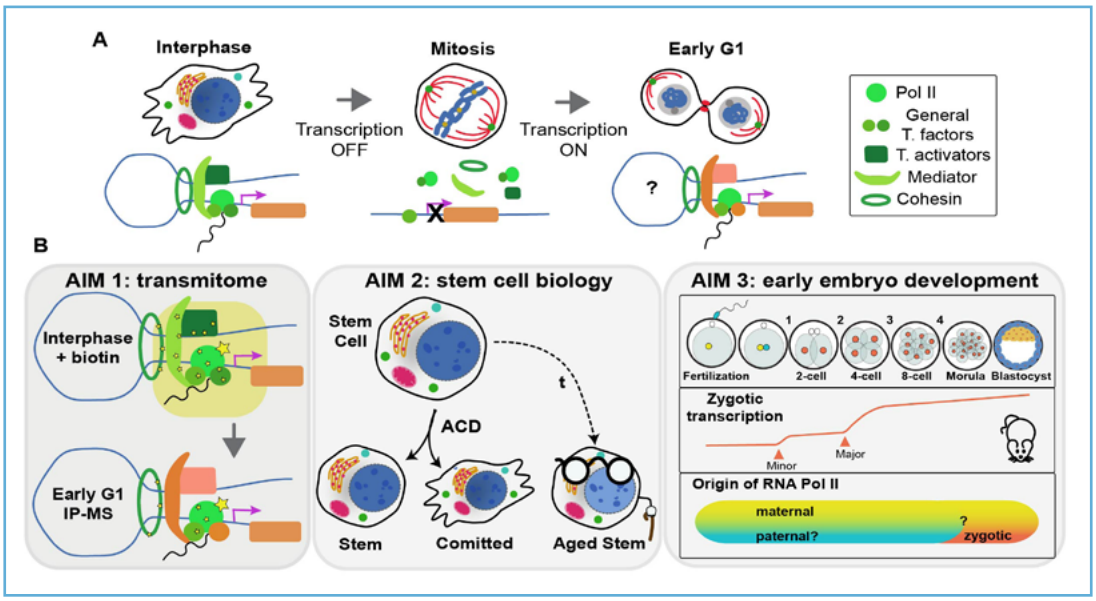
Principal Investigator:
Carlos Perea-Resa (since May 2022)

Undergraduate students:
Estrella Sayago (since September 2022)
Juan Ginés (since January 2023)
Irene Álvarez (since January 2023)

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

List of publications

These articles were published before incorporation to CBMSO.

» Ardehali MB, Damle M, Perea-Resa C, Blower MD, Kingston RE. (2021). Elongin A associates with actively transcribed genes and modulates enhancer RNA levels with limited impact on transcription elongation rate *in vivo*. J Biol Chem. 296:100202. doi: 10.1074/jbc.RA120.015877.

» Hekman RM, Hume AJ, Goel RK, Abo KM, Huang J, Blum BC, Werder RB, Suder EL, Paul I, Phanse S, Youssef A, Alysandratos KD, Padhorny D, Ojha S, Mora-Martin A, Kretov D, Ash PEA, Verma M, Zhao J, Patten JJ, Villacorta-Martin C, Bolzan D, Perea-Resa C, Bullitt E, Hinds A, Tilston-Lunel A, Varelas X, Farhangmehr S, Braunschweig U, Kwan JH, McComb M, Basu A, Saeed M, Perissi V, Burks EJ, Layne MD, Connor JH, Davey R, Cheng JX, Wolozin BL, Blencowe BJ, Wuchty S, Lyons SM, Kozakov D, Cifuentes D, Blower M, Kotton DN, Wilson AA, Mühlberger E, Emili A. Actionable Cytopathogenic Host Responses of Human Alveolar Type 2 Cells to SARS-CoV-2. Mol Cell. 2021 Jan 7;81(1):212. doi: 10.1016/j.molcel.2020.12.028. Erratum for: Mol Cell. 2020 Dec 17;80(6):1104-1122.e9. PMID: 33417854; PMCID: PMC7831449.

» Perea-Resa C, Bury L, Cheeseman IM, Blower MD. Cohesin Removal Reprograms Gene Expression upon Mitotic Entry. Mol Cell. 2020 Apr 2;78(1):127-140.e7. doi: 10.1016/j.molcel.2020.01.023. Epub 2020 Feb 7. PMID: 32035037; PMCID: PMC7178822.

» Perea-Resa C, Wattendorf L, Marzouk S, Blower MD. (2021). Cohesin: behind dynamic genome topology and gene expression reprogramming. Trends Cell Biol. 31(9):760-773. doi: 10.1016/j.tcb.2021.03.005.

Participation in projects

» Talento proposal, funded by Comunidad de Madrid (May 2022-June 2027). PI: Carlos Perea-Resa.

Other activities

» Awarded with the Talento program funded by Comunidad de Madrid and CSIC (May 2022-June 2027).

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

Group Members

Principal Investigator:
José Fernández Piqueras
(retired 25/8/22)

CoPI:
Javier Santos Hernández

Scientific Staff:
María del Consuelo Villa Morales
María del Pilar López Nieva
Concepción Vaquero Lorenzo
Alfonso Blázquez Castro

Postdoctoral fellows:
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(until september 2022)

Predoctoral fellows:
Antonio Lahera Alonso
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Laura Vela Martín
Sara Ruiz García.

Technicians:
Mª Ángeles Cobos Fernández
(until june 2022)
Isabel Merlín Sastre

Undergraduate students

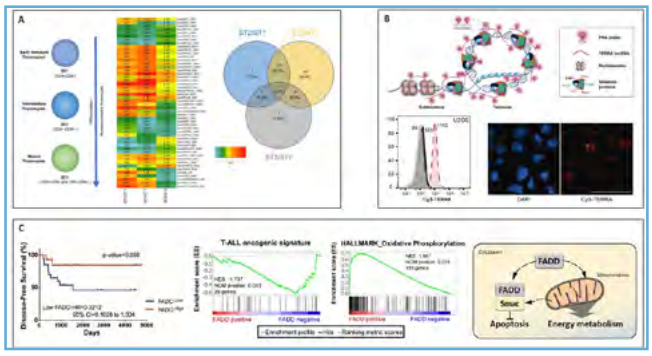
TFGs:
Natalia Ansede Bordonaba
(2021/2022)
Laura Pérez Gómez
(2021/2022)
Daniel Parra Sánchez
(2021/2022)

TFM:
Damián Stodulski Cielsa
(2021/2022)

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

Summary

T-cell lymphoblastic leukaemia/lymphoma (T-LBL and T-ALL) are haematological diseases with an urgent need for reliable prognostic biomarkers that allow therapeutic stratification and dose adjustment. Therefore, the major aim of our work is to decipher new molecular biomarkers and to propose more effective and less toxic treatments. To this end, we integrate data from genomics, transcriptomics and proteomics approaches as a start point to identify new driver-molecular mechanisms. During the 2021-2022 period we have continued with the identification of new mutations and changes in gene expression that have allowed us to propose new therapy strategies. In this sense, we have evidenced that the efficacy of γ-secretase inhibitors depends on the gene dosage of the MYC gene. We have also performed a proteomic analysis that reveals new non-apoptotic functions of FADD protein in these neoplasms. In order to improve our understanding on the efficacy of radiation, we have demonstrated the advantages of combined radiation regimens in controlling tumorigenesis that allow better control of healthy tissue homeostasis and facilitate tumour cell death. In addition, we are currently interested in evaluating the dysregulation of circular RNAs and long and short ncRNAs, to achieve a comprehensive view of the complex regulatory lncRNA/circRNA-miRNA-mRNA axes dysregulated in T-cell lymphoblastic neoplasms in the context of a personalized precision medicine. To this end, we have investigated the differential expression patterns of circRNAs in different development stages of human thymocytes to perform predictions in silico regarding the ability of specific circRNAs when controlling the expression of genes involved in thymocyte differentiation. Our study provides, for the first time, significant insights into the usefulness of circRNAs in discriminating between different stages of thymocyte differentiation and provides new potential circRNA-miRNA-mRNA networks capable of controlling the expression of genes involved in T-cell differentiation in the thymus. Regarding the analysis of lncRNAs, we have reported a new easy-to-use method, by coupling the specificity of a peptide nucleic acid (PNA)-labelled probe with flow cytometry (RNA-Flow FISH method that allows a reliable quantification of long lncRNAs, in particular those related to telomeres (TERRA and TERC) in cell lines and blood, with broad applications in basic research and clinical diagnostics.



A. Pairwise comparison analysis and differential expression of circRNAs in thymocytes at three stages of intra-thymic differentiation.
B. TERRA Quantification of lncRNA TERRA using RNA FISH-Flow methodology.
C. A dual role for FADD in human precursor T-cell neoplasms.

List of publications

» Marín-Rubio, J.L., Vela-Martín, L., Gudgeon, J., Pérez-Gómez, E., Sidgwick, F.R., Trost, M., Cunningham, D.L., Santos, J., Fernández-Piqueras, J., Villa-Morales, M. (2022). A dual role for FADD in human T-cell lymphoblastic neoplasms. Int. J. Mol. Sci. 23(23): 15157; https://doi.org/10.3390/ijms232315157.

» González-Vasconcellos, I., Cobos Fernández, M.A., Atkinson, M.J., Fernández- Piqueras, J., Santos, J. (2022) Quantifying telomeric lncRNAs using PNA-labelled RNA Flow-FISH (RNA-Flow). Commun. Biol. (Nature). 5(1): 513. doi: 10.1038/s42003-022-03452-3.

» López-Nieva, P., Fernández-Navarro, P., Cobos-Fernández, M.A., González-Vasconcellos, I., Sánchez-Pérez, R., Aroca, A., Fernández-Piqueras, J., Santos, J. (2022). Patterns of differentially expressed circRNAs in human thymocytes. Non-Coding RNA. 8(2): 26. doi: 10.3390/ncrna8020026. Cover Volume 8 Issue 2 April 2022.

» López-Nieva, P., González-Vasconcellos, I., González-Sánchez, L., Cobos-Fernández, M.A., Ruiz-García, S., Sánchez-Pérez, R., Aroca, A., Fernández-Piqueras, J., Santos, J. (2022). Differential molecular response in mice and human thymocytes exposed to a combined-dose radiation regime. Sci. Rep. 12(1): 3144. doi: 10.1038/s41598-022-07166-8.

» Lopez-Nieva, P., Gonzalez-Sanchez, L., Cobos-Fernandez, MA., Cordoba, R., Santos, J., and Fernandez-Piqueras, J. (2021) More insights on the use of γ-secretase inhibitors in cancer treatment. Oncologist. 25(2): e298-e305. doi: 10.1002/onco.13595.

Participation in projects and networks

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

» New biomarkers in precursor T-cell lymphoblastic neoplasms: intratumoral heterogeneity, editing of mRNA and exosomes (RTI2018-093330-B-I00). (1/1/2019 to 07/12/2022). Ministry of Science and Innovation PI1: José Fernández Piqueras. PI2: Javier Santos Hernández.

Doctoral theses

» Antonio Lahera Alonso (2022). "La desregulación de STAT5 en las neoplasias linfoblásticas de células T: bases moleculares subyacentes y posibles líneas de tratamiento. Universidad Autónoma de Madrid. Supervisors: José Fernández Piqueras y María Villa Morales.

Patents

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

» In-vitro method for quantifying the cellular content of specific RNAs. Inventors: Iria González Vasconcellos, María Angeles Cobos Fernández, José Fernández Piqueras, Javier Santos Hernández. Type: European patent. Reference: EP21382615.9. Date of register: 07/07/21.

» 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. Date of Presentation: 21/07/2021. On June 9, 2022, the Patent Cooperation Treaty (PCT) international application was filed with the European Patent Office (EPO), claiming priority of patent application No. EP21382663. The granted numbering was PCT/EP2022/065750. This PCT has received a favourable report and now it will continue the process; next step will be the National Phase Entry.

Other activities

» José Fernández-Piqueras. President of the Animal Experimentation Ethics Committee of the Severo Ochoa Center for Molecular Biology (CEEA-CBMSO) (2010-2022). Member of the External Scientific Committee of the Research Institute 12 October Hospital and its Permanent Commission (2016-2022). Member of the Board of Trustees of the Severo Ochoa Foundation (/2019-2022).

» Javier Santos. Member of Multidisciplinary Low Dose Initiative (MELODI). An European association studying the genetic effects of low dose ionizing radiation exposure in the European population (since 2010 to date).

REGULATION OF GENE EXPRESSION IN *LEISHMANIA*

Group Members

Principal Investigator:
Jose María Requena Rolanía

Scientific Staff
Manuel Soto Álvarez

Postdoctoral fellows
Alba Sebastián Martín (2021)
Jose Carlos Solana

Predoctoral fellows
Esther Camacho Cano
Alejandro Sánchez Salvador (sep-2022)

Technicians
Javier Adán Jiménez (2022)

Undergraduate and Master Students
Andrea Clemente Ureña (2021)
Carmen Palomino Cano (2021)
Mauricio Reinoso Dueñas (2021)
Stefania Merino (2022)

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

Summary

The early-diverging protozoan parasite *Leishmania* causes leishmaniasis in many regions of the world. This disease is ranked second (after malaria) among parasitic diseases. No acceptable vaccine for preventing leishmaniasis exists and treatment options are limited. Moreover, *Leishmania* is an atypical eukaryote regarding genome organization and gene expression regulation: genes are expressed as long transcription units requiring extensive post-transcriptional processing. Thus, *Leishmania* is an adequate model for studying post-transcriptional regulation without the interference of transcriptional regulation. The main research activity of our group is focused on genome organization and gene expression studies. Still, we maintain some activity in developing vaccines to prevent leishmaniasis and improving diagnosis/typing methods.

In the last coup of years, our group has continued improving the genomic assemblies of prototypical *Leishmania* species, by a combination of second and third-generation NGS methodologies. In this regard, the collaboration with the Genomics & Massive Sequencing service at CBMSO (headed by Dr Begoña Aguado) is paramount. In particular, we have generated a *de novo* assembly for *Leishmania* major (Friedlin) genome, which was the first *Leishmania* genome sequenced (in 2005) and from then it became a reference and source for many molecular studies. In consequence, the new assembly generated by our group has gained general acceptance (TriTrypDB). Also, our group has determined the poly-A+ transcriptome for this species and generated complete gene models, paving the way for addressing differential gene expression. Genomic annotations require a continuous process of curation, derived from new studies that uncover functional roles of genes/proteins previously unknown or by the characterization of new transcripts/peptides that obligate to reconsider some gene models. Thus, the alliance of proteomics, genomics, and transcriptomics has resulted in a powerful combination for improving the annotation of the *Leishmania* genomes. For hosting the genomics/transcriptomics data generated (and curated) by our group for four *Leishmania* species *(L. major, L. infantum, L. donovani y L. braziliensis)*, a web page was created: http://leish-esp.cbm.uam.es.

Finally, as members of the Tropical Diseases network (ISCIII; http://www.ricet.es/es/), which moved to a CIBER in infectious diseases (CIBERinfec; https://www.ciberinfec.es) in 2022, our group was engaged in collaborative research dealing with the molecular diagnosis and typing of *Leishmania* strains isolated from patients. In particular, we have characterized the *Leishmania* mitochondrial genome (aka, kinetoplast DNA or kDNA) which usually is ignored in genomic studies due to its structural complexity. After a characterization of the kDNA for several *Leishmania* species (and other trypanosomatids), we demonstrated that the kDNA maxicircle is a superior molecular marker for taxonomic and typing purposes in trypanosomatids.

List of publications

» Nocua, P.A., Requena, J.M., and Puerta, C.J. (2021). Identification of the interactomes associated with SCD6 and RBP42 proteins in *Leishmania braziliensis*. J. Proteomics 233, 104066. **doi:** 10.1016/j.jprot.2020.104066

» Soto, M., Ramírez, L., Solana, J.C., Cook, E.C.L., Hernández-García, E., Requena, J.M., and Iborra, S. (2021). Inoculation of the *Leishmania infantum* HSP70-II Null Mutant Induces Long-Term Protection against L. amazonensis Infection in BALB/c Mice. Microorganisms 9, 363. **doi:** 10.3390/microorganisms9020363

» Gómez, I., López, M.C., Rastrojo, A., Lorenzo-Díaz, F., Requena, J.M., Aguado, B., Valladares, B., and Thomas, M.C. (2021). Variability of the Pr77 sequence of L1Tc retrotransposon among six *T. cruzi* strains belonging to different discrete typing units (DTUs). Acta Trop. 222, 106053. **doi:** 10.1016/j.actatropica.2021.106053

» Camacho, E., González-de la Fuente, S., Solana, J.C., Rastrojo, A., Carrasco-Ramiro, F., Requena, J.M., and Aguado, B. (2021). Gene annotation and transcriptome delineation on a *de novo* genome assembly for the reference *Leishmania* major friedlin strain. Genes (Basel). 12, 1359. **doi:** 10.3390/genes12091359

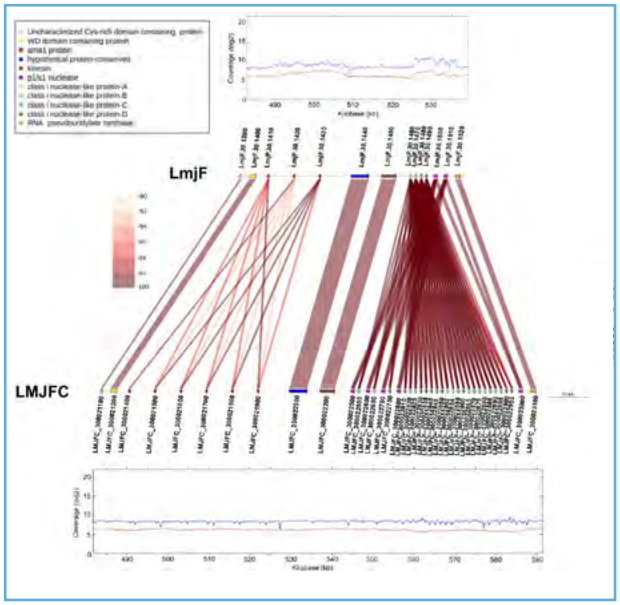
» Fernández, L., Solana, J.C., Sánchez, C., Jiménez, M.Á., Requena, J.M., Coler, R., Reed, S.G., Valenzuela, J.G., Kamhawi, S., Oliveira, F., Fichera, E., Glueck, R., Bottazzi, M.E., Gupta, G., Cecilio, P., Pérez-Cabezas, B., Cordeiro-da-Silva, A., Gradoni, L., Carrillo, E., and Moreno, J. (2021). Protective Efficacy in a Hamster Model of a Multivalent Vaccine for Human Visceral *Leishmaniasis* (MuLeVaClin) Consisting of the KMP11, LEISH-F3+, and LJL143 Antigens in Virosomes, Plus GLA-SE Adjuvant. Microorganisms 9, 2253. **doi:** 10.3390/MICROORGANISMS9112253

» Sacristán-Horcajada, E., González-de la Fuente, S., Peiró-Pastor, R., Carrasco-Ramiro, F., Amils, R., Requena, J.M., Berenguer, J., and Aguado, B. (2021). ARAMIS: From systematic errors of NGS long reads to accurate assemblies. Brief. Bioinform. 22, bbab170. **doi:** 10.1093/BIB/BBAB170

» Solana, J.C., Moreno, J., Iborra, S., Soto, M., and Requena, J.M. (2022). Live attenuated vaccines, a favorable strategy to provide long-term immunity against protozoan diseases. Trends Parasitol. 38, 316–334. doi: 10.1016/J.PT.2021.11.004

» Solana, J.C., Bernardo, L., Moreno, J., Aguado, B., and Requena, J.M. (2022). The Astonishing Large Family of HSP40/DnaJ Proteins Existing in *Leishmania*. Genes (Basel). 13, 742. doi: 10.3390/GENES13050742

» Solana, J.C., Chicharro, C., García, E., Aguado, B., Moreno, J., and Requena, J.M. (2022). Assembly of a Large Collection of Maxicircle Sequences and Their Usefulness for *Leishmania* Taxonomy and Strain Typing. Genes (Basel). 13, 1070. doi: 10.3390/GENES13061070



A new assembly of the L. major genome allowed the correction of complex genomic regions that were miss-assembled in the previous genome version for this species.

Participation in projects and networks

» Transcriptomics and genomics studies as molecular basis to design control strategies for leishmaniasis in Spain. Ministerio de Economía, Industria y Competitividad. Ref. SAF2017-86965-R. PIs: Jose M. Requena & Begoña Aguado. 2018-Jun, 2021

» Integrating OMICS data to decipher *Leishmania* gene organization and expression: clues for tackling leishmaniasis (Leish-OMICs). Agencia Estatal de Investigación. Ref. PID2020-117916RB-I00. PIs: Jose M. Requena & Begoña Aguado. Sep, 2021-2024

» Red de investigación colaborativa en enfermedades Tropicales (RICET). Instituto de Salud Carlos III. Ref. RD16/0027/0008. PI: Jose M. Requena. 2017-2021.

» CIBER en Área Enfermedades Infecciosas (CIBERINFEC). Instituto de Salud Carlos III. Ref. CB21/13/00018. PI: Javier Moreno. From 2022.

Doctoral theses

Esther Camacho Cano (2022) "Estudios genómicos y transcriptómicos en *Leishmania*". Universidad Autónoma de Madrid. Supervisors: Jose M. Requena & Begoña Aguado.

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

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

Group Members

Principal Investigator:
Iván Ventoso
Juan José Berlanga
Miguel Ángel Rodríguez
Margarita Cabrera
(since May 2022)

Postdoctoral fellows:
Tania Matamoros

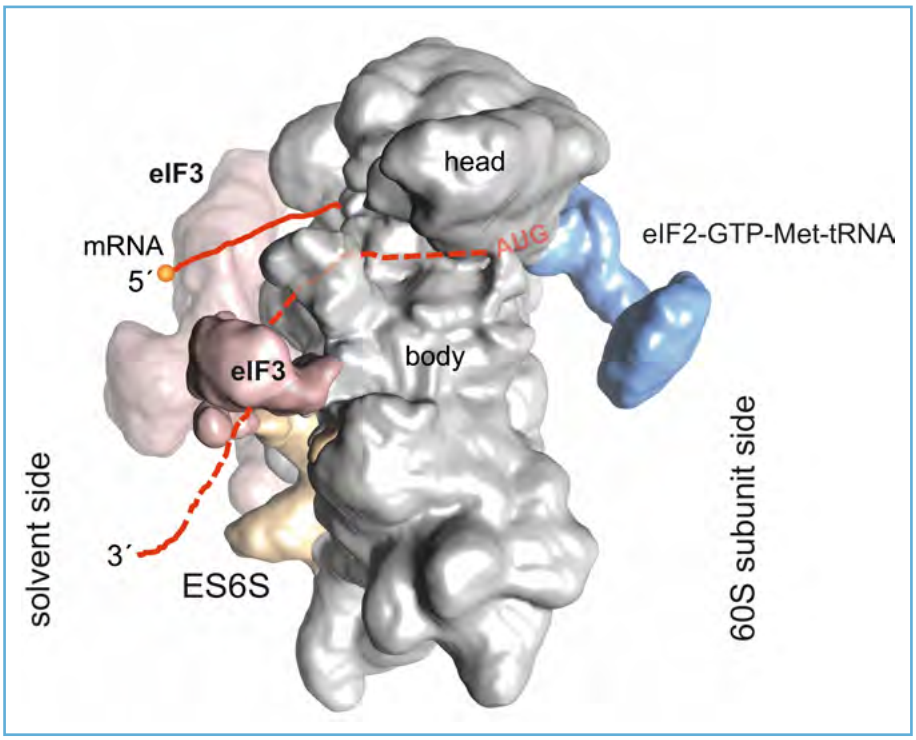
Technicians:
José Alcalde
Cristina Blanco

Undergraduate and Master Students:
Darío Aguilar
Alazne Rubio

Summary

We continue to investigate how eukaryotic systems (mammals, yeast and RNA viruses) regulate translation initiation at both global and message-specific manner, trying to identify new elements in ribosomes and mRNAs, and new initiation factor (eIFs) activities involved in the differential translation of mRNAs during cell proliferation and stress response. In the last two years, we further characterized the role of ES6S region of 40S ribosomal subunit in mRNA entry and ribosome scanning in different eukaryotic species (mammals, plants, insects and yeast). Our data suggest the ES6S region could be serving as a platform for the recruitment of RNA helicases (eIF4A and DDX3, among others) involved in RNA secondary structure unwinding. By using the nsp1 protein of SARS-CoV-2 as a tool, we are also studying how the base composition of 5' UTR and CDS regions shape translation and mRNA stability in human cells.

We continue to study how cells reprogramme translation during the stress response in yeast and mammals by modulating the activity of eIF2 and eIF2A factors, and the physiological impact of this response on cell and organismal adaptation, survival and aging. Thus, we recently found that preventing eIF2α phosphorylation not only impaired stress response, but also accelerated aging in yeast by a mechanism that involves proteostasis disruption. In this context, our research will focus on the study of key processes regulating proteostasis such as protein aggregation and autophagy and how they affect cell longevity.



Involvement of ribosomal ES6S region and eIF2 in mRNA threading and initiation codon recognition, respectively. Model of pre-initiation 43S complex. mRNA (red) penetrates through the ES6SA and ES6SB helices of the 40S subunit (yellow). The positions of eIF3 (pink) and the ternary complex (eIF2-GTP-Met-tRNAi, blue) are also shown.

List of publications

» Lafarga V, Sirozh O, Díaz-López I Galarreta A,Hisaoka M, Zarzuela E, Boskovic J, Jovanovic B, Fernandez-Leiro R, Muñoz J, Stoecklin G, Ventoso I, Fernandez-Capetillo O. (2021) Widespread displacement of DNA- and RNA-binding factors underlies toxicity of arginine-rich cell-penetrating peptides. EMBO J. 40(13):e103311. doi: 10.15252/embj.2019103311.

» Jiménez-Saucedo T, Berlanga JJ, Rodríguez-Gabriel M. (2021). Translational control of gene expression by eIF2 modulates proteostasis and extends lifespan. Aging (Albany NY). 13(8):10989-11009. doi: 10.18632/aging.203018.

» Vega M, Castillo D, de Cubas L, Wang Y, Huang Y, Hidalgo E, Cabrera M. (2022) Antagonistic effects of mitochondrial matrix and intermembrane space proteases on yeast aging. BMC Biology. 20(1):160. doi: 10.1186/s12915-022-01352-w.

Participation in projects

» Plataformas y modelos preclínicos para el abordaje multidisciplinar en COVID-19 y en respuesta a futuras pandemias (COVTRAVI-19-CM). Fondos REACT-EU. Coordinator, M Fresno, I Ventoso WP leader, 01-07-2021 al 31-12-2022

Genome Dynamics and Function

Genome Maintenance and Instability Unit

Luis Blanco

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

Luis Blanco group further delineated the mechanism of DNA primer synthesis by human PrimPol, a specific primase alleviating DNA replication stress, characterizing a motif crucial to stabilize the incoming 3´deoxynucleotide during replication fork restart, its discrimination against dideoxynucleotides, and the mechanism of PolDIP2-dependent stimulation of dNTP binding and processivity of human PrimPol.

Miguel de Vega group characterized a novel DNA polymerase (IEE) mainly present within enterohemorrhagic, enteropathogenic and enterotoxigenic *E. coli* strains. It has been shown that IEE is endowed with error-prone DNA polymerase activity able to promote dislocations of primer and template strands. Importantly, IEE is competent for DNA end-joining suggesting its potential to repair double-strand breaks.

José A. Tercero group has shown a differential recruitment of the central DNA damage tolerance protein Rad5/HLTF into two types of nuclear foci in response to cellular stress. This relocalization is mediated by the conserved E2-ubiquitin conjugating enzyme Rad6 through different pathways, uncovering a link between the maintenance of genome stability and proteostasis that, in turn, helps to better understand cellular homeostasis as a whole.

Applying a combination of sophisticated genomic approaches and single molecule analyses, María Gómez group unveiled unexpected regulatory roles of histone H1 on non-coding RNA turnover and m6A deposition, highlighting the intimate relationship between chromatin conformation, RNA metabolism, and DNA replication to maintain genome performance.

The group of Emilio Lecona has shown that the AAA ATPase VCP/p97 limits the basal activation of the replication stress response through the extraction of the DNA polymeras alpha/Primase complex from chromatin and they have proposed that ubiquitin and SUMO work as timers to control DNA replication.

Aura Carreira group has uncovered the RNA helicase DDX5, as an interacting partner of the tumor suppressor and DNA repair mediator BRCA2. Both proteins cooperate in the resolution of DNA-RNA hybrids at DNA double-strand breaks (DSBs) of highly transcribed regions. This activity in turn enhances high-fidelity repair of DSBs by homologous recombination in human cells. This function is impaired in certain BRCA2-associated breast cancer variants.

Ignacio Flores group has discovered unique patterns of telomere fusions related to the alternative lengthening of telomeres (ALT) in various types of cancer. A notable finding was the identification of telomere fusions in the blood of cancer patients, which could be used as a highly specific and sensitive method for early cancer detection.

As described in more detail in this report, de Vega and Blanco labs have continued their collaborative projects to develop a novel diagnostic method of SARS-CoV-2 infection, based on the isothermal amplification of specific padlock probes. This novel method is being protected by a CSIC patent.

Luis Blanco Dávila
GENOME MAINTENANCE AND VARIABILITY: ENZYMOLOGY OF DNA REPLICATION AND REPAIR

Aura Carreira Moreno
GENOME INSTABILITY AND CANCER PREDISPOSITION

Miguel de Vega
MAINTENANCE OF BACTERIAL GENOME STABILITY

Ignacio Flores
TELOMERES IN CANCER AND REGENERATION

María Gómez
FUNCTIONAL ORGANIZATION OF THE MAMMALIAN GENOME

Emilio Lecona
CHROMATIN, CANCER AND THE UBIQUITIN SYSTEM

José Antonio Tercero Orduña
CHROMOSOME REPLICATION AND GENOME STABILITY

GENOME MAINTENANCE AND VARIABILITY: ENZYMOLOGY OF DNA REPLICATION AND REPAIR

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

Group Members

Principal Investigator:
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Postdoctoral fellow:
María Isabel Martínez Jiménez

Predoctoral fellows:
Nieves Calero Muñoz
Cristina Velázquez Ruiz
Ana Martínez Carrón
Marcos Jiménez Juliana

Technician:
Susana Guerra González

Visiting scientist:
Paola Libertad García Medel
(February-October, 2022)

Summary

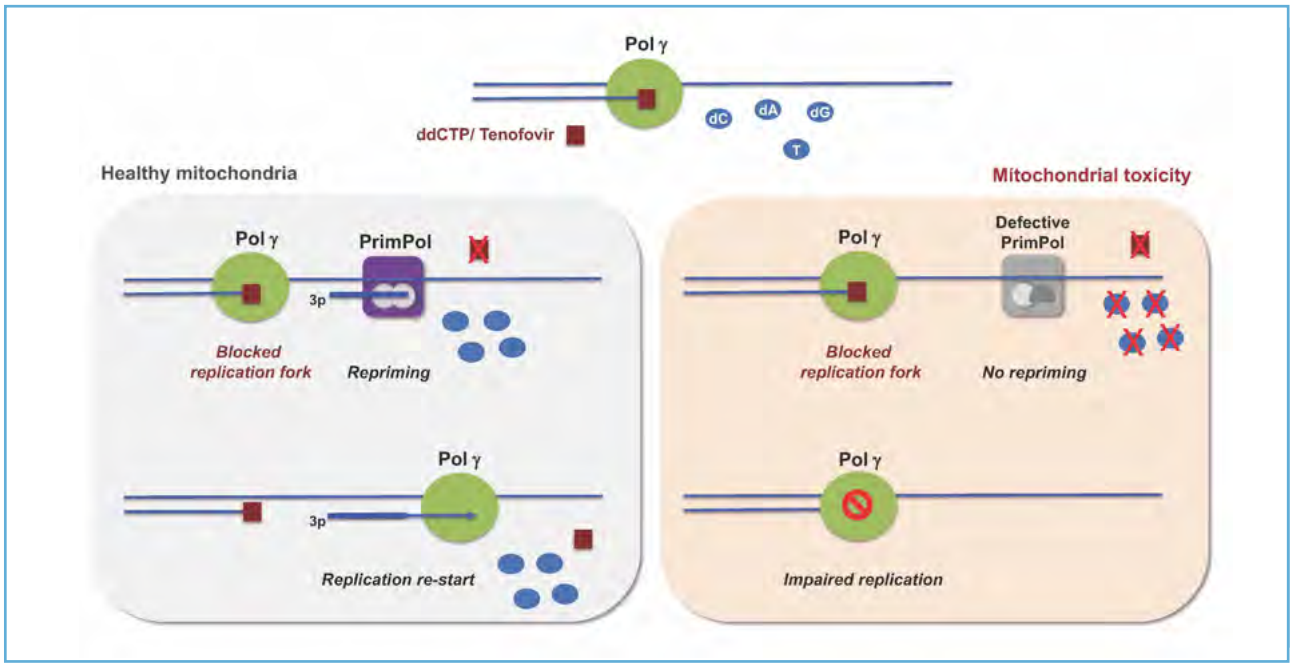
Replicative DNA polymerases are primarily responsible for the copying of template DNA. Overall DNA replication is assisted by other enzymes and factors that prevent the collapse of the replication fork when encountering unrepaired lesions and blocking structures on the template DNA, or upon insertion of unnatural nucleotides that can act as chain terminators. All these problems are defined as "replication stress" (RS), which can lead to genome instability.

More than a decade ago we described the relevance of a new enzyme, PrimPol, in the DNA replication process, and more specifically in tolerance to RS. In response to replication fork blockage, the unique DNA primase activity of PrimPol creates a new replicative starting point beyond the lesion or blockage point, which allows DNA replicase to continue replication, thus relieving RS, but leaving a gap behind that needs to be filled later.

Previous findings in collaboration with Juan Méndez lab (CNIO; Madrid), showed a significant reduction of replication fork speed during nuclear DNA replication in the absence of PrimPol, that promoted firing of new replication origins. We have recently shown in collaboration with Karen Anderson (Yale Univ. USA) that this PrimPol-mediated repriming mechanism has also relevance for mitochondrial DNA maintenance, especially under therapeutic conditions with antiretroviral nucleotide analogues. This is because PrimPol is able to discriminate various nucleotide analogues as tenofovir or ddC (Carvalho et al., 2021), but the absence of repriming due to a dysfunctional PrimPol hinders successful mitochondrial DNA replication, ultimately leading to mitochondrial toxicity. Therefore, PrimPol re-priming becomes a key element for DNA replication specially under RS conditions at both nuclear and mitochondrial DNA replication.

As part of our detailed structure-function studies of PrimPol, we have identified a key amino acid motif, WFYY, crucial to bind the incoming 3´dNTP at the dimer formation step of primer synthesis (Calvo et al., 2021). In collaboration with Sjoerd Wanrooij lab (Univ Umea, Sweden), we have recently shown that the accessory replication protein PolDIP2 is a PrimPol interactor which stimulates PrimPol activity by increasing its affinity for the dNTP substrates (Kasho et al., 2021).

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



Mitochondrial DNA replication stress provoked by nucleotide analog therapy or lesion in the template is alleviated by PrimPol. Left panel: In a functional PrimPol scenario (healthy mitochondria), blocked mitochondrial DNA replication is recovered by PrimPol re-priming. Right panel: the absence of functional PrimPol (mutated or inhibited) leads into impaired mtDNA replication (mitochondrial toxicity).

List of publications

» Carvalho, G., Díaz-Talavera, A., Calvo, P.A., Blanco, L. and Martínez-Jiménez, M.I. (2021) Human PrimPol discrimination against dideoxynucleotides during primer synthesis. Genes 24;12(10):1487. doi: 10.3390/genes12101487.

» Calvo, P.A., Martínez-Jiménez, M.I., Díaz, M., Stojkovic, G., Kasho, K., Guerra, S., Wanrooij, S., Méndez, J. and Blanco, L. (2021) Motif WFYY of human PrimPol is crucial to stabilize the incoming 3'-nucleotide during replication fork restart. Nucleic Acids Res. 49, 8199-8213. doi: 10.1093/nar/gkab634

» González-Acosta, D., Blanco-Romero, E., Ubieto-Capella, P., Mutreja, K., Míguez, S., Llanos, S., García, F., Muñoz, J., Blanco, L., Lopes, M., & Méndez, J. (2021). PrimPol-mediated repriming facilitates replication traverse of DNA interstrand crosslinks. The EMBO Journal, 40(14), e106355. doi: 10.15252/embj.2020106355

» Kasho, K., Stojkovič, G., Velázquez-Ruiz, C., Martínez-Jiménez, M. I., Doimo, M., Laurent, T., Berner, A., Pérez-Rivera, A.E., Jenninger, L., Blanco, L., Wanrooij, S. (2021). A unique arginine cluster in PolDIP2 enhances nucleotide binding and DNA synthesis by PrimPol. Nucleic Acids Research, 49(4), 2179-2191. doi:10.1093/nar/gkab049

Participation in projects

» IND2018/BMD-9984. Doctorado Industrial. Principal Investigator: Luis Blanco.

» CONVENIO CAIXAIMPULSE (CF01-00005). Simple and rapid SARS-CoV2 diagnostic test by phi29 polymerase amplification. Principal Investigator: Luis Blanco.

» PGC2018-093576-B-C21 (FEDER). Deciphering new roles of specialized human DNA polymerases in DNA replication and repair (until September 2022). Principal Investigator: Luis Blanco.

» PID2021-125966OB-I00 (FEDER-UE). Optimización de metodologías isotérmicas de amplificación de ácidos nucleicos (from September 2022). Principal Investigator: Luis Blanco.

Research Networks

» Genomic Instability. Red CONSOLIDER

» PTI+ Salud Global. Subproject WP3-IVD

GENOME INSTABILITY AND CANCER PREDISPOSITION

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

Group Members

Principal Investigator:
Aura Carreira Moreno
Since January 2022-

Predoctoral fellow:
Rady Chaaban
Since April 2022-

Predoctoral fellow:
Lucia Alvaro Aranda
Since March 2022-

Predoctoral fellow:
Anna Minello
(at Institut Curie, FR)

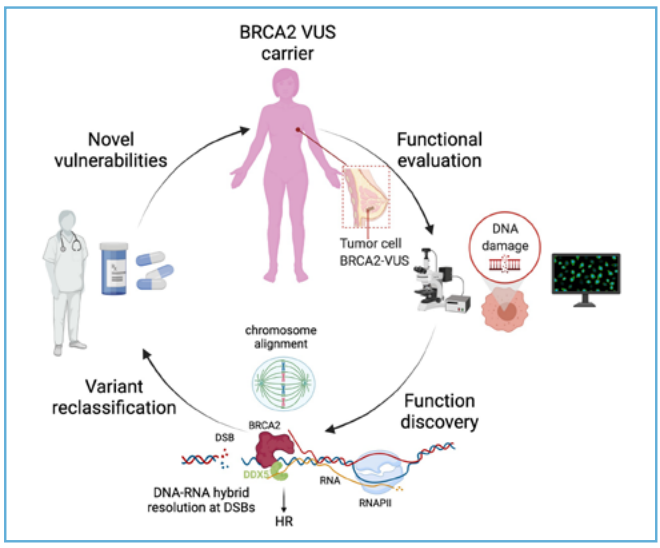
Postdoctoral fellow:
Jesus Gomez Escudero
Since May 2022-

Technician:
Aida Contreras Perez
Since March 2022-

Summary

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

Through a BRCA2-N-terminus 'interactome' performed in our lab, we have identified and characterized novel interacting partners of BRCA2 involved in other DNA repair pathways. This screening revealed RNA helicases and splicing factors among the most enriched. Interestingly, BRCA2 deficient cells accumulate R-loops providing evidence for its role in either R-loop prevention or processing. During this period, we have shown that BRCA2 and the RNA helicase DDX5 cooperate to resolve DNA-RNA hybrids DNA double-strand breaks located in transcribed regions (Sessa et al EMBOJ 2021). This work was performed in collaboration with the group of A. Aguilera (Cabimer, Seville, SP). I was invited to present this work in multiple occasions including the "DNA damage, Mutation and Cancer" Gordon Conference in Ventura, CA (US) in March 2020 or the Jacques Monod Conference in Roscoff, (FR) in 2019. Other highlights of this period are listed below:



Schematic representation of the possible impact of the functional characterization of BRCA2 VUS detected in breast cancer patients on function discovery. In addition to the better understanding of the mechanisms of genome maintenance, these novel functions may have important clinical implications for the reclassification of VUS and therefore for the genetic counseling of cancer patients and their families. In turn, these new activities of BRCA2 or new partners may lead to the discovery of vulnerabilities that could be exploited for treatment. DSBs: DNA double-strand breaks; RNAPII, RNA polymerase II. Figure created with BioRender.com.

List of publications

» Biswas B, Chaaban R, Chakraborty S, Devaux A, Dian AL, Minello A, Singh JK, Vagner S, Uguen P, Lambert S, Dutertre M, Carreira A. (2022) At the crossroads of RNA biology, genome integrity and cancer. Bull Cancer. Jun;109(6):728-735. doi: 10.1016/j.bulcan.2022.02.014.

» Sessa G, Ehlén Å, von Nicolai C, Carreira A (2021). Missense Variants of Uncertain Significance: A Powerful Genetic Tool for Function Discovery with Clinical Implications. Cancers (Basel) (review). 2021 Jul 23;13(15):3719. doi: 10.3390/cancers13153719

» Ehlén Å, Sessa G, Zinn-Justin S, Carreira A. (2021) The phospho-dependent role of BRCA2 on the maintenance of chromosome integrity. Cell Cycle 20(8):731-741. doi: 10.1080/15384101.2021.1892994

» Sessa G*, Gómez-González B*, Silva S, Pérez-Calero C, Beaurepere R, Barroso S, Martineau S, Martin C, Ehlén Å, Martínez JS, Lombard B, Loew D, Vagner S, Aguilera A#, Carreira A#.(* equal contribution, # corresponding author) (2021) BRCA2 promotes DNA-RNA hybrid resolution by DDX5 helicase at DNA breaks to facilitate their repair. EMBO J; 40(7):e106018. doi: 10.15252/embj.2020106018

» D Vugic, Å Ehlén, A Carreira (2021): Book: Homologous Recombination: Monitoring Homologous Recombination Activity in Human Cells Methods in Molecular Biology 2153, 115-126 (invited contribution, co-editor) Humana Press Springer ISBN 978-1-0716-0643-8. doi: 10.1007/978-1-0716-0644-5_9

» Julien M, Ghouil R, Petitalot A, Caputo SM, Carreira A, Zinn-Justin S. (2021) Intrinsic Disorder and Phosphorylation in BRCA2 Facilitate Tight Regulation of Multiple Conserved Binding Events. Biomolecules. Jul 20;11(7). doi: 10.3390/biom11071060.

» Gómez-González B, Sessa G, Carreira A, Aguilera A. (2021) A new interaction between BRCA2 and DDX5 promotes the repair of DNA breaks at transcribed chromatin. Mol Cell Oncol. ;8(3):1910474. doi: 10.1080/23723556.2021.1910474.

» Co-edited with Andres Aguilera the book Methods in Molecular Biology, "Homologous Recombination"

» Co-edited with Sergey Korolev the Special Issue of the journal Genes, "The Key of DNA Recombination and Replication: Recombination Mediator Proteins"

Participation in projects

National:

» 2021-2023: BRCAFORK AEI (Spanish Agency of Science and Innovation) grant (coordinator)

International:

» 2022-2024: Unveiling mechanisms of tumor formation linked to BRCA2 mutation; Worldwide Cancer Research-AECC grant (Coordinator)

» 2022-2027: RNAREP International Research Project (IRP) CNRS-CSIC (co-coordinator)

Other activities

» 2021: organization of the 5th Curie international course on "Post-transcriptional gene regulation joint with 3rd course on "Genome instability and Human Disease".

» 2022: organization of the biannual Institut Curie International PhD course on "Genome Instability and human Disease"

MAINTENANCE OF BACTERIAL GENOME STABILITY

Group Members

Principal Investigator:
Miguel de Vega

Postdoctoral fellow:
Patricia A. Calvo
(until August 2021)
Carlos D. Ordoñez
(since February 2022)
Alicia del Prado
(since October 2022)

Research Assistant:
Silvia Díaz
(since May 2021)

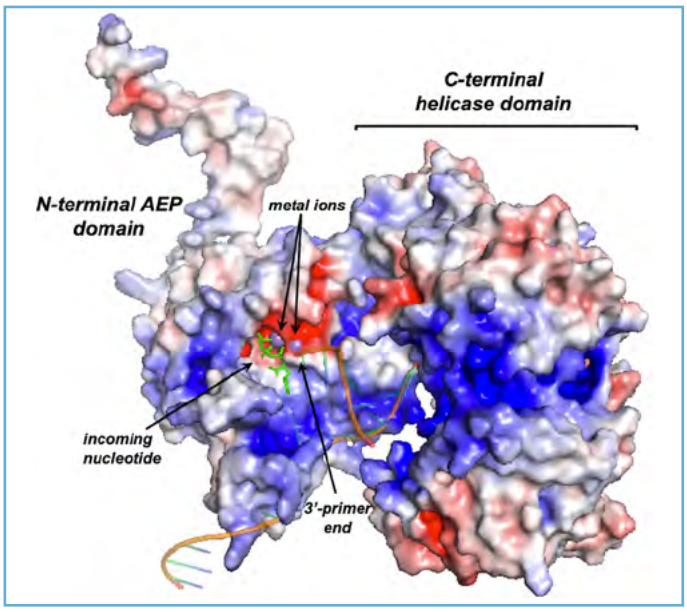
Undergraduate student:
Amalia Buitrago
(until June 2021)
Iza Oliwia Bienkowska
(since January 2022 until June 2022)

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

Summary

Bacterial genomes contain an abundance of transposable insertion sequence (IS) elements responsible for the generation of beneficial mutations that cause both genome evolution and bacterial adaptation, although the unrestrained expansion of IS elements could be harmful. IS629 is present in most strains of enterohemorrhagic *Escherichia coli* O157 and provokes polymorphisms associated with gene inactivation and/or genomic deletions. Previous results showed that excision of IS629 is promoted by the IS-excision enhancer (IEE) protein in a transposase dependent manner. We have shown that IEE is present in more than 30% of all available *E. coli* genome assemblies, mainly within enterohemorrhagic, enteropathogenic and enterotoxigenic genomes. We showed that IEE is endowed with an error-prone DNA polymerase activity in its N-terminal archaeo-eukaryotic primase (AEP) domain able to promote dislocations of the primer and template strands. Importantly, IEE has the ability to carry out an end-joining reaction of 3'-single-strand DNA overhangs. 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 that once the DSBs are created by the transposase, IEE would be instrumental to search for microhomologies between both ends allowing their repair.

During these years we collaborated with Thomas Helleday lab (Karolinska Institute) in the analysis of human OGG1 (hOGG1) activity in the presence of small activators. hOGG1 is a bifunctional glycosilase that removes 8-oxoguanine from the DNA generating an abasic site (AP) and has a very weak AP lyase that cleaves the AP site releasing a 3'-PUA end. Both, the AP sites and the 3'-PUA ends are subsequently removed by human AP endonuclease 1. Previous analysis from the Helleday's group showed that a small compound TH10785 activated the AP lyase activity of hOGG1. Our in vitro analyses allowed us to conclude that the small activator was conferring a new activity to hOGG1 that enabled the enzyme to break AP sites releasing a product with a terminal 3' phosphate that should be eliminated by a phosphatase to complete the repair process. This result was confirmed *in vivo* by the Helleday's group that showed that TH10785 created a dependency of PNPK1 (the main phosphatase involved in base excision repair) to repair the oxidative damage in cells. This work opened the possibility to reroute or control DNA repair pathways.



Modeling of the interaction of IEE with a DNA substrate. The incoming nucleotide, metal ions A and B and DNA are shown as sticks, purple spheres and ribbons, respectively. IEE is shown as electrostatic surface representation. The figure was done by superposition of the catalytic active sites of IEE and the ternary M. smegmatis LigD1-DNA-NTP complex [PDB 6SA0]. The predicted structure of IEE from E. coli O157:H7 was obtained through the Uniprot web site (https://www.uniprot.org/) from the Alphafold Database ID A0A0H3JF09, and used under CC BY4.0 License. The original colors were changed as described.

List of publications

» Michel, M., Benitez-Buelga, C., Calvo, P.A., Hanna, B.M.F., Mortusewicz, O., Masuyer, G., Davies, J., Wallner, O., Sanjiv, K., Castañeda-Zegarra, S., Jemth, A-S., Visnes, T., Sastre-Perona, A., Albers, J.J., Danda, A.N., Homan, E.J., Marimuthu, K., Zhenjun, Z., Chi, C., Sarno, A., Wiita, E., von Nicolai, C., Komor, A., Rajagopal, V., Müller, S., Hank, E.C., Varga, M., Scaletti, E.R., Pandey, M., Karsten, S., Haslene-Hox, H., Loevenich, S., Martilla, P., Rasti, A., Mamonov, K., Ortis, F., Schömberg, F., Loseva, O., Stewart, J., D'Arcy-Evans, N., Koolmeister, T., Henriksson, M., Michel, D., de Ory, A., Acero, L., Calvete, O., Scobie, M., Hertweck, C., Vilotijevic, I., Kalderén, C., Osorio, A., Perona, R., Stolz, A., Stenmark, P., Berglund, U.W., de Vega, M., Helleday, T. (2022) Small-molecule activation of OGG1 increases oxidative DNA damage repair by gaining a new function. Science, 376: 1471-1476. doi: 10.1126/science.abf8980

Participation in projects

» Novel functions and mechanisms of bacterial Archaeo/Eucaryotic primase superfamily enzymes". Funding organism MCIN/AEI/10.13039/501100011033 and by "ERDF A way of making Europe" grant BFU2017-83900-P. Principal Investigator: Miguel de Vega. Period: 01/01/2018-30/09/2021

» 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. Funding organism MCIN/AEI/10.13039/501100011033 grant PID2020-115978GB-I00. Principal Investigator: Miguel de Vega. Period: 01/09/2021-30/09/2024.

Other activities

» Organizer of the symposium "DNA replication and repair" held during the 44th National Congress of the Spanish Society of Biochemistry and Biotechnology (September 2022)

TELOMERES IN CANCER AND REGENERATION

Group Members

Principal Investigator:
Ignacio Flores

Predoctoral fellow:
Javier Lucas de la Fuente

Technician:
Andreea Baradau

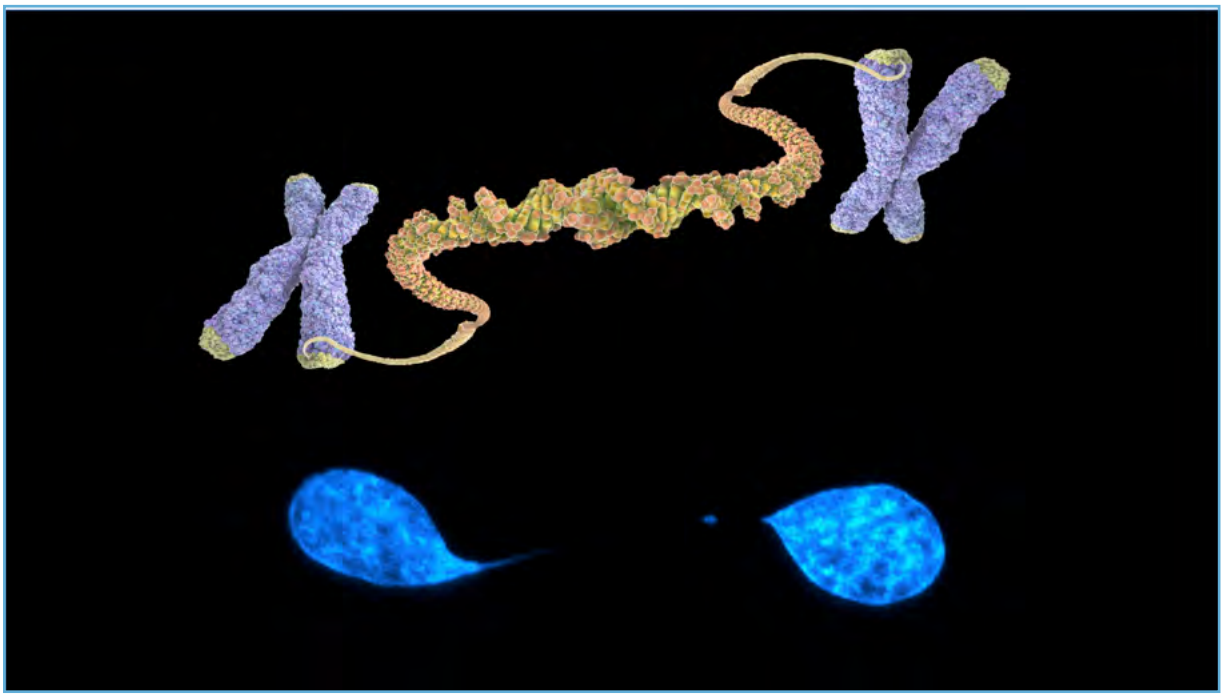
https://www.cbm.uam.es/iflores

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).

List of publications

» Francesc Muyas, Manuel José Gómez Rodriguez, Isidro Cortes-Ciriano, Ignacio Flores.The ALT pathway generates telomere fusions that can be detected in the blood of cancer patients. bioRxiv 2022.01.25.477771; doi: https://doi.org/10.1101/2022.01.25.477771

» Marielle Breau, Christelle Cayrou, Dmitri Churikov, Charles Fouillade, Sandra Curras-Alonso, Serge Bauwens, Frederic Jourquin, Laura Braud, Frederic Fiore, Rémy Castellao, Emmanuelle Josselin, Carlota SánchezFerrer, Giovanna Giovinazzo, Eric Gilson, Ignacio Flores, Arturo Londono-Vallejo, Serge Adnot, Vincent Géli. Telomerase Prevents Emphysema in Old Mice by Sustaining Subpopulations of Endothelial and AT2 Cells. bioRxiv 2021.01.07.425708; doi: https://doi.org/10.1101/2021.01.07.425708

Proyectos financiados

» TITLE: Role of telomerase and telomeres in polyploid induction and heart regeneration . Ref: PID2019-110339RB-I00 (2020-2024)

AGENCY: Ministerio de Ciencia, Innovación y Universidades

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

PI: Dr. Ignacio Flores

» TITLE: REANIMA (Molecular and cellular basis of cardiac regeneration: from animal models to the human heart). Ref B2017/BMD-3875

AGENCY: Biomedicine R&D Program. Community of Madrid (CAM)

DATES FROM: 01/01/2018 TO: 31/12/2022

PI: Dr. Ignacio Flores

Patents

» INVENTORS: Muyas, F, Gómez M, Cortes-Ciriano, I, Flores I.
TITLE: Detection of telomere fusion events.
APPLICATION NO.: 21217571.5 PRIORITY : Europe
PRIORITY DATE: 23/11/2021
HOLDER ENTITY: CSIC (CBMSO), CNIC, EMBL
COUNTRIES TO WHICH IT HAS BEEN EXTENDED: USA, Japan

FUNCTIONAL ORGANIZATION OF THE MAMMALIAN GENOME

Group Members

Principal Investigator:
María Gómez

Postdoctoral fellow
José Miguel Fernández Justel (until August 2021)

Predoctoral fellows:
Cristina Santa María Tobías
Sara Martín Vírgala
Javier Isoler Alcaraz
(co-IP: César Cobaleda)
Sara Tur Gracia
(co-IP: Carlos Estella)

Master and undergraduate students
Álvaro Eugenio Álvarez Montero
(until June 2021;
co-IP: Emilio Lecona)
Jesús Garzón Ganaza
(until June 2021)
Sasha Wilisz
(August 2021-June 2022)
Alicia Gallego Jiménez
(september 2021-June 2022)

Visiting scientist:
Shreya Ramesh
(until August 2021)

www.cbm.uam.es/mgomez

Summary

Copying and decoding the genomic information in a timely and accurate fashion is essential for life. Both tasks (DNA replication and transcription) are remarkably complex in scale and regulation, and the molecular machineries involved in them translocate on the same substrate, the chromatin. The general aim of our laboratory is understanding how the processes of DNA replication and transcription are coordinated in mammalian cells to maintain genome stability. This knowledge is fundamental for basic biology and have enormous implications for cell fate specification in physiology and pathology. To investigate this we combine the application of genomics, epigenomics, epitranscriptomics, molecular biology and imaging techniques to a variety of cell scenarios with altered chromatin structure and/or transcription dysregulation.

In our recent work we showed that appropriate RNA turnover in chromatin is essential to avoid replicative stress and limit replication-transcription conflicts, and unveiled an unexpected role of histone H1 on RNA post-transcriptional modifications. In particular, we found that non-coding RNA retention in chromatin associates with reduced levels of m6A in histone H1-depleted cells, resulting in increased stability of chromatin-bound RNAs and R-loops accumulation, which in turn generates problems with incoming DNA replication forks. Accordingly, altering the m6A RNA methylation pathway rescues the replicative phenotype of H1 loss. Our findings uncover a novel link between chromatin conformation and non-coding RNA turnover and m6A deposition, with important implications for understanding genome functionality. In addition, in this period we have investigated the usage of replication origins upon stress challenges. We found that is the frequency of activation of the same pool of replication origins what is mainly increased upon stress, and that, in unchallenged cells, the frequency of activation correlated with the 3D organization of the origins in the nucleus. These results support the proposed architectural organization of origins in DNA replication factories and highlight the tight similarities between replication and transcription multimolecular assemblies in the 3D nucleus.

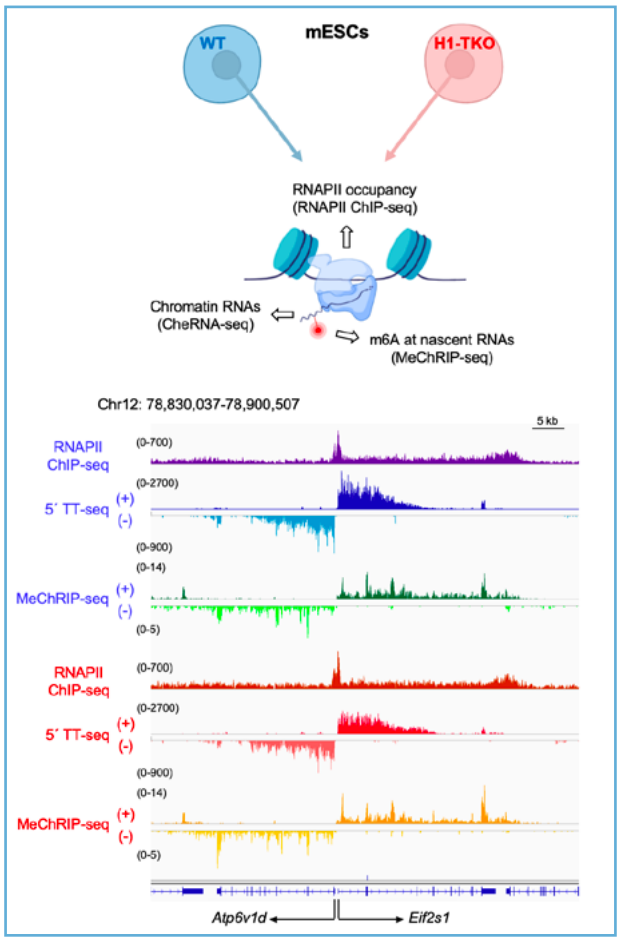


Figure legend: Integrative analyses of transcription dynamics and m6A deposition on nascent RNAs in mouse embryonic stem cells (mESCs) with reduced levels of histone H1 (H1-TKO).

List of publications

» Fernández-Justel, JM., Santa-María, C., Martín-Vírgala, S., Ramesh, S., Ferrera-Lagoa, A., Salinas-Pena, M., Isoler-Alcaraz, J., Maslon, MM., Jordan, A., Cáceres, J and Gómez, M. (2022) Histone H1 regulates non-coding RNA turnover in chromatin in a m6A-dependent manner. Cell Rep 40: 111329. doi: 10.1016/j.celrep.2022.111329

» Gallego, A., Fernández-Justel, JM., Martín-Vírgala, S., Maslon, MM. And Gómez, M. (2022) Slow RNAPII transcription elongation rate, low levels of RNAPII pausing, and elevated histone H1 content at promoters associate with higher m6A deposition on nascent mRNAs. Genes 13: 1652. doi: 10.3390/genes13091652

» Jodkowska, K., Pancaldi, V., Almeida, R., Rigau, M., Fernández-Justel, JM., Graña, O., Rubio, M., Rodríguez-Acebes, S., Carrillo, E., Pisano, D., Al-Sharour, F., Valencia, A., Gómez, M*. and Méndez, J*. (2022) 3D chromatin connectivity underlies replication origin efficiency in mouse embryonic stem cells. Nucl. Acids. Res 50:12149-12165. *CO-CORRESPONDING AUTHORS. doi: 10.1093/nar/gkac1111

Participation in projects

» DNA replication as a driver for cell fate decisions (RepliFate) (GA101072903) European Research Executive Agency. MSCA Doctoral Network | Horizon Europe (HORIZON-MSCA-2021-DN-01). 2022-2026 (Co-PI)

» Replication-transcription crosstalk and its impact on genome homeostasis (Re-Talk) (PID2019-105949GB-I00). Ministry of Science, Innovation and Universities | Proyectos Excelencia. 2021-2023 (PI)

Other activities

» Organization of the Symposium of the MSCA Doctoral Network “RepliFate: DNA replication at the heart of cell fate decisions and cancer development” 21st-22nd November 2022. CBMSO. Madrid, Spain.

» Coordination of the “Regulation of Gene Expression and Genome Dynamics” group of the Spanish Society of Biochemistry and Molecular Biology (SEBBM), since 2021.

CHROMATIN, CANCER AND THE UBIQUITIN SYSTEM

Group Members

Principal Investigator:
Emilio Lecona

Predoctoral fellow:
Rodrigo Martín Rufo

Master and undergraduate students:
Tomás Viuda
(from October 2022)
Ángel Mora
(October 2021-July 2022)
Guillermo de la Vega Barranco
(October 2020-July 2021)
Clara Plaza
(from October 2022)
Alejandra Perona
(from October 2022)
Yolanda Andrés
(October 2021-June 2022)
Alejandro Fernández Llorente
(September 2020-June 2021)

Técnicos de laboratorio:
Daniel Hernández
(from September 2022)

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

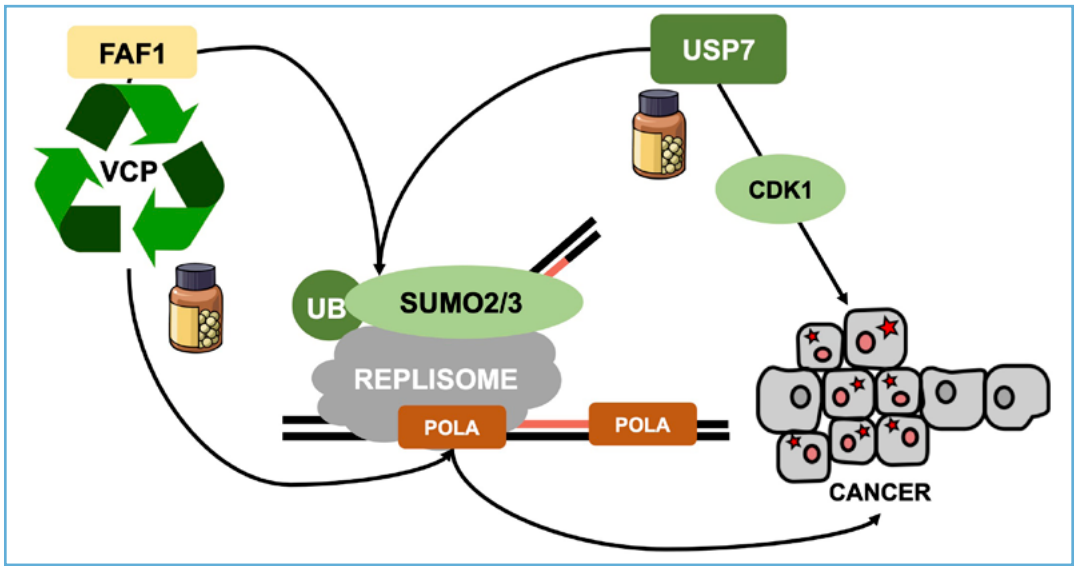
Summary

DNA replication is a central process in cell biology that mediates the copy of cellular DNA to ensure the faithful transmission of genetic information through cell division. Our group is interested in understanding how the small protein modifiers ubiquitin and SUMO control DNA replication. In the last two years we have provided new insights into the role of the SUMO/ubiquitin equilibrium in chromatin during DNA replication and cell cycle progression. Building up on our observation that the deubiquitylase USP7 maintains the SUMO rich environment at active replication forks (Lecona *et al.*, NSMB 2016; Lecona and Fernández Capetillo, Bioessays 2016), we now show that USP7 links S phase progression and the activation of the mitotic program through the regulation of CDK1 (Galarreta *et al.*, EMBO J 2021). USP7 inhibition leads to a premature activation of the mitotic program that contributes to cell death induced by USP7 inhibitors and is relevant for their application in cancer treatment (Galarreta *et al.*, IJMS 2021).

The SUMOylation and ubiquitylation of replication factors modulates their functions or targets them for extraction by the AAA ATPase VCP/p97 (Martín-Rufo *et al.*, Sem Cell Dev Biol 2022). Our work has shown that VCP/p97 associates with its adaptor FAF1 in S phase to extract SUMOylated and ubiquitylated factors from chromatin. Thus, VCP/p97 cooperates with the deubiquitylase USP7 to coordinately control the SUMO/ubiquitin landscape during DNA replication (Franz *et al.*, Cell Rep 2021). Further, we have observed that VCP/p97 limits the basal activation of the replication stress response (RSR) in an unperturbed S phase. In this context, VCP/p97 removes the DNA polymerase α/Primase complex from chromatin and prevents the accumulation of primed DNA structures that activate the RSR (Rodríguez-Acebes, Martín-Rufo *et al.*, bioRxiv 2022).

Finally, we have explored the functions of the group SUMOylation of the replisome and its interplay with the ubiquitylation of replication factors. Our results show that ubiquitin and SUMO play independent functions during DNA replication. While depleting protein SUMOylation alters DNA replication dynamics without inducing replication stress and DNA damage, the inhibition of the ubiquitin pathway activates the RSR without altering the speed of DNA replication forks.

In the last two years, our advances have established the relevance of the ubiquitin/SUMO landscape in chromatin during DNA replication. Now we want to explore how the ubiquitin/SUMO pathways shape replisome composition to control DNA replication, cell identity and cancer.



The SUMO-rich environment in the replisome is maintained by the action of USP7 and VCP/p97, in association with FAF1. In addition, USP7 controls the activation of CDK1 and VCP/p97 limits the replication stress response extracting the DNA polymerase α/primase complex (POLA) from chromatin. USP7 and VCP/p97 inhibitors are being explored in cancer treatment.

List of publications

» Rodríguez-Acebes S, Martín-Rufo R, Fernández-Llorente A, de la Vega-Barranco G, Oroz P, Martín-Doncel E., Toledo LI, Méndez J, Lecona E. (2022) VCP/p97 extracts DNA polymerase α/Primase to limit the activation of the replication stress response. bioRxiv. doi: https://doi.org/10.1101/2022.07.25.501345

» Martín-Rufo R., de la Vega-Barranco G., Lecona E. (2022) Ubiquitin and SUMO as timers during DNA replication. Semin. Cell Dev. Biol. 21: S1084-9521(22)00052-0. doi: 10.1016/j.semcdb.2022.02.013.

» Franz V, Valledor P, Ubieto-Capella P, Pilger D, Galarreta A, Lafarga V, Fernández-Llorente A, de la Vega-Barranco G, den Brave F, Hoppe T, Fernández-Capetillo O, Lecona E. (2021) USP7 and VCP^{FAF1} define the SUMO/Ubiquitin landscape at DNA replication forks. Cell Rep. 37: 109819. doi:10.1016/j.celrep.2021.109819

» Galarreta A, Valledor P, Fernández-Capetillo O, Lecona E. (2021) Coordinating DNA Replication and Mitosis through Ubiquitin/SUMO and CDK1. Int. J. Mol. Sci. 22: 8796. doi: 10.3390/ijms22168796.

» Galarreta A, Valledor P, Ubieto-Capella P, Lafarga V, Zarzuela E, Muñoz J, Malumbres M, Lecona E*, Fernández-Capetillo O. (2021) USP7 limits CDK1 activity throughout the cell cycle. EMBO J. 40:e99692. doi: 10.15252/embj.201899692 **Co-corresponding autor*

» El Motiam A, de la Cruz-Herrera CF, Vidal S, Seoane R, Baz-Martínez M, Bouzaher YH, Lecona E, Esteban M, Rodríguez MS, Vidal A, Collado M, Rivas C. (2021) SUMOylation modulates the satbility and function of PI3K-p110β. Cell. Mol. Life Sci. 78:4053-4065. doi: 10.1007/s00018-021-03826-6.

Projects and Networks

» RepliFate-Coordinator
DNA replication at the heart of cell fate decisions and cancer development
MSCA Doctoral Networks 2021
Ref-101072903.
October 2022-September 2026

» ProteoCure COST Action CA20113. Participant.
October 2021-October 2025

Other activities

» Symposium "DNA replication at the heart of cell fate decisions and cancer development" CBMSO (21/11/2022).

» Severo Ochoa Meetings Committee, Sociedad Española de Bioquímica y Biología Molecular (SEBBM).

CHROMOSOME REPLICATION AND GENOME STABILITY

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

Group Members

Principal Investigator:
José Antonio Tercero Orduña

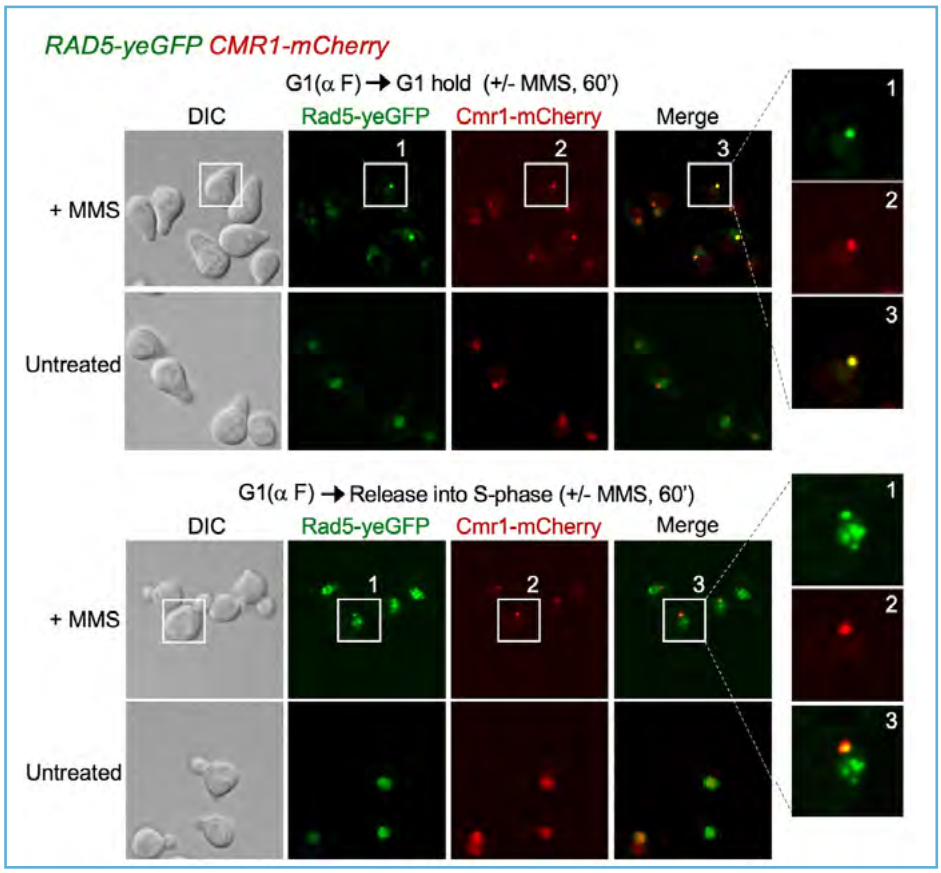
Predoctoral fellows:
Paula González Fernández
Carl P. Lehmann

Master students:
Alejandro Aznar Casado (until June 2021)
Miguel Ángel Prados Sánchez (Jan. 2022- Sep. 2022)

Summary

The aim of our group is to contribute to the understanding of the mechanisms that help prevent genomic instability, which is a hallmark of cancer as well as one of the causes of developmental and neurological disorders, premature ageing or inflammatory signalling. Our main goal is to better understand how genome stability is maintained during chromosome replication, especially in the presence of DNA damage or replicative stress. The molecular bases of these processes are evolutionarily conserved, allowing us to study them in detail using the genetically tractable yeast *Saccharomyces cerevisiae* as a eukaryotic model organism.

During this period we have focused our studies on the analysis of the regulation and function of proteins involved in the mechanisms of DNA damage tolerance (DDT). These mechanisms overcome unrepaired DNA lesions that block replication forks, thereby contributing to the completion of chromosome replication in every cell cycle, and are therefore important for the maintenance of genome integrity. A central DDT protein is the E3 ubiquitin ligase and ATPase/helicase Rad5 (HTLF/SHPRH in human cells), which mediates template-switch recombination, an error-free mode of bypassing DNA lesions, and also influences the recruitment and activity of translesion DNA synthesis polymerases. We have found that Rad5 is regulated in different ways, including relocalization, post-translational modifications and oscillation of protein levels throughout the cell cycle. We have shown that Rad5 is regulated in response to cellular stress by intranuclear relocalization via differential recruitment into two types of foci, both oftentimes coexisting within the same cell. We have found that one type of foci represents sites of protein activity while the other marks places of sequestration of damaged or misfolded protein destined for subsequent refolding. We have deciphered the common and distinct pathways and mechanisms involved in the formation of each type of foci and have uncovered a link between the maintenance of genome stability and proteostasis, which in turn helps to understand cellular homeostasis as a whole. We have also shown that Rad5 reaches maximum protein levels during the S phase of the cell cycle. These levels are directly related to the timing of this protein's activity and are strictly regulated by the ubiquitin proteasome pathway. Moreover, we have found that Rad5 is a phosphoprotein, which may have implications for its activity and mode of action.



Live-cell fluorescence microscopy image showing differential recruitment of the Rad5 protein into two types of nuclear foci in response to cellular stress during the G1 and S phases of the cell cycle.

List of publications

» Lehmann, C. P., Saugar, I. and Tercero, J. A. (2021) Fluorescence microscopy for analysis of relocalization of structure-specific endonucleases. Methods Mol. Biol. 2153: 521-534. doi: 10.1007/978-1-0716-0644-5_535.

Research networks

» RED2018-102372-T (MICINN). Genome Instability (INESGEN). 2019-2021.

Participation in projects

» PID2020-119708GB-I00 (MICINN). DNA damage tolerance in eukaryotic cells: regulation, mechanisms and importance for genome integrity maintenance (DDT-RMGIM). 2021-2024. Principal Investigator: José A. Tercero.

PROGRAM

Tissue and Organ Homeostasis

Tissue and Organ Homeostasis

Cell-Cell Communication & Inflammation Unit

María Yañez-Mó

Cell-cell communication through electric signals, soluble mediators, extracellular vesicles, or adhesion to the extracellular matrix network is essential for differentiation during development and maintenance of tissue homeostasis. Tumour progression, aging and some genetic diseases related to loss of tissue homeostasis, deregulated intercellular communication and increased inflammation and fibrosis. The Cell-Cell communication & Inflammation Unit combines efforts of several groups using cellular or animal models, both mammals and *Drosophila* to study cell-cell and cell-matrix interactions during development, homeostasis or age-associated diseases.

Highlights of the unit

- Identification of the protein PLLP as part of the trafficking machinery that determines the apical polarization of intercellular adhesion molecule (ICAM)-1, the main epithelial adhesion receptor involved in epithelium-leukocyte interactions (Cacho-Navas et al. Cell Mol Life Sci, 2022)
- The proposal of the mechanism for long-distant signal transport and transfer through direct cell-to-cell contact (Gradilla & Guerrero, Curr Top Dev Biol. 2022), and in silico model of this mechanism for morphogen gradient formation (Aguirre-Tamaral & Guerrero, Plos Comput Biol, 2022).
- The description of the interaction between glypicans and the adhesion molecule Ihog in cytoneme-mediated Hedgehog signaling (Simon et al., Elife 2021), and an in silico model for the implication of this interaction in cytoneme guidance (Aguirre-Tamaral et al., Nature Comm. 2022).
- The description of the relevance of the adhesion molecule ALCAM/CD166 in the interactions of cancer-derived Extracellular Vesicles (EVs) and their uptake by recipient cells with potential implications in the peritoneal metastasis cascade promoted by EVs in colorectal and ovarian cancer (Cardeñes et al., Int J Mol Sci 2022).
- Evidence for the connection of Extracellular Vesicles biogenesis routes with those regulating mitophagy and cellular metabolism homeostasis in melanoma cells (Suarez et al., JEV 2021 and Toribio & Yáñez-Mó EJCB, 2022)
- The mechanisms of chromatin remodeling during embryonic development and regeneration in *Drosophila* and how heterochromatin marks disappear and are recovered when regeneration is complete, correlating in time with levels of transcription of two retrotransposons, Roo and F-element (Azpiazu & Morata Dev. Biol 2022).

- The decoding of the molecular mechanisms by which aged T cells contribute to inflammaging and age-related diseases: (a) Th1-Cytokines induce cellular senescence (b) Loss of Self-tolerance mechanisms. (c) Defective immuno-surveillance of senescent cells. (d) Altered gut microbiota. (Soto-Heredero et al., Cell Metabolism 2021; Carrasco et al., Nature Rev Immunol, 2022).
- The proposal of new therapeutic targets to reverse aortic aneurysms and prevent sudden death due to aortic dissections (Oller et al., Circulation, 2021 and Atherosclerosis, Thrombosis Vascular Biology, 2022)
- The delineation of the signaling pathway activated by nitric oxide that leads to aortic aneurysm formation in Marfan syndrome, identifying potential biomarkers and therapeutic targets for this disease (de la Fuente-Alonso et al., Nat Comm 2021) that have been protected by a European patent (PCT/EP2022/060201).
- The identification of genes and pathomechanisms underlying hydrocephalus, the pathological enlargement of cerebral ventricles (del Puerto et al., Mol. Psychiatry 2021 and PCT/EP2022/061794).
- The understanding through a multidisciplinary genetic, cellular and molecular approach of the key roles of T-cell intracellular antigens in physiopathological events and the connection between abnormal dynamics of TIA1-dependent stress granules and Welander distal myopathy (Carrascoso et al., Int. J. Mol. Sci. 2021, Fernández-Gómez & Izquierdo Int. J. Mol. Sci. 2022, Velasco &Izquierdo Int. J. Mol. Sci. 2022; Fernández-Gómez et al., Cells, 2022).
- The development and preclinical study of two novel peritoneal dialysis fluids with reduced impact on the epithelial to mesenchymal transition of mesothelial cells and reduced peritoneal damage compared with standard dialysis fluids (Kopytina V, et al., Front Pharmacol. 2022; Herzog R, et al., Sci Transl Med. 2021). A new dialysis fluid using as osmotic agent a natural flavonoid (Troxerutin), with cardioprotective effects and reduced mesothelial insult was patented (P202230612)
- The identification of proteases processing the matrix-remodeling enzymes of the lysyl oxidase family as well as the regulation of their enzymatic activity (Rosell-García et al., Int. J. Mol. Sci. 2022; Rodríguez-Pascual & Rosell-García, Analytical Biochemistry 2022).

Carlos Cabañas Gutiérrez
FUNCTIONAL INTERACTIONS BETWEEN TETRASPANINS 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
MOLECULAR PATHOPHYSIOLOGY OF PERITONEAL INFLAMMATION AND FIBROSIS (PERINFIB)

Jaime Millán
CELL BIOLOGY OF INFLAMMATION

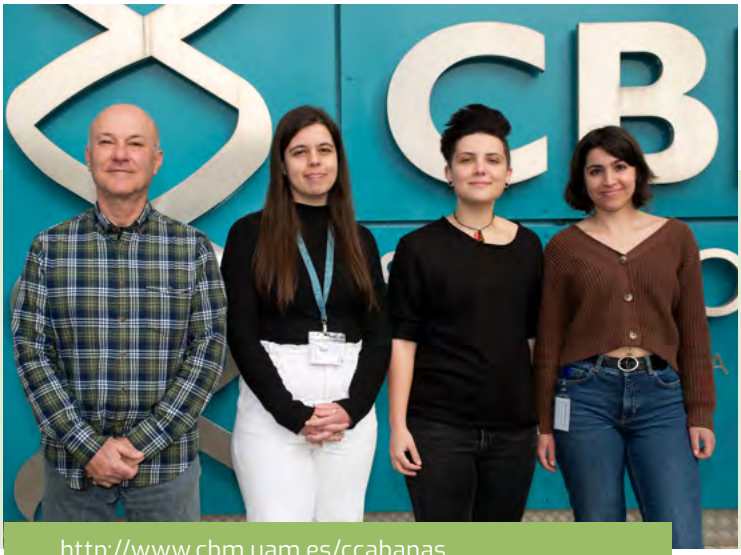
María Mittelbrunn Herrero
IMMUNOMETABOLISM AND INFLAMMATION

Ginés Morata Pérez / Natalia Azpiazu Torres
MECHANISMS OF TUMORIGENESIS IN DROSOPHILA

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

FUNCTIONAL INTERACTIONS BETWEEN TETRASPANINS AND CELL ADHESION MOLECULES



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

Group Members

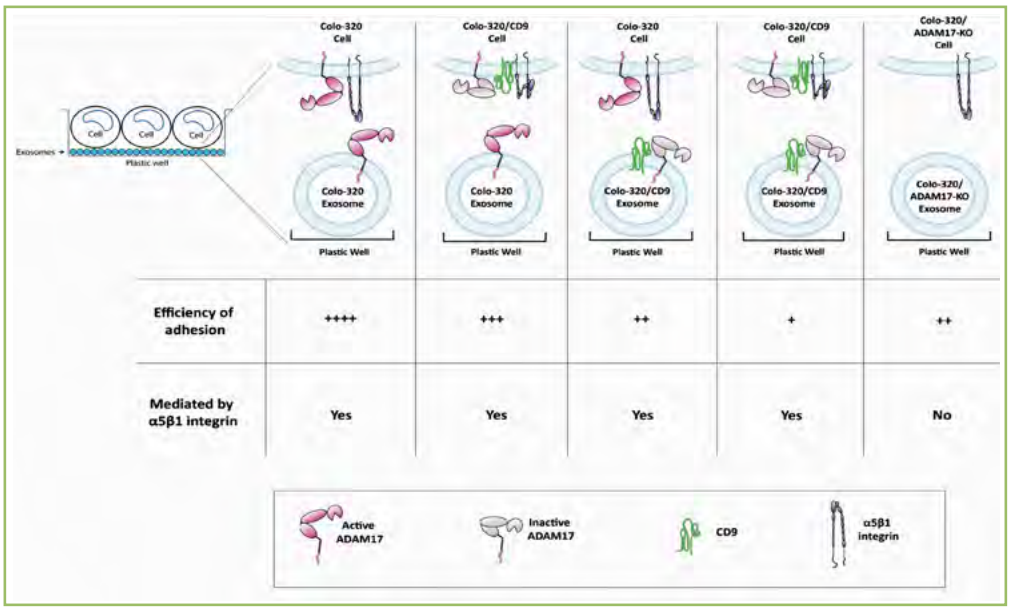
Principal Investigator:
Carlos Cabañas Gutiérrez

Predoctoral fellows:
Beatriz Cardeñes Pérez (until January, 2022)
Irene Clares Pedrero
Tamara Bezos Carmona (since April, 2021)
Almudena Rocha Mulero (since September, 2021)

Summary

Tetraspanins are a family of highly conserved proteins that function as membrane-organizers and control fundamental cell functions, including adhesion, migration and proliferation. The tetraspanin CD9 associates specifically with different cell adhesion receptors of the immunoglobulin and integrin families in tetraspanin-enriched microdomains (TEMs). Through these interactions, CD9 exerts important regulatory effects on the function of associated adhesion molecules. 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 all multicellular organisms 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 these proteins are amongst the best markers for these EVs.

Exosomes produced by cancer cells have been shown to influence many processes related to cancer progression and metastasis but the specific molecules that mediate their interaction and uptake by distinct target cells have remained elusive. We have isolated exosomes produced by colorectal carcinoma (CRC) and ovarian cancer (OvC) cells and performed cell-adhesion assays on immobilized exosomes and also measured the uptake of fluorescently-labelled exosomes by using flow cytometry and confocal microscopy techniques. We found that the interaction between cellular integrin $\alpha 5\beta 1$ and its ligand ADAM17 on the exosomal surface mediated the binding and uptake of cancer-derived exosomes. Interestingly, this process was negatively regulated by the expression of tetraspanin CD9 on exosomes. The adhesion molecule ALCAM/CD166 is also involved in the interaction of cancer-derived EVs with recipient cancer cells and in their subsequent uptake. The identification of ALCAM/CD166 as a molecule mediating the docking and uptake of CRC and OvC-derived EVs could be potentially exploited to block the peritoneal metastasis cascade promoted by EVs in CRC and OvC patients.



Schematic representation of the involvement of ADAM17, CD9 and integrin α5β1 in the interactions between exosomes and recipient cells. The interaction of exosomes with cells is mediated by integrin α5β1 (in cells) and ADAM17 (in exosomes) and is further regulated by CD9.

List of publications

» Cardeñes B, Clares I, Bezos T, Toribio V, López-Martín S, Rocha A, Peinado H, Yáñez-Mó M, Cabañas C. (2022). ALCAM/CD166 Is involved in the binding and uptake of cancer-derived extracellular vesicles. International Journal of Molecular Sciences **23:**5753. doi: 10.3390/ijms23105753.

» Torres-Gomez A, Fiyouzi T, Guerra-Espinosa C, Cardeñes B, Clares I, Toribio V, Reche PA, Cabañas C, Lafuente EM. (2022). Expression of the phagocytic receptors αMβ2 and αXβ2 is controlled by RIAM, VASP and Vinculin in neutrophil-differentiated HL-60 cells. Frontiers in Immunology **13:**951280. doi: 10.3389/fimmu.2022.951280

» Pascual-Antón L, Cardeñes B, Sainz de la Cuesta R, González-Cortijo L, López-Cabrera M, Cabañas C, Sandoval P. (2022). Mesothelial-to-Mesenchymal transition and exosomes in peritoneal metastasis of ovarian cancer. International Journal of Molecular Sciences **22:**11496. doi: 10.3390/ijms222111496.

» Cardeñes B, Clares I, Toribio V, Pascual L, López-Martín S, Torres-Gomez A, Sainz de la Cuesta R, Lafuente EM, López-Cabrera M, Yáñez-Mó M, Cabañas C. (2021). Cellular Integrin α5β1 and exosomal ADAM17 mediate the binding and uptake of exosomes produced by colorectal carcinoma cells. International Journal of Molecular Sciences **22:**9938. doi: 10.3390/ijms22189938.

» Arimori T, Miyazaki N, Mihara E, Takizawa M, Taniguchi Y, Cabañas C, Sekiguchi K, Takagi J. (2021). Structural mechanism of laminin recognition by integrin. Nature Communications **12:**4012. doi: 10.1038/s41467-021-24184-8.

» Torres-Gómez Á, Cardeñes B, Díez-Sainz E, Lafuente EM, Cabañas C. (2021). Functional Integrin regulation through Interactions with tetraspanin CD9. Methods in Molecular Biology **2217:**47-56. doi: 10.1007/978-1-0716-0962-0_5.

Participation in projects

» "Functional regulation of integrins and other cell adhesion molecules of leukocytes and tumor cells; their relevance in the binding, fusion and uptake of exosomes". SAF2016-77096-R. Spanish Ministry of Economy and Competitiveness (MINECO). (2017-2021). PI: Carlos Cabañas.

» "Molecular determinants involved in the binding/uptake of tumor exosomes by immune and non-immune recipient target cells". PID2021-123199OB-I00. Spanish Ministry of Science and Innovation (MICINN) (2022-2025). PI: Carlos Cabañas.

Doctoral theses

Beatriz Cardeñes Pérez (2022). Regulatory role of cell adhesion molecules in the binding, fusion and uptake of tumor-derived exosomes. Universidad Autónoma de Madrid. Supervisor: Carlos Cabañas. Outstanding *cum Laude.*

MOLECULAR CHARACTERIZATION OF VASCULAR PATHOGENESIS AND LYMPHOMAGENESIS

Group Members

Principal Investigator:
Miguel R. Campanero

Postdoctoral fellows:
Cristina Clemente Toribio (from March 2022)

Predoctoral fellows:
Alberto Hernández Alcántara (from July 2018)
Yilin Sun (from March 2019)
Carolina Gutiérrez Martínez (from March 2022)

Technicians:
Patricia Martínez Núñez
Estela Herranz Martín
María José Méndez Olivares (July 2021 – June 2022)

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

Summary

This group has investigated, in collaboration with numerous researchers from USA and European countries, the molecular mechanisms underlying lymphomagenesis and some of the most relevant cardiovascular diseases. The group identified mediators of pathological vascular wall remodeling that are involved in atherosclerosis, restenosis, intramural hematoma, arterial hypertension, thoracic aortic aneurysm and dissection (TAAD), and abdominal aortic aneurysm (AAA). Indeed, the team has recently identified novel genes that mediate aortic diseases, including *RCAN1, C/EBPβ, ADAMTS1* or *NOS2*. They have shown that these genes are essential to maintain aortic homeostasis and that their deficiency leads to aortic aneurism and dissections *(Nat Med 2017; Nat Commun 2018)*. Their findings have allowed the identification of new pathophysiological mechanisms and therapeutic targets in aortic diseases. Their studies showing that deficiency of the metalloproteinase Adamts1 leads to aortic pathology in mice due to increased Nos2-dependent NO production *(Nat Med 2017)* were particularly pioneering, changed the current view of the pathophysiology of TAAD, and opened new exciting avenues of research into the pathogenesis of TAADs that led to the identification of new targets for pharmacological intervention and potential biomarkers for the follow up of Marfan syndrome *(Nat Commun 2021)*.

This group has also made important contributions to the field of lymphomagenesis and leukemogenesis, discovering a tumor suppressor role for E2F4 in B-cell lymphoma as a critical transcriptional repressor of the *E2F1* oncogene (*Leukemia 2012)*; identifying MAZ as a critical activator of *MYB* expression and uncovering a novel mechanism for overcoming transcriptional repression by the E2F-pRB pathway *(Nucleic Acids Res 2017)*. By using gene expression profiling they identified *CDCA7* as a gene overexpressed in lymphoid tumors and discovered that its high expression is required for lymphoma/leukemia growth in soft gels, but not in liquid media; for tumor formation in immunodeficient mice; and for lymphoma migration and invasion through its capacity to modulate tubulin and actomyosin cytoskeleton dynamics *(Haematologica 2018 & 2020)*. The selective role of CDCA7 in soft gels is of special interest because tumor initiating cells (TICS), which are particularly resistant to current chemotherapy or radiotherapy, can be selected from a pool of tumor cells by culturing them in these gels. The group is currently funded by the *Spanish Association Against Cancer* to identify additional genes critical for lymphoid tumor growth in soft gels because these genes are potentially critical for TICs growth *in vivo* and hence facilitate the design of therapies that efficiently target tumor initiating cells.

List of publications

» A. de la Fuente-Alonso, M. Toral, A. Alfayate, M.J. Ruiz-Rodríguez, E. Bonzón-Kulichenko, G. Teixido-Tura, M.J. Méndez-Olivares, S. Martínez-Martínez, D. López-Maderuelo, I. González-Valdés, E. Garcia-Izquierdo, S. Mingo, C.E. Martín, L. Muiño-Mosquera, J. De Backer, J.F. Nistal, A. Forteza, A. Evangelista, J. Vázquez, M.R. Campanero*, and J.M. Redondo*. *Co-senior and corresponding authors. (2021). Aortic disease in Marfan syndrome is caused by overactivation of sGC-PRKG signaling by NO. Nat. Commun. 12(1):2628. doi: 10.1038/s41467-021-22933-3

» M.R. Campanero*, and J.M. Redondo*. *Corresponding authors. (2021). Letter by Campanero and Redondo regarding Brief Report, "Jugular vein injection of high-titer lentiviral vectors does not transduce the aorta". Arterioscler Thromb Vasc Biol. 41(4): e238-e239. doi: 10.1161/ATVBAHA.121.315934

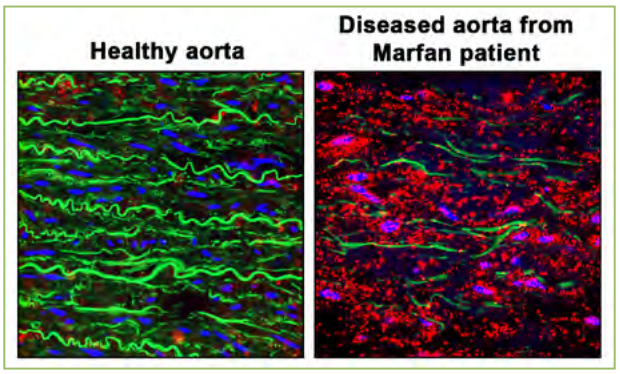
» A. del Puerto, J. Pose-Utrilla, A. Simón-García, C. López-Menéndez, A.J. Jiménez, E. Porlan, L. Sánchez-Miranda Pajuelo, G. Cano-García, B. Martí-Prado, A. Sebastián-Serrano, M.P. Sánchez-Carralero, F. Cesca, G. Schiavo, I. Ferrer, I. Fariñas, M.R. Campanero, and T. Iglesias. (2021). Kidins220 deficiency 1 causes ventriculomegaly via SNX27-retromer-dependent AQP4 degradation. Mol. Psychiatry 26(11):6411-6426. doi: 10.1038/s41380-021-01127-9

» M. Toral, A. de la Fuente-Alonso, M.R. Campanero*, and J.M. Redondo*. *Co-senior and corresponding authors. (2022). The Nitric Oxide Signalling Pathway in Aortic Aneurysm and Dissection. Brit. J. Pharmacol. 179:1287–1303. doi: 10.1111/bph.15694.

Patents

» Inventors: Juan Miguel Redondo, Jesús M. Vázquez, Marta Toral, Andrea de la Fuente-Alonso, Mª Jesús Ruiz Rodríguez, Álvaro Alfayate, and Miguel R. Campanero. Method for the diagnosis, prognosis, and/or treatment of thoracic aortic aneurysm. Country of priority: Europe. Priority date: April 16, 202. Owners: CNIC, CSIC, UAM. Licensing: Under process.

» Inventors: Teresa Iglesias Vacas, Miguel R. Campanero, Julia Pose Utrilla, Ana Simón García, Celia López Menéndez, Luis Sánchez-Miranda Pajuelo y Ana Mª del Puerto del Pino. Title: Methods and compositions for the treatment of disorders characterized by a KIDINS220 dysfunction in a subject. Country of priority: Europe. Priority date:May 6, 2021. Owners: CSIC, UAM, CIBERNED. Licensing: Under process.



Images showing nuclei (blue staning), increased pVASP-S239 staining (red), and disorganized elastic fibers (green) in the aortic wall of a patient with Marfan Syndrome as compared with a control donor (Healthy Aorta)

Participation in projects

» Title: Roles of smooth muscle and endothelial calcineurin in arterial hypertension and aortic aneurysm. Reference PID2020-115217RB-I00 from Spanish Agencia Estatal de Investigación. PI: Miguel R. Campanero. Duration: 1/9/2021 – 31/08/2024

» Title: Mediadores y mecanismos moleculares de patologías aórticas y valvulares. Consortium reference B2017/BMD3676 from Comunidad de Madrid. PI: Miguel R. Campanero. Duration: 1/9/2017 – 30/06/2022

» Title: Nitric Oxide Signaling and proteoglycans in Marfan syndrome's aortopathy: mechanisms and new therapeutic drugs. Reference LCF/PR/HR19/52160008 from La Caixa Health Research. PI: Miguel R. Campanero. Duration: 15/09/2019 – 14/09/2022

» Title: Identification of efficient therapeutic targets for lymphoid tumors. Reference PROYE20060CAMP from Fundación AECC. PI: Miguel R. Campanero. Duration: 01/12/2020 – 30/11/2023

» **IIS IdiPAZ;** member of the research group "Animal and cellular models for the detection and characterization of leukemia stem cells".

» **CIBERCV.** Member of the group led by Juan Miguel Redondo.

Other activities

» 19th Meeting of the Spanish Society for Cell Biology. Boadilla del Monte (Madrid). October 26–29, 2021. Member of the Scientific Committee; Chair of Symposium 6 "Metabolic homeostasis and disease"

» Elected member of the International Basic/Translational Panel for GenTAC, a public (NHLBI/NIH) alliance committed to advancing our understanding of genetic thoracic aortic diseases and their treatment.

MECHANISMS OF CELL-CELL SIGNALING DURING DEVELOPMENT

Group Members

Group Leader:
Isabel Guerrero Vega

Associated Researcher:
Ana Busturia Jimeno
Ana-Citlali Gradilla Castellanos

Postdoctoral Fellows:
Adrián Aguirre Tamaral

Graduated Students:
Carlos Jiménez-Jiménez
Clara Fernández Pardo
Víctor Mendoza Grimau.

Technical Assistance:
Irene Sánchez-Platero
Sandra Villatoro Gómez

Undergraduate Students:
Claudia Pedreira Conchado
Nerea Gómez Villalón

Master student:
Clara Fernández Pardo

Invited Professor:
Pedro Ripoll Quintas

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

Summary

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

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

Our research is interdisciplinary; we use diverse experimental systems in a variety of *Drosophila* tissues, state-of-the-art methodologies for genetic, cellular and molecular analyses, superresolution and 4D *in vivo* imaging confocal microscopy and electron microscopy. The quantitative information generated is allowing the development of mathematical models for the signalling processes that, in turn, let us explore different hypotheses. The knowledge of the mechanisms underlying cell-cell signaling and cell signaling integration during normal development will also contribute to understand other cell communication processes such as the maintenance of adult homeostasis and tumor progression.

In the same group, there is another line of investigation led by Dr. Ana Busturia with the title: "*Drosophila* Developmental Epigenetics". This research aims to understand how the epigenetic mechanisms impact

the development and homeostasis of an organism. This is done by studying the roles played by the expression levels and/or activity of the Polycomb (PcG) and trithorax (trxG) group proteins in normal and pathological development. We use *Drosophila* as a model system to deepen our understanding of these processes in vertebrates. Three research areas are being developed. 1) We study organ communication mediated by hemolymph exosomes containing microRNAs that modify PcG/trxG levels in target organs. 2) We analyze the effect of the apoptosis mediated by the immune derived Antimicrobial Peptides on the modulation of PcG induced tumor formation. 3) We investigate the PcG- mediated trans-generational inheritance of the physiological traits observed upon supplementing *Drosophila* diet with enriched milk fat globule membrane (MFGM).

List of publications

» Aguirre-Tamaral A, et al., (2022). Predictive model for cytoneme guidance in Hedgehog signaling based on Ihog-Glypicans interaction. *Nat Commun*. Sep 26;13(1):5647. doi: 10.1038/s41467-022-33262-4. PMID: 36163184; PMCID: PMC9512826.

» Gradilla AC, Guerrero I. (2022). Hedgehog on track: Long-distant signal transport and transfer through direct cell-to-cell contact. *Curr* Top Dev Biol.;150:1-24. doi: 10.1016/bs.ctdb.2022.03.002. Epub 2022 Apr 30. PMID: 35817500.

» Simon E, Jiménez-Jiménez C, et al., (2021). Glypicans define unique roles for the Hedgehog co-receptors Boi and Ihog in cytoneme-mediated gradient formation. *eLife*. Aug 6;10:e64581. doi: 10.7554/eLife.64581. PMID: 34355694; PMCID: PMC8410076.

» Aguirre-Tamaral A, Guerrero I. (2021). Improving the understanding of cytoneme-mediated morphogen gradients by in silico modeling. *PLoS Comput Biol*. Aug 3;17(8):e1009245. doi: 10.1371/journal.pcbi.1009245. PMID: 34343167; PMCID: PMC8362982.

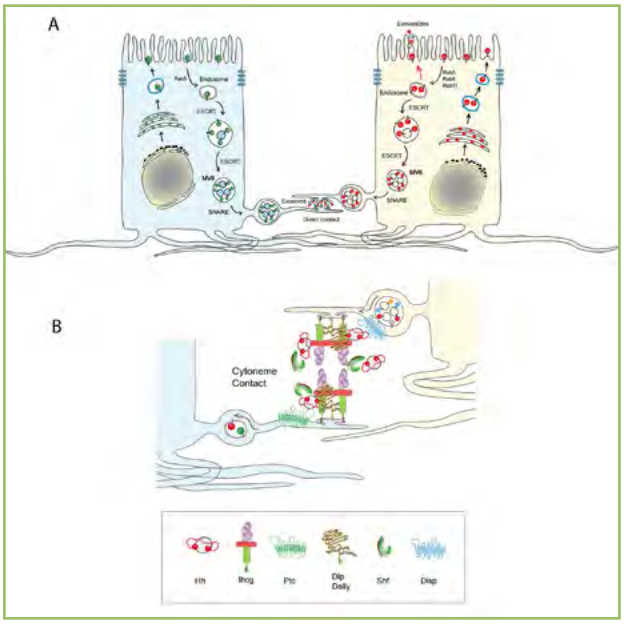
» Royo F, et al., (2021) Extracellular Vesicles From Liver Progenitor Cells Downregulates Fibroblast Metabolic Activity and Increase the Expression of Immune-Response Related Molecules. *Front Cell Dev Biol*. Jan 12;8:613583. doi: 10.3389/fcell.2020.613583. PMID: 33511119; PMCID: PMC7835421.

» Solorzano J, et al., (2022). A genome-wide computational approach to define microRNA-Polycomb/trithorax gene regulatory circuits in *Drosophila*. *Developmental Biology,* Mar;495:63-75. doi: 10.1016/j. ydbio. 2022.12.008.

Participation in projects

» Cellular Communication by Direct Contact: Cytoneme formation in the Hedgehog signaling function during development. Ministerio de Ciencia, Innovación y Universidades -PID2020-114533GB-C21. Sept 2021- Oct 2024. PI: Isabel Guerrero Vega. Amount: 330.000 €.

» Network of Excellence in Research and Innovation in Extracellular Vesicles (TENTACLES). RED18-102411-T. Ministerio de Ciencia, Innovación y Universidades 2019-2021. Coordinator: María Yañez Mó. PI Isabel Guerrero Vega.



A) Hedgehog (Hh) signaling conceived as a synapse-like process between Hh producing and receiving cells through cytoneme contacts. For Hh presentation, after an intracellular recycling process in the Hh producing cells Hh travels in multivesicular bodies (MVBs) along cytonemes and is released to interact with the reception complex at the contact sites. The Hh receptor Patched (Ptc) also has an intracellular recycling process in the receiving cells and travels in multivesicular bodies (MVBs) along cytonemes to interact with Hh.

B) The adhesion protein and Hh co-receptors Ihog and the glypicans Dlp and Dally are recruited for ligand release and receptor interaction. The soluble factor Shifted (Shf) (ortholog the vertebrate Wif-1) helps the Hh release from the producing cell to the receiving cell cytonemes. The transmembrane proteins Dispatched (Disp), required for Hh release from the producing cells, and Ptc in the receiving cytonemes also interact with some of the other proteins during the release and reception processes.

Other activities

» EMBO Workshop in Long distance cell-cell signalling in development and disease.
https://meetings.embo.org/event/21-cell-signallin.

Isabel Guerrero (Co-organizer); University of Exeter (UK) (10-13 April 2022).

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

Group Members

Principal Investigator:
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Beatriz Ramos Velasco (GARJUV-CAM)

Undergraduate and Master Students:
Rocío Naranjo Sánchez (INVESTIGO-CAM)
(Since September 2022)
Isabel Alcalde Rey (January 2022-June 2022) (TFG)
Andrea Fernández-Gómez (January 2021-July 2021) (TFM)

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Summary

T-cell intracellular antigen 1 (TIA1) and TIA1-like/related protein (TIAL1/TIAR) are RNA-binding proteins involved in the control of gene expression through influencing the origin and fate of cellular RNAs and proteins. These multifunctional master regulators play prevalent roles in the expression/function of the human transcriptome, translatome, proteome and interactome in order to prevent the development of deleterious cellular phenotypes in compromised cellular situations. For example, changes in the expression/function and/or subcellular localization of TIA1 and/or TIAR have been associated with important physio(patho)logical consequences on human biology, including embryogenesis, inflammation, tumorigenesis, neuronal homeostasis, tauopathies, myopathies, cell stress responses and viral infections. The participation of TIA proteins in these complex programs points to their direct involvement in the regulation of myriad cellular pathways among other apoptosis, autophagy/mitophagy, immune system, membrane dynamics, axonal regeneration, activity and localization of cellular translational machinery, cell cycle, proteostasis, dynamics of stress granules (SGs) as well as oxidative and metabolism challenges (Figure 1, upper panel). Further, they are key components of SGs, RNA and protein aggregates that are formed in response to stressful stimuli to reduce cellular activity as a survival mechanism. For example, TIA1 p.E384K mutation is the genetic cause of Welander distal myopathy (WDM), a late-onset muscular dystrophy whose pathogenesis has been related with altered SGs dynamics. Thus, we have expanded our understanding of the main regulatory role of T-cell intracellular antigens in physio(patho)logical events as well as the connection between abnormal dynamics of TIA1-dependent stress granules and WDM through a multidisciplinary genetic, cellular and molecular approaches. Our observations show distinct dynamics between the formation, assembly, and disassembly of TIA1$^{WT/WDM}$-dependent SGs in molecular grammar-dependent way. These observations have allowed us to expand the existing knowledge on the role of TIA1 and the WDM mutation in SGs dynamics (Figure 1, bottom panel). To point out, eventually, that our working line also fits within the priorities of the European Union, objectives and goals, of the "2030 Agenda for Sustainable Development" and specifically Goal 3: "Good Health and Well-being" to "ensure healthy lives and promote well-being for all at all ages".

List of publications

» Velasco, B. R. and Izquierdo, J. M. (2022) T-cell intracellular antigen 1-like protein in physiology and pathology. Int. J. Mol. Sci. 23, 7836. doi: 10.3390/ijms23147836.

» Fernández-Gómez, A., Velasco, B.R. and Izquierdo, J. M. (2022) Dynamics of T-cell intracellular antigen 1-dependent stress granules in proteostasis and Welander distal myopathy under oxidative stress. Cells 11, 884. doi: 10.3390/cells11050884.

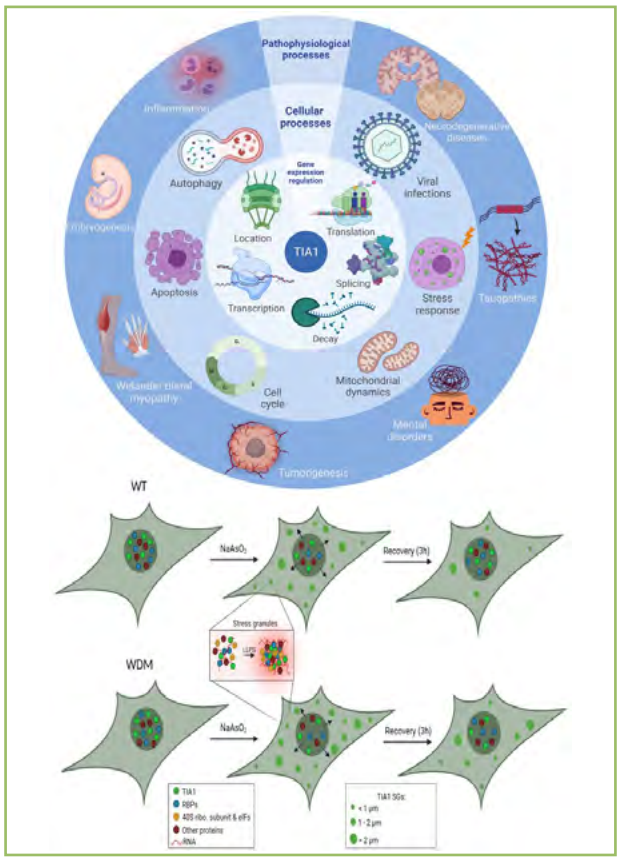
» Fernández-Gómez, A. and Izquierdo, J. M. (2022) The multifunctional faces of T-cell intracellular antigen 1 in health and disease. Int. J. Mol. Sci. 23, 1400. doi: 10.3390/ijms23031400.

» Izquierdo, J. M. (2022) SDHA: a key player in T cell-mediated intestinal disease severity. Cell. Mol. Immunol. 19, 139-141. doi: 10.1038/s41423-021-00820-7.

» Carrascoso, I., Vela, B. R. and Izquierdo, J. M. (2021) Deficiency of T-cell intracellular antigen 1 in murine embryonic fibroblasts is associated with changes in mitochondrial morphology and respiration. Int. J. Mol. Sci. 22, 12775. doi: 10.3390/ijms222312775.

» Miguel-Arribas, A., Val-Calvo, J., Gago-Córdoba, C., Izquierdo, J. M., Abia, D., Wu, L. J., Errington, J. and Meijer, W. J. J. (2021) A novel bipartite antitermination system widespread in conjugative elements of Gram-positive bacteria. Nucleic Acids Res. 49, 5553-5567. doi: 10.1093/nar/gkab360.

» Klionsky, D.J., Abdel-Aziz, A.K., (...), Izquierdo, J.M., (...), Stallings, C. L. and Tong, C. K. (2021) Guidelines for the use and interpretation of assays for monitoring autophagy (4th edition). Autophagy 17, 1-382. doi: 10.1080/15548627.2020.1797280.



The hallmarks of TIA1 on gene expression regulation/flux, and cellular and pathophysiological processes. Schematic representation of the main processes of gene expression regulation, cellular events and pathophysiological processes associated with TIA1 expression (upper panel). Dynamics of $TIA1^{WT/WDM}$-dependent stress granules (SG) under oxidative stress by sodium arsenite (bottom panel). This figure was created with BioRender.com.

Participation in projects

» "Study of the molecular grammar of TIA1-dependent stress granules in proteostasis and Welander distal myopathy". Ministry of Science and Innovation-AEI-FEDER (PID2021-126152OB-100). September 2022-August 2025. PI: José María Izquierdo Juárez.

» "Molecular characterization of TIA1-dependent stress granules. Pathophysiological implications in proteostasis and Welander distal myopathy". Funding entity: Ministry of Science and Innovation-AEI-FEDER (RTI2018-098517-B-100). January 2019-September 2022. PI: José María Izquierdo Juárez.

» CSIC Extraordinary Grant: "Molecular characterization of TIA1-dependent stress granules. Pathophysiological implications in proteostasis and Welander distal myopathy". Funding entity: CSIC2021AEP009. Participating entities: CSIC. Time period: January 2022-August 2022. PI: José María Izquierdo Juárez.

MOLECULAR PATHOPHYSIOLOGY OF PERITONEAL INFLAMMATION AND FIBROSIS (PERINFIB)

Group Members

Principal Investigator:
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Guadalupe González Mateo

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Lucia Pascual Antón
Valeria Kopytina
Eva María Arriero País

Project Manager:
Javier Carrero Liroa

Visiting scientists:
Esra Cetin (Cardiff University, United Kingdom): June 1st- July 8th 2022.
Vanessa Marchant (Fundación Jiménez Diaz, UAM): February 1st-April 30th 2021.

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

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 PD-induced 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 (China patent: 107073022), in which the glucose has been substituted by Stevioside as osmotic agent. We could demonstrate that this PD fluid induces less MMT and causes less peritoneal damages, when compared with classical glucose-based PD fluids (Kopitina et al., 2022). Recently, we have patented a new PD fluid (Spanish Patent Reference: ES1641.1754), in which the osmotic agent is Troxerutin, a flavonoid with potent antioxidant activity, which has been shown to be cardioprotective 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.

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).

List of publications

» Kopytina V, Pascual-Antón L, Toggweiler N, Arriero-País EM, Strahl L, Albar-Vizcaíno P, Sucunza D, Vaquero JJ, Steppan S, Piecha D, López-Cabrera M, González-Mateo GT. (2022). Steviol glycosides as an alternative osmotic agent for peritoneal dialysis fluid. Front Pharmacol. 13:868374. doi: 10.3389/fphar.2022.868374.

» Del Rio D, Masi I, Caprara V, Spadaro F, Ottavi F, Strippoli R, Sandoval P, López-Cabrera M, Sainz de la Cuesta R, Bagnato A, Rosanò L. (2021). Ovarian Cancer-Driven Mesothelial-to-Mesenchymal Transition is Triggered by the Endothelin-1/β-arr1 Axis. Front Cell Dev Biol. 9:764375. doi: 10.3389/fcell.2021.764375.

» Pascual-Antón L, Cardeñes B, Sainz de la Cuesta R, González-Cortijo L, López-Cabrera M, Cabañas C, Sandoval P. (2021). Mesothelial-to-Mesenchymal Transition and Exosomes in Peritoneal Metastasis of Ovarian Cancer. Int J Mol Sci. 22(21):11496. doi: 10.3390/ijms222111496

» Cardeñes B, Clares I, Toribio V, Pascual L, López-Martín S, Torres-Gomez A, Sainz de la Cuesta R, Lafuente EM, López-Cabrera M, Yáñez-Mó M, Cabañas C. (2021). Cellular Integrin α5β1 and Exosomal ADAM17 Mediate the Binding and Uptake of Exosomes Produced by Colorectal Carcinoma Cells. Int J Mol Sci. 22(18):9938. doi: 10.3390/ijms22189938.

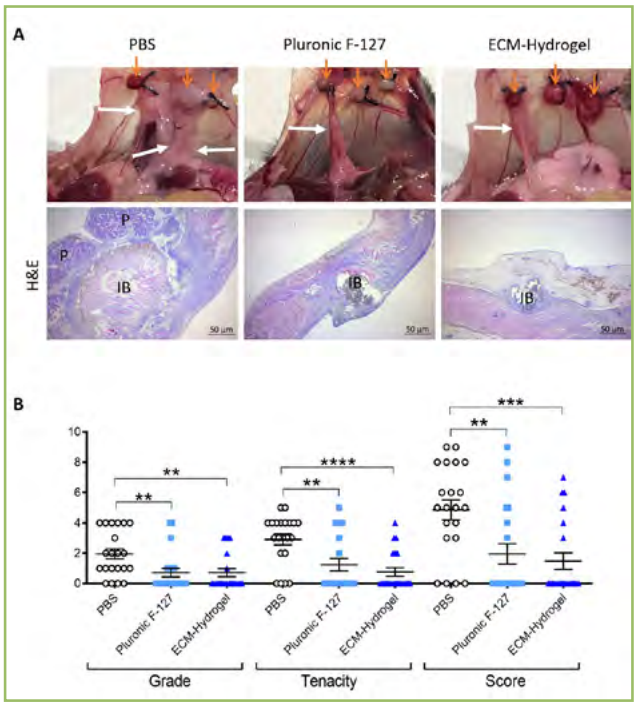
» Herzog R, Sacnun JM, González-Mateo G, Bartosova M, Bialas K, Wagner A, Unterwurzacher M, Sobieszek IJ, Daniel-Fischer L, Rusai K, Pascual-Antón L, Kaczirek K, Vychytil A, Schmitt CP, López-Cabrera M, Alper SL, Aufricht C, Kratochwill K. (2021). Lithium preserves peritoneal membrane integrity by suppressing mesothelial cell αB-crystallin. Sci Transl Med. 13(608):eaaz9705. doi: 10.1126/scitranslmed.aaz9705.

» Terri M, Trionfetti F, Montaldo C, Cordani M, Tripodi M, Lopez-Cabrera M, Strippoli R. (2021). Mechanisms of Peritoneal Fibrosis: Focus on Immune Cells-Peritoneal Stroma Interactions. Front Immunol. 12:607204. doi: 10.3389/fimmu.2021.607204.

Participation in 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 Mesotelial a Mesénquimal: Un Proceso Patológico Importante en Enfermedades que Afectan al Peritoneo. Project Ref.: PID2019-110132RB-I00. MINISTERIO DE CIENCIA E INNOVACIÓN. 2020-2023. Principal Investigator: Manuel López Cabrera



*ECM-hydrogel treatment ameliorates peritoneal adhesion formation in mice. (A) Representative pictures of peritoneal adhesions and Haematoxylin & Eosin (H&E) staining in the three mouse groups. P: pancreas. IB: ischemic button. Orange arrows depict the three IBs. On the PBS group, white arrows point three severe adhesions between visceral and parietal peritoneum. On the Pluronic F-127 there is no visceral adhesion in two IBs and the white arrow shows one adhesion with high score. In the ECM-hydrogel group, white arrow points to one adhesion with low score and there are two IBs without adhesion formation. Left H&E image shows a parietal IB adhered to pancreas in a PBS mouse. (B) Quantification of adhesions shows significant differences between PBS group with the Pluronic F-127 and ECM-hydrogel groups. Bars represent the mean ± SEM. **p<0.01 compared with PBS group. ***p<0.001 compared with PBS group.*

Doctoral theses

» Lucia Pascual Antón (2022). La transición mesotelio-mesénquima como diana terapéutica común para el tratamiento de las adhesiones peritoneales postquirúrgicas y la metástasis peritoneal. Universidad Autónoma de Madrid. 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. Peritoneal dialysis solutions containing a natural flavonoid as an osmotic agent. Spanish Patent Reference: ES1641.1754 Country of Priority: Spain. Date of Priority: 05 July 2022

CELL BIOLOGY OF INFLAMMATION

Group Members

Group Leader:
Jaime Millán

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(from 06-22)

Predoctoral Fellows:
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Natalia Colás Algora
(until 05-22)
Gema Cerro Tello
Carmen López Pujante

Technicians:
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Gema de Rivas Hidalgo

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Mª Almudena Gómez Martínez

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Summary

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

The vascular endothelium controls the passage of cells and solutes between the blood and the parenchyma in the inflamed tissue. During these two years we have identified new molecular mechanisms whereby human endothelial cells control their barrier properties, which has enabled us to reinforce the endothelial monolayer integrity in an inflammatory context. We are addressing the role of the RhoA subfamily of GTPases in regulating VE-cadherin expression levels and endothelial barrier function. We are exploring the role of this subfamily as a therapeutic target to prevent organ edema during systemic inflammation. Finally, we are applying our expertise to try to prevent pulmonary endothelial barrier disruption caused by the cytokine storm induced by SARS-Cov-2 in seriously ill COVID19 patients.

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

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

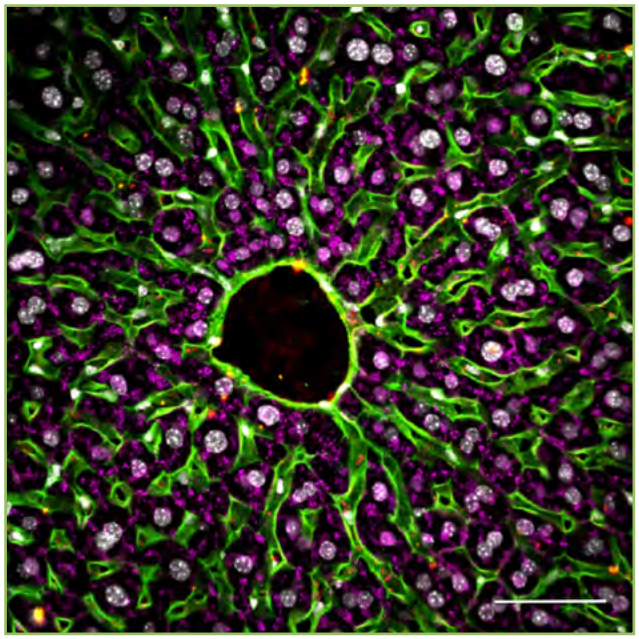

Figure 1. *Organization of the hepatic parenchyma. The intercellular adhesion molecule (ICAM-1) is highly expressed in sinusoidal endothelial cells (green). PLLP (purple) is expressed in the subapical compartment of hepatocytes (Cacho-Navas et al. 2022). Nuclei are labelled in white. Cells forming this spherical barrier can be distinguished thank to the staining of ZO-1, a component of tight junctions (green), the filamentous actin (F-actin), in red, and nuclei (blue). Scale bar, 50 µm*

List of publications

» Cacho-Navas C, Reglero-Real N, Colás-Algora N, Barroso S, de Rivas G, Stamatakis K, Feito J, Andrés G, Fresno M, Kremer L, Correas I, Alonso MA, Millán, J. Plasmolipin regulates basolateral-to-apical transcytosis of ICAM-1 and leukocyte adhesion in polarized hepatic epithelial cells. (2022) Cell. Mol. Life. Sci. Jan 9;79(1):61. doi: 10.1007/s00018-021-04095-z.

» Maeso-Alonso L., Alonso-Olivares H, Martínez-García N, López-Ferreras L, Villoch-Fernández J, Puente-Santamaría L, Colas-Algora N, Fernández-Corona A, Lorenzo-Marcos ME, Jiménez B, Holmgren L, Wilhelm M, Millán J, Del Peso L, Claesson-Welsh L, Marques MM, Marin M.C. (2022) p73 is required for vessel integrity controlling endothelial junctional dynamics through Angiomotin. Cell. Mol. Life. Sci. Oct 1;79(10):535. doi: 10.1007/s00018-022-04560-3

Participation in projects

» Functional characterization of endothelial surface mesoscopic domains regulated by the RhoA subfamily of GTPases during the inflammatory response (EndoBarrier). PID2020-119881RB-I00. AEI. PI: Jaime Millán. 01/01/2021 – 31/01/2023.

» Strategic initiative "Immune response to infection and vaccination in COVID-19".PTI+ Global Health CSIC. SGL2103017. PI: Jaime Millán. 01/03/21-31/12/22.

» 2020AEP021. Generación de tejido hepático humano para investigar la función de barrera celular durante la respuesta inflamatoria.PI: Jaime Millán. 01/01/21-01/09/21.

» Searching for new therapeutic targets to prevent lung microvascular endothelial barrier disruption and pulmonary edema caused by SARS-CoV2-induced cytokine storm. Proyecto Intramural COVID19. CSIC-COV19-194. PI: Jaime Millán. 01/12/2020-30/11/2021.

» Búsqueda de biomarcadores en la córnea humana para el diagnóstico temprano del Queratocono. bases moleculares de la enfermedad. Doctorado industrial Comunidad de Madrid. IND2019/BMD-17139. PI: Jaime Millán. 01/01/2020-31/12/2022.

» TOMOXLIVER : Estudio de la disfunción del hepatocito desde un abordaje multidisciplinar. B2017/BMD-3817. Comunidad de Madrid. PI: Jaime Millán. 01/01/2018 – 31/12/2022.

» Building human hepatic tissue to investigate cell barriers during the inflammatory response. SAF2017-88187-R. Programa estatal de investigación, desarrollo e innovación orientada a los retos de la sociedad. PI: Jaime Millán. 01/01/2018 – 31/12/2021.

Doctoral theses

» Natalia Colás Algora (2022). Estudio de la regulación de la barrera endotelial por las Rho GTPasas de la subfamilia RhoA. Sobresaliente Cum Laude. Universidad Autónoma de Madrid. Director, Jaime Millán.

IMMUNOMETABOLISM AND INFLAMMATION

Group Members

Principal Investigator:
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Isaac Francos Quijorna
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Enrique Gabandé Rodríguez

Predoctoral fellows:
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Gonzalo Soto Heredero
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Predoctoral fellows:
José Ignacio Escrig Larena
(from September 2021)
Sandra Delgado Pulido
(from July 2022)

Technicians:
Eva María Blanco Ruiz

Undergraduate and Master Students:
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Álvaro Fernández Almeida (TFM)

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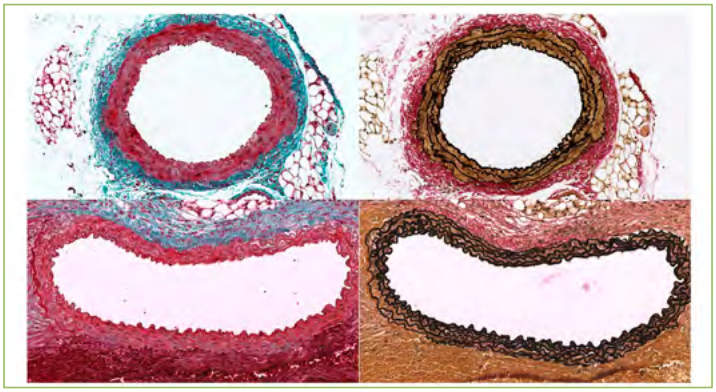
Summary

Our research goal is to identify new strategies to targeting immune cells for boosting systemic resilience to inflammaging, cellular senescence and age-related multimorbidity. Our most important discoveries in the last years are:

1. To demonstrate that mimicking age-associated mitochondrial dysfunction in T cells does not only recapitulate immunosenescence, but causes a general, body-wide deterioration of health with multiple aging-related features, including metabolic, musculoskeletal, cardiovascular and cognitive alterations, altogether resulting in premature death (Science, 2020). These results place the metabolism of T cells at the crossroad between inflammation, senescence and aging, highlighting that immunometabolism can be a therapeutic target to delay aging.
2. To decode the molecular mechanisms by which aged T cells contribute to inflammaging and age-related diseases. (a) Th1-Cytokines induce cellular senescence (b) Loss of Self-tolerance mechanisms. (c) Defective immuno-surveillance of senescent cells. (d) Altered gut microbiota. (Cell Metabolism 2021; Nature Rev Immunol, 2022; Annual Rev Immunol, in press).
3. To propose new therapeutic to reverse aortic aneurysms and prevent sudden death due to aortic dissections by boosting mitochondrial metabolism using NAD Precursors (Circulation, 2021; Atherosclerosis, Thrombosis Vascular Biology, 2022; Br J Pharmacol. 2022).

Awards

» Maria Mitelbrunn has been awarded with the PREMIO BANCO SABADELL for Biomedical Research (2022). Jorge Oller has been awarded with the PINP prize to best



Targeting vascular metabolism as a novel therapeutic strategy for managing aortic aneurysms and preventing sudden death. Representative histological images from aortas from Control (Top) and Marfan Mice (botton). Original work: Oller J, Circulation, 2021 and Oller J, Arterioscler Thromb Vasc Biol 2022.

List of publications

» Roldán-Montero R, Pérez-Sáez JM, Cerro-Pardo I, Oller J, Martinez-Lopez D, Nuñez E, Maller SM, Gutierrez-Muñoz C, Mendez-Barbero N, Escola-Gil JC, Michel JB, Mittelbrunn M, Vázquez J, Blanco-Colio LM, Rabinovich GA, Martin-Ventura JL. (2022). Galectin-1 prevents pathological vascular remodeling in atherosclerosis and abdominal aortic aneurysm Sci Adv. 8(11):eabm7322. doi: 10.1126/sciadv.abm7322.

» Oller J, Gabandé-Rodríguez E, Roldan-Montero R, Ruiz-Rodríguez MJ, Redondo JM, Martín-Ventura JL, Mittelbrunn M (2022). Rewiring Vascular Metabolism Prevents Sudden Death due to Aortic Ruptures-Brief Report. Arterioscler Thromb Vasc Biol. 42(4):462-469. doi: 10.1161/ATVBAHA.121.317346

» Del Rey MJ, Meroño C, Municio C, Usategui A, Mittelbrunn M, García-Consuegra I, Criado G, Pablos JL (2021). TFAM-deficient mouse skin fibroblasts - an *ex vivo* model of mitochondrial dysfunction. Dis Model Mech. 14(8):dmm048995.doi: 10.1242/dmm.048995.

» Carrasco E, Gómez de Las Heras MM, Gabandé-Rodríguez E, Desdín-Micó G, Aranda JF, Mittelbrunn M. (2022). The role of T cells in age-related diseases. Nat Rev Immunol. 22(2):97-111. doi: 10.1038/s41577-021-00557-4.

» Mittelbrunn M, Kroemer G. (2021). Hallmarks of T cell aging. Nat Immunol. 22(6):687-698. doi: 10.1038/s41590-021-00927-z.

» Navarro MN, Gómez de Las Heras MM, Mittelbrunn M (2022). Nicotinamide adenine dinucleotide metabolism in the immune response, autoimmunity and inflammageing. Br J Pharmacol. 179(9):1839-1856. doi: 10.1111/bph.15477.

» Oller J, Gabandé-Rodríguez E, Ruiz-Rodríguez MJ, Desdín-Micó G, Aranda JF, Rodrigues-Diez R, Ballesteros-Martínez C, Blanco EM, Roldan-Montero R, Acuña P, Forteza Gil A, Martín-López CE, Nistal JF, Lino Cardenas CL, Lindsay ME, Martín-Ventura JL, Briones AM, Redondo JM, Mittelbrunn M. (2021). Extracellular Tuning of Mitochondrial Respiration Leads to Aortic Aneurysm. Circulation. 143(21):2091-2109. doi: 10.1161/CIRCULATIONAHA.120.051171.

» Soto-Heredero G, Desdín-Micó G, Mittelbrunn M. (2021). Mitochondrial dysfunction defines T cell exhaustion. Cell Metab. 33(3):470-472. doi: 10.1016/j.cmet.2021.02.010.

Participation in projects

» Letting up senescence and inflammaging through T cells. ERC CoG 101044248 Let T Be. European Research Council. 2023-2028.PI: Maria Mittelbrunn

» Estrategias nutricionales de precisión para reactivar el sistema inmune deteriorado como consecuencia de la edad, la obesidad o la quimioterapia. CAM-Y2020/BIO-6350. Comunidad de Madrid. Pis: Ana Ramírez and Elisa Carrasco.2022-2024

» Cellular Metabolism as a New Therapeutic Target to Prevent Multimorbidity and Cardiovascular Diseases. PI19/00855. Instituto de Salud Carlos III-FEDER. PI: María Mittelbrunn Herrero. 2020-2022

» Endolysosomal Mitochondria Crosstalk in Cell and Organism Homeostasis. ERC StG 715322 EndoMitTalk. European Research Council (ERC). PI: María Mittelbrunn Herrero. 2017-2022

» Inmunometabolismo como diana para retrasar el envejecimiento cutáneo (SI1/PJI/2019-00073). Comunidad de Madrid (convocatoria de Proyectos para Jóvenes Doctores 2019) (2020-2022).

Doctoral theses

» Gabriela Grisel Desdín Micó (2021). El metabolismo mitocondrial en los linfocitos T regula el envejecimiento y las enfermedades asociadas a la edad. Universidad Universidad Autónoma de Madrid. Supervisor: María Mittelbrunn. ''Cum Laude''.

» Omar Alberto Amorocho Domínguez (2022). Role of Sirtuin 1 in CD4+ T cells activation and differentiation in a murine model of obesity and transplantation. Universidad Universidad de São Paulo (Brasil) and Universidad Autónoma de Madrid. Supervisors: Niels Olsen Saraiva Câmara (Instituto de Ciencias Biomédicas de la Universidad de São Paulo) and María Mittelbrunn.

MECHANISMS OF TUMORIGENESIS IN *DROSOPHILA*

Group Members

Principal Investigators (PI, co-PI):
Ginés Morata Pérez
Natalia Azpiazu Torres

Scientific Staff:
Manuel Calleja Requena

Visiting scientists:
Viviana Veladez Graham
(from September 2022)

Postdoctoral fellows:
Noelia Pinal Seoane
(until May 2021)

Predoctoral fellows:
Izarne Medina Azpiazu
(until May 2021)
Juan Manuel García Arias

Technicians:
Angélica Cantarero Mateo
Rosa M ª González Herrera

Undergraduate and Master Students:
Laura Serrano (TFM)
Celia Contreras (TFG)
Alexandra Rico (TFG)

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

Summary

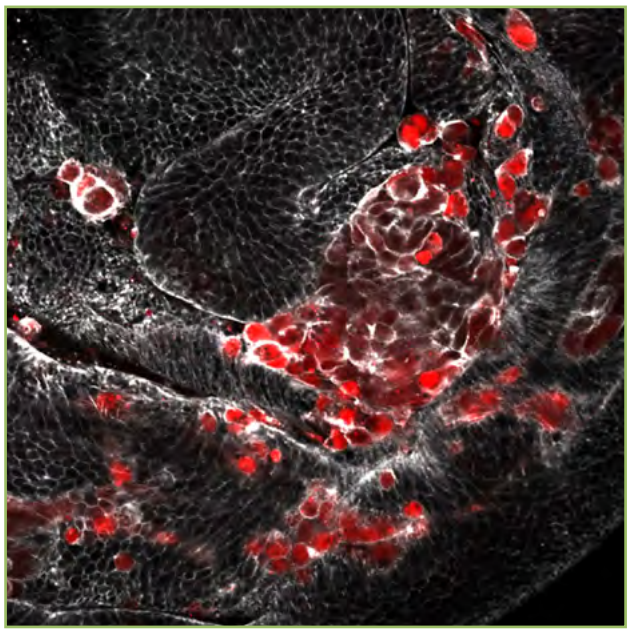
Using *Drosophila* as a model system our work has focused primarily on the study of two major processes, regeneration and tumorigenesis, and in particular the role of the Jun N-terminal Kinase (JNK) pathway, a conserved pathway known to be associated with tumorigenesis and regeneration in metazoans.

The JNK signaling pathway is a principal inducer of apoptosis in *Drosophila*, an autocrine function aimed to eliminate damaged or aberrant cells. This pro-apoptotic function includes the elimination by cell competition of oncogenic cells that may appear in development. Recent work has shown that JNK also has a pro-proliferation function, mediated by paracrine signaling and associated with the activity of the JAK/STAT, Wingless (Wg) and Decapentaplegic (Dpp).

Regarding the role of JNK function in tumorigenesis, we have found that it is a major factor in the process. We are presently investigating the role of JNK in the tumorigenic processes generated by mutations like scribble, erupted or polyhomeotic. Some to this work has already been published (Medina et al 2021). We have also found evidence for a new mechanism of interactions between cell populations, such as that overgrowing tumour cells are able to restrain the growth of neighbour normal tissues. This process may provide new hints as to how tumour cells colonise normal tissues.

Another critical function of the JNK pathway is the induction of senescence in *Drosophila* cells: Just a transient stress given to cells refractory to apoptosis results in their transformation into senescent cells that contain indefinite JNK activity. Under these conditions those cells secrete proliferative signals (Upd, Dpp, Wg) that cause tumorigenic overgrowths in neighbour non-senescent tissue. This research line is being actively investigated in the lab.

Another goal in the laboratory is to study chromatin remodeling during tissue regeneration. Surviving cells of damaged tissues readapt their status to reconstruct the lost tissue. They undergo chromatin remodeling to activate or repress new batteries of genes, a process that may be affected by the activation of retrotransposon LINE elements, which regulate global chromatin accessibility. The role of retrotransposons had not been studied during regeneration of *Drosophila*. We have found (Azpiazu and Morata 2022) that during regeneration, nuclear chromatin acquires an "intermediate" state that resembles the young embryonic totipotent state. Moreover, Roo and F-Element retrotransposons of *Drosophila* suffer profound changes in expression in the first hours of embryonic development and also during regeneration.



Portion of a wing imaginal disc of Drosophila containing a number of senescent cells, labelled red. Note that the senescent cells are bigger than the surrounding non-senescent ones, as indicated by F-actin staining (white)

List of publications

» Morata, G. (2021) Cell competition: a historical perspective Develop. Biol. 476, 33-40 doi:10.1016/j.ydbio.2021.02.012

» Medina, I. Calleja, M. and Morata G. (2021) Tumorigenesis and cell competition in *Drosophila* in the absence of polyhomeotic function PNAS 118, 45 doi.org/10.1073/pnas.2110062118

» Morata, G and Affolter M. (2021) Walter Jacob Gehring, W. Biogr. Mems Fell. R. Soc. 71, 197-212 doi.org/10.1098/rsbm.2021.0011

» Azpiazu, N. and Morata, G. (2022) Chromatin remodeling and retrotransposons activities during regeneration in *Drosophila* Develop. Biol 482, 7-16 doi: 10.1016/j.ydbio.2021.11.005

» Morata, G. and Lawrence, P. (2022) An exciting period of *Drosophila* developmental biology: Of imaginal discs clones, compartments, parasegments and homeotic genes Develop. Biol 482, 12-21 doi.org/10.1016/j.ydbio.2022.01.008

» Pinal, N. Azpiazu, N. and Morata, G. (2022). Size compensation in *Drosophila* after generalised cell death. bioRxiv 2022.11.04.515194; doi:10.1101/2022.11.04.515194

Participation in projects

» "Estudio del papel de la vía JNK en los procesos de apoptosis/competición celular, tumorogenesis y regeneración en *Drosophila*". Ministerio de Ciencia e Innovación. PGC2018-095151-B-I00. PI: Ginés Morata. 2019-2021

» "La via JNK en los procesos de tumoración y regeneración en *Drosophila*". Ministerio de Ciencia e Innovación. PID2021-125377NB-I00. PIs: Ginés Morata – Natalia Azpiazu. 2022-2025

Doctoral theses

» Izarne Medina Azpiazu (2022). Tumorogénesis y competición celular en células con falta de función del gen polyhomeotic en *Drosophila melanogaster* ". Universidad Autónoma de Madrid. Advisor: Ginés Morata

EXTRACELLULAR MATRIX REMODELING IN THE CARDIOVASCULAR SYSTEM

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

Group Members

Principal Investigator:
Fernando Rodríguez Pascual

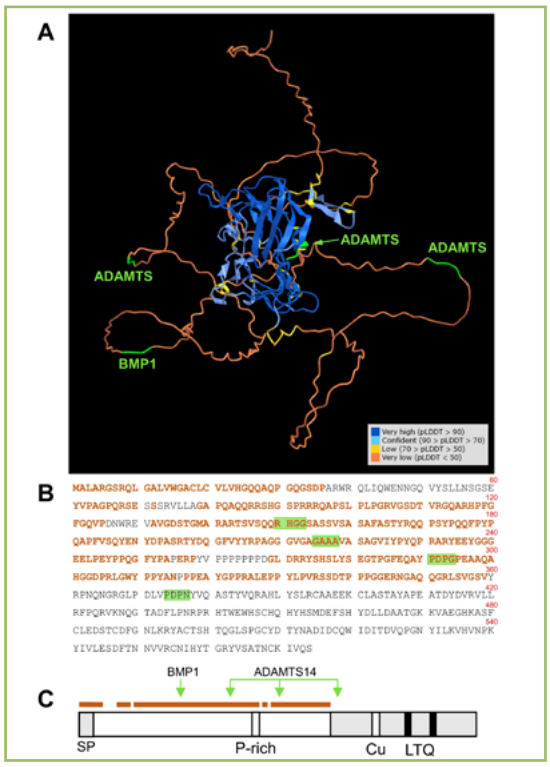
Postdoctoral Fellow:
Tamara Rosell García (1/1/2021 al 29/10/2021)

Postdoctoral Fellow:
Sergio Rivas Muñoz (1/12/2021 al 30/11/2022)

Master Student:
Silvia Alcaraz Romero (22/10/2021 al 15/07/2022)

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 protein. 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 post-translational modification that largely determines the biomechanical properties of the collagen. The LOX family of matrix-remodeling enzymes is composed by 5 members, a canonical LOX and four LOX-like isoforms from 1 to 4 (LOXL1, LOXL2, LOXL3 and LOXL4). The activity of the canonical LOX and the isoform LOXL1 are regulated by proteolysis and in the laboratory we have analyzed the molecular determinants of this proteolytic regulation. Our results have shown the existence of a complex pattern for the proteolysis of these LOX members, with multiple proteases contributing to the extracellular processing of these enzymes. Additionally, we have studied the regulation by hypoxia of several collagen remodeling enzymes, including lysyl- and prolyl-hydroxylases and analyzed the contribution of this hypoxic regulation to the development of fibrosis in several pathological contexts.



Location of BMP1 and ADAMTS14 cleavage sites within the tridimensional structure of LOXL1 protein. A) AlphaFold prediction model for LOXL1 protein showing the degree of confidence (predicted local distance difference test, pLDDT) with a color code as indicated. N-terminal region up to position 360 mostly displays an unstructured folding (pLDDT<50, brown), a predictor of disordered region. This unstructured segment is highlighted within the LOXL1 protein sequence (B, C). The position of the BMP1 and ADAMTS14 cleavage sites is indicated in green. Note that these processing sites are within the disordered region (BMP1 at 151-152, ADAMTS14 at 216-217 and 292-293), or solvent-exposed within the structure C-terminal domain (ADAMTS14 at 375-376).

List of publications

Artículos

- Rodriguez-Pascual, F., Rosell-Garcia, T. (2022) The challenge of determining lysyl oxidase activity: Old methods and novel approaches. Anal Biochem. 639:114508. doi: 10.1016/j.ab.2021.114508.
- Rosell-García, T., Rivas-Muñoz, S., Colige, A., Rodriguez-Pascual, F. (2022) Cleavage of LOXL1 by BMP1 and ADAMTS14 Proteases Suggests a Role for Proteolytic Processing in the Regulation of LOXL1 Function. Int J Mol Sci. 23(6):3285. doi: 10.3390/ijms23063285.
- García-Izquierdo, E., Mingo-Santos, S., Olivo-Rodríguez, C., Moñivas-Palomero, V., Rivas-Lasarte, M., Martín-López, C.E., Rosado-García, S., Sánchez-López, A.J., Redondo, J.M., Rodríguez-Pascual, F., Segovia-Cubero, J., Forteza-Gil, A. (2022) Exploring the potential relationship between collagen cross-linking and impaired myocardial relaxation in Marfan syndrome: An observational study using serum biomarkers. Int J Cardiol. 352:125-130. doi: 10.1016/j.ijcard.2022.01.050.

Capítulos de libros

- Rosell-García, T, Rodriguez-Pascual, F. (2021) Techniques to Assess Collagen Synthesis, Deposition, and Cross-Linking In Vitro. Methods Mol Biol. 2299:115-122. doi: 10.1007/978-1-0716-1382-5_8.
- Rodríguez-Pascual, F. (2021) The Evolutionary Origin of Elastin: Is Fibrillin the Lost Ancestor? In book: Extracellular Matrix: Developments and Therapeutics. doi: 10.5772/intechopen.95411.

Participation in projects

- RTI2018-095631-B-I00. "Activación proteolítica de lisil oxidasas. Aplicaciones biotecnológicas y contribución al remodelado de la matriz extracelular en el desarrollo de enfermedades humanas". Proyectos I+D+i "Retos Investigación" del Programa Estatal de I+D+i orientada a los retos de la sociedad. Convocatoria 2018. PI: Fernando Rodríguez Pascual. 01/01/2019-30/09/2022.

Other activities

- Fernando Rodríguez Pascual. Member of the Editorial Board of the journals "Scientific Reports" (Molecular Biology Section), "Asia-Pacific Journal of Ophthalmology" (Visual Sciences Section) and the "Molecular Biology Reports" (Extracellular Matrix Biology Section).

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

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

Group Members

Principal Investigator:
María Yáñez-Mó

Postdoctoral fellows:
Fernando Gutiérrez del Burgo (from January 2022)

Predoctoral fellows:
Victor Toribio Serrano
Beatriz Benayas López
Miguel Palma Cobo

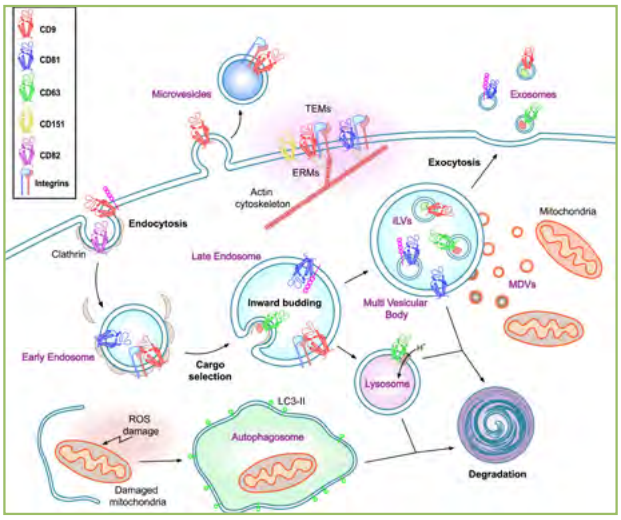
Technicians:
Soraya López-Martín

Undergraduate and Master Students:
Joaquín Morales
Diego Matas

Summary

Our group is focused on the role of tetraspanin-enriched membrane nanodomains in extracellular vesicles (EVs). We pursue both biotechnological applications for EV detection, isolation or characterization as well as fundamental knowledge on tetraspanin involvement in the molecular mechanisms of EV biogenesis, cargo selection and uptake.

Our latest data suggest that tetraspanins finely regulate the dynamics of endosomal compartments and their interrelation with autophagy and mitophagy pathways. Thus, tetraspanins emerge as potent regulators of the metabolic fitness of the cell, with novel therapeutic implications in the fight against cancer.



Scheme of the role of tetraspanins in the dynamics of endosomal compartments and their connection with autophagy and mitophagy. Extracted from Toribio and Yáñez-Mó, Eur J Cell Biol 2022

Other activities

Secretary of the Instituto Universitario de Biología Molecular (IUBM) since January 2022. Member of the Scientific committee of the 6th International GEIVEX Symposium 2022 (Santiago de Compostela October 26-28th 2022). Member of the organizing committee of the GEIVEX/TeNTaCLES 2021 Minisymposium (November 18th, 2021, online). Coordinator of the UAM node and the Exosome Characterization platform in CIVIS Cancer and Immunology Blended Mobility Network "Technical innovations in basic and translational research: applications to immunology-oncology". Teacher at the 2nd and 3rd online Specialization Course on Extracellular Vesicles (GEIVEX/UFV). Member of the Board of the non-profit association "Grupo español de innovación e investigación en vesículas extracelulares GEIVEX". President since November 18th, 2021. Member of the Editorial Board of Scientific Reports and Frontiers in Immunology. Guest Editor for European Journal of Cell Biology and International Journal of Mollecular Science. Research and Development contract between the CSIC, the FUAM and IMMUNOSTEPS.

List of publications

» Cardeñes B, Clares I, Bezos T, Toribio V, López-Martín S, Rocha A, Peinado H, Yáñez-Mó M, Cabañas C. (2022) ALCAM/CD166 Is Involved in the Binding and Uptake of Cancer-Derived Extracellular Vesicles. Int J Mol Sci. May 20;23(10):5753. doi: 10.3390/ijms23105753.

» Toribio V, Yáñez-Mó M. (2022) Tetraspanins interweave EV secretion, endosomal network dynamics and cellular metabolism. Eur J Cell Biol. Jun-Aug;101(3):151229. doi: 10.1016/j.ejcb.2022.151229.

» Campos-Silva C, Cáceres-Martell Y, Sánchez-Herrero E, Sandúa A, Beneitez-Martínez A, González Á, Provencio M, Romero A, Jara-Acevedo R, Yáñez-Mó M, Valés-Gómez M. (2022) A simple immunoassay for extracellular vesicle liquid biopsy in microliters of non-processed plasma. J Nanobiotechnology. Feb 8;20(1):72. doi: 10.1186/s12951-022-01256-5.

» Cardeñes B, Clares I, Toribio V, Pascual L, López-Martín S, Torres-Gomez A, Sainz de la Cuesta R, Lafuente EM, López-Cabrera M, Yáñez-Mó M, Cabañas C. (2021) Cellular Integrin α5β1 and Exosomal ADAM17 Mediate the Binding and Uptake of Exosomes Produced by Colorectal Carcinoma Cells. Int J Mol Sci. Sep 14;22(18):9938. doi: 10.3390/ijms22189938.

» Calle A, Toribio V, Yáñez-Mó M, Ramírez MÁ. (2021) Embryonic Trophectoderm Secretomics Reveals Chemotactic Migration and Intercellular Communication of Endometrial and Circulating MSCs in Embryonic Implantation. Int J Mol Sci. May 26;22(11):5638. doi: 10.3390/ijms22115638.

» Suárez H, Andreu Z, Mazzeo C, Toribio V, Pérez-Rivera AE, López-Martín S, García-Silva S, Hurtado B, Morato E, Peláez L, Arribas EA, Tolentino-Cortez T, Barreda-Gómez G, Marina AI, Peinado H, Yáñez-Mó M. (2021) CD9 inhibition reveals a functional connection of extracellular vesicle secretion with mitophagy in melanoma cells. J Extracell Vesicles. May;10(7):e12082. doi: 10.1002/jev2.12082.

» Campos-Silva C, Cáceres-Martell Y, López-Cobo S, Rodriguez MJ, Jara R, Yáñez-Mó M, Valés-Gómez M. (2021) An Immunocapture-Based Assay for Detecting Multiple Antigens in Melanoma-Derived Extracellular Vesicles. Methods Mol Biol; 2265:323-344. doi: 10.1007/978-1-0716-1205-7_24.

» Royo F, Azkargorta M, Lavin JL, Clos-Garcia M, Cortazar AR, Gonzalez-Lopez M, Barcena L, Del Portillo HA, Yáñez-Mó M, Marcilla A, Borras FE, Peinado H, Guerrero I, Váles-Gómez M, Cereijo U, Sardon T, Aransay AM, Elortza F, Falcon-Perez JM. (2021) Extracellular Vesicles From Liver Progenitor Cells Downregulates Fibroblast Metabolic Activity and Increase the Expression of Immune-Response Related Molecules. Front Cell Dev Biol. Jan 12;8:613583. doi: 10.3389/fcell.2020.613583.

» López de Las Hazas MC, Gil-Zamorano J, Cofán M, Mantilla-Escalante DC, Garcia-Ruiz A, Del Pozo-Acebo L, Pastor O, Yañez-Mo M, Mazzeo C, Serra-Mir M, Doménech M, Valls-Pedret C, Rajaram S, Sabaté J, Ros E, Sala-Vila A, Dávalos A. (2021) One-year dietary supplementation with walnuts modifies exosomal miRNA in elderly subjects. Eur J Nutr. Jun;60(4):1999-2011. doi: 10.1007/s00394-020-02390-2.

» 10. Calle A, Gutiérrez-Reinoso MÁ, Re M, Blanco J, De la Fuente J, Monguió-Tortajada M, Borràs FE, Yáñez-Mó M, Ramírez MÁ. (2021) Bovine peripheral blood MSCs chemotax towards inflammation and embryo implantation stimuli. J Cell Physiol. Feb;236(2):1054-1067. doi: 10.1002/jcp.29915.

Participation in projects

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

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

» Microdominios de membrana, exosomas, virus y vacunas. PID2020-119627GB-I00, Proyectos I+D Generación de Conocimiento. Principal Investigator: María Yáñez-Mó. 01/09/2021-31/08/2024.

» VALIDACION CLINICA DE UN SISTEMA PARA DETECTAR TRANSLOCACIONES DE ALK EN VESICULAS EXTRACELULARES EN PLASMA. DTS21/00134, Instituto de Salud Carlos III. Principal Investigator: María Yáñez-Mó. 01/01/2022-31/12/2023.

» Vacunas basadas en exosomas miméticos. PDC2021-121052-I00. Ministerio de Ciencia e Innovación. Principal Investigator: María Yáñez-Mó. 01/12/2021-30/11/2023.

Tissue and Organ Homeostasis

Cell Architecture and Organogenesis Unit

Fernando Martín Belmonte

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

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

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

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

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

Highlights of the unit

» Studies from the Cellarch unit demonstrate that in mouse models of acute and chronic neuro-inflammation, astrocyte-derived SFRP1 promotes and sustains microglial activation via the upregulation of components of HIF-dependent inflammatory pathway, thereby acting as an astrocyte to microglia amplifier of neuroinflammation (Rueda-Carrasco et al. 2021, EMBO Rep). Our unit studies have advanced our understanding of how cells in the mammalian embryo transit from pluripotency to lineage specification, most unexpectedly by the use of the same set of factors, such as Oct4 (Andreu MJ et al. 2022, Cell Report; Tiana et al. 2022; Sci Adv; Alvarez-Franco A et al. 2021, Cardiovasc Res).

» Other studies focused on identifying mechanisms that ensure the formation of the subcellular cytoskeleton capable of supporting the cellular structure. We characterized that the SMTNL2 protein has an essential role in the formation of microvilli that decorate the luminal membrane of mammalian enterocytes through the regulation of the subapical actin cytoskeleton (Hachimi et al. 2021, Current Biol)). Also, one of our groups demonstrated that While the binding of Rho GTPases to the N-terminal region of most formins activates actin polymerization, calmodulin does so in the formin INF2 (Labat-de-Hoz et al., CMLS 2022). Furthermore, our unit has developed a coarse-grained model of a two-dimensional actomyosin cortex that reproduces many essential aspects of actin filament and actomyosin network formation, such as dynamics of polymerization and depolymerization, treadmilling, network formation, and the autonomous oscillatory dynamics of actomyosin (Hernández-Del-Valle M et al. 2022, BMC Biol).

» A significant part of the work carried out in our unit uses the drosophila model for its experiments. We are interested in the molecular and cellular mechanisms that control cell fate specification and morphogenesis during development. We discovered an essential molecular link connecting cell cycle progression and p53's proapoptotic activity, which coordinates the appropriate response following DNA damage (Velarde SB et al. 2021; Plos Biol). Another study also identified the genetic and molecular mechanisms that control glial response after nervous system damage (Ruiz-Losada M et al. 2022, Cell Death Differ). Additional analyses in drosophila have shown that the differences in signalling downstream of the Hedgehog morphogen measured between developing *Drosophila* organs are simply different states along the temporal dynamics of the pathway (Miguez et al., 2022, Development). A genome-wide genetic screen in the *Drosophila* wing using ARN interference described the loss-of-function phenotype of 80% of *Drosophila* genes. Similarly, a study uncovered a specific requirement of CUBAM in slit diaphragm degradation and recycling in the fly nephrocyte. Interestingly, this function might be conserved in vertebrate podocytes, as suggested by some clinical parameters present in patients with Imerslund-Gräsbeck syndrome carrying CUBN mutations (Atienza-Manuel et al. 2021, Development).

Miguel Angel Alonso Lebrero / Isabel Correas Hornero
CELL POLARITY

Paola Bovolenta
MORPHOGENESIS AND NEURODEGENERATION OF THE VERTEBRATE CNS

Jose F. de Celis
GENETIC ANALYSES OF SIGNALING PATHWAYS DURING EPITHELIAL DEVELOPMENT IN DROSOPHILA MELANOGASTER

Carlos Estella / Antonio Baonza
CELLULAR RESPONSE TO STRESS AND MORPHOGENESIS

Miguel Manzanares
FUNCTIONAL GENOMICS

Fernando Martin Belmonte
GROUP OF INTESTINAL MORPHOGENESIS AND HOMEOSTASIS

Nuria Martínez Martín
METABOLISM AND B CELL FUNCTION

David G. Míguez
SYSTEMS BIOLOGY

Mar Ruiz Gómez / Joaquim Culí Espigul
GENETIC AND FUNCTIONAL ANALYSIS OF THE RENAL FILTRATION DIAPHRAGM IN HEALTH AND DISEASE

Ernesto Sánchez-Herrero Arbide
SEGMENTAL SPECIFICATION AND PATTERN FORMATION IN DROSOPHILA

Esther Serrano-Saiz
TRANSCRIPTIONAL CONTROL OF SEXUAL DIFFERENTIATION OF THE NERVOUS SYSTEM

CELL POLARITY

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Summary

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

Previous work in our laboratory established a critical role for the midbody remnant in primary cilium formation in polarized epithelial cells. We have now observed that the majority of midbody remnants are physically connected to the plasma membrane through a membranous stalk derived from an intact arm of the cytokinetic bridge. Thanks to this physical continuity, the midbody remnant delivers a specialized membrane patch that the centrosome uses to build the ciliary membrane. Our study shows how the ciliary membrane and the primary cilium originate in polarized epithelial cells.

Formins are a family of proteins involved in the assembly of actin filaments. Most formins, such as mDia1, contain a diaphanous inhibitory domain (DID) at the N-terminal region that interacts with the C-terminal region to maintain the molecule in an inactive state. mDia1 and other formins are regulated by the binding of Rho GTPases to the DID. INF2 is a formin linked to inherited renal and neurological disease in humans. INF2 possesses an N-terminal extension of unknown structure and function that precedes the DID. Our work has demonstrated that this extension is organized into two α-helices, the first of which interacts directly with Ca^{2+}/calmodulin through a peptide motif that is conserved in vertebrates. Consistent with this interaction, INF2 produces massive actin polymerization in response to increased Ca2+ levels. Our study reveals that, unlike other formins, INF2 is regulated by interaction of Ca^{2+}/calmodulin with the INF2 N-terminal extension.

The MAL family of proteins has been the focus of our laboratory's research for a long time. Our third project has dealt with the characterization of MALL, a membrane tetraspanning member of this family. We have found endogenous MALL in membranes and, unexpectedly, in nuclear-membraneless structures, the PML bodies. Our study suggests that MALL can adopt a membrane-embedded or a water-soluble conformation depending on its physical environment —lipidic or aqueous— in the cell. During mitosis, overexpressed MALL aggregates at the cytokinetic bridge into large solid structures that produce cytokinesis failure and lead to cells with aberrant chromosome content. Since MALL is overexpressed in some types of cancer, an excess of MALL could contribute to malignancy by inducing chromosome instability.

List of publications

» Labat-de-Hoz, L., Rubio-Ramos, A., Casares-Arias, J., Bernabé-Rubio, M., Correas, I., and Alonso, M.A. (2021) A model for primary cilium biogenesis by polarized epithelial cells: Role of the midbody remnant and associated specialized membranes. Front. Cell Dev. Biol. 8, 622918. doi: 10.3389/fcell.2020.622918

» Rubio-Ramos, A., Labat-de-Hoz. L., Correas, I., Alonso, M.A. (2021) The MAL protein, an integral component of specialized membranes, in normal cells and cancer. Cells 10, 1065., doi: 10.3390/cells10051065.

» Bernabé-Rubio, M., Bosch-Fortea, M., García, E., Bernardino de la Serna, J., and Alonso, M.A. (2021) Adaptive lipid immiscibility and membrane remodeling are active functional determinants of primary ciliogenesis. Small Methods 5, 2000711. doi: 10.1002/smtd.202000711

» Bernabé-Rubio. M., Bosch-Fortea, M., Alonso, M.A., Bernardino de la Serna J. (2021) Multi-dimensional and spatiotemporal correlative imaging at the plasma membrane of live cells to determine the continuum nano-to-micro scale lipid adaptation and collective motion. Methods 193, 136-147. doi: 10.1016/j.ymeth.2021.06.007

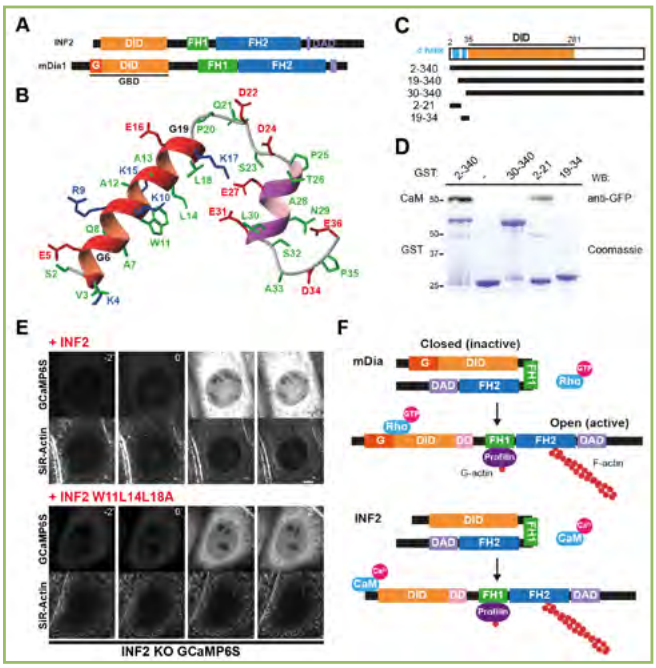
» Casares-Arias, J., Alonso, M.A., San Paulo, A., González, M.U. (2021) Correlative confocal and scanning electron microscopy of cultured cells without using dedicated equipment. STAR Protoc. 2, 100727. doi: 10.1016/j.xpro.2021.100727.

» Cacho-Navas, C., Reglero-Real, N., Colás-Algora, Barroso, S., de Rivas, G., Stamatakis, K., Feito, J., Andrés, G., Fresno, M., Kremer, L., Correas. I., Alonso, M.A., and Millán, J. (2022). Plasmolipin regulates basolateral-to-apical transcytosis of ICAM-1 and leukocyte adhesion in polarized hepatic epithelial cells. Cell. Mol. Life Sci. 79, 61. doi:10.1007/s00018-021-04095-z.

» Labat-de-Hoz, L., and Alonso, M.A. (2021) Formins in human disease. Cells 10, 2554, doi: 10.3390/cells10102554.

» Rubio-Ramos, A., Bernabé-Rubio, M., Labat-de-Hoz, L., Casares-Arias, J., Kremer, L., Correas, I., and Alonso, M.A. (2022) MALL, a membrane-tetraspanning proteolipid overexpressed in cancer, is present in membraneless nuclear biomolecular condensates. Cell. Mol. Life Sci. 79, 236. doi: 10.1007/s00018-022-04270-w.

» Labat-de-Hoz, L., Comas, L., Rubio-Ramos, A., Casares-Arias, J., Fernández-Martín, L., Pantoja-Uceda, D., Martín, M.T., Kremer, L., Jiménez, M.A., Correas, I., and Alonso, M.A. (2022) Structure and function of the N-terminal extension of the formin INF2. Cell. Mol. Life Sci. 79, 571. doi: 10.1007/s00018-022-04581-y.



Structure and regulation of INF2. (A) INF2 has an extension N-terminal to the DID that is shorter than that of mDia1. (B) Ribbon representation of the NMR solution structure of the N-terminal extension of INF2. (C) INF2 fragments fused to GST used in the pull-down assay experiment. (D) Pull-down assay showing the binding of calmodulin to the first α-helix of the N-terminal extension of INF2. (E) Actin dynamics in INF2 KO cells expressing the GCaMP6S calcium biosensor and wild type INF2 or INF2 with the W11L14L18A mutation that inactivates the binding of calmodulin. Cells were treated with A23187 (0 min) and analyzed by videomicroscopy before and after treatment. Actin was visualized with SirR-Actin. (F) Whereas mDia1 is activated by the binding of GTP-loaded, INF2 activation takes place by binding Ca^{2+}/calmodulin.

Participation in projects

» "Biogenesis of the primary cilium: characterization of the midbody remnant and regulation of its inheritance". MCIN, PGC2018-095643-B-100 (2019-2022). PI: Miguel A. Alonso

» "Regulation of normal and pathogenic INF2 formin". MCIN, PID2021-123179NB-I00 (2022-2025). PI: Miguel A. Alonso

» "TomoXLiver: Study of the dysfunction of the hepatocyte from a multidisciplinary approach". Comunidad de Madrid, B2017/BMD-3817 (2018-2022). PI: Isabel Correas

Doctoral theses

» Armando Rubio Ramos (2022). La proteína MALL: identificación en los cuerpos PML y su implicación en la organización nuclear. Univ. Autónoma de Madrid. Supervisors: Isabel Correas and Miguel A. Alonso

» Leticia Labat de Hoz (2022) Regulación de la formina INF2 normal y patogénica: papel del extremo amino terminal. Univ. Autónoma de Madrid. Supervisor: Miguel A. Alonso

MORPHOGENESIS AND NEURODEGENERATION OF THE VERTEBRATE CNS

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Margarita Dillinger
(11, 2021-03, 2022)
Sergio Cruz (01-06, 2022)
Eva Pajda (01-06, 2022)
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Summary

A functional CNS depends on a correct development and the maintenance of optimal homeostatic conditions. Variations in the necessary developmental programs often cause congenital malformations, whereas loss of an efficient CNS homeostasis leads to progressive aging or neurodegenerative conditions. Our team seeks to understand these events focusing, on one side, on early vertebrate eye morphogenesis and, on the other, on the implication that Secreted Frizzled Related Protein 1 (SFRP1) has in Alzheimer's disease (AD) pathogenesis.

We have demonstrated that the presumptive retinal pigment epithelium (RPE) of the eye is required for eye morphogenesis with a species-specific mechanism that varies between teleost and amniotes. In teleosts, RPE cells undergo a profound cell shape change by strongly reducing their apico-basal axis while enlarging their surface area through an important cytoskeletal reorganization. This transformation occurs in the absence of cell proliferation and generates mechanical forces that contribute eye morphogenesis. The gene regulatory network that governs this transformation include transcription factors that control the expression onset of cell adhesion and cytoskeletal components (collaboration with JR Martinez-Morales, CABD, CSIC-UPO). This mechanism differs in amniotes, in which the RPE grows mostly through cell proliferation, which, in different species, is inversely proportional to the length of the apico-basal axis, so that in human RPE progenitors are highly proliferative and quite elongated in shape. We thus propose that zebrafish RPE stretching and fast differentiation are two efficient mechanisms that enable the RPE to reach the size needed for optic vesicle folding when this process takes less time than the cell cycle.

Related to neurodegeneration, we pursued our observation that SFRP1, a small secreted protein found in the brain matrisome and mostly produced by astrocytes and choroid plexus cells, is involved in AD pathogenesis. We have now shown that astrocyte-derived SFRP1 promotes and sustains microglial activation via the upregulation of components of HIF-dependent inflammatory pathway, thereby acting as an astrocyte to microglia amplifier of neuroinflammation. By generating a transgenic mouse model that overexpresses Sfrp1 in astrocytes, we have also shown that high SFRP1 levels cause an age-dependent loss of hippocampal dendritic spines with poor cognitive performance and reduced response to long-term potentiation.

EM analysis shows smaller presynaptic terminals with a reduced cargo of synaptic vesicles in the transgenics, the synaptosomes of which also are enriched in synaptic adhesion molecules and proteins involved in synaptic vesicle cycle (proteome analysis in collaboration with A. Smit, Vrije Universiteit, Amsterdam). Thus, high SFRP1 levels in the brain are sufficient to induce synaptic alterations, suggesting that this protein might have pleiotropic function in AD.

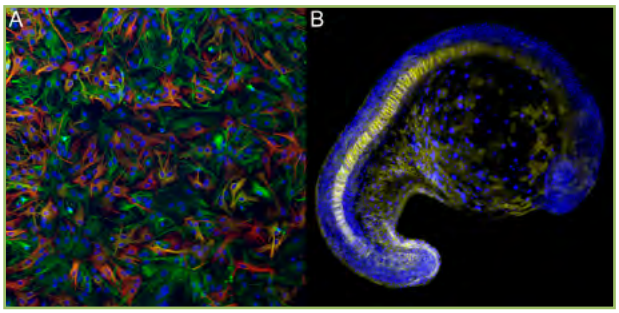

Figure 1. *A) Primary mixed culture of astrocyte and microglial cells from Sfrp1 knock out mice stained for CD45 (green), GFAP (red) and DAPI (blue). B) Transgenic zebrafish larva at 20 hpf in which the notochord and few mesenchymal cells are labelled in yellow whereas histone staining (blue) highlights the nuclei.*

List of publications

» Buono L, Corbacho J., Naranjo S., Almuedo-Castillo M, Moreno-Marmol T., de la Cerda B, Sanbria-Reinoso E, Polvillo R, Díaz-Corrales FJ, Bogdanovic O, Bovolenta P*, Martínez-Morales JR* (2021) Analysis of gene network bifurcation during optic cup morphogenesis in zebrafish. Nat Comm. 12: 3866 doi: 10.1038/s41467-021-24169-7. *co-correspondence.

» Rueda-Carrasco, J, Martin-Bermejo MJ*, Pereyra, G*, Mateo, MI*, Borroto, A, Brosseron, F., Kummer, MP, Schwartz, S, Lopez-Atalaya, JP; Alarcon, B., Esteve, P. Heneka, MT, and Bovolenta, P. (2021) SFRP1 modulates astrocyte to microglia cross-talk in acute and chronic neuroinflammation EMBO Rep. Sep 27:e51696. doi: 10.15252/embr.202051696. *equal contribution

» Moreno-Marmol, T, Ledesma M, Tabanera N, Martin-Bermejo MJ, Cardozo, MJ, Cavodeassi F, and Bovolenta P. (2021) Stretching of the retinal pigment epithelium contributes to zebrafish optic cup morphogenesis. eLife 10:e63396. doi: 10.7554/eLife.63396

» Camacho-Macorra C, Sintes M, Tabanera N, Grasa I, Bovolenta P*, Cardozo MJ* (2021) Mosmo is required for zebrafish craniofacial development. Front Cell &Dev Biol. 9:767048. doi: 10.3389/fcell.2021.767048 *co-correspondence.

» Camacho-Macorra C, Tabanera N, Bovolenta P*, Cardozo MJ* (2021) Maternal vgll4a promotes blastoderm cohesion enabling yap1-mediated mechano-transduction during zebrafish epiboly. bioRxiv doi. 10.1101/ 2020.12.01.407478. *co-correspondence

» Hernández-Bejarano M., Gestri G., Monfries C., Tucker L., Dragomir E.I., Bianco IH, Bovolenta P., Wilson SW and Cavodeassi E. (2022) Foxd1 dependent induction of temporal retinal character is required for visual function. Development 15;149(24) dev200938. doi: 10.1242/dev.200938

Doctoral theses

» Carlos Camacho de la Macorra (2022). Yap-Taz en la especificación del epitelio pigmentado de vertebrados. Facultad de Ciencias, Universidad Autónoma de Madrid. Co-Dirección: Dra. Paola Bovolenta / Dr. Marcos Cardozo.

Participation in projects

» Deconstructing gene regulatory networks for improving sight and brain disabilities (Brains4Sight). (ERA-NET Neuron, NEURON_NDD-255) 2022-2024. Coordinator

» New approaches to understand prevalent and neurodegenerative diseases. Agencia Estatal de Investigacion (AEI, PID2019-104186RB-100) 2020-2023.

» RedDevNeural 3.0. An integrative approach to understand the logic of neural development. (AEI, RED2018-102553-T). 2020-2022. Coordinator

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

» Our group belongs to the Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER).

Other activities

» During this period Paola Bovolenta has been a member of the ERC Scientific Council, Chair of the Open Access Working Group of the ERC; Member of the Scientific Advisory Board of the ERA-NET NEURON; Armenise-Harvard, Bettencourt-Schueller, Telethon and Gadea Foundations; Senior Editor of the Eur. J. Neurosci. and reviewing Editor of eLife; SENC President, among other duties. PB is member of EMBO and the Academia Europæa.

» Several members our group participate in outreach activities, i.e. Semana de la Ciencia 2022, 4ºESO+Empresa 2021.

GENETIC ANALYSES OF SIGNALING PATHWAYS DURING EPITHELIAL DEVELOPMENT IN *DROSOPHILA MELANOGASTER*

Group Members

Principal Investigator:
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Predoctoral fellow:
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Cristina Martínez Ostalé (until September 2022)

Technician:
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Master Student 2021:
Jousef Issa García

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Summary

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

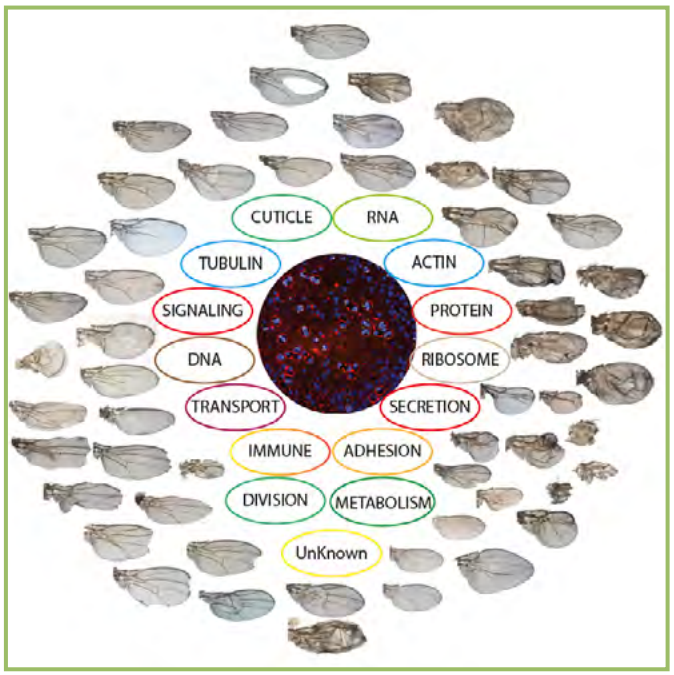

Image of the wing imaginal disc surface (central circle) showing the DNA (blue), Actine (red) and Tubulin (light blue) of mitotic cells. This image is surrounded by the names of the main molecular classes that include most of the 14.000 Drosophila genes (colored ovals), and by wings in which the expression of individual genes has been knockdown by RNA interference

List of publications

» Soler-Beatty, J., Molnar, C., Luque, C. de Celis, J.F. and Martín-Bermudo, M.D. (2021) EGFRAP encodes a new negative regulator of the EGFR acting in both normal and oncogenic EGFR/Ras-driven tissue morphogenesis. PLoS Genet 17(8): e1009738. doi: 10.1371/journal.pgen.1009738

» López-Varea, A., Ostalé, C.M., Vega-Cuesta, P., Ruiz-Gómez, A., Organista, M.F., Martín, M., Hevia, C.F., Molnar, C., de Celis, J., Culi, J., Esteban, N. and de Celis, J.F. (2021) Genome-wide phenotypic RNAi screen in the *Drosophila* wing: Global parameters. Genes|Genomes|Genetics jkab351, doi: 10.1093/g3journal/jkab351

» López-Varea, A., Vega-Cuesta, P., Ruiz-Gómez, A., Ostalé, C.M., Hevia, C.F., Molnar, C., Martín, M., Organista, M.F., Culí, J., Esteban, N. and de Celis J.F. (2021). Genome-wide phenotypic RNAi screen in the *Drosophila* wing: phenotypic description of functional classes. Genes|Genomes|Genetics jkab349 doi: doi.org/10.1093/g3journal/jkab349.

» Ostalé, C.M., Esteban, N., López-Varea, A. and de Celis, J.F. (2021). Functional requirements of protein kinases and phosphatases in the development of the *Drosophila melanogaster* wing. Genes|Genomes|Genetics. 2021; jkab349, doi: 10.1093/g3journal/jkab348.

Participation in projects

» Búsqueda genética a escala genómica y análisis de genes con relevancia biomédica / PGC2018-094476-B-100. PI:Jose F. de Celis / 01-01-2019 to 30-09-2022.

CELLULAR RESPONSE TO STRESS AND MORPHOGENESIS

Group Members

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Sara Tur-Gracia
Marina Pérez Aguilera
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Clara Agudo
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Alejandra García
(TFG until June 2021)
Javier Pereira
(TFG until June 2022)
Sara Cristobal
(TFM until June 2022)
Gonzalo García Girón
(TFM until June 2022)
Sara de Pablo Vinader
(TFG)
Paula Gil Cortés (TFM)
Daniel Felipe
(JAE contract, TFG)
Inés Kelleher
(JAE contract, TFM)

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Summary

During development a precise balance between cell proliferation and cell death (or apoptosis) is crucial in maintaining tissue homeostasis and ensuring the proper formation of functional three-dimensional organs. This becomes particularly important after cellular or tissue injury, as organs must compensate for cell loss during the regeneration process. Defects in the precise coordination between cell proliferation and cell death or apoptosis could trigger tumor development and organ malformations.

Our main general aim is to understand how cells respond to different stresses to maintain tissue homeostasis. In addition, we are interested in the molecular and cellular mechanisms that control cell fate specification and morphogenesis during development. To this end we use *Drosophila* as our model organism because its genomic conservation (*Drosophila* genome is 60% homologous to that of humans) and its powerful genetic tools that allow us to easily manipulate gene expression making this organism ideal for modelling human diseases.

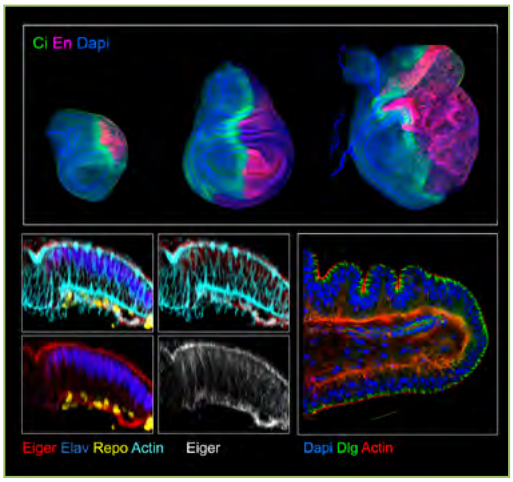
Our main research lines are:

1. How appendages are formed? We study the molecular and cellular mechanisms that control appendage specification and morphogenesis. At cellular and molecular level, a small number of signaling pathways, transcription factors and cell behaviors are reiteratively used throughout development to generate a three-dimensional appendage in *Drosophila* and in vertebrates.

2. Coordination between cell proliferation and apoptosis during tissue homeostasis. The mechanisms that control cell proliferation and apoptosis in response to different types of stress must be tightly coordinated and balance to maintain genomic integrity and prevent tumor development. Defective cells must be rapidly eliminated to prevent the transmission of mutations and the formation of malignant cells. What are the molecular mechanisms that link together cell division and cell death is an important question with huge implications in the field of cancer biology.

3. Regeneration mechanisms in epithelial and nervous system. Regeneration is the ability that some organisms present to repair damaged organs or tissues. This ability differs, not only between different species, but also between different stages of development of the same organism. One of our goals is to identify the cellular signals that promote and limit the regenerative capacity of an organism during development.

When neural tissue is damaged, a regenerative response is induced, aiminig to preserve the structural integrity and function of the nervous system. This regenerative response is mostly mediated by glial cells. We investigate the genetic and molecular mechanisms that control glial response after damage.



An overview of the different projects carried out in our lab. Imaginal discs stained to visualized different proteins implicated in regeneration, tissue homeostasis and morphogenesis.

List of publications

» Velarde, S. B., Quevedo, A., Estella, C., Baonza, A. (2021) Dpp and Hedgehog promote the glial response to neuronal apoptosis in the developing *Drosophila* visual system. PLoS Biol. 2021 Aug 11;19(8):e3001367. doi: 10.1371/journal.pbio.3001367.

» Ruiz-Losada, M., Pérez-Reyes, C., Estella, C. (2021) Role of the Forkhead Transcription Factors Fd4 and Fd5 During *Drosophila* Leg Development. Front Cell Dev Biol. Aug 2;9:723927. doi: 10.3389/fcell.2021.723927.

» Ruiz-Losada, M., González, R., Peropadre, A., Gil-Gálvez, A., Tena, J. J., Baonza, A., Estella, C. (2022) Coordination between cell proliferation and apoptosis after DNA damage in *Drosophila*. Cell Death Differ. Apr;29(4):832-845. doi: 10.1038/s41418-021-00898-6.

» Clarembaux-Badell, L., Baladrón-de-Juan, P., Gabilondo, H., Rubio-Ferrera, I., Millán, I., Estella, C., Valverde-Ortega, F. S., Cobeta, I. M., Thor, S., Benito-Sipos, J. (2022) Dachshund acts with Abdominal-B to trigger programmed cell death in the *Drosophila* central nervous system at the frontiers of Abd-B expression. Dev Neurobiol. Sep;82(6):495-504. doi: 10.1002/dneu.22894.

» Baonza, A., Tur-Gracia, S., Pérez-Aguilera, M., Estella, C. (2022) Regulation and coordination of the different DNA damage responses in *Drosophila*. Front Cell Dev Biol. Sep 6;10:993257. doi: 10.3389/fcell.2022.993257.

Book chapter:

» Hernández-Munain, C., Estella, C., Garcia, S., Reyes, J. C., Serrano, E., Gutierrez, C. (2021) The non-coding genome. Consejo Superior de Investigaciones Científicas (España). pp 78-101

Participation in projects

» Estudio de la red de regulación génica inducida por el daño en el ADN y su coordinación en diferentes contextos celulares. PID2021127114NBI00 (2022-2025). PIs Carlos Estella and Antonio Baonza.

» Estudio de los mecanismos moleculares que controlan la especificación celular y la homeostasis tisular. PGC2018-095144BI00 (20192022). PI: Carlos Estella.

Other activities

» Antonio Baonza and Carlos Estella are Associated Editors of the research topic "Regulation and Coordination of the Different DNA Damage Responses and their Role in Tissue Homeostasis Maintenance" in Frontiers in Cell and Developmental Biology.

» Antonio Baonza is Associate Editor of the Journal Heliyon (Cell press),

» Antonio Baonza was a Visiting Researcher (10/2019-03/2021) at Sir William Dunn School of Pathology, University Oxford. Funded by a Salvador de Madariaga, visiting fellowship.

Doctoral theses

» Mireya Ruiz-Losada (2022). "Coordination between cell proliferation and apoptosis after DNA damage". Universidad Autónoma de Madrid. Supervisor: Carlos Estella

» Sara Ahmed de Prado (2021) . "Estudio de los mecanismos que regulan la re- especificacion cellular y la perdida de la capacidad proliferative en respuesta al daño en el disco imaginal de ala de *Drosophila melanogaster*". Universidad Autónoma de Madrid. Supervisor: Antonio Baonza.

FUNCTIONAL GENOMICS

Group Members

Principal Investigator:
Miguel Manzanares

Postdoctoral fellow:
Maria Tiana (since September 2021)
Elva Martin Batista (since November 2021)

Predoctoral fellow:
Antonio Barral (since December 2022)

Visiting scientist :
Antonio Barral (March 2021-November 2022)
Marta Portela (since March 2021)
Alba Alvarez-Franco (March-November 2021)

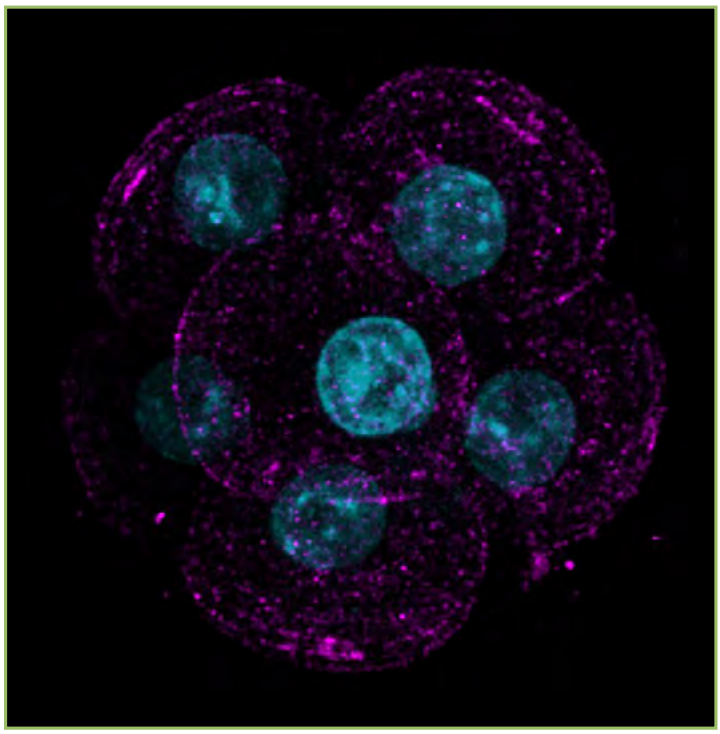
http://www.cbm.uam.es/functional-genomics

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.



Confocal image of a 6-cell mouse embryo, where the nuclei are stained width DAPI (cyan), and the GATA3 protein is detected by a specific antibody (magenta)

List of publications

» Andreu MJ, Alvarez-Franco A, Portela M*, Gimenez-Llorente D, Cuadrado A, Badia-Careaga C, Tiana M, Losada A, Manzanares M (2022). Establishment of 3D chromatin structure after fertilization and the metabolic switch at the morula-to-blastocyst transition require CTCF. Cell Rep 41, 111501. doi: 10.1016/j.celrep.2022.111501

» Portela M, Jimenez-Carretero D, Labrador V, Andreu MJ, Arza E, Caiolfa VR, Manzanares M (2022). Chromatin dynamics through mouse preimplantation development revealed by single molecule localisation microscopy. Biol Open 11, bio059401. doi: 10.1242/bio.059401

» Tiana M, Lopez-Jimenez E, Sainz de Aja J, Barral A, Victorino J, Badia-Careaga C, Rollan I, Rouco R, Santos E, Sanchez-Iranzo H, Acemel RD, Torroja C, Adan J, Andres-Leon E, Gomez-Skarmeta JL, Giovinazzo G, Sanchez-Cabo F, Manzanares M (2022). Pluripotency factors regulate the onset of Hox cluster activation in the early embryo. Science Adv 8, eabo3583. doi: 10.1126/sciadv.abo3583

» Alvarez-Franco A, Rouco R, Ramirez RJ, Guerrero-Serna G, Tiana M, Cogliati S, Kaur K, Saeed M, Magni R, Enriquez JA, Sanchez-Cabo F, Jalife J, Manzanares M (2021). Transcriptome and proteome mapping in the sheep atria reveal molecular features of atrial fibrillation progression. Cardiovasc Res 117, 1760-75. doi: 10.1093/cvr/cvaa307

» Victorino J, Alvarez-Franco A, Manzanares M. Functional genomics and epigenomics of atrial fibrillation (2021). J Mol Cell Cardiol 157, 45-55. doi: 10.1016/j.yjmcc.2021.04.003

» Bleckwehl T, Crispatzu G, Schaaf K, Respuela P, Bartusel M, Benson L, Clark SJ, Dorighi KM, Barral A, Laugsch M, van IJcken WFJ, Manzanares M, Wysocka J, Reik W, Rada-Iglesias A (2021). Enhancer-associated H3K4 methylation safeguards in vitro germline competence. Nat Commun 12, 5771. doi: 10.1038/s41467-021-26065-6

Participation in projects

» Genome homeostasis and cellular fates in the early mouse embryo - GENHOME (AEI, PID2020-115755GB-I00, PI Miguel Manzanares (2021-24)

Other activities

» Co-organizer of the virtual conference “Embryonic Extra-embryonic Interfaces: Engineering Development” (July 2021)

INTESTINAL MORPHOGENESIS AND HOMEOSTASIS

Group Members

Principal Investigators (PI, co-PI):
Fernando Martin Belmonte

Postdoctoral fellows:
Covadonga Diaz
Catalina Grabowski
Tatiana Alfonso

Predoctoral fellows:
Gabriel Baonza
Gonzalo Herranz

Technicians:
Tamara Gonzalez

Undergraduate and Master Students:
Diego Alonso Larre
(TFM;JAE intro 2022)
Alejandra Ramos Marco
(TFM; JAE intro 2021)
Carlos Quintana Quintana
(TFG)
Belén Seco Estrada
(TFG)

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

Summary

Our main scientific interest is understanding intestinal morphogenesis and cellular polarity during morphogenesis, homeostasis, and regeneration, as well as their implications for human diseases, such as intestinal bowel diseases (IBD), obesity, diabetes, and cancer.

We use organotypic cell models as basic model systems and different zebrafish and mouse epithelial tissues as more physiological models for these investigations. In addition, my lab has initiated a new line of research using mouse embryonic stem cells (mES) to address epithelial formation and study asymmetric division in the development of the intestine.

The intestinal tract plays a fundamental role in development, regeneration, immunity and nutrition. Many critical aspects of intestinal development and function remain unexplored despite their relevance. For instance, the contribution of neonatal enterocytes in the metabolic crosstalk with microbiota and immune cells to reach homeostasis is fairly unknown. In our laboratory, we propose a multidisciplinary approach to understanding better the molecular mechanisms associated with formation, patterning and metabolism in intestinal morphogenesis and homeostasis.

A complete understanding of the molecular processes we intend to address will yield important new insights into intestinal development and homeostasis in vertebrates. It will also reveal new opportunities to discover pharmaceutical targets in human disease. Cancer is one of the leading causes of mortality and morbidity worldwide, with colorectal cancer (CRC) being especially prevalent among the elderly. Therefore, the investigation of this pathology will contribute to increasing the life expectancy of the affected population.

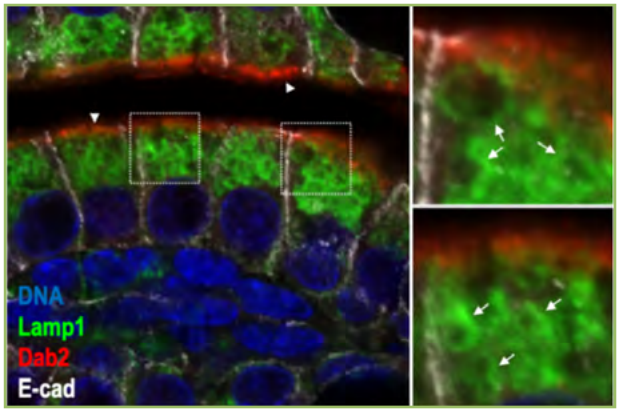


Figure 1. Confocal image of an Intestinal epithelial cell (IEC) in neonatal gut (p7). *Confocal image of a seven-day mice gut showing the enrichment of lysosomal vesicles (arrows) in Lysosome-rich-enterocytes (LREs).*

In summary, with our research, we aim to clarify the mechanisms associated with intestinal homeostasis and CRC at different levels:

- I Characterize the role of smoothelin-like2 (smtnl2) in the reorganization of the cytoskeleton and the progression of carcinoma in intestinal tissues.

- II Characterize the role of neonatal lysosome-rich enterocytes (LRE) in the early stages of development and metabolism.

- III Characterize the metabolic crosstalk among microbiota, epithelial cells, and immune cells controlling intestinal homeostasis.

- IV Analysis of epithelialization of stem cells in embryonic and intestinal lumen formation in health and disease.

In summary, combining these gut-on-a-chip models with *in vivo* physiological systems will offer new ways to understand gut morphogenesis, patterning, homeostasis, and its relationship to human diseases such as cancer. Moreover, to translate this basic research into possible studies on tumour progression, our research will be transferred to clinical research by analyzing the expression of these proteins in human epithelial tumours in collaboration with the Ramón y Cajal Hospital Institute for Health Research (IRYCIS-Madrid).

Participation in projects

» Mecanismos moleculares de formación, diferenciación y homeostasis de túbulos intestinales y su relación con patologías humanas. PI: Fernando Martin-Belmonte. Ministerio de Ciencia e Innovación (PID2020-120367GB-I00). 09/2021 -08/2024.

» Understanding the role of CyclinB1-NuMA interaction in spindle orientation and epithelial morphogenesis (NuMA_CyclinB1_EM). PI: Fernando Martin-Belmonte. Granting Body: EU (H2020-MSCA-IF-2019-897948). 09/2022 -08/2024.

List of publications

» Herranz G, Martín-Belmonte F. (2022) Cadherin-mediated adhesion takes control. EMBO J. 41(24):e112662. doi: 10.15252/embj.2022112662.

» Naß J, Koerdt SN, Biesemann A, Chehab T, Yasuda T, Fukuda M, Martín-Belmonte F, Gerke V. (2022) Tip-end fusion of a rod-shaped secretory organelle. Cell Mol Life Sci. 79(6):344. doi: 10.1007/s00018-022-04367-2.

» Alfonso-Pérez T, Baonza G, Herranz G, Martín-Belmonte F. (2022) Deciphering the interplay between autophagy and polarity in epithelial tubulogenesis. Semin Cell Dev Biol. 131:160-172. doi: 10.1016/j.semcdb.2022.05.015.

» Manzano AR, Martín-Belmonte F. (2022) Actomyosin fibers DApPLE epithelial apical junctions. J Cell Biol. 221(5):e202203035. doi: 10.1083/jcb.202203035.

» Hachimi M, Grabowski C, Campanario S, Herranz G, Baonza G, Serrador JM, Gomez-Lopez S, Barea MD, Bosch-Fortea M, Gilmour D, Bagnat M, Rodriguez-Fraticelli AE, Martin-Belmonte F. (2021) Smoothelin-like 2 Inhibits Coronin-1B to Stabilize the Apical Actin Cortex during Epithelial Morphogenesis. Curr Biol. 31(4):696-706.e9. doi: 10.1016/j.cub.2020.11.010.

» Baonza G, Herranz G, Martin-Belmonte F. (2021) Intercalate or invaginate: PI(3,4,5)P3 governs a membrane constriction switch in cell shaping. Dev Cell. 56(18):2542-2544. doi: 10.1016/j.devcel.2021.09.006.

» Alfonso-Pérez T, Baonza G, Martin-Belmonte F. (2021) Breast cancer has a new metabolic Achilles' heel. Nat Metab. 3(5):590-592. doi: 10.1038/s42255-021-00394-8.

» Bosch-Fortea M, Martín-Belmonte F. (2021) Methods to Generate Tube Micropatterns for Epithelial Morphogenetic Analyses and Tissue Engineering. Methods Mol Biol.;2179:227-242. doi: 10.1007/978-1-0716-0779-4_18.

Other activities

» Associated editor of Nephron (Journal) (2015-2022); Scientific panel of French agency of research (ANR) (Cell Biology, Development and Evolution, from 2020); Master professor: Coordination of sorting, routing and distribution of proteins in polarized cells, Universidad Autonoma de Madrid; Molecular Biology of the Cell course, Institute Pasteur, Paris; Cytoskeleton course at Institute Curie, Paris; Cell adhesion and migration, Universidad Autonoma de Madrid.

METABOLISM AND B CELL FUNCTION

Group Members

Principal Investigator:
Nuria Martínez Martín

Predoctoral Fellow:
Marta Iborra Pernichi
Jonathan Ruiz
Belén Seco (Oct 2022-)

Technician:
María Velasco de la Esperanza

Master student:
Laura Díaz (Feb 2021-Jun 2021)
Javier Velázquez (Feb 2021-Jun 2021)
Belén Seco (Feb 2022-Sept 2022)

www.cbm.csic.es/nmartinez

Summary

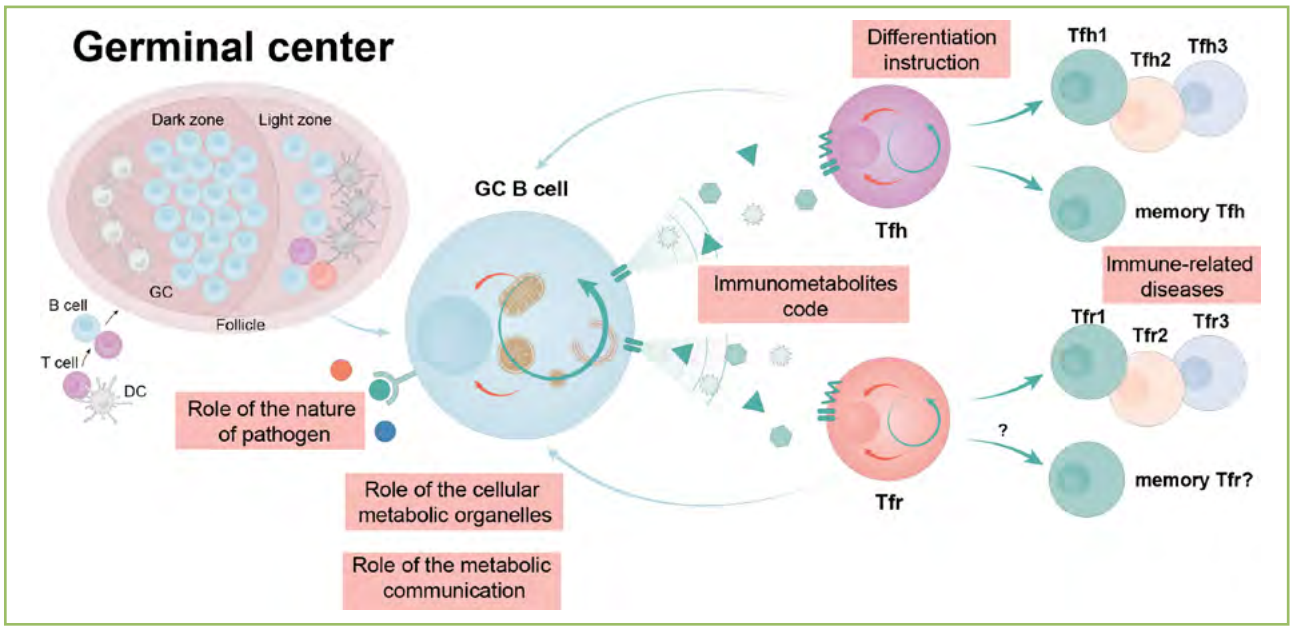
In our organism, infection leads to the orchestration of a fine-tuned immune response. This response relies on the activation of several immune cells; among them, activation of B lymphocytes is crucial, giving place to the production of specific high-affinity antibody-producing cells (plasma cells, PC) 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 state-of-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.

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.



Study of the cell-intrinsic and paracrine mechanism of B cell metabolism during the immune response.

List of publications

» Iborra-Pernichi M, Ruíz J, Martínez-Martín N. Quantification of intracellular ATP content in ex-vivo GC B cells. 2022. *Accepted in Methods in Molecular Biology.*

Participation in projects

» ¨Deciphering how B cell metabolism shapes germinal centre shutdown and its relation with immune system disorders¨. National Research Agency (AEI). (PID2021-126298OB-100), PI : Nuria Martínez Martín. 2022-2025.

» ¨Desentrañar un mecanismo paracrino para la homeostasis intestinal regido por ¨huellas digitales de la autofagia¨. ¨Europa Investigación¨. National Research Agency (AEI). (EIN2020-112225). PI : Nuria Martínez Martín. 2020-2022.

» ¨ Finetuning of autophagy nature in intestinal homeostasis and its relationship to inflammatory bowel diseases¨. National Research Agency (AEI). (RTI2018101586AI00). PI : Nuria Martínez Martín. 2019-2022.

SYSTEMS BIOLOGY

Group Members

Principal Investigator:
David G. Míguez

Postdoctoral fellows:
Mario Ledesma Terrón

Predoctoral fellows:
Ahmed Fayad
Diego Pérez Dónes

Technicians:
Ainara Ballesteros Losa

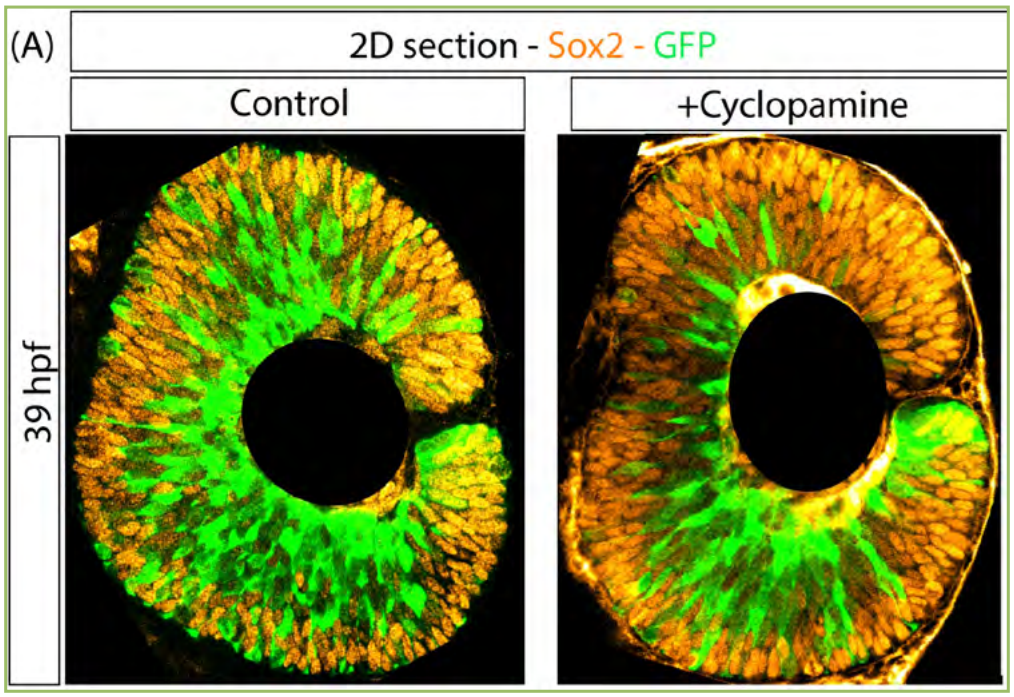
Undergraduate and Master Students:
Diego Mazo Durán

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

Summary

The cellular machinery is governed by interacting proteins, genes and metabolites that form complex and highly interconnected networks of interactions. This way, extracellular stimuli triggers pathways of biological events that regulate gene expression, protein activity, and ultimately, cell response. The architecture of these signaling cascades is highly nonlinear, integrating multiple layers and loops of feedback and feedforward regulation. These nonlinearities strongly affect the dynamics of activation and de-activation of the signaling cascades, inducing emerging properties such as bistability, oscillations or ultra-sensitivity.

To understand cellular decisions, it is not sufficient to understand the function of each of the proteins in a pathway, and a deep understanding of the consequences of the nonlinear wiring of the pathway is required. We use *in vivo* experiments and theoretical approaches to understand how the wiring of the pathways affects the role of the proteins that regulate these decision, in the context of balance between proliferation and differentiation of stems cells during neurogenesis.



Sections of developing zebrafish retinas stained with Sox2 (orange) and Atho5 (green) for contron (left) and treatment with the Hh inhibitor Cyclopamin (right)

List of publications

» Hernández-Del-Valle, M., Valencia-Expósito, A., Gorfinkiel, N., Martín-Bermudo, M.D., Míguez, D.G., (2022). Analysis of Actomyosin Oscillatory Dynamics Using a Coarse-Grained Model. Front. Phys. 10, 881384. doi:10.3389/fphy.2022.881384

» Hernández-del-Valle, M., Valencia-Expósito, A., López-Izquierdo, A., Casanova-Ferrer, P., Tarazona, P., Martín-Bermudo, M.D., Míguez, D.G., (2022). A coarse-grained approach to model the dynamics of the actomyosin cortex. BMC Biol 20, 90. doi:10.1186/s12915-022-01279-2

» Míguez, D.G., Iannini, A., García-Morales, D., Casares, F., (2022). The effects of Hh morphogen source movement on signaling dynamics. Development dev.199842. doi:10.1242/dev.199842

» Pérez-Dones, D., Ledesma-Terrón, M., Míguez, D.G., (2021). Quantitative Approaches to Study Retinal Neurogenesis. Biomedicines 9, 1222. doi:10.3390/biomedicines9091222

Participation in projects

» "Cuantificacion del papel de la via de señalizacion tgfbeta en la regulacion de la neurogenesis de vertebrados" RTI2018-096953-B-I00. Plan Nacional I+D+i Ministry of Science of Spain. PI: David Míguez. 2019-2022.

Doctoral theses

» Mario Ledesma Terrón (2021). A systems biology study to understand the dynamics of progenitor cells populations in the tissue development, Universidad Autónoma de Madrid. Director David G Míguez.

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

Group Members

Principal Investigators:
Mar Ruiz Gómez (PI)
Joaquim Culí Espigul (co-PI)

"Ad Honorem" Professor:
Juan Modolell Mainou

Scientific Staff:
Sonsoles Campuzano Corrales

Postdoctoral fellows:
Marta Carrasco Rando

Predoctoral fellows:
Vicente Castillo Mancho

Technicians:
Raúl Somavilla Cabrero (from May 2021)

Master Students:
Jorge Sarmiento Jiménez (2021)

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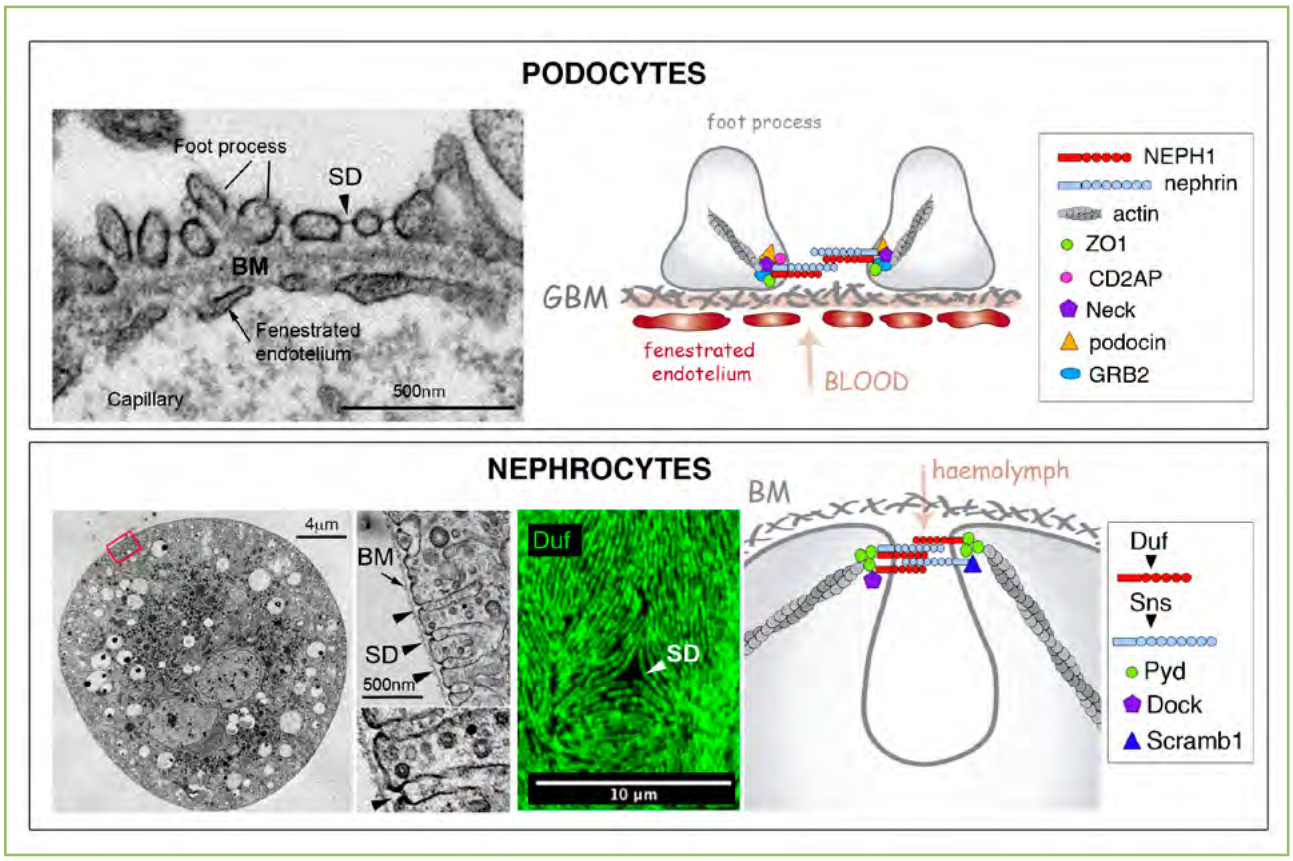
Summary

The slit diaphragm (SD) is a modified cell junction, present in excretory cells from invertebrates to humans, which works as a molecular filter during the process of haemolymph/plasma ultrafiltration. SDs are a major target of injury leading to chronic kidney disease (CKD), a prevalent pathology whose incidence is globally increasing despite the efforts invested in improving standard treatments and implementing state-of-the-art clinical resources. The major reason being the scarcity of early markers of kidney damage, which has hampered CKD early detection and its effective prevention and/or treatment. Hence, the challenge and the main goal of our research is to advance in understanding the molecular mechanisms involved in SD formation and stability, by identifying novel SD components and dissecting the major signalling events that regulate SD dynamics in normal and pathological conditions. These studies should facilitate the detection of novel therapeutic targets, thus helping to reach an early CDK diagnosis. Due to the conservation of SDs across species, in the laboratory we opted to combine the use of two model organisms: *Drosophila melanogaster* and the zebrafish *Danio rerio* to achieve these main goals. First, we take advantage of the versatility of the genetic techniques used and the ease of obtaining transgenic animals quickly and cheaply in *Drosophila*, to identify novel SD components and regulators in nephrocytes, the fly excretory cells involved in haemolymph ultrafiltration, which our previous work and that of other researchers has proved to be a suitable model to study nephropathies and SD dynamics. And subsequently, we validate the novel genes and proposed mechanisms in vertebrates using the zebrafish.

In the last two years we have focused in dissecting the requirement of the endocytic receptor CUBAM for the correct positioning of SDs, suggesting its possible and conserved role in SD recycling (Atienza-Manuel et al., 2021) and in the functional characterisation of a novel gene, *scramb1*, which encodes a phospholipid scramblase essential for the assembly of the nephrocyte SD. Our studies revealed that Scramb1 is a component of the fly SD interactome, required for the recruitment of a main structural SD protein, that Scramb1 activity is regulated by Ca^{2+}, and suggest that the correct targeting of Scramb1 to lipid raft microdomains is critical for SD assembly. We have also characterised the Scramb1 interactome by immunoprecipitation under native conditions and the identification of its interactants by mass spectrometry techniques, and are now validating these interactants.



TEM micrographs, immunostainings and schemes, showing the location and composition of the slit diaphragm (SD) in vertebrate podocytes and Drosophila nephrocytes

List of publications

» Atienza-Manuel, A., Castillo-Mancho, V., De Renzis, S., Culi, J. and Ruiz-Gómez, M. (2021) Endocytosis mediated by an atypical CUBAM complex modulates slit diaphragm dynamics in nephrocytes. Development 148, dev199894. doi: 10.1242/dev.199894

» Carrasco-Rando, M. and Ruiz-Gómez, M. (2022) Desarrollo embrionario del riñón. In: Hernando. Nefrología Clínica, 5ª Ed.; Madrid; Editorial Médica Panamericana, pp. 27-37.

Participation in projects

» Building a renal slit diaphragm: a developmental and proteomic approach. MICINN. PID2019-105492GB-I00. (2020-23). PI: M. Ruiz-Gómez & J. Culi.

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" (2022).

SEGMENTAL SPECIFICATION AND PATTERN FORMATION IN *DROSOPHILA*

Group Members

Principal Investigator:
Ernesto Sánchez-Herrero Arbide

Postdoctoral Fellow:
David Foronda Alvaro

Technicians:
María Paloma Martín Fernández
Daniel Tovar Manzano (since October 2022)

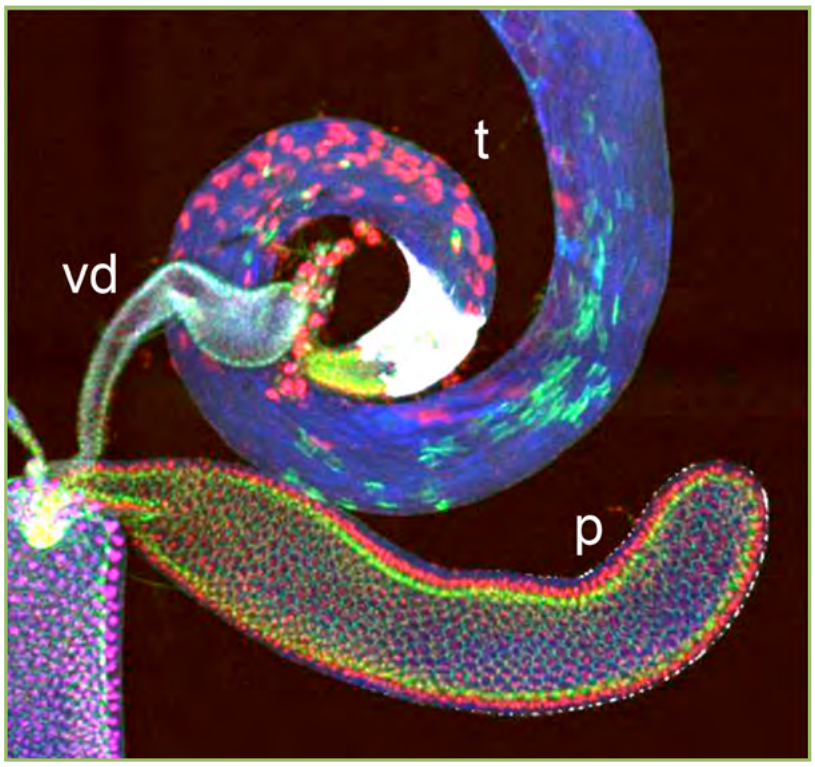
Undergraduate and Master students:
Juan Lombardo Hernández (until June 2021)
Eduardo Cazalla Ibáñez (until June 2021)
Marina Blanco Rubio (October 2021 - June 2022)
Lorena Rico García (October 2021 - June 2022)
Blanca López Arrabal (from September 2022)
Sergio Palomino Gálvez (from September 2022)

http://www.cbm.uam.es/ernesto_sanchez-herrero

Summary

The Hox genes are a group of genes required for the specification of the antero-posterior axis in bilaterians. We have studied in *Drosophila* the role of the Hox gene Abdominal-B (Abd-B) in the development of the posterior abdomen and in testes development. The seventh abdominal segment (A7) of the *Drosophila* male requires Abd-B and also the activity of the Hox cofactors Extradenticle (Exd) and Homothorax (Hth). Hth is needed to maintain Abd-B expression in this segment and Abd-B can repress exd and hth when expressed at high levels. A domain N-terminal to the homeodomain, the DNA-binding domain of Hox proteins, contains a tryptophane conserved in all Hox proteins that contacts the Exd protein. Although we have shown by Bifluorescence Complementation that Exd and Abd-B physically interact, the tryptophane is not required for this interaction or for Abd-B activity. The change of this aminoacid to an Alanine, however, increases Abd-B activity, as do other changes in the Abd-B protein, especially modifications in a sequence just C-terminal to the homeodomain.

The male gonads show a spherical shape in larva but elongate and coil during pupal stages to achieve their adult size and shape. This change depends on the interaction between the testis and muscle cells from the genital disc, a structure that develops at the back of the larva. These muscle cells migrate and surround the gonad in pupa, and at the same time the testis elongate and coil. We have found that Abd-B is essential for the migration of muscle cells from the genital disc and the elongation of testes. This elongation also requires the activity of the Notch, FGF and the sex determination pathways. Abd-B codes for two proteins, both required for testes development. The Abd-BM protein is expressed in the testes, and needed autonomously for their initial short elongation, and also expressed in the genital disc, and required there non-autonomously for the posterior major elongation and coiling of the testes. The Abd-BR protein is expressed in the genital disc and needed for the contact between genital disc and testes. Abd-B also regulates the dextral direction of coiling by controlling the expression, in the testes, of MyosinID. In the absence of this Hox gene testes can coil dextrally, sinistrally or do not coil. These results show that Abd-B is a key gene for the elongation and dextral coiling of *Drosophila* testes.



Detail of a testis (t), vas deferens (vd) and paragonia (p) of a Drosophila male stained with tropomyosin (white), Abdominal-B (blue), phalloidin (green) and Topro (red).

List of publications

» Eden W. McQueen, Mehrnaz Afkhami, Joel Attalah, John M. Belote, et al. (2022). A standardized nomenclature and atlas of the female terminalia of *Drosophila melanogaster.* Fly, 16, 128-151, doi: 1080/19336934.2022.2058309.

» Marie Kmita, Edwina McGlinn and Ernesto Sánchez-Herrero (2022). Editorial: Mechanisms of hox-driven patterning and morphogenesis . Front. Cell Dev. Biol. 10:992341, doi: 10.3389/fcell.2022.992341.

Participation in projects

» "Study of the activity of *Drosophila melanogaster* Hox genes in organ development"/ PID2020-113318GB-I00. PI: Ernesto Sánchez-Herrero Arbide / October 2021 - October 2024.

TRANSCRIPTIONAL CONTROL OF SEXUAL DIFFERENTIATION OF THE NERVOUS SYSTEM

Group Members

Principal Investigator:
Esther Serrano-Saiz

Predoctoral fellows:
Rafael Casado-Navarro
Ana Bermejo-Santos

Technician:
Rodrigo Torrillas de la Cal

Undergraduate and Master Students:
Miguel Rubio
Julia Berges
Jose Ignacio Gómez Blanco
(co-directed with Dr. Claudio Toma)

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 are approaching this question using genetic and genomic techniques in mice. We propose that DMRTs control sex-specific neuron identities and numbers, connectivity and constriction of sexual transcriptional differences in cooperation with sex-linked factors and sex-specific splicing mechanisms. We are also interested in conserved *DMRT* functions in humans and how specific *DMRTs* genetic variants could contribute to the onset and sexual bias of psychiatric disorders. Our work will unravel novel principles of brain sexual differentiation and will generate mouse models to better understand genetic mechanisms that either afford protection or generate vulnerability in the etiology and sexual bias of mental disorders.

The gates of the rodent behavior (credit Rafael Casado-Navarro).

List of publications

» Casado-Navarro R and Serrano-Saiz E. (2022). DMRT transcription factors in the control of nervous system sexual differentiation. Front in Neuroanat. 16:937596. doi: 10.3389/fnana.2022.937596.

» Martín-Fernández F, Bermejo-Santos A, Bragg-Gonzalo L, Briz CG, Serrano-Saiz E, Nieto M. (2022). Role of Nrp1 in controlling cortical inter-hemispheric circuits. Elife. 11:e69776. doi: 10.7554/eLife.69776.

» Serrano-Saiz E, Isogai Y. Single-cell molecular and developmental perspectives of sexually dimorphic circuits underlying innate social behaviors. (2021). Curr Opin Neurobiol. 68:159-166. doi: 10.1016/j.conb.2021.03.010.

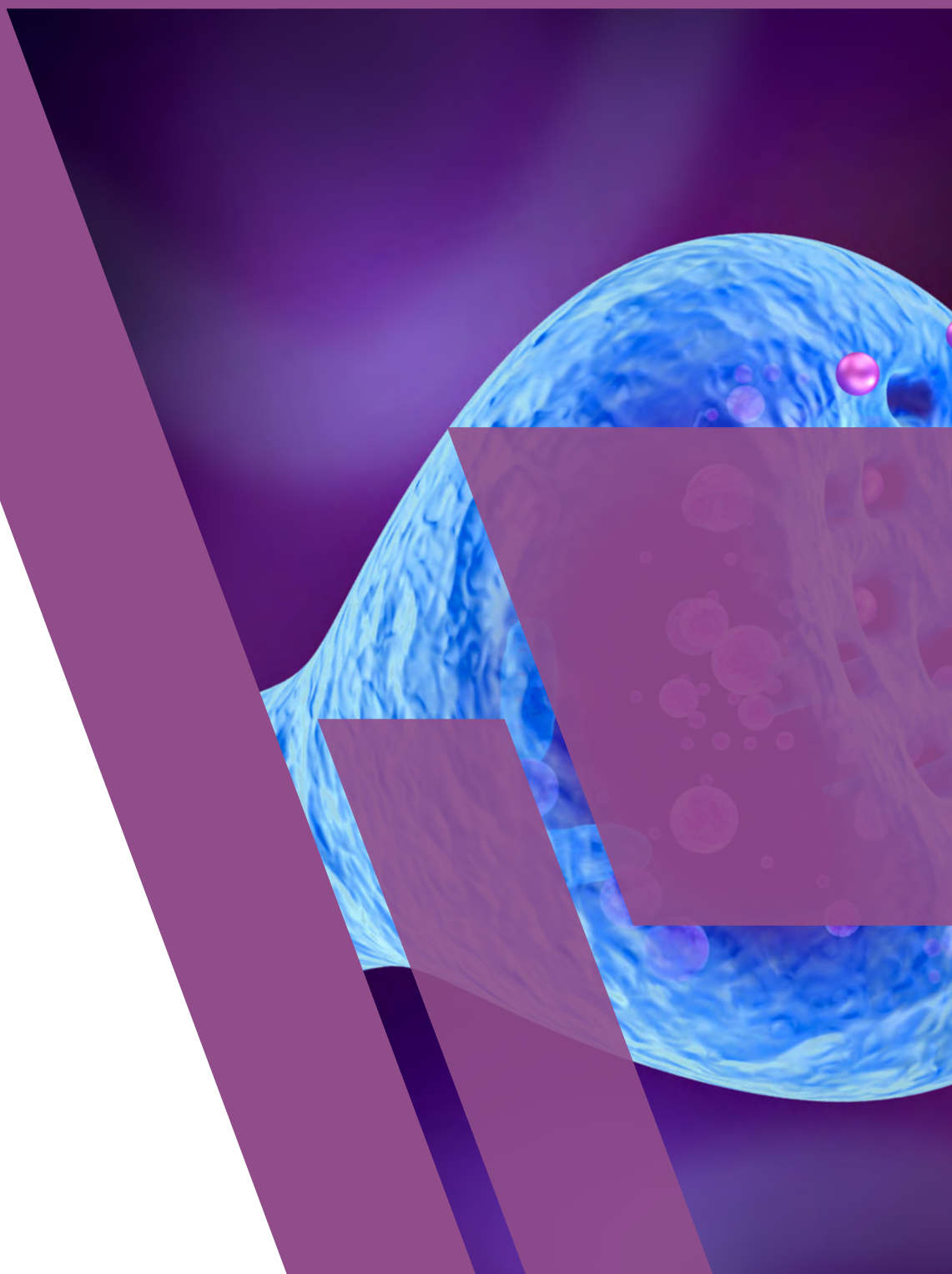
Participation in projects

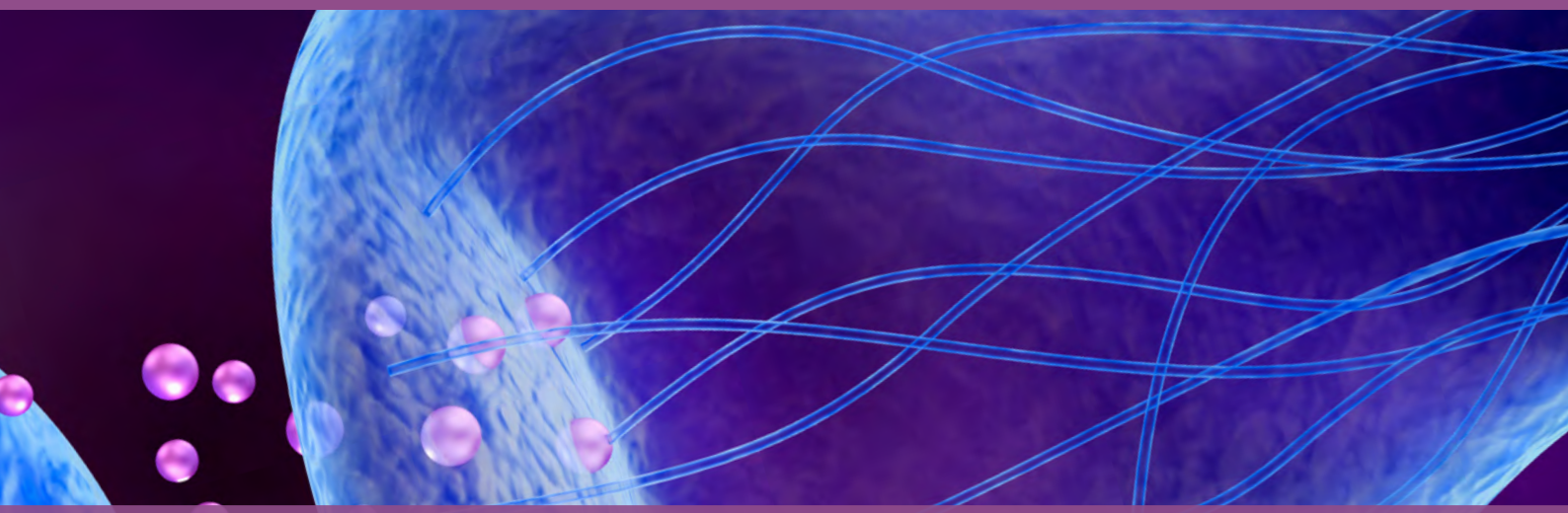
» Transcriptional control of sexual differentiation across the mouse nervous system by Dmrt2, Dmrt3 and Dmrt5 (DMRTSexDif). (2022-2025) PID2021-127235NB-I0. PI: Esther Serrano Saiz.

» Combined genetic and functional studies to establish the role of DMRTA1 and DMRTA2 in susceptibility to attention deficit-hyperactivity disorder and its sexual skewing. (2022-2024) PI: Esther Serrano Saiz, Co-PI: Claudio Toma. Fundación Alicia Koplowitz.

» ReDevNeural "An integrative approach to understand the logic of neural development". RED2018-102553-T (AEI). (2020-2022) Research Network. Coordinator: Paola Bovolenta. 12 PIs, including Esther Serrano-Saiz

PROGRAM





Physiological and Pathological Processes

Physiological and Pathological Processes

Molecular Neuropathology Unit

Beatriz López Corcuera

The scientific interest of the investigators at the Molecular Neuropathology Unit, includes basic research aimed at understanding the detailed mechanisms of healthy brain function and its deviations causing human nervous system disorders. The scientific expertise of the members of our unit covers all areas of neurobiology, including genetics, biochemistry, cell and molecular biology, electrophysiology, animal behavior, and computational biology. We count with expert technical staff driving cutting edge facilities that allow our researchers to undertake studies at different levels including molecular, cellular -both neuronal and non-neuronal- as well as circuit levels, which are aimed to generate knowledge connecting brain activities to whole brain function leading to complex behavior. Hence, our recent research has made valuable contributions addressing fundamental neurobiological questions of brain physiology, but above all, we emphasize on the transfer to the clinics and to the pharmaceutical and biotechnology industry the scientific information we obtain. Some key contributions from the last two years are:

- The discovery of a new tau isoform raised by intron retention (Avila's/Hernandez´s group).
- The finding of cholesterol metabolism alterations in peripheral blood cells of Alzheimer's disease patients as a potential prodromal indicator of the disease (Bullido´s group).
- The contribution of R-Ras function loss to hypomyelinating diseases and the generation of Ras deficient mice as models for the study of myelin pathologies (Cubelos´group).
- The establishing an in vitro model reproducing critical periods of brain development, which could be manipulated to counteract the synaptic dysfunction in the adult life (Díez-Guerra´s group).
- The identification of ageing-related changes in brain membrane lipids and receptors leading to increased responses to survival and decreased responses to learning and memory stimuli (Dotti´s group).
- The elucidation of signaling cascades that link synaptic plasticity with brain development and its alteration by some environmental contaminants (Esteban´s group).

» The discovery of sphingomyelin and cholesterol regulatory role on glucocorticoid and endocannabinoid systems, and on glutamate-mediated synaptic plasticity. The contribution of alterations of these lipids to the psychiatric condition in Niemann Pick diseases. Launch of a clinical trial reducing cholesterol in Niemann Pick type C patients (Ledesma´s group).

» The detection of neural stem cells in the adult human hippocampus, and the discovery of specific adult hippocampal signatures in patients with distinct neurodegenerative diseases such as ALS, Huntington´s and Parkinson´s diseases, Dementia with Lewy bodies, and frontotemporal dementia (Llorens-Martín´s group).

» The identification of glycine transporter 2 variants found in hyperekplexia patients as amenable to rescue from its trafficking defect by chemical chaperones. The discovery of transporter regulation by signaling cascades involved in brain development (López-Corcuera´s group).

» The discovery of altered RNA polyadenylation of the thiamine transporter and thiamine deficiency in Huntington's disease patients that originated a clinical trial to explore a thiamine supplementation therapy (Lucas´s group).

» The neurobiology of stem cells and their performance as regenerative and replacement therapy in experimental Parkinson's disease (Pereira´s group).

» The establishment of a new ideal approach to pinpoint new loci and pathways implicated in bipolar disorder. The foundation of Madrid Manic Group (MadManic), which provides phenotypic records and biospecimens for bipolar disorder (Toma´s group).

» The identification of the mTORC1 element as a central regulator of metabolism in Alzheimer's disease (Wandosell´s group).

» The role of several neurotransmitter transporters and sodium channels in the control of neuronal excitability and its dysfunction in various neurological diseases (Zafra´s group).

Understanding the laws that govern these aspects is crucial for pursuing and revealing the fundamental principles and processes underlying memories, learning, thoughts and complex behaviors.

Jesús Avila / Félix Hernández
TAU FUNCTION AND DYSFUNCTIONS IN ALZHEIMER DISEASE

María Jesús Bullido Gómez-Heras
PATHOGENIC MECHANISMS OF ALZHEIMER'S DISEASE

Beatriz Cubelos
MOLECULAR MECHANISMS OF OLIGODENDROCYTE-NEURON INTERACTION AND MYELIN PATHOLOGIES

Javier Díaz-Nido
PHYSIOPATHOLOGY AND THERAPY OF NEURODEGENERATIVE DISEASES: FRIEDREICH'S ATAXIA

Fco. Javier Díez Guerra
MOLECULAR BASES OF NEURONAL PLASTICITY

Carlos Dotti
SURVIVAL AND PLASTICITY IN THE AGING BRAIN

José Antonio Esteban García
MECHANISMS OF SYNAPTIC PLASTICITY, AND CONTRIBUTION TO COGNITIVE FUNCTION

María Dolores Ledesma Muñoz
LIPIDS IN NEURONAL PHYSIOLOGY AND PATHOLOGY

Beatriz López Corcuera
PHYSIOPATHOLOGY OF GLYCINE TRANSPORTERS IN GLYCINERGIC NEUROTRANSMISSION: HYPEREKPLEXIA AND PAIN

José J. Lucas
MOLECULAR BASIS OF HUNTINGTON'S DISEASE AND OTHER CENTRAL NERVOUS SYSTEM DISORDERS

María Llorens-Martín
ADULT NEUROGENESIS AND NEURODEGENERATIVE DISEASES

Marta P. Pereira
HUMAN STEM CELL BIOLOGY IN TRANSLATIONAL NEUROSCIENCE

Eva Porlan
NEURAL STEM CELLS IN THE ADULT BRAIN: INTRINSIC AND EXTRINSIC FACTORS THAT REGULATE THEIR SELF-RENEWAL AND DIFFERENTIATION

Claudio Toma
THE GENETICS OF PSYCHIATRIC DISEASES

Francisco Wandosell Jurado
MOLECULAR MECHANISMS OF NEURODEGENERATION

Francisco Zafra
MOLECULAR BASIS OF NEUROTRANSMISSION AND ITS IMPLICATION IN NEUROPATHOLOGY

TAU FUNCTION AND DYSFUNCTIONS IN ALZHEIMER DISEASE

Group Members

Principal Investigators (PI, co-PI):
Jesús Avila de Grado,
Félix Hernández Pérez

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Summary

Tau is mainly a neuronal protein that could be involved in several cellular functions in addition of being a microtubule associated protein. At the present, we are analyzing those functions and we continue with the study of the possible role of tau in Alzheimer disease (AD) and other tauopathies. During 2021-2022, we have tested: a) the presence of tau in non-neuronal cells, b) the presence of new tau isoform and c) we have started to look for a possible specific role of tau in neuronal aging, since aging is the main risk factor for Alzheimer disease.

About the presence of tau in non-neuronal cells, we have found that tau could be present in microglia cells, being that presence the consequence of the endocytosis of extracellular (neuronal) tau into microglia cells. Also, we have found that the effect of tau in microglia cells results in the activation of p38 kinase that could be toxic for the cell. In that way, inhibition of p38 kinase decreases tau toxicity in microglia cells and improves microglia phagocytic function. Additionally, tau expression was found in kidney cells playing, this kidney tau, a role in podocyte architecture.

A new tau isoform raised by intron 12 retention has been described. This new tau isoform contains an extra 16aa peptide, only present in humans, with two tryptophan residues and we named it as W-Tau isoform. Looking at W-Tau isoform, we have found that it has a low capacity for self-aggregation, unlike of the previously known human tau isoforms. Also, W-Tau isoform could prevent the aggregation of the other tau isoforms. In vitro experiments, using the 16aa peptide, specific for W-Tau, have indicated that such peptide could prevent not only tau self-assembly but also beta amyloid peptide aggregation.

During this period, we have studied aging at the dentate gyrus (DG) and hippocampal region of old mice, since DG is one of the regions that are earlier affected in AD. Previously, we described, *in vivo*, that aging features in DG from old mice could be ameliorated expressing the so-called Yamanaka Factors (YF). Now, we are looking for more simple factors that could replace the action of YF and starting to look for a specific tau function in neuronal aging.

Finally, our group has done several collaborations, located inside and outside of the CBMSO, in aspects related to Alzheimer disease and other neurodegenerative disorders like aging.

List of publications

» Anton-Fernandez A, Valles-Saiz L, Avila J, Hernandez F (2022) Neuronal nuclear tau and neurodegeneration. Neuroscience S0306-4522: 00368-00362 Doi 10.1016/j.neuroscience.2022.07.015

» Avila J, Perry G (2021) A Multilevel View of the Development of Alzheimer's Disease. Neuroscience 457: 283-293 Doi 10.1016/j.neuroscience.2020.11.015

» Cuadros R, Perez M, Ruiz-Gabarre D, Hernandez F, Garcia-Escudero V, Avila J (2022) Specific Peptide from the Novel W-Tau Isoform Inhibits Tau and Amyloid beta Peptide Aggregation In Vitro. ACS Chem Neurosci 13: 1974-1978 Doi 10.1021/acschemneuro.2c00188

» Draffin JE, Sanchez-Castillo C, Fernandez-Rodrigo A, Sanchez-Saez X, Avila J, Wagner FF, Esteban JA (2021) GSK3alpha, not GSK3beta, drives hippocampal NMDAR-dependent LTD via tau-mediated spine anchoring. EMBO J 40: e105513 Doi 10.15252/embj.2020105513

» Garcia-Escudero V, Ruiz-Gabarre D, Gargini R, Perez M, Garcia E, Cuadros R, Hernandez IH, Cabrera JR, Garcia-Escudero R, Lucas JJet al (2021) A new non-aggregative splicing isoform of human Tau is decreased in Alzheimer's disease. Acta neuropathologica 142: 159-177 Doi 10.1007/s00401-021-02317-z

» Hernandez F, Ferrer I, Perez M, Zabala JC, Del Rio JA, Avila J (2022) Tau Aggregation. Neuroscience S0306-4522: 00220-00222 Doi 10.1016/j.neuroscience.2022.04.024

» Perea JR, Bolos M, Cuadros R, Garcia E, Garcia-Escudero V, Hernandez F, McManus RM, Heneka MT, Avila J (2022) p38 Inhibition Decreases Tau Toxicity in Microglia and Improves Their Phagocytic Function. Mol Neurobiol 59: 1632-1648 Doi 10.1007/s12035-021-02715-0

» Perea JR, Garcia E, Valles-Saiz L, Cuadros R, Hernandez F, Bolos M, Avila J (2022) p38 activation occurs mainly in microglia in the P301S Tauopathy mouse model. Sci Rep 12: 2130 Doi 10.1038/s41598-022-05980-8

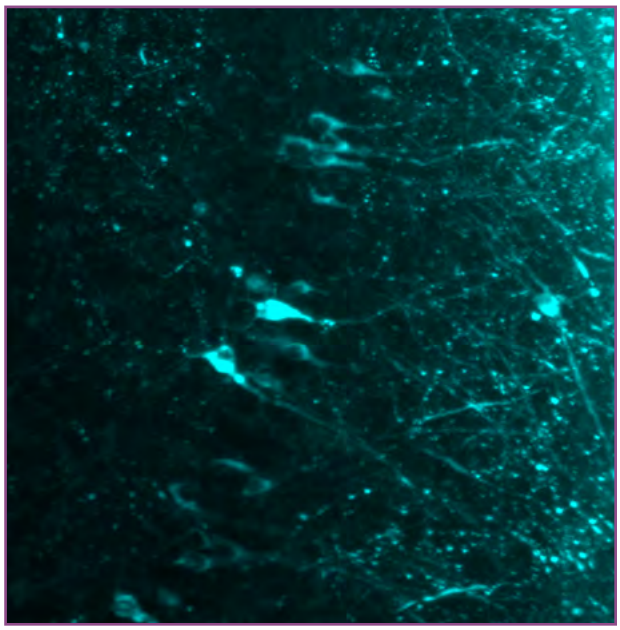
» Sayas CL, Avila J (2021) GSK-3 and Tau: A Key Duet in Alzheimer's Disease. Cells 10: Doi 10.3390/cells10040721

» Valles-Saiz L, Peinado-Cahuchola R, Avila J, Hernandez F (2022) Microtubule-associated protein tau in murine kidney: role in podocyte architecture. Cell Mol Life Sci 79: 97 Doi 10.1007/s00018-021-04106-z

Awards

» Mayo 2022, Jesús Avila, Académico Correspondiente de Bioquímica y Biología Molecular, Real Academia Nacional de Medicina.

» Premio Medalla Margarita Salas a la mejor trayectoria en supervision investigadora, CSIC 2022



Using "Clarity methodology", we have detected the presence of tau in the brain cortex of a FTD model of tau (tau P301S) by staining tau protein with abAT8.

Participation in projects

» Part of the Networking Research Center on Neurodegenerative Diseases (CIBERNED).

» PID2020-113204GB-I00: Neuroregeneración en la enfermedad de Alzheimer a través de la expression de factores pluripotencia *in vivo*. IP: Félix Hernández

» PID2021-123859OB-I00: Funciones de las Isoformas de Tau. Ministerio de Ciencia e Innovación. IP: Jesús Avila

Doctoral theses

» Laura Vallés Saiz (2022). Nuevas funciones para la proteína tau en el sistema nervioso central y tejidos periféricos. Félix Hernández. Universidad Autónoma de Madrid.

Patents

» Jesús Avila, José J Lucas, Raquel Cuadros, Esther García-García, Vega García-Escudero, Félix Hernández, Daniel Ruiz-Gabarre. A new non-aggregative splicing isoform of human Tau is decreased in Alzheimer's disease. Application N.: EP21382283.6 Title holder: CSIC. Priority country: Spain Priority date: 05 April 2021

PATHOGENIC MECHANISMS OF ALZHEIMER'S DISEASE

Group Members

Principal Investigator:
María Jesús Bullido Gómez-Heras

Scientific Staff:
Jesús Aldudo Soto
María Recuero Vicente

Technicians:
Isabel Sastre Merlín (co-IP José Férnandez Piqueras)

Predoctoral Fellows:
Blanca Salgado Fuentes (October 2022)

Undergraduate and Master Students
Jaime Morales García (until September 2021)
Víctor Mejías Pérez (until September 2021)
Blanca Salgado Fuentes (November 2021-September 2022)
Beatriz Izquierdo Alarcón (February-June 2021)
Daniel González Díaz (February-June 2022)

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

Summary

We continue the study of mechanisms mediating the neurodegenerative cascade leading to Alzheimer's disease (AD) in neuronal cell models. Based on previous results of degeneration induced by herpes simplex 1 virus (HSV-1), we are focused on the endo-lysosomal pathway and cholesterol metabolism as major functions mediating HSV-1 induced, AD-like neurodegeneration.

To expand the screening of these pathways, we have recently started to develop 2D and 3D neural models derived from the human progenitors LUHMES and ReN cell lines. We have been able to obtain different neural types (oligodendrocytes, astrocytes and neurons), and proved the ability of both progenitors and differentiated cells to resemble an AD-like phenotype that includes intracellular accumulation of Aβ and hyperphosphorylated tau, inhibition of Aβ secretion, alterations in autophagy-lysosomal pathway and changes in cholesterol levels, following HSV-1 infection. These preliminary results pave the way for the development of interesting research platforms, in which we plan to validate candidate genes like LAMP2 and MMP14 and to deepen our knowledge about the link between cholesterol and lysosome alterations and the AD like neurodegeneration induced by HSV-1.

To evaluate the translational potential of findings obtained in the models, we are analyzing genes and biomarkers in samples of controls and AD patients at dementia and pre-dementia stages. Putting together the urgent need of peripheral biomarkers and the widely supported involvement of the immune system in AD, we have selected peripheral blood mononuclear cells (PBMCs) as the material to be screened. Measurement of PBMC free cholesterol levels in two independent case-control samples totaling 480 participants revealed decreased cholesterol content in patients from early, pre-dementia disease stages, as well as in control subjects bearing the APOE4 allele. We also detected increased CD16+ and decreased CD8+ cell percentages in patients with moderate or severe dementia. These results support the presence of changes in peripheral blood cells of AD patients and suggest that alterations of cholesterol metabolism in these cells could be prodromal events. In addition, they suggest a relationship between APOE genotype and cholesterol levels in PBMC, which could manifest even in healthy individuals and perhaps partly explain the involvement of APOE4 in AD.

Therefore, our studies in cell models and patients continues supporting that failures of lysosomal function and cholesterol homeostasis are relevant in neurodegeneration, with the candidates under study having the potential to be early biomarkers or pharmacological targets for AD.

List of publications

» Martín-Montes A, Recuero M, Sastre I, Vilella E, Rosich-Estragó M, Atienza M, Cantero JL, Frank-García A, Bullido MJ. (2022) Cholesterol dysregulation in peripheral blood mononuclear cells of Alzheimer's disease. J Neuroimmunol. 15;373:577996. doi: 10.1016/j.jneuroim.2022.577996.

» Le Guen Y, Belloy ME, Grenier-Boley B, de Rojas I, Castillo-Morales A, Jansen I, Nicolas A, Bellenguez C, Dalmasso C, Küçükali F, et al. (2022) Association of Rare APOE Missense Variants V236E and R251G With Risk of Alzheimer Disease. JAMA Neurol. 79, 652-663. doi: 10.1001/jamaneurol.2022.1166.

» Bellenguez C, Küçükali F, Jansen IE, Kleineidam L, Moreno-Grau S, Amin N, Naj AC, Campos-Martin R, Grenier-Boley B, Andrade V, et al. (2022) New insights into the genetic etiology of Alzheimer's disease and related dementias. Nat Genet. 54, 412-436. doi: 10.1038/s41588-022-01024-z.

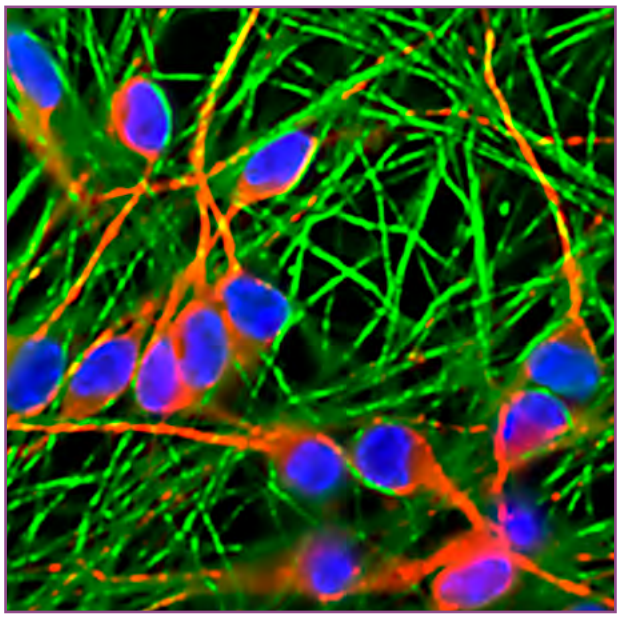
» de Rojas I, Hernández I, Montrreal L, Quintela I, Calero M, Royo JL, Huerto Vilas R, González-Pérez A, Franco-Macías E, et al. (2021) Genomic Characterization of Host Factors Related to SARS-CoV-2 Infection in People with Dementia and Control Populations: The GR@ACE/DEGESCO Study. J Pers Med. 11, 1318. doi: 10.3390/jpm11121318.

» de Rojas I, Moreno-Grau S, Tesi N, Grenier-Boley B, Andrade V, Jansen IE, Pedersen NL, Stringa N, Zettergren A, Hernández I, et al. (2021) Common variants in Alzheimer's disease and risk stratification by polygenic risk scores. Nat Commun. 12, 3417. doi: 10.1038/s41467-021-22491-8.

» Moreno-Grau S, Fernández MV, de Rojas I, Garcia-González P, Hernández I, Farias F, Budde JP, Quintela I, Madrid L, González-Pérez A, Montrreal L, Alarcón-Martín E, Alegret M, Maroñas O, Pineda JA, Macías J; GR@ACE study group; DEGESCO consortium, et al. (2021) Long runs of homozygosity are associated with Alzheimer's disease. Transl Psychiatry. 11, 142. doi: 10.1038/s41398-020-01145-1.

» Llorente P, Mejías V, Sastre I, Recuero M, Aldudo J, Bullido MJ. (2021) Matrix metalloproteinase 14 regulates HSV-1 infection in neuroblastoma cells. Antiviral Res. 192:105116. doi: 10.1016/j.antiviral.2021.105116.

» Kristen H, Sastre I, Aljama S, Fuentes M, Recuero M, Frank-García A, Martin A, Sanchez-Juan P, Lage C, Bullido MJ, Aldudo J. (2021) LAMP2 deficiency attenuates the neurodegeneration markers induced by HSV-1 infection. Neurochem Int. 146, 105032. doi: 10.1016/j.neuint.2021.105032.



LUHMES neurons on day 7 of differentiation labeled with MAP2 (red) and B-III tubulin (green) specific antibodies. DAPI-stained nuclei are also shown.

Networks, Consortia, International Proyects

» Networked Center of Biomedical Research. Neurodegenerative diseases -CIBERNED- (https://www.ciberned.es/grupos/grupo-de-investigacion?id=28805)

» Hospital la Paz Institute for Health Research - IdiPaz. (http://www.idipaz.es) Group "Neurology and cerebrovascular diseases", PI E Díez-Tejedor

» Dementia Genetics Spanish Consortium (DEGESCO). Participant group. (https://www.ciberned.es/plataformas/degesco)

» European Alzheimer's Disease BioBank & International Genomics Alzheimer's Project (IGAP). Associated group. (https://consortiapedia.fastercures.org/consortia/igap/)

National Proyects

» Cholesterol homeostasis and lysosome pathway in HSV-1 induced neurodegeneration and in Alzheimer's disease: Pathogenic mechanisms and biomarkers. PID2020-113921RB-I00. PI Bullido MJ & Frank-Garcia A. 01/09/2021-31/08/2024.

» Progressive Supranuclear Palsy: identification of susceptibility loci, implicated cellularity and molecular pathways for drug development. ISCIII CIBERNED. PI Bullido MJ. Rábano A; Sanchez-Juan P; Ruiz-Laza A (coord.). 01/01/2020-31/12/2022.

» Role of the lysosomal protein LAMP2 in the AD-like phenotype induced by HSV-1: Study of the LAMP2 interactome in virus infected neurons. Fundación Ramón Areces. PI Bullido MJ. 01/03/2017- 01/03/2021.

MOLECULAR MECHANISMS OF OLIGODENDROCYTE-NEURON INTERACTION AND MYELIN PATHOLOGIES

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

Group Members

Principal Investigator:
Beatriz Cubelos

Predoctoral fellows
Berta Alcover Sánchez (since 2019)
Gonzalo García Martín (since 2021)
Brian Ernie Quinones (since 2022)

Undergraduate and Master Students
Rosa Plaza Clavero (2022)
Andrea Alcaraz Ramírez (2022)
Gonzalo García Martín (2021)
Jorge Navarro Nadal (2021)

Summary

In our laboratory we study the neurological component of demyelinating pathologies and investigate the molecular mechanisms responsible for the processes of myelination in the Central Nervous System (CNS). Adequate myelination is essential for the correct transmission of the nerve impulse. In the CNS, oligodendrocytes (OLs) are the responsible cells for myelination of neuronal axons, through a complex process that requires multiple cellular interactions. In the absence of a correct myelination, diseases such as Multiple Sclerosis or leukodystrophies appear, currently orphans of an effective treatment. The possibility of generating therapies based on the neurological component of these diseases could stimulate the regeneration of new oligodendrocytes or increase the capacity of the remaining set of oligodendrocytes to produce more myelin and reestablish correct myelination.

Our group has demonstrated the importance of the GTPases R-Ras1 and R-Ras2, essential proteins in the differentiation and survival of OLs. Moreover, we have described the relevance of their presence for the maintenance of energetic homeostasis and for the correct functioning of the nerve impulse transmission. Models lacking R-Ras1 and/or R-Ras2 faithfully reproduce the symptomatological characteristics of myelin diseases and could be used as models for the development of new treatments based on the neurological component.

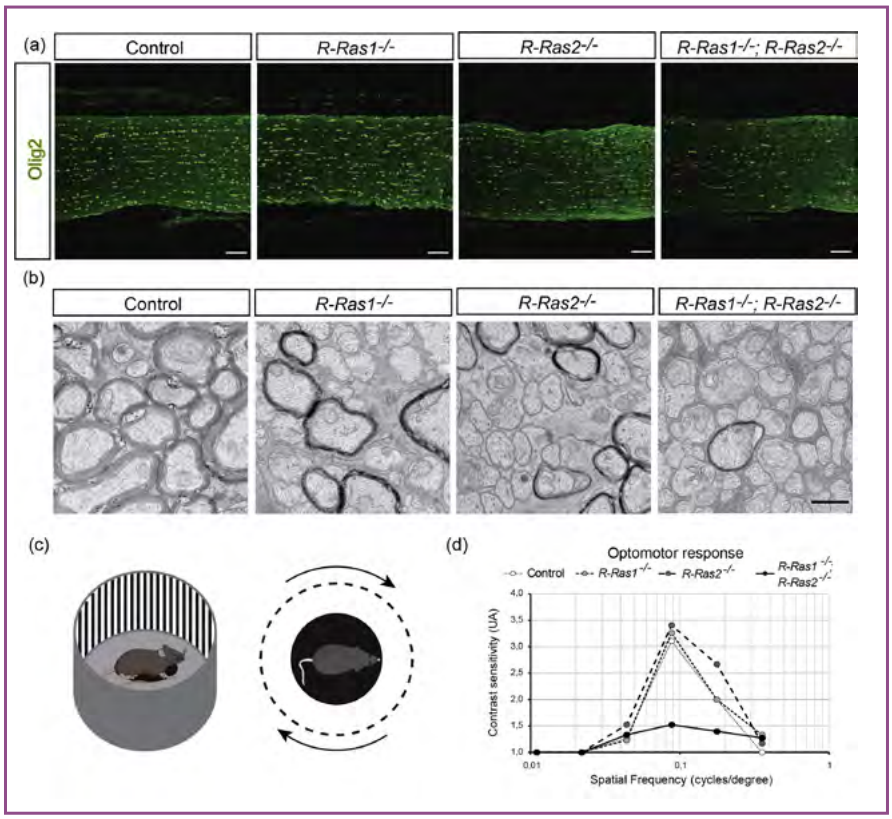


Figure: (a) Loss of oligodendrocytes in mutant mice lacking from R-Ras1, R-Ras2, or both. (b) Axons lose their protective myelin sheath when they lack R-Ras1 and/or R-Ras2. (c) and (d) Loss of visual function generated by the loss of oligodendrocytes and myelin in the mutant mice.

List of publications

Articles

» Garcia-Martin G., Sanz-Rodriguez M., Alcover-Sanchez B., Pereira MP., Wandosell F., Cubelos B. (2022) R-Ras1 and R-Ras2 Expression in Anatomical Regions and Cell Types of the Central Nervous System. I International Journal of Molecular Sciences. 23(2):978. doi: 10.3390/ijms23020978.

» Alcover-Sanchez B., Garcia-Martin G., Escudero-Ramirez J., Gonzalez-Riano C., Lorenzo P., Gimenez-Cassina A., Formentini L., de la Villa-Polo P., Pereira MP., Wandosell F., Cubelos B (2021). Absence of R-Ras1 and R-Ras2 causes mitochondrial alterations that trigger axonal degeneration in a hypomyelinating disease model. Glia. 69(3):619-637. doi: 10.1002/glia.23917.

» Garcia-Martin G., Alcover-Sanchez B., Wandosell F., Cubelos B. (2022) Pathways Involved in Remyelination after Cerebral Ischemia. Current Neuropharmacology 2022;20(4):751-765. doi: 10.2174/1570159X19666210610093658.

Book chapters

» Alcover-Sanchez B. and Cubelos B. (2022). Chapter 30 - R-Ras1−/− and R-Ras2−/− mice as models for investigating multiple sclerosis. Handbook of Animal Models in Neurological Disorders. Elsevier Academic Press. Pages 369-376. ISBN 9780323898331.

Participation in projects

» Project Title: Role of R-Ras1 and R-Ras2 in oligodendrocyte differentiation and specification. Proposal number: PID2021-123269OB-I00
Funding source: Spanish Ministry of Economy and Competitiveness.
Duration: 2022-2024. PI: Beatriz Cubelos

» Project Title: Role of RRAS1/2-PI3K-AKT pathway in myelination process.
Proposal number: RTI2018-096303-B-C33. Funding source: Spanish Ministry of Economy and Competitiveness.
Duration: 2019-2021. PI: Beatriz Cubelos

» Project Title: Opto-Electronic Neural Connectoid Model Implemented for Neurodegenerative Disease. Proposal number: 101047177
Funding source: European Project - HORIZON-EIC-2021-PATHFINDEROPEN-01 (EIC Pathfinder Open 2021)
Duration: 2022-2024. Team member: Beatriz Cubelos

Other activities

» Guest Editor of International Journal of Molecular Science (IJMS) (B. Cubelos)

PHYSIOPATHOLOGY AND THERAPY OF NEURODEGENERATIVE DISEASES: FRIEDREICH´S ATAXIA

Group Members

Principal Investigator:
Javier Díaz-Nido

Postdoctoral Researchers
Frida Loría Salinas
Saúl Herranz Martín

Predoctoral Researcher
Andrés Vicente Acosta

Technician
Jorge de los Santos Galán Cruz

https://www.cbm.uam.es/en/research/programs/physiological-and-pathological-processes/molecular-neuropathology/neuronal-repair-and-molecular-therapy-in-neurodegeneration-spinocerebellar-ataxias

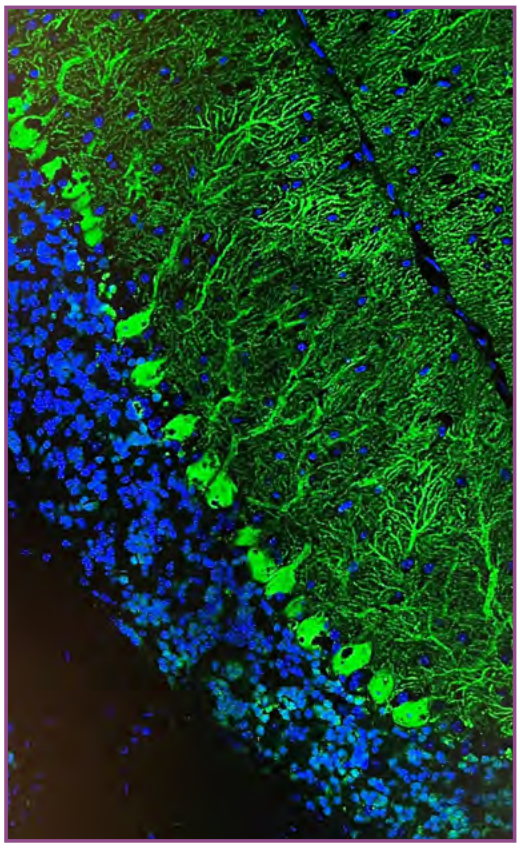
Summary

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.



Mouse cerebellar cortex

List of publications

» Moreno-Lorite J, Pérez-Luz S, Katsu-Jiménez Y, Oberdoerfer D, Díaz-Nido J. (2021) DNA repair pathways are altered in neural cell models of frataxin deficiency. Mol Cell Neurosci. 2021 Mar;111:103587. doi:10.1016/j.mcn.2020.103587.

» Ocana-Santero G, Díaz-Nido J, Herranz-Martín S. (2021) Future Prospects of Gene Therapy for Friedreich's Ataxia. Int J Mol Sci. 2021 Feb 11;22(4):1815. doi:10.3390/ijms22041815.

» Vicente-Acosta A, Giménez-Cassina A, Díaz-Nido J, Loria F. (2022) The smoothened agonist SAG reduces mitochondrial dysfunction and neurotoxicity of frataxin-deficient astrocytes. J Neuroinflammation. 2022 Apr 12;19(1):93. doi:10.1186/s12974-022-02442-w.

Other activities

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

» Javier Diaz-Nido is involved in the teaching of different courses at the Bachelor in Biochemistry and the Master in Molecular Biomedicine at UAM.

MOLECULAR BASES OF NEURONAL PLASTICITY

Group Members

Principal Investigator:
Fco. Javier Díez Guerra

Predoctoral fellow:
Raquel de Andrés Hernáiz
Elena Martínez Blanco

Laboratory technician:
Lucía Baratas Álvarez,

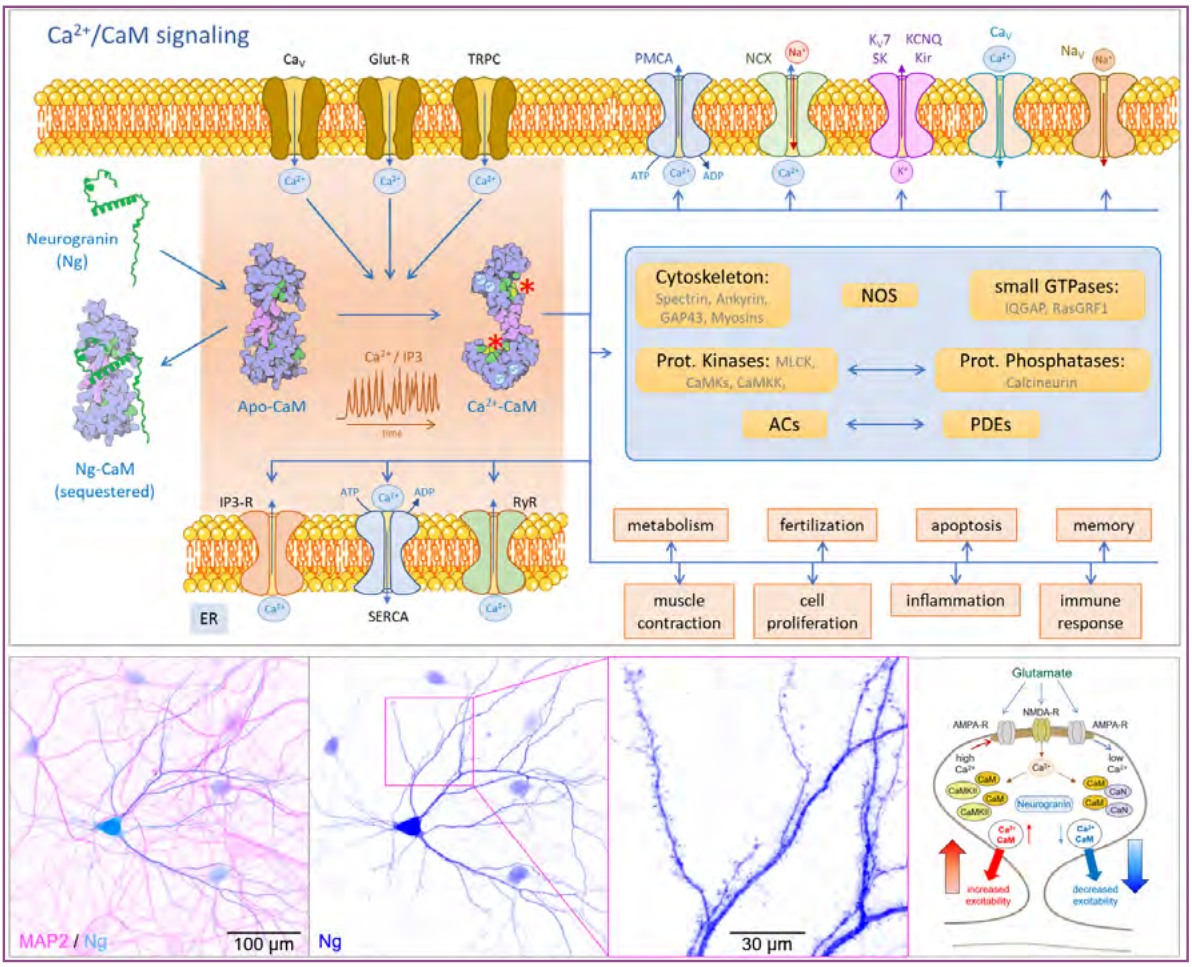
Undergraduate:
Miguel Angel Serrano Lope (2021)

Research assistant:
Sara Muñoz López (until Feb 2022)

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

Summary

Higher cognitive functions differentiate humans from other animal species. These functions depend on the activity of complex neural networks in our forebrain. As we age or due to neurological or psychiatric disorders, these networks lose functionality caused by the dysfunction and loss of synapses and eventually neurodegenerative events. In the development of the mammalian central nervous system there are periods of active synaptogenesis and synaptic remodeling, driven by sensory experience and essential for the proper configuration of adult neural networks. Until we understand in depth how these high synaptic plasticity events work and how to induce them, it will be difficult to imagine actions to prevent, alleviate or recover the loss of cognitive functionality associated with age or pathologies. In our laboratory, we have been able to reproduce several features of these periods of high plasticity using in vitro models based on primary cultures of dissociated embryonic neurons. We have found that neuronal electrical activity is critical and want to understand the underlying molecular and cellular mechanisms. To this end, we are focused on determining how synaptic activity, and more specifically, local intracellular calcium (Ca+2) oscillations modulate signaling pathways leading to synapse production and reinvigoration. Calmodulin (CaM), a calcium-binding protein, transduces Ca+2 oscillations into intracellular signaling events that lead to short-term and long-term effects, including the modulation of gene expression patterns. CaM activity is locally regulated by proteins such as Neurogranin (Ng), an abundant CaM-sequestering protein in the post-synaptic compartment of forebrain neurons. Ng brain levels and cognitive performance are closely and directly correlated in the human brain. Therefore, we have set as our research goals the study of the regulation of Ng expression and its functional role in the postsynaptic environment. We use techniques in the areas of biochemistry, cellular and molecular biology, gene expression manipulation, transcriptomics, proteomics and advanced light microscopy and image analysis, and build genetically-encoded biosensors, to identify the actors and the interactions that are relevant for synaptic generation and remodeling. We propose Ng as a target for strategies to prevent, alleviate or cure impaired cognitive function. Since Ng expression is restricted to some forebrain areas and late developmental stages, interventions to promote Ng expression will be likely devoid of undesired side-effects. In summary, a deeper understanding of the role of CaM-sequestering proteins in synaptic plasticity will make it possible to develop new therapies to improve cognitive functions and quality of life of aging individuals and patients of neurological diseases.



Upper panel: Calcium/Calmodulin (Ca+2/CaM)-mediated signaling. Intracellular Ca+2 oscillations are decoded in space and time through the calcium-binding protein CaM, leading to different outcomes depending on the local context, the nature of calcium oscillations and the presence of CaM-sequestering proteins, such as Neurogranin (Ng). Lower panel: Neurogranin (Ng) expression in mature hippocampal neurons in culture (DIV18). Neurogranin present in dendrites and spines controls post-synaptic excitability by regulating local Calmodulin (CaM) availability.

Participation in projects

» REFERENCIA: RTI2018-098712-B-I00. TÍTULO: "Papel de los microRNAs y los exosomas en la induccion de la tolerancia isquemica en el cerebro". AREA: Biomedicina. SUBAREA: Enfermedades del sistema nervioso. Entidad beneficiaria: Universidad Autónoma de Madrid. Centro: Centro de Biología Molecular Severo Ochoa (CSIC-UAM)
Investigador/a principal 1 (IP1): FRANCISCO ZAFRA GOMEZ
Investigador/a principal 2 (IP2): FRANCISCO JAVIER DÍEZ GUERRA
Fecha de inicio del proyecto: 1 enero 2019. Fecha de finalización del proyecto: 30 septiembre 2022

Patents

» "ELECTRODOS NANOESTRUCTURADOS PARA LA ESTIMULACIÓN ELÉCTRICA DE CÉLULAS EN CULTIVO, DISPOSITIVOS, SISTEMAS Y PROCEDIMIENTOS ASOCIADOS", Spanish Patent granted on 28 December 2022 under number ES-2887832-B2.

- Titulares:

CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS (CSIC) (80.0%),

UNIVERSIDAD AUTÓNOMA DE MADRID (10.0%)

UNIVERSITY OF FLORIDA RESEARCH FOUNDATION, INC. (UFRF) (10.0%)

- Inventores:

MOBINI, Sahba; GARCÍA MARTÍN, José Miguel; GONZÁLEZ SAGARDOY, María Ujué; MARTÍN GONZÁLEZ, Maria Soledad; CABALLERO CALERO, Olga; GARCÍA MARTÍNEZ, Jorge M; DÍEZ GUERRA, Francisco Javier & PATRICK, Erin E

SURVIVAL AND PLASTICITY IN THE AGING BRAIN

Group Members

Principal Investigator:
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Inés Berenguer López

Predoctoral fellows
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Andrea González Carrascosa (2021)
Alba Montoro Canelo (2021)
Miguel Ángel Serrano Lope (2022)
Violeta Enriquez Zarralanga (2022)

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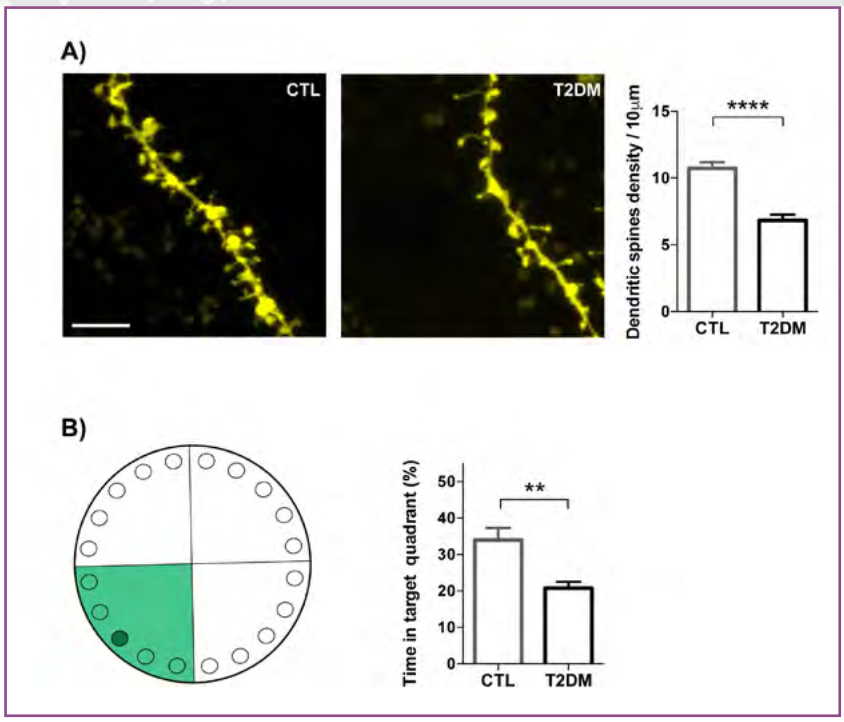
https://www.cbm.uam.es/cdotti

Summary

Aging determines the occurrence of changes in a panoply of biochemical / molecular hubs, each contributing to the typical deficits of age. In fact, aging comes with alterations in genomic and non-genomic activities, including among the latter, but not only, defects in mitochondrial function, intracellular trafficking, proteostasis, in the response to cellular stress and in the control of intracellular calcium. What mechanism could act upstream of these defects, responsible for each of them occurring? In other words: is there a "master" change with age, upstream of all (or some) defects that occur with age? A defect that could fulfill the “upstream” role is membrane signaling. In fact, changes in membrane signaling start to appear early after cell maturation and worsen with time, they occur in all cells of our organs and tissues and influence genomic and non-genomic activities. Although a widely studied example of defective membrane signalling with age is brain insulin resistance, age is also accompanied by resistance to thyroid stimulating hormone (TSH), to growth hormone, orexin, corticosteroid hormones, fibroblast growth factor and more. Moreover, defective membrane signalling is also evident for neurotransmitter signalling and G-protein coupled receptors. Therefore, understanding the mechanisms underlying the changes in plasma membrane signalling with age could open avenues of intervention to reduce the sensory, motor and cognitive deficits that occur with age. In my group we have demonstrated that aging produces steady changes in the lipid composition of the neuronal plasma membrane, making it a more rigid structure in which membrane receptors have reduced dynamism, both in their mobility in the plane of the membrane and in their exchange. In turn, we showed that receptors affected by age produce weaker signalling when faced with learning and memory stimuli but, at the same time, effectively mediate survival responses, suggesting that the loss of cognitive abilities that we are experiencing with age is, to put it in some way, a “toll” to keep our neurons alive. The projects that we are currently developing are aimed at determining the molecular mechanisms behind these changes, both physiological and pathological brain aging.



*A. Analysis of the shapes of the dendritic spines of hippocampal neurons of 14-month-old mice, control (CTL) or affected by chronic type 2 diabetes (T2DM). The graph bar on the right is the quantitative analysis of the number of dendritic spines shown in the immunofluorescence image, demonstrating the significant decrease in the number of spines in the T2DM mice.mean statistical significance. Bar: 10µm. *** p<0.001*

*B. Learning behavior of 14-month-old mice, control (CTL) or affected by chronic type 2 diabetes (T2DM). The test consisted of determining the time that the mice spent in the quadrant where the escape route is located (in green). The bar graph shows that mice with T2DM spend less time in the appropriate quadrant (and more in unrelated areas). ** p<0.005.*

List of publications

» Guix FX, Capitán AM, Casadomé-Perales Á, Palomares-Pérez I, López Del Castillo I, Miguel V, Goedeke L, Martín MG, Lamas S, Peinado H, Fernández-Hernando C, Dotti CG. Increased exosome secretion in neurons aging in vitro by NPC1-mediated endosomal cholesterol buildup.

Life Sci Alliance. 2021 Jun 28;4(8):e202101055. doi: 10.26508/lsa.202101055.

» Almansa D, Peinado H, García-Rodríguez R, Casadomé-Perales Á, Dotti CG, Guix FX. Extracellular Vesicles Derived from Young Neural Cultures Attenuate Astrocytic Reactivity In Vitro.

Int J Mol Sci. 2022 Jan 25;23(3):1371. doi: 10.3390/ijms23031371.

» Martín MG, Dotti CG. Plasma membrane and brain dysfunction of the old: Do we age from our membranes?

Front Cell Dev Biol. 2022 Oct 6;10:1031007. doi: 10.3389/fcell.2022.1031007.

» 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., and Dotti, C.G. (2022). Diabetes-associated cognitive decline in mice with genetic predisposition to Alzheimer's disease, not in wild type. Research Square: https://doi.org/10.21203/rs.3.rs-1869956/v1

Participation in projects

» 2018-2021 (extended 12/2022). EU Joint Program -Neurodegenerative Disease Research (JPND). Principal (External) Investigator. Effect of early and adult-life stress on the brain epigenome: relevance for the occurrence of Alzheimer's Disease and Diabetes-related dementia (Acronym: EpiAD).

» 2020-2023. Principal Investigator. Effect of age on intracellular signaling for plasticity and survival . Ministry of Science and Innovation/AEI. PID2019-104389RB-I00.

» 2021-2022. Principal Investigator. Neuronal plasticity-to-survival shift in the aging mouse EU. CSIC NeuroAging Platform (PTI).

Doctoral theses

» Álvaro Casadomé Perales.
Título: Cambios en la composición de las vesículas extracelulares de la corteza cerebral del ratón durante el envejecimiento: enfoque en la ceramida sintasa 2. Biociencias Moleculares. Universidad Autónoma de Madrid. Fecha de defensa: 18 Nov. 2022.

Other activities

Popular science book: La ciencia del buen envejecer.

Authors: Carlos Dotti and Pablo Gonz.
ISBN: 9788413611679
Editorial: Shackleton Books
Date of edition: 2022

MECHANISMS OF SYNAPTIC PLASTICITY, AND CONTRIBUTION TO COGNITIVE FUNCTION

Group Members

Principal Investigator:
José Antonio Esteban García

Postdoctoral Fellows:
Víctor Briz Herrezuelo

Predoctoral Fellows:
Sergio López García (until October 2022)
Alba Fernández Rodrigo
Esperanza López Merino
Eneko Merino Casamayor (from November 2021)
Celia García Vilela (from September 2022)

Technician:
Silvia Gutiérrez Eisman

Undergraduate:
Jessie Jiang (until June 2021)
Vittoria Nisticò (October 2021 – May 2022)
Izascun Los Arcos (from June 2022)

www.cbm.csic.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 have investigated how environmental contaminants shift some of these signaling mechanisms, altering neurodevelopmental programs with long-lasting consequences in the adult life. In particular, using rats as animal model, we have found that chronic exposure to low concentrations of common pesticides during embryonic development and early postnatal life changes the activation of intracellular signaling and impairs synaptic plasticity. We believe these changes are responsible for behavioral deficits observed in these animals later in life.

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

List of publications

» Sánchez-Castillo, C.*, Cuartero, M. I.*, Fernández-Rodrigo, A., Briz, V., López-García, S., Jiménez-Sánchez, R., López, J. A., Graupera, M. and Esteban, J. A. (2022) Functional specialization of different PI3K isoforms for the control of neuronal architecture, synaptic plasticity and cognition. Science Adv, 8:eabq8109. doi: 10.1126/sciadv.abq8109. *Equal contributions.

» López-Merino, E., Cuartero, M. I., Esteban, J. A.* and Briz, V.* (2022) Perinatal exposure to pesticides alters synaptic plasticity signaling and induces behavioral deficits associated with neurodevelopmental disorders. Cell Biol Toxicol. doi: 10.1007/s10565-022-09697-2. *Co-corresponding authors.

» Brachet, A., Lario, A., Fernández-Rodrigo, A., Heisler, F. F., Gutiérrez, Y., Lobo, C., Kneussel, M. and Esteban, J. A. (2021) A Kinesin 1-protrudin complex mediates AMPA receptor synaptic removal during long term depression. Cell Rep 36, 109499. doi: 10.1016/j.celrep.2021.109499.

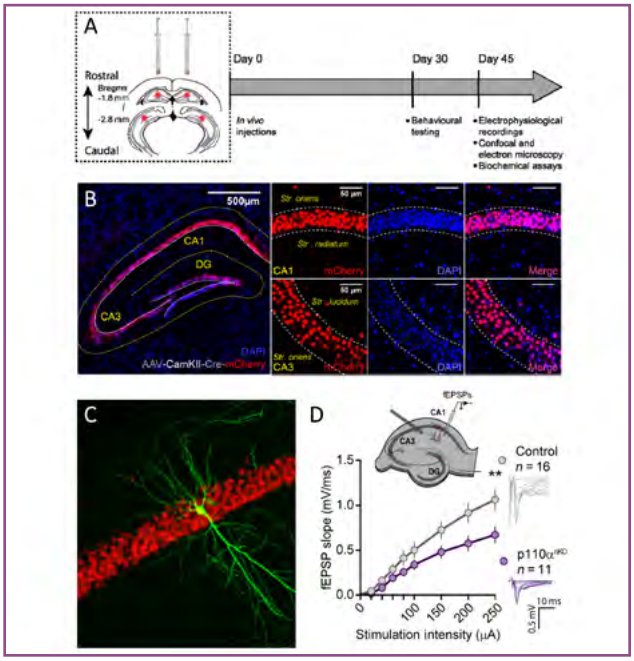
» Gutiérrez, Y., López-García, S., Lario, A., Gutiérrez-Eisman, S., Delevoye, C. and Esteban, J. A. (2021) KIF13A drives AMPA receptor synaptic delivery for long-term potentiation via endosomal remodeling. J Cell Biol, 220:e202003183. doi: 10.1083/jcb.202003183.

» Draffin, J. E., Sánchez-Castillo, C., Fernández-Rodrigo, A., Sánchez-Sáez, X., Ávila, J., Wagner, F. F. and Esteban, J. A. (2021) GSK3α, not GSK3β, drives hippocampal NMDAR-dependent LTD via tau-mediated spine anchoring. EMBOJ 40, e105513. doi: doi: 10.15252/embj.2020105513.

» Esparza-Moltó, P. B., Romero-Carramiñana, I., Núñez de Arenas, C., Pereira, M. P., Blanco, N., Pardo, B., Bates, G. R., Sánchez-Castillo, C., Artuch, R., Murphy, M. P., Esteban, J. A. and Cuezva, J. M. (2021) Generation of mitochondrial reactive oxygen species is controlled by ATPase inhibitory factor 1 and regulates cognition. PLoS Biol 19:e3001252. doi: 10.1371/journal.pbio.3001252.

» Ribeiro, L. F., Catarino, T., Carvalho, M., Cortes, L., Santos, S. D., Opazo, P. O., Ribeiro, L. R., Oliveiros, B., Choquet, D., Esteban, J. A., Peça, J. and Carvalho, A. L. (2021) Ligand-independent activity of the ghrelin receptor modulates AMPA receptor trafficking and supports memory formation. Sci Signal 14, eabb1953. doi: 10.1126/scisignal.abb1953.

» Ruiz-Pérez, G., Ruiz de Martín Esteban, S., Marqués, S., Aparicio, N., Grande, M. T., Benito-Cuesta, I., Martínez-Relimpio, A. M., Arnanz, M. A., Tolón, R. M., Posada-Ayala, M., Cravatt, B. F., Esteban, J. A., Romero, J. and Palenzuela, R. (2021) Potentiation of amyloid beta phagocytosis and amelioration of synaptic dysfunction upon FAAH deletion in a mouse model of Alzheimer's disease. J Neuroinflammation 18, 223. doi: 10.1186/s12974-021-02276-y.



Analysis of neuronal function of PI3K isoforms. A. Experimental strategy for knocking-out specific genes using in vivo viral injection to express the cre recombinase in adult mice, followed by behavioral assays, and terminated with electrophysiological, biochemical and morphological analysis. B. Representative hippocampal section showing the extent of viral infection (red) together with nuclear staining (DAPI). C. Representative confocal image of a GFP-expressing CA1 pyramidal neuron for morphological analysis. D. Assessment of basal synaptic strength from hippocampal slices of saline-injected (control) and cre-expressing (knockout) mice, using electrophysiological techniques with the experimental configuration shown in the inset.

Participation in projects

» 2022-2023 Ministerio de Ciencia e Innovación/ NextGenerationEU, PDC2021-120815-I00. "GSK3alpha, an unsuspected therapeutic target for Alzheimers disease". Principal Investigator: José A. Esteban.

» 2021-2024 Ministerio de Ciencia e Innovación, PID2020-117651RB. "Interplay between synaptic and metabolic plasticity. Relevance for mental disease". Principal Investigator: José A. Esteban.

» 2018-2021 Ministerio de Ciencia, Innovación y Universidades, SAF2017-86983-R. "Subcellular and molecular regulation of PI3K/PTEN signaling during synaptic plasticity. Strategies for Alzheimer's disease". Principal Investigator: José A. Esteban.

Doctoral theses

» Carla Sánchez Castillo (2021). "Role of Class IA PI3K isoforms in synaptic plasticity and cognitive function". Universidad Autónoma de Madrid. Director: José A. Esteban

LIPIDS IN NEURONAL PHYSIOLOGY AND PATHOLOGY

Group Members

Principal Investigator:
María Dolores Ledesma Muñoz

Postdoctoral fellows:
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Ángel Gaudioso Guirado

Predoctoral fellows:
Beatriz Soto Huelín (until October 2022)
Ana Toledano Zaragoza,
Sara Naya Forcano (since May 2021),
Elena Melgarejo de la Peña (since September 2022)

Technicians:
Mercedes Hernández del Cerro

Undergraduate and Master Students:
Miguel Parra Martínez (until June 2021)
Mario Díaz García (until June 2021)
Jaime Mulero Franco (until June 2022)
Sofía Fajardo Callejón (October 2021-March 2022)

Visiting scientists:
Ameer Taha (University of California Davis) (January-August 2022)

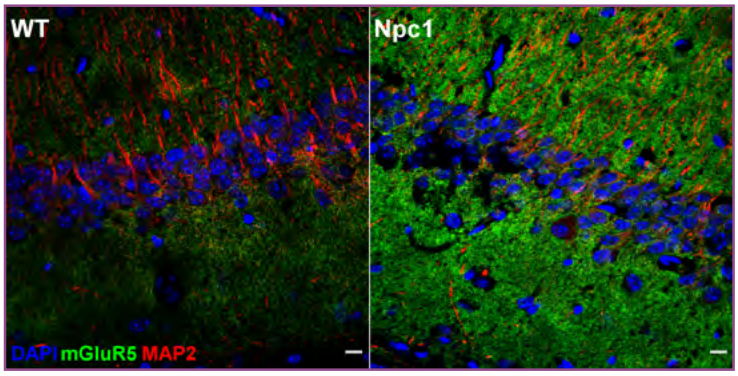
http://www.cbm.uam.es/dledesma

Summary

Our research focuses on understanding the contribution of lipids, in particular cholesterol and sphingomyelin that are especially abundant in neurons, to neuronal physiology and aging. We also investigate the pathological consequences of their alterations in lipid storage disorders such as Niemann Pick diseases (NPDs). To this aim we use mice in which the metabolism of these lipids has been genetically altered allowing *in vivo* analysis and the preclinical assessment of potential therapies. In the period 2021-2022, our studies unveiled the important role of sphingomyelin and cholesterol in neuronal synaptic plasticity (by influencing the metabotropic glutamate receptor 5) and on the regulation of the glucocorticoid and endocannabinoid systems. These roles link alterations in sphingomyelin and cholesterol distribution and levels to psychiatric conditions, like anxiety and depression, which characterize NPDs and show increased incidence with age. Moreover, we have obtained evidence supporting sphingomyelin overload as a primary cause of myelin alterations by affecting oligodendrocyte differentiation, which may be an early pathological event leading to neurodegeneration in NPDs. In addition to acquire basic knowledge about the role of lipids in neurons and glial cells our research has a relevant translational side. Thus, during 2021-2022 we have indentified a specific sphingomyelin species in blood as a potential biomarker for brain pathology in NPD type A. We have successfully assessed in the NPD mouse models a nutraceutical strategy based on dietary polyphenols that ameliorates brain phenotypes by increasing extracellular vesicle release. As part of the translational efforts we have been in contact with and received support from national and international NPD patient associations (in Spain, US, Germany and Switzerland) and Biotech companies (Takeda (Japan)); we have filed a patent on a potential treatment for NPDs that target the endocannabinoid system; and participate in a clinical trial in NPD type C patients (EudraCT: 2019-004498-18), which is assessing a cholesterol-reducing drug that showed benefits in a preclinical study conducted by our laboratory.

Patents

Inventors: M.D. Ledesma, A. Gaudioso, E.H. Schuchman.
Title: "Compositions and methods for diagnosing, treating and preventing lysosomal storage diseases".
Number: U.S. Patent Application No. 63/369,721.
Country: United States
Filing date: July 28, 2022.
Owner: Wylder Nation Foundation



The levels of the metabotropic glutamate receptor 5 are increased in the brain of the mouse model for Niemann Pick disease type C.

Representative images of immunofluorescences against mGluR5 (green), the neuronal marker MAP2 (red) and the cell nuclei marker DAPI (blue) in the hippocampus of age-matched wild type (WT) mice and mutant mice for the cholesterol transport protein NPC1 (Npc1) that model NPD type C.

List of publications

Articles:

- Crivelli, S.M. et al. (2021) CERT reduces C16 ceramide, amyloid-β levels, and inflammation in a model of Alzheimer's disease. Alzheimer Res Ther 13:45 doi: 10.1186/s13195-021-00780-0
- Schuchman, E.H. et al. (2021) New paradigms for the treatment of lysosomal storage diseases: targeting the endocannabinoid system as a therapeutic strategy. Orphanet J Rare Dis 16:151 doi: 10.1186/s13023-021-01779-4.
- Kalinichenko, L.S. et al. (2021) Neutral sphingomyelinase mediates the co-morbidity trias of alcohol abuse, major depression and bone defects. Mol Psychiatry. 26:7403-7416. doi: 10.1038/s41380-021-01304-w.
- Kalinichenko, L.S. et al. (2022) Cerebral Cortex. bhac106. doi: 10.1093/cercor/bhac106
- Gaudioso, Á. et al. (2022) Models to study basic and applied aspects of lysosomal storage disorders. Adv Drug Deliv Rev. 190:114532. doi: 10.1016/j.addr.2022.114532.
- Gascón-Bayarri, J. et al. (2022) Efficacy and safety clinical trial with efavirenz in patients diagnosed with adult Niemann-pick type C with cognitive impairment. Medicine (Baltimore). 101: e31471. doi: 10.1097/MD.0000000000031471.

Book chapters:

- Ledesma, M.D., and Bortolozzi, A. Understanding Mental Disorders. In: Brain, mind & Behaviour, CSIC scientific challenges: towards 2030. Editorial CSIC. (2021) Vol 5, pp.89-95

Participation in projects

- European Commission–NextGeneration EU CSIC's Thematic Platforms (PTI+ Neuro-Aging) (May 2021-December 2022)
- "Efficacy assessment of FAAH inhibitors to induce sphingomyelin hydrolysis and treat brain pathology in the acid sphingomyelinase knock out mice". Wylder Nation Foundation (USA) (September 2019- August 2023)
- "Efficacy assessment of the synthetic retinoid Fenretinide to treat brain pathology in the acid sphingomyelinase knock out mice" Niemann-Pick Selbsthilfegruppe Deutschland (Germany) (March 2022-September 2022)
- "Assessment of pharmacological mTOR inhibition in a mouse model for Niemann Pick disease type C" Niemann Pick Swiss association (April 2021-September 2021).
- "Aproximaciones no convencionales para comprender y tratar las enfermedades de Niemann Pick". Plan Nacional I+D, Ministerio Español de Ciencia e Innovación, PID2020-112830RB-I00 (Septiembre 2021-Agosto 2023)
- "Los receptores metabotrópicos de glutamato en la patología y terapia de las alteraciones psiquiátricas en la enfermedad de Niemann Pick tipo C" Fundación Koplowitz (Octubre 2020-Septiembre 2022).
- "Influencia de la esfingomielina y el colesterol sinápticos en alteraciones psiquiátricas. Relevancia para las enfermedades de Niemann Pick". Plan Nacional I+D, Ministerio Español de Ciencia e Innovación, SAF2017-87698-R (Enero 2018-Septiembre 2021)

Doctoral theses

- Beatriz Soto Huelin (2022) "Aproximaciones terapéuticas para tratar la patología cerebral en la enfermedad de Niemann Pick tipo A", Universidad Autónoma Madrid. Directora: María Dolores Ledesma
- Ana Toledano Zaragoza (2022) "Alteraciones de mGluR5 y el sistema endocannabinoide en la patología psiquiátrica de Niemann Pick tipo C", Universidad Autónoma Madrid. Directora: María Dolores Ledesma

PHYSIOPATHOLOGY OF GLYCINE TRANSPORTERS IN GLYCINERGIC NEUROTRANSMISSION: HYPEREKPLEXIA AND PAIN

Group Members

Principal Investigator (PI)
Beatriz López Corcuera

Predoctoral fellows
Raquel Felipe Mendía
Jorge Sarmiento Jiménez (from September 2021)

Students
Araceli Vázquez Medel (from September 2022)

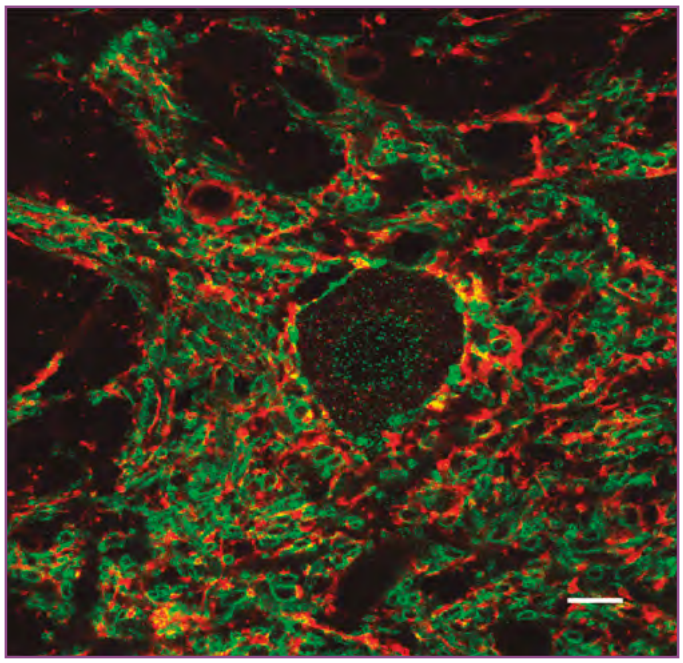
Technical Assistance
Enrique Núñez Balbuena (50% dedication)

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

Summary

At present, the group studies the physiology and pathologies of glycinergic neurotransmission including hyperekplexia and pain. Hyperekplexia is a rare sensorimotor disorder provoked by defective glycinergic inhibition that may have severe consequences in neonates. The neuronal GlyT2 glycine transporter, which is crucial for the recycling of synaptic neurotransmitter and supplies glycine for synaptic vesicle refilling, is nonfunctional in the presynaptic form of the disease. One of our aims is to identify and characterize new mutations in the human GlyT2 gene *(SLC6A5)* found in hyperekplexia patients. After the identification and assessment of the pathogenic mechanisms of several hyperekplexia-associated GlyT2 variants, we have now shown some of them are amenable to rescue from its trafficking defect by chemical chaperones. This may help developing more specific pharmacochaperones as candidate therapeutic tools for hyperekplexia with the help of 3D computational models we have developed. Some other hyperekplexia mutations have revealed interesting unknown aspects on transporter oligomerization we study with refined oligomer modeling. Moreover, we have found new components of GlyT2 interactoma, some of which are candidate hyperekplexia genes that remain to be identified, besides revealing a role in ion homeostasis for GlyT2 and its partners.

An additional aspect of our research led us advance in the knowledge of the mechanisms of GlyT2 regulation. First, we have shown GlyT2 is regulated by the Hedgehog pathway in vitro and *in vivo*. GlyT2 control by this signaling cascade, clearly involved in development, moved us to investigate a possible role for GlyT2 in the development of glycinergic neurotransmission. We have also studied the role of transporters in the processing of nociceptive information by exploring mechanisms of GlyT2 regulation by M2 muscarinic acetylcholine receptors, the most clearly involved in pain processing in the spinal cord. Finally, we explored modulators of GlyT2 activity to obtain information applicable to analgesia. We analyzed the comparative docking of the two selective GlyT2 inhibitors with nanomolar affinity and defined their differential interactions with the transporter protein. Structural information about the interactions with GlyT2 may provide useful tools for new drug discovery applicable to analgesia.



Immunolocalization of GlyT2 and M2 muscarinic acetylcholine receptors in rat spinal cord.

List of publications

» de la Rocha-Muñoz A, Núñez E, Vishwanath AA, Gómez-López S, Dhanasobhon D, Rebola N, López-Corcuera B, de Juan-Sanz J, Aragón C. (2021) The presynaptic glycine transporter GlyT2 is regulated by the Hedgehog pathway in vitro and *in vivo*. Commun Biol. 4(1):1197. doi: 10.1038/s42003-021-02718-6.

» Benito-Muñoz, C., Perona, A., Felipe, R., Pérez-Siles, G., Núñez, E., Aragón, C. and López-Corcuera, B. (2021) Structural determinants of the glycine transporter 2 (GlyT2) for the selective inhibitors ALX1393 and ORG25543. ACS Chem Neurosci. 12(11):1860-1872. doi: 10.1021/acschemneuro.0c00602.

» Danbolt NC, López-Corcuera B, Zhou Y. (2022) Reconstitution of GABA, Glycine and Glutamate Transporters. Neurochem Res. 47(1):85-110. doi: 10.1007/s11064-021-03331-z.

» de la Rocha-Muñoz A, Melgarejo E, Aragón C, López-Corcuera B. (2021) Rescue of two trafficking-defective variants of the neuronal glycine transporter GlyT2 associated to hyperekplexia. Neuropharmacology. 189:108543. doi: 10.1016/j.neuropharm.2021.108543.

» Jiménez E, Fornés A, Felipe R, Núñez E, Aragón C, López-Corcuera B. (2022) Calcium-Dependent Regulation of the Neuronal Glycine Transporter GlyT2 by M2 Muscarinic Acetylcholine Receptors. Neurochem Res. 47(1):190-203. doi: 10.1007/s11064-021-03298-x.

Participation in projects

» PID2020-119399RB-I00. The neuronal glycine transporter GlyT2 in pain and in hyperekplexia: pathological implications in development. Ministry of Science and Innovation. PI: B. López Corcuera. 2021-2024.

» 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.

Other activities

The group belongs to the Institute of Investigación Biosanitaria IdiPAZ from November 2010 as research group "Implication of glycinergic and glutamatergic systems in pathologies of the Central Nervous System".

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

www.cbm.uam.es/lineas/lucasgroup.htm

Group Members

Principal Investigator:
José J. Lucas

Postdoctoral fellows
Ainara Elorza Peregrina
(until June 2021)
Sara Picó del Pino

Predoctoral fellows:
Ivana Ollà
Claudia Rodríguez López
David Lozano Muñoz

Technicians:
María Santos Galindo
(until September 2022)
Miriam Lucas Santamaría

Summary

Research in our laboratory focuses on elucidating the molecular basis of neurological diseases, particularly Huntington's disease (HD), Alzheimer's disease, autism and epilepsy. For this, we have had continuous funding from the Spanish Science and Health Ministries and the European Union, as well as from private Foundations and Institutions.

A key methodological approach for research in our laboratory is the generation and characterization of mouse models of disease that truly recapitulate pathogenesis, as well as neuropathology and symptoms (construct and face validity) and that often allow to characterize existing therapies (predictive validity) and to assay novel ones.

Altered RNA processing in the pathogenesis of neural disease has been a central theme of research in of our laboratory in recent years. More precisely, we have found alteration of specific splicing factors and a role of aberrant alternative splicing in HD (Fernández-Nogales et al. Nat Med 20:881-5, 2014; Cabrera JR & Lucas. Brain Pathol. 27:181-9, 2017; Hernández et al. Brain 143:2207-19, 2020) and in Alzheimer's (García-Escudero et al. Acta Neuropathol. 142:159-77, 2021). The relevance of the human/mouse intersect RNAseq analysis that we have developed and applied to stablish the global mis-splicing signature in HD human postmortem tissue (Elorza et al. Brain 144:2009-23, 2021) is highlighted by a recent spotlight review on our study (Xing et al. Trends Neurosci 2236:00209-5, 2021).

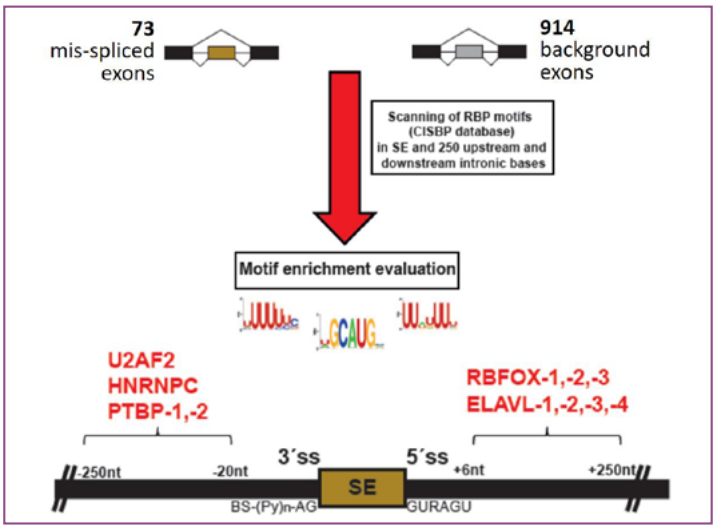
We have also found alteration in microexon splicing and regulated polyadenyation of the transcriptome in autism (Parras et al. Nature 560:441-6, 2018), in epilepsy (Parras et al. Brain 143:2139-53, 2020) as well as in HD (Picó S, et al. Sci Transl Med 13:eabe7104, 2021). The latter led to the discovery of a deficit of thiamine transport through brain endothelium in HD patients, which originated a clinical trial to explore its therapeutic relevance (https://clinicaltrials.gov/ct2/show/NCT04478734).

Participation in projects

» Part of the H2020 ITN Consortium PurinesDX

» Part of the Networking Research Center on Neurodegenerative Diseases (CIBERNED) (https://www.ciberned.es/grupos/grupo-de-investigacion?id=28582)

» PID2021-123141OB-I00, Altered RNA processing at the convergence of Huntington's disease and other neural diseases. Ministerio de Ciencia e Innovación. Principal Investigator: José J. Lucas.



Motif Scan analysis of candidate splicing factors responsible for the global mis-splicing detected in Huntington's disease striatum, as reported in Elorza et al. Brain 2020

List of publications

» Elorza A, Marquez Y, Cabrera JR, Sanchez-Trincado JL, Santos-Galindo M, Hernandez IH, Pico S, Diaz-Hernandez JI, Garcia-Escudero R, Irimia M et al (2021) Huntington's disease-specific mis-splicing unveils key effector genes and altered splicing factors. Brain 144: 2009-2023 Doi 10.1093/brain/awab087

» Garcia-Escudero V, Ruiz-Gabarre D, Gargini R, Perez M, Garcia E, Cuadros R, Hernandez IH, Cabrera JR, Garcia-Escudero R, Lucas JJ et al (2021) A new non-aggregative splicing isoform of human Tau is decreased in Alzheimer's disease. Acta Neuropathol 142: 159-177 Doi 10.1007/s00401-021-02317-z

» Migazzi A, Scaramuzzino C, Anderson EN, Tripathy D, Hernandez IH, Grant RA, Roccuzzo M, Tosatto L, Virlogeux A, Zuccato C et al (2021) Huntingtin-mediated axonal transport requires arginine methylation by PRMT6. Cell Rep 35: 108980 Doi 10.1016/j.celrep.2021.108980

» Otero A, Betancor M, Erana H, Fernandez Borges N, Lucas JJ, Badiola JJ, Castilla J, Bolea R (2021) Prion-Associated Neurodegeneration Causes Both Endoplasmic Reticulum Stress and Proteasome Impairment in a Murine Model of Spontaneous Disease. Int J Mol Sci 22: Doi 10.3390/ijms22010465

» Pico S, Parras A, Santos-Galindo M, Pose-Utrilla J, Castro M, Fraga E, Hernandez IH, Elorza A, Anta H, Wang N et al (2021) CPEB alteration and aberrant transcriptome-polyadenylation lead to a treatable SLC19A3 deficiency in Huntington's disease. Sci Transl Med 13: eabe7104 Doi 10.1126/scitranslmed.abe7104

» Yu D, Zarate N, White A, Coates D, Tsai W, Nanclares C, Cuccu F, Yue JS, Brown TG, Mansky RH et al (2022) CK2 alpha prime and alpha-synuclein pathogenic functional interaction mediates synaptic dysregulation in huntington's disease. Acta Neuropathol Commun 10: 83 Doi 10.1186/s40478-022-01379-8

Patents

» Lourdes Ruiz-Desviat, Ainoha Martínez-Pizarro, Sara Picó, José J Lucas, Brage S Andresen. Splice shifting oligonucleotides for use in the treatment of diseases characterized by altered inclusion of microexons. Application N.: EP21382898.1 Title holder: UAM/CSIC/University of Southern Denmark Priority country: Spain Priority date: 07 October 2021

» Jesús Avila, José J Lucas, Raquel Cuadros, Esther García-García, Vega García-Escudero, Félix Hernández, Daniel Ruiz-Gabarre. A new non-aggregative splicing isoform of human Tau is decreased in Alzheimer's disease. Application N.: EP21382283.6 Title holder: CSIC. Priority country: Spain Priority date: 05 April 2021

Doctoral theses

» Estudio de la enfermedad de Huntington como una deficiencia de tiamina asociada a SLC19A3 y sus implicaciones terapeúticas. Sara Picó del Pino. Universidad Autónoma de Madrid. Julio 2022.

ADULT NEUROGENESIS AND NEURODEGENERATIVE DISEASES

Group Members

Principal Investigator:
María Llorens-Martín

Postdoctoral Fellows:
Berenice Márquez Valadez
Julia Terreros-Roncal (From Nov 10, 2022)

Predoctoral Fellows:
Julia Terreros-Roncal (Until Nov 9, 2022)
Elena Moreno Jiménez
Miguel De La Flor García
Marta Gallardo Caballero (From Feb 16, 2022)
Ana Victoria Prádanos Senén (From Oct 1st, 2022)

Technicians:
Carla Rodríguez Moreno

Undergraduate And Master Students:
Evgenia Kokosali (From Dec 1st, 2022)
Javier Molina Hernández (From Sept 1st, 2022; Co-Supervised With. David Míguez).

Visiting Scientists
Fabio Cafini Barrado

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

Summary

The hippocampus is a brain region that hosts one of the most striking forms of neural plasticity, namely the generation of new neurons throughout life, or adult hippocampal neurogenesis (AHN). AHN participates in hippocampal-dependent learning and mood regulation, and it encompasses the birth and functional integration of newborn neurons. The generation of new neurons is compromised during physiological aging and under neurodegenerative conditions in rodents. Our group demonstrated that AHN persists during physiological aging until the tenth decade of human life (Terreros-Roncal et al., Science 2021). Moreover, we showed that AHN is critically impaired in patients with distinct neurodegenerative diseases, such as Alzheimer´s disease (Moreno-Jiménez et al., Nature Medicine, 2019), ALS, Huntington´s disease, Parkinson´s disease, Dementia with Lewy Bodies, and Frontotemporal dementia (Terreros-Roncal et al., Science, 2021). Progress in the field of human AHN has been hindered by technical limitations related to the quality of available human brain samples. In this regard, our group overcame these technical difficulties and demonstrated that specific tissue processing methodologies are necessary to observe the presence of new neurons in the adult human hippocampus (Flor-García et al., Nature Protocols, 2020).

Supported by the recent award of the ERC Consolidator Grant "ERC-CoG-2020-101001916-Hum*AN*", our research group seeks to investigate the basic biology of neural stem cells (the cell population that gives rise to new neurons in the adult mammalian hippocampus) as well as the mechanisms that control the modulation of adult hippocampal neurogenesis under physiological and pathological conditions in humans. We also perform *in vivo* and *in vitro* studies on animal models of different diseases. In particular, we are interested in determining the therapeutic potential of increasing adult hippocampal neurogenesis for the treatment of neurodegenerative and psychiatric diseases. Importantly, in most of these disorders, the hippocampus is one of the most affected areas. Therefore, other research lines of our lab are focused on developing strategies capable of increasing the functionality of newborn neurons. For that purpose, we use novel viral tools and distinct non-pharmacological approaches aimed at increasing hippocampal plasticity.

Doctoral theses

» Julia Terreros-Roncal (2022). Neurogénesis hipocampal adulta en sujetos neurológicamente sanos y pacientes con enfermedades neurodegenerativas. Universidad Autónoma de Madrid. Directora: María Llorens-Martín.

List of publications

» Terreros-Roncal J, Flor-García M, Moreno-Jiménez EP, Rodríguez-Moreno CB, Márquez-Valadez B, Gallardo-Caballero M, Rábano A, Llorens-Martín M. Methods to study adult hippocampal neurogenesis in humans and across the phylogeny. Hippocampus. 2022 Oct 18. doi: 10.1002/hipo.23474.

» Márquez-Valadez B, Rábano A, Llorens-Martín M. Progression of Alzheimer's disease parallels unusual structural plasticity of human dentate granule cells. Acta Neuropathologica Communications. 2022 Aug 29;10(1):125. doi: 10.1186/s40478-022-01431-7.

» Terreros-Roncal J, Moreno-Jiménez EP, Flor-García M, Rodríguez-Moreno CB, Trinchero MF, Cafini F, Rábano A, Llorens-Martín M. Impact of neurodegenerative diseases on human adult hippocampal neurogenesis. SCIENCE. 2021 Nov 26;374(6571):1106-1113. doi: 10.1126/science.abl5163.

» Terreros-Roncal J, Moreno-Jiménez EP, Flor-García M, Rodríguez-Moreno CB, Trinchero MF, Cafini F, Rábano A, Llorens-Martín M. Response to comment on "Impact of neurodegenerative diseases on human adult hippocampal neurogenesis". SCIENCE. 2022 Apr 15;376(6590):eabo0920. doi: 10.1126/science.abo0920.

» Terreros-Roncal J, Moreno-Jiménez EP, Flor-García M, Rodríguez-Moreno CB, Trinchero MF, Cafini F, Rábano A, Llorens-Martín M. Response to comment on "Impact of neurodegenerative diseases on human adult hippocampal neurogenesis". SCIENCE. 2022 Apr 15;376(6590):eabn7270. doi: 10.1126/science.abn7270.

» Flor-García M; Ávila J; Llorens-Martín M. GSK-3β S9A overexpression leads murine hippocampal neural precursors to acquire an astroglial phenotype *in vivo*. Developmental Neurobiology. 2021 Jul;81(5):710-723. DOI:10.1002/dneu.22823.

» Terreros-Roncal J; Flor-García M; Rábano A; Llorens-Martín M. Evidences for Adult Hippocampal Neurogenesis in Humans. Moreno-Jiménez EP; The Journal of Neuroscience. 2021 Mar 24;41(12):2541-2553.

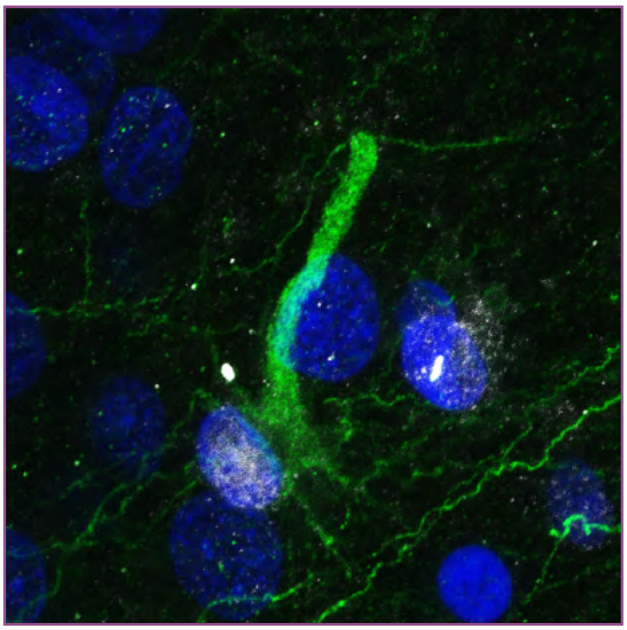


Image of the human dentate gyrus showing the presence of neural stem cells in the adult human hippocampus.

Participation in projects

» ERC Consolidator Grant 2020 (European Commission), ERC-CoG-2020-101001916. "HumAN: Interrogating human adult hippocampal neurogenesis". Type: European. PI: María Llorens-Martín. 10/01/2021 – 09/30/2026.

» Spanish Ministry of Science and Innovation: Programa Estatal I+D+i orientada a los retos de la sociedad. PID2020-113007RB-I00. "Maturation of new granule neurons in Alzheimer´s disease (MAGNA)". PI: María Llorens-Martín. 09/01/2021 – 08/31/2024.

» The Alzheimer´s Association RAPID Grant, AARG-17-528125-RAPID. "Novel methods to interrogate the subcellular machinery of AD models *in vivo*". Country: USA. PI: María Llorens-Martín. 01/01/2021 - 12/31/2022.

» The Alzheimer´s Association 2017 Research Grant, AARG-17-528125. "Novel methods to interrogate the subcellular machinery of AD models *in vivo*". Country: USA. PI: María Llorens-Martín. 01/01/2018 - 06/30/2021.

Other activities

» Third position, National Award to the best scientific paper of the year: Spanish National Newspaper "La Vanguardia" (Spain). 2022. (Terreros-Roncal et al Science. 2022).

» Spain National Young Researcher Award "Gabriela Morreale in Medicine and Health Sciences". Spanish Ministry or Research, Innovation and Universities (2022). María Llorens-Martín.

» Pfizer Foundation Young Investigator Award: Pfizer Foundation. 2022. María Llorens-Martín.

» Spain Royal Academy on Medicine Translational Medicine Award. 2022. María Llorens-Martín.

» CIBERNED National Young Investigator Award 2021. Julia Terreros-Roncal. Salamanca (Spain). 2022.

» Organización del Simposio internacional "Adult hippocampal neurogenesis in physiology and pathology (S27)" en el FENS Forum 2022. María Llorens-Martín.

HUMAN STEM CELL BIOLOGY IN TRANSLATIONAL NEUROSCIENCE

Group Members

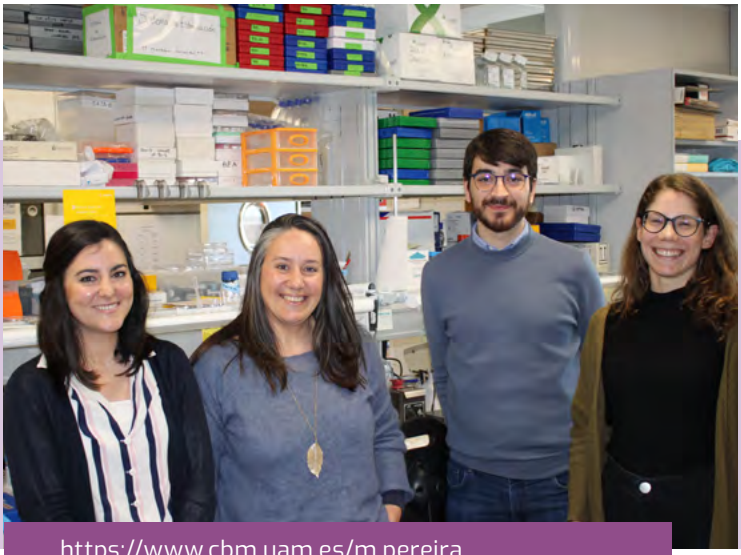
Principal Investigator:
Marta P. Pereira
Alberto Martínez Serrano
(until September 2022)

Scientific Staff:
Silvia García López
(until Dec 2021)

Postdoctoral Fellow:
Cristina Ulecia-Morón
(since Dec. 2022)

Predoctoral fellows:
Miguel Esteban Lucía
Marina Rodriguez Rubio
Brina Stančič

Undergraduate and Master Students:
Irene Párraga Borrell
Andrés Pordomingo González



https://www.cbm.uam.es/m.pereira

Summary

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

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

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

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

List of publications

» Vasudevan S, Dotti A, Kajtez J, Martínez-Serrano A, Gundlach C, Maçãs SC, Lauschke K, Vinngaard AM, López SG, Pereira M, Heiskanen A, Keller SS, Emnéus J. (2022) Omnidirectional leaky opto-electrical fiber for optogenetic control of neurons in cell replacement therapy. Bioelectrochemistry. 2023 Feb;149:108306. doi: 10.1016/j.bioelechem.2022.108306.

» Kajtez J, Wesseler MF, Birtele M, Khorasgani FR, Rylander Ottosson D, Heiskanen A, Kamperman T, Leijten J, Martínez-Serrano A, Larsen NB, Angelini TE, Parmar M, Lind JU, Emnéus J. (2022) Embedded 3D Printing in Self-Healing Annealable Composites for Precise Patterning of Functionally Mature Human Neural Constructs. Adv Sci (Weinh). 2022 Sep;9(25):e2201392. doi: 10.1002/advs.202201392

» Sánchez-González C, Herrero Martín JC, Salegi Ansa B, Núñez de Arenas C, Stančič B, Pereira MP, Contreras L, Cuezva JM, Formentini L. (2022) Chronic inhibition of the mitochondrial ATP synthase in skeletal muscle triggers sarcoplasmic reticulum distress and tubular aggregates. Cell Death Dis. 2022 Jun 22;13(6):561. doi: 10.1038/s41419-022-05016-z.

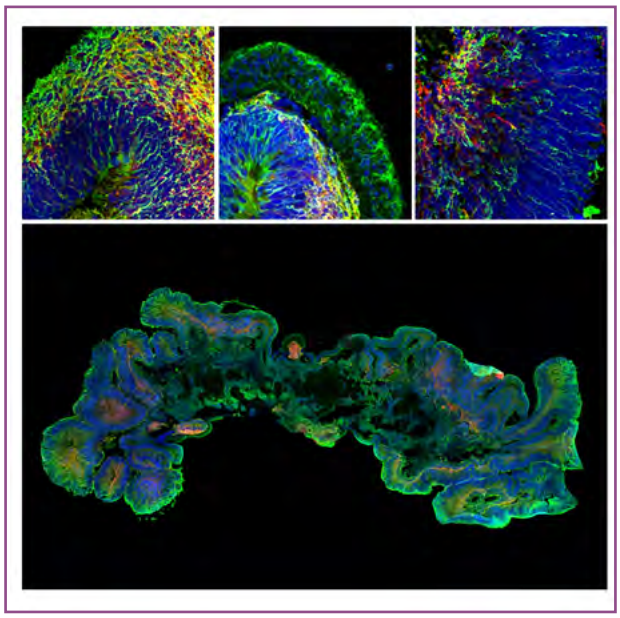
» Nelke A, García-López S, Martínez-Serrano A, Pereira MP. (2021) Multifactoriality of Parkinson's Disease as Explored Through Human Neural Stem Cells and Their Transplantation in Middle-Aged Parkinsonian Mice. Front Pharmacol. 2021;12:773925. doi: 10.3389/fphar.2021.773925.

» Shah FJ, Caviglia C, Zór K, Carminati M, Ferrari G, Sampietro M, Martínez-Serrano A, Emnéus JK, Heiskanen AR. (2021) Impedance-based Real-time Monitoring of Neural Stem Cell Differentiation. J Electr Bioimpedance. 2021 Nov 20;12(1):34-49. doi: 10.2478/joeb-2021-0006

» Rothenbücher TSP, Gürbüz H, Pereira MP, Heiskanen A, Emneus J, Martinez-Serrano A. (2021) Next generation human brain models: engineered flat brain organoids featuring gyrification. Biofabrication. 2021 Mar 16;13(1):011001. doi: 10.1088/1758-5090/abc95e.

» Alcover-Sanchez B, Garcia-Martin G, Escudero-Ramirez J, Gonzalez-Riano C, Lorenzo P, Gimenez-Cassina A, Formentini L, de la Villa-Polo P, Pereira MP, Wandosell F, Cubelos B. (2021) Absence of R-Ras1 and R-Ras2 causes mitochondrial alterations that trigger axonal degeneration in a hypomyelinating disease model. Glia. 2021 Mar;69(3):619-637. doi: 10.1002/glia.23917

» D. Martín-Hernández, C. Ulecia-Morón, AG. Bris, MP. Pereira, JR. Caso. (2021) Monoaminergic system and Antidepressants. In: CR. Martin, LA. Hunter, VB. Patel, VR. Preedy, R. Rajendram. The Neuroscience of Depression. Academic Press, Elsevier, ISBN 978-0-12-817933-8, pp. 345-355. doi: 10.1016/B978-0-12-817933-8.00093-1



Whole-brain organoid derived from human stem cells. Organized populations of neurons in different stages of maturation are present, with a variety of histological patterns (green, beta-III-tubulin; red, Map-2; blue,DAPI counterstain)

Participation in projects

» OpenMIND (Opto-Electronic Neural Connectoid Model Implemented for Neurodegenerative Disease). HORIZON-EIC-2021-PTHAFINDEROPEN-01 (European Innovation Council). From 2022 to 2025. Principal Investigator (CBMSO): Marta P. Pereira.

» ASCTN-Training (Training for Advanced Stem Cell Technologies in Neurology). H2020-MSCA-ITN-2018 (European Union). From 2018 to 2023. Principal Investigator (CBMSO): Alberto Martínez Serrano (co-leading Marta P. Pereira).

» TerCel. Red Temática de Investigación en Terapia Celular. RD16/0011/0032 (RETICS). Instituto de Salud Carlos III. From 2017 to 2021. Principal Investigator (CBMSO): Alberto Martínez Serrano.

» Brainfolding (Brain organoids: development and complexity). Ref. PDI2020-118189RB-I00 MICIN/AEI/10.13039/50110001133

Doctoral theses

» Camille Baumlin (2021) “Effects of a neuroprotective stem cell therapy involving Mesenchymal Stem Cells secreting GDNF in Parkinsonian mice from short to long-term”. Co-supervisors: Marta Pérez Pereira, Alberto Martínez Serrano

NEURAL STEM CELLS IN THE ADULT BRAIN: INTRINSIC AND EXTRINSIC FACTORS THAT REGULATE THEIR SELF-RENEWAL AND DIFFERENTIATION

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

Group Members

Principal Investigator:
Eva Porlan

Predoctoral fellows:
Ana L. Barrios Muñoz (until 09/2021)
Coral López Fonseca

Technicians:
Berta Alcover Sánchez (01/2022 – 04/2022)
Beatriz Ortigosa Fernández (from 01/2022)

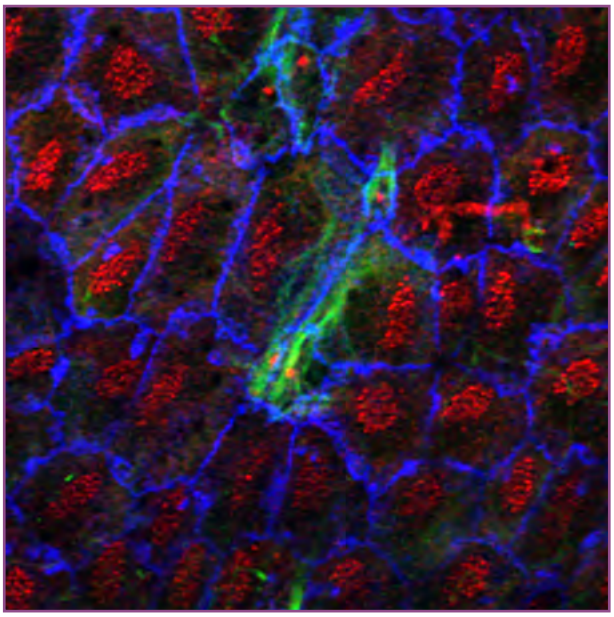
Undergraduate and Master Students:
Edurne Mugica Urruzola (09/2021-09/2022)

Summary

In adult vertebrates, somatic stem cells (SC) are self-renewing 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. Impairing the 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 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.

Polo like kinase 1 *(PLK1)* is an essential gene coding a druggable serine/threonine kinase with crucial roles. Although the canonical functions of PLK1 are related to cell proliferation, as to drive mitotic cell cycle and serving to establish a functional bipolar spindle during mitosis, unexpected mitosis-independent roles are emerging. In this regard, we have contributed to describing the role of Plk1 in the control of cell fate during embryonic neural progenitor division (Gonzalez-Martinez, et al., 2022), and we have established that Plk1 is a key player in adult NSC self-renewal and neuronal differentiation (Barrios-Muñoz, PhD Thesis, 2021).



Neural stem cells (green) at the surface of the lateral ventricle in the adult murine brain.

List of publications

» del Puerto, A., J. Pose-Utrilla, A. Simón-García, C. López-Menéndez, A. J. Jiménez, E. Porlan, L. S. M. Pajuelo, G. Cano-García, B. Martí-Prado, Á. Sebastián-Serrano, M. P. Sánchez-Carralero, F. Cesca, G. Schiavo, I. Ferrer, I. Fariñas, M. R. Campanero and T. Iglesias (2021). "Kidins220 deficiency causes ventriculomegaly via SNX27-retromer-dependent AQP4 degradation." Mol Psy 26(11): 6411-6426. doi: 10.1038/s41380-021-01127-9

» Gonzalez-Martinez, J., A. W. Cwetsch, J. Gilabert-Juan, J. Gomez, G. Garaulet, P. Schneider, G. de Carcer, F. Mulero, E. Caleiras, D. Megias, E. Porlan and M. Malumbres (2022). "Genetic interaction between PLK1 and downstream MCPH proteins in the control of centrosome asymmetry and cell fate during neural progenitor division." Cell Death Differ 29(8): 1474-1485. doi: 10.1038/s41418-022-00937-w

Participation in projects

» PID2019-104763RB-I00. Novel druggable regulators of adult neurogenesis and direct lineage reprogramming: implications for regeneration. Acronym: NeuroRepReg

AEI and FEDER.

Duration: 06/2020 - 12/2023.

PI: Eva Porlan.

Doctoral theses

» Ana Laura Barrios Muñoz (2021). Plk1, un nuevo regulador de la neurogénesis adulta. UAM. Eva Porlan.

Other activities

» The group belongs to the Instituto de Investigación Biosanitaria Hospital Universitario La Paz (IdiPaz) within the Research Group "Implication of glycinergic and glutamatergic systems in pathologies of the Central Nervous System".

THE GENETICS OF PSYCHIATRIC DISEASES

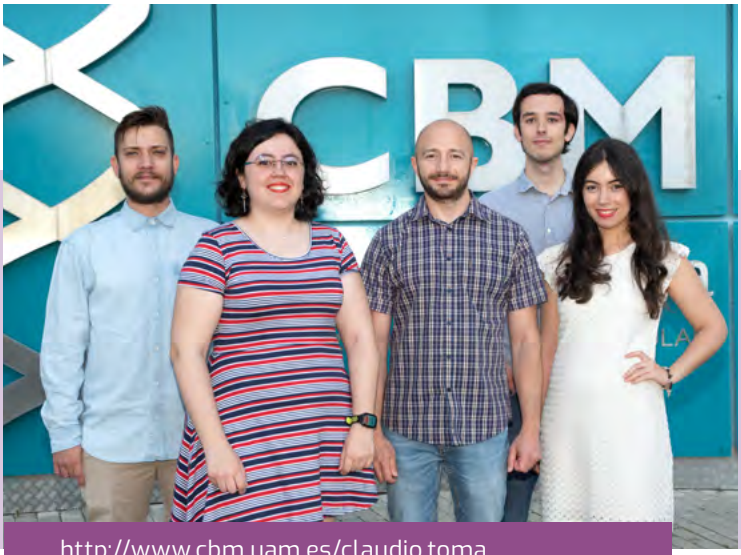
Group Members

Principal Investigator:
Dr Claudio Toma

PhD students:
Miriam Martínez Jiménez
Inés Garcia Ortiz

Undergraduate and Master Students:
José Ignacio Gómez Blanco (co-supervisor Serrano E)
Nerea Regueira Acebedo (until June 2021)

Technicians:
Sergio Espartero Boza



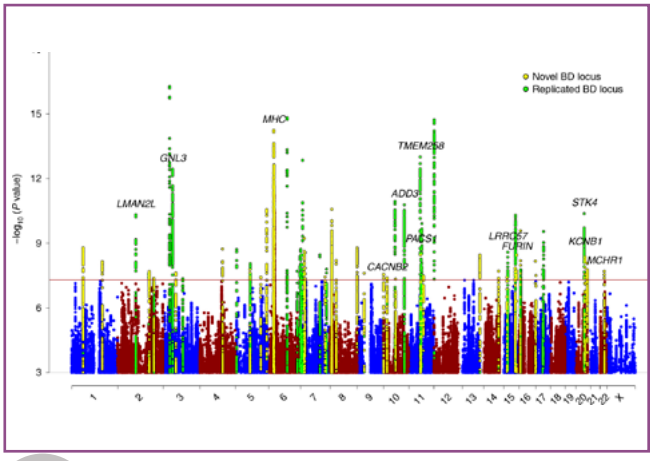
http://www.cbm.uam.es/claudio.toma

Summary

The identification of genes implicated in psychiatric diseases is our front-line research. Family studies have established a strong genetic contribution to psychiatric diseases, but the specific genes involved still remain largely unknown. Psychiatric disorders are caused by a combination of common variants, each with a small effect, and multiple rare variants of higher penetrance. Our aim is to identify susceptibility genes implicated in autism spectrum disorder (ASD), bipolar disorder (BD), and schizophrenia. In our lab we perform genomic approaches applied to psychiatry via next-generation sequencing (whole-exome sequencing, whole-genome sequencing, total RNA-sequencing), the examination of copy number variants, and the investigation of common variants through case-control and family-based association studies.

In the last years, we adopted a combination of genomic approaches using one of the largest cohort of families with multiple members affected by bipolar disorder. In these studies, we employed: 1) A novel powerful genomic approach by combining linkage analysis with exome sequencing showing that this method is effective for gene discovery. We performed a non-parametric linkage analysis and found a linkage peak (LOD score>3) on chromosome 10q11-q21, which is explained by rare pathogenic coding variants, including those from the BD-associated *ANK3* gene; 2) A combination of both transcriptomic and whole-genome sequencing in multiplex bipolar families, pinpointing novel putative susceptibility genes and pathways with biological significance for the disorder.

Recently, we established a novel Spanish node for psychiatric genetics and founded the Madrid Manic Group (MadManic) that brings together geneticists, bioinformaticians, clinicians, and psychiatrists aiming to develop novel translational tools applied in clinical practise combining genetic risk from genomic arrays, digital records from ecological momentary assessment (EMA) and machine learning approaches. Currently, we established one of the largest cohort of BD patients and controls in Spain with several biospecimens (DNA, RNA, and plasma). We created an electronic clinical protocol for recording in a unique platform (MeMind) clinical history of patients, response to medications, and behavioural scales. These instruments are allowing the contribution of our group to global consortia efforts for gene discovery, pharmacogenomics and clinical presentations for BD research. Recently, we participated in the largest genomic study in BD through a genome-wide association study (GWAS) meta-analysis of 57 cohorts (41,000 cases and 371,549 controls) (Figure 1), which identified 64 loci implicated in the disorder.



From Mullins et al., 2021 Nature Genetics. The x axis shows genomic position (chromosomes 1–22 and X), and the y axis shows statistical significance as –log10 (P value). The red line shows the genome-wide significance threshold (P < 5 × 10−8). Genome-wide significant loci are colored green for loci previously associated with BD and yellow for novel associations from this study.

List of publications

- Ollà I, et al. (2022) Pathogenic mis-splicing of CPEB4 in schizophrenia. BioRxiv. doi: 10.1101/2022.09.22.508890
- Zwicker A, et al. (2022) Polygenic scores and onset of major mood or psychotic disorders among offspring of affected parents. American Journal of Psychiatry, https://orca.cardiff.ac.uk/id/eprint/153290/
- Hesam-Shariatia S, et al. (2022) Epigenetic signatures relating to disease-associated genotypic burden in familial risk of bipolar disorder. Translational Psychiatry doi: 10.1038/s41398-022-02079-6
- Mullins N, et al. (2022) Dissecting the Shared Genetic Architecture of Suicide Attempt, Psychiatric Disorders, and Known Risk Factors. Biological Psychiatry; 91(3):313–327; doi: 10.1016/j.biopsych.2021.05.029
- Overs BJ, et al. (2021) Effects of polygenic risk for suicide attempt and risky behavior on brain structure in young people with familial risk of bipolar disorder. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 186(8):485-507 doi.org/10.1002/ajmg.b.32879
- Mullins N, et al. (2021) Genome-wide association study of more than 40,000 bipolar disorder cases provides new insights into the underlying biology. Nature Genetics; 53(6):817-829. doi: 10.1038/s41588-021-00857-4.
- Toma C*, et al. (2021) A linkage and exome study of multiplex families with bipolar disorder implicates rare coding variants of ANK3 and additional rare alleles at 10q11-q21. Journal of Psychiatry and Neuroscience; 46(2):247-257 doi:10.1503/jpn.200083. (*, corresponding author)
- Overs BJ, et al. (2021) Cortical mediation of relationships between dopamine receptor D2 and cognition are absent in youth at-risk Bipolar Disorder. Psychiatric Research doi: 10.1016/j.pscychresns.2021.111258.

Cover Image

- Volume 186B, Number 8, December 2021; Overs BJ, et al. (2021) Effects of polygenic risk for suicide attempt and risky behavior on brain structure in young people with familial risk of bipolar disorder. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics doi: 10.1002/ajmg.b.32879, https://onlinelibrary.wiley.com/toc/1552485x/2021/186/6

Participation in 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". PIs: Serrano E, Toma C (2022-24).
- Ministerio de Ciencia e Innovación (Proyectos I+D+i: PID2020-114996RB-I00): "Advances in understanding bipolar disorder through the combination of digital phenotyping and genome-wide approaches". PI: Toma (2021-2024).
- I-LINK program CISC (LINK-B-20068): "Whole-genome sequencing and pharmacogenomic studies in a large cohort of bipolar patients with electronic administrative health records". PIs: Toma C, Fullerton J, Schofield P (2021-22).
- MRFF-EPCDR Pharmacogenomics (Australia): "A multifaceted approach to the pharmacogenomic signatures of bipolar disorder for improving treatment outcomes phenotyping and genome-wide approaches". PIs: Fullerton J, Green M, Scholfied P, Toma C (2021-25)

Awards and recognition

- Associate editor for Frontiers in Psychiatry
- Associate editor for Frontiers in Genetics
- Associate editor for Frontiers in Neuroscience
- Editorial board member for Journal of Clinical Medicine (section psychiatry)
- Honorary appointment at Neuroscience Research Australia (NeuRA)
- Adjunct senior lecturer at School of Medicine, University of New South Wales Sydney
- PhD Fellowship in Neuroscience to Inés García Ortiz (Fundación Tatiana Pérez de Guzmán el Bueno)

Networks

- Psychiatric Genomics Consortium Bipolar Disorder (PGC-BD)
- Bipolar Sequencing Consortium (BSC)
- Global Bipolar Cohort (GBC)
- Consortium on Lithium Genetics (ConLiGen) (http://www.conligen.org/mem-es.html)
- Mood stabilizer Genomics Consortium (MoStGen)

MOLECULAR MECHANISMS OF NEURODEGENERATION

Group Members

Principal Investigator:
Francisco Wandosell Jurado

Postdoctoral fellows
Lara Ordoñez Gutierrez

Predoctoral fellows
Mario Villa
Sergio Rivas (until 2021)
Alba Orantes
Marta Garcia Juan

Undergraduate and Master Students
Laura Martín Martínez (until 2021)

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

Summary

Our group is interested in a series of neurodegenerative diseases, such as Alzheimer's, cerebral ischemia or brain tumours that are mostly associated with age. From the beginning as group leader, our studies focused on neuronal neurodegeneration in three brain pathologies (Alzheimer. Ischemia and glia-glioma transformation) related to age. We have focused on a signalling pathway modified in the three pathologies, PI3K-Akt-mTORC1.

We are analysing the role of the mTORC1 element as a central regulator of catabolism and anabolism. Regarding Alzheimer's disease, we have verified that mTORC1 inhibition has a therapeutic effect both *in vivo* and *ex vivo* (in primary cultures of neurons and glia) in a transgenic mouse model of AD. This inhibition produces a certain activation of autophagy that leads to a reduction in the amyloid load both in transgenic mice and in neurons and glia from these mice. At this time our work is focused on the analysis of how the different autophagy regulators can modify the generation/secretion of amyloid in this transgenic model.

Second, we focus on glioblastomas, age-related tumours, and recently published that some Akt-regulated elements (such as WIP and YAP/TAZ) are responsible for cell division of cancer stem cells and maintenance of their tumour phenotype.

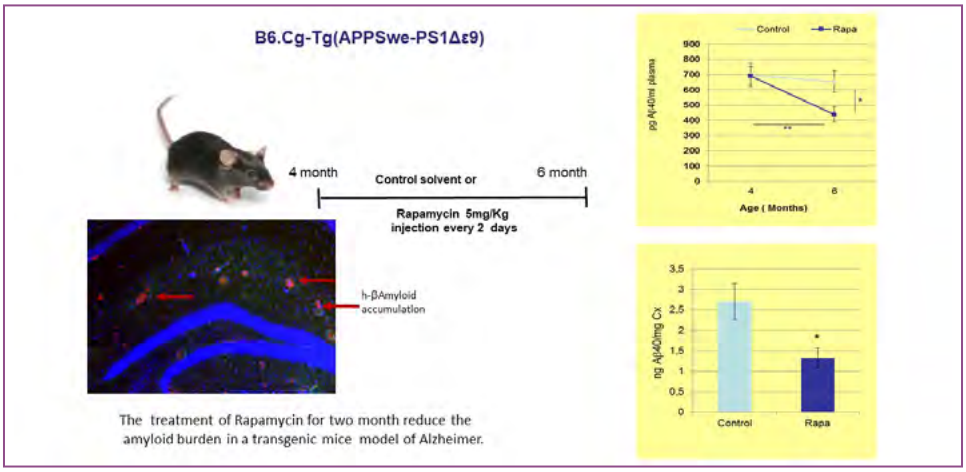
Our work now focuses on astrocyte-astrocytoma-glioma conversion trying to define the role of WIP as a regulator of the actin cytoskeleton and glioma proliferation. We have characterized by RNA-seq the differential expression corresponding to the elimination of WIP, YAP or TAZ in gliomas and we try to identify and study the elements regulated by them that control proliferation and the tumour phenotype in glioma.

Our project on Ischemia has been transferred to Dr. Mª José Pérez, who was the main responsible of this issue in our group, and who has become PI and project manager. We maintaining an active collaboration on this issue from a secondary role.

Doctoral theses

» Sergio Rivas Muñoz (16 de Diciembre de 2021), Unv. Autónoma de Madrid

» Directores: Dr. Francisco Wandosell y Drª Ines M. Anton



Therapeutic effect of rapamycin in a mouse model of AD. Chronic treatment of rapamycin (5mg/kg, every two days), during two months, reduced the human amyloid burden in plasma and in the brain of AD mice.

List of publications

» Benito-Cuesta I., Ordoñez L. and Wandosell F. (2021). "AMPK activation does not enhance autophagy in neurons in contrast to mTORC1 inhibition: different impact on β-amyloid clearance". Autophagy-17(3):656-671. doi.org/10.1080/15548627.2020.1728095.

» Klionsky, D. J., et al., (2021). "Guidelines for the use and interpretation of assays for monitoring autophagy (4th edition). Autophagy. 17-1: 1-382.ISSN 1554-8635

» Garcia-Martin,G., Alcover-Sanchez, B., Wandosell F. and B. Cubelos (2021). "Pathways involved in remyelination after cerebral ischemia". Current Neuropharmacology, .doi:10.2174/1570159X19666210610093658

» Antón I.M. and Wandosell F. (2021). WIP, YAP/TAZ and Actin Connections Orchestrate Development and Transformation in the Central Nervous System ". Frontiers in Cell and Develop. Biol. 9- 2021 | https://doi.org/10.3389/fcell.2021.673986.

» Otero-Losada M., Wandosell F.G. and Capani F. (2021). "Neuroprotection in Synaptic Signalling During Neurological Disorders ". Front. Synaptic Neurosci. -doi: 10.3389/fnsyn.2021.746487. (Editorial)

» Ordóñez-Gutiérrez L., Fábrias G., Casas J., and Wandosell F. (2021). "Diets with Higher ω-6/ω-3 Ratios Show Differences in Ceramides and Fatty Acid Levels Accompanied by Increased Amyloid-Beta in the Brains of Male APP/PS1 Transgenic Mice ". Int. J. Mol. Sci. 22, 10907. doi.org/10.3390/ijms222010907

» Benito-Cuesta, I., Ordoñez L. and Wandosell F.(2021). "Trehalose Reduces the Secreted Beta-Amyloid Levels in Primary Neurons Independently of Autophagy Induction ". Metabolites.; 11(7): 421. doi 10.3390/metabo11070421.

» Garcia-Martin,G., Sanz-Rodriguez,M.,Alcover-Sanchez, B. Pereira, M.P., Wandosell, F. & B.z Cubelos B.(2022) "R-Ras1 and R-Ras2 expression in anatomical regions and cell types of the central nervous system". Int. J. Mol. Sci. 2022, 23(2), 978; https://doi.org/10.3390/ijms23020978

» de la Cueva, M. Antequera, D., Ordoñez, L Wandosell, F., Camins, A., Carro E. and Bartolome F. (2022) "Amyloid-β impairs mitochondrial dynamics and autophagy in Alzheimer's disease experimental models". Sci Rep 12, 10092 (2022). https://doi.org/10.1038/s41598-022-13683-3.

Libros /Books

» Antón I. M., Wandosell, F. and Vicente-Manzanares M. (2022). "Cancer cell development, migratory response, and the role of the tumor microenvironment in invasion and metastasis ". Book in "Cell movement in Health and Disease", Chapter 15:". Edited by M. Schnoor, L.-M. Yin & S. X. Sun, Elsevier Editorial. eBook ISBN: 9780323901963

Participation in projects

NATIONAL GRANTS

» I+D+i-RETOS- RTI2018-096303-B-C1. "Papel de la vía AKT/YAP/TAZ en Patologias del Sistema Nervioso Central". (2019-2021) IP1- Francisco Wandosell; IP2- Inés M. Antón Gutiérrez

» I+D+i-RETOS-PID2021-124801NB-I00. "NUEVOS COMPONENTES DE LA RUTA WIP, YAP/TAZ EN SISTEMA NERVIOSO CENTRAL: PROLIFERACION VERSUS DIFERENCIACION" (2022-2024). IP1- Dr. F.Wandosell; IP2-Drª I. M. Antón Gutiérrez

» ISCIII-CIBERNED nº: CB06/05/0067 "Mecanismos Moleculares de Neurodegeneración" IP: Dr. F. Wandosell

UE GRANTS

» ERA-NET NEURON 2016 (PCIN-2016-20)*. "Regulation of the voiding reflex after spinal cord injury: abolition by silencing of hyper-excited bladder C-fiber afferents by gene therapy to restore continence and voiding"- ELPIS. Coordinator: Pr. François Giuliano (Francia); PI-(CBM-CSIC): F Wandosell (Spain) *Proyectos prorrogados por Pandemia

MOLECULAR BASIS OF NEUROTRANSMISSION AND ITS IMPLICATION IN NEUROPATHOLOGY

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

Group Members

Principal Investigator:
Francisco Zafra

Predoctoral fellows:
Dolores Piniella

Technicians:
Enrique Núñez

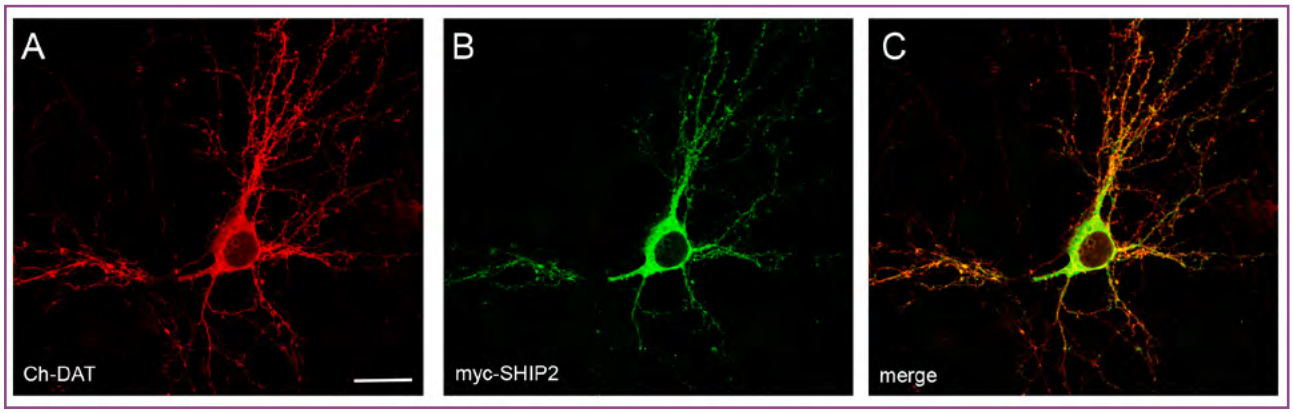
Master and undergraduate students:
Ania Canseco Rodríguez
Alejandro Maestre Guillén.

Summary

Our laboratory is interested in the molecular mechanisms of neurotransmission and its relationship with various pathologies of the nervous system. Communication between neurons implies an interplay between excitatory pathways mediated by glutamate and inhibitory neurotransmission mediated by GABA and glycine. The modulating action of less abundant neurotransmitters, such as dopamine, is added on top of these major pathways. This neurotransmitter crosstalk is controlled by a series of membrane proteins including ion channels, receptors and transporters. Alterations in these proteins are associated with diseases such as stroke, epilepsy or schizophrenia. Our objectives include the study of the interactions between some of these proteins and their environment, represented by the interactome and the lipidome. By using proximity labeling proteomic techniques we have been able to find new proteins and lipids that control the activity of the dopamine transporter. On the other hand, we have been able to find new interactions with the AKT1 signaling pathway with the sodium ion channel (NaV1.1), which controls the activity of GABAergic neurons and whose alteration is associated with a form of severe epilepsy called Dravet syndrome. We have also investigated regulatory mechanisms of glycine transporters, which participate in the transmission of pain in the spinal cord and of sensory information in the retina. In a model of neuropathic pain, we have obtained evidence indicating a readjustment of the levels of the glycinergic marker GlyT2 in response to microglia activation. In the retina, we have described a new regulatory mechanism by microRNAs that adjusts the levels of the GlyT1 to the intensity of light.

During this time, we have maintained collaborations with the groups of Dr. F.J. Díez-Guerra, Drs. B. López-Corcuera (CBMSO) and C. Avendaño (UAM) on the role of glycinergic pathways; Drs. A. Rodríguez Artalejo (UCM) and D. Bartolomé-Martín (la Laguna) in the study of the activity of the sodium and potassium channels. We have also collaborated with clinicians of the Sant Joan de Déu Hospital in Barcelona (Dr. J. Armstrong), the San Sebastián University Hospital (Dr. I. Martí-Carrera) and the University Hospital of Mostoles (Dr. A. Díaz de Bustamante) with whom we have participated in the investigation of the pathogenic mechanisms underlying a form of epilepsy due to mutations in the GABA transporter.

In summary, our work deepens our understanding of the mechanisms of communication in the brain under physiological and pathological conditions.



The dopamine transporter (Ch-DAT) and phosphoinositide phosphatase SHIP2 (in green) show extensive colocalization when co-expressed in neurons

List of publications

» Martinez-Lozada, Z., Hewett, S.J., Zafra, F. and Ortega, A. (2022) The known, the unknown, and the future of glutamate transporters. Front. Cell Neurosci. 16, 1005834. doi: 10.3389/fncel.2022.1005834

» Zafra, F. and Piniella, D. (2022) Proximity labeling methods for proteomic analysis of membrane proteins. J. Proteomics. 264. 104620. doi: 10.1016/j.jprot.2022.104620

» Jiménez, E., Piniella, D., Giménez, C. and Zafra, F. (2022) Regulation of the Glycine Transporter GLYT1 by microRNAs. Neurochem. Res. 47(1), 138-147. doi: 10.1007/s11064-021-03228-x

» Piniella, D., Martínez-Blanco, E., Bartolomé-Martín, D., Sanz-Martos, A.B. and Zafra, F. (2021) Identification by proximity labeling of novel lipidic and proteinaceous potential partners of the dopamine transporter. Cell. Mol. Life Sci. 78(23), 7733-7756. doi: 10.1007/s00018-021-03998-1

» Arribas-Blázquez, M., Piniella, D., Olivos-Oré, L.A., Bartolomé-Martín, D., Leite, C., Giménez, C., Artalejo, A.R. and Zafra, F. (2021) Regulation of the voltage-dependent sodium channel NaV1.1 by AKT1. Neuropharmacology 197, 108745. doi: 10.1016/j.neuropharm.2021.108745

» García-Magro, N., Martin, Y.B., Negredo, P., Zafra, F. and Avendaño, C. Microglia and Inhibitory Circuitry in the Medullary Dorsal Horn: Laminar and Time-Dependent Changes in a Trigeminal Model of Neuropathic Pain. Int. J. Mol. Sci. 22(9), 4564. doi: 10.3390/ijms22094564

Participation in projects

» 2019-2021. “Papel de los microRNAs y los exosomas en la inducción de la tolerancia isquémica en el cerebro”. Ministerio de Ciencia e Innovación RTI2018-098712-B-I00. PI: Francisco Zafra.

Doctoral theses

» Dolores Piniella Alcalde (2021). “Identificación de nuevas proteínas que interaccionan con los transportadores de glutamato y dopamina (GLT-1 y DAT) mediante alteraciones en sus respectivos entornos”. Universidad Autónoma de Madrid. Supervisors: F. Zafra and C. Giménez.

Physiological and Pathological Processes

Metabolic and Signaling Networks in disease Unit

Laura Formentini

Complementary and joint research activities within the Metabolic and Signaling Networks in Disease Unit aim to decipher the molecular, cellular, and signaling pathways involved in metabolic homeostasis, in the context of human diseases. These activities include unveiling the molecular mechanisms of both prevalent and rare disorders, with the goal of identifying new therapeutic targets and molecules that regulate or are regulated by metabolism.

Using diverse cellular and animal models, as well as patient samples, we have contributed to the elucidation of the role of membrane signaling pathways during the onset and progression of metabolic, cardiovascular, skin and inflammatory diseases, as well as cancer. We have also studied cellular processes that promote the dysregulation of mitochondrial function and other underlying pathophysiological events in cancer, metabolic syndrome, kidney fibrosis, cardiovascular diseases, and rare metabolic diseases. We are also committed to transfer our results to the clinic, to advance in the diagnosis, prognosis, and treatment of these pathologies. This is reflected for example in our studies towards repurposing of drugs for the treatment of cancer, insulin resistance, and metabolic disorders.

The following aims represent our core research interests:

- Understanding the role of mitochondrial metabolites (NAD+, ATP) in the regulation of innate and adaptive immune responses.
- Elucidating the crosstalk between metabolism and organ fibrosis.
- Clarifying how cancer cells adapt to unfavorable tumor microenvironment by rewiring their mitochondrial metabolism.
- Decoding the molecular mechanism underlying metabolic sex-differences in health and disease.
- Unveiling the mechanistic implication of IF1 in regulating the activity and super-assembly of the mitochondrial H+-ATP-synthase in different systems and animal models.

- Understanding calcium regulation of mitochondrial function by way of the calcium-dependent mitochondrial carriers of aspartate-glutamate (Aralar, citrin) and ATP-Mg/Pi (SCaMCs).
- Deciphering the GRK2-based signalosomes on cell cycle dynamics and breast cancer proliferation versus senescence, instability and dissemination under the influence of hormonal, metabolic and micro-environmental stresses
- Elucidating the role of muscular mitochondrial perturbations in metabolic disorders and during ageing.
- Revealing the connection between non-canonical Gq signaling and cell homeostasis by deciphering the new interactome of Gαq, with emphasis on autophagy/exosome trafficking, endothelial dysfunction/inflammation and tumor microenvironment/cancer progression
- Studying the impact of GRK2 deletion in keratinocytes in the skin immune cell landscape, barrier function, skin-microbiome interaction, hair follicle homeostasis and squamous cell carcinoma progression.
- Using multiomic layers and genomics in the diagnosis of patients with rare diseases. Research in advanced therapies (small molecules, RNA, etc.)
- Investigating redox-dependent mechanisms of cardioprotection in ischemia-reperfusion injury.
- Modulating GRK2-dependent adipose-liver crosstalk in high fat diet-induced obesity.
- Studying the physiopathology of neurometabolic diseases in iPSC generated from patients' fibroblasts and differentiated to relevant cellular lineages.
- Generating new CRISP/Cas gene edited cellular and animal models of disease with patient specific splicing mutations, to develop correcting RNA therapies.
- Implementing a novel technological platform that allows to analyze the N-glycome of biological samples.

Eduardo Balsa Martinez
MITOCHONDRIAL DYSFUNCTION IN METABOLIC DISEASES

Pedro Bonay Miarons
FUNCTIONAL GLYCOGENOMICS

Susana Cadenas Álvarez
MITOCHONDRIAL PATHOPHYSIOLOGY

Sara Cogliati
MOLECULAR MECHANISMS OF SEX-DIFFERENCES IN METABOLISM PHYSIOLOGY AND DISEASE

José M. Cuezva
THE ROLE OF MITOCHONDRIA IN HUMAN PATHOLOGY

Laura Formentini
ROLE OF MITOCHONDRIAL METABOLISM ON THE PATHOPHYSIOLOGY OF SKELETAL MUSCLE

Santiago Lamas Peláez
MOLECULAR PATHOPHYSIOLOGY OF FIBROSIS

Federico Mayor Jr
PATHO-PHYSIOLOGICAL IMPLICATIONS OF G PROTEIN-COUPLED RECEPTORS SIGNALING NETWORKS

Petronila Penela
CELLULAR SIGNALING NETWORKS IN CANCER (ONCO-RESECEL)

Belén Pérez
TRANSLATIONAL MEDICINE IN INBORN ERRORS OF METABOLISM AND OTHER RARE GENETIC DISEASES

Catalina Ribas
REGULATORY FUNCTIONS AND MECHANISMS OF CELL SIGNALING PATHWAYS THROUGH G PROTEINS: A NEW INTERACTOME

Lourdes Ruiz Desviat
PHYSIOPATHOLOGY STUDIES AND THERAPEUTIC APPROACHES IN ANIMAL AND CELLULAR MODELS OF NEUROMETABOLIC DISEASES

Jorgina Satrústegui Gil-Delgado / Beatriz Pardo Merino / Araceli del Arco Martínez / Cayetano von Kobbe Alonso
CALCIUM SIGNALING IN MITOCHONDRIA: METABOLIC CONTROL AND MITOCHONDRIAL PHYSIOPATHOLOGY

Javier Traba Domínguez
MITOCHONDRIAL BIOLOGY IN IMMUNE MODULATION

MITOCHONDRIAL DYSFUNCTION IN METABOLIC DISEASES

Group Members

Principal Investigators (PI, co-PI):
Eduardo Balsa Martinez

Postdoctoral fellows:
Alba Roca Portoles, Sara Laine Menendez and Mauro Agro (10/01/2020-01/10/2021)

Postdoctoral fellows:
Alba Roca Portoles
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https://www.cbm.uam.es/balsalab

Summary

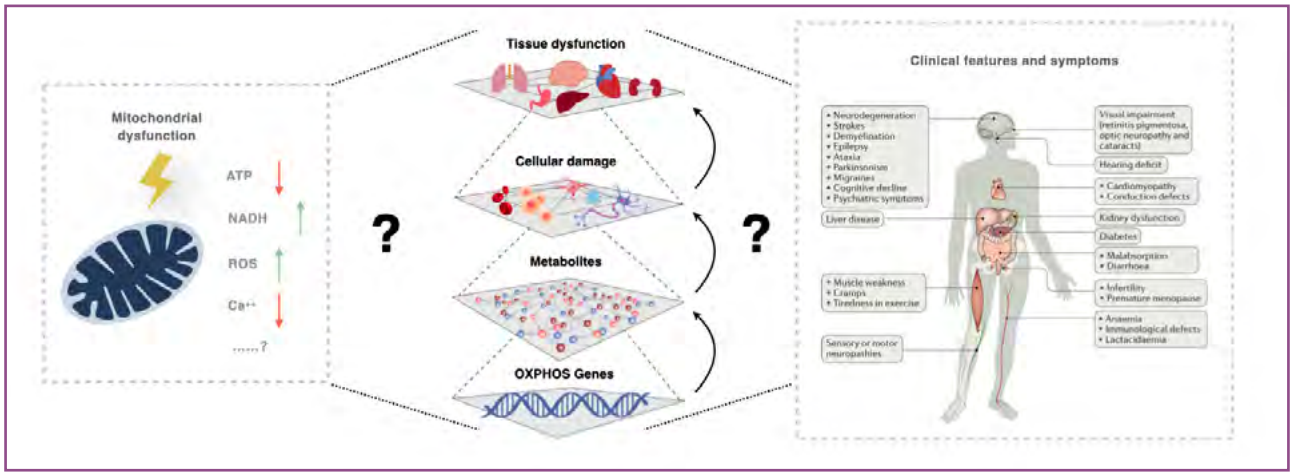
Mitochondria are unique and complex organelles that carry out critical metabolic functions within the cells. Once considered to be mere sites of ATP generation, it is now evident that these organelles participate in a wide range of cellular processes including calcium homeostasis, apoptosis, redox balance or cell fate. Because of this multifaceted contribution of mitochondria to key biologic and metabolic pathways it is not surprising that mitochondrial dysfunction has been linked to many human disorders including neurodegeneration, diabetes, cancer or aging.

Specifically, our lab focuses on defects in the oxidative phosphorylation system (OXPHOS) occurring from mitochondrial disease mutations that compromise cellular fitness and survival. This biochemical failure is thought to underlie pathologies associated with mitochondrial dysfunction. However, the precise metabolic processes, signaling pathways and compensatory responses resulting from a defective mitochondrial Electron Transport Chain (ETC) that drive these fatal disorders are not entirely understood. Although diminished ATP production has been considered a hallmark of mitochondrial dysfunction, our recent discoveries highlighted that other metabolic failures such as disturbed redox hemostasis due to accumulated levels of NADH can be equally detrimental. Moreover, which cell types contribute the most to the disease and whether disease-carrying cells negatively impact the function of its surrounding wild-type neighbors or distant organs remain poorly characterized.

The long-tern goal of our lab is to understand the molecular components that regulate mitochondrial metabolism, in the context of physiology and diseases, and use this knowledge to develop successful therapies.

We are currently exploring two central areas. First, we aim to elucidate the molecular mechanisms whereby mitochondrial dysfunction compromise cellular fitness and leads to organ failure in the context of human diseases. Second, we focus on understanding the metabolic vulnerabilities of metastasizing cancer cells and to define novel therapeutic approaches to prevent cancer progression.

To accomplish these goals, we are employing cutting-edge technologies such CRISPR/Cas9-based genetic screenings, multi-omics platform, single cell clonal tracking and preclinical mouse models.



Mitochondrial dysfunction leads to abnormalities such decreased ATP levels, overproduction of Reactive Oxygen Species (ROS) or accumulation of NADH that drive human pathologies. However, the precise metabolic processes, signaling pathways and compensatory responses resulting from these biochemical abnormalities are not entirely understood. Our work focused on developing a holistic understanding of the molecular and metabolic components that contribute to cell and tissue deterioration in the context of mitochondrial dysfunction.

List of publications

Articles:

» Sevilla-Montero J, Munar-Rubert O, Pino-Fadón J, Aguilar-Latorre C, Villegas-Esguevillas M, Climent B, Agrò M, Choya-Foces C, Martínez-Ruiz A, Balsa E, Muñoz-Calleja C, Gómez-Punter RM, Vázquez-Espinosa E, Cogolludo A, Calzada MJ. (2022) Cigarette smoke induces pulmonary arterial dysfunction through an imbalance in the redox status of the soluble guanylyl cyclase. Free Radic Biol Med. DOI: 10.1016/j.freeradbiomed.2022.09.026. Afiliación al CBM

» Bennett CF, O'Malley KE, Perry EA, Balsa E, Latorre-Muro P, Riley CL, Luo C, Jedrychowski M, Gygi SP, Puigserver P. (2021) Peroxisomal-derived ether phospholipids link nucleotides to respirasome assembly. Nature Chemical Biology. DOI: 10.1038/s41589-021-00772-z

» Latorre-Muro P, O'Malley KE, Bennett CF, Perry EA, Balsa E, Tavares CDJ, Jedrychowski M, Gygi SP, Puigserver P. (2021) A cold-stress-inducible PERK/OGT axis controls TOM70-assisted mitochondrial protein import and cristae formation. Cell Metabolism. DOI: 10.1016/j.cmet.2021.01.013

» Perry EA, Bennett CF, Luo C, Balsa E, Jedrychowski M, O'Malley KE, Latorre-Muro P, Ladley RP, Reda K, Wright PM, Gygi SP, Myers AG, Puigserver P. (2021) Tetracyclines promote survival and fitness in mitochondrial disease models. Nature Metabolism. DOI: 10.1038/s42255-020-00334-y

Participation in projects

» ERC Starting Grant (2020 ERC-Stg) 948478 -MitoCure-. Funded by the EC-European Research Council. 01/01/2021 - 31/12/2025. Coordinator/PI: Eduardo Balsa Martinez.

» Regulación molecular y metabólica de las enfermedades mitocondriales. PID2019-110766GA-I00, funded by AEI: 01/01/2020 end 31/12/2022. PI: Eduardo Balsa Martínez.

FUNCTIONAL GLYCOGENOMICS

Group Members

Principal Investigator:
Pedro Bonay Miarons

Undergraduate and Master Students:
Raquel Lorenzo Marco
Maria del Rosario Luaces Serrano
Silvia Novo Acevedo
Antonio Perez Gonzalesz

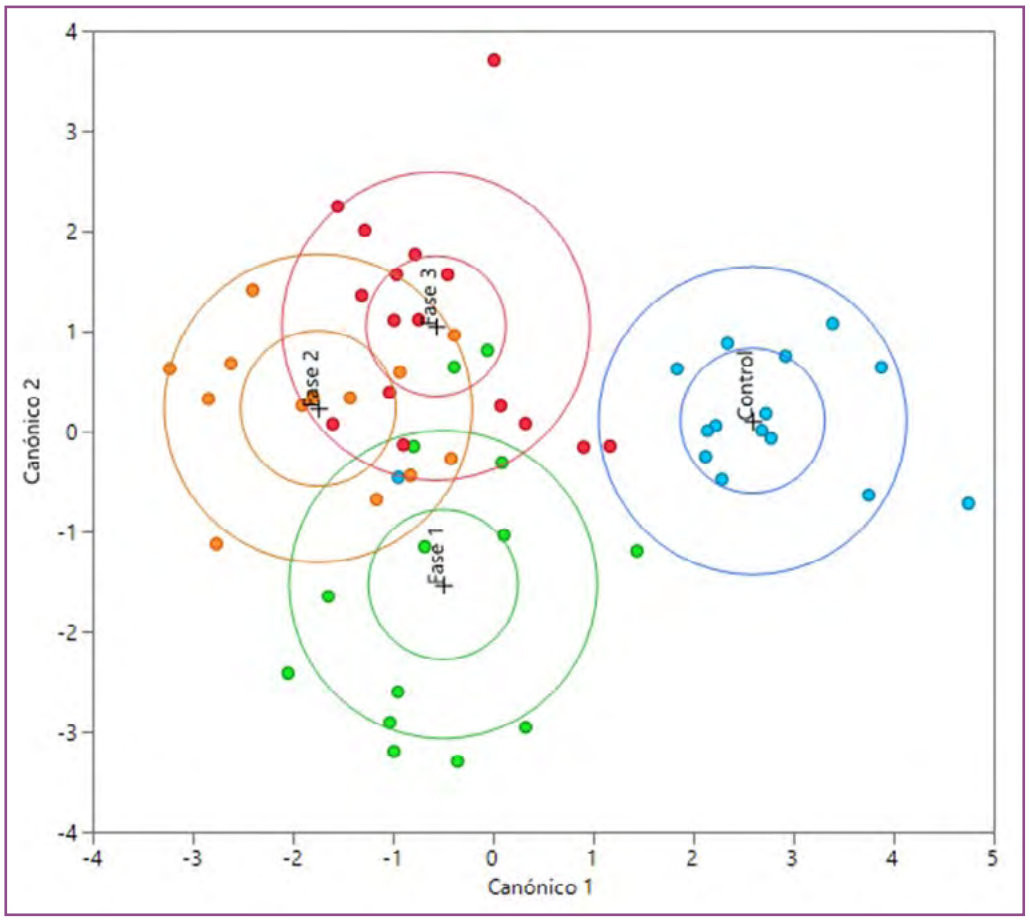
https://www.cbm.uam.es/pbonay

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 Croatia 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.



Discriminant analysis derived from 24 structural glycan traits (Fig. 1) showing the clear resolution between Control healthy subjects (blue dots) and the different stages of Cardiac symptomatic stages patients from American trypanosomiasis.

Other activities

The PI has been awarded one more year as the national representative from Spain at the International Glycoconjugate Organization (IGO, associated to IUB-MB since 1989) and The Societyy of Glycobiology The roles inside the IGO and now at the SG, are to further international collaboration for the study of glycoconjugates, survey the academic activities on Glycosciences around the globe and to define the roadmap to integrate the glycosciences as a significant part of the science curriculum in the high-school, undergraduate and graduate education.

MITOCHONDRIAL PATHOPHYSIOLOGY

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

Group Members

Principal Investigator:
Susana Cadenas Álvarez

Predoctoral fellow
Ana Mata Villanueva

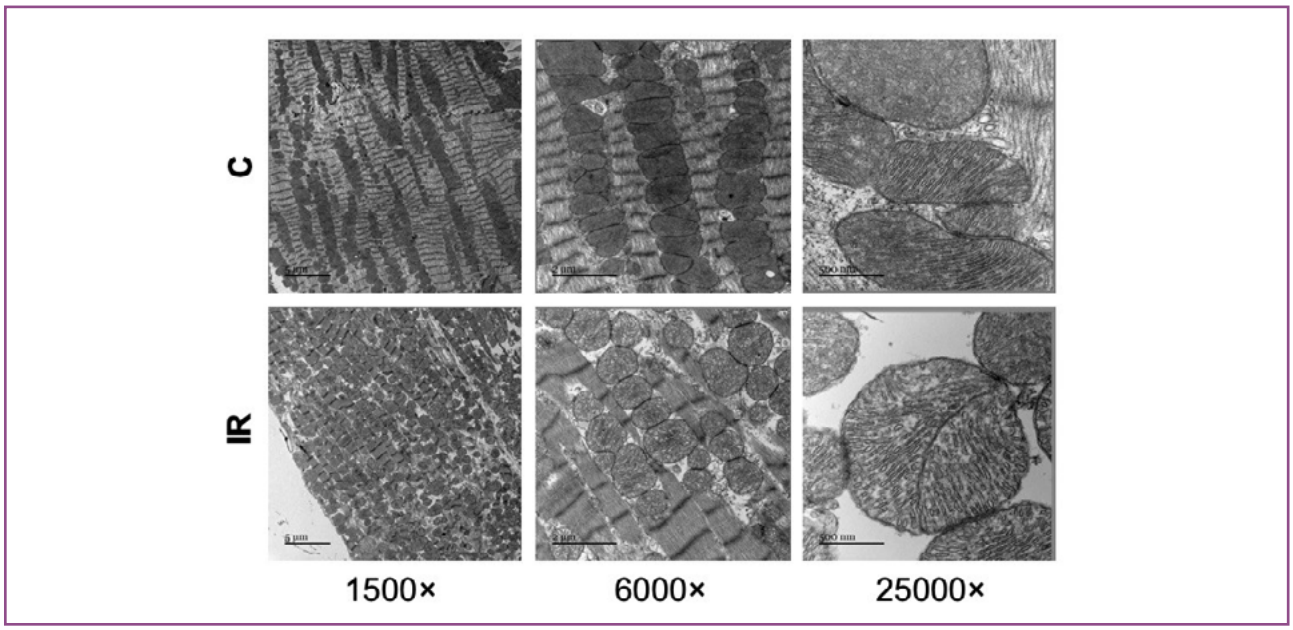
Undergraduate students
Sara Natalia Jaroszewicz (September 2021-June 2022)

Summary

Mitochondria are an important source of reactive oxygen species (ROS) in mammalian cells. The production of ROS by mitochondria underlies oxidative damage in many pathologies and contributes to redox signaling. Research in our lab is focused on the role of mitochondria and mitochondria-derived ROS in cell physiology and in the development of pathological conditions. In particular, we are interested in myocardial ischemia-reperfusion (IR) injury, which contributes to adverse cardiovascular outcomes after myocardial ischemia, cardiac surgery or circulatory arrest. During myocardial infarction, the lack of oxygen and nutrients induces ischemic injury. The most effective treatment to limit infarct size is early reperfusion. Reperfusion injury consist of the paradoxical exacerbation of cellular damage following restoration of blood flow to previously ischemic tissues. Myocardial IR injury is mediated by several factors including excessive ROS production, which are generated mainly at reperfusion. The combined effects of ROS and elevated calcium concentration lead to the opening of the mitochondrial permeability transition pore, which plays a critical role in reperfusion injury.

Our recent studies have focused on the protective role of the mitochondrial uncoupling protein UCP3 in cardiac IR injury. UCP3 is involved in the control of the production of mitochondrial ROS and in the protection against oxidative damage. We have found that hearts from mice lacking UCP3 subjected to IR have larger infarct size than those from wild-type mice. However, suppression of superoxide production in UCP3 deficient hearts decreases infarct size. UCP3 deficiency affects cardiac metabolism after IR, particularly fatty acid oxidation. Myocardial IR adversely affects mitochondrial ultrastructure, reduces the number of inter-mitochondrial junctions and increases the accumulation of lipid droplets, effects that are more evident in hearts lacking UCP3.

The transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) is a critical regulator of the cellular stress response. In response to different activation stimuli, Nrf2 translocates from the cytoplasm to the nucleus, where it activates the transcription of its downstream targets by binding to the antioxidant response element (ARE) or the electrophile response element (EpRE). Nrf2 is believed to control de basal and inducible expression of a large number of genes involved in antioxidant defense, detoxification, inflammatory response, proteasomal and autophagic degradation, and metabolism, reflecting its multiple cellular functions. We are currently studying the cardioprotective role of Nrf2 in myocardial IR injury and its possible involvement in ischemic preconditioning. These studies have potential clinical value as they might help develop therapeutic strategies for cardioprotection.



Electron micrographs depicting mitochondrial morphology of mouse hearts following ex vivo ischemia-reperfusion. C, control; IR, ischemia-reperfusion.

List of publications

» Cadenas S. (2022) Mitochondria rescue cells from ischemic injury. Science 377, 579-580. doi: 10.1126/science.add4629

» Jiménez-Villegas J., Kirby J., Mata A., Cadenas S., Turner M.R., Malaspina A., Shaw P.J., Cuadrado A., Rojo A.I. (2022). Dipeptide repeat pathology C9orf72-ALS is associated with redox, mitochondrial and NRF2 pathway imbalance. Antioxidants 11, 1897. doi: 10.3390/antiox11101897

» Mata A., Cadenas S. (2021). The antioxidant transcription factor Nrf2 in cardiac ischemia-reperfusion injury. Int. J. Mol. Sci. 22, 11939. doi: 10.3390/ijms222111939

Participation in projects

» "Redox-sensitive mechanisms for cardioprotection in ischemia-reperfusion injury" (PI19/01030). Spanish Ministry of Health. Fondo de Investigación Sanitaria (FIS), Instituto de Salud Carlos III. PI: S Cadenas. Funded period: 2020-2022

» We participate in the COST Action BenBedPhar CA20121 (2021-2025).

» Our group belongs to the "Instituto de Investigación Sanitaria del Hospital Universitario de La Princesa". http://www.iis-princesa.org

MOLECULAR MECHANISMS OF SEX-DIFFERENCES IN METABOLISM PHYSIOLOGY AND DISEASE

Group Members

Principal Investigator:
Sara Cogliati

Scientific Staff:
Asier Collado, Research assistant, Programa Yo Investigo (from October 2022)

Predoctoral fellows:
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Technicians:
Ana Sagrera Aparisi (from January 2021)

Undergraduate and Master Students :
Lucía Gonzalez Colmenarejo TFG (February- June 2022)
José García-Consuegra Martínez TFM (February-June 2022)
Stefano Vecchione, Erasmus Student TFM (from October 2022)

www.cbm.uam.es/scogliati

Summary

Our laboratory aims to understand the molecular mechanisms of metabolic sex differences in health and disease, explicitly exploring mitochondria's role. Indeed, mitochondria are the central hub of metabolism and targets of sexual hormones, with a suggested role in modulating sex-specific differences in many physio-pathological conditions.

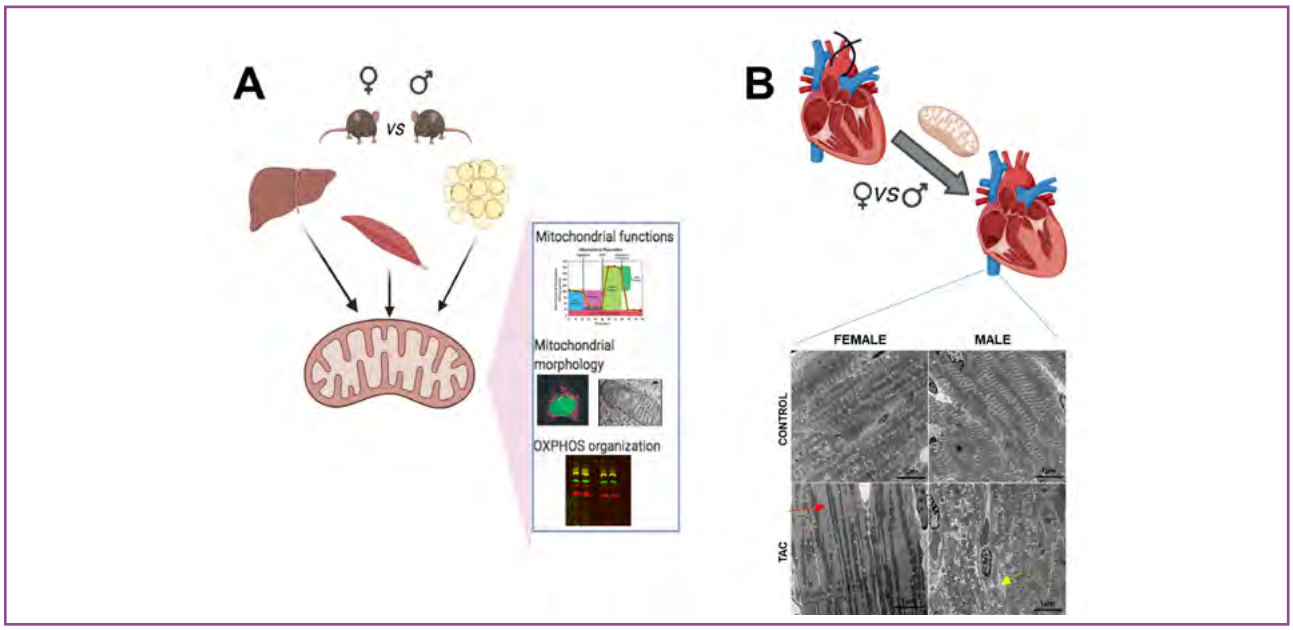
We are currently running two projects: one considering metabolism and one cardiovascular disease.

Since metabolism shows considerable differences between the two sexes, we are exploring the hypothesis that mitochondrial functions can be differently modulated in males and females and therefore determine important physiological differences.

To prove this, we are performing a wide analysis of mitochondrial functions and morphology, together with gene expression analysis and metabolomics approach (in collaboration with Christian Frezza's laboratory, CECAD, Cologne). Our target tissues are the liver, muscle, and white adipose tissue of males and females mice, further clustered according to the estrous phase.

In parallel, to be able to study the role of sex steroids and different growth conditions on mitochondrial functions in vitro, we are generating two fibroblast cell lines from female and male mice that constitutively over-express the estrogen and androgen receptors.

Cardiovascular disease is the first death cause in women worldwide. Nowadays, we know that biological sex has a strong impact on cardiovascular performance however the molecular mechanisms are still unknown. In our lab, we aim to understand the mitochondrial role in the development of heart failure, a pathological condition that presents important clinical sex differences. Applying a biochemistry approach, electronic microscopy, and gene expression analysis, we are mapping the mitochondrial differences between male and female mice during the progression of heart failure. The ultimate goal is to identify some mitochondrial pathways that could be potential therapeutic targets for heart failure. Our preliminary data suggest that after transaortic constriction, fertile female mice are protected from cardiac hypertrophy and this correlates with less fibrosis and the maintenance of the mitochondrial network analyzed by electron microscopy.



(A)Characterization of the mitochondrial functions in liver, muscle, and white adipose tissue of male and female mice. This project aims to identify the mitochondrial functions responsible for the sex differences in metabolism.

(B)Characterization of the mitochondrial role in the sex differences of heart failure. After inducing heart failure by the trans-aortic constriction technique, we analyze the mitochondrial function in the molecular mechanisms leading to heart failure in male and female mice. Preliminary data from electron microscopy micrographs show that female mice after trans-aortic constriction preserve the mitochondrial network (red arrow) while males develop more fibrosis (yellow arrow). (Figure created with Biorender.com)

List of publications

» Cogliati S, Cabrera-Alarcón JL and Enriquez JA (2021). Regulation and functional role of the electron transport chain supercomplexes. Bioch Soc Trans. 49(6):2655-2668 doi: 10.1042/BST20210460

» Cogliati S*, Herranz F, Ruiz-Cabello J and Enríquez JA * (*co-correspondent authors) (2021). Digitonin concentration is determinant for mitochondrial supercomplexes analysis by BlueNative Page. BBA-Bioenergetics, 1862:1-7. doi: 10.1016/j.bbabio.2020

» Benegiamo G, Bou Sleiman M, Wohlwend M, Rodríguez-López S, Goeminne LJE, Laurila PP, Klevjer M, Salonen MK, Lahti J, Jha P, Cogliati S, Enriquez JA, Brumpton BM, Bye A, Eriksson JG, Auwerx J. (2022). COX7A2L genetic variants determine cardiorespiratory fitness in mice and human. Nat Metab. 4(10):1336-1351. doi: 10.1038/s42255-022-00655-0.

Participation in projects

» Sex-differences in glucose metabolism: characterization of the mitochondrial role. (MEMIX). Agencia estatal de investigación. PI: Sara Cogliati. From 01/09/2021 to 31/08/2024.

THE ROLE OF MITOCHONDRIA IN HUMAN PATHOLOGY

Group Members

Principal Investigator:
José M. Cuezva

Postdoctoral Fellows
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Alba Roca Portoles (until June 2021)
Jesús Vallejo Diaz (until March 2022)

Predoctoral Fellows
Laura Torresano Cicuendez (until July 2022)
Inés Romero Carramiñana
Sonía Dominguez Zorita

Technicians
Cristina Núñez de Arenas
Helena Vázquez Gámez
Brenda Sánchez Garrido (until December 2021)
Rocío Moreno Palomares (April-December 2022)
Verónica Romero Albillo (September-December 2022)

Undergraduate and ERASMUS students
Teresa Manchón Campillo (June 2021)
Cristian Andres Carmona Carmona (University of Verona, Italy, 2021)
Katarina Majerik Behinska (Slovak University in Nitra, 2022)

http://www.cbmso.es/jmcuezva

Summary

Mitochondria play key roles in cellular metabolism, bioenergetics, the execution of cell death and intracellular signaling. Consistent with its prime physiological roles mitochondrial dysfunction is involved in the genesis and progression of ageing and of a plethora of human pathologies including cancer, metabolic syndrome, neurodegeneration and rare disorders. The mitochondrial ATP synthase is a key transducer in energy conservation by oxidative phosphorylation (OXPHOS), in the execution of cell death and in intracellular signaling by calcium and reactive oxygen species (ROS). Previously, we documented the mechanisms and role-played by the ATP synthase in metabolic reprogramming during liver development and in human carcinomas. More recently, we demonstrated that the inhibitor of the ATP synthase, named ATPase Inhibitory Factor 1 (IF1), is highly overexpressed in carcinomas playing a pivotal role in metabolic reprogramming of cancer and stem cells. We showed that binding of IF1 to the ATP synthase inhibits the enzyme under normal physiological conditions and this binding is prevented by phosphorylation of IF1-S39 through the activity of a cAMP-dependent protein kinase A like activity. Inhibition of the ATP synthase is required for adaptation to hypoxia, cell cycle progression and in cancer. Contrariwise, dephosphorylation of IF1 is required to increase the mitochondrial output of ATP in response to an increase in energy demand. Moreover, the IF1-mediated inhibition of the ATP synthase triggers a ROS signal that promotes the activation of nuclear programs of proliferation and resistance to cell death. Hence, IF1 is a most relevant mitochondrial protein that participates in defining the cellular phenotype.

A main objective of our group is to deepen into the cellular biology and role of the ATP synthase/IF1 axis in cancer and other metabolic disorders, neuronal and immune functions and in ageing. To cover these aims, we have developed transgenic mice (Tg-IF1) that conditionally overexpress human IF1 in neurons, liver, colon, heart and skeletal muscle, and generated the ATP5IF1 lox/lox mouse which has been successfully used to knock-out IF1 (IF1-KO) in neurons, enterocytes and immune cells. With these models, we have demonstrated *in vivo* the role of the ATP synthase/IF1 in metabolic reprogramming and in signaling adaptive cellular and tissue responses in normal and pathophysiological situations (Fig. 1). Moreover, we have developed (i) the PROTEOmAb Platform for the identification of metabolic proteins as biomarkers of disease and (ii) identified FDA-approved small molecules that regulate OXPHOS for targeting mitochondria and effective bedside translation of the drugs to patients affected by mitochondrial dysfunction.

List of publications

» Escós A et al., (2022) TPL2 kinase expression is regulated by the p38γ/p38δ-dependent association of aconitase-1 with TPL2 mRNA. Proc Natl Acad Sci U S A. 119: e2204752119. doi: 10.1073/pnas.2204752119.

» Peñuelas-Haro I et al., (2022) The NADPH oxidase NOX4 regulates redox and metabolic homeostasis preventing HCC progression. Hepatology. doi: 10.1002/hep.32702.

» Carmona-Carmona CA et al., (2022) Mitochondrial Elongation and OPA1 Play Crucial Roles during the Stemness Acquisition Process in Pancreatic Ductal Adenocarcinoma. Cancers (Basel).;14:3432. doi:10.3390/cancers14143432.

» Sánchez-González C et al., (2022) Chronic inhibition of the mitochondrial ATP synthase in skeletal muscle triggers sarcoplasmic reticulum distress and tubular aggregates. Cell Death Dis. 13:561. doi:10.1038/s41419-022-05016-z.

» Domínguez-Zorita S et al., (2022) The ATPase Inhibitory Factor 1 is a Tissue-Specific Physiological Regulator of the Structure and Function of Mitochondrial ATP Synthase: A Closer Look Into Neuronal Function. Front Physiol. 13:868820. doi:10.3389/fphys.2022.868820.

» Torresano L et al., (2022) Analysis of the metabolic proteome of lung adenocarcinomas by reverse-phase proteinarrays (RPPA) emphasizes mitochondria as targets for therapy. Oncogenesis. 11:24. doi: 10.1038/s41389-022-00400-y.

» Mascaraque-Checa M et al., (2022) Metformin overcomes metabolic reprogramming-induced resistance of skin squamous cell carcinoma to photodynamic therapy. Mol Metab. 60:101496. doi: 10.1016/j.molmet.2022.101496.

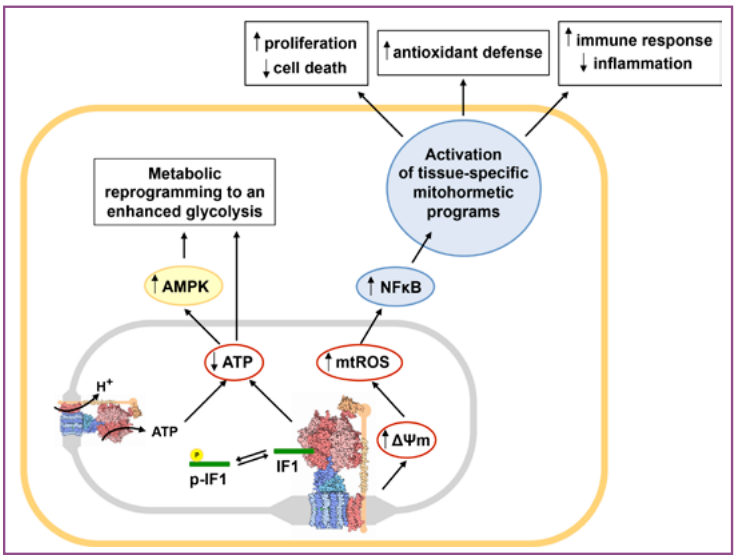
» Nuevo-Tapioles C et al., (2021) Effective therapeutic strategies in a preclinical mouse model of Charcot-Marie-Tooth disease. Hum Mol Genet. 30:2441-2455. doi: 10.1093/hmg/ddab207.

» García-Navas R et al., (2021) Critical requirement of SOS1 RAS-GEF function for mitochondrial dynamics, metabolism, and redox homeostasis. Oncogene. 40:4538-4551. doi: 10.1038/s41388-021-01886-3.

» Esparza-Moltó PB et al., (2021) Generation of mitochondrial reactive oxygen species is controlled by ATPase inhibitory factor 1 and regulates cognition. PLoS Biol. 19:e3001252. doi: 10.1371/journal.pbio.3001252.

Patents

» Co-inventores: Cristina Nuevo-Tapioles, Jorgina Satrústegui, Francisco Palau y José M. Cuezva. Título: Tratamiento de la Enfermedad de Charcot-Marie-Tooth. N° patente: P202130576. País de prioridad: España. Fecha prioridad: 21/06/2021. Propietarios: 68% Universidad Autónoma de Madrid; 20% consorcio público estatal CIBER y 12% Hospital Sant Joan de Deu.



Main metabolic and redox circuits regulated by the inhibition of the ATP synthase by dephosphorylated IF1. Figure taken from reference Front Oncol. 2018 Mar 7;8:53.

Participation in projects

» "Mitochondria and its dysfunction in pathology: The role of IF1". PID2019-108674RB-100. Ministerio de Ciencia, Innovación y Universidades. IP: José M. Cuezva. 01/06/20 a 31/07/23.

» "Genotipado y fenotipado proteómico, metabolómico y funcional en el síndrome de Kearns-Syre para la identificación de nuevos biomarcadores y opciones terapeuticas". Ministerio de Sanidad. ACCI-CIBERER 2021-2022.

» José M. Cuezva is leader of unit "U713" of the CIBER of Enfermedades Raras (CIBERER) and of the Research Group "Metabolismo Energético Traslacional" of the Instituto Universitario Hospital 12 de Octubre (i+12), both are initiatives of the "Instituto de Salud Carlos III". In addition, forms part of the Network of Excellence RED2018-102379-T METABOCANCER.

Doctoral theses

» Laura *Torresano* Cicuéndez. (2022) "El proteoma del metabolismo en cáncer de pulmón: nuevas dianas terapéuticas". Universidad Autónoma de Madrid. Directores: José M. Cuezva.

» Sonia Domínguez Zorita (2022). "Implicaciones fisiopatológicas de la ATP sintasa mitocondrial en la homeostasia del colon" Universidad Autónoma de Madrid. Director: José M. Cuezva.

ROLE OF MITOCHONDRIAL METABOLISM ON THE PATHOPHYSIOLOGY OF SKELETAL MUSCLE

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

Group Members

Principal Investigator:
Laura Formentini

Predoctoral fellows:
Cristina Sánchez González, PhD student (until February 2022)
Juan Cruz Herrero Martín, PhD student (until July 2022)
Beñat Salegi Ansa, PhD student (starting January 2022)

Students:
Francisco Javier Arenas Cerro, TFM student (starting December 2022)

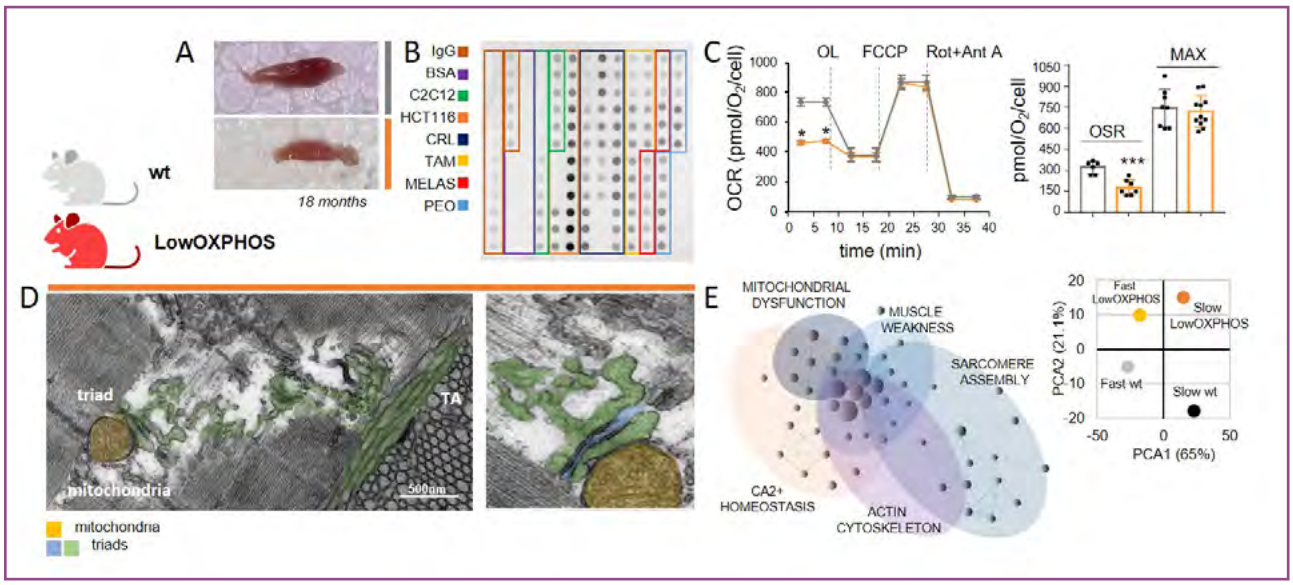
Summary

Our investigation aims at understanding how mitochondrial bioenergetics participate in the integration of different cellular functions. Complex regulatory mechanisms enable mitochondrial metabolism to match cell demands, which extend beyond the production of ATP: during the last decade we demonstrated that mitochondrial oxidative phosphorylation (OXPHOS) plays further roles in controlling cell death (*EMBO J,* 2014, 33(7):762-78); immunity (*Cell Reports*, 2017, 19(6):1202-1213) and oncogenesis (*Mol Cell*, 2012, 45(6):731-42; *Nat Comm*, 2020, 11:3606). Impaired mitochondrial function also deeply alters lipid species and metabolism (*Diabetologia*, 2017, 60(10):2052-2065; *EMBO J.* 2020, e103812) and is emerging as a pivotal hallmark of metabolic disorders. Understanding which products of metabolism are limiting for correct cell function, and how cells obtain or transform them in physiological tissue environments, is crucial to exploit mitochondrial metabolism for therapy.

The main achievement of our research over the last two years was to deepen our knowledge of mitochondrial metabolism in the pathophysiology of skeletal muscle, the highest oxidative tissue in mammals. We defined how chronic mitochondrial dysfunctions drive the formation of muscular tubular aggregates (TA), honeycomb-like arrays of sarcoplasmic reticulum (SR) tubules that induce severe sarcomere disorganization and muscular pain. TA develops in the skeletal muscle of patients with Tubular Aggregate Myopathy (TAM, ORPHA:2593; OMIM:160565, 615883) as well as in other disorders, including endocrine syndromes, diabetes, and aging, although their primary cause is unknown. We investigated the molecular mechanisms of TA onset and a potential therapy in a preclinical mouse model of the disease. We showed that upon chronic *in vivo* inhibition of the mitochondrial ATP synthase, oxidative soleus muscle experiments a metabolic and structural switch towards glycolytic fibers, increases mitochondrial fission, and activates mitophagy to recycle damaged mitochondria. TA results from the over-response of the fission controller DRP1, which upregulates the Store-Operate-Calcium-Entry and increases the mitochondria-SR interaction in a futile attempt to buffer calcium overloads upon prolonged OXPHOS inhibition. Accordingly, hypoxic muscles cultured *ex vivo* show an increase in mitochondria/SR contact sites and autophagic/mitophagic zones, where TA clusters grow around defective mitochondria. Moreover, hypoxia triggered a stronger TA formation upon ATP synthase inhibition, and this effect was reduced by the DRP1 inhibitor mDIVI.

In vivo edaravone treatment in mice with restrained OXPHOS restored a healthy phenotype by prompting mitogenesis and mitochondrial fusion. Altogether, our data provide a functional link between the ATP synthase/DRP1 axis and the setting of TA, and repurpose edaravone as a possible treatment for TA-associated disorders (Cell Death Dis. 2022 Jun 22;13(6):561).

Ultimately, our investigation aims to provide knowledge based on new mitochondrial aspects for better prevention, diagnosis, and therapy of metabolic and rare diseases that target skeletal muscle.



Several approximations to define mitochondrial dysfunction in LowOXPHOS mice: A) soleus muscle dimension in wt and LowOXPHOS animals. B) high-throughput Reverse Phase Protein Array (RPPA) of mitochondrial proteins. C) Oxygen consumption measurements by Seahorse technology. D) Transmission electron microscopy of longitudinal soleus slices from LowOXPHOS mice. E) Proteomics analysis revealed perturbation in metabolic pathways in LowOXPHOS mice in comparison to wt.

List of publications

» Sánchez-González C, Herrero Martín JC, Salegi Ansa B, Núñez de Arenas C, Stančič B, Pereira MP, Contreras L, Cuezva JM, Formentini L. Chronic inhibition of the mitochondrial ATP synthase in skeletal muscle triggers sarcoplasmic reticulum distress and tubular aggregates. Cell Death Dis. 2022 Jun 22;13(6):561. doi: 10.1038/s41419-022-05016-z.

» Sánchez-González C, Formentini L. An optimized protocol for coupling oxygen consumption rates with β-oxidation in isolated mitochondria from mouse soleus. STAR Protoc. 2021 Aug 12;2(3):100735. doi: 10.1016/j.xpro.2021.100735.

» Alcover-Sanchez B, Garcia-Martin G, Escudero-Ramirez J, Gonzalez-Riano C, Lorenzo P, Gimenez-Cassina A, Formentini L, de la Villa-Polo P, Pereira MP, Wandosell F, Cubelos B. Absence of R-Ras1 and R-Ras2 causes mitochondrial alterations that trigger axonal degeneration in a hypomyelinating disease model. Glia. 2021 Mar;69(3):619-637. doi: 10.1002/glia.23917.

Participation in projects

» Funding: "Role of mitochondrial metabolism on the pathophysiology of skeletal muscle: effect of the FAD-dependent dehydrogenases associated to oxidative phosphorylation". Acronym: mitoFAD. PID2019-104241RB-I00 Ministerio de Ciencia e Innovación, Spain. 2020-2023. PI: Laura Formentini

» Participating in CIBER-ER Unit 713.

» Participating in Translation of Energy Metabolism" Group (Instituto de Investigación Hospital 12 de Octubre (i+12)).

Doctoral theses

» Cristina Sánchez González. (2022) "Papel de la bioenergética mitocondrial sobre el metabolismo del músculo esquelético durante el ejercicio y en patología". *Cum laude* - UAM University. Director: Laura Formentini

» Juan Cruz Herrero Martín (2022) "Papel de las deshidrogenasas FAD-dependientes en la fisiopatología del músculo esquelético". *Cum laude* - UAM University. Director: Laura Formentini

MOLECULAR PATHOPHYSIOLOGY OF FIBROSIS

Group Members

Principal Investigator:
Santiago Lamas Peláez

Postdoctoral fellows:
Verónica Miguel Herranz (until July 2021)
Carlos Rey Serra

Predoctoral Fellow:
Belén Sirera Conca

Technicians:
Jessica Paola Tituaña Fajardo
José Ignacio Herrero Lahuerta (until January 2022)

Undergraduate and Master Students:
Irene Ranz Fernández. 09/2020- 06/2021, final degree project (co-supervisor Verónica Miguel Herranz)
Laura Fernández Hernández. 09/2020-06/2021, final degree project (co-supervisor Verónica Miguel Herranz)
María Madejón Sánchez. (09/2022-), final degree project, (co-supervisor Carlos Rey Serra)

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

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 and repair focusing on: a) the importance of fatty acid oxidation 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 are complemented by cellular models and biochemical approaches directed towards the study of mitochondrial biogenesis and function. We have found that overexpression of the enzyme Cpt1a in kidney tubules promotes enhanced fatty acid oxidation, restores mitochondrial homeostasis and protects from fibrosis. By dissecting the role of specific components of the circadian clock through the employment of genetically modified mouse models, we have investigated the cross-regulation between the circadian rhythm and kidney inflammation and fibrosis, with a particular emphasis on the metabolic component. We have complemented this approach by evaluating the impact of circadian-related metabolically healthy diets, such as time restriction feeding, on kidney fibrosis and function.

List of publications

» Miguel V, Tituaña J, Herrero JI, Herrero L, Serra D, Cuevas P, Barbas C, Rodríguez-Puyol D, Márquez-Exposito L, Ruiz-Ortega M, Castillo C, Sheng X, Susztak K, Ruiz-Canela M, Salas-Salvadó J, Hu FB, Martínez Gonzalez MA, Ortega S, Ramos R and Lamas S. (2021). Renal tubule Cpt1a overexpression mitigates kidney fibrosis by restoring mitochondrial homeostasis. Journal of Clinical Investigation.140695. doi: 10.1172 /JCI140695.

» Rey-Serra C, Tituaña J, Lin T, Herrero JI, Miguel V, Barbas C, Meseguer A, Ramos R, Firsov D, Chaix A, Panda S, Lamas S. (2022). Cross regulation between the molecular clock and kidney inflammatory, metabolic and fibrotic responses. bioRxiv doi: 10.1101/2022.05.18.492458.

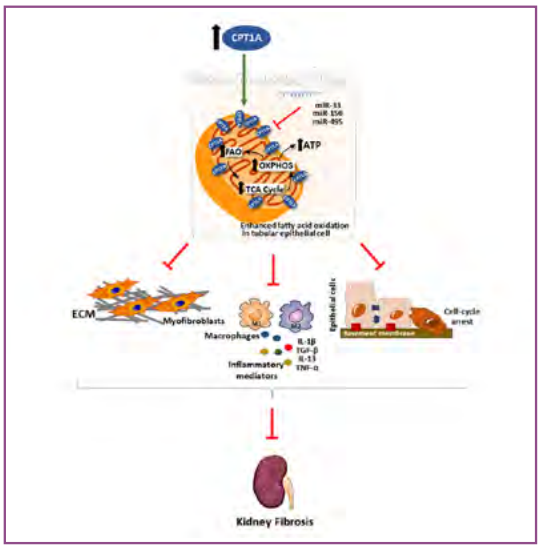
» Perramón M, Carvajal S, Reichenbach V, Fernández-Varo G, Boix L, Macías-Muñoz L, Melgar-Lesmes P, Bruix J, Melmed S, Lamas S and Jiménez W. (2022). The pituitary tumour-transforming gene 1/delta-like homologue 1 pathway plays a key role in liver fibrogenesis. Liver Int. 42(3):651-662. doi: 10.1111/liv.15165.

» Ruiz-Ortega M, Lamas S and Ortiz A. (2022). Antifibrotic agents for the management of CKD: a review. Am J Kidney Dis. 80(2):251-263. doi: 10.1053/j.ajkd.2021.11.010.

» Miguel V and Lamas S. (2021). Linking transcription to energy: the path to understand kidney injury. Kidney Int 100(6):1165-1167. doi: 10.1016/j.kint.2021.09.018.

» Miguel V, Ramos R, García-Bermejo L, Rodríguez-Puyol D and Lamas S. (2021). The program of renal fibrogenesis is controlled by microRNAs regulating oxidative metabolism. Redox Biology 40:101851 doi: 10.1016/j.redox.2020.101851.

» Márquez-Exposito L, Rodrigues-Díez RR, Rayego-Mateos S, Fierro-Fernandez M, Rodrigues-Díez R, Orejudo M, Santos-Sánchez L, Blanco EM, Laborda J, Mezzano S, Lamas S, Lavoz C and Ruiz-Ortega M. (2021). Deletion of delta-like 1 homologue accelerates renal inflammation by modulating the Th17 immune response. FASEB J. 35(1):e21213. doi: 10.1096/fj.201903131.



The enhancement of fatty acid oxidation in renal tubular epithelial cells protects against kidney fibrosis

Participation in projects

» Red de Investigación Renal (ISCiii) REDinREN. RD16/0009/0016. (2017-2021) (RETIC), PI: Santiago Lamas.

» Enfermedad Renal Crónica: nuevas estrategias para la prevención, diagnóstico y tratamiento (NOVELREN-CM). S2017/BMD-3751. (2018-2021). (Programa Biomedicina Comunidad de Madrid) PI and Coordinator: Santiago Lamas.

» Fibrosis pulmonar post CoVid19: marcadores y opción terapéutica con Metformina. CSIC-COV19-096. (2020-2021), PI: Santiago Lamas.

» Mesenchymal metabolic rewiring to cure organ fibrosis (FIBROMET). EIN2020-112282. (2020-2022), PI: Santiago Lamas.

» Combating kidney fibrosis by metabolic reprogramming (RENFIBMET). PID2019-104233RB-I00. (2020-2023), PI: Santiago Lamas.

Other activities

» Coordination of the NOVELREN-CM consortium of the Biomedicine programme from the Comunidad de Madrid for the study on chronic renal failure. 2018-2022.

» Group leader in the research network RedinRen, RETICS programme, Instituto de Salud Carlos III. 2020-2022

» Collaboration with M2R laboratories for the establishment of an experimental model of acute respiratory distress syndrome.

Awards

» 2021: Doctor Honoris Causa, Facultad de Medicina, Universidad de la República, Uruguay

» 2022: Basic Research Award, Society for Free Radical Research-Europe

Doctoral theses

» Carlos Rey Serra (2021). Impact of the circadian regulation on the metabolic basis of kidney fibrosis. Universidad Autónoma de Madrid. Director: Santiago Lamas.

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

Group Members

Principal Investigator:
Federico Mayor Jr

Co-Principal Investigator
Cristina Murga

Scientific Staff
Irene García Higuera
Natalia Reglero-Real (from April 2022)

Postdoctoral fellows
Alba Concepción Arcones (until December 2021)
Carmen Vida
Yadileiny Portilla (from January 2023)
Dafne García Mateos (from March 2022, co-supervised with C. Ribas and P. Penela)

Predoctoral Fellows
Alejandro Asensio (co-supervised with C. Ribas)
Maria Margarida Martins Neves (until March 2021, co-supervised with P. Penela)
Angela Albitre (co-supervised with P. Penela)
Viviana Marolda (co-supervised with P. Penela)
Rocío Santos (from July 2022)

Technicians
Cristina Delgado-Arévalo (from January 2022)
Estefanía Vázquez de Oro (from September 2022)

Undergraduate and Master students
Daniel Oña Sánchez
Ana Romo Gallo (co-supervised with C. Ribas)

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

Summary

Our laboratory is interested in understanding the role of regulatory hubs in the maladaptive rewiring of cell signaling networks that takes place during the onset or progression of metabolic, cardiovascular and inflammatory diseases and in cancer. G protein-coupled receptor kinase 2 (GRK2) acts as a relevant node by modulating signaling mediated by many GPCRs and via phosphorylation or scaffolding interactions with a growing array of cellular partners. We have pioneered the research on such complex "interactome" and on the mechanisms regulating GRK2 levels and functionality. In obesity and insulin resistance-related contexts, we have described that age and sex-related factors modulate maladaptive changes in cardiac GRK2 levels and tissue-specific roles of GRK2 in the modulation of metabolic homeostasis. In collaboration with P.Penela and C.Ribas, we have shown that GRK2 acts as an oncomodulator in breast cancer and in stratified epithelial tumors, by interacting with networks related to the hallmarks of cancer in a cell type-dependent manner, and that the GRK2 interactor Gαq regulates the autophagic machinery upon nutrient fluctuations.

Complex intercommunication among cell types from different tissues and with the cells of the immune system is essential for cellular and organismal homeostasis. We aim to elucidate how cell type-specific GRK2 interactomes are involved in cell-cell communication in defined physiological and pathological conditions. We will use cellular and animal models with altered GRK2 levels or functionality in specific cell types to study:

-Maladaptive reshaping of metabolic and inflammatory networks in different tissues in physio-pathological situations related to aging, obesity, insulin resistance and cardiovascular alterations, with emphasis on the role of GRK2 in myeloid (neutrophils and macrophages) and other immune cells in inter-organ crosstalk (objectives led by C. Murga, I. García-Higuera and N. Reglero-Real).

-Autophagy, endothelial dysfunction and inflammation. N.Reglero-Real and C. Ribas will collaborate in elucidating the modulation of these processes by Gq and other signalling networks.

-Crosstalk of signaling cascades within the tumor microenvironment. Connections among tumor microenvironment stresses, chemokine and growth factor receptors and GRK2 interactors (HDAC6, HuR, Mdm2, Lyn) in the rewiring of breast cancer cell phospho-proteome, acetylome and ubiquitome leading to metastatic features (in collaboration with P. Penela and the Oncornet 2.0 consortium).

-Epidermal homeostasis and keratinocyte-immune cells crosstalk. Impact of GRK2 deletion in keratinocytes in the skin immune cell landscape, barrier function, skin-microbiome interaction and hair follicle homeostasis, and in the susceptibility to inflammatory diseases and squamous cell carcinomas (in collaboration with C. Ribas).

List of publications

» Cabezudo S, Sanz-Flores M, Caballero A, Tasset I, Rebollo E, Diaz A, Aragay AM, Cuervo AM, Mayor F Jr *, Ribas C * (*, corresponding authors) (2021). Gαq activation modulates autophagy by promoting mTORC1 signaling. Nature Communications 12(1):4540. doi: 10.1038/s41467-021-24811-4.

» Arcones AC, Vila-Bedmar R, Mirasierra M, Cruces-Sande M, Vallejo M, Jones B, Tomas A, Mayor F Jr*, Murga C* (*, corresponding authors) (2021). GRK2 regulates GLP-1R-mediated early phase insulin secretion *in vivo*. BMC Biol. 19(1):40. doi: 10.1186/s12915-021-00966-w

» Smit MJ, Schlecht-Louf G, Neves M, van den Bor J, Penela P, Siderius M, Bachelerie F, Mayor F Jr. (2021). The CXCL12/CXCR4/ACKR3 Axis in the Tumor Microenvironment: Signaling, Crosstalk, and Therapeutic Targeting. Annu Rev Pharmacol Toxicol. 61:541-563. doi: 10.1146/annurev-pharmtox-010919-023340.

» Reglero C, Ortiz Del Castillo B, Rivas V, Mayor F Jr, Penela P. (2021). Mdm2-Mediated Downmodulation of GRK2 Restricts Centrosome Separation for Proper Chromosome Congression. Cells. 10(4):729. doi: 10.3390/cells10040729.

» Arcones AC, Murga C, Penela P, Inserte J and Mayor F Jr (2021). G protein–coupled receptor kinase 2 at crossroads of metabolic and cardiovascular diseases. Current Op Endo Metabol Research 16:75-85 doi:10.1016/j.coemr.2020.09.004

» Arcones AC, Martínez-Cignoni MR, Vila-Bedmar R, Yáñez C, Lladó I, Proenza AM, Mayor F Jr, Murga C. (2021). Cardiac GRK2 Protein Levels Show Sexual Dimorphism during Aging and Are Regulated by Ovarian Hormones. Cells. 10(3):673. doi: 10.3390/cells10030673

» Cuadrado, M, Garzón, J, Moreno, S, García-Higuera, I. (2022). Efficient terminal erythroid differentiation requires the APC/C cofactor Cdh1 to limit replicative stress in erythroblasts. Sci Rep., 12(1):10489. doi: 10.1038/s41598-022-14331-6.

» Mayor F Jr, Murga C. (2022). G Protein-Coupled Receptor Kinases Take Central Stage. Cells. 12(1):23. doi: 10.3390/cells12010023.

Participation in projects

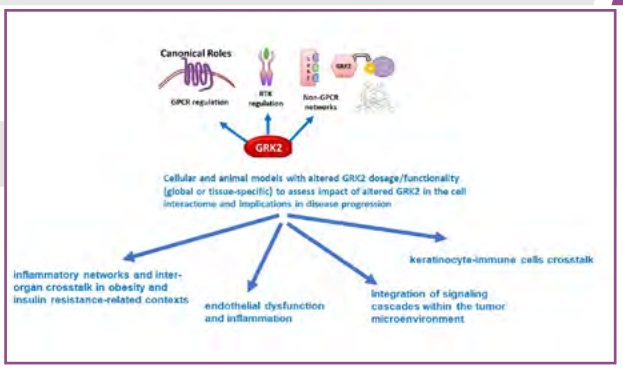
» H2020-MSCA Programme, Grant agreement 860229-ONCORNET2.0 (ONCOgenic Receptor Network of Excellence and Training) -Coordinator: Martine Smit, Amsterdam, F. Mayor Network group PI, 2020-2024.

» CIBER CARDIOVASCULAR, Instituto de Salud Carlos III, Group CB16/11/00278 (PI, F Mayor) 2017-2023.

» Comunidad de Madrid–Programa de Actividades I+D en BIOMEDICINA. INFLAMUNE-B2017/BMD-3671/ New molecular and cellular mechanisms in immune physiopathology and inflammatory diseases. 2018 -2022, and INTEGRAMUNE-CM/P2022/BMD-7209. Integrated cellular and molecular systems in immune-inflammatory pathophysiology. Coordinator PI: F Mayor. 2023-2026.

» Instituto de Investigación Sanitaria Hospital La Princesa. Group 11 (PI: F. Mayor) and Group 17 (PI: C. Murga)

» Agencia Estatal Investigación. PID2020-117218RB-I00. Integrated GRK2 signaling networks and molecular mechanisms of disease. PI: Federico Mayor Jr. and Cristina Murga (co-PI). 2021-2024.



Investigation of the role of GRK2-governed networks in the maladaptive rewiring of signaling pathways in diverse pathological conditions.

» Neves M, Marolda V, Mayor F Jr*, Penela P* (*, corresponding authors)(2022). Crosstalk between CXCR4/ACKR3 and EGFR Signaling in Breast Cancer Cells". Int J Mol Sci. 23 (19), 11887; doi.org/10.3390/ijms231911887

» Cacho-Navas C, Reglero-Real N, Colás-Algora N, Barroso S, de Rivas G, Stamatakis K, Feito J, Andrés G, Fresno M, Kremer L, Correas I, Alonso MA, Millán J. (2022). Plasmolipin regulates basolateral-to-apical transcytosis of ICAM-1 and leukocyte adhesion in polarized hepatic epithelial cells. Cell Mol Life Sci. 2022 Jan 9;79(1):61. doi: 10.1007/s00018-021-04095-z.

» Perdices-Lopez C, Avendaño MS, Barroso A, Gaytán F, Ruiz-Pino F, Vázquez MJ, Leon S, Song YB, Sobrino V, Heras V, Romero-Ruiz A, Roa J, Mayor F Jr, Murga C, Pinilla L, Kaiser UB, Tena-Sempere M. (2022). Connecting nutritional deprivation and pubertal inhibition via GRK2-mediated repression of kisspeptin actions in GnRH neurons. Metabolism. 129:155141. doi: 10.1016/j.metabol.2022.155141.

Doctoral theses

» Alvaro Caballero Lombraña (2021). Nuevo interactoma de la proteína Gq y su implicación en la homeostasis vascular. Departamento de Biología Molecular. UAM. Supervisors: Catalina Ribas and Federico Mayor. "Cum laude".

» María Margarida Martins Neves (2021). Molecular mechanisms underlying the role of GRK2 in Breast Cancer progression. Departamento de Biología Molecular. UAM. Supervisors: Petronila Penela and Federico Mayor. "Cum laude".

Other activities

» Federico Mayor: Director, Institute of Molecular Biology, Universidad Autónoma, Madrid (re-elected 2021). Member of Scientific advisory boards of the Lilly Foundation Spain, IDIBAPS-Clinic (Barcelona, Spain) and IIS "Fundación Jiménez Díaz" (Madrid, Spain). Honorary Member of the Spanish Society for Biochemistry and Molecular Biology-SEBBM (2022).

» Cristina Murga: Member of the Rector Board of the IIS "La Princesa" (Madrid, Spain). Member of the "Claustro" of the UAM. President, Senior University of UAM since 2022.

CELLULAR SIGNALING NETWORKS IN CANCER (ONCO-RESECEL)

Group Members

Principal Investigator:
Petronila Penela

Postdoctoral fellows
Teresa González Muñoz (since September 2022)
Dafne García Mateos (since March 2022, co-supervised with Catalina Ribas)

Predoctoral Fellows
Belén Ortiz del Castillo
Maria Margarida Martins Neves (until April 2021, co-supervised with Federico Mayor)
Angela Albitre (co-supervised with Federico Mayor)
Viviana Marolda (co-supervised with Federico Mayor)

Technicians
Estefanía Vázquez de Oro (until September 2022)

Undergraduate and Master students
Paula Casasano Mateos (2022)

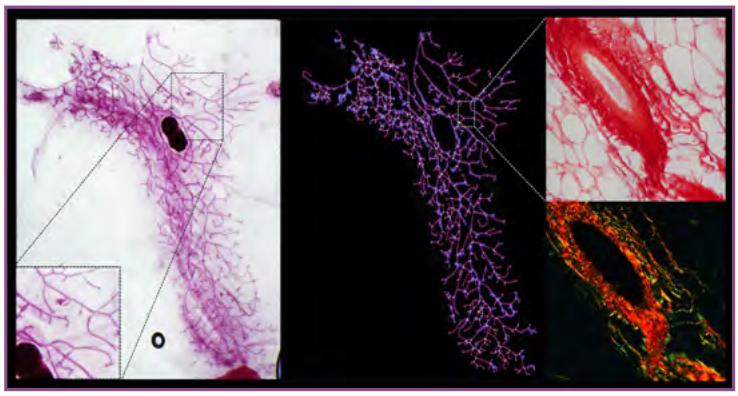
https://www.cbm.uam.es/ppenela

Summary

Intratumor cell heterogeneity and the intrinsic adaptability of tumour cells are fundamental features of cancer that have major consequences for the tumour evolution and the emergence of resistances. Cellular heterogeneity may result from either clonal evolution driven by genetic instability and/or from differentiation of stem-like cells, whereas the dynamic rewiring of signaling networks contributes to cell adaptability. Moreover, lifestyle factors also affect the incidence of cancer and the efficacy of treatments. Therefore, it is urgent to find out the mechanisms involved and the molecular dependencies of tumours for better tackling with cancer treatments. Even though oncogenes and tumour suppressors are main players in transformation and malignant progression, they only account for a limited proportion of the differential gene expression observed in tumoral tissues, pointing out the involvement of additional factors in supporting and scaling up tumours. In this regard, dysregulation of proteins acting as signaling nodes and eliciting post-translational modifications might play a relevant role in different aspects of tumour cell behaviour (motility, cell cycle control, stress responses). Our group is interested in the role of kinases and other posttranslational modifiers in the dynamic rewiring of cell signaling networks, which could be susceptible to being therapeutically targeted.

Results of our laboratory showed that G protein-coupled receptor kinase2 (GRK2) is a versatile molecular hub that modulates signalling mediated by many GPCRs and a growing 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 or cytosolic protein deacetylases (HDAC6) to foster EGF signalling and motility, and the concurrent up-regulation of GRK2 and these activities emerges as a functional signature in breast cancer types beyond the hormone-dependent status.

Our research aims are to identify a) GRK2-governed signalling circuits involved in breast cancer progression and resistance, deciphering the relevant 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 processes to differentiation, proliferation or senescence; c) influence of hormonal (adrenergic, estrogenic), metabolic stresses and micro-environmental conditions on GRK2 intertwinement with relevant partners for genomic stability an stroma remodelling, analysing changes in mammary gland morphology and epithelial organization, altered angiogenic processes and tissular fibrosis that facilitate tumour growth and dissemination.



Imaging analysis of mouse mammary glands. Carmine-stained whole-mount histology of mammary glands (left panel) for gross morphological analysis of ducts and terminal end buds. Skeletonized image for the quantification of branching complexity (middle panel). Picrosirius red staining of mammary gland sections (right upper panel) viewed with polarized light microscopy (right bottom panel) to evaluate the organization of collagen fibers.

List of publications

- Jiménez-Reinoso A, Tirado N, Martinez-Moreno A, Díaz VM, García-Peydró M, Hangiu O, Díez-Alonso L, Albitre Á, Penela P, Toribio ML, Menéndez P, Álvarez-Vallina L, Sánchez Martínez D (2022). Efficient preclinical treatment of cortical T cell acute lymphoblastic leukemia with T lymphocytes secreting anti-CD1a T cell engagers. J Immunother Cancer. 10: e005333. doi: 10.1136/jitc-2022-005333
- Neves M, Marolda V, Mayor F Jr, Penela P. (2022). Crosstalk between CXCR4/ACKR3 and EGFR Signaling in Breast Cancer Cell. Int J Mol Sci. 23:11887. doi: 10.3390/ijms231911887
- Reglero C, Ortiz Del Castillo B, Rivas V, Mayor F Jr, Penela P (2021). Mdm2-Mediated Downmodulation of GRK2 Restricts Centrosome Separation for Proper Chromosome Congression. Cells. 10: 729. doi: 10.3390/cells10040729
- Smit MJ, Schlecht-Louf G, Neves M, den Bor JV, Penela P, Siderius M, Bachelerie F, Mayor F Jr. (2021) The CXCL12/CXCR4/ACKR3 Axis in the Tumor Microenvironment: Signaling, Crosstalk, and Therapeutic Targeting. Annu Rev Pharmacol Toxicol. 61:541-56. doi: 10.1146/annurev-pharmtox-010919-023340.
- Arcones AC, Murga C, Penela P, Inserte J and Mayor F Jr (2021) G protein–coupled receptor kinase 2 at crossroads of metabolic and cardiovascular diseases. Current Op Endo Metabol Research 16:75-85 doi:10.1016/j.coemr.2020.09.004

Doctoral theses

- Adolfo J. Molejón García. (2021.) "G Protein Coupled Receptor Kinase (GRK)-2: Involvement in DNA Damage Response and Functional Repercussions in Senescence". Department of Molecular Biology, UAM. Supervisor: Petronila Penela
- Maria Margarida Martín Neves. (2021). "Molecular mechanisms underlying the role of GRK2 in Breast Cancer progression". Department of Molecular Biology, UAM. Supervisors: Petronila Penela and Federico Mayor

Participation in projects

- European Union:H2020-MSCA Programme, Grant agreement 860229-ONCORNET2.0 (ONCOgenic Receptor Network of Excellence and Training) -Coordinator: Martine Smit, Amsterdam. P Penela, Ethical Board Network and trainee supervisor, 2020-2024.
- CIBER CARDIOVASCULAR, Instituto de Salud Carlos III, Group CB16/11/00278 (PI, F Mayor) 2017-2023. Group member
- INTEGRAMUNE-CM / P2022/BMD-7209. COM. MADRID–Programa de Actividades I+D en BIOMEDICINA/ Coordinator: F. Mayor, UAM (Researcher, P Penela, 2023-2026)
- "Exploring post-translational regulation of angiogenic and inflammatory-related processes during colorectal cancer progression and differential recurrence". CIVP20A6618. Fundacion Ramón Areces, 2021-2024. Principal Investigator: P. Penela
- "Exploring the role of GRK2 in BRCA1 dysfuntion and mechanisms of PARP-inhibitor resistance in breast tumor models beyond BRCA status". PI21/01834 Carlos III Institute of Health (FIS). 2022-2024. Principal Investigator: P. Penela
- REACT-UE_CONTRAVI-19-CM. "Plataformas y modelos preclínicos para el abordaje multidisciplinar en COVID-19 y en respuesta a futuras pandemias" (Universidad Autónoma de Madrid). Coordinator: M. Fresno. 01/03/2022 -31/12/2022. PO Penela, PI of WP.1.2.2.
- INFLAMUNE-B2017/BMD-3671. Comunidad de Madrid–Programa de Actividades I+D en BIOMEDICINA. Coordinator: M. Fresno, UAM (Researcher, P Penela, 2018 -2021).
- Instituto de Investigación Sanitaria Hospital La Princesa. Area 1. Group 13 (PI: P. Penela)

Other activities

- Petronila Penela, Erasmus Academic Coordinator of the Faculty of Sciences, UAM (Universidad Autónoma de Madrid)

TRANSLATIONAL MEDICINE IN INBORN ERRORS OF METABOLISM AND OTHER RARE GENETIC DISEASES

Group Members

Principal Investigators (PI, co-PI):
Belén Pérez
Pilar Rodríguez-Pombo

Scientific Staff:
Alejandra Gámez Abascal

Postdoctoral fellows:
Alvaro Briso-Montiano
(until December 2021)

Predoctoral fellows:
Laura Arribas
Diana Gallego
Obdulia Sánchez Lijarcio
Cristina Segovia Falquina
Alejandro Soriano Sexto
Alicia Vilas Lagoa

Technicians:
Elena Montalvo
Blanca Rodríguez
(From November 2022)
Fátima Leal
Rosa Navarrete

Undergraduate and Master Students
Olga Ramírez 2021- 2022 (TFG)
Luis Calderón 2011-2022 (TFM)
Aikaterini Stamatelaki, Erasmus+ (Greece)

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

Summary

Inborn errors of metabolism (IEM) are one of the major groups of rare diseases (1 every 800 newborns have one). Genomic high-throughput sequencing has dramatically accelerated gene discovery and transformed metabolic precision medicine. However, more than half of patients remain without a genetic cause identified. From the more than 1400 pathologies categorized as IEM, there are only therapies for hundreds of IEMs. Using a multi-omic layer approach in combination with functional genomics, we have contributed to identify new defects associated with pathology. We are highlighting identification of pathogenic variants: i) in a gene involved in the synthesis of coenzyme A associated with a severe dilated cardiomyopathy (PPCDC), ii) in the moonlighting H-protein encoded by GCSH that has a dual role in protein lipoylation required for bioenergetic enzymes iii) in genes other than SLC2A1 that usually has been associated with GLUT1 transporter deficiency and iv) in YIF1B associated with a severe neurodevelopment delay describing a new defect that combines a ciliopathies with a Golgiphatie.

Regarding therapies, we have been involved in the development of small chemical drugs targeted to rescue the activity of destabilizing mutant proteins as a common mechanism for loss and gain of function mutations in IEM. We have published evidence regarding the possible use of small chemical molecules such as celastrol in combination with pharmacological chaperones (PC) in the treatment of the severe rare diseases PMM2-CDG and methylmalonic aciduria, this last in combination with vitamin B12. Advancing the development of pharmaco-chaperoning, we have obtained five complete crystal structures of hPMM2 (free and bound to the essential activator glucose-1,6-bisphosphate), three for the wild type and two for the destabilizing p.Thr237Met variant. Using the structure, we have proposed that ~80% and ~50% of the missense variants of the core and cap domains respectively are potential candidates for treatment with PC. Furthermore, we have developed a drug discovery and screening platform using recombinant proteins and cellular models generated by CRISPRcas9 gene editing.

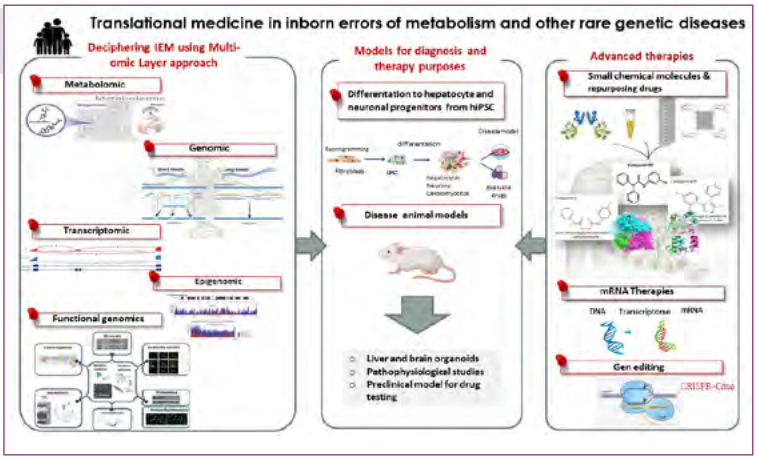
For preclinical evaluation, we have generated cellular models from patients and healthy hiPSC. We highlight the development of a protocol for the differentiation of iPSCs to hepatocyte-like cells (HLC) and their use as a preclinical model for the evaluation of PCs. In MMA, the HLC exhibited MMA disease hallmarks, and the pharmaco-chaperon treatment in combination with B12 significantly reduced the methylmalonic acid levels and rescue the liver damage associated with the disease.

Doctoral theses

» Irene Bravo Alonso (2021). Caracterización genética de la acidosis láctica congénita: un ejemplo de análisis integral de una enfermedad rara. Autónoma de Madrid. Supervisors: Belén Pérez / Pilar Rodríguez Pombo.

List of publications

- Bravo-Alonso, I., et al. (2022) Pathogenic variants of the coenzyme A biosynthesis-associated enzyme phosphopantothe-noylcysteine decarboxylase cause autosomal-recessive dilated cardiomyopathy. J Inherit Metab Dis. doi: 10.1002/jimd.12584.
- Soriano-Sexto, A., et al. (2022) Identification of Clinical Variants beyond the Exome in Inborn Errors of Metabolism. Int. J. Mol. Sci. 23(21):12850. doi: 10.3390/ijms232112850.
- Arribas-Carreira, L. et al. (2022) Pathogenic variants in GCSH encoding the moonlighting H-protein cause combined Nonketotic Hyperglycinemia and Lipoate Deficiency. Hum Mol Genet. ddac246. doi: 10.1093/hmg/ddac246.
- Martín-Rivada, Á., et al. (2022) Newborn screening for propionic, methylmalonic acidemia and vitamin B12 deficiency. Analysis of 588,793 newborns. J Pediatr Endocrinol Metab. 35(10):1223-1231. doi: 10.1515/jpem-2022-0340.
- Martínez-Pizarro A., et al. (2022) Antisense Oligonucleotide Rescue of Deep-Intronic Variants Activating Pseudoexons in the 6-Pyruvoyl-Tetrahydropterin Synthase Gene. Nucleic Acid Ther. 32(5):378-390. doi: 10.1089/nat.2021.0066
- Segovia-Falquina, C., et al. (2022) A functional platform for the selection of pathogenic variants of PMM2 amenable to rescue via the use of pharmacological chaperones. Hum Mutat. 43(10):1430-1442. doi: 10.1002/humu.24431.
- Stanescu, S., et al. (2022) Mitochondrial bioenergetic is impaired in Monocarboxylate transporter 1 deficiency: a new clinical case and review of the literature. Orphanet J Rare Dis. 17(1):243. doi: 10.1186/s13023-022-02389-4.
- Alcaide, P., et al. (2022) Lymphocyte Medium-Chain Acyl-CoA Dehydrogenase Activity and Its Potential as a Diagnostic Confirmation Tool in Newborn Screening Cases. J Clin Med. 11(10):2933. doi: 10.3390/jcm11102933.
- Briso-Montiano, A., et al. (2022) Hepatocyte-like cells differentiated from methylmalonic aciduria cblB type induced pluripotent stem cells: A platform for the evaluation of pharmacochaperoning. Biochim Biophys Acta Mol Base Dis. 1868(9):166433. doi: 10.1016/j.bbadis. 2022.166433.
- Sánchez Lijarcio O., et al. (2022) The clinical and biochemical hallmarks generally associated with GLUT1DS may be caused by defects in genes other than SLC2A1. Clin Genet. 102(1):40-55. doi: 10.1111.
- López-Márquez, A., et al. (2022) Modeling Splicing Variants Amenable to Antisense Therapy by Use of CRISPR-Cas9-Based Gene Editing in HepG2 Cells. Methods Mol Biol. 2434:167-184. doi: 10.1007/978-1-0716-2010-6_10.
- Briso-Montiano A., et al. (2022) Insight on molecular pathogenesis and pharmacochaperoning potential in phosphomannomutase 2 deficiency, provided by novel human phosphomannomutase 2 structures. J Inherit Metab Dis. 45(2):318-333. doi: 10.1002.
- Alonso-Barroso, E., et al. (2021) Cardiomyocytes Derived from Induced Pluripotent Stem Cells as a Disease Model for Propionic Acidemia. Int J Mol Sci. 22(3):1161. doi: 10.3390/ijms22031161.
- Jabato, F.M. et al. (2021) Gene expression analysis method integration and co-expression module detection applied to rare glucide metabolism disorders using ExpHunterSuite. Sci Rep. 11(1):15062. doi: 10.1038/s41598-021-94343-w.
- Medico Salsench, E., et al. (2021) Expanding the mutational landscape and clinical phenotype of the YIF1B related brain disorder. Brain. 144(10): e85. doi: 10.1093/brain/awab297.
- Picó, S. et al. (2021) CPEB alteration and aberrant transcriptome-polyadenylation unveil a treatable SLC19A3 deficiency in Huntington's disease. Sci Transl Med. 13(613):eabe7104. doi: 10.1126/scitranslmed.abe7104.
- Rojano, E. et al. (2021) Evaluating, filtering and clustering genetic disease cohorts based on Human Phenotype Ontology data with Cohort Analyzer. J Pers Med. 11(8):730. doi: 10.3390/jpm11080730.



Graphical abstract including the aims of the group: Multiomic approach to identified pathogenic genes and for pathophysiological studies, application of advanced therapies, and development of cellular and animal models.

Participation in projects

- Belen Pérez is Head of a CIBERER group (CB06/07/0004) and head of an IdiPAZ group
- Cross-omic approach for the discovery of the genetic basis of inborn errors of metabolism and for a personalized therapeutic intervention. (PI19/01155). 2020- 2023. PI Belén Pérez. Fondo de Investigaciones Sanitarias. Instituto de Salud Carlos III
- Genomic Medicine – IMPACT Genómica" (IMP/00009). 2021-2023. Inst. Salud Carlos III. Participant group. PI: Angel Carracedo
- Network of genomic, functional, clinical and therapeutic resources for the study of rare neurological diseases. 2018-2021. Coordinator Carmen Ayuso. PI Belén Pérez. Comunidad de Madrid B2017/BMD3721.
- Development of therapies with pharmacological chaperones for the congenital defect of glycosylation PMM2-CDG. 2018-2021. PI Belén Pérez. Ciberer-ISCIII.
- Molecular bases of neurometabolic diseases and development of mutation-specific therapies. 2016-2021. PI: Belén Pérez. Fundación Isabel Gemio.
- Towards a new era for the identification and characterization of inborn errors of glycosylation. 2019-2021. IP: Belén Pérez. E-Rare-18-117 EUROGLYCAN (Associated partner not eligible for funding).

Other activities

- Belén Pérez: master coordinator Molecular Biomedicine, Minister of Health expert, Vice-president of the Spanish Human Genetic Society (AEGH), Vice-director of Centro de Diagnóstico de Enfermedades Moleculares (CEDEM https://cedem.cbm.uam.es/). Scientific Committee III Annual Meeting AEGH, Member of the Internal Scientific Committee IdiPAZ.
- Pilar Rodriguez-Pombo International Mobility Coordinator and member of the CIVIS Health Hub.
- Alejandra Gámez: Representative in the Chemistry Commission for the Chemist Grade, member of the CIVIS Health Hub, Member of the CBM Equality Working Group, Scientific Mentor in Plan STEMadrid (Consejería de Educación e Innovación), External scientist consultant for different biotech companies.

REGULATORY FUNCTIONS AND MECHANISMS OF CELL SIGNALING PATHWAYS THROUGH G PROTEINS: A NEW INTERACTOME

Group Members

Principal Investigator:
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Dafne García Mateos (from March 2022)

Predoctoral Fellows
Alejandro Asensio López (co-supervised with Federico Mayor)

Research assistant
Isabel Jiménez López (from September 2022)

Undergraduate and Master students
Gemma Paredes García
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https://www.cbm.uam.es/cribas

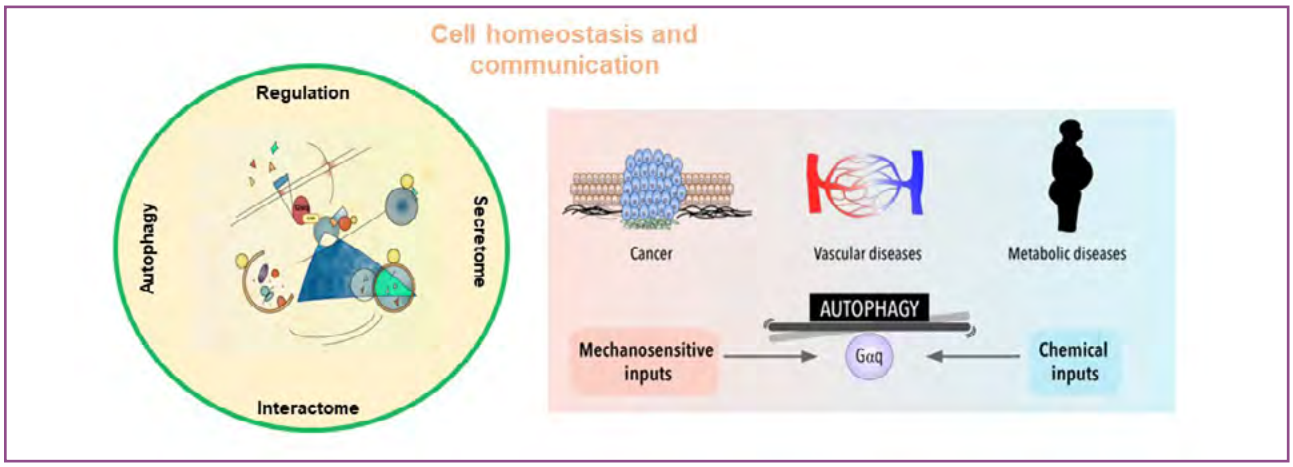
Summary

Our laboratory is investigating key nodes in signaling networks involved in both physiological and pathological conditions and the molecular mechanisms involved. G-protein-coupled receptors (GPCRs) are a family of membrane proteins with great physiological and pharmacological importance. In particular, Gq protein-coupled receptors (Gq-GPCR) are increasingly involved in pathologies such as cardiovascular/metabolic diseases and cancer. In recent years, the Gαq interactome has expanded considerably with the description of new effectors, helping to improve our understanding of the cellular and physiological events controlled by this Gα subunit. Recently, our group has contributed in the last year to unveil a new adapter role for Gq, through a new interaction region in Gαq, different from the classical effector-binding region. Our recent results reveal an unforeseen connection between non-canonical Gq signaling and cell homeostasis. Furthermore, Gαq is known to interact with various components of the cytoskeleton, as well as with important membrane microdomain organizers, suggesting the existence of signaling complexes that could be limited to specific subcellular environments.

The main objective of our group is to understand how changes in Gq-GPCR signaling (involving different types of cells and tissues) are integrated at the cellular and organism level, and how they can promote the progression of pathologies, using cell and animal models with altered expression/activity of this protein, as well as samples from patients or animal models of disease. We will focus particularly on the functional impact of this new interactome of Gαq and its modulation by accessory proteins (such as GRKs, Caveolins, AGS, RGS, EBP50, Ric8), with emphasis on how chemical and/or mechanical inputs on Gq signaling play a role in autophagy/exosome trafficking, endothelial dysfunction/inflammation and tumor microenvironment/cancer progression. The identification of new signaling pathways that relate Gαq to the crosstalk between different cell homeostasis and communication machineries will provide a better understanding of the impact of maladaptive Gq-coupled GPCR activation in pathological conditions.

In addition, and in close collaboration with other members of our CBMSO Programme, we use cellular and animal models with altered GRK2 levels or functionality to study:

- Epidermal homeostasis and keratinocyte-immune cells crosstalk. Impact of GRK2 deletion in keratinocytes in the skin immune cell landscape, barrier function, skin-microbiome interaction and hair follicle homeostasis, and in the susceptibility to inflammatory diseases and squamous cell carcinomas (in collaboration with F.Mayor).



Unveiling new Gαq-dependent functions. Gαq as a potential integrator of chemical and mechanical signals modulating autophagic process. Involvement of Gαq interactome-autophagy control in pathophysiological settings. Scheme modified from "Thesis" Sofia Cabezudo (2018) and from "Gq Signaling in Autophagy Control: Between Chemical and Mechanical Cues" Antioxidants (2022).

List of publications

» Navarro-Lérida I, Aragay AM, Asensio A, Ribas C*. (*, corresponding author). Gq Signaling in Autophagy Control: Between Chemical and Mechanical Cues. (2022) Antioxidants 18;11(8):1599. doi: 10.3390/antiox11081599.

» Cabezudo S, Sanz-Flores M, Caballero A, Tasset I, Rebollo E, Diaz A, Aragay AM, Cuervo AM, Mayor F Jr *, Ribas C * (*, corresponding authors) (2021). Gαq activation modulates autophagy by promoting mTORC1 signaling. Nature Communications 12(1):4540. doi: 10.1038/s41467-021-24811-4.

Doctoral theses

» Alvaro Caballero Lombraña (2021) "Nuevo interactoma de la proteína Gq y su implicación en la homeostasis vascular". Departamento de Biología Molecular. Universidad Autónoma de Madrid.. Supervisors: Catalina Ribas and Federico Mayor. "Cum laude".

Other activities

» Catalina Ribas: Academic Secretary of Molecular Biology Department, Universidad Autónoma, Madrid (until September 2021).

Participation in projects

» Member of the Instituto de Investigación Sanitaria Hospital La Princesa. Group 11: "Animal models of inflammatory diseases and tissue remodeling"

» Participation in ERNEST (European Research Network on Signal Transduction) COST ACTION (European cooperation in Science and Technology)

» CIBER CARDIOVASCULAR, Instituto de Salud Carlos III, Group CB16/11/00278 (PI, F Mayor) 2017-2023. Group member.

» Comunidad de Madrid–Programa de Actividades I+D en BIOMEDICINA. INFLAMUNE-B2017/BMD-3671/ New molecular and cellular mechanisms in immune physio-pathology and inflammatory diseases. 2018-2021. Group member.

» REACT-UE_CONTRAVI-19-CM. "Plataformas y modelos preclínicos para el abordaje multidisciplinar en COVID-19 y en respuesta a futuras pandemias" (Universidad Autónoma de Madrid). 01/03/2022 -31/12/2022. PI of WP.1.2.1

» Un nuevo interactoma de Gq implicado en la modulación de autofagia y estrés oxidativo: repercusión en disfunción endotelial y patologías relacionadas. PI18/01662. Principal investigator: Catalina Ribas. Instituto de Salud Carlos III. (FUNDACION PARA LA INVESTIGACION BIOMEDICA DEL HOSPITAL UNIVERSITARIO "LA PRINCESA"-UAM). 01/01/2019-31/12/2021.

» Redes de señalización de Galfaq en la homeostasis y comunicación celular: repercusión en disfunción endotelial e inflamación. PI22/00966. Instituto de Salud Carlos III. Principal investigator: Catalina Ribas (FUNDACION PARA LA INVESTIGACION BIOMEDICA DEL HOSPITALUNIVERSITARIO "LA PRINCESA"-UAM). 01/01/2023-31/12/2025.

PHYSIOPATHOLOGY STUDIES AND THERAPEUTIC APPROACHES IN ANIMAL AND CELLULAR MODELS OF NEUROMETABOLIC DISEASES

Group Members

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https://www.cbm.uam.es/lab220

Summary

Our research is focused in neurometabolic diseases, propionic acidemia (PA) and hyperphenylalaninemias (HPAs) among others, enzymatic deficiencies of autosomal recessive inheritance, characterized by the toxic accumulation of precursors and lack of downstream metabolites.

Previous results from our group include the generation of patient-derived iPSCs from PA patients' fibroblasts and a CRISPR/Cas edited isogenic control. In this period, we have differentiated them to cardiomyocytes which have been characterized in relation to the biochemical phenotype, expression of miRNAs and genes in different signaling pathways related to mitochondrial function and cardiac alterations, which are one of the major life-threatening complications in patients with the disease. We have also successfully differentiated iPSC to neuronal precursors and astrocytes, which in PA samples show a maturation defect, mitochondrial dysfunction, increased ROS levels and altered miRNAs expression signatures.

In a collaborative study and using a hypomorph PA mouse model, we have demonstrated that pharmacological inhibition of O-GlcNAcase, the enzyme removing O-GlcNAc from proteins, and specifically from CPS1, catalysing the first step in the urea cycle, resulted in clinically relevant reductions of systemic ammonia, hallmark of PA disease, a strategy that can also be applied to other genetic and acquired liver diseases.

Ongoing projects related to splice modulation include providing the proof of concept of the therapeutic use of antisense oligonucleotides (ASO) for a number of neurometabolic gene defects. For this aim, we have generated by CRISPR/Cas novel cellular and animal models with specific splicing variants in the *PAH* gene, responsible for phenylketonuria. The aim is to study the molecular pathogenesis of patient specific mutations and to identify therapeutic ASO to correct the splice defect. In other gene defects, we have focused on the correction of the aberrant insertion in the mRNA of pseudoexons activated by deep-intronic mutations. Such events are heavily underreported, a situation that may change with the incorporation of transcriptome sequencing as part of the diagnostic pipeline. Thus, we have identified several pseudoexons prone to activation by different point mutations in the PTS gene, responsible for a tetrahydrobiopterin (BH4) synthesis defect resulting in hyperphenylalaninemia and monoamine neurotransmitter deficiency. We have identified several ASO that can correct the aberrant insertion of four overlapping pseudoexons activated by different mutations, resulting in protein recovery.

In addition to our work with splicing defects in neurometabolic diseases, we have recently identified a series of ASO that modulate missplicing of a neuronal microexon involved in autism spectrum disorders and schizophrenia (collaboration with Dr. Jose J Lucas, CBMSO and Dr. Brage S. Andresen, University of Southern Denmark; patent PCT/EP2022/077882).

List of publications

» Soria LR, et al. (2022) O-GlcNAcylation enhances CPS1 catalytic efficiency for ammonia and promotes ureagenesis. Nat Commun. 13(1):5212. doi: 10.1038/s41467-022-32904-x.

» Martinez-Pizarro A, Desviat LR. (2022) RNA solutions to treat inborn errors of metabolism. Mol Genet Metab. 136(4):289-295. doi: 10.1016/j.ymgme.2022.07.006.

» Martínez-Pizarro A, Leal F, Holm LL, Doktor TK, Petersen USS, Bueno M, Thöny B, Pérez B, Andresen BS, Desviat LR.(2022) Antisense Oligonucleotide Rescue of Deep-Intronic Variants Activating Pseudoexons in the 6-Pyruvoyl-Tetrahydropterin Synthase Gene. Nucleic Acid Ther. 32(5):378-390. doi: 10.1089/nat.2021.0066.

» Briso-Montiano Á, Vilas A, Richard E, Ruiz-Sala P, Morato E, Desviat LR, Ugarte M, Rodríguez-Pombo P, Pérez B. (2022) Hepatocyte-like cells differentiated from methylmalonic aciduria cblB type induced pluripotent stem cells: A platform for the evaluation of pharmacochaperoning. Biochim Biophys Acta Mol Basis Dis.;1868(9):166433. doi: 10.1016/j.bbadis.2022.166433

» López-Márquez A, Martínez-Pizarro A, Pérez B, Richard E, Desviat LR.(2022) Modeling Splicing Variants Amenable to Antisense Therapy by Use of CRISPR-Cas9-Based Gene Editing in HepG2 Cells. Methods Mol Biol. 2434:167-184. doi: 10.1007/978-1-0716-2010-6_10.

» Luque J, et al. (2022) CIBERER: Spanish national network for research on rare diseases: A highly productive collaborative initiative. Clin Genet. 101(5-6):481-493. doi: 10.1111/cge.14113.

» Hammond S, et al. (2021) Delivery of oligonucleotide-based therapeutics: challenges and opportunities. EMBO Mol Med.;13(4):e13243. doi: 10.15252/emmm.202013243.

» Alonso-Barroso E, Pérez B, Desviat LR, Richard E. (2021) Cardiomyocytes Derived from Induced Pluripotent Stem Cells as a Disease Model for Propionic Acidemia. Int J Mol Sci. 22(3):1161. doi: 10.3390/ijms22031161.

Patents

» Inventors : Lourdes Ruiz-Desviat, Ainoha Martínez-Pizarro, Sara Picó, José J Lucas, Brage S Andresen
Title : Splice shifting oligonucleotides for use in the treatment of diseases characterized by altered inclusion of microexons
REF. PCT/EP2022/077882, Priority date 07 October 2021
Title holder: UAM/CSIC/University of Southern Denmark

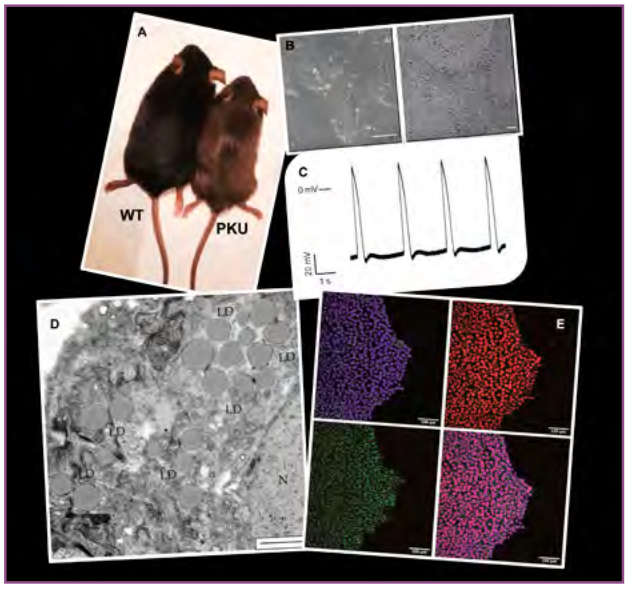

Figure: Disease models used in our research. A) PKU mouse model generated by CRISPR/Cas. B) PA iPSCs and differentiated cardiomyocytes C) Action potentials and (D) EM image of PA cardiomyocytes. E) Pluripotency markers in iPSCs.

Participation in projects

» "Elucidation of cardiac electrophysiological alterations in propionic acidemia: Towards the identification of targets for therapeutics". Propionic Acidemia Foundation (PAF-113). (2022-2023). PI: Eva María Richard Rodríguez.

» "Acidemia propiónica: impacto en el epigenoma y el proteoma en relación con el fenotipo cardiaco y neurológico". Fundación Ramón Areces, XX concurso nacional para la adjudicación de ayudas a la investigación en ciencias de la vida y de la materia 2020 (May 2021-May 2024). PI: Eva María Richard Rodríguez.

» "Mecanismos responsables del fenotipo patológico en enfermedades neurometabólicas raras y aproximaciones terapéuticas personalizadas" PID2019-105344RB-I00. MICINN (2020-2022). PIs: Lourdes Ruiz Desviat & Eva María Richard Rodríguez.

» "Red de recursos genómicos, funcionales, clínicos y terapéuticos para el estudio de las enfermedades raras neurológicas". Programas de actividades de I+D entre grupos de investigación de la Comunidad de Madrid (Biomedicina S2017/BMD-3721). (2018-2021). Coordinador: Carmen Ayuso.

» "Cardiomyocytes derived from induced pluripotent stem cells as a new model for therapy development in propionic acidemia". Propionic Acidemia Foundation (PAF-107). (2019-2021). PI: Eva María Richard Rodríguez.

» European cooperation in Science and Technology (COST) Action CA17103. Delivery of RNA therapy (2018-2023). Lourdes R Desviat, Management Committee member Spain.

Networks:

» CIBER DE ENFERMEDADES RARAS (CIBERER). ISCIII CB06/07/0017.

» Instituto de Investigación Sanitaria Hospital La Paz (IdiPaz).

CALCIUM SIGNALING IN MITOCHONDRIA: METABOLIC CONTROL AND MITOCHONDRIAL PHYSIOPATHOLOGY

Group Members

Principal Investigators:
Jorgina Satrústegui Gil-Delgado
Beatriz Pardo Merino
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Cayetano von Kobbe Alonso

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Elena Bogónez Peláez (until September 2022)

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Teresa Mingo Morcillo (from October 2022)

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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.

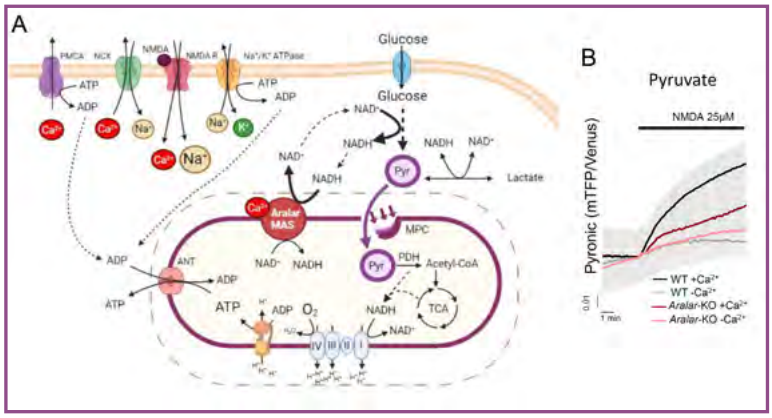
In neurons, calcium is thought to regulate neuronal activation, by adjusting ATP production to ATP consumption. This occurs thanks to stimulation of glycolysis and OXPHOS. The mitochondrial calcium uniporter (MCU) was thought to play a major role by increasing mitochondrial calcium and OXPHOS in response 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 an 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) 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 Ca^{2+} 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) Scheme of the cytosolic and mitochondrial processes involved. (B) Cytosolic sensor used to image pyruvate levels during NMDA stimulation. In Aralar-KO neurons, NMDA fails to activate glycolysis, and increase pyruvate levels. These responses are Ca2+-dependent (compare + /- Ca2+ recordings).

List of publications

» Pérez-Liébana, I., Juaristi, I., González-Sánchez, P., González-Moreno, L., Rial, E., Podunavac, M., Zakarian, A., Molgó, J., Vallejo-Illarramendi, A., Mosqueira-Martín, L., Lopez de Munain, A., Pardo, B., Satrústegui, J., del Arco, A. (2022) A Ca2+-Dependent Mechanism Boosting Glycolysis and OXPHOS by Activating Aralar-Malate-Aspartate Shuttle, upon Neuronal Stimulation. J Neurosci. 42(19):3879-3895. doi: 10.1523/JNEUROSCI.1463-21.2022

» Pardo, B., Herrada-Soler, E., Satrústegui, J., Contreras, L., del Arco, A. (2022) AGC1 Deficiency: Pathology and Molecular and Cellular Mechanisms of the Disease. Int J Mol Sci. 23(1):528. doi: 10.3390/ijms23010528.

» Sánchez-González, C., Herrero Martín, J.C., Salegi Ansa, B., Núñez de Arenas, C., Stančič, B., Pereira, M.P., Contreras, L., Cuezva, J.M., Formentini, L. (2022). Chronic inhibition of the mitochondrial ATP synthase in skeletal muscle triggers sarcoplasmic reticulum distress and tubular aggregates. Cell Death Dis 13(6):561. doi: 10.1038/s41419-022-05016-z.PMID: 35732639

» García-Catalán S, González-Moreno L, del Arco A. Ca2+-regulated mitochondrial carriers of ATP-Mg2+/Pi: Evolutionary insights in protozoans. (2021) Biochim Biophys Acta Mol Cell Res. 1868(7):119038. doi:10.1016/j.bbamcr.2021.119038

» Esparza-Moltó, P.B., Romero-Carramiñana, I., Núñez de Arenas, C., Pereira, M.P., Blanco, N., Pardo, B., Bates, G.R., Sánchez-Castillo, C., Artuch, R., Murphy, M.P., Esteban, J.A., Cuezva, J.M. (2021) Generation of mitochondrial reactive oxygen species is controlled by ATPase inhibitory factor 1 and regulates cognition. PLoS Biol. 19(5):e3001252. doi: 10.1371/journal.pbio.3001252.

» Nuevo-Tapioles, C., Santacatterina, F., Sánchez-Garrido, B., de Arenas., C.N., Robledo-Bérgamo, A., Martínez-Valero, P., Cantarero, L., Pardo, B., Hoenicka, J., Murphy, M.P., Satrústegui, J., Palau, F., Cuezva, J.M. (2021) Effective therapeutic strategies in a preclinical mouse model of Charcot-Marie-Tooth disease. Hum Mol Genet. 30(24):2441-2455. doi: 10.1093/hmg/ddab207.

» Civera-Tregón, A., Domínguez, L., Martínez-Valero, P., Serrano, C., Vallmitjana, A., Benítez, R., Hoenicka, J., Satrústegui, J., Palau, F. (2021) Mitochondria and calcium defects correlate with axonal dysfunction in GDAP1-related Charcot-Marie-Tooth mouse model. Neurobiol Dis. 152:105300. doi: 10.1016/j.nbd.2021.105300.

» Rubio, C., Lizárraga, E., Alvarez-Cilleros, D., Pérez-Pardo, P., Sanmartín-Salinas, P., Toledo-Lobo, M.V., Alvarez, C., Escrivá, F., Fernández-Lobato, M., Guijarro, L.G., Valverde, A. M., Carrascosa, J.M. (2021) Aging in male Wistar rats associates with changes in intestinal microbiota, gut structure, and cholecystokinin-mediated gut-brain axis function. J Gerontol A Biol Sci Med Sci, 76: 1915-1921. doi: 10.1093/gerona/glaa313

Participation in projects

» This group is Member of the Instituto de Investigación Sanitara Fundación Jiménez Díaz (IIS FJD) (from 2013) as "Señalización mitocondrial del calcio" unit.

» Citrin replacement with Aralar: dissecting Citrin-Aralar interactions. CITRIN Foundation Research Grants. PI: Jorgina Satrústegui. 04/2020-06/2022.

» Generation of a new human-like citrin-deficiency mouse model to study Citrin deficiency. CITRIN Foundation Research Grants. PIs: Laura Contreras & Araceli del Arco. 06/2022-10/2024

MITOCHONDRIAL BIOLOGY IN IMMUNE MODULATION

Group Members

Principal Investigator:
Javier Traba Domínguez

Predoctoral Fellow:
Carolina Meroño Ortega (March 2021 - present)

Predoctoral Fellow:
Aurea Oliva Herrero (September 2021 - present)

Technician:
Rocío Moreno Palomares (April 2022 - December 2022)

Yara Cuesta Valero (Undergraduate Student, April 2021 – July 2021; Master Student, February 2022 – June 2022)

Ana Alcalá Lalinde (Undergraduate Student, January 2022 – June 2022)

Master Student:
Alejandra Carrancho Arroyo (January 2021 – July 2021)

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Summary

The mitochondria are essential organelles that carry out diverse functions in the eukaryotic cell. In addition to ATP production by oxidative phosphorylation, they participate in many pathways such as heat production, calcium signaling, detoxification of reactive oxygen species, synthesis of heme and other molecules, and regulation of cell death. Emerging functions include their role in the regulation of innate and adaptive immune responses, which happens largely by two mechanisms:

1) The mitochondria regulate immunometabolism, which studies how the metabolism of the immune cell changes when they are activated or differentiate into effector cell, and how those metabolic changes are essential for their effector function. For instance, M1 or proinflammatory macrophages in culture are largely glycolytic, whereas M2 or reparatory macrophages utilize oxidative phosphorylation to meet their ATP requirements.

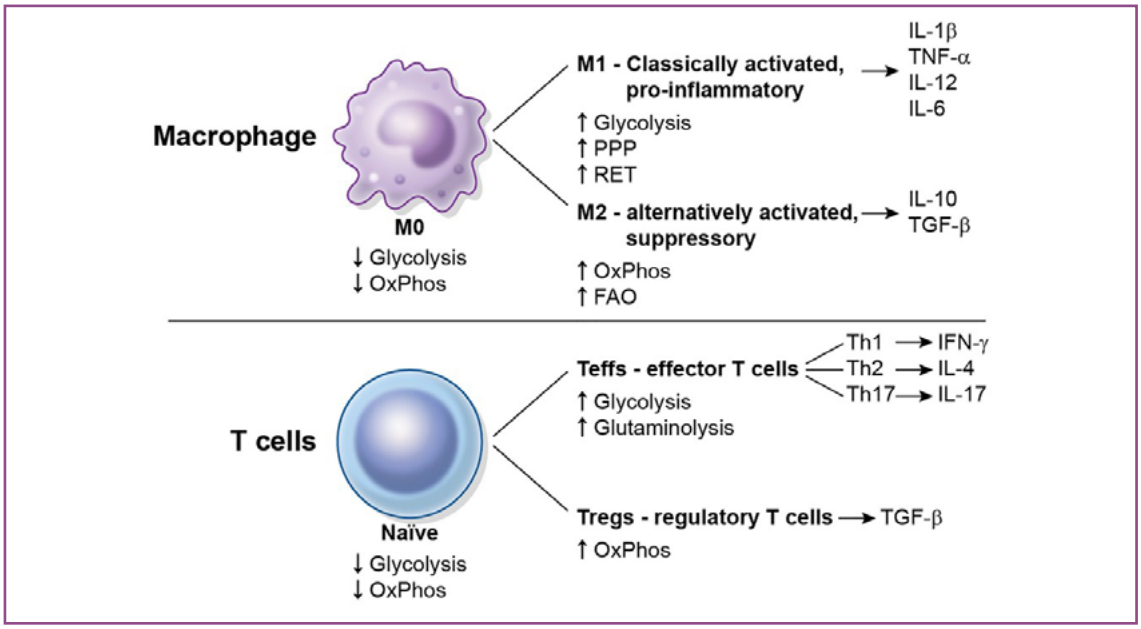
2) During dysfunction, the mitochondria—due to their prokaryotic origin—can produce and release molecules (mitochondrial DNA, formyl peptides, etc.) that activate diverse routes of innate immune signaling, such as the NLRP3 inflammasome or the cGAS-STING pathway. This leads to the secretion of proinflamatory cytokines, including interleukin-1β or type I interferons.

The mitochondria thus play a key role in immune regulation. The overarching goal of our group is to study the role of the mitochondria in the modulation of innate and adaptive immune pathways.

We are particularly interested in how posttranslational modifications (PTMs) of mitochondrial proteins—such as lysine acetylation or succinylation—will regulate the activation and polarization of the macrophage. For this project, we focus on the roles of mitochondrial nicotinamide adenine dinucleotide (NAD+)-dependent deacetylases (like Sirtuin 3, SIRT3) or desuccinylases (like Sirtuin 5, SIRT5). Indeed, we have recently found that SIRT3 controls the secretion of type I interferons, which are antiviral molecules.

In the laboratory, we also study the role of mitochondrial metabolites, including adenine nucleotides (adenosine mono-, di- or triphosphate) or NAD+, in the activation and differentiation of macrophages and T lymphocytes. As transport of metabolites across the inner mitochondrial membrane is carried out by proteins of the Mitochondrial Carrier Family, we are altering the expression of mitochondrial carriers specific for those metabolites in immune cells, including SLC25A24/SCaMC-1, a calcium-dependent mitochondrial transporter for ATP-Mg/Pi, and SLC25A51/MCART1, the recently identified mitochondrial transporter for NAD+.

Since autoimmune or degenerative diseases are associated with imbalances in macrophage polarity or lymphocyte lineage differentiation, the study of how mitochondrial metabolites affect the differentiation of immune cells, and its potential modulation by compounds such as NAD+ precursors, might be interesting for potential future therapies.



Overview of metabolic changes in macrophages and CD4+ T cells upon activation. Mitochondrial metabolites such as adenine nucleotides or nicotinamide adenine dinucleotide (NAD+) might alter polarization of the macrophages into the M1 or M2 phenotypes, or differentiation of the T cell into the different lineages.

List of publications

Articles:

- Geiger, S. S., Traba, J., Richoz, N., Farley, T. K., Brooks, S. R., Petermann, F., Wang, L., Gonzalez, F. J., Sack, M. N. and Siegel, R. M. (2021) Feeding-induced resistance to acute lethal sepsis is dependent on hepatic BMAL1 and FXR signalling. Nat. Commun. 12, 2745.doi: 10.1038/s41467-021-22961-z
- Traba, J., Sack, M. N., Waldmann, T. A. and Anton, O.M. (2021) Immunometabolism at the Nexus of Cancer Therapeutic Efficacy and Resistance. Front. Immunol. 12, 657293.doi: 10.3389/fimmu.2021.657293
- Araki, D., Fu, J. F., Huntsman, H., Cordes, S., Seifuddin, F., Alvarado, L. J., Cheruku, P. S., Cash, A., Traba, J., Li, Y., Pirooznia, M., Smith, R. H. and Larochelle, A. (2021) NOTCH-mediated *ex vivo* expansion of human hematopoietic stem and progenitor cells by culture under hypoxia. Stem Cell Reports. 16, 2336-2350.doi: 10.1016/j.stemcr.2021.08.001
- Oliva, A., Meroño, C and Traba, J. (2022) Mitochondrial function and dysfunction in innate immunity. Curr. Opin. Physiol. 28, 100571.doi: 10.1016/j.cophys.2022.100571

Book Chapters:

- Antón, O. M. and Traba, J. (2022) Measurement of Cytosolic Mitochondrial DNA After NLRP3 Inflammasome Activation. In: Abdul-Sater, AA (ed) Methods in Molecular Biology 2459, The Inflammasome, Methods and Protocols. Humana Press, United States, pp. 117-129.
- Traba, J. and Antón, O.M. (2022) Assessing Changes in Human Natural Killer Cell Metabolism Using the Seahorse Extracellular Flux Analyzer. In: Shimasaki, N (ed) Methods in Molecular Biology 2463, Natural Killer (NK) Cells, Methods and Protocols. Humana Press, United States, pp. 165-180.

Participation in projects

- Regulación inmunometabólica a través de la proteína mitocondrial Sirtuina 3. PID2019-105665RA-I00, Agencia Estatal de Investigación. Principal Investigator: Javier Traba Domínguez. 01/06/2020- 31/05/2023.
- Ramón y Cajal. Agencia Estatal de Investigación. RYC2018-026050-I, Principal Investigator: Javier Traba Domínguez (Universidad Autónoma de Madrid). 01/09/2020-31/08/2025.
- COVTRAVI-19-CM, Plataformas y modelos preclínicos para el abordaje multidisciplinar en COVID-19 y en la respuesta a futuras pandemias. Comunidad de Madrid. PI: Manuel Fresno (Universidad Autónoma de Madrid). 01/01/2022-31/12/2022. Group member

PROGRAM

Interactions with the Environment

Interactions with the Environment

Immune System Development and Function Unit

César Cobaleda

The immune system is in charge of maintaining the integrity of living organisms, protecting them both from infectious diseases and from endogenous alterations of tissue homeostasis. However, its deregulation can lead to both immunodeficiencies and autoimmune disease and, furthermore, the cells of the immune system can become malignant themselves. In our Unit, basic and translational scientists are focused on the study of the molecular, cellular, genetic and epigenetic mechanisms regulating the development and function of the cells of the immune system, and the bases of their interactions with self-components and pathogens, with the final goal of understanding its malfunction in pathological conditions as a key requirement for developing novel therapeutic strategies.

In these two years, studying normal lymphocyte development, it was found that efficient signaling via the earliest form of the T-cell receptor (TCR) was critical for generation of a diverse TCR repertoire, and mice with impaired signaling can generate hyperactive T-cells and may suffer from autoimmune reactions. Also, novel insights were provided into the mechanisms that control thymic involution during aging; indeed, a new role for Notch1 signaling in thymic epithelial cells was identified, paving the way for novel therapies to improve T-cell production in aging and other clinical settings.

Regarding lymphocyte activation and its role in normal and aberrant immune responses, we have discovered that interleukin-23 controls protein synthesis and cellular metabolism to induce pathogenic functions in T cells, with implications for autoimmune and inflammatory disorders. Our groups have also identified novel unconventional roles of the autophagic machinery in the regulation of cytokine signaling and inflammation. Also, novel targets of nitroalkylation by nitro-fatty acids have been found involved in controlling T-cell activation, transcriptional induction and production of proinflammatory cytokines.

In the field of hematopoietic malignancies, our groups have validated critical physiological pathways of T-cell development as suitable targets for novel immunotherapies, including Chimeric Antigen Receptors. They have also found that preventive pharmacological treatment of mice predisposed to childhood B-cell acute leukemia with JAK/STAT inhibitors protects them from the appearance of the disease. Our groups are also studying the metabolic and immune features of aggressive High Grade B Cell Lymphomas, to identify new targeted therapies against them.

In the context of the response against pathogens, a model of Trypanosoma cruzi infection has shown a relationship between HCN4 overexpression and the risk of sudden death in Chagas disease; also, autoantibodies against the human protein sCha have been identified as biomarkers for the risk of such sudden death. Regarding viruses, metagenomic sequencing studies have uncovered the evolutionary history of ectromelia virus, which causes a mouse disease similar to human smallpox, and identified unexpected genetic variability of human herpes simplex viruses. Other groups are studying the immune response to viral infections, trying to develop strategies that can contribute to vaccine development.

Finally, with the global emergence of novel viral pandemics, several groups of the Unit are studying SARS-CoV-2 and monkeypox viruses from different perspectives, like the evaluation of airborne virus transmission in public places, the development of new assays for detection of antiviral antibodies, the characterization of the nature of the immune response and the degree of protection conferred by infection or vaccination, or the characterization of the new virus clades.

Balbino Alarcon
SIGNAL TRANSDUCTION BY THE T-CELL ANTIGEN RECEPTOR

Antonio Alcami
IMMUNITY AND VIROMICS

Cesar Cobaleda Hernández
CELLULAR PLASTICITY IN DEVELOPMENT AND CANCER

Margarita Del Val Latorre
VIRAL IMMUNOLOGY

Manuel Fresno Escudero
IMMUNE DEVELOPMENT AND INFLAMMATORY-MEDIATED DISEASES

Nuria Girones
IMMUNOREGULATORY MECHANISMS IN THE DEVELOPMENT OF CHAGAS DISEASE: TRANSLATIONAL APPLICATIONS

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

Juan Manuel Serrador / Miguel Ángel Íñiguez
NITRIC OXIDE AND BIOACTIVE LIPIDS IN THE IMMUNE RESPONSE

María Luisa Toribio García
DEVELOPMENT OF THE HUMAN LYMPHOHEMATOPOIETIC SYSTEM

Hisse Martien van Santen
TCR DOMAINS IN T CELL DIFFERENTIATION AND PATHOPHYSIOLOGICAL AND THERAPEUTIC RESPONSES

SIGNAL TRANSDUCTION BY THE T-CELL ANTIGEN RECEPTOR

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Valentina Blanco
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Undergraduate and Master Students
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Paula Cárdenas

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Summary

T-lymphocyte activation requires recognition of antigenic peptides on MHC (pMHC) by the T cell antigen receptor (TCR). The TCR contacts the antigen extracellularly through its variable α and β chains, which have very short cytoplasmic tails, so that signal initiation is passed on to the CD3 subunits, which contain longer tails that can contact intracellular interactors. We have found that the TCR is organized in the plasma membrane of resting T cells as oligomers of up to 20 TCRs that we have named as TCR nanoclusters. We believe that the organization of the TCR in nanoclusters can explain in part the high sensitivity of T cells for antigen in spite the low affinity of the monovalent TCR for pMHC. Indeed, we have recently found that TCR nanoclustering allows cooperativity phenomena between pMHC-engaged and non-engaged TCRs. In addition, we have proposed that conformational changes mediate the conversion of pMHC-TCR contacts into CD3-driven intracellular signals, thus triggering the TCR. One of the consequences of the conformational change in the TCR is the exposure of a proline-rich sequence (PRS) of CD3ε that becomes available for binding to the adaptor protein Nck. We have developed small molecular weight inhibitors of the recruitment of Nck to the TCR as immunomodulatory agents. These inhibitors are orally available and show a potent prophylactic and therapeutic effect in different models of autoimmune diseases while sparing the T cell response to pathogens. Another direct effector of the TCR is the small GTPase RRas2 (also known as TC21), which binds constitutively to the non-phosphorylated TCR and plays important roles in homeostatic signaling through PI3K. RRas2 is also a direct effector of the B cell antigen receptor (BCR). We are studying the role of RRas2 in physiological processes of T and B lymphocytes such as homeostatic control of the populations, formation of the immunological synapse, thymic selection, germinal center formation, as well as in pathological processes such as formation of T and B cell lymphomas and leukemias. Our most exciting recent findings suggest that RRas2 is an oncogenic driver in the generation of chronic lymphocytic leukemias and some types of breast cancer. RRas2 drives the generation of cancer in the absence of activating mutations which are common in KRas and other classic Ras GTPases. We are trying to figure out the mechanisms and the relevance of RRas2 for human cancer.

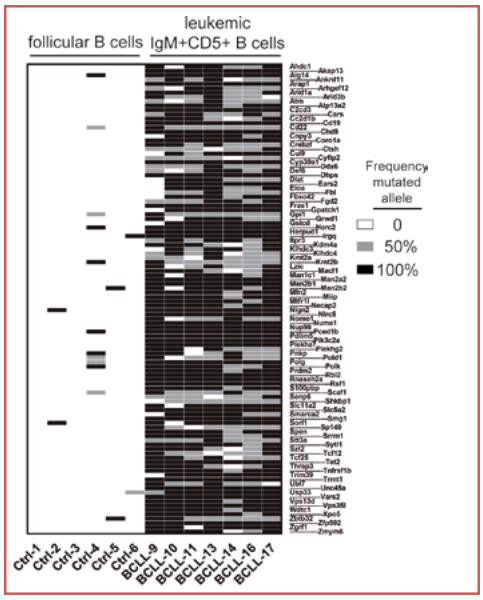
Other activities

Books

» Kumari, S., Schamel, W.W. and Alarcon, B. (2021). Editorial: Cytoskeletal Regulation of Immune Response. Front. Cell. Dev. Biol. 9:791327. doi: 10.3389/fcell.2021.791327

Workshops

» Understading TCR structure and signaling for efficient Immunotherapy". Org.: B. Alarcón, W. Schamel and O. Acuto. Current Trends in Biomedicine Workshops, International University of Andalucia, Baeza 18-20 October 2022.



Heatmap of mutation rates of 107 mutated genes detected in leukemic cells from 7 independent mice overexpressing human RRAS2 in B cells and 6 control mice. Black: 100% of the mRNA sequences bear the same mutation; grey: 50% of mRNA sequences bear the same mutation; white: unmutated

Participation in projects

- Recreación de interacciones T:T y T:B in vitro como sistema para entender nuevas funciones linfoides tanto fisiológicas como patológicas. PID2019-104935RB-100. 2020-2022. PI: Balbino Alarcón.
- Desarrollo ultrarrápido de anticuerpos humanos neutralizantes del SARS-CoV-2 mediante aproximaciones in silico e in vitro. CSIC PIE-202020E081. 2020-2021. 202020E081.
- Modelo en ratón de leucemia linfocítica crónica (LLC) para la validación de nuevas intervenciones terapéuticas. PDC2021-121170-I00. 2022-2023. PI: Balbino Alarcón.

List of publications

- Horndler, L., Delgado, P., Abia, D., Balabanov, I., Martínez-Fleta, P., Cornish, G., Llamas, M.A., Serrano-Villar, S., Sánchez-Madrid, F., Fresno, M., van Santen, H.M. and Alarcón, B. (2021). Flow cytometry multiplexed method for the detection of Neutralizing human antibodies to the native sars-cov-2 spike protein. EMBO Mol. Med. 13(3):e13549. doi: 10.15252/emmm.202013549
- Viola L Boccasavia, V.L., Borroto, A., Oeste, C.L., Prieto, C., Alonso-López, D., Diaz-Muñoz, M.D., Batista, F.D. and Alarcón, B. (2021). Antigen presentation between T-cells drives Th17 polarization under conditions of limiting antigen. Cell Rep. 34(11):108861. doi: 10.1016/j.celrep.2021.108861
- Piñero, P., Marco De La Calle, F.M., Horndler, L., Alarcón, B., Uribe Barrientos, M., Sarmiento, H. and Tarín, F. (2021). Flow cytometry detection of sustained humoral immune response (IgG + IgA) against native spike glycoprotein in asymptomatic/mild SARS-CoV-2 infection. Sci. Rep. 2021 11(1):10716. doi: 10.1038/s41598-021-90054-4
- Rueda-Carrasco, J., Martin-Bermejo. M.J., Pereyra, G., Mateo, M.I., Borroto, A., Brosseron, F,, Kummer, M,P,, Schwartz, S., López-Atalaya, J.P., Alarcon, B., Esteve, P., Heneka, M,T, and Bovolenta, P. (2021). SFRP1 modulates astrocyte-to-microglia crosstalk in acute and chronic neuroinflammation. EMBO Rep. 2021 27:e51696. doi: 10.15252/embr.202051696
- Almendro-Vázquez, P,, Laguna-Goya, R., Ruiz-Ruigomez. M., Utrero-Rico, A., Lalueza, A., Maestro de la Calle, G., Delgado, P., Perez-Ordoño, L., Muro, E., Vila, J., Zamarron, I., Moreno-Batanero, M., Chivite-Lacaba, M., Gil-Etayo, F.J., Martín-Higuera, C., Meléndez-Carmona, M.Á., Lumbreras, C., Arellano, I., Alarcon, B., Allende, L.M., Aguado, J.M. and Paz-Artal E. (2021). Longitudinal dynamics of SARS-CoV-2-specific cellular and humoral immunity after natural infection or BNT162b2 vaccination. PLoS Pathog. 17(12):e1010211. doi: 10.1371/journal.ppat.1010211
- Hortal, A.M., Oeste, C.L., Cifuentes, C., Alcoceba, M., Fernández-Pisonero, I., Clavaín, L., Tercero, R., Mendoza, P., Domínguez, V., García-Flores, M., Pintado, B., Abia, D., García-Macías, C., Navarro-Bailón, A., Bustelo, X.R., González, M. and Alarcón, B. (2022). Overexpression of wild type RRAS2, without oncogenic mutations, drives chronic lymphocytic leukemia. Mol. Cancer 21(1):35. doi: 10.1186/s12943-022-01496-x
- Maza, M.D.C., Úbeda, M., Delgado, P., Horndler, L., Llamas, M.A,, van Santen, H.M., Alarcón, B., Abia, D., García-Bermejo, L., Serrano-Villar, S., Bastolla, U. and Fresno, M. (2022). ACE2 Serum Levels as Predictor of Infectability and Outcome in COVID-19. Front. Immunol. 13:836516. doi: 10.3389/fimmu.2022.836516
- Romero-Pinedo, S., Quesada, M., Horndler, L., Álvarez-Fernández, S., Olmo, A., Abia, D., Alarcón, B. and Delgado, P. (2022). Vaccine Type-, Age- and Past Infection-Dependence of the Humoral Response to SARS-CoV-2 Spike S Protein. Front. Immunol. 13:809285. doi: 10.3389/fimmu.2022.809285
- Fernández-Pisonero, I., Clavaín, L., Robles-Valero, J., Lorenzo-Martín, L.F., Caloto, R., Nieto, B., García-Macías, C., Oeste, C.L., Sánchez-Martín, M., Abad, A., Hortal, A., Caballero, D., González, M., Dosil, M., Alarcón, B and Bustelo, X,R. (2022). A hotspot mutation targeting the R-RAS2 GTPase acts as a potent oncogenic driver in a wide spectrum of tumors. Cell Rep. 38:110522. doi: 10.1016/j.celrep.2022.110522
- Borroto, A., Alarcón, B. and Navarro, M.N. (2022). Mutation of the Polyproline Sequence in CD3ε evidences TCR signaling requirements for differentiation and function of pro-Inflammatory Tγδ17 cells. Front. Immunol. 13:799919. doi: 10.3389/fimmu.2022.799919

Patents

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

IMMUNITY AND VIROMICS

Group Members

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Undergraduate and Master Students
Miguel Angel Salazar (01/02/2021-30/07/2021)

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Summary

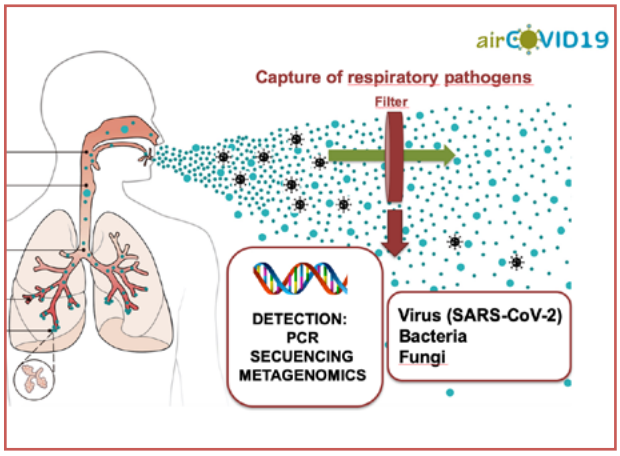
We work on poxvirus proteins that modulate the host immune and inflammatory responses. We are characterizing poxvirus proteins secreted from infected cells that interact with type I interferon, tumor necrosis factor or chemokines, and modulate their activity. We also work on a viral mechanism blocking DNA sensing (poxin/schlafen) that prevents the activation of type I interferon induction. The contribution of these viral proteins to pathogenesis is tested in a mouse model of infection (mousepox). Understanding the mechanism of action of these viral proteins provide information on the function of the immune system and new strategies to control the immune response. We are using this information to develop new anti-inflammatory therapeutic approaches that we may take to the clinic to block inflammatory responses that cause human diseases.

Our projects on viral metagenomics have reconstructed the natural history of ectromelia virus isolates causing mousepox outbreaks in animal house facilities around the world and provided the first genomic sequence from a natural isolate. Studies on herpesviruses showed that herpes simplex virus 1 (HSV-1) and HSV-2 generate greater diversity than expected for DNA viruses. HSV-2, infecting genital mucosa, generates more variants than HSV-1, infecting oronasal mucosa, and this may have contributed to the evolutionary divergence of this viruses adapting to different anatomical niches. We are extending our environmental viral metagenomic projects to 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 may identify common properties of viruses that inhabit polar and alpine regions, similar extreme ecosystem separated geographically.

The COVID19 pandemic has illustrated the importance of airborne virus transmission and the need to improve the microbiological quality of the air. During the COVID19 pandemic we optimized a filtration technology to capture airborne viruses and have used it to monitor the presence of SARS-CoV-2 in the air of hospitals and to sequence complete genomes of viruses present in air samples. We are extending these studies to other human respiratory viruses. Using this technology, we demonstrated the presence of *mpox* (monkeypox) virus in air samples during the recent 2022 international *mpox* outbreak, suggesting that airborne transmission may play a role in future outbreaks of mpox. In collaboration with technologists, we are developing new methods to sample airborne viruses and to inactivate viruses in the air.

Doctoral theses

» Carlos Fernández Linares (2021). Análisis bioinformático de metagenomas de virus polares. Universidad Autónoma de Madrid. Co-supervised: A. Alcamí and A. Rastrojo.



Air sampler based on filtration through teflon or nanofiber filters.

Patents

» Alcamí, A, Hernaez, B. and Alvarez de Miranda, J. Interferon inhibitor and its use in the treatment of autoinflammatory diseases. Spanish Patent EP2238282 06/09/2022. Owner: CSIC.

» Alcamí, A., Sanchiz, A., Lagarón, J. M., Prieto, C. and Pardo, M. Capture equipment for airborne biological particles. Spanish Utility Model 21/07/2022. Owner: CSIC.

» Pérez-Díaz, J. L. and Alcamí, A. Apparatus and methods for sampling airborne particles. J.L. US Patent Application 02/2020. Owner: Counterfog Co. and CSIC.

» Rastrojo, A. and Alcamí, A. Device and method for capturing and analysing airborne organisms. International PCT/EP2021/065932 14/06/2021. Owner: CSIC.

List of publications

» Hernaez, B. et al. (2022) Monitoring monkeypox virus 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.

» Del Álamo, C. et al. (2022) Fast Air-to-Liquid sampler detects surges in SARS-CoV-2 aerosol levels in hospital rooms. Int. J. Environ. Res. Public Health 20(1):576. doi: 10.3390/ijerph20010576.

» López-Muñoz, A. D. et al. (2022) High-throughput engineering of cytoplasmic- and nuclear-replicating large dsDNA viruses by CRISPR/Cas9. J. Gen. Virol. 103(10). doi: 10.1099/jgv.0.001797.

» Del Álamo, C. et al. (2022) Assessment of surface disinfection effectiveness of decontamination system COUNTERFOG® SDR-F05A+against bacteriophage ϕ29. Food Environ. Virol. 14(3):304-313. doi: 10.1007/s12560-022-09526-z.

» Martín-Delgado, M. C. et al. (2022) Monkeypox in humans: a new outbreak. Rev. Esp. Quimioter. 35(6):509-518. doi: 10.37201/req/059.2022.

» Alvarez-de Miranda, F. J. et al. (2021) TNF decoy receptors encoded by poxviruses. Pathogens 10(8):1065. doi: 10.3390/pathogens10081065.

» López, M. G. et al. (2021) The first wave of the COVID-19 epidemic in Spain was associated with early introductions and fast spread of a dominating genetic variant. Nat. Genet. 53(10):1405-1414. doi: 10.1038/s41588-021-00936-6.

» López-Muñoz, A. D. et al. (2021) Herpes simplex virus 2 (HSV-2) evolves faster in cell culture than HSV-1 by generating greater genetic diversity. PLoS Pathog. 17(8):e1009541. doi: 10.1371/journal.ppat.1009541.

» Mavian, C. et al. (2021) Comparative pathogenesis, genomics and phylogeography of mousepox. Viruses 13(6):1146. doi: 10.3390/v13061146.

» López-Muñoz, A. D. et al. (2021) Combination of long- and short-read sequencing fully resolves complex repeats of herpes simplex virus 2 strain MS complete genome. Microb. Genom. 7(6):000586. doi: 10.1099/mgen.0.000586.

Research projects

» Ancient viruses and microorganisms in polar lakes. EU Horizon 2020 EASIGenomics (PID10643 POLARPALEOVIRUS). PI A. Alcamí. 07/2020-06/2022.

» Diversity of DNA and RNA viruses in Antarctica. Joint Genome Institute, U.S. Department of Energy (JGI Proposal ID: 505820; Contract No. DE-AC02- 05CH11231). PIs: A. Alcamí and C. Cary 01/2020-12/2023.

» NexGenerationEU: Strategic Line Transmission and Containment. CSIC Global Health Platform. Coordinators: A. Alcamí and C. Prieto. 04/21-12/23.

» Healthy environmental-friendly and resilient farm to work (HE-FARM) (101084097). Horizon EU. Coordinator: JL Pérez-Díaz. PI: A. Alcamí.11/22-10/25.

» High-efficient technology for clean and safe air: development of advanced air purifiers to inactivate pathogens in aerosols (SafeAir) (HR22-00813). Fundacion La Caixa. Coordinator: M.A.Bañares. PI: A. Alcamí.12/22-11/25.

Other activities

» Advisor to the World Health Organization Advisory Committee on Variola Virus Research.

CELLULAR PLASTICITY IN DEVELOPMENT AND CANCER

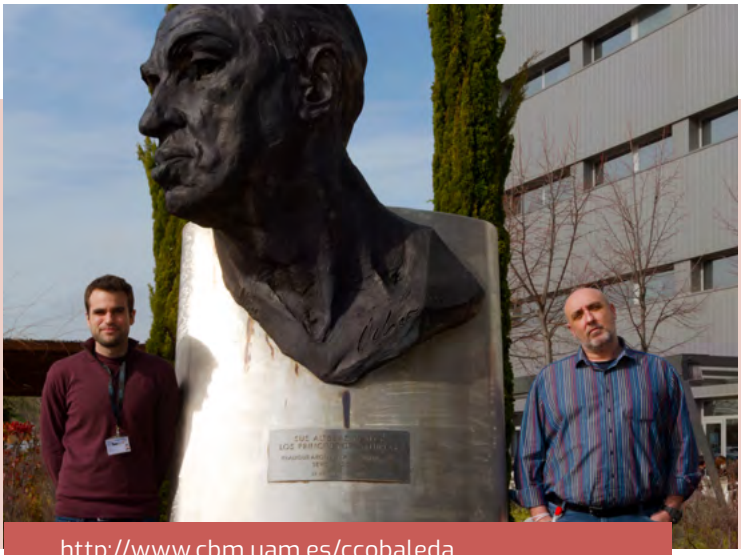
Group Members

Principal Investigator
César Cobaleda Hernández

Postdoctoral fellows
Alba Azagra Rodríguez (October 2021-October 2022)

Predoctoral fellows
Jorge Martínez Cano
Javier Isoler Alcaraz (co-IP: María Gómez)

Undergraduate and Master Students
Antonio Giráldez Trujillo (January-June 2022)
Sofía Gutiérrez Rodríguez (January-June 2021)



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

Summary

Our group wants to understand how cellular identity is determined and how it is deregulated in pathological conditions such as leukemias and immunodeficiencies. As experimental tools we use: i) samples from patients and ii) genetically engineered mouse models (GEMMs) in which we modify the expression of transcription factors and epigenetic regulators, either normal or oncogenic (Figure 1).

B-cell acute lymphoblastic leukemia (B-ALL) is the most common form of childhood cancer. Interestingly, almost 5% of all newborn children present an inborn genetic predisposition to develop B-ALL. Luckily, very few (<1%) of these predisposed children will progress to B-ALL, by suffering a second hit that leads to full-blown disease. The causes that trigger this progression are still unclear, but B-ALL incidence seems to be increasing in parallel with the adoption of modern lifestyles. A stress on the immune system has been postulated to be involved in the progression to B-ALL, and this stress could be triggered by exposure to common infections (under certain circumstances), or by other stressors like antibiotics, diet, or alterations in the microbiota. Therefore, understanding the interaction between the immune stress and the preleukemic cells existing in so many newborns could provide us with strategies aimed at preventing childhood B-ALL.

We have generated mice expressing the oncogenic lesions responsible for the initiation of childhood B-ALLs, and we have demonstrated that common infections are indeed one of the main triggering factors leading to leukemia development. Furthermore, we have shown that preventive pharmacological treatment of predisposed mice with inhibitors of the JAK/STAT oncogenic signaling pathway protects them from the appearance of the disease, showing that B-ALL could be a preventable disease. Since the presence of latent premalignant cells is a common characteristic of many types of cancers, our results offer a general proof-of-principle for the development of similar preventive strategies for other malignancies.

Wolf Hirschhorn Syndrome (WHS) is a complex rare disease, and affected patients present many severe problems, including life-threatening immunodeficiencies. One of the genes affected in WHS is *WHS-Candidate-1 (WHSC1)*, an epigenetic regulator involved in many processes affecting genome function and that, interestingly, is also mutated in childhood B-ALLs and other malignancies of B-cell origin like Multiple Myeloma. We are using GEMMs with deregulated WHSC1 function to gain insight into the molecular biology of WHS-associated hematopoietic pathologies, in order to better understand the role of epigenetics in normal and aberrant hematopoietic development, and to develop future prospective therapeutic interventions.

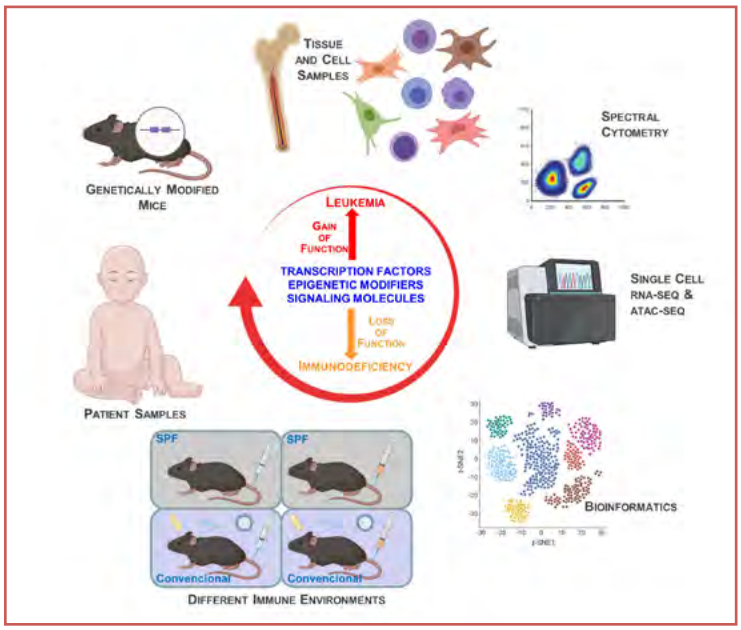


FIGURE. A COMPREHENSIVE APPROACH TO THE STUDY OF LEUKEMIAS AND IMMUNODEFICIENCIES. We use genetically modified mice where we express the molecular lesions associated with human hematopoietic pathologies, such as leukemias, myeloma or immunodeficiencies. We characterize these mice using state-of-the-art technologies like spectral cytometry, single-cell transcriptomics and epigenomics, or advanced bioinformatic approaches. We expose these animals to different types of environmental stress to better understand the interactions leading from genetic predisposition to the appearance of the disease. Finally, we validate our findings on human samples and propose new therapeutic possibilities.

List of publications

» Cobaleda, C, Vicente-Dueñas C and Sanchez-Garcia I. (2021) Infectious triggers and novel therapeutic opportunities in childhood B cell leukaemia. Nat Rev Immunol. 21:570-581. doi: 10.1038/s41577-021-00505-2.

» Cobaleda, C, Vicente-Dueñas, C and Sanchez-Garcia, I. (2021) An immune window of opportunity to prevent childhood B cell leukemia. Trends Immunol. 42:371-374. doi: 10.1016/j.it.2021.03.004.

» Cobaleda, C., Vicente-Dueñas, C., Ramirez-Orellana, M. and Sanchez-Garcia, I. (2022) Revisiting the concept of childhood preleukemia. Trends Cancer 8:887-889. doi: 10.1016/j.trecan.2022.06.012.

» Isidro-Hernandez, M., Aleman-Arteaga, S., Casado-Garcia, A., Ruiz-Corzo, B., Riesco, S., Prieto-Matos, P., Martinez-Cano, J., Sanchez, L., Cobaleda, C, Sanchez-Garcia, I. and Vicente-Dueñas C. (2022) Childhood B-Cell Preleukemia Mouse Modeling. Int. J. Mol. Sci. 23:7562. doi: 10.3390/ijms23147562.

» Casado-Garcia, A., Isidro-Hernandez, M., Oak, N., Mayado, A., Mann-Ran, C., Raboso-Gallego, J., Aleman-Arteaga, S., Buhles, A., Sterker, D., Sanchez, E.G., Martinez-Cano, J., Blanco, O., Orfao, A., Alonso-Lopez, D., De Las Rivas, J., Riesco, S., Prieto-Matos, P., Gonzalez-Murillo, M., Garcia Criado, F.J., Garcia Cenador, M.B., Radimerski, T., Ramirez-Orellana, M., Cobaleda, C., Yang, J.J., Vicente-Dueñas, C., Weiss, A., Nichols, K.E. and Sanchez-Garcia, I. (2022) Transient Inhibition of the JAK/STAT Pathway Prevents B-ALL Development in Genetically Predisposed Mice. Cancer Res. 82:1098-1109. doi: 10.1158/0008-5472.CAN-21-3386.

» Azagra A. and Cobaleda C. (2022) NSD2 as a Promising Target in Hematological Disorders. Int. J. Mol. Sci. 23:11075. doi: 10.3390/ijms231911075.

Other activities

» Member of the Scientific Advisory Board of “Fundación Unoentrecienmil”.

» 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) (since August 2022).

Participation in projects

» Participation in the EU COST Action CA16223 “LEGEND” (Leukemia Gene Discovery).

» Coordinated Project “PREVENT” from the “Asociación Española Contra el Cáncer” (since October 2021).

» IP Project SAF2017-83061-R (Programa Estatal de Investigación, Desarrollo e Innovación Orientada a los Retos de la Sociedad, until October 2021).

» IP Project PID2021-122787OB-I00 (Proyectos de Generación de Conocimiento, from September 2022).

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
Andrea C. Méndez (until July 10th 2022)

Predoctoral Fellows:
Cristina Rodríguez Rojas
Víctor Muñoz Abad
Andrés Soto Zaragoza

Predoctoral Fellows:
Beatriz Tejedor Sáez-Bravo (until August 28th 2022)
Ignacio Ruiz Fernández (from May 1st 2021 to November 30th 2021)
Carlos Jiménez García (from January 10th 2022 to September 30th 2022)

Project Manager:
Astrid Valencia Quiñónez (since November 1st 2022)

Technician:
Beatriz Mena Romero

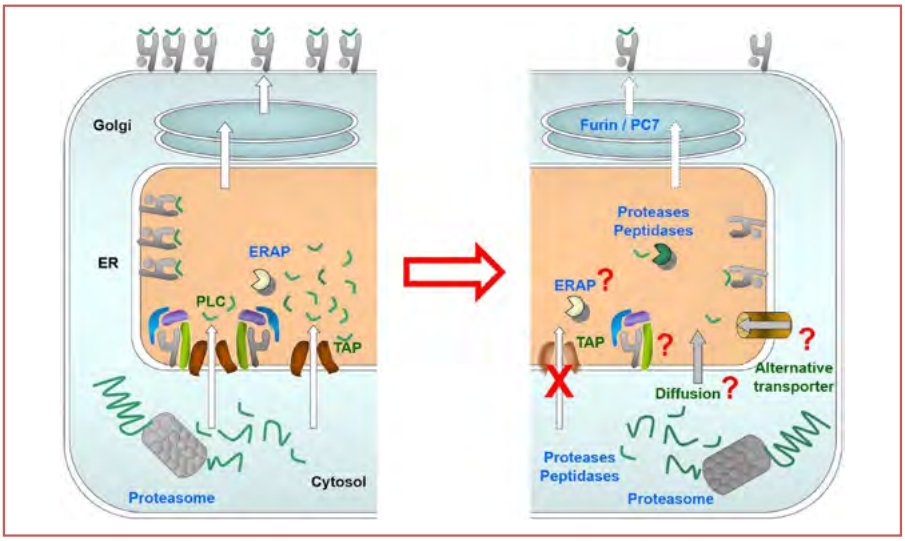
http://www.cbm.uam.es/viralimmunology

Summary

Our field of interest aims to improve 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, long-lasting 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 two different mouse models, deficient in: 1) 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. 2) The GTPase N-ras, a deficiency that we have shown to lead to a defective anti-viral memory $CD8^+$ T-lymphocyte response. The viral infection models we are using are vaccinia virus (VACV) and murine cytomegalovirus (MCMV). VACV is the vaccine vector used for the complete eradication of variola virus, the agent causing smallpox. MCMV, as a model for human cytomegalovirus, remains latent and leads to the generation for life of a massive number of viral epitope-specific T lymphocytes, a phenomenon termed “inflationary memory.”

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* outbreak, analyzing the protective immune response in the elderly from smallpox vaccinations performed more than fifty years earlier.



Viral antigen processing and presentation pathway. The left panel depicts the MHC class I antigen processing pathway, showing the central role played by TAP transporter. The right panel shows the impact of TAP inactivation on MHC class I antigen presentation. The panel also points to potential routes that may mediate the processing of MHC class I antigenic peptides that, although at low levels, are presented by MHC class I in these cells.

List of publications

» Alvarez I, Antón LC, James EA. (2022) Editorial: alternative antigen processing and presentation in immune disorders. Front Immunol. 13: 993393. doi: 10.3389/fimmu.2022.993393.

Other activities and Awards

» Luis C. Antón: Co-editor of a 2022 Special Topic of Frontiers in Immunology. doi: 10.3389/978-2-83250-286-0, ISBN 978-2-83250-286-0.

» Margarita Del Val. Coordinator, CSIC Platform for Interdisciplinary Research on Global Health (PTI Salud Global). It comprises 144 research groups of all disciplines. https://pti-saludglobal-covid19.corp.csic.es/en/

» Margarita Del Val. Coordinator, Master on Pandemics, Global Health and COVID 19. Universidad Internacional Menéndez Pelayo. 2020-2021 and 2021-2022.

» Margarita Del Val. Intense science dissemination activity related to the pandemic: over 60,000 contributions in all types of mass media (not counting social networks), and over 100 invited conferences.

» Margarita Del Val. Over 30 prizes and awards (2021-22), including Science Dissemination Prize awarded by CSIC and FBBVA (2021) and Honour Medal for Social Values by Universidad Internacional Menéndez Pelayo (2021).

Participation in projects

» HORIZON-European Innovation Council-PATHFINDER-OPEN-2021. Intracellular Carriers Against Resistant microOrganisms (ICARO) GA 101046927. 2022-2026. Coordinator: Arrays-for-cell Nanodevices. Hub PI: M Del Val.

» PID2019-110407RB-I00. Ministry of Economy and Competitiveness. Programa Estatal de Investigación, Desarrollo e Innovación Orientada a los Retos de la Sociedad. Strengthening of immune memory and antigen presentation as tools to overcome immunodeficiencies in $CD8^+$ T cell responses (IMPRIMEM). 2020-2024. PI: M. Del Val.

» Ministry of Science and Innovation. CSIC PTI Salud Global. Immune response to infection and vaccination to COVID-19. SGL2103032. 2020-2022. PI: M Del Val.

» Ministry of Science and Innovation. CSIC PTI Salud Global. Comprehensive multidisciplinary study of the vulnerability to COVID-19 of the elderly population. Immunity to SARS-CoV-2 infection and vaccination of elderly people housed in residences (BRANYAS). SGL2103056. 2020-2022. PI: M Del Val.

» Ministry of Science and Innovation. CSIC. Ayudas Extraordinarias a Proyectos de Investigación en el marco de las Medidas Urgentes Extraordinarias para hacer frente al Impacto Económico y Social del Covid19 (Ayudas CSIC-COVID-19). Characterization of the immune response to SARS-CoV-2 for control during the epidemic phase. CSIC-COV19. 2020-2021. PI and coordinator of 7 research groups: M. Del Val.

» B2017/BMD-3731. Programas de actividades de I+D entre grupos de investigación de la Comunidad de Madrid en Biomedicina 2017. Nanotherapy for trained immunity to improve organ transplant take. 2018-2021. Coordinator: J. Cano Hub. PI: M. Del Val.

IMMUNE DEVELOPMENT AND INFLAMMATORY-MEDIATED DISEASES

Group Members

Principal Investigator
Manuel Fresno Escudero

Scientific Staff
Konstantinos Stamatakis Andriani

Postdoctoral fellows
Maria Pilar Ubeda Cantera
Angela Rodriguez Garcia Rendueles
Javier Galán Martínez. Until 21.4.2022

Predoctoral fellows
Inés Sánchez Gomez. Until 1.4.2021
Javier Merino Valverde
Patricia Torres Gérica
Mª Teresa García Prieto
Sergio Polo Nicoli
Alfonso Herreros Cabello

Technicians
Mª de los Angeles Chorro. Retirement 13.8.2022
Mª del Carmen Maza Delgado. Until 31.8.2022
Carolina Maroto González
Paula Martínez Cenalmor
Marta García Sánchez. Gestora proyecto REACT

Undergraduate and Master Students
Théo Sarrazin. Academic year 2021-22.
Lucía Cermeño. Academic year 2021-22.
Paula Lozano Barrios. Academic year 2021-22.

Visiting scientists
Héctor O. Rodríguez Angulo. U. Caracas. Venezuela

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Summary

Deletion of TCFL5, a bHLH transcription factor, drastically reduces the tumor properties of colon cancer cells. Interestingly, the 2 major isoforms, TCFL5_E1/E8 and TCFL5_E2b/E8 (CHA) had a different promoter and opposite functions, being TCFL5_E2b/E8 protumoral function. TCFL5_E1/E8 is essential for NFKB2 activity regulating the expression of anti-apoptotic genes as BCL2 whereas TCFL5_E2b/E8 controls the expression of the pluripotency markers SOX2, NANOG and KLF4. We have identified some genes regulated by TCFL5_E1/E8 and TCFL5_E2b/E8 and established its role in some leukemias and in normal lymphopoiesis. TCFL5_E2b/E8 expression was associated with greater severity in lymphoma and myeloma samples from patients. Using *Tcfl5* deficient mice, we found Tcfl5 is required for the formation of germinal centers and differentiation of pro-B to pre-B cells by affecting the levels of SYK and BCR signalling, resulting in an inability to respond to stimuli and an increase in cell death. TCFL5 is also expressed during early mouse embryonic development, the preimplantation period and plays a role in the differentiation of embryonic cells to germline precursors by controlling the expression of genes important in their differentiation, as shown in Tcfl5 deficient mice.

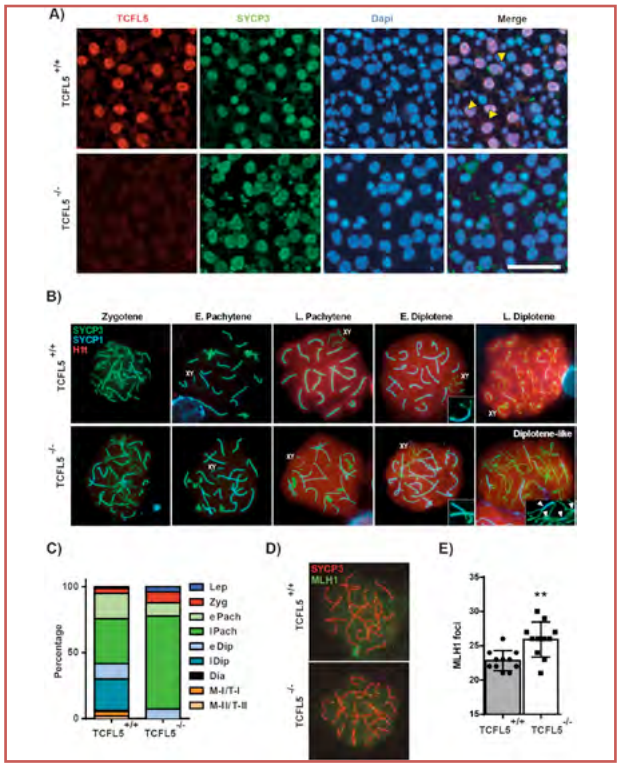
Toll-like receptors (TLRs) play a crucial role in pathogen recognition. However, signaling via TLR4 and TLR2 was different, as TLR2 ligands activated NF-κB and MAPKs earlier and exhibited a higher IL-10 /IL-12 ratio compared to TLR4 ligands. Furthermore, p38 MAPK is critical for IL-10 expression in response to TLR2 ligands, which triggers the macrophage change to a M2 and regulatory phenotype in contrast to the M1 phenotype induced by TLR4 activation. TRIF was required for IFN-β induction and consequent expression of IL-12 in response to TLR2. Moreover, *in vivo* administration of TLR2 ligands exert a modulatory effect on cytokines with beneficial effects on the prevention of Listeria dissemination in a murine model of neonatal listeriosis.

TLR4 is considered the major receptor to recognize all LPSs. However, some atypical LPS's depart from the well-studied *E. coli* LPS and induce a TLR2-dependent inflammatory response in immune cells. Molecular docking analysis of O. intermedium LPS predicts a favorable formation of a TLR2/TLR4/MD-2 heterodimer, further confirmed by FRET. These imply that atypical LPSs may induce TLR4/TLR2 heterodimerization to decrease the bacteria activation of the innate immune system.

Finally, we were also working on Chagas' disease caused by Trypanosoma cruzi (see N. Gironès page) and contributed to understand some immunological aspects of COVID 19 infection (see corresponding page).

List of publications

- Front Cell Infect Microbiol. 2021 Aug 27;11:737364. doi: 10.3389/fcimb.2021.737364
- Front Cell Infect Microbiol. 2021 Jun 29;11:672448. doi: 10.3389/fcimb.2021.672448.
- J Invest Dermatol. 2021. Jun;141 (6): 1522-1532.e3. doi: 10.1016/j.jid.2020.10.020.
- Microorganisms. 2021. Oct 23;9(11):2208. doi: 10.3390/microorganisms9112208
- Vaccines (Basel). 2021 Mar 17;9(3):269. doi: 10.3390/vaccines9030269.
- Ann N Y Acad Sci. 2021 Aug;1497(1):27-38. doi: 10.1111/nyas.14586
- EMBO Mol Med. 2021. Mar 5;13(3):e13549. doi: 10.15252/emmm.202013549.
- Front Immunol. 2021. Jun 21;12:660065. doi: 10.3389/fimmu.2021.660065
- Front Immunol. 2021. Apr 19;12:632304. doi: 10.3389/fimmu.2021.632304.
- Biomedicines. 2022. Sep 19;10(9):2326. doi: 10.3390/biomedicines10092326.
- Int J Mol Sci. 2022. Dec 10;23(24):15682. doi: 10.3390/ijms232415682.
- Front Immunol. 2022. Jan 24;12:748303. doi: 10.3389/fimmu.2021.748303.
- Front Pharmacol. 2022. Jan 28;12:806395. doi: 10.3389/fphar.2021.806395.
- Sci. Rep. 2022. Jun 29;12(1):10956.doi:10.1038/s41598-022-15167-w.
- Front Immunol. 2022. Mar 23;13:836516. doi: 10.3389/fimmu.2022.836516.
- Cell Mol Life Sci. 2022 Jan 9;79(1):61. doi: 10.1007/s00018-021-04095-z.
- Mol Oncol. 2022. May;16(9):1876-1890. doi: 10.1002/1878-0261.13085.
- Int J Mol Sci. 2022. Oct 31;23(21):13275. doi: 10.3390/ijms232113275.
- Frontiers in Drug Discovery. 2022. Vol 1-2021. DOI: 10.3389/fddsv.2021.789710



TCFL5 deficiency arrest cells in pachytene/diplotene transition. (A) Immunofluorescence of squashed seminiferous tubules (B) Immunofluorescence of spread spermatocytes. (C) Quantification of meiotic stages (D) Immunofluorescence of spread spermatocytes (E) MLH1 quantification

Participation in projects

- Fresno Escudero, Manuel. Red de Investigación Colaborativa en Enfermedades Tropicales (RICET). Instituto de Salud Carlos III. RD16/0027/0006. ISCIII. 2017-2021.
- Manuel Fresno (Coordinador). B2017/BMD-3671. INFLAMUNE-CM. NUEVOS MECANISMOS MOLECULARES Y CELULARES IMPLICADOS EN LA FISIOPATOLOGÍA INMUNE Y ENFERMEDADES INFLAMATORIAS. Comunidad de Madrid. 2018-Junio 2022.
- Fresno Escudero, Manuel. TCFL5/CHA EN LA DIFERENCIACIÓN Y ACTIVACIÓN DE LINFOCITOS B Y T Y EN LA GENERACIÓN DE LEUCEMIAS. PIB2019-104760RB-100. Ministerio de Ciencia e Innovación. Julio 2020-Junio 2023.
- Fresno Escudero, Manuel. Jefe Grupo 12. ISS La Princesa.
- Fresno Escudero, Manuel (Coordinador). PLATAFORMA Y MODELOS PRECLINICOS PARA EL ABORDAJE MULTI-DISCIPLINAR EN COVID19 Y EN RESPUESTAS A FUTURAS PANDEMIAS. Comunidad de Madrid. Noviembre 2021-Diciembre 2022.

IMMUNOREGULATORY MECHANISMS IN THE DEVELOPMENT OF CHAGAS DISEASE: TRANSLATIONAL APPLICATIONS

Group Members

Principal Investigator (PI):
Núria Gironès

Predoctoral Fellows:
Alfonso Herreros Cabello
Javier del Moral Salmoral
Inés Sánchez Gómez

Technicians:
Carolina Maroto González
Javier del Moral Salmoral
Diana Karolina Santos Peñaloza

Undergraduate and Master Students:
Mario Gómez Montes
Andrea Rodrigo Castro

Visiting scientists:
Héctor Omar Rodríguez Angulo

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

Summary

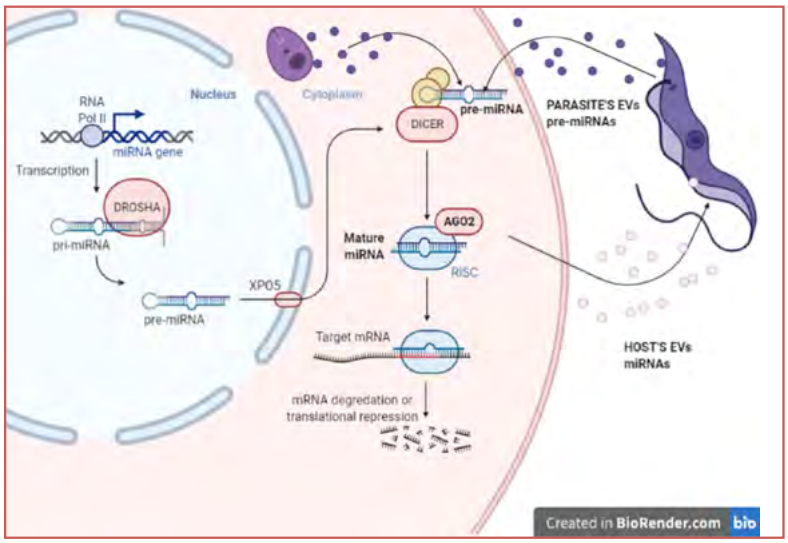
During 2021 and 2022 the objectives of our research group were: (i) to further study the role of the SLAMF1 immune receptor in the infection *Trypanosoma cruzi*, the causative agent of Chagas disease; (ii) to evaluate the prognostic value of an isoform of TCFL5 (sCha) in Chagas disease patients; (iii) to study the effects of *T. cruzi* infection in cardiac remodeling; and (iv) to contribute to the understanding of *T. cruzi* mitochondrial genome, transcriptome and proteome.

We pursued the research on the SLAMF1 immune receptor during infection using proteomics, and identified target genes that are being studied for their relevance in the context of the infection as therapeutic targets. We also studied the prognostic value of circulating miRNAs in Chagas disease patients, and the alterations in miRNA expression in macrophages infected with *T.* cruzi, and identified for the first time the presence of *T. cruzi* miRNAs in the infected culture that localize in extracellular vesicles (EVs).

We studied cardiac remodeling in the experimental mouse model of *T. cruzi* infection, and found overexpression of HCN4 channels, suggesting that arrythmogenic treatments should be administered with caution in Chagas disease patients since they can have secondary effects (Rodriguez-Angulo H.O. et al. 2021).

We determined the complete genome of mitochondrial maxicircle and minicircles of *T. cruzi*. It was previously thought that minicircles were circular dsDNA composed of 4 repetitive elements, but using NGS sequencing we also found minicircles of 3, 2 and 1 repeats in the mitochondrial DNA. These findings are relevant because little is known about the role of the *T. cruzi* mitochondrial genome, and these findings may help to understand better the molecular biology of the parasite for fighting the infection (Callejas-Hernández F. et al. 2021).

Finally, we showed that autoantibodies against the immunodominant sCha (a TCFL5 isoform) epitope discriminate the risk of sudden death in chronic Chagas cardiomyopathy. Autoantibody levels correlated with the alterations found in 24h Holter ECG recording for the detection of arrhythmias and prevention of sudden death, and thus having a potential application as an alternative when Holter ECG is not available (Rodríguez-Angulo H.O. et al. 2021). We also collaborated in studies about the role of TCFL5 in spermatogenesis (Galán-Martínez J, Berenguer I.et al. 2022) and colorectal cancer (Galán.Martínez J., Stamatakis K. et al., 2022) with the research group of Dr. Manuel Fresno.



Schematic representation of the host-parasite interaction by means of EVs containing host cell and T. cruzi miRNAs.

List of publications

» Galán-Martínez J, Berenguer I, Del Carmen Maza M, Stamatakis K, Gironès N, Fresno M. TCFL5 deficiency impairs the pachytene to diplotene transition during spermatogenesis in the mouse. Sci Rep. 2022 Jun 29;12(1):10956. doi: 10.1038/s41598-022-15167-w.

» Rodríguez-Angulo HO, Colombet-Naranjo D, Maza MC, Poveda C, Herreros-Cabello A, Mendoza I, Perera JC, Goyo JD, Gironès N, Fresno M. Molecular Remodeling of Cardiac Sinus Node Associated with Acute Chagas Disease Myocarditis. Microorganisms. 2021 Oct 23;9(11):2208. doi: 10.3390/microorganisms9112208.

» Galán-Martínez J, Stamatakis K, Sánchez-Gómez I, Vázquez-Cuesta S, Gironés N, Fresno M. Isoform-specific effects of transcription factor TCFL5 on the pluripotency-related genes SOX2 and KLF4 in colorectal cancer development. Mol Oncol. 2022 May;16(9):1876-1890. doi: 10.1002/1878-0261.13085.

» Fresno M, Gironès N. Myeloid-Derived Suppressor Cells in *Trypanosoma cruzi* Infection. Front Cell Infect Microbiol. 2021 Aug 27;11:737364. doi: 10.3389/fcimb.2021.737364.

» Callejas-Hernández F, Herreros-Cabello A, Del Moral-Salmoral J, Fresno M, Gironès N. The Complete Mitochondrial DNA of *Trypanosoma cruzi:* Maxicircles and Minicircles. Front Cell Infect Microbiol. 2021 Jun 29;11:672448. doi: 10.3389/fcimb.2021.672448

» Rodríguez-Angulo HO, Lamsfus-Calle A, Isoler-Alcaráz J, Galán-Martínez J, Herreros-Cabello A, Callejas-Hernández F, Chorro-de-Villaceballos MA, Maza MC, Santi-Rocca J, Poveda C, Moral-Salmoral JD, Marques J, Mendoza I, Ramírez JD, Guhl F, Carrillo I, Pérez-Tanoira R, Górgolas M, Pérez-Ayala A, Monge-Maillo B, Norman F, Pérez-Molina JA, López-Vélez R, Fresno M, Gironès N. Autoantibodies against the immunodominant sCha epitope discriminate the risk of sudden death in chronic Chagas cardiomyopathy. Ann N Y Acad Sci. 2021 Aug;1497(1):27-38. doi: 10.1111/nyas.14586.

Participation in projects

» Title: *Plataformas y modelos preclínicos para el abordaje multidisciplinar en COVID-19 y en respuesta a futuras pandemias* (COVTRAVI-19-CM). Financed by: *Comunidad de Madrid / REACT UE/ FEDER*. Duration: from Jan 2020 to Dec2022. Coordinators of *Plataforma de modelos preclínicos de infección y patogénesis*: Iván Ventoso and Núria Gironès. Coordinator COVTRAVI-19-CM: Manuel Fresno.

» Title: *Redes Moleculares y celulares en enfermedades inflamatorias (INFLAMUNE).* Financed by: *Comunidad de Madrid/FEDER.* Scientific manager of the RedLab 323 (BIOLUMLAB): Núria Gironès. Duration: From Jan 2021 to 31 Dec 2021 (extended until Jun 2022). Coordinator INFLAMUNE. Manuel Fresno.

» Title: *Red de Investigación Colaborativa en Enfermedades Tropicales* (RICET) (0027/0006). Financed by: RETICS ISCIII. Duration: from Jan 2017 to 31 Dec 2021. Participant: Núria Gironès. Coordinator UAM-B: Manuel Fresno.

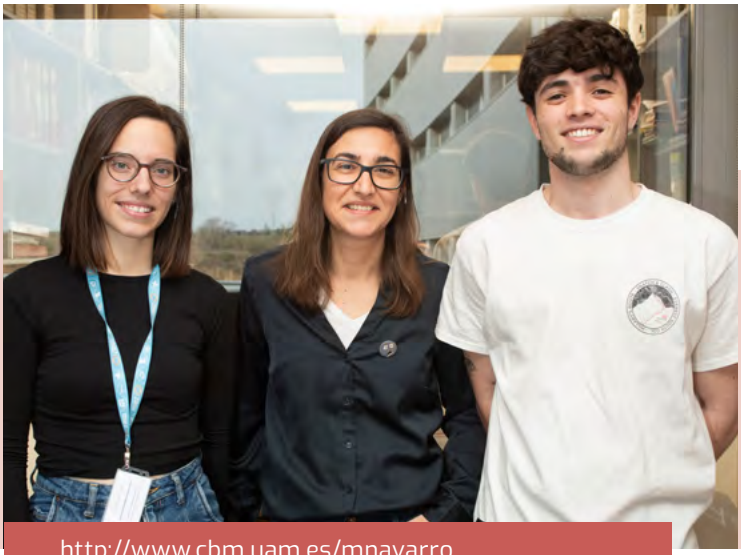
» Title: *Role of the Trypanosoma cruzi SLAMF1 receptor ligand and microRNAs during infection: applications in diagnosis and therapy* (SLAMIRNA), (PID2021-123389OB-I00). Financed by: MICINN/FEDER. Duration: from Sept 2022 to Aug 2025. PI: Núria Gironès.

» Title: *Multiomic approach to the study of Chagas disease immunopathogenesis* (CHAGOMICS). (PGC2018-096132-B-I00). Financed by: MICINN/FEDER. Duration: from Jan 2019 to Dec 2021 (extended to Sept 2022). PI: Núria Gironès.

Doctoral theses

» Inés Sánchez Gómez (2021). The role of the transcription factor TCFL5 in the physiopathology of B lymphocytes. Dr. in Molecular Biosciences UAM. Co-directors: Manuel Fresno and Núria Gironès.

INTRACELLULAR SIGNALLING IN INFLAMMATORY PROCESSES



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

Group Members

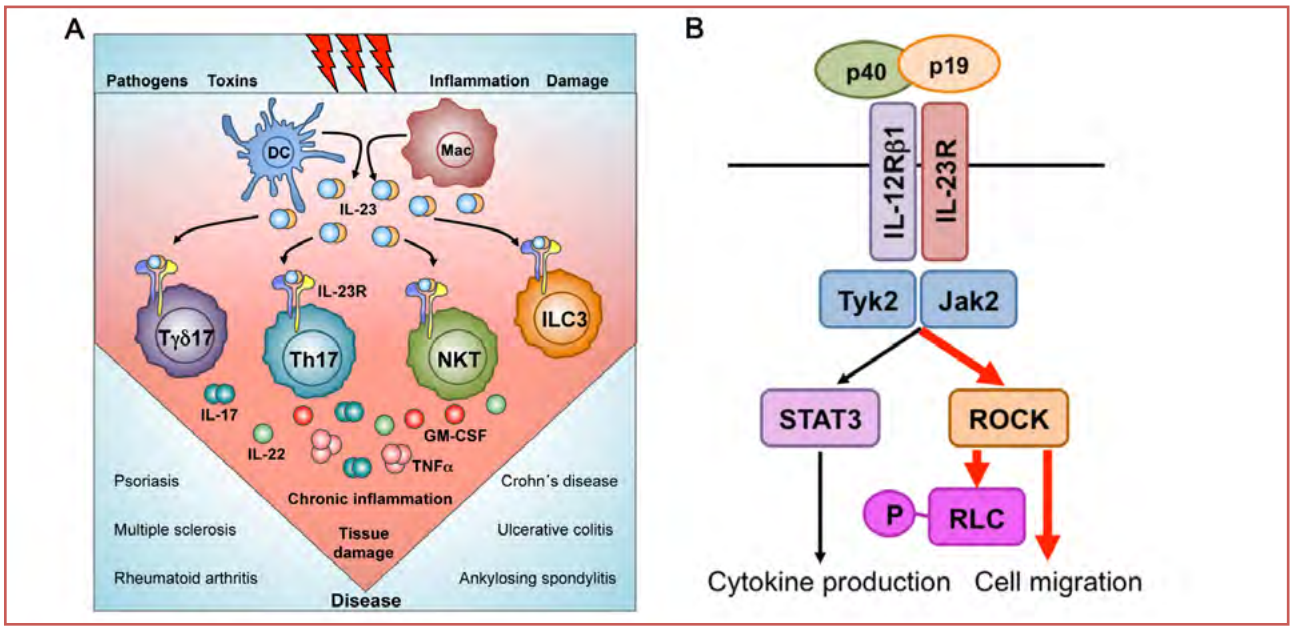
Principal Investigator:
María N. Navarro

Predoctoral fellow:
Gloria Pastor Fernández (until june 2022)

Research assistant:
María Jesús Valle Pastor
(from may 2021)

Summary

The incidence of chronic inflammatory diseases has significantly increased among developed countries in the past 30 years. Therefore, there is a need for development of novel treatments for this type of conditions. The chronic inflammatory diseases are initiated by an uncontrolled immune response that triggers the hyper-activation of signalling cascades, promoting the accumulation of pro-inflammatory mediators and finally, the manifestation of clinical symptoms. In different chronic inflammatory diseases, the axis formed by interleukin 23 and interleukin 17 (IL-23/IL-17) has emerged as a key signalling hub in pathologies such as psoriasis, inflammatory bowel diseases, multiple sclerosis or rheumatoid arthritis in murine models and more importantly, in humans. The pathogenic effects of IL-23 have been linked to its ability to promote the production of inflammatory mediators, mainly interleukin 17 and 22 (IL-17/IL-22). These secreted mediators amplify intercellular communication networks, causing a chronic inflammation and finally, clinical symptoms. IL-23 pathogenic actions are mostly restricted to distinct subpopulations of T lymphocytes: the CD4 helper subset Th17 and the TCRγδ subpopulation Tγδ17. Therapeutic strategies aimed at inhibiting the intracellular signalling networks triggered by different receptors have been successfully applied for treatment of inflammatory diseases and cancer. Despite the prominent role of IL-23 in diseases, these therapeutic approaches have not been fully exploited in the context of inflammatory pathologies since the IL-23 signalling cascade remains largely unknown. Our lab is interested in the characterisation of the signalling network triggered by IL-23 and other pro-inflammatory cytokines, as a strategy to uncover novel mediators of cytokine signalling for the development of therapeutic tools based on the interference with intracellular signalling pathways. Recently, the lab has discovered how interleukin 23 promotes pathogenic T cell migration to the inflamed site, 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.



Role of IL-23 signalling in chronic inflammatory diseases. A) Schematic representation of the role of IL-23 in inflammatory diseases [Cells. 2020 Sep 7;9(9):2044]. B) Working model for IL-23 signalling pathway [PLoS Biol. 2020 Mar 23;18(3):e3000646].

List of publications

» Navarro MN, Gómez de Las Heras MM, Mittelbrunn M. (2022) Nicotinamide adenine dinucleotide metabolism in the immune response, autoimmunity and inflammageing. Br J Pharmacol. May;179(9):1839-1856. doi: 10.1111/bph.15477.

» Borroto A, Alarcón B, Navarro MN. (2022). Mutation of the Polyproline Sequence in CD3ε Evidences TCR Signaling Requirements for Differentiation and Function of Pro-Inflammatory Tγδ17 Cells. Front Immunol. Mar 31;13:799919. doi: 10.3389/fimmu.2022.799919.

» Castillo-González R, Cibrian D, Fernández-Gallego N, Ramírez-Huesca M, Saiz ML, Navarro MN, Fresno M, de la Fuente H, Sánchez-Madrid F. (2021). Galectin-1 Expression in CD8+ T Lymphocytes Controls Inflammation in Contact Hypersensitivity. J Invest Dermatol. Jun;141(6):1522-1532. e3. doi: 10.1016/j.jid.2020.10.020.

Participation in projects

» 2020-2023. PID2019-110511RB-I00, Ministerio de Ciencia e Innovación. "Intracellular Signalling Blockage for inflammatory diseases (iSigB)". Principal investigator.

METABOLISM IN CANCER AND AGING

Group Members

Principal Investigator:
Ana Ortega Molina (since September 2021)

Predoctoral Fellow:
Beatriz Lopez Martín-Lunas (since October 2022)

Technicians:
Cristina Lebrero Fernández
(November 2021-November 2022)
Nerea Delgado Mayenco (July 2022-Sepember 2022)
Marta Jiménez Muñoz (since November 2022)

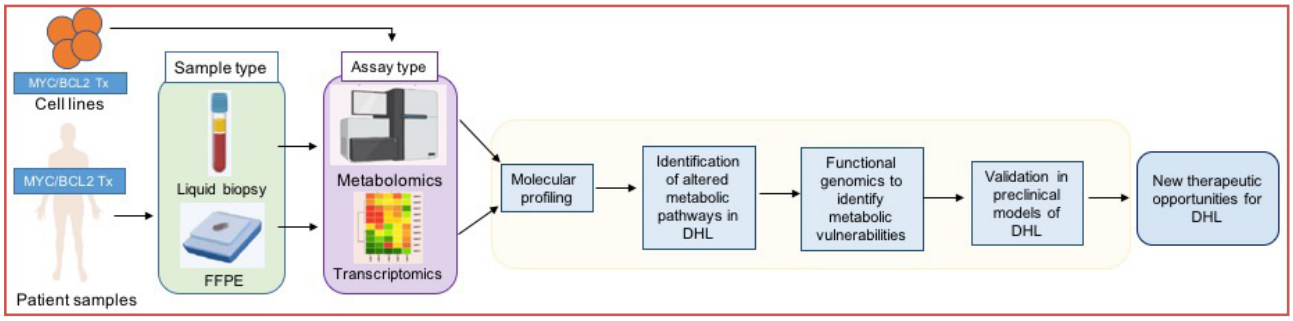
Undergraduate and Master Students:
Nerea Regueira Acebedo (February 2022-July 2022)
Sara Salazar Ortego (since December 2022)
Marta González Pérez (since December 2022)

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

Summary

Diffuse large B cell lymphoma (DLBCL) is the most common lymphoid malignancy in adults and represents a heterogeneous group of tumors with distinct subtypes that differ in genetic alterations, clinical outcome, response to treatment and prognosis. Approximately 5-15% of DLBCL are high grade B cell lymphomas (HGBL) with rearrangements of MYC and BCL2 and/or BCL6, and they are known as "double-hit" lymphoma (DHL) or "triple hit lymphoma" (THL). Due to simultaneous activation of these driver oncogenes, DHLs are among the most aggressive and chemoresistant lymphoma subtype with minimal treatment options and have poor outcomes. Furthermore, the co-expression of MYC and BCL2 proteins without underlying rearrangements is considered a new adverse prognostic indicator termed double-expressor lymphoma (DEL). Deregulated MYC expression contributes to metabolic reprogramming in tumor cells through a complex network of factors. Specifically, c-MYC-transformed lymphoma B cells rely on glutamine metabolism for bioenergetics and redox homeostasis. In addition, serine metabolism, through the enhanced activity of SHMT2 enzyme, contributes to the biology of MYC-aggressive lymphomas. However, the metabolic features of the HGBL-DH lymphomas are not fully understood and could imply potentially targetable vulnerabilities. Understanding the unique biology of these tumors, including metabolism, could provide a rationale for exploring targeted agents beyond the current focus on BCL2 and MYC inhibitors.

Our lab, which opened its doors on September 2021, aims to characterize the metabolic features of these particular tumors using transcriptomic and metabololomic tools in preclinical models and patient samples, and screen new metabolic vulnerabilities that could be used as new targeted therapies for these aggressive lymphomas with very poor outcomes with the standard therapies.



Research workflow

List of publications

» Ortega-Molina A, Efeyan A. (2021) From mouse genetics to targeting the Rag GTPase pathway. Mol Cell Oncol. 24;8(5):1979370. Doi: 10.1080/23723556.2021.1979370.

» Ortega-Molina A#, Lebrero-Fernández C, Sanz A, Deleyto-Seldas N, Plata-Gómez AB, Menéndez C, Graña-Castro O, Caleiras E, Efeyan A#. (2021). Inhibition of Rag GTPasesignaling in mice suppresses B cell responses and lymphomagenesis with minimal detrimental trade-offs. Cell Rep. 36(2):109372. Doi: 10.1016/j.celrep.2021.109372.

#Co-corresponding authors

» de la Calle Arregui C, Plata-Gómez AB, Deleyto-Seldas N, García F, Ortega-Molina A, Abril-Garrido J, Rodriguez E, Nemazanyy I, Tribouillard L, de Martino A, Caleiras E, Campos-Olivas R, Mulero F, Laplante M, Muñoz J, Pende M, Sabio G,Sabatini DM, Efeyan A. (2021). Limited survival and impaired hepatic fasting metabolism in mice with constitutive Rag GTPase signaling. Nat Commun. 12(1):3660. Doi: 10.1038/s41467-021-23857-8.

Other activities

» I FERO BMS FELLOWSHIP in Hemato-Oncology 2021.

» *Beca Leonardo a Investigadores y Creadores Culturales 2022.*

» "Margarita Salas" Award 2022 for young scientists under 40 from Regional Government of Madrid.

Participation in projects

» Estudio de posibles alternativas para superar la resistencia a inmunoterapia de los linfomas B agresivos. Beca Leonardo 2022 (Project. LEO22-2-2003). Fundación BBVA. 01/11/2022-30/04/2024. Principal investigator.

» Deciphering metabolic dependencies as targetable vulnerabilities in double-hit lymphomas. Ministerio de Ciencia e Innovación. Agencia Estatal de Investigación (PID2021-124601OB-I00). 01/09/2022- 31/08/2025. Principal investigator.

» Deciphering and targeting unfolding protein response in high-grade B cell lymphoma. III Ayuda de Investigación en Oncología +QUEUNTRAIL·Alcoi & Asociación Española de Investigación sobre el Cáncer (ASEICA). 01/12/2021-30/11/2022. Principal investigator.

» Targeting unfolding protein response in high-grade B cell lymphomas. 2021 I FERO-BMS. Fundación FERO (BFERO2021.05). 01/12/2021-30/11/2023. Principal investigator.

» Targeting metabolic dependencies in high-grade B cell lymphomas. Proyecto Intramural Especial. CSIC (20212AT020). 01/09/2021-31/08/2024. Principal investigator.

» Functional genomics and new therapeutic interventions in Follicular Lymphoma. Programa Ramón y Cajal. Agencia Española de Investigación (RYC2019-027280-I). 01/09/2021-31/08/2026. Principal investigator.

UNCONVENTIONAL AUTOPHAGY IN HEALTH AND DISEASE

Group Members

Principal Investigators (PI, co-PI):
Felipe X. Pimentel Muiños

Postdoctoral fellows:
Pilar Delgado Cañaveras (from 01/03/2022)

Predoctoral fellows:
Elena Terraza Silvestre
Julia Bandera Linero (from 01/12/2021)
Elena Blanco Arribas (from 16/10/2022)

Technicians:
Cristina Martín Vílchez (from 01/05/2021)

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Summary

Autophagy is a degradative process that eliminates obsolete or damaged cytoplasmic components and is critical for the cellular homeostasis. Judging from the pathological phenotypes observed in mice lacking members of the autophagic machinery (the so-called ATGs), autophagy has an important role in protection against a variety of pathologies, including cancer, inflammatory diseases or neurodegenerative disorders. This protective capacity suggests that manipulation of autophagy could hold therapeutic value, a possibility that is being intensely studied by a variety of laboratories around the world. However, it is nowadays becoming 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, but their characterization is important because it could determine the way ATG activity could be manipulated with therapeutic purposes.

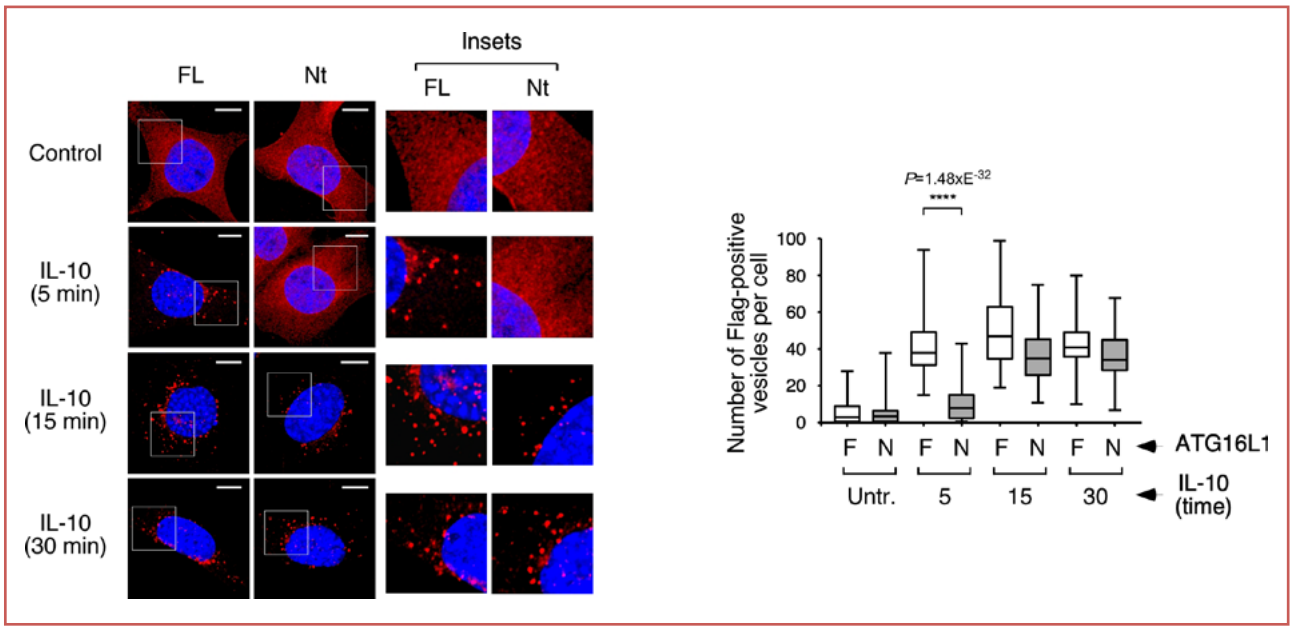
With this goal in mind, the lab focuses on ATG16L1 as a paradigm to discover new unconventional activities of the autophagic machinery. In its most canonical function, ATG16L1 is critical for proper formation of autophagosomes, double-membrane vesicles that sequester the cellular material that will eventually 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 2021-22 period have focused on the role of unconventional autophagy in:

1) The regulation of endocytosis, intracellular trafficking and signaling output of cytokine receptors

2) The regulation of immunogenic cell death and its impact in cancer biology

3) The innate immune response against intracellular bacterial infections

To identify and characterize these processes we use a variety of genetically manipulated experimental systems, proteomics and bioinformatics approaches. We are trying to characterize these mechanisms and explore how they may be influenced by a coding polymorphic allele of ATG16L1 (T300A) whose presence in homozygosis increases the risk of suffering Crohn's disease, a serious intestinal inflammatory disease. Exploring and establishing ways to manipulate atypical autophagy with therapeutic intentions is also a central 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).

List of publications

Articles:

» Serramito-Gómez, I., Terraza-Silvestre, E., Fernández-Cabrera, A., Villamuera, R., Pimentel-Muiños, FX. (2022) ATG16L1 WD40 domain-dependent IL10R signaling is insensitive to the T300A Crohn disease risk polymorphism. Autophagy, 18:3023-3030. doi: 10.1080/15548627.2022.2054241

» Villamuera, R., Fernández-Cabrera, A., Serramito-Gómez, I., Terraza-Silvestre, E., Taouil, R., Pimentel-Muiños, FX. (2021) Unconventional WD40 domain-dependent role of ATG16L1 in the regulation of IL10R endocitosis, trafficking and signaling. Autophagy, 17:2639-2641. doi: 10.1080/15548627.2021.1947606

» Klionsky, DJ., et al., Pimentel-Muiños, FX., et al., Tong, CK. (2021) Guidelines for the use and interpretation of assays for monitoring autophagy (4th edition). Autophagy, 17:1-382. doi: 10.1080/15548627.2020.1797280

Book chapters:

» Pimentel-Muiños, FX. (2022) Autophagy in the gastrointestinal system and crosstalk with the microbiota. In: Rohtermel, BA. and Diwan, A. (Eds) Autophagy in health and disease (Second Edition). Elsevier, Academic Press, USA, pp.321-333. ISBN: 978-0-12-822003-0.

Participation in projects

» Title and Reference: Unconventional activities of the autophagic mediator ATG16L1 and its relevance in health and disease (Ref. PID2020-114699RB-100)
Financing Agency: Ministerio de Ciencia e Innovación.
Principal Investigator: Felipe X. Pimentel Muiños (CSIC)
Dates: 01/09/2021 – 31/08/2024

NITRIC OXIDE AND BIOACTIVE LIPIDS IN THE IMMUNE RESPONSE

Group Members

Principal Investigators:
Juan Manuel Serrador
Miguel Ángel Íñiguez

Predoctoral Fellows:
Ángel Bago (until October 2021)
Alba Gil (from April 2021)
Claudia Guerra (from September 2022)

Technicians:
Ana Renshaw
Laura Cayuela (until February 2022)

Undergraduate and Master Students:
Valentina Canino Avilés (From July 2021 to July 2022)

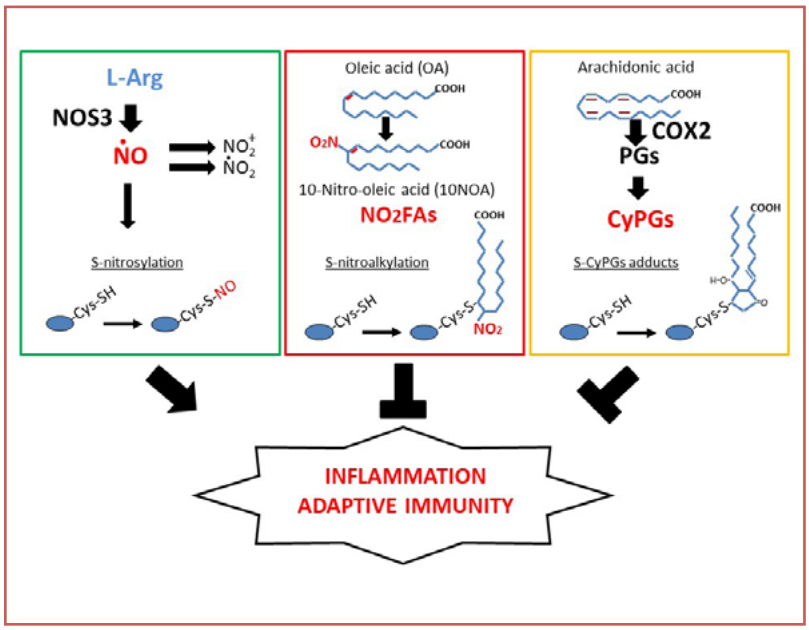
htttps://www.cbm.uam.es/immune_NO_bioactive_lipids

Summary

Nitric oxide (NO) and bioactive lipids as nitro-fatty acids (NO_2-FA) or prostaglandins, are key mediators for maintaining cellular homeostasis, with an essential role in inflammation. Our research lines are dedicated to the study the role played by NO as well as nitro and oxo modified fatty acids in inflammation and in the activation and differentiation of T lymphocytes. We are currently studying the actions exerted by these agents on the activation of human T lymphocytes, analysing their involvement in the regulation of gene expression and activation of transcription factors. We are also interested in the analysis of chemotaxis, intercellular adhesion and the organization of adhesion and signalling receptors at the immune synapse. In addition, we are also examining the potential actions of these compounds on the selection of the adaptive immune response in human T lymphocytes.

NO is a key messenger in the pathogenesis of inflammation. In the immune system, NO has been considered to be a cytotoxic molecule associated with the response of phagocytic cells to pathogens as part of the first line of host defence against infection. However, NO can also regulate the adaptive immune response, linking innate and adaptive immunity. NO affects T helper cell differentiation and the effector functions of T lymphocytes, and is a potential target for therapeutic manipulation. In the last years, our group has been interested in the study of the regulatory actions exerted by NO in T cell functions, focusing on protein S-nitrosylation and fatty acid nitro-alkylation, leading to the formation NO_2-FA, as important post-translational modifications by which NO can act as a signalling molecule during T cell-mediated immunity.

Fatty acid oxidative modifications result in the production of bioactive lipids including prostaglandins and NO_2-FA, important signalling molecules that can modulate the inflammatory process and the immune response. We are interested in the analysis of their influence on diverse parameters of T lymphocyte function, focusing on their effects on transcriptional activation and gene expression and their consequences on cell activation and differentiation. Their anti-inflammatory and immunomodulatory effects take place mainly through their ability to covalently modify transcriptional regulatory proteins and enzymes and to activate various nuclear and membrane receptors, finally modifying protein function and altering patterns of gene expression. Research on the molecular and cellular basis of the actions of electrophilic fatty acids in inflammation and the immune response, will contribute to the understanding of the potential therapeutic benefits of these compounds.



Nitric oxide (NO), Nitro fatty acids (NO2FA) and Cyclopentenones (CyPG) modulate inflammation and adaptive immunity by means of their ability to promote post-translational modification (PTM) of key proteins. NO, synthesized enzymatically from L-Arg by the action of nitric oxide synthases (NOS) mediates redox-based PTM as S-nitrosylation of Cys residues of proteins. Nitration products of unsaturated fatty acids (nitro fatty acids; NO2FA) as oleic acid, are formed via NO-dependent oxidative reactions. These nitrated unsaturated fatty acids are potent electrophiles that mediate nitroalkylation reactions with Cys. Cyclopentenone prostaglandins (cyPG) are also potent electrophilic lipid mediators that can form covalent adducts with nucleophiles as Cys residues, thus modifying regulation and function of certain proteins. Recent findings have shown that these agents can regulate different steps in the inflammatory and T cell activation processes, which accounts for some of their immunoregulatory properties.

List of publications

» Cacheiro-Llaguno, C., Hernández-Subirá, E., Díaz-Muñoz, D.M., Fresno, M., Serrador, J.M., and Íñiguez, M.A. (2022). Regulation of Cyclooxygenase-2 Expression in Human T Lymphocytes by Glucocorticoid Receptor–Mediated Transrepression of Nuclear Factor of Activated T Cells. Int. J. Mol. Sci. 23, 13275. doi:10.3390/ijms232113275. 5/6

» Bago, A., Íñiguez M.A., (CA), and Serrador, J.M. (CA) (2021) Nitric Oxide and Electrophilic Cyclopentenone Prostaglandins in Redox Signaling, Regulation of Cytoskeleton Dynamics and Intercellular Communication. Frontiers. Cell Dev. Biol. 9, 673973. doi:10.3389/fcell.2021.673973.

» Hachimi, M., Grabowski, C., Campanario, S., Herranz, G., Baonza, G., Serrador, J.M., Gomez-Lopez, S., Barea, M.D., Bosch-Fortea, M., Gilmour, D., Bagnat, M., Rodriguez-Fraticelli A.E., and Martin-Belmonte, F. (2021) Smoothelin-like 2 Inhibits Coronin-1B to Stabilize the Apical Actin Cortex during Epithelial Morphogenesis. Curr. Biol. 31, 696-706.e9. doi: 10.1016/j.cub.2020.11.010

Other activities

Associate Faculty Member of Faculty Opinions:

» Sánchez-Madrid F and Serrador J: Faculty Opinions Recommendation of [Dyck L et al., J Exp Med 2022 219(3:)]. In Faculty Opinions, 12 Dec 2022; doi: 10.3410/f.741564540.793596966

» Sánchez-Madrid F and Serrador J: Faculty Opinions Recommendation of [Otsuka S et al., J Clin Invest 2022 131(11:)]. In Faculty Opinions, 04 Apr 2022; doi: 10.3410/f.739892410.793592189

» Sánchez-Madrid F and Serrador J: Faculty Opinions Recommendation of [Avery L et al., Front Immunol 2022 12(:726406)]. In Faculty Opinions, 04 Apr 2022; doi: 10.3410/f.741842325.793592190

» Sánchez-Madrid F and Serrador J: Faculty Opinions Recommendation of [Leithner A et al., J Cell Biol 2021 220(4:)]. In Faculty Opinions, 27 Apr 2021; 10.3410/f.739482053.793584660

IIS Hospital Universitario de la Princesa Group 18: Actions of bioactive lipids in the immune and inflammatory response.

Participation in projects

» Nitric Oxide and Nitro-fatty acid signalling in T cell-mediated immunity (RTI2018-100815-B-100). Ministerio de Ciencia, Innovación y Universidades. From: 01/01/2019 To: 12/31/2021. PIs: J.M. Serrador and M.A. Íñiguez.

Doctoral theses

» Ángel Bago Plaza (2022). Nitro Fatty acids actions on T cell activation. Department of Molecular Biology. Universidad Autónoma de Madrid. Directors: J.M. Serrador and M.A. Íñiguez.

DEVELOPMENT OF THE HUMAN LYMPHOHEMATOPOIETIC SYSTEM

Group Members

Principal investigator:
María Luisa Toribio García

Postdoctoral fellows:
Marina García Peydró
Patricia Fuentes Villarejo
Sara González García

Predoctoral fellows:
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Fátima Bayón Calderón
Carmela Cela Rodríguez

Technicians:
Juan Alcain Sánchez
Eloísa Castillo Gutiérrez

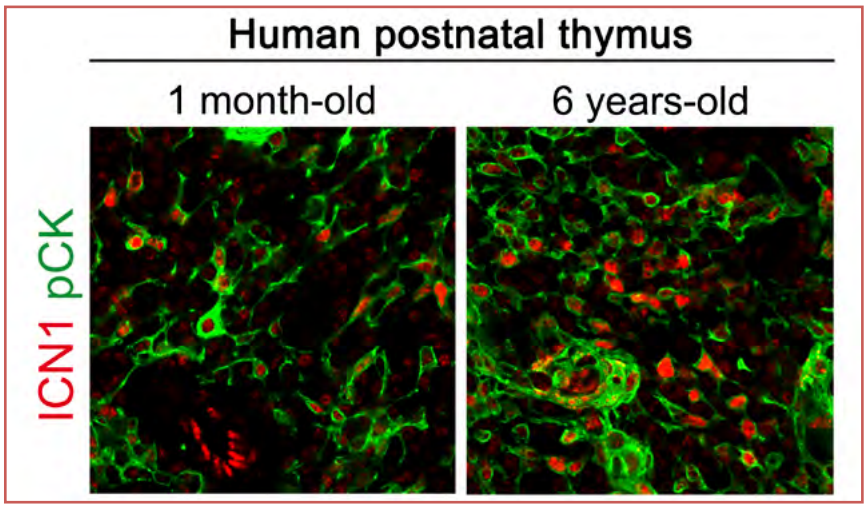
Undergraduate and Master Students:
Adrián Fernández Rego (until June 2021)

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

Summary

Our group studies the cellular and molecular mechanisms that control the commitment and differentiation along the T-cell lineage of progenitors seeding the human thymus. Our goal is to obtain mechanistic information about how dysregulation of particular intrathymic physiological pathways leads to T-cell acute lymphoblastic leukemia (T-ALL), with the final aim of identifying specific molecular targets for therapeutic intervention. Focusing on the NOTCH1 pathway as a major driver of T-ALL, we recently developed a novel *in vivo* model of *de novo* generation of human T-ALL, which revealed the contribution of several NOTCH1 targets at distinct stages of T-ALL pathogenesis. In the last two years, this information was used together with proprietary monoclonal antibodies (mAbs) and patented technology for *de novo* generation of human effector T cells, to develop state-of-the-art therapeutic strategies targeting T-ALL-specific molecules, including chimeric antigen receptors-(CAR-) armed T cells, therapeutic antibody-drug conjugates (ADCs), and secreting T-cell engaging antibodies (STAbs). We also generated cutting-edge preclinical models for *in vivo* validation of our T-ALL immunotherapy strategies, as an obligatory step preceding clinical translation. Our results have provided promising preclinical results as proof of concept of the suitability of new targets for implementation of next-generation efficacious and safe strategies overcoming current challenges of T-ALL immunotherapy.

On the other hand, we have studied the spatiotemporal regulation of NOTCH1 activation in the postnatal human thymus and have uncovered a novel role for NOTCH1 signaling in thymus biology. We found that *in vivo* activation of NOTCH1 is not confined to thymic hematopoietic cells as expected; rather, NOTCH1 signaling is also induced in thymic epithelial cells (TECs), and increases significantly with age, mostly in the medullary TEC (mTEC) compartment, in humans and also in mice, suggesting a conserved role for NOTCH1 in TEC homeostasis during thymus aging. Accordingly, we found that organization and integrity of the postnatal thymus critically depends on Notch activation, as specific abrogation of canonical Notch signaling in epithelial cells (Foxn1Cre/+ x RBPjκfl/fl mutant mice), led to a significant disruption of the medullary thymic microenvironment, accompanied by a decrease of mTEC numbers, resulting in an accelerated thymus involution. These data uncover a new role for NOTCH1 activation in the control of adult TEC homeostasis, and point toward Notch signaling manipulation as a novel strategy for thymus regeneration therapies to improve T-cell production in aging and other clinical settings. This possibility is currently under investigation.



Confocal microscopy of 1 month- and 6 years-old human thymus sections showing activation of the NOTCH1 pathway in the nucleus (red) of thymic epithelial cells (green). ICN1, Intracellular Notch1; pCK, pancytokeratine.

List of publications

» Toribio ML, González-García S. Notch Partners in the Long Journey of T-ALL Pathogenesis. Int J Mol Sci. 2023; 24:1383. doi: 10.3390/ijms24021383.

» Jiménez-Reinoso et al. Efficient preclinical treatment of cortical T-cell acute lymphoblastic leukemia with T lymphocytes secreting anti-CD1a T cell engagers. J Immunother Cancer. 2022;10:e005333. doi: 10.1136/jitc-2022-005333.

» Santamaria S et al. Therapeutic potential of an anti-CCR9 mAb evidenced in xenografts of human CCR9+ tumors. Front Immunol. 2022; 13:825635. doi: 10.3389/fimmu.2022. 825635.

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» Fuentes P, Toribio ML, González-García S. Human T-ALL Xenografts. Methods Mol Biol. 2021;2185:215-239. doi: 10.1007/978-1-0716-0810-4_13.

Projects

» MICINN. PLEC2022-009312. Targeting CCR7-mediated homeostasis of Tregs to Break the Immune Tolerance in Solid tumors (Treg-less). 2022-2024. PI.

» MICINN PDC2021-121238-I00. Towards a new Immunotherapy specific for T-cell Acute Lymphoblastic Leukemia (T-ALL) based on a unique anti-pre-TCR antibody-drug conjugate (PreTCR-ADC). 2021-2023. PI.

» MICINN PID2019-105623RB-I00. Molecular bases of T-cell acute lymphoblastic leukemia (T-ALL): Novel immunotherapeutic strategies (Immuno-T-ALL). 2020-2022. PI.

Patents

» Toribio, Alarcón, Alcain, Bayón, Fuentes, García-Peydró, González-García, Murcia. Therapeutic treatment for T-cell acute lymphoblastic leukemias using a monoclonal antibody against the pre-T cell receptor. PCTES2021/070254. P202030309. Spain, USA. April 17, 2020. CSIC and Fundación Uno Entre Cien Mil.

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TCR DOMAINS IN T CELL DIFFERENTIATION AND PATHOPHYSIOLOGICAL AND THERAPEUTIC RESPONSES

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Summary

T cells use their T cell receptor (TCR) to continuously scan Major Histocompatibility Complex (MHC) molecules presenting a repertoire of peptides derived from the proteome present in the host. Presentation of pathogen-derived peptides gives rise to protective immune responses, while responses against peptides from host proteins can lead to auto-immunity. We study how the organization of the TCR into nanoclusters allows T cells to become activated upon recognition of only a few peptide-bound MHC molecules and apply our knowledge of the molecular mechanisms of TCR signaling to development of new versions of recombinant immune receptors for cancer immunotherapy. Using transgenic mouse models that impair TCR nanoclusters formation and signaling strength of the TCR, we described a previously unknown, TCR signaling strength-regulated set point at the first stage of thymic T cell development that determines the diversity of the mature T cell repertoire. This set point permits early T cells with reduced signaling capacity to progress, albeit less efficiently, to the next developmental stages and these hypo-responding cells may contribute to the occurrence of self-reactive clones in the mature T cell repertoire.

Our research on miniTCRs, that combine antigen recognition domains of the TCR with signaling domains similar to Chimeric Antigen Receptors (CARs) and should allow for development of tumor-specific recombinant T cells, shows that the identity and topology of the signaling domains of these miniTCRs determines their efficiency with respect to activating the T cells. These results have a direct implication for improving CAR-based cellular cancer immunotherapy. They also have provided insight into the mechanisms that underlie full TCR function, which we are currently following-up upon.

We also have generated a new type of CAR-T cells against Acute Myeloid Leukemia (AML). Cellular Immunotherapy against this type of cancer has been severely hampered by unacceptable toxicity against the patient's myeloid precursors that express the same antigen as the ones targeted on AMLs. We use a logical gate formed by an activating CAR recognizing an antigen expressed by precursors and most AMLs and an inhibitory immune receptor that recognizes a ligand only expressed by the myeloid precursors. We obtained evidence 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. We will test optimized versions of these CAR-T cells in pre-clinical models of cancer immunotherapy, a first and necessary step to test its potential for clinical application.

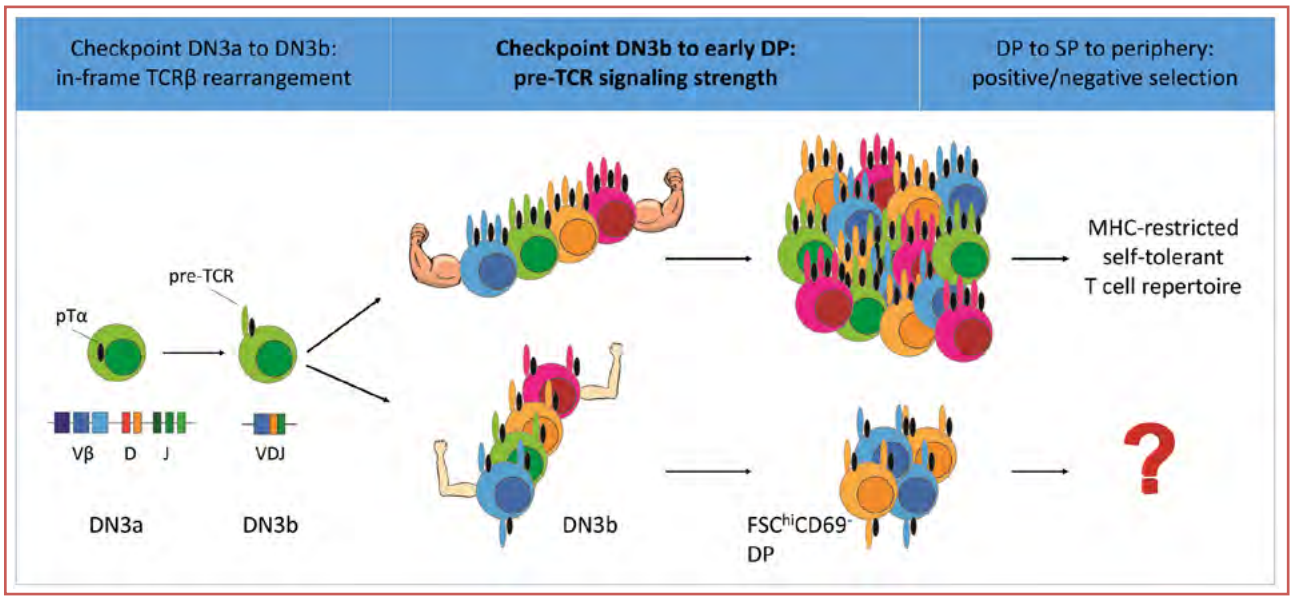

Figure legend: Signaling strength via the earliest version of the TCR ('pre-TCR') determines the size and diversity of the full TCR repertoire. The potential autoimmune impact of inclusion of weakly signaling clones in the full repertoire is being investigated.

List of publications

» Bovolenta ER, García-Cuesta EM, Horndler L, Ponomarenko J, Schamel WA, Mellado M, Castro M, Abia D, van Santen HM (2022). A set point in the selection of the αβTCR T cell repertoire imposed by pre-TCR signaling strength. Proc Natl Acad Sci USA 119(22): e2201907119. doi: 10.1073/pnas.2201907119.

» Maza MDC, Úbeda M, Delgado P, Horndler L, Llamas MA, van Santen HM, Alarcón B, Abia D, García-Bermejo L, Serrano-Villar S, Bastolla U, Fresno M (2022). ACE2 Serum Levels as Predictor of Infectability and Outcome in COVID-19. Front Immunol. 13, 836516. doi: 10.3389/fimmu.2022.836516.

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Participation in projects

» European Network on Anti-Cancer Immuno-Therapy Improvement by modification of CAR and TCR Interactions and Nanoscale Geometry (EN-ACTI2NG). Coordinator: HM van Santen; H2020-MSCA-ITN-2016, 721358; funding agency: European Commission (until 30/04/2021).

» Identidad, topología y geometría de los dominios de señalización del TCR en respuestas inmunes protectoras, terapéuticas e auto-inmunes. PID2019-104703GB-I00. 2020 – 2023. PI: HM van Santen.

» Preclinical evaluation of CAR-T cells with KIR-based logic gates for selectively targeting myeloid leukemias. Fundación LAIR. 2022 – 2024. PI: HM van Santen.

» Member of the 'Grupo de Inmunoterapia y Terapias Avanzadas' of the 'Sociedad Española de Hematología y Oncología Pediátricas' (since 2018).

Patents

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

Interactions with the Environment

Microbes in Health and Welfare unit

José Berenguer

As evidenced with COVID-19, infectious diseases remain an important cause of human morbidity and mortality, and are responsible for huge economic losses all over the world. Also, cellular and acellular microbes are suitable models to gain insight in eukaryotic cell biology and tools and source of biotechnological and biomedical products that improve economy and health. Accordingly, the overall scientific objective of the "Microbes in Health and Welfare" (MHW) unit is to study basic and applied aspects of the interactions of microbes with their hosts and the environment.

In the last two years, research groups of our unit have made the following relevant advances. Regarding basic science, our virologists have obtained evidences of how anti-HIV-1 drugs modulate mechanical properties of the viral capsid by using atomic force microscopy, and the way viruses evade the host immune system through viral proteases. Bacteriologists of the unit have studied environmental adaptations like how biogeochemical cycles operate in deep subsurface environments, or the identification of proteins and sRNAs involved in cold adaptation in a relevant food pathogen. Also, studies on conjugation in Gram-positive bacteria have led to the discovery of a novel two-component antitermination system and the molecular mechanism of covalent binding between mating pairs, whereas studies in extreme thermophiles have revealed the role of recombination-related proteins in defense against invading DNA.

On more applied fields, biomedical aspects were addressed on topics such as the use of biosafe viruses as therapeutic agents against human brain tumours in orthotopic preclinical models, the development of new vaccine prototypes, either attenuated viral forms lacking several genes or protective synthetic dendrimeric peptides vaccines and noncoding sRNA-based antivirals against animal and human viruses. Relevant achievements in the applied branch include new antiviral therapeutic strategies against HIV, SARS-Cov2 and other RNA viruses, and the development of new methods for the in-silico discovery of new carbapenemases inhibitors.

Finally, the MHW unit has been involved in biotechnology-focused research with applications outside the biomedical field, like the engineering of capsid-based nanostructured proteins with increased mechanical resistance and self-healing, the discovery of new enzymes and the thermostabilization of known ones through (ultra)high-throughput methods, or the description and use of new enzymes for the production of rare oligosaccharides and glycosylated compounds for the food industry and biomedical applications.

José M. Almendral del Río / Alberto López-Bueno
DNA VIRUS EVOLUTION, PATHOGENESIS, and ANTI-CANCER POTENTIAL

Ricardo Amils Pibernat
MOLECULAR ECOLOGY OF EXTREME ENVIRONMENTS

José Berenguer Carlos / Mario Mencía Caballero
BIOTECHNOLOGY AND GENETICS OF EXTREME THERMOPHILES

Esteban Domingo Solans
GENETIC VARIABILITY OF RNA VIRUSES

María Fernández Lobato
YEAST ENZYMES BIOENGINEERING TO GENERATE BIOACTIVE COMPOUNDS

Mauricio García Mateu
VIRUS ENGINEERING AND NANOBIOTECHNOLOGY

Paulino Gómez-Puertas
MOLECULAR MODELING GROUP

Aurelio Hidalgo Huertas
ULTRAHIGH-THROUGHPUT DISCOVERY AND ENGINEERING OF ENZYMES FOR BIOTECHNOLOGICAL APPLICATIONS

Wilfried J.J. Meijer
CONJUGATION IN GRAM-POSITIVE BACTERIA

Luis Menendez Arias
HUMAN IMMUNODEFICIENCY VIRUS REPLICATION AND ANTIRETROVIRAL THERAPY

Manuel Pazos Don Pedro
BACTERIAL CELL ENVELOPE DURING PRESEPTAL GROWTH

M. Graciela Pucciarelli Morrone
REGULATION BY RNA IN THE STRESS AND VIRULENCE

Yolanda Revilla Novella
VIRUS-CELL INTERACTION AND VACCINES DEVELOPMENT: THE ASFV MODEL

Margarita Sáiz Zalabardo
MODULATION OF ANTIVIRAL IMMUNITY BY VIRAL PROTEASES AND NONCODING RNAS

Francisco Sobrino Castelló
NEW STRATEGIES FOR PREVENTION AND CONTROL OF VIRAL DISEASES: FOOT-AND-MOUTH DISEASE VIRUS AS A MODEL

DNA VIRUS EVOLUTION, PATHOGENESIS, AND ANTI-CANCER POTENTIAL

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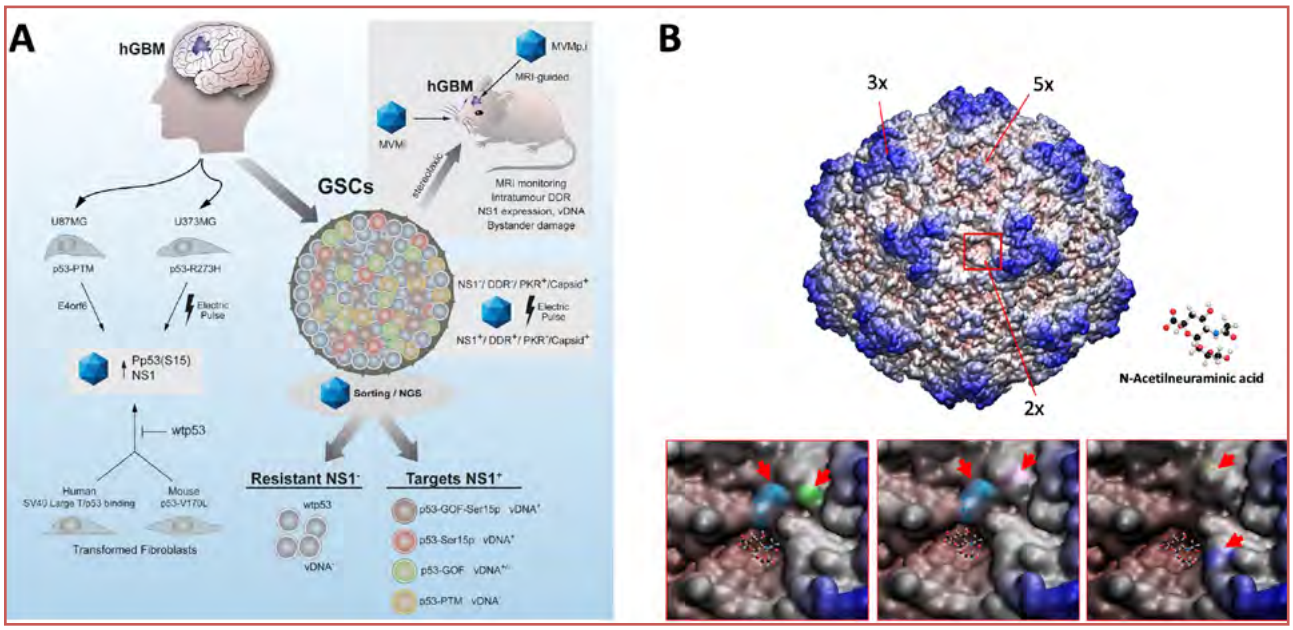
Summary

We have focused our research over the last two years in reliably testing the anti-cancer capacity of the Minute Virus of Mice (MVM), a member of the Parvoviridae. In our recent report, we describe that human glioblastoma stem cells (GSCs), with patient-specific p53 mutants and p53-Ser15 phosphorylation, are selective targets for two MVM strains (p, i) that are non-pathogenic for humans. These MVM strains induced a DNA Damage Response (DDR) in GSCs growing as neurospheres and disrupted the architecture of GSC-derived brain tumors in orthotopic rodent models (see Figure 1A), showing promise for biosafe personalized therapy against human cancers with p53 deregulations.

Other major related issues being explored include attempting physical and chemotherapeutic treatments to overcome cellular innate responses of cancer cells against MVM infection, and targeting MVM infection to the tumour vasculature by engineering the MVM capsid with VEGF peptides. Further, major efforts are being dedicated to exploring evolutionary strategies to develop parvoviruses with improved anticancer properties by optimizing their cytotoxicity and replication capacity in human tumour cells. For this, we are exploring the phenotypic features of (i) naturally evolved MVM variants with distinct tropism and pathogenicity, (ii) chimeric viruses spontaneously emerging after coinfection with two MVM strains, and (iii) a collection of MVM mutants affected at the capsid domain recognizing sialic acid receptors that were obtained from directed evolution strategies (Figure 1B).



Parvovirus MVM as oncolytic agent against human glioblastoma. A) Graphical abstract of the obtained experimental data supporting the oncolytic capacity of the parvovirus Minute Virus of Mice (MVM) against primary human glioblastoma (taken from Gil-Ranedo et al., Cell Reports 36, 109673, 2021). B) Viruses adapted to glioblastoma cells were obtained by directed evolution strategies. Upper: 3D-structure of the MVM capsid. Lower: Close-up images at the twofold axis for three MVM double mutants showing the proximity of the changed residues (red arrows) to the N-Acetilneuraminic acid (sia) binding site (in preparation).

List of publications

» Gil-Ranedo, J., Gallego-García, C., and J.M. Almendral (2021). Viral targeting of glioblastoma stem cells with patient-specific genetic and post-translational p53 deregulations. Cell Reports Vol 36, Issue 10, 109673. doi: 10.1016/j.celrep.2021.109673 .

» Mavian C., López-Bueno A, Martín R, Nitsche A, Alcamí A. (2021). Comparative Pathogenesis, Genomics and Phylogeography of Mousepox. Viruses. 15;13(6):1146. doi: 10.3390/v13061146 .

Doctoral theses

» Carlos Gallego-García (2021), "Terapia de glioblastoma por el parvovirus MVM: implicación de p53 y su modulación por quimioterapia genotóxica". Director: José M. Almendral. Dpto. Biología Molecular (UAM).

» Tania Calvo-López (2021), "Manipulación de la cápsida de parvovirus en tropismo e inducción de anticuerpos anti VEGF". Director: José M. Almendral. Dpto. Biología Molecular (UAM).

Participation in projects

» Entitled: "Directed parvovirus evolution aimed at human cancer therapy". PID2019-111146RB-I00. I+D+i Retos de Investigación 2019, Ministerio de Ciencia, Innovación y Universidades". Principal investigators: A. López-Bueno and J.M. Almendral. Jun 2020-May 2023.

» Entitled: "Plataformas y modelos preclínicos para el abordaje multidisciplinar en COVID-19 y en respuesta a futuras pandemias". COVTRAVI-19-CM. Coordinator: Manuel Fresno. Principal investigators: A. López-Bueno and J.M. Almendral. Feb 2022-Dec 2022.

» Entitled: "Use of nonionizing radiation (modulation) to enhance cancer treatment with oncolytic viruses: effects on cell viability and signaling pathways in tumor models". Paso Alto Biophysics and Biomedical Engineering S.L., Principal investigators: Y. Revilla and J.M. Almendral. Since Nov 2022.

MOLECULAR ECOLOGY OF EXTREME ENVIRONMENTS

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Summary

This area of research has the following objectives:

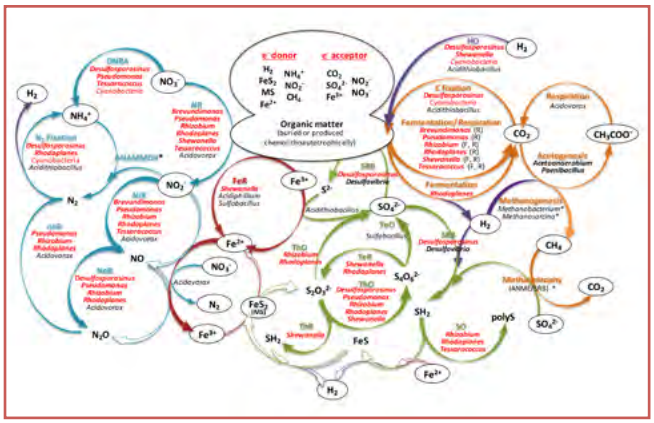
-Geomicrobiology of the Iberian Pyrite Belt (IPB): characterization of the underground bioreactor responsible of the origin of the extreme conditions detected in the Río Tinto basin. This objective is developed in collaboration with Professor J.L. Sanz from the Department of Molecular Biology (UAM). The development of 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 ecology, 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.

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.

Participation in projects

- The physicochemical nature of water on early Mars (Mars-FirstWater). ERC Consolidator Grant 818602 (2019-2023).
- Decifering the metabolism of Fe(II) oxidation associated to the reduction of nitrate (NRFeOx) and its utilization for the bioremediation of nitrate contaminated waters. TED2021-129563B-I00 (2023-2024). PI: R. Amils.
- Biodiversity characterization of the Río Tinto basin and the subsurface of the Iberian Pyrite Belt responsible of its origin, biotechnological applications. PID2019-104812GB-I00 (2020-2023). PI: R. Amils.
- Red Nacional de Microorganismos Extremófilos (RedEx, 2021, 2022).



Geomicrobiological model of the coupled C, H, N, S and Fe biogeochemical cycles operating in the deep subsurface of the IPB.

Doctoral theses

» Mahshid Sedghi (2021). Microalgae based wastewater treatment. Universidad Autónoma de Madrid. Ricardo Amils.

» José Manuel Martínez (2022). Ecología microbiana del Salar de Uyuni (Bolivia). Efectos de la caotropicidad como factor limitante para la vida. Univesidad Autónoma de Madrid. Ricardo Amils.

List of publications

» Martínez, J.M. et al. (2021) Draft genome sequence of *Pseudomonas* sp. strain T2.31D-1 isolated from 414 meters deep in the subsyrface of the Iberian Pyrite Belt. Microbiol. Resource Announc. 11, e572104. doi:10.3389/fmicb.2020.572104.

» Kristin Bashir, A. et al. (2021) Taxonomic and functional analysis of intact microbial communities thriving in extreme, astrobiology-relevant, anoxic sites. Microbiome 9, 50. doi:10.1186/s40168-20-00989-5.

» Martínez, J.M. et al. (2021) Subsurface and surface halophile communities of the chaotropic Salar de Uyuni. Environ. Microbiol. 23(7), 3987-4001. doi:10.1111/1462-2920.15411.

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» Amils, R. and Gómez, F. (2021) Editorial: Extremophiles 2.0. Microorganisms 9(4), 784. doi: 10.3390/microorganisms9040784.

» Sacristán-Horcajada, E. et al. (2021) ARAMIS: From PacBio systematic error detection to accurate assemblies. Briefings in Bioinformatics 22(6), bbab170. doi:10.1093/bib/bbab170.

» Sanz, J.L. et al. (2021) Biological production of H2, CH4 and CO2 in the deep subsurface of the Iberian Pyrite Belt. Environ. Microbiol., 23(7), 3913-3922. doi:10.1111/1462-2920-15561

» Sanz, J.L. et al. (2021). Methanogenesis at high temperatura, high ionic strength and low pH in the volcanic area of Dallol, Ethiopia. Microorganisms 9(6), 1231. doi:10.3390/microorganisms9061231.

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» Allman, C.J. et al. (2021) Hydrogeochemical variability of the acidic springs in the Rio Tinto headwaters. Water 13, 2861. doi: 10.3390/w13202861.

» Fernández-Remolar, D. et al. (2021). Unveiling microbial preservation under hyperacidic and oxidizing conditions in the Neogene Río Tinto deposits. Sci. Rep. 11, 21543. doi:10.1038/s41598-021-00730-8.

» Abramov, S.M. et al. (2022) Biogeochemical Niches of Fe-cycling Communities Influencing Heavy Metal Transport along the Rio Tinto, Spain. Appl. Environ, Microbiol. 88(4), e02290-21. doi: 10.1128/aem.02290-21

» Schwendner, P. et al. (2022) Microbial degradation of amino acids as a potential biosignature. Front. Astron. Space Sci. 9, 781542. doi:10.3389/fspas.2022.781542.

» Anglés, A. et al. (2022) Endokarstic edifices formed by fungal activity during the early Holocene in the "Salar de Uyuni". Front. Microbiol. 13, 913452. doi:10.3389/fmicb.2022.913452

» Huang, T. et al. (2022) *Salinibacillus dalangtanensis* sp. nov., a moderate halophile isolated from hypersaline sediments of the Qaidam Basin in NW China. Int. J. Syst. Evol. Microbiol. 71, 005501. doi:10.1099/ijsem.0.005501.

» Mateos, G. et al. (2022) *Shewanella* sp. T2.3D-1.1 a novel microorganism subtaining the Iron cycle in the deep subsurface of the Iberian Pyrite belt. Microoorganisms 10, 1585. doi:10.3390/microorganisms10081585.

» Huang-Lin, E. et al. (2022) Potential applications of an exopolysaccharide produced by *Bacillus xiamenesis* RT6 isolated from an ecidic environment. Polymers 14, 3918. doi:10.3390/polym14183918.

BIOTECHNOLOGY AND GENETICS OF EXTREME THERMOPHILES

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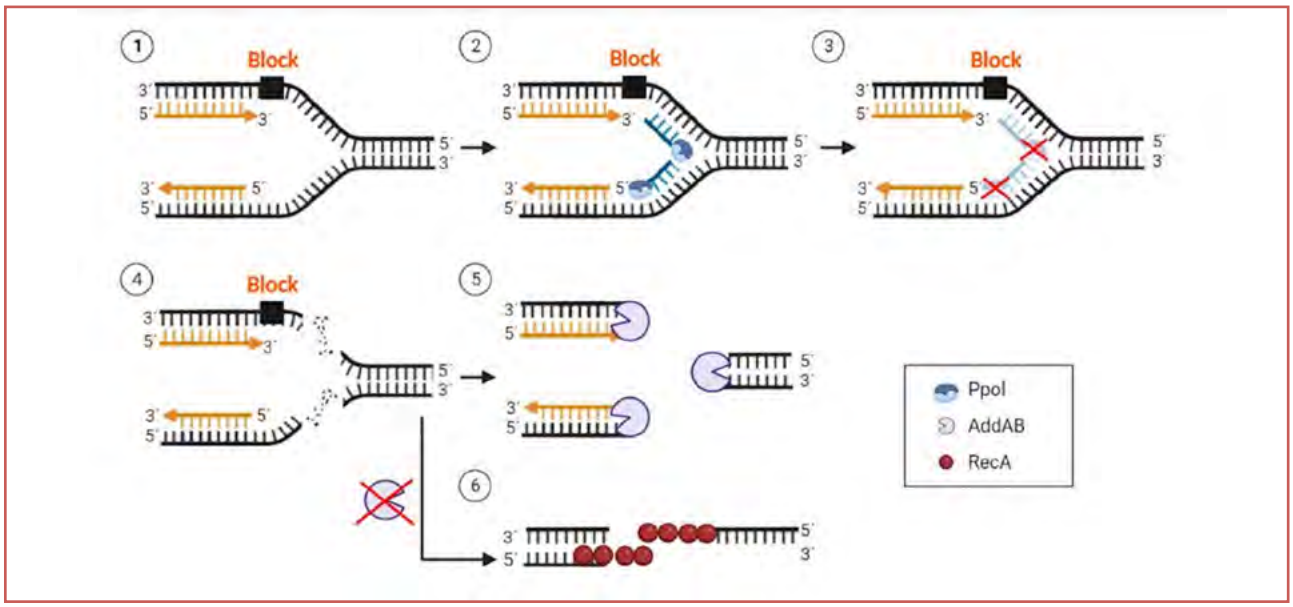
Summary

The main objective of our group during this period has been to analyze the mechanisms of DNA transfer and those acting as defense barriers in thermophilic bacteria that could render biotechnological applications. DNA transfer and repair is enhanced in thermal environments due to the strong selective factor appointed by high temperatures against replication fidelity that results in the selection of small genomes. For *Thermus thermophilus* (Tth), this selection has leaded to the evolution of a polyploid genome and the most efficient natural competence apparatus (NCA) so far described. In addition, Tth can exchange DNA by direct cell contacts using "transjugation", a process in which a DNA donation apparatus (DDA) allows the ejection of DNA from a "donor" strain with the concomitant incorporation by a competent recipient mate through its NCA.

The main focus of our research in the last two years has been the analysis of the mechanisms involved in protection against the integration of environmental DNA (eDNA) in the genome. In this context we have studied the role of a DNA primase-polymerase (Ppol) encoded by mobile element ICETh2, as anti-eDNA barrier. We found that Ppol loss of function mutations increase dramatically the transformability of the cells with eDNA by 2-3 orders of magnitude, playing apparently a defensive role that is not active against DNA transferred by a mating pair in transjugation. This differential protective activity is similar to that provided by ThAgo, a homologue of the human Argonaute protein that uses DNA-DNA interference to cleave exogenous DNA entering the cells by transformation, suggesting a role for Ppol as putative generator of ssDNA guides for ThAgo. Further work of our group has shown the relevance of Ppol for plasmid stability and the existence of Ppol-independent mechanisms for the generation of ssDNA guides for ThAgo. Our most recent work has shown a deep imbrication of Ppol in DNA repair in Tth, keeping an unexpected equilibrium between its activity and that of the excinuclease AddAB, required to generate the 3' overhands needed to repair dsDNA breaks by homologous recombination. In such equilibrium Ppol compensates for an apparently lethal overactivity of AddAB, in such a way that only *addAB* loss-of-function mutants survive to the inactivation of Ppol. Future work of the group will focus on the relation of HGT and recombination pathways and use this knowledge to develop new tools for the directed evolution of proteins in Tth.



Model for the in vivo interplay between Primpol and AddAB. Primpol polymerase (Ppol) performs DNA synthesis on ssDNA regions to continue DNA replication (1-2). In the absence of Ppol double-strand breaks occur at the ssDNA regions (3-4). The AddAB complex processively degrades the dsDNA ends (5). Tth mutants lacking AddAB present DNA fragmentation but still keep a less efficient system for recombinative repair of the double-strand breaks by archaeal-like pathways encoded by its genome (6).

List of publications

» Mencía, M. (2022) Acid digestion and symbiont: Proton sharing at the origin of mitochondriogenesis? BioEssays, 2200136. doi: 10.1002/bies.202200136

» Verdú, C., Pérez-Arnaiz, P., Peropadre, A., Berenguer, J., and Mencía, M. (2022) Deletion of the primase-polymerases encoding gene, located in a mobile element in *Thermus thermophilus* HB27, leads to loss of function mutation of addAB genes. Front. Microbiol. 13, 1005862. doi: 10.3389/fmicb.2022.1005862.

» Miguel-Ruano, V., Rivera, I., Rajkovic, J., Knapik, K., Torrado, A., Otero, J.M., Beneventi, E., Becerra, M., Sanchez-Costa, M., Hidalgo, A., Berenguer, J., Gonzalez-Siso, M.I., Cruces, J., Rúa, M.L., and Hermoso, J. (2021) Characterization of a novel thermophilic esterase EstD11 provide catalytic insights for the HSL family. Comput Struct Biotechnol J. 19: 1214-1232. doi: 10.1016/j.csbj.2021.01.047. PMID: 33680362;

» Orrego, A.H., Andrés-Sanz, D., Velasco-Lozano, S., Sanchez-Costa, M., Berenguer, J., Guisán, J.M., Rocha-Martin, J., and López-Gallego, F. (2021) Self-sufficient asymmetric reduction of β-ketoesters catalyzed by a novel and robust thermophilic alcohol dehydrogenase co-immobilised with NADH. Catal. Sci. Technol., 11, 3217-3230. doi: 10.1039/d1cy00268f

» Bosch, S., Sánchez-Freire, E., del Pozo, M.L., Česnik, M., Quesada, J., Mate, D. M. Hernández, K., Qi, Y., Clapés, P., Vasić-Rački, Đ., Findrik, Z., Berenguer, J., Hidalgo, A. (2021) Thermostability engineering of a class II pyruvate aldolase from *Escherichia coli* by *in vivo* folding interference. ACS Sustainable Chem. Eng. 9, 15, 5430–5436. doi:10.1021/acssuschemeng.1c00699

Research networks and projects

» National Network of Extremophilic Microorganisms REDEX2019. Coordinator: J. M. Gonzalez-Grau

» New tools derived from DNA transfer and interference systems from thermophilic bacteria. PID2019-109073RB-I00. Co-PIs J. Berenguer and M. Mencía

» A new approach for the sustainable bioconversion of plastic waste into high value products based on thermophilic microorganisms and enzymatic synthesis. TED2021-130430B-C22. PI: M. Mencía.

Awards and recognitions

» Editor in Chief of International Microbiology, the journal of the Spanish Society for Microbiology (Springer-Nature group).

GENETIC VARIABILITY OF RNA VIRUSES

Group Members

Principal Investigator:
Esteban Domingo Solans

Scientific Staff:
Celia Perales Viejo

Postdoctoral fellows:
Pilar Somovilla Crespo
(since January 2022)

Predoctoral fellows:
Carlos García Crespo
Victoria Castro Illana
(until July 2021)
Brenda Martínez González
Rebeca Lobo Vega
(until June 2021)

Technicians:
Ana Isabel de Ávila Lucas
Isabel Gallego Jiménez
(until December 2021)
María Eugenia Soria Benito

Undergraduate and Master Students:
Miriam Herráez Moncusí
(until June 2021)
Antoni Durán i Pastor
(since September 2021)
Lucía Vázquez Sirvent
(until April 2022)

Visiting scientists:
Ana Grande Pérez,
Universidad de Málaga
(February-June 2022)
Josep Sardanyes, Centre de Recerca Matemàtica
(November 2022).

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

Summary

We investigate mutant spectra of RNA viruses, and we search for new treatments for their associated diseases. We work with hepatitis C virus (HCV) and SARS-CoV-2. Despite predictions that this coronavirus would vary little due to its genome size, our work and work from others has revealed a remarkable complexity of individual isolates. This has placed the new pathogen under the quasispecies focus to interpret its behavior and to develop effective treatments. Our previous work with HCV documented synergies between nucleoside analogues, partly due to their difference preference for mutation sites. This encouraged the search for synergistic treatments for COVID-19. To this aim, we have designed stricter evaluation protocols, particularly use of high fitness virus and antiviral administration once the infection is ongoing.

We carry our work in collaboration with the group of Celia Perales (CNB-CSIC) who is associated with Fundación Jiménez Díaz, an institution that advises us regarding COVID-19 patient monitoring, and provides us with well characterized virus isolates. Thanks to such collaboration, and that of experts on bioinformatics, we have quantified the complexity of SARS-CoV-2 isolates, with high resolution. Virus from patients who developed mild disease may be the source of more variants than virus from patients who suffered severe disease. These studies are carried out in collaboration also with the groups of Nuria Verdaguer (IBMB, Barcelona, as part of our participation in the platform PTI Salud Global from CSIC) and Jordi Gómez (Instituto López Neyra, CSIC, Granada, with a joint Plan Nacional project).

Jointly with the groups of Soledad Delgado (UPM), Federico Morán (UCM) and Cecilio López-Galíndez (ISCIII) we are currently applying artificial intelligence methods (derivation of self-organized neural network maps) to quantify haplotypes in viral populations and their modifications. Our hypothesis is that detailed information on genetic variation dynamics will help us to find more effective treatments.

We participate in additional collaborations with other teams, and we have been asked to write review articles on viral quasispecies, as shown in the publication list.

Doctoral theses

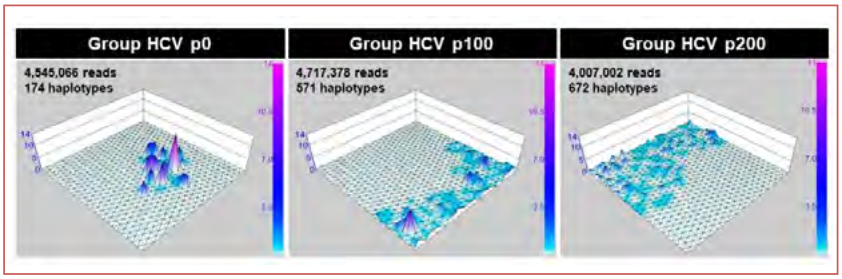
» Victoria Castro Illana (2021). Estudio de determinantes virales y celulares para el diseño de terapias frente al virus de la hepatitis C. Universidad Autónoma de Madrid. Directores/as: Celia Perales, Pablo Gastaminza.

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.



Self-organized haplotype maps in the mutant spectrum of hepatitis C virus upon evolution in human hepatoma cells. Reads obtained by deep sequencing, and deduced haplotype numbers are indicated [from Delgado et al. Microbiol. Spectr. 9(3):e01459-21, 2021].

List of publications

Artículos en revistas científicas:

- Domingo, E. & Perales, C. (2021). The time for COVID-19 vaccination. J. Virol. 95(8):e02437-20. doi:10.1128/JVI.02437-20.
- García-Crespo, C., et al. (2021). Population disequilibrium as promoter of adaptive explorations in hepatitis C virus. Viruses. 13(4):616. doi: 10.3390/v13040616.
- Domingo, E., et al. (2021). Historical perspective on the discovery of the quasispecies concept. Annu. Rev. Virol. 8(1):51-72. doi: 10.1146/annurev-virology-091919-105900.
- Llorens-Revull, M., et al. (2021). Partial restoration of immune response in hepatitis C patients after viral clearance by direct-acting antiviral therapy. PLoS One. 16(7):e0254243. doi: 10.1371/journal.pone.0254243.
- Domingo, E., et al. (2021). Mutation rates, mutation frequencies, and Proofreading-Repair Activities in RNA virus genetics. Viruses. 13(9):1882. doi: 10.3390/v13091882.
- Sabariegos, R., et al. (2021). Akt phosphorylation of HCV NS5B regulates polymerase activity and HCV infection. Front. Microbiol. 12:754664. doi: 10.3389/fmicb.2021.754664.
- Llorens-Revull, M., et al. (2021). Study of quasispecies complexity and liver damage progression after liver transplantation in hepatitis C virus infected patients. Genes (Basel). 12(11):1731. doi: 10.3390/genes12111731.
- Delgado, S., et al. (2021). A two-level, intra-mutant spectrum haplotype profile of hepatitis C virus revealed by self-organized maps. Microbiol. Spectr. 9(3):e0145921. doi: 10.1128/Spectrum.01459-21.
- Soria, M.E., et al. (2021). High SARS-CoV-2 viral load is associated with a worse clinical outcome of COVID-19 disease. Access Microbiol. 3(9):000259. doi: 10.1099/acmi.0.000259.
- Caridi, F., et al. (2021). Adaptive value of foot-and-mouth disease virus capsid substitutions with opposite effects on particle acid stability. Sci. Rep. 11(1):23494. doi: 10.1038/s41598-021-02757-3.
- Ariza-Mateos, A., et al. (2022). Application of archaeological concepts to the interpretation of RNA virus quasi species evolution. Theor. Biol. Forum. 115(1-2):133-143. doi: 10.19272/202211402009.
- García-Crespo, C., et al. (2022). Eficacy decrease of antiviral agents when administered to ongoing hepatitis C virus infections in cell culture. Front. Microbiol. 13:960676. doi: 10.3389/fmicb.2022.960676.
- Sabariegos, R., et al. (2022). Guanosine inhibits hepatitis C virus replication and increases indel frequencies, associated with altered intracellular nucleotide pools. PLoS Pathog. 18(1):e1010210. doi: 10.1371/journal.ppat.1010210.
- Martínez-González, B., et al. (2022). SARS-CoV-2 point mutation and deletion spectra and their association with different disease outcomes. Microbiol. Spectr. 10(2):e0022122. doi: 10.1128/spectrum.00221-22.
- Torres-Vázquez, B., et al. (2022). In vitro selection of high affinity DNA and RNA aptamers that detect hepatitis C virus core protein of genotypes 1 to 4 and inhibit virus production in cell culture. J. Mol. Biol. 434(7):167501. doi: 10.1016/j.jmb.2022.167501.
- Martínez-González, B., et al. (2022). SARS-CoV-2 mutant spectra at different depth levels reveal an overwhelming abundance of low frequency mutations. Pathogens. 11(6):662. doi: 10.3390/pathogens11060662.
- Martínez-González, B., et al. (2022). Vaccine breakthrough infections with SARS-CoV-2 Alpha mirror mutations in Delta Plus, Iota, and Omicron. J. Clin. Invest. 132(9):e157700. doi: 10.1172/JCI157700.
- Hufsky, F., et al. (2022). The International Virus Bioinformatics Meeting 2022. Viruses. 14(5):973. doi: 10.3390/v14050973.
- Gregori, J., et al. (2022). Quasispecies fitness partition to characterize the molecular status of a viral population. Negative effect of early ribavirin discontinuation in a chronically infected HEV patient. Int. J. Mol. Sci. 23(23):14654. doi: 10.3390/ijms232314654.

Participation in projects

- Combinaciones de antivirales frente al SARS-CoV-2. CSIC-COV19-014. Funding: Agencia Estatal CSIC. (2020-2021). IPs: Nuria Verdaguer, Esteban Domingo y Celia Perales.
- Plataforma para el desarrollo de estrategias de control de salud animal. PLATESA2, S2018/BAA-4370. Funding: Comunidad de Madrid/FEDER. (2019-2022). IP: Noemí Sevilla. IP Work Package VIRNA: Esteban Domingo.
- Desorganización molecular durante la mutagénesis letal de SARS-CoV-2: afectación de regiones de RNA estructuradas y no estructuradas. PID2020-113888RB-I00. Funding: Ministerio de Ciencia e Innovación. (2021-2024). IPs: Jordi Gómez y Esteban Domingo.
- Mutagénesis letal sinérgica para SARS-CoV-2 con muestras de virus de pacientes. Funding: PTI+ Salud Global, dentro del WP9 Antivirales. Agencia Estatal Consejo Superior de Investigaciones Científicas (CSIC) (2022-2023). IPs: Nuria Verdaguer, Esteban Domingo y Celia Perales.
- Towards understanding the molecular mechanisms of lethal mutagenesis in SARS-CoV-2. 525/C/2021. Funding: Fundació La Marató TV3 (30/07/2021- 29/07/2024). IP: Nuria Verdaguer y Celia Perales. Colaborador: Esteban Domingo.
- Exosomas como biomarcadores de progresión de la enfermedad hepática tras la curación del virus de la hepatitis C. PI18/00210. Funding: Instituto de Salud Carlos III. (01/2019 - 12/2022). IP: Celia Perales. Colaborador: Esteban Domingo.
- Pertenencia a la Red Centro de Investigación Biomédicas en Red de Enfermedades Hepáticas y Digestivas (CIBERehd). Grupo CB06/04/0086.
- Pertenencia a Instituto de Investigación Sanitaria (Fundación Jiménez Díaz).

YEAST ENZYMES BIOENGINEERING TO GENERATE BIOACTIVE COMPOUNDS

Group Members

Principal Investigator:
María Fernández Lobato

Scientific Staff:
Miguel Remacha Moreno

Postdoctoral Fellows:
Peter Kidibule. From May 2021

Predoctoral fellows:
David Piedrabuena
Peter Kidibule. Until April 2021
Zoran Merdzo. Until Nov. 2021
Martín García. Until June 2022
Marina Minguet
Egle Narmontaite
María Martínez. From Oct. 2022

Technicians:
Mª Pilar Sanchez Pozo
Laura Barahona. From Oct. 2022

Undergraduate and Master Students:
Helena Soto Pérez. 2021
Sandra Redondo Cueto. 2021
Sergio Izquierdo Gea. 2021
María Martínez Ranz. 2022
Laura Cuesta Ramos. 2022
Raquel González Jabardo. 2022
Laura Barahona. 2022
Sofia López Solís. 2022

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

Summary

We work with microorganisms producing bioactive compounds for application in functional and nutraceutical food. We try to connect the generation of knowledge to the development of biotechnological applications. Basically, we focus on the characterization of enzymes producing new compounds, the analysis of their structural-functional determinants, their operational improvement using molecular biology tools, the characterization of the new molecules produced and the evaluation of their potential biological activity.

During the last years we have continued with the characterization and study of proteins from non-conventional yeast showing glycosyltransferase activity, applicable in the production of new heterooligosaccharides and glycoconjugate derivatives with putative prebiotic or antioxidant properties. We have also characterized new fungal chitinases that can hydrolyze different chitinolytic materials, waste from the industrial activity, to generate chitooligosaccharides with promising bioactive properties. In general, most of the characterized proteins are glycosyl hydrolases (GH) structurally included in families GH32, 31, 13 or 18. Recently we have found that some of the characterized enzymes can glycosylate compounds with aromatic rings such as hydroxytyrosol or phloretin, which gives them a special biotechnological interest. We have obtained numerous variants of enzymes that increase or alter the pattern of biosynthetic products. Objectives included in those of the Glicoenz consortium (http://www.glicoenz.org/p/glicoenz.html).

Participation in projects

- EMFF-BlueEconomy-2018. Proposal number: 863697. FISH chitinolytic biowastes FOR FISH active and sustainable packaging material (FISH4FISH). 11/2019-11/2022.
- 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. 04/2019-04/2023.
- PID2019-105838RB-C32: Prospecting and development of microbial enzymes to obtain new glycosylated compounds of pharmacological interest. MCI-Programa Estatal de Investigación, I+D+i-Orientada a los Retos de la Sociedad. 06/2020-05/2023.
- REACT-UE-COVTRAVI-19-CM: Plataformas y modelos preclínicos para el abordaje multidisciplinar en COVID-19 y en respuesta a futuras pandemias. UAM. Coordinator: Manuel Fresno. From Jan to Dec 2022.
- TED2021-129288B-C22: Simplification of the use of chitin-enriched waste for the enzymatic production of bioactive chitooligosaccharides. AEI- Projects oriented towards the ecological transition and the digital transition. 11/2022-10/2024.
- PDC2022-133134-C22: Scaling up the production of glycosidases to obtain modified flavonoids and their evaluation in biomedical applications. EAI-Proof of Concept Projects. 11/2022-10/2024.

List of publications

» Kidibule et al. (2021) Production and Characterization of Chitooligosaccharides by the Fungal Chitinase Chit42 Immobilized on Magnetic Nanoparticles and Chitosan Beads: Selectivity, Specificity, and Improved Operational Utility" RSC Advances, 11, 5529-5536. doi: 10.1039/D0RA10409D

» Rubio et al. (2021) Aging in male Wistar rats associates with changes in intestinal microbiota, gut structure and cholecystokinin-mediated gut-brain axis function. J. Gerontol. A Biol. Sci. Med. Sci. 76 (11), 1915-1921. doi: 10.1093/gerona/glaa313

» Rodrigo-Frutos et al. (2021) New insights into the molecular mechanism behind mannitol and erythritol fructosylation by β-fructofuranosidase from *Schwanniomyces occidentalis*. Sci. Report, 11, 7158. doi: 10.1038/s41598-021-86568-6

» Gimeno-Pérez et al. (2021) The β-Fructofuranosidase from *Rhodotorula dairenensis*: Molecular Cloning, Heterologous Expression, and Evaluation of Its Transferase Activity. Catalysts 11(4), 476. doi: 10.3390/catal11040476

» Miguez et al., (2021) Enzymatic Synthesis and Characterization of Different Families of Chitooligosaccharides and Their Bioactive Properties. Applied Sciences 11, 3212; (pp 1-14). doi: 10.3390/app11073212

» Piedrabuena et al. (2021) Enzymatic synthesis of novel fructosylated compounds by Ffase from *Schwanniomyces occidentalis* in green solvents. RSC Advances, 11, 24312-24319. doi: 10.1039/d1ra01391b

» Jiménez-Ortega et al. (2021) Structural inspection and protein motions modelling of a fungal Family 18 chitinase by crystallography depicts the molecular determinants of its dynamic enzymatic mechanism. Comput. Struct. Biotechnol. J. 19, 5466-5478. doi: 10.1016/j.csbj.2021.09.027

» Gonzalez-Alfonso et al. (2021) Polyglucosylation of rutin catalyzed by cyclodextrin glucanotransferase from *Geobacillus* sp. Optimization and chemical characterization of products. Ind. Eng. Chem. Res. 60, 51, 18651-18659. doi: 10.1021/acs.iecr.1c03070

» Cervantes et al. (2022) Reuse of immobilized *Komagataella phaffii* cells for the selective elimination of D-glucose in syrups of bioactive carbohydrates. ACS Food Sci. Technol. 2, 4, 682-690. doi: 10.1021/acsfoodscitech.2c00008

» Braga et al. (2022) Tailoring fructooligosaccharides composition with engineered *Zymomonas mobilis* ZM4. Appl. Microbiol. Biotechnol. 106, 4617-4626. doi: 10.1007/s00253-022-12037-3

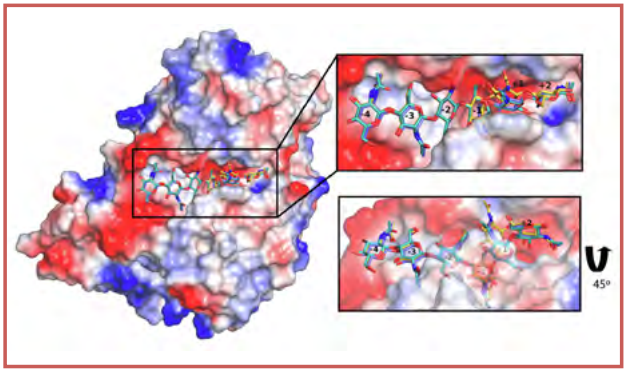
» Jiménez-Ortega et al. (2022) Structure-function insights into the fungal endo-chitinase Chit33 depict its mechanism on chitinous material. Int. J. Mol. Sci. 23 (14), 7599, (pp-1-15). doi: 10.3390/ijms23147599

» Capecchi et al. (2022) Nanoparticles of lignin and saccharides from fishery wastes as sustainable UV-shielding, antioxidant and antimicrobial bio-fillers. Biomacromolecules, 23, 8, 3154-3164. doi: 10.1021/acs.biomac.2c00236

» Garcia-Gonzalez et al. (2022) Isomelezitose overproduction by alginate-entrapped recombinant *E. coli* cells and in-vitro evaluation of its potential prebiotic effect. Int. J. Mol. Sci. 23(20), 12682. doi: 10.3390/ijms232012682

» Jiménez-Ortega et al. (2022) Insights into the structure of the highly glycosylated Ffase from *Rhodotorula dairenensis* enhance its biotechnological potential. Int. J. Mol. Sci. 23(23), 14981. doi:10.3390/ijms232314981

» Amorim et al. (2022) Engineering Saccharomyces for the one-step production of a functional sweetening mixture towards food applications. Food Bioprod. Process. 135,124-134. doi: 10.1016/j.fbp.2022.07.006



Structural traits of complexes into chitinase Chit42 machinery. Superposition of Chit42 D169A/E171A complexed with NAG6 (teal) and NAG4/NAG2 (gold/slate)

Awards and recognitions

» María Fernández Lobato. Director of the Molecular Biology Department, Universidad Autónoma, Madrid (from 06/2021).

» Miguel Remacha Moreno. Director of the Doctoral School, Universidad Autónoma, Madrid (from 12/2021).

Doctoral theses

» Peter Kidibule (2021) Using chitinolytic materials to obtain bioactive oligosaccharides. Characterization of biocatalysts and products. UAM. Director: María Fernández Lobato. International Mention. Honor Mention.

» Ángel García Horstmann (2021) Estudio de microorganismos halófilos moderados productores de exopolisacáridos pertenecientes a salinas de interior en Castilla-La Mancha. UAM. Co-directors: Adrián García de Marina Bayo and María Fernández Lobato. Industrial Mention.

» Zoran Merdzo Kunovac (2021) Estudio de la α-glucosidasa de *Schwanniomyces occidentalis* para su aplicación en la síntesis de isomaltooligosacáridos y otros compuestos glucosilados. UAM. Director: María Fernández Lobato.

» Diego Martin Garcia Gonzalez (2022) Study of the α-glucosidase from the yeast *Metschnikowia reukaufii* and its use in biocatalytic processes to produce bioactive oligosaccharides from honey. UAM. Director: María Fernández Lobato. International Mention.

VIRUS ENGINEERING AND NANOBIOTECHNOLOGY

Group Members

Principal Investigator:
Mauricio G. Mateu

Scientific staff:
Alejandro Valbuena Jiménez

Predoctoral Fellows:
Santos Domínguez Zotes (until may 2022)
Luis Valiente Martínez-Sicluna
Judith Escrig Traver

Master Student:
Joseph Mcgrail Gámez

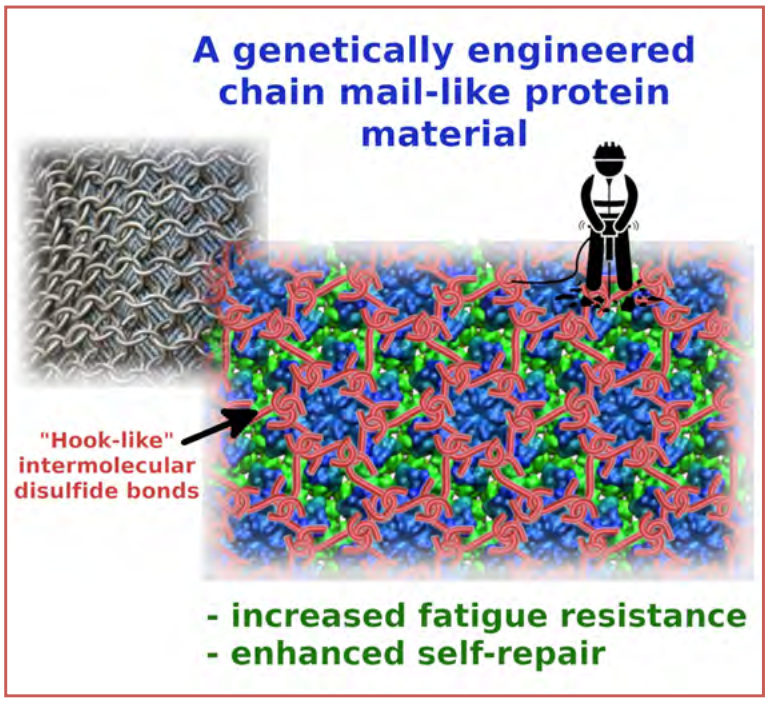
http://www.cbm.uam.es/mgmateu

Summary

Major research goals: We use protein engineering techniques and biochemical, biophysical and virological analyses to study the assembly, conformational stability and dynamics and physical properties of viruses, and their biological relevance (Mateu (ed.) (2013) *Structure and Physics of Viruses*, Springer 2013; new edition under way). Based on these studies, we aim at providing novel insights into key processes for viral infection, including virus morphogenesis, structural rearrangements and uncoating; and to provide guidelines and proof of concept for the application of this knowledge for the design of vaccines, antiviral drugs, biomaterials or modified nanoparticles for biomedical or bionanotechnological uses (see Mateu (2016). In *Protein-based Engineered Nanostructures*, Springer 2016, pp.83-120).

Scientific relevance and technological implications: Some major scientific contributions in the last years include: i) experimental evidence on the biological relevance of mechanical properties of viruses; ii) insights into the intimate relationship between virus mechanical elasticity and conformational dynamics at equilibrium; iii) detailed descriptions of virus capsid self-assembly routes, including the actual visualization in real time using high-speed atomic force microscopy of single molecules during capsid lattice assembly; iv) the discovery of the possibility to develop new antiviral drugs acting on the mechanical properties of viral particles; v) the genetic design of novel biomaterials with improved mechanical properties.

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, and of mechanically resistant virus-based nanostructured materials.



A nanostructured protein material was genetically modified to permanently increase its resistance to fatigue and enhance self-healing. Each protein molecule was mutated to become covalently linked to each adjacent molecule, analogous to the rivetting of iron rings in a medieval knight´s chain mail.

List of publications

- Maity, S., Valbuena, A., Mateu, M.G. and Roos, W.H. (2021). High-speed AFM unveils assembly dynamics in real time. Eur. Biophys. J. 50, suppl. 1, 51-52. doi.org/10.1007/s00249-021-01558-w.
- Domínguez-Zotes, S., Fuertes, M.A., Rodríguez-Huete, A., Valbuena, A.* and Mateu, M.G.* (2022). A Genetically engineered, chain mail-like nanostructured protein material with increased fatigue resistance and enhanced self-healing. Small 18: e2105456. doi: 10.1002/smll.202105456.
- Domínguez-Zotes, S., Valbuena, A.* and Mateu, M.G.* (2022). Antiviral compounds modulate elasticity, strength and material fatigue of a virus capsid framework. Biophys. J. 121, 919-931. doi: 10.1016/j.bpj.2022.02.014.
- Valiente, L., López-Argüello, S., Rodríguez-Huete, A., Valbuena, A.* and Mateu, M.G.* (2022). Molecular determinants of human rhinovirus infection, assembly and conformational stability at capsid protein interfaces. J. Virol. 96, 00840-22. doi: 10.1128/jvi.00840-22. *corresponding authors.

Doctoral theses

- Santos Domínguez Zotes (2022). Modulación de las propiedades mecánicas y dinámicas del entramado proteico que forma la cápsida del virus de la inmunodeficiencia humana. Universidad Autónoma de Madrid. Supervisors: Mauricio G. Mateu and Alejandro Valbuena.

Participation in projects

- MICINN RTI 2018-096635-B-100. "Autoensamblaje, mecánica y fluctuaciones conformacionales de cápsidas de virus. Implicaciones para el desarrollo de biorrecubrimientos, nanopartículas y fármacos antivirales". 2019-2022. PI. M. G. Mateu.
- MICINN PID 2021-126973OB-I00. "Biomecánica y dinámica de virus humanos para el desarrollo de fármacos antivirales y materiales modificados por ingeniería de proteínas". 2022-2025. PI. M. G. Mateu.

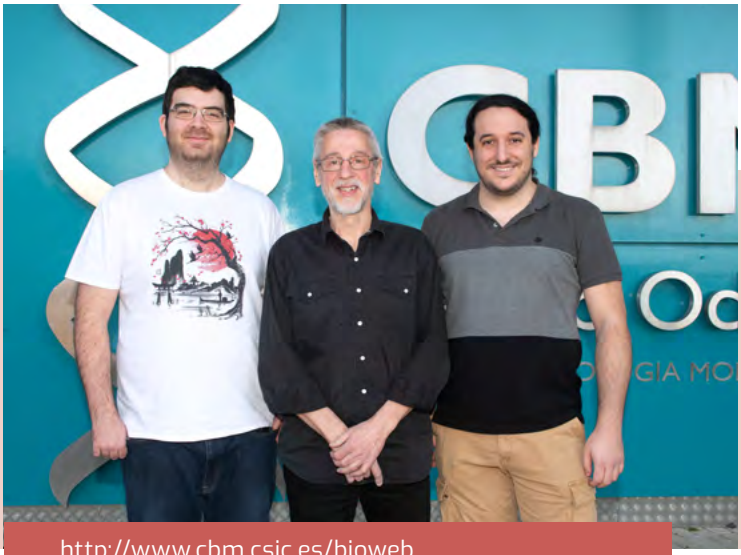
Research Networks

- Red Temática Nacional de Excelencia en Física Virológica

Other activities

- Mauricio G. Mateu, member of the Editorial Board of Virus Research.

MOLECULAR MODELING GROUP



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

Group Members

Principal Investigator
Paulino Gómez-Puertas

Scientific Staff
Íñigo Marcos-Alcalde

Predoctoral fellow:
David Ros-Pardo

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. 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) 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

» 2022. Patent "MiniACE2 proteins as antivirals against SARS-CoV-2 infection", registered in collaboration with IDCBIS - Bogotá, Colombia.

Other activities

» Paulino Gómez-Puertas, member of the editorial board of "International Journal of Molecular Sciences" and “Biophysica”.

List of publications

» Dahdouh et al. (2022). Computational Modeling and Design of New Inhibitors of Carbapenemases: A Discussion from the EPIC Alliance Network. International Journal of Molecular Sciences 23, 9746. doi: 10.3390/ijms23179746

» Christensen et al. (2022). Biallelic variants in ZNF142 lead to a syndromic neurodevelopmental disorder. Clinical Genetics 102, 98-109. doi: 10.1111/cge.14165

» Levy et al. (2022). Neurodevelopmental disorders associated with PSD-95 and its 2 interaction partners. International Journal of Molecular Sciences 23, 4390. doi: 10.3390/ijms23084390

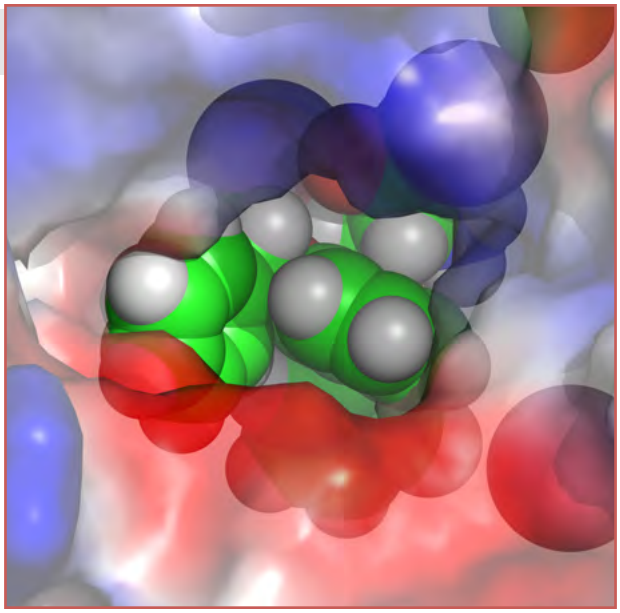
» Fernández-Justel et al. (2022). Diversity of mechanisms to control bacterial GTP homeostasis by the mutually exclusive binding of adenine and guanine nucleotides to IMP dehydrogenase. Protein Science 31, e4314. doi: 10.1002/pro.4314

» Arnedo et al. (2022). Molecular Basis of the Schuurs–Hoeijmakers Syndrome: What We Know about the Gene and the PACS-1 Protein and Novel Therapeutic Approaches. International Journal of Molecular Sciences 23, 9649. doi: 10.3390/ijms23179649

» Lucía-Campos, C., Valenzuela, I., Latorre-Pellicer, A., Ros-Pardo, D., Gil-Salvador, M., Arnedo et al. (2022). A novel intragenic duplication in HDAC8 gene underlying a case of Cornelia de Lange syndrome. Genes 13, 1413. doi: 10.3390/genes13081413

» Kumble et al. (2022). The clinical and molecular spectrum of QRICH1 associated neurodevelopmental disorder. Human Mutation 43, 266–282. doi: 10.1002/humu.24308

» Latorre-Pellicer et al. (2021). Things are not always what they seem: From Cornelia de Lange to KBG phenotype in a girl with genetic variants in NIPBL and ANKRD11. Molecular Genetics & Genomic Medicine 9, e1826. doi: 10.1002/mgg3.1826



Binding of a ligand to the active site of an enzyme during a computational inhibitor screening process.

» Morejón-García et al. (2021). Dysfunctional Homozygous VRK1-D263G variant impairs the assembly of Cajal bodies and DNA damage response in hereditary spastic paraplegia. Neurology Genetics 7, e624. doi: 10.1212/NXG.0000000000000624

» Latorre-Pellicer et al. (2021). Clinical relevance of postzygotic mosaicism in Cornelia de Lange syndrome and purifying selection of NIPBL variants in blood. Scientific Reports 11, 15459. doi: 10.1038/s41598-021-94958-z

» Rodríguez-Palmero et al. (2021). DLG4-related synaptopathy: A new rare brain disorder. Genetics in Medicine 23, 888–899. doi: 10.1038/s41436-020-01075-9

» García-Hernández et al. (2021). Pathogenic convergence of CNVs in genes functionally associated to a severe neuromotor developmental delay syndrome. Human Genomics 15, 11. doi: 10.1186/s40246-021-00309-4

Participation in projects

» COMPUDRUG (2018-2021): Development of a new and efficient drug design system based on computational dynamic calculation of macromolecular structures. AEI, RTC-2017-6494-1.

» CARE (2020-2022): Combating Antibiotic-Resistant Enterobacteriaceae; structure-based discovery of clinical trial-ready inhibitors. ISCIII, DTS20-00024.

» DRUGCOHESIN (2019-2022): New drugs for chromosome biology. Design of specific inhibitors of the Cohesin complex". AEI, RTI2018-094434-B-I00

» AEPIC (2020-2023): Alliance for the Exploration of Pipelines for Inhibitors of Carbapenemases. Joint Programming Initiative on Antimicrobial Resistance - EU-JPIAMR Network Plus (http://www.jpiamr.eu/aepic)

» DRUGCARBAPENEM (2022-2025): “New drugs to inhibit Carbapenemases. Discovery of compounds by structural design". AEI, PID2021-126625OB-I00.

» Dr. Gomez-Puertas, associated researcher to the Institute for Health Research of La Paz University Hospital (IdiPAZ) since 2012.

» Molecular Modeling Group, member of the "Conexión Cáncer" - CSIC since 2022.

ULTRAHIGH-THROUGHPUT DISCOVERY AND ENGINEERING OF ENZYMES FOR BIOTECHNOLOGICAL APPLICATIONS

Group Members

Principal Investigators
Aurelio Hidalgo

SCIENTIFIC STAFF:

Postdoctoral fellows
Sandra Bosch (from 5/2021)
Davide Cecchini
María Gimeno (from 1/2021)
Diana Maté (until 12/2021)
Mercedes Sánchez (until 2/2021)

Predoctoral fellows
Laura Blas (from 10/2021)
Sandra Bosch (until 4/2021)
Jorge Bravo (until 5/2021)
John Martínez (from 10/2021)

Technicians
Carmen Ortega
Jorge Martínez (from 2/2022, w/ José Almendral & Alberto López-Bueno)

Undergraduate and Master Students
Laura Blas (until 9/2021)
Andrés Hernando (until 6/2021)
Isabel Martín (until 6/2021)

www.cbm.uam.es/ahidalgo

Summary

Microbial diversity is a vast reservoir of genetic information that can be valorized through industrial application, from biosynthetic gene clusters to novel enzyme catalysts. The synergy between new experimental discovery tools based on biology and those based on nanotechnologies are instrumental to find relevant genes faster and more efficiently, particularly enabling academic labs to undertake screening campaigns until now costly and limited to large enterprises.

In the HT Discovery lab, we develop methods to discover and engineer industrially relevant enzymes and biosynthetic gene clusters 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.

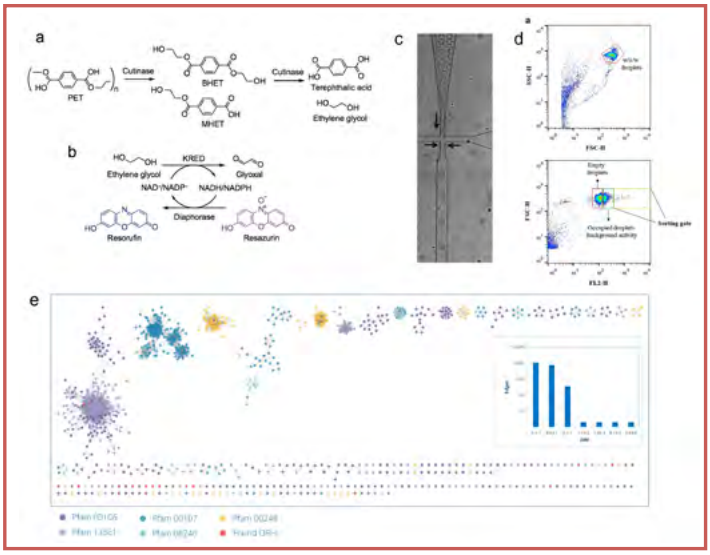
However, the complexity of cellular metabolism limits the applicability of biological selections. For this reason, we also work on screening methods, which involve individual enzymatic assays in vitro of each enzyme variant generated. To shorten this long, tedious and expensive process, droplet microfluidics enables the miniaturization of assays with throughput of kHz rates as well as a 1000x reduction of volume and assay costs. Moreover, microfluidics enables the conversion of general lab operations (additions, aliquoting, detection of a given property) into a particular chip design. Using ultrahigh-throughput screening, we have developed screening methods for esterases, KREDs, lyases and other enzymes in the EU-funded projects MetaFluidics, RadicalZ and CC-TOP. The enzymes discovered using these methods are not only located in unexplored regions of sequence space, but sometimes exhibited much coveted properties for subsequent engineering, such as catalytic promiscuity.

Research Networks

» Red Nacional de Microorganismos Extremófilos.

Other activities

» Participation in the European Night of Researchers, Madrid 2022.



From enzyme discovery to insight. A and B: Functional assays are developed to screen the metagenomic diversity for industrially relevant enzymes, e.g. for PET degradation. These assays are often coupled to a fluorescence readout for ultrahigh-throughput screening. C: metagenomic clones are encapsulated in water-in-oil-in-water picoliter droplets and incubated. D: picodroplets are sorted by FACS. E: hit sequences are analyzed and often found to be very different from members of known families, as evidenced from sequence similarity networks.

List of publications

Artículos:

» Gimeno-Pérez, M., Finnigan, J., Echeverria, C., Charnock, S. J., Hidalgo, A. & Mate, D. M. (2022). A coupled ketoreductase-diaphorase assay for the detection of polyethylene terephthalate-hydrolyzing activity. ChemSusChem e202102750. DOI:10.1002/cssc.202102750

» Hageskal, G., Heggeset, T. M. B., Nguyen, G.-S., Haugen, T., Jønsson, M., Egas, C., Hidalgo, A., Wentzel, A. & Lewin, A. S. (2022). Flow-based method for biofilm microbiota enrichment and exploration of metagenomes. AMB Express 12, 36. DOI: 10.1186/s13568-022-01377-y

» Miguel-Ruano, V., Rivera, I., Rajkovic, J., Knapik, K., Torrado, A., Otero, J. M., Beneventi, E., Becerra, M., Sánchez-Costa, M., Hidalgo, A., Berenguer, J., González-Siso, M.-I., Cruces, J., Rúa, M. L. & Hermoso, J. A. (2021). Biochemical and Structural Characterization of a novel thermophilic esterase EstD11 provide catalytic insights for the HSL family. Comput. Struct. Biotechnology J 19, 1214–1232. DOI: 10.1016/j.csbj.2021.01.047

» Bosch, S., Sanchez-Freire, E., Pozo, M. L. del, Česnik, M., Quesada, J., Mate, D. M., Hernández, K., Qi, Y., Clapés, P., Vasić-Rački, Đ., Blažević, Z. F., Berenguer, J. & Hidalgo, A. (2021). Thermostability Engineering of a Class II Pyruvate Aldolase from *Escherichia coli* by in Vivo Folding Interference. ACS Sustain. Chem. Eng. 9, 5430–5436. DOI: 10.1021/acssuschemeng.1c00699

» Wessel, J., Petrillo, G., Estevez-Gay, M., Bosch, S., Seeger, M., Dijkman, W. P., Iglesias-Fernández, J., Hidalgo, A., Uson, I., Osuna, S. & Schallmey, A. (2021). Insights into the molecular determinants of thermal stability in halohydrin dehalogenase HheD2. FEBS J. 288, 4683–4701. DOI: 10.1111/febs.15777

Capítulos de libros:

» Cecchini, D., Sanchez-Costa, M., Herrera-Orrego, A., Fernández-Lucas, J., & Hidalgo, A. In Magnani, F. Mirabelli, C. & Paradisi, F. (eds). Methods in Molecular Biology 2397 (2022). Springer, pp 19–32. DOI: 10.1007/978-1-0716-1826-4_2

Doctoral theses

» Sandra Bosch Reñé (2021). Métodos de evolución dirigida y diseño racional para la termoestabilización de una aldolasa relevante a nivel industrial. Universidad Autónoma de Madrid. Aurelio Hidalgo. Mención Internacional.

» Jorge Bravo Villanueva (2022). Desarrollo de un sistema basado en microgotas, independiente de vectores, y de alto rendimiento para el cribado funcional in vitro de metagenotecas. Universidad Autónoma de Madrid. Aurelio Hidalgo.

Patents

» Licensing agreement for 7 ketoreductases to Prozomix, Ltd. 11/03/2021.

Participation in projects

» Innovative tools for sustainable exploration of marine microbiome innovative tools for sustainable exploration of marine microbiomes: towards a circular blue bioeconomy and healthier marine environments (HE-CL6, GA 101081957, BlueTools). European Commission. 01/12/2022-30/11/2026. Role: coordinator

» Rapid discovery and development of enzymes for novel and greener consumer products (H2020-SC2, GA 10100560 RadicalZ). European Commission. 01/06/2021- 31/05/2025. Role: coordinator

» C-C Bond Formation Using Top Performing Enzymes (MSCA-ITN, GA 956631 CC-TOP). European Commission. 01/02/2021- 31/01/2025.

» Búsqueda y mejora de 2-desoxirribosil transferasas mediante métodos de ultra-alto rendimiento para la síntesis sostenible de nuevos análogos de nucleósido terapéuticos. Ministerio de Ciencia e Innovación. (UltraNDTs, Project. PID2020-117025RB-I00). 01/09/2021- 31/08/2024.

» New strategies to study virus-host interactions from humans to the ecosystem: a "one-health perspective" (VIRHOS). Generalitat Valenciana (01/01/22 - 31/12/25).

» Plataformas y modelos preclínicos para el abordaje multidisciplinar en COVID-19 y en respuesta a futuras pandemias (COVTRAVI 19-CM). Comunidad de Madrid (01/01/22 - 31/12/22)

CONJUGATION IN GRAM-POSITIVE BACTERIA

Group Members

Principal Investigator:
Wilfried J.J. Meijer

Postdoctoral fellow:
Andrés Miguel Arribas
(since 01 January 2022)

Predoctoral fellows:
Fernando Freire Gómez
(since 01 September 2021)
Daniel González Alvarez
(since 01 June 2022)
César Gago Córdoba
(until 07 April 2022)
Jorge Val Calvo
(until 25 February 2021)

Undergraduate and Master students
Roos Ligtvoet (February –September 2022)
Ana Martín María
(until July 2021)
Minerva Bravo Velasco
(until September 2021)

Visiting scientists
Nuria Quiles Puchalt
(Feb – June 2021)
Alfonso Felipe Ruíz
(June –July 2021)
Javier Mancheño Bonillo
(June - July 2021)

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

Summary

Bacterial conjugation is the process by which a conjugative DNA element is transferred from a donor to a recipient cell via a connecting channel. Conjugative elements are often located on plasmids, named conjugative plasmids. Besides containing all genes necessary for conjugation, conjugative elements often contain antibiotic resistance genes, and conjugation is the main horizontal gene transfer route responsible for the spread of antibiotic resistance (AR) genes, which is a serious problem worldwide. Very little is known about conjugation in Gram-positive (G+) bacteria. Despite of its notorious role in AR, there is also a positive side: conjugative plasmids can be used to generate tools to genetically modify bacteria that are relevant in industrial, clinical and scientific settings that are reluctant to modification by other methods.

The two main objectives in our lab are developing tools to modify G+ bacteria and studying different stages of the conjugation process. The final goal is to use this information to generate drugs or strategies to impede conjugation [long-term strategy]. Most of our studies are based on multidisciplinary research involving collaborations with other groups that contribute with specific expertise. Bioinformatics plays a crucial role in all our research lines. Recently, we have found that pLS20 is the prototype of a family of related plasmids that can be divided into four clades. This finding is important because it permits us to identify conserved genes/features and protein domains/motifs. Another goal of our investigations is to provide the scientific community with tools allowing genetic modification of relevant G+ bacteria. We have developed a system, based on the pLS20 conjugation machinery, allowing the exchange of chromosomal regions of >100 kb between strains. Another goal is to gain insights into the different stages of the conjugation process that may lead to ways to impede conjugation-mediated spread of AR. Results obtained in the last years have shown that the scientific significance of our research is much wider than these goals, which is illustrated by the following two examples. First, by studying the regulatory circuitry controlling expression of the conjugation genes we have identified a novel two-component processive antitermination system that is present on almost all conjugation operons in G+ bacteria. Second, by studying how donor cells contact recipient cells we discovered that they achieve this by a special adhesin allowing covalent attachment between the two cells.

Participation in projects

» Study of proteins involved in different aspects of the conjugation process of the *Bacillus subtilis* plasmid pLS20. PID2019-108778GB-C21P. Acronym: ConJuFun. Spanish Ministry of Science and Innovation. 2020-2023. PI Wilfried J.J. Meijer.

List of publications

» Erkelens, A.M., Qin, L., van Erp, B., Miguel-Arribas, A., Abia, D., Keek, H.G.J., Markus, D., Cajili, M.K.M., Schwab, S., Meijer, W.J.J. and Dame, R.T. (2022) The *B. subtilis* Rok protein is an atypical H-NS-like protein irresponsive to physico-chemical cues. Nucleic Acids Res. 50(21), 12166-12185. doi: 10.1093/nar/gkac1064.

» Amatsu, R., Mori, K., Ishikawa, S., Meijer, W.J.J. and Yoshida K-I. (2022) A new tool for the flexible genetic manipulation of *Geobacillus kaustophilus*. Bio Protoc. 12(17), e4502. doi: 10.21769/BioProtoc.4502.

» Miguel-Arribas, A., Wu, L.J., Michaelis, C., Yoshida, K-I., Grohmann, E., and Meijer, W.J.J. (2022) Conjugation operons in Gram-positive bacteria with and without antitermination systems. Microorganisms. 10(3), 587. doi: 10.3390/microorganisms 10030587.

» Mori, K., Fukui, K., Amatsu, R., Ishikawa, S., Verrone, V., Wipat, A., Meijer, W.J.J. and Yoshida, K-I. (2022) A novel method for transforming *Geobacillus kaustophilus* with a chromosomal segment of *Bacillus subtilis* transferred via pLS20-dependent conjugation. Microb Cell Fact. 21(1), 34. doi: 10.1186/s12934-022-01759-8.

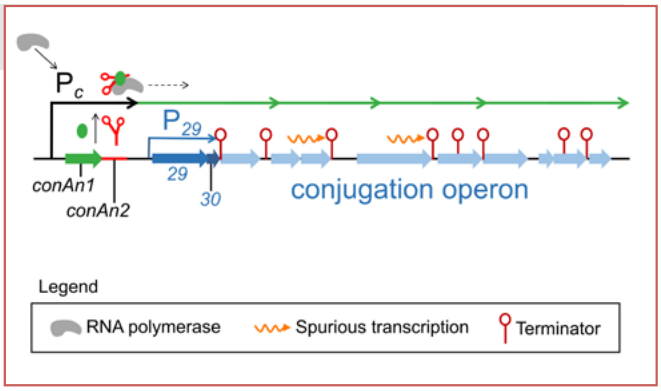
» Crespo, I., Bernardo, N., Cuppari, A., Calisto, B.M., Val-Calvo, J., Miguel-Arribas, A., Meijer, W.J.J., Carpena, X., Gil-Ortiz, F., Malfois, M. and Boer, D.R. (2022) Structural and biochemical characterization of the relaxosome auxiliary proteins encoded on the *Bacillus subtilis* plasmid pLS20. Comput Struct Biotechnol J. 20, 757-765. doi: 10.1016/j.csbj.2021.12.041.

» Val-Calvo, J., Miguel-Arribas, A., Freire, F., Abia, D., Wu, L.J. and Meijer W.J.J. (2021). Establishment genes present on pLS20 family of conjugative plasmids are regulated in two different ways. Microorganisms. 9(12), 2465. doi: 10.3390/microorganisms 9122465.

» Val-Calvo, J., Miguel-Arribas, A., Abia, D., Wu, L.J. and Meijer W.J.J. (2021) pLS20 is the archetype of a new family of conjugative plasmids harboured by Bacillus species. NAR Genom Bioinform. 3(4), lqab096. doi: 10.1093/nargab/lqab096.

» Mori, K., Verrone, V., Amatsu, R., Fukui, K., Meijer, W.J.J., Ishikawa, S., Wipat, A. and Yoshida K-I. (2021) Assessment of *Bacillus subtilis* plasmid pLS20 conjugation in the absence of quorum sensing repression. Microorganisms. 9(9), 1931. doi: 10.3390/microorganisms9091931.

» Brady, A., Quiles-Puchalt, N., Gallego Del Sol, F., Zamora-Caballero, S., Felipe-Ruíz, A., Val-Calvo, J., Meijer, W.J.J., Marina, A. and Penadés, J.R. (2021) The arbitrium system controls prophage induction. Curr Biol. 31(22), 5037-5045. e3. doi: 10.1016/j.cub.2021.08.072.



Schematic view of the two-component antitermination system that is present near the start of the pLS20 conjugation operon, as well as on most other conjugative operons present in Gram-positive bacteria.

» Ferreira, W.T., Hong, H.A., Hess, M., Adams, J.R.G., Wood, H., Bakun, K., Tan, S., Baccigalupi, L., Ferrari, E., Brisson, A., Ricca, E., Teresa Rejas, M., Meijer, W.J.J., Soloviev, M. and Cutting, S.M. (2021) Micellar antibiotics of Bacillus. Pharmaceutics. 13(8), 1296. doi: 10.3390/pharmaceutics13081296.

» Miguel-Arribas, A., Val-Calvo, J., Gago-Córdoba, C., Izquierdo, J.M., Abia, D., Wu, L.J., Errington, J. and Meijer, W.J.J. (2021) A novel bipartite antitermination system widespread in conjugative elements of Gram-positive bacteria. Nucleic Acids Res. 49(10), 5553-5567. doi: 10.1093/nar/gkab360.

» Meijer, W.J.J, Boer, D.R., Ares, S., Alfonso, C., Rojo, F., Luque-Ortega, J.R. and Wu, L.J. (2021) Multiple layered control of the conjugation process of the *Bacillus subtilis* plasmid pLS20. Front Mol Biosci. 8, 648468. doi: 10.3389/fmolb.2021.648468.

» Gago-Córdoba, C., Val-Calvo, J., Abia, D., Díaz-Talavera, A., Miguel-Arribas, A., Aguilar Suárez, R., van Dijl, J.M., Wu, L.J. and Meijer, WJJ. (2021) A conserved class II type thioester domain-containing adhesin is required for efficient conjugation in *Bacillus subtilis*. mBio. 12(2), e00104-21. doi: 10.1128/mBio.00104-21.

Doctoral theses

» César Gago Córdoba (2022). The initial steps of the pLS20 conjugation process: insights into the mechanism of recipient cell selection and attachment by donor cells. Universidad Autónoma Madrid. Director Wilfried J.J. Meijer. International Mention

» Jorge Val Calvo (2021). The pLS20 family of plasmids and regulation of their establishment genes. Universidad Autónoma Madrid. Director Wilfried J.J. Meijer. International Mention. Received in addition the extraordinary prize by the autonomous university of Madrid of honourable mention.

HUMAN IMMUNODEFICIENCY VIRUS REPLICATION AND ANTIRETROVIRAL THERAPY

Group Members

Principal Investigator:
Luis Menéndez Arias

Postdoctoral fellows:
Estrella Frutos Beltrán

Predoctoral fellows:
Samara Martín Alonso
Javier Martínez del Río

Technicians
Nerea López Carrobles
(since May 2021)

Undergraduate and Master students:
Adrián Sánchez Ibáñez
(until January 2021)
David Borrego García
(until May 2021)
Miruna A. Martin
(November 2021 – July 2022)
Matilda Devlin (since September 2022)
Marta Cogoni
(October – December 2022)

Visiting scientists:
Moisés A. Árquez Mendoza
(August – December 2021)

www.cbm.csic.es\retrovir

Summary

Infections caused by human immunodeficiency viruses (HIV) constitute a major burden to human health worldwide. Despite significant advances in antiretroviral therapy, HIV still causes around 650,000 deaths each year. The HIV genome is composed of two copies of single-stranded RNA. The viral reverse transcriptase (RT) is responsible for the replication of the HIV genome. RT inhibitors constitute the backbone of current 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. We have engineered HIV-1 RT variants with increased thermal stability and DNA polymerization accuracy, currently marketed as biotechnological tools. Present and future research in our lab focuses on obtaining novel RTs with increased accuracy of DNA synthesis and high nucleic acid binding affinity, suitable for RNA amplification from single cells.

Nucleoside analog RT inhibitors (NRTIs) play a central role in antiretroviral therapy. However, HIV type 1 (HIV-1) and HIV type 2 (HIV-2) differ in their mutational pathways of NRTI resistance. Unlike in HIV-1 RT, thymidine analogue resistance mutations (TAMs) such as M41L, D67N, K70R and S215Y do not confer resistance to AZT and other NRTIs when found in HIV-2, due to amino acid differences located in the fingers subdomain of its RT. We have recently demonstrated that the accumulation of TAMs in HIV-2 RT renders an enzyme with defective strand displacement activity.

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 important for the future clinical management of the disease. To address coming challenges in antiretroviral research, we are extending our biochemical studies to other catalytic activities of the RT (e.g. ribonuclease H). Collaborations with medicinal chemists have been established to identify potentially useful RNase H inhibitors. These drugs, specific for HIV-1 and HIV-2, could also be developed into broader spectrum inhibitors blocking infection of other viruses (e.g. hepatitis B virus, herpesviruses).

Research in our laboratory has also highlighted the role of the HIV-1 RT's RNase H activity in template switching and strand displacement during reverse transcription. We have recently shown that dual inhibitors of the DNA polymerase and RNase H activities of HIV-1 RT are particularly effective in blocking strand displacement DNA synthesis when RNA was used as template.

Doctoral theses

» Samara Martín Alonso (2022). Molecular determinants of strand displacement polymerization by HIV reverse transcriptases. Faculty of Sciences, Autonomous University of Madrid. Director: Luis Menéndez Arias. Thesis with International Mention.

List of publications

» Martín-Alonso, S., Frutos-Beltrán, E. and Menéndez-Arias, L. (2021) Reverse transcriptase: from transcriptomics to genome editing. Trends Biotechnol. 39, 194-210; doi:10.1016/j.tibtech.2020.06.008

» Ma, Y., et al. (2021). Medicinal chemistry strategies for discovering antivirals effective against drug-resistant viruses. Chem. Soc. Rev. 50, 4514-4540; doi:10.1039/d0cs01084g

» Gao, P., et al. (2021) Novel indolylarylsulfone derivatives as covalent HIV-1 reverse transcriptase inhibitors specifically targeting the drug-resistant mutant Y181C. Bioorg. Med. Chem. 30, 115927, doi:10.1016/j.bmc.2020.115927

» Fu, Z., Menéndez-Arias, L., Liu, X. and Zhan, P. (2021) Advances in natural products-based antiviral agents. In: ul-Islam, S and Banday, JA (eds.). Chemistry of biologically potent natural products and synthetic compounds (Series: Emerging trends in medicinal and pharmaceutical chemistry). Scrivener Publishing LLC, pp. 21-42.

» Álvarez, M., Sapena-Ventura, E., Luczkowiak, J., Martín-Alonso, S. and Menéndez-Arias, L. (2021) Analysis and molecular determinants of HIV RNase H cleavage specificity at the PPT/U3 junction. Viruses 13, 131; doi:10.3390/v13010131.

» Menéndez-Arias, L. (2021) Decoding molnupiravir-induced mutagenesis in SARS-CoV-2. J. Biol. Chem. 297, 100867; doi:10.1016/j.jbc.2021.100867

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» Huang, T., et al. (2021) Search, identification and design of effective antiviral drugs against pandemic human coronaviruses. Adv. Exp. Med. Biol. 1322, 219-260; doi:10.1007/978-981-16-0267-2_9

» Kang, D., et al. (2021) Discovery, optimization, and target identification of novel coumarin derivatives as HIV-1 reverse transcriptase-associated ribonuclease H inhibitors. Eur. J. Med. Chem. 225, 113769; doi:10.1016/j.ejmech.2021.113769

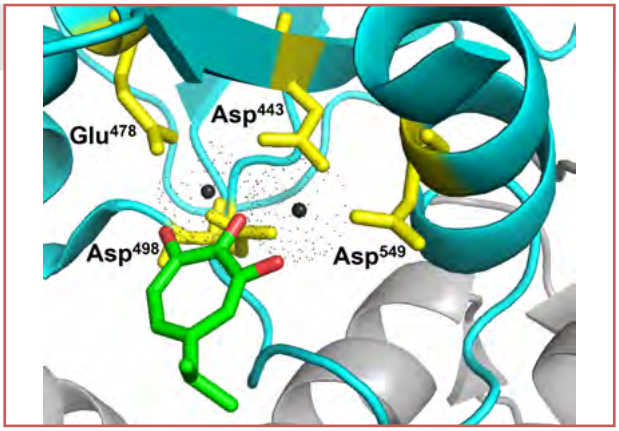
» Martín-Alonso, S., et al. (2022) Novel RNase H inhibitors blocking RNA-directed strand displacement DNA synthesis by HIV-1 reverse transcriptase. J. Mol. Biol. 434, 1677507; doi:10.1016/j.jmb.2022.167507

» Menéndez-Arias, L. and Delgado, R. (2022) Update and latest advances in antiretroviral therapy. Trends Pharmacol. Sci. 43, 16-29. doi: 10.1016/j.tips.2021.10.004.

Participation in projects

» PID2019-104176RB-I00: Novel HIV reverse transcriptases with improved biochemical properties for biotechnological applications (2020-2023), Spanish Ministry of Science and Innovation.

» COOPA20299: Resistance to reverse transcriptase inhibitors in HIV variants, circulating in Northern Colombia: mutations and mechanisms involved (2019-2021), Spanish National Research Council.



Crystal structure of HIV-1 RT bound to β-thujaplicinol at the RNase H active site (PDB file 3IG1). Tbe effects of β-thujaplicinol and other RNase H inhibitors in RNA-dependent strand displacement DNA synthesis are described in Martín-Alonso et al. (2022) J. Mol. Biol. 434, 1677507.

» Kellner, M. J., et al. (2022) A rapid, highly sensitive and open-access SARS-CoV-2 detection assay for laboratory and home testing. Front. Mol. Biosci. 9, 801309; doi:10.3389/fmolb.2022.801309

» Hadj Hassine, I., Ben M'hadheb, M. and Menéndez-Arias, L. (2022) Lethal mutagenesis of RNA viruses and approved drugs with antiviral mutagenic activity. Viruses 14, 841; doi:10.3390/v14040841

» Zhang, L., et al. (2022) Current medicinal chemistry strategies in the discovery of novel HIV-1 ribonuclease H inhibitors. Eur. J. Med. Chem. 243, 114760; doi:10.1016/j.ejmech.2022.114760

» Zhang, L., et al. (2022) Design, synthesis, and biological evaluation of novel double-winged galloyl derivatives as HIV-1 RNase H inhibitors. Eur. J. Med. Chem. 240, 114563; doi:10.1016/j.ejmech.2022.114563.

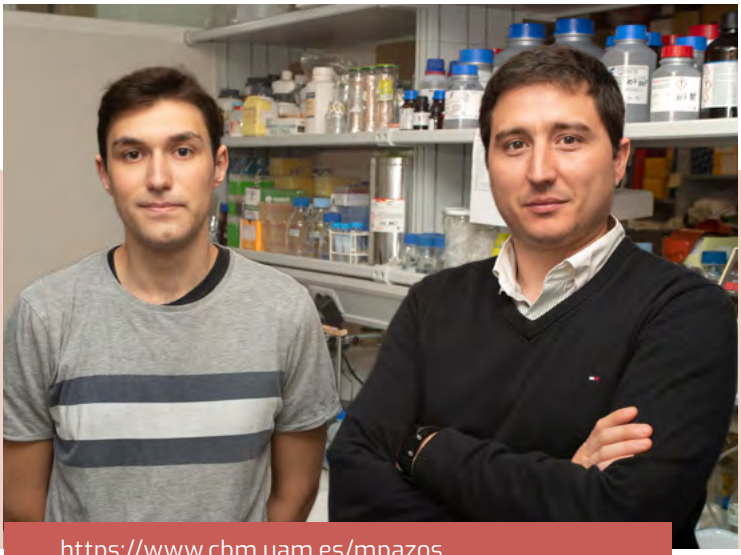
Other activities

» 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, Viruses, Virus Research and Word Journal of Translational Medicine.

» Luis Menéndez Arias has been co-editor with Drs. Hervé Galons (Paris, France), Peng Zhan (Jinan, P. R. China) and Bin Yu (Zhengzhou, P. R. China) of a special issue on 'Medicinal chemistry in seeking metalloprotein inhibitors' of the European Journal of Medicinal Chemistry (2021), and with Drs. Xinyong Liu and Peng Zhan (Jinan, P. R. China) and Vasanthanathan Poongavanam (Odense, Denmark) of a book on Antiviral Drug Discovery and Development, published by Springer (2021).

» Member of the Global Virus Network (www.gvn.org)

BACTERIAL CELL ENVELOPE DURING PRESEPTAL GROWTH



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

Group Members

Principal Investigator
Manuel Pazos

Technician
Daniel Ballesteros (from Nov. 2022)

Undergraduate
Paula de la Fuente (from Oct. 2022)

Summary

The research in the laboratory focuses on understanding the molecular mechanisms underpinning the biogenesis of the bacterial cell envelope of model and pathogenic gastrointestinal organisms (e.g. *Escherichia coli*) during preseptal growth, in the context of physiology and pathogenicity.

The peptidoglycan (PG) sacculus is an essential structural component of most bacterial cell envelopes, and its synthesis is one of the most frequently targeted process by antibiotics. Since the rise of antimicrobial resistance is making infections harder to treat, with special emphasis to those caused by Gram-negative bacteria due to their extra outer membrane barrier, it is crucial to understand how the cell envelope integrity is maintained during the bacterial cell cycle. The cell envelope integrity is essential for the physiology and pathogenicity of bacteria, and therefore both peptidoglycan and membrane synthesis machineries are coordinated and regulated to ensure the robust growth of the cell envelope.

Contrary to cell elongation and cell division, which have been studied in greater depth, the transition between both stages called preseptal growth remains poorly characterized. This switch involves the incorporation of new PG material at mid-cell before the septum is formed. In previous work we described the connection between the cytosolic FtsZ polymers and the PG synthases (PBP1A and PBP1B) through the partially redundant proteins ZipA and FtsA-FtsN. The disruption of both connections disables the incorporation of new preseptal PG, leading to non-viable filamented cells, identifying the preseptal PG growth as an essential process occurring prior cell division (Pazos et al. 2018, Nat Commun 9:5090). These results would answer the long-standing question about the mechanism by which single-point mutations in FtsA are able to bypass the requirement of ZipA for cell division and preseptal PG synthesis.

In addition, we described for the first time how the PG-binding SPOR proteins DamX and DedD are required for full functionality of the PG synthases PBP1A and PBP1B, and the lethal effect of an unbalanced DedD:DamX protein ratio (Pazos *et al.* 2020, mBio 11:e02796-20). SPOR domains are widely spread and conserved in bacterial proteins, making them promising target to interfere with bacterial cell envelope synthesis.

To accomplish our ultimate goal of identifying new potential antimicrobial targets, we aim to use a multidisciplinary approach combining genetics, biochemistry, cell biology and different microscopy techniques to characterize the molecular components and cell envelope features during this potentially vulnerable stage of the bacterial cell cycle.

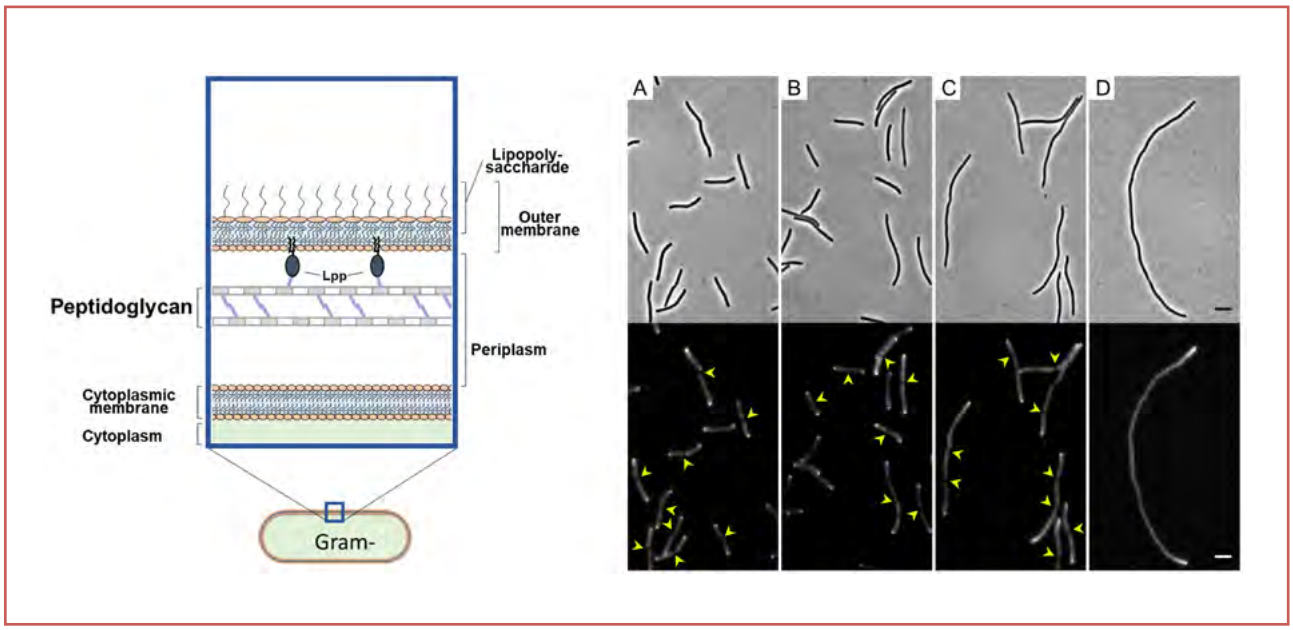


Figure. Schematic structure of the bacterial cell envelope in Gram-negative bacteria (left). Phase contrast and fluorescence microscopy images of Escherichia coli conditional mutants pulse labelled with fluorescent d-amino acid. The non-fluorescently labelled preseptal PG synthesis bands are indicated by yellow arrows (right). Adapted from: Pazos and Peters 2019. Subcell Biochem 92:127–168, Pazos et al. 2018. Nat Commun 9:5090.and fluorescence microscopy images of Escherichia coli conditional mutants pulse labelled with fluorescent d-amino acid. The non-fluorescently labelled preseptal PG synthesis bands are indicated by yellow arrows (right). Adapted from: Pazos and Peters 2019. Subcell Biochem 92:127–168, Pazos et al. 2018. Nat Commun 9:5090.

List of publications

Articles with CBM-UAM affiliation:

» Mamou, G., Corona, F., Cohen-Khait, R., Housden, N.G., Yeung, V., Sun, D., Sridhar, P., Pazos, M., Knowles, T.J., Kleanthous, C. and Vollmer, W., (2022) Peptidoglycan maturation controls outer membrane protein assembly. Nature. 606, 953-959. doi: 10.1038/s41586-022-04834-7.

Articles without CBM-UAM affiliation:

» Wacnik, K., Rao, V.A., Chen, X., Lafage, L., Pazos, M., Booth, S., Vollmer, W., Hobbs, J.K., Lewis, R.J. and Foster S.J. (2022) PBP1 of *Staphylococcus aureus* has multiple essential functions in cell division. mBio. 13, e0066922. doi: 10.1128/mbio.00669-22.

» Sassine, J., Pazos, M., Breukink, E. and Vollmer, W. (2021) Lytic transglycosylase MltG cleaves in nascent peptidoglycan and produces short glycan strands. Cell Surf. 7, 100053. doi: 10.1016/j.tcsw.2021.100053.

» Pazos, M. and Vollmer, W. (2021) Regulation and function of class A Penicillin-binding proteins. Curr. Opin. Microbiol. 60, 80-87.doi: 10.1016/j.mib.2021.01.008.

Participation in projects

» 2022-2026: 2020-T1/BMD-19970. Programa de Atracción de Talento (CAM-UAM). Principal Investigator: Manuel Pazos.

REGULATION BY RNA IN THE STRESS AND VIRULENCE

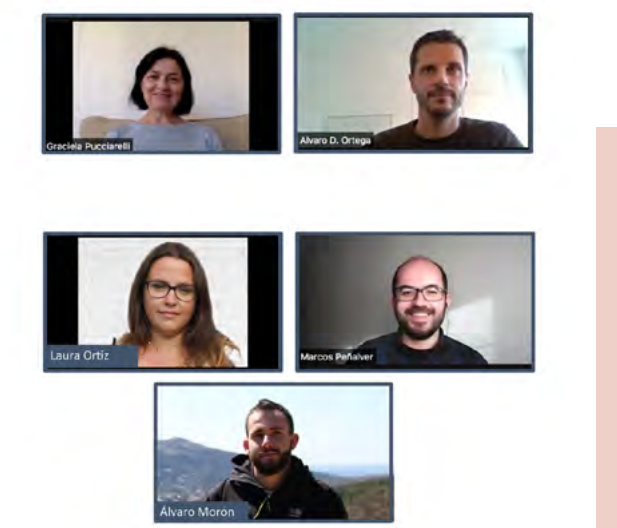
Group Members

Principal Investigator (PI):
M. Graciela Pucciarelli Morrone

Co-principal investigador (co-IP):
Álvaro Darío Ortega Moreno

Predoctoral fellows:
Marcos Peñalver Medina
Álvaro Morón García (June 2021 / June 2022)

Technician:
Laura Ortíz Miravalles (from March 2021)



https://www.cbm.uam.es/mg.pucciarelli

Summary

Listeria monocytogenes is a foodborne bacterial pathogen that causes listeriosis, a severe disease mostly affecting pregnant women, elderly, and immunocompromised individuals as well as livestock. *L. monocytogenes* exhibits an outstanding capacity to tolerate widely used practices in the food industry that control microbial growth in food, including refrigeration. Our main objective is to understand the regulatory mechanisms and the adaptive strategies that allow *L. monocytogenes* to grow at refrigeration temperatures (0-4°C).

During these last two years, our experimental approach has relied on systems-level approaches to identify transcriptional and post-transcriptional regulatory networks at 4°C. We performed transcriptomics analyses along the acclimation from 37°C to 4°C, which showed the participation of transcriptional regulators and small non-coding regulatory RNAs (sRNAs) in two defined phases catalogued as early and late responses (Fig. 1A B). We are currently characterizing the precise functional role of these regulators in cold adaptation and, in addition, focusing on cell wall proteome changes occurring specifically at 4°C. The available data indicate that at least two surface proteins are produced only in cold. The next studies are designed to investigate the contribution of these proteins to the adaptive response and to characterize the mechanisms that control their expression.

We expect that the understanding of the regulatory mechanisms governing the *L. monocytogenes* capacity to tolerate cold temperature will provide the field with novel targets useful to prevent its growth in refrigerated food. These new antimicrobial preventive practices during food processing and storage will ultimately reduce the risk of *L. monocytogenes* infections in both humans and livestock.

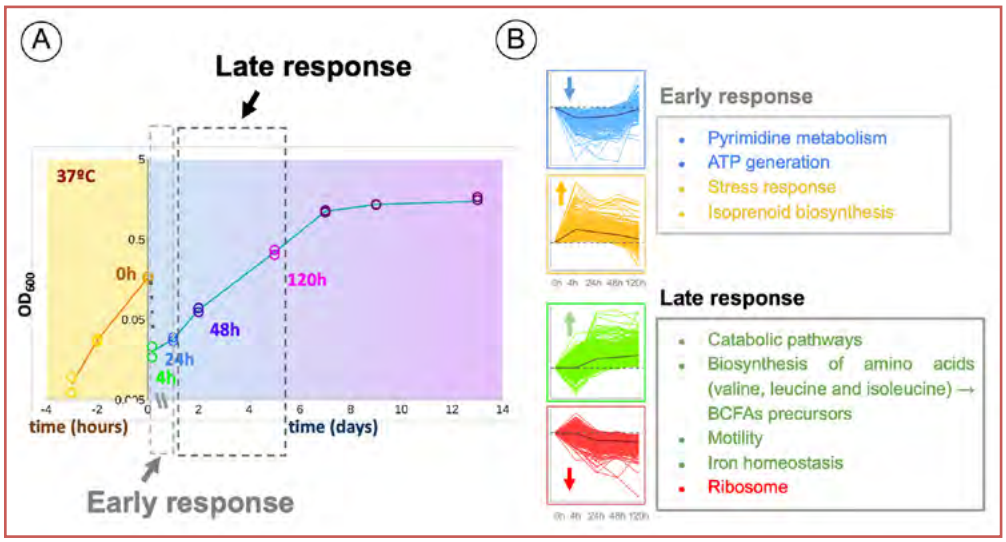


Figure 1: (A) Growth of Listeria monocytogenes during the downshift from 37°C to 4°C in which early and late responses are differentiated; (B) Physiological changes found by genome-wide transcriptional analysis along the adaptation of the pathogen to 4°C.

List of publications

» Dessaux, C., Pucciarelli, M.G., Guerreiro, D.N., O'Byrne, C.P., García-Del Portillo, F. (2021) Activation of the *Listeria monocytogenes* Stressosome in the Intracellular Eukaryotic Environment. Appl. Environ. Microbiol. 87(12): e0039721. doi: 10.1128/AEM.00397-21.

» Cestero, J.J., Castanheira, S., Pucciarelli, M.G., García-Del Portillo, F. (2021) A Novel *Salmonella* Periplasmic Protein Controlling Cell Wall Homeostasis and Virulence. Front. Microbiol. 12:633701. doi: 10.3389/fmicb.2021.633701.

» Quereda, J.J., Morón-García, A., Palacios-Gorba, C., Dessaux, C., García-Del Portillo, F., Pucciarelli, M.G., Ortega, A.D. (2021) Pathogenicity and virulence of *Listeria monocytogenes*: A trip from environmental to medical microbiology. Virulence 12(1):2509-2545. doi: 10.1080/21505594.2021.1975526.

» Huesa, J., Giner-Lamia, J., Pucciarelli, M.G., Paredes-Martínez, F., García-del Portillo, F., Marina, A., Casino, P. (2021) Structure-based analyses of *Salmonella* RcsB variants unravel new features of the Rcs regulon. Nucleic Acids Res. 49(4):2357-2374. doi: 10.1093/nar/gkab060.

» Guerreiro, D.N., Pucciarelli, M.G., Tiensuu, T., Gudynaite, D., Boyd, A., Johansson, J., García-Del Portillo, F., O'Byrne, C.P. (2022) Acid stress signals are integrated into the σB-dependent general stress response pathway via the stressosome in the food-borne pathogen *Listeria monocytogenes*. PLoS Pathog. 18(3): e1010213. doi: 10.1371/journal.ppat.1010213.

» Hernández, S.B., Castanheira, S., Pucciarelli, M.G., Cestero, J.J., Rico-Pérez, G., Paradela, A., Ayala, J.A., Velázquez, S., San-Félix, A., Cava, F., García-Del Portillo, F. (2022) Peptidoglycan editing in non-proliferating intracellular *Salmonella* as source of interference with immune signaling. PLoS Pathog. 18(1): e1010241. doi: 10.1371/journal.ppat.1010241.

Participation in projects

» Title: Training network to understand and exploit mechanisms of sensory perception in bacteria (PATHSENSE). Starting / end dates: 01-04-2017 / 31-03-2021. Ref.: H2020-MSCA-ETN-721456 Marie-Sklodowska-Curie. Innovative Training Network (ITN) Actions. European Commision. Coordinator: Conor O'Byrne (Galway University, Ireland). Role: Associated partner UAM.

» Title: Regulation by RNA in the adaptation of Listeria to cold. Starting / end dates: 01-01-2019 to 30-06-2022. Ref: PGC2018-096364-B-I00. Spanish Ministry of Science and Innovation. IP: M. Graciela Pucciarelli. Co-IP: Álvaro Dario Ortega.

Doctoral theses

» Emilija Ivanova Stojcheva (2021). Title: Antibacterial properties of plant extracts and isolated phytocompounds against *Listeria monocytogenes* and their effects on the activation of the general stress response. Universidad Autonoma de Madrid (UAM). Directors: José Carlos Quintela & M. Graciela Pucciarelli.

VIRUS-CELL INTERACTION AND VACCINES DEVELOPMENT: THE ASFV MODEL

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

Group Members

Principal Investigator:
Yolanda Revilla Novella

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Elena Riera Laguna
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Technician:
Cecilia Flores

Undergraduate and Master Students
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Visiting scientists:
Roberta Piredda

Summary

Viruses have adapted multiple actions to disrupt host defense pathways and hijack host mechanisms for their advantage. African swine fever virus (ASFV) is one of the most complex DNA viruses due to its large genome, encoding between 150 and 170 genes, which are involved in multiple functions, such as evasion of the host immune response. ASFV is the causative agent of African swine fever (ASF), one of the most lethal diseases affecting domestic pigs and wild boar, which is seriously endangering the global swine industry due to its quick and uncontrolled spread.

Interferons (IFN) are cytokines playing a key role against infections, and the early induction of IFN-I, is a central event of the innate immune defense against viruses. Specifically, type I IFNs are involved in induction of antimicrobial state both in infected and neighboring cells, activating the innate immune response and leading to the adaptive immune response. Our recent work has established the different ability to control the host IFN-β production as a key element for virulence. Differences in virulence among different ASFV strains have been related to their ability to counteract the innate immune response, specifically, in the control of IFN-I. We have described that virulent ASFV inhibit both the cGAS/STING and the IFN-I-induced JAK/STAT pathways, thus controlling the production of IFN-I and its dependent genes.

Inactivated and subunit ASFV-vaccines have failed to confer effective protection to vaccinated animals, as it seems like that viral replication is needed. The generation of live attenuated vaccines (LAVs) is currently the most promising strategy, through the attenuation of ASFV strains by genetic manipulation. For the generation of effective and safe LAVs, it is necessary to identify the molecular determinants of ASFV virulence.

Our group has generated several LAVs prototypes from the Arm/07/CBM/c2 genotype II strain, by deleting genes previously shown to have a role in virulence. As an example of that, we have specifically deleted the EP402R (CD2v) and A238L genes by an “in house” CRISPR/Cas9 adapted-technology in COS-1 cells, without detectable further genetic changes. The successful immunization of pigs has proven this vaccine to be safe and fully protective against the circulating Korean Paju genotype II strain, opening the possibility of a new vaccine on the market in the near future.

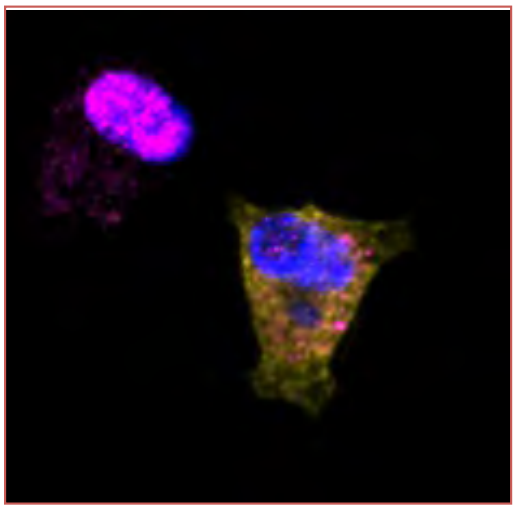

Figure legend: pSTAT2 subcellular location in IFN-I stimulated mock-infected (left) or infected (right) porcine alveolar macrophages cells (PAM).

List of publications

- Recombinant African Swine Fever Virus Arm/07/CBM/c2 Lacking CD2v and A238L Is Attenuated and Protects Pigs against Virulent Korean Paju Strain. Pérez-Núñez D, Sunwoo SY, García-Belmonte R, Kim C, Vigara-Astillero G, Riera E, Kim DM, Jeong J, Tark D, Ko YS, You YK, Revilla Y. Vaccines (Basel). 2022 Nov 23;10(12):1992. doi: 10.3390/vaccines10121992.
- Non-Invasive Sampling in the Aspect of African Swine Fever Detection-A Risk to Accurate Diagnosis. Walczak M, Szczotka-Bochniarz A, Żmudzki J, Juszkiewicz M, Szymankiewicz K, Niemczuk K, Pérez-Núñez D, Liu L, Revilla Y. Viruses. 2022 Aug 11;14(8):1756. doi: 10.3390/v14081756.
- African Swine Fever Virus induces STAT1 and STAT2 degradation to counteract IFN-I signaling. Elena Riera, Daniel Pérez-Núñez, Raquel García Belmonte, Lisa Miorin, Adolfo García Sastre and Yolanda Revilla. Frontiers in Microbiology, August 2021. DOI 103389/fmicrobiol, 2021.

Patents

File No. to be used for priority declarations EP21382940

Date of receipt 19 October 2021

Coinventors. Yolanda Revilla, Daniel Pérez Núñez

Títtle. RECOMBINANT AFRICAN SWINE FEVER VIRUS AND USES THEREOF

Submission number 300427317. Application number EP21382940.1

Priority Country: Spain. Owners: CSIC/CARESIDE. License, CARESIDE

Participation in projects

- ERA-NET. ICRAD European Commission. PI Yolanda Revilla Novella, COORDINATOR

  "Characterization of virus and host-specific modulation of type I IFN in African Swine Fever Virus virulence or attenuation. Acronym (IFNASF)"

  Start: 15/03/2021 -15/03/2024 (3 years)

- PLAN NACIONAL PID2020c-117300RB-100. PI: Yolanda Revilla. Co PI Daniel Pérez-Núñez. 2021-2024. Duración (años): 3

  "Modulación de la respuesta innata por el virus de la peste porcina africana (vppa) en relación con el desarrollo de vacunas" Acrónimo: INNATE/ASFVAC

- RESEARCH AND DEVELOPMENT CONTRACT BETWEEN AGENCIA ESTATAL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS and Laboratorios Hipra, S.A. PI Yolanda Revilla, CoPI: Daniel Pérez Núñez

  Development of new tools for ASFV vaccine. 14/10/2019-14/10/2021.

- RESEARCH AND DEVELOPMENT CONTRACT BETWEEN AGENCIA ESTATAL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS, M.P. AND CARESIDE Co. Ltd. April 15, 2020-April 15 2022. PI Yolanda Revilla. "ASFV LAVs candidates as vaccine prototypes".

- Paso Alto Biophysics and Biomedical Engineering S.L. Principal investigator: Y. Revilla and J.M. Almendral (since Nov 2022). "Use of nonionizing radiation (modulation) to enhance cancer treatment with oncolytic viruses: effects on cell viability and signaling pathways in tumor models".

Doctoral theses

- Raquel García Belmonte, diciembre 2022. El Virus de la Peste Porcina Africana modula la vía de señalización de la respuesta innata cGAS-STING: Papel del gen viral MGF505-2R. Universidad. Autónoma de Madrid

MODULATION OF ANTIVIRAL IMMUNITY BY VIRAL PROTEASES AND NONCODING RNAS

Group Members

Principal Investigator
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Scientific Staff
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Postdoctoral Fellow
Miguel Ramón Rodríguez Pulido

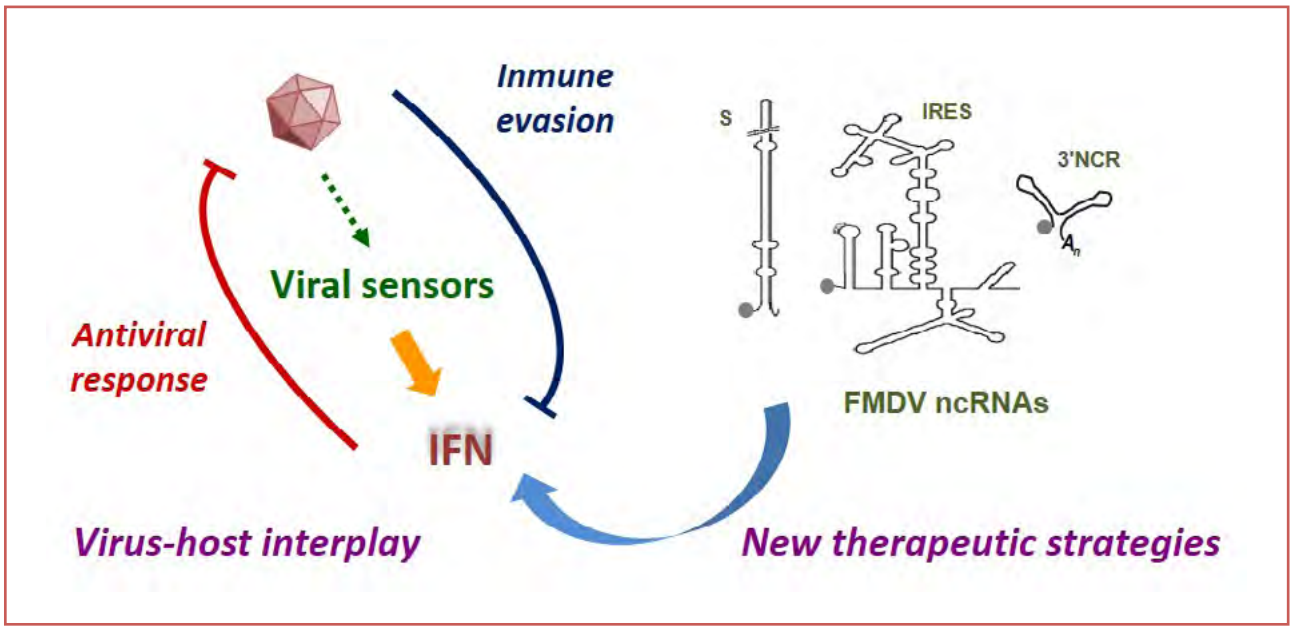
Predoctoral Fellow
Miryam Polo Hernández

Undergraduate Student
Lucía Camacho Pulido (until 30/06/21)

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

Summary

Our research interest is focused on i) the interplay between the host innate immunity system and RNA viruses. We use foot-and-mouth disease virus (FMDV), a highly infectious and worldwide distributed pathogen, as a model. Our studies include the identification of proteins involved in detection of the viral RNA genome, as well as the characterization of the immune evasion mechanisms exerted by the virus to counteract the host antiviral response based on triggering of the type-I interferon pathway in infected cells. The outcome of the balance between the antiviral response and viral antagonism may determine the onset of disease and pathogenesis and we are actively working on the identification of host innate effector proteins which are targets for the viral proteases. We have reported a novel mechanism of viral evasion based on the dual cleavage of LGP2 and MDA5, immune sensors of the RIG-I-like receptors family, at a conserved helicase motif by the FMDV Leader protease (L_{pro}). The proteolytic activity of the two virally encoded proteases (L_{pro} and $3C_{pro}$) is being tested for interference with different signaling routes known to respond to infection by RNA viruses (RIG-I-like receptors, *Toll*-like receptors, cGAS/STING, RNA interference). The impact of these interactions on viral infection and antiviral immunity is under study; ii) the biotherapeutic applications of synthetic non-coding RNAs derived from the FMDV genome (ncRNAs), known to induce a broad spectrum antiviral activity and to enhance specific B- and T-cell mediated immune responses elicited after vaccination. We have reported the inhibitory effect of the ncRNAs against infection by pathogens of different viral families including relevant zoonotic viruses (FMDV, West Nile virus, Rift Valley fever virus, African swine fever virus) and shown their biological activity in cells from different species including farm and wild animals, as well as *in vivo* in mice and swine. The use of these ncRNAs against human coronaviruses has been 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 to improve stability and efficacy in RNA 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.



Schematic representation of the interplay between host viral sensors and viral strategies to counteract the antiviral response in infected cells. The use of FMDV noncoding RNAs (ncRNAs) as IFN-I inducers eliciting the host antiviral defense is also depicted.

List of publications

» Saiz, M. and Martinez-Salas, E. (2021). Uncovering targets of the Leader protease: Linking RNA-mediated pathways and antiviral defense. WIREs RNA 12(4):e1645. doi: 10.1002/wrna.

» Forner, M., Cañas-Arranz, R., Defaus, S., de León, P., Rodríguez-Pulido, M., Ganges, L., Blanco, E., Sobrino, F., Andreu, D. (2021) Peptide-Based Vaccines: Foot-and-Mouth Disease Virus, a Paradigm in Animal Health. Vaccines (Basel) 8;9(5):477. doi: 10.3390/vaccines9050477.

» Rodriguez Pulido, M., Polo, M., Borrego, B. and Saiz, M. (2022) Use of foot-and-mouth disease virus non-coding synthetic RNAs as vaccine adjuvants. In Brun, A. (ed) Vaccine Technologies for Veterinary Viral Diseases. Methods in Molecular Biology vol. 2465, Humana, New York, NY., pp.125-135. doi: 10.1007/978-1-0716-2168-4_7.

» Albiol, F. J., Briones, C., Camacho, J., Carrión, M., Elvira, L., Eritja, R., Fernández, C., de Frutos, M., García Hernández, M., García Gomez-Tejedor, G., García, R., González, A. J., Herranz, F., Horrillo, M. C., Llosá, G., Martín, J. A., Nacher, E., de la Puerta, A., Sáiz, M., Sanz, Y., Sobrino, F. and Villa, R. (2021) New Methods for Diagnostic Tools and Prevention. In Delgado, M. and Moros, M. (ed) Challenges in Biomedicine & Health, Challenge 8. In S. Marco, J. and Moreno-Arribas, V. (coord) Scientific Challenges: towards 2030, vol. 4. Editorial CSIC. ISBN Vol. 4: 978-84-00-10744-4.

Participation in projects

» Peptide vaccines, antiviral strategies and viral immune evasion mechanisms. Biotherapeutic applications. Ministerio de Ciencia e Innovación. Ref. PID2020-113184RB-C21. PI: F. Sobrino and M. Sáiz (01/09/2021 - 31/08/2024)

» Antiviral activity of immunomodulatory non-coding RNAs and cell-targeted compounds against human coronaviruses. CSIC Ref. 202020E162. PI: M. Sáiz and F. Sobrino (11/05/2020 - 10/05/2021)

» Plataforma para el desarrollo de estrategias de control de Salud Animal (PLATESA2CM). Comunidad de Madrid/ FEDER S2018/BAA-4370 (2019-2021). Coord: N. Sevilla (INIA), PI subproject: F. Sobrino

» Global Foot-and-mouth Research Alliance (GFRA) http://www.ars.usda.gov/GFRA

Patents

» A. López- Carrascosa, F. Sobrino, E. Torres, R. Cañas, M. Sáiz, P. de León, MJ Bustos. Lauryl gallate for use as antiviral agent. EP1641.1681. (2021) PCT. CSIC

Other activities

» Associate editor of Frontiers in Molecular and Infection Microbiology-Virus and Host section (since 2018).

NEW STRATEGIES FOR PREVENTION AND CONTROL OF VIRAL DISEASES: FOOT-AND-MOUTH DISEASE VIRUS AS A MODEL

Group Members

Group Leader (IP)
Francisco Sobrino

Postdoctoral fellows
Patricia de León

Technicians
María José Bustos
Laura Cerrada

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

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 B- and T- cell epitopes as feasible field vaccines. We have also analyzed the functional role of FMDV proteins on the viral particle stability and internalization, the replication cycle and the mechanisms mediating the pathogenesis of FMDV and other related viruses causing vesicular diseases, such as swine vesicular disease virus (SVDV), vesicular stomatitis virus (VSV) and a zoonotic virus such as West Nile virus. As part of these studies, 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 potential of the modular approach based on dendrimer peptides vaccines against SARS-CoV-2. In addition, the antiviral synergy against different coronaviruses, and eventually other emerging viruses, of combinations of broad antiviral spectrum host-targeted compounds such as VPA or LG and direct acting antivirals (i.e. remdesivir) is under study.

Patents

» Patent Pending: EP21382535.9 (17-06-2021). Lauryl gallate for use as antiviral agent. Inventores: de León, A. L. Carrascosa, M.J. Bustos, F. Sobrino. E. Torres, M. Sáiz, R. Cañas.

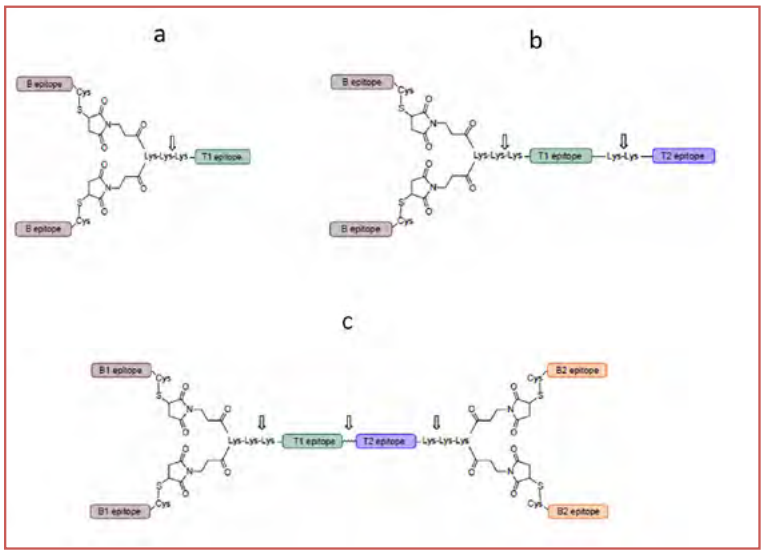


Figure 1. Dendrimer peptide vaccines: a modular approach. Scheme of the prototypes of FMDV dendrimeric peptide vaccine that conferred solid protection against homologous virus challenge in the pig. Bivalent-branched B-cell epitope immunogens (B_2T) conjugated to one (a) or two (b) T-cell epitopes in tandem via thiol-maleimide linkages at both α- and ε-amino ends of a branched Lys core. (c) Tail-to-tail fusion of two B_2T molecules via orthogonal chemical ligation (Click chemistry), leading to a B_2T-TB_2 multivalent platform. The arrows indicate a target for cathepsine D, a lysosomal protease suggested to be involved in MHC class II antigen processing.

List of publications

» de León, P., Cañas-Arranz, R., Torres, E., Forner, M. Defaus, S., Bustos, M.J., Revilla, C., Domínguez, J., Andreu, D., Blanco, E. and Sobrino, F. Phenotype of swine T-cell response evoked by B2T peptide dendrimers displaying two FMDV T-cell epitopes in tandem. Front. Immunol. 11:621537. doi: 10.3389/fimmu.2020.621537 (2021).

» Forner, M., Cañas-Arranz, R., Defaus, S., de León, P., Rodríguez-Pulido, M., Ganges, L., Blanco, E., Sobrino, F*. and Andreu, D. Peptide-based vaccines: foot-and-mouth disease virus, a paradigm in animal health. Vaccines 2021, 9, 477; 10.3390/vaccines9050477 (2021).

» Cañas-Arranz, R., de León, P., Defaus, S., Torres, E., Forner, M., Bustos, M.J., Andreu, D., Blanco, E. and Sobrino, F. Immunogenicity of foot-and-mouth disease virus dendrimer peptides: need for a T-cell epitope and ability to elicit heterotypic responses. Molecules 26(16), 4714; 10.3390/molecules26164714 (2021).

» Caridi, F., Cañas-Arranz, R., Vázquez-Calvo, A., de León, P., Calderón, K.I., Domingo, E., Sobrino, F*. and Martín-Acebes, M.A. Compensatory mutations with opposite effects on acid-stability of foot-and mouth disease virus capsid induce fitness gain and promote rapid adaptation. Sci. Rep. 11:23494; 10.1038/s41598-021-02757-3 (2021). * F. Sobrino corresponding author.

Edited books

» J. Blazquez, J. Gómez, E. Gómez-Diaz and F. Sobrino. Evolution of Health and Diseases. In Origins, (co)evolution, diversity and synthesis of life (Volume 2). P. Bovolenta, M. Manzanares, J. Buceta (Topic Coordinators). S. Elena and I. Cómas (Challenge Coordinators). CSIC Scientific Challenges Towards 2030. ISBN: 978-84-00-10737-6 (2021).

» F-J.A., C. Briones., J. Camacho., M. Carrión., L. Elvira., R. Eritja., C. Fernández., M. de Frutos., M. García., G. García., R. García., A.J. González., F. Herranz., M.C. Horrillo., G. Llosá., J.A. Martín., E. Nacher., M. Sáiz-Zalabardo., Y. Sanz., F. Sobrino and R. Villa. New Methods for Diagnostic tools and prevention. In Challenges in Biomedicine and Health (Volume 4). M. Delgado, M. Matamoros (Topic Coordinators). S. Marcos, A. de Marco (Challenge Coordinators). CSIC Scientific Challenges: Towards 2030. ISBN: 978-84-00-10744-4.

Participation in projects

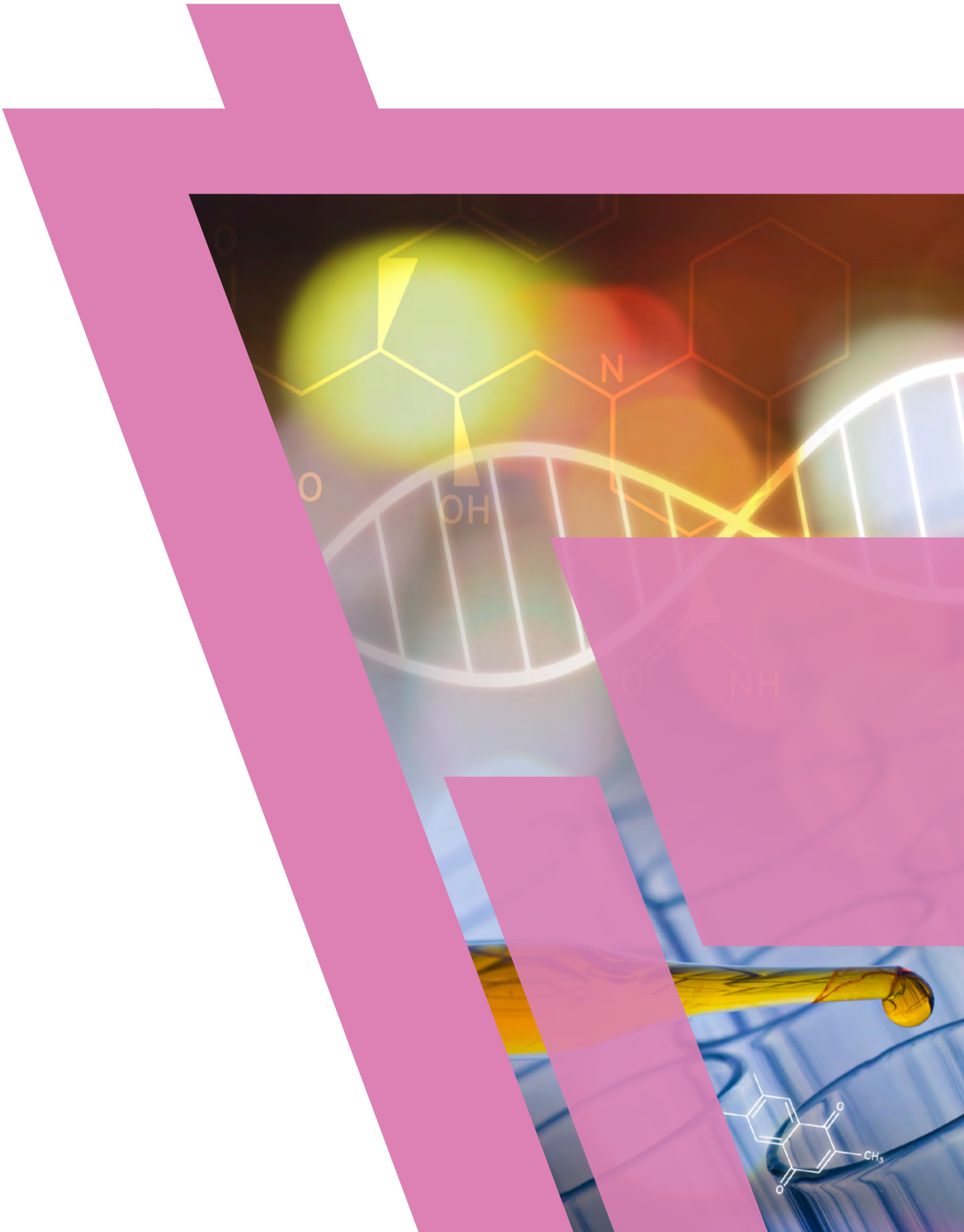
» PID2020-113184RB-C2 (AEI-MCI) 1. Proyecto coordinado con la U. Pompeu Fabra (D. Andreu). Vacunas peptídicas, estrategias antivirales y mecanismos de evasión inmune. Aplicaciones bioterapeuticas. (2021- 23). PI: Francisco Sobrino.

» CAM S2018/BAA-4370. Plataforma para el desarrollo de estrategias de control de salud animal (PLATESA2-CM) Entidades participantes: INIA, CSIC (CBMSO), UCM, UA. Investigador responsable del subproyecto NESTRANVIR (CBMSO): F. Sobrino. (2019-2022).

» Proyecto CSIC 202020E162. Antiviral activity of immunomodulatory non-coding RNAs and cell-targeted compounds against human coronaviruses. PIs: M Sáiz y F. Sobrino (2020-2021).

» INFRAFRONTIER2020 COVID-19 (EU). Immunongenicity in mice of COVID-19 dendrimer peptide vaccines (2021). Principal Investigator (CBMSO): F. Sobrino.

» VetBioNet (EU). REF: VBN_21_52. Characterization of the protection and responses elicited in pigs by a dendrimeric peptide (B2T) FMD vaccine including a new T cell epitope (2021–2022). Principal Investigator (CBMSO): F. Sobrino.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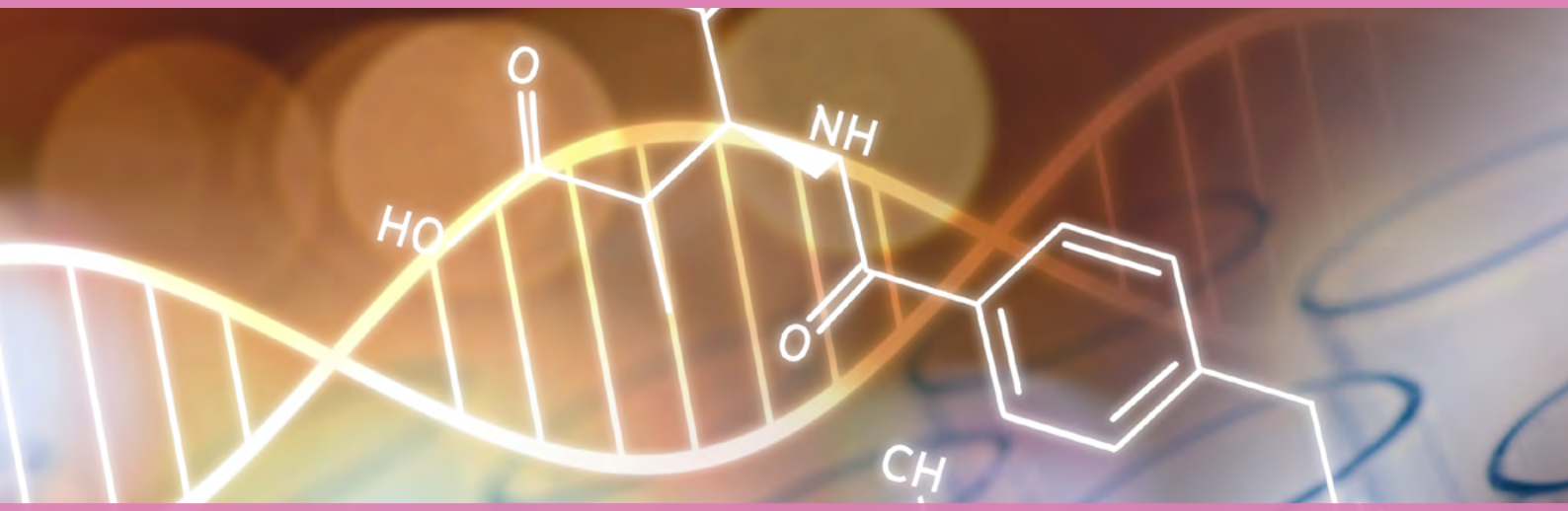


Science and Society

SCIENCE AND SOCIETY

http://www.cbm.uam.es/joomla-rl/index.php/en/science-and-society

Group Members

Director:
José Antonio López Guerrero

Technical Responsible:
Almudena Hernando

Collaborators:
Begoña Aguado
Mar Ruiz
Noemí Tabanera

Activities

Within the framework of the CBMSO Scientific Culture Program, The Department of Scientific Culture (DCC) of the CBMSO has collaborated in the following activities 2021-2022: 1) Scientific Weeks in Madrid (workshop and seminars in 2021 and webinars in 2022). 2) Annual program of 30 guided visits of Secondary School students –about 1000 students- to our Research Centre (activity canceled -2020-2022- due to the pandemic). 3) Weblog "Bio(Ciencia+Tecnología)" of the Madri+d Foundation. 4) Social Networks: Facebook and Twitter. 5) Organization and coordination of the "Biotechnology Explorer" courses for secondary school teachers (Webinar in 2021). 6) Educational Program "4ESO + EMPRESA" of the Community of Madrid. 7) Stay at the CBMSO of the Students "Finalist of the Spanish Biology Olympiad". 8) Participation in the "Science With You" congress at the Student Residence "Severo Ochoa Foundation". 9) Participation in Mass Media about Scientific Dissemination and Disclosure: Spanish National Radio (Radio 5 –"Entre Probetas" and "El Laboratorio de JAL"-, Radio 1 –"A Hombros de Gigantes- and Radio Exterior –"Marca España"-), Spanish National TV -TVE-1-, Spanish TV -La Sexta-; Collaborations with the magazines of scientific culture "El Cultural".

Scientific Weeks in Madrid

Research

The NeuroVirology (UAM) group aims at the study of the effect of HSV-1 on neurodegeneration, demyelinating disease and new antiherpetic compounds in both immature and differentiated myelin-producing oligodendrocytic cells. The features of shedding microvesicles released by HOG cells infected with HSV-1 and their participation in the viral cycle are being described. It has been detected for the first time microvesicles containing HSV-1 virions. Since 2021 the group is also interested in the characterization of the role and modulation of autophagy in HSV-1 infected cells.

Finally, as of 2020, the group started a new project on the effect of new antivirals and viricides on coronavirus infection. Some Dextran Sulfate-based extrapolymeric substances exert potent antiviral activity against SARS-CoV-2 in vitro and *in vivo*.

List of publications

Scientific (http://www2.cbm.uam.es/~jalopez/lab/english.htm)

» López Guerrero, J.A. (2021) Coronavirus: anatomía de una pandemia. Ed. Guadalmazán. ISBN: 978-84-17547-45-5

» Ripa I, Andreu S, López-Guerrero JA and Bello-Morales R. Membrane Rafts: Portals for Viral Entry. Front. Microbiol., 04 February 2021, Volumen 12 | https://doi.org/10.3389/fmicb.2021.631274

» Daniel J. Klionsky et al. Guidelines for the use and interpretation of assays for monitoring autophagy (4th edition). Autophagy. 2021 Feb 8:1-382. doi: 10.1080/15548627.2020.1797280. Online ahead of print. PMID: 33634751

» Andreu, S., Ripa, I., Praena, B., López-Guerrero, J.A., and Bello-Morales, R. The Valproic Acid Derivative Valpromide Inhibits Pseudorabies Virus Infection in Swine Epithelial and Mouse Neuroblastoma Cell Lines. Viruses 2021, 13(12), 2522; https://doi.org/10.3390/v13122522

» Bello-Morales R, Andreu S, Ripa I, López-Guerrero JA. HSV-1 and Endogenous Retroviruses as Risk Factors in Demyelination. Int J Mol Sci. 2021 May 27;22(11):5738. doi: 10.3390/ijms22115738.

» Andreu S, Ripa I, Bello-Morales R, López-Guerrero JA. Nebulized CLODOS Technology Shows Clear Virucidal Properties against the Human Coronavirus HCoV-229E at Non-Cytotoxic Doses. Viruses. 2021 Mar 23;13(3):531. doi: 10.3390/v13030531.

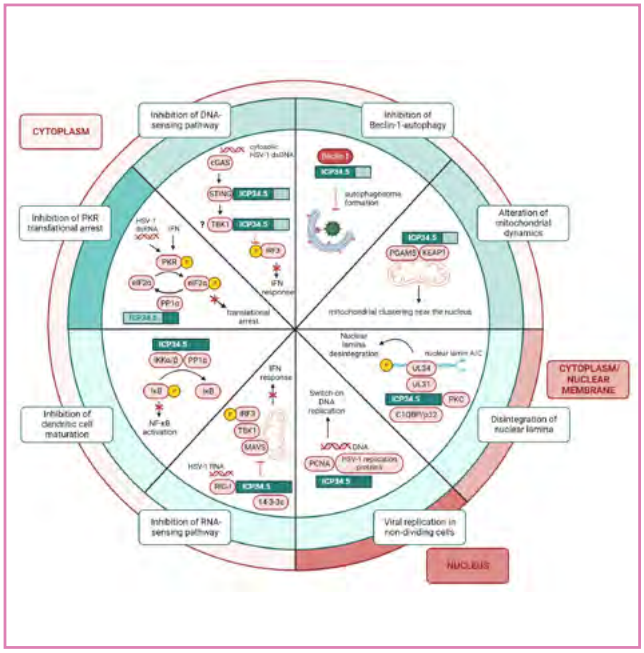
» Bello-Morales, R, Andreu, S, Ruiz-Carpio, V, Ripa, I and López-Guerrero, J.A. Extracellular Polymeric Substances: Still Promising Antivirals. Viruses 2022, 14(6), 1337; doi. org/10.3390/v14061337

» Ripa I, Andreu S, López-Guerrero JA, Bello-Morales R. Interplay between Autophagy and Herpes Simplex Virus Type 1: ICP34.5, One of the Main Actors. Int J Mol Sci. 2022, 23(21):13643. doi: 10.3390/ijms232113643

» Praena B, Mascaraque M, Andreu S, Bello-Morales R, Abarca-Lachen E, Rapozzi V, Gilaberte Y, González S, López-Guerrero JA, Juarranz Á. Potent Virucidal Activity In Vitro of Photodynamic Therapy with Hypericum Extract as Photosensitizer and White Light against Human Coronavirus HCoV-229E. Pharmaceutics. 2022, 14(11):2364. doi: 10.3390/pharmaceutics14112364

Social communication of science

López-Guerrero, J.A. El Cultural:
08/05/2021 “¿Puede alguien negarse a aceptar la evidencia (científica en el caso de la pandemia)?
31/12/2021 “Hitos del 2021”
23/12/2022 “Hitos del 2022”



The virulence factor of HSV-1 known as ICP34.5 is a viral multifunctional protein that blocks many facets of the cellular antiviral responses (Int J Mol Sci. 2022 Nov 7;23(21):13643. doi: 10.3390/ijms232113643)

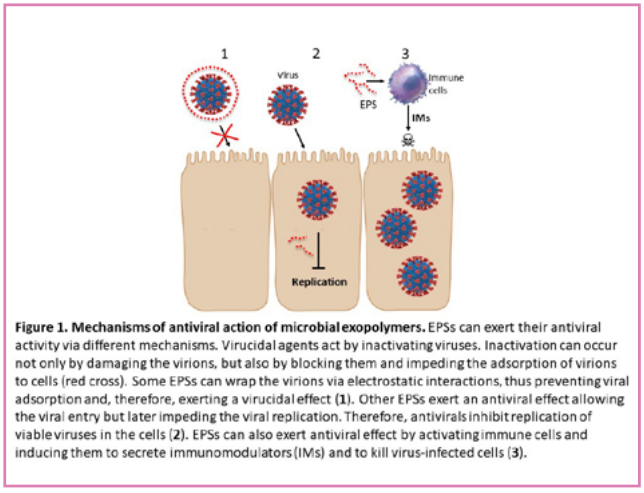


Figure 1. Mechanisms of antiviral action of microbial exopolymers. EPSs can exert their antiviral activity via different mechanisms. Virucidal agents act by inactivating viruses. Inactivation can occur not only by damaging the virions, but also by blocking them and impeding the adsorption of virions to cells (red cross). Some EPSs can wrap the virions via electrostatic interactions, thus preventing viral adsorption and, therefore, exerting a virucidal effect (**1**). Other EPSs exert an antiviral effect allowing the viral entry but later impeding the viral replication. Therefore, antivirals inhibit replication of viable viruses in the cells (**2**). EPSs can also exert antiviral effect by activating immune cells and inducing them to secrete immunomodulators (IMs) and to kill virus-infected cells (**3**).

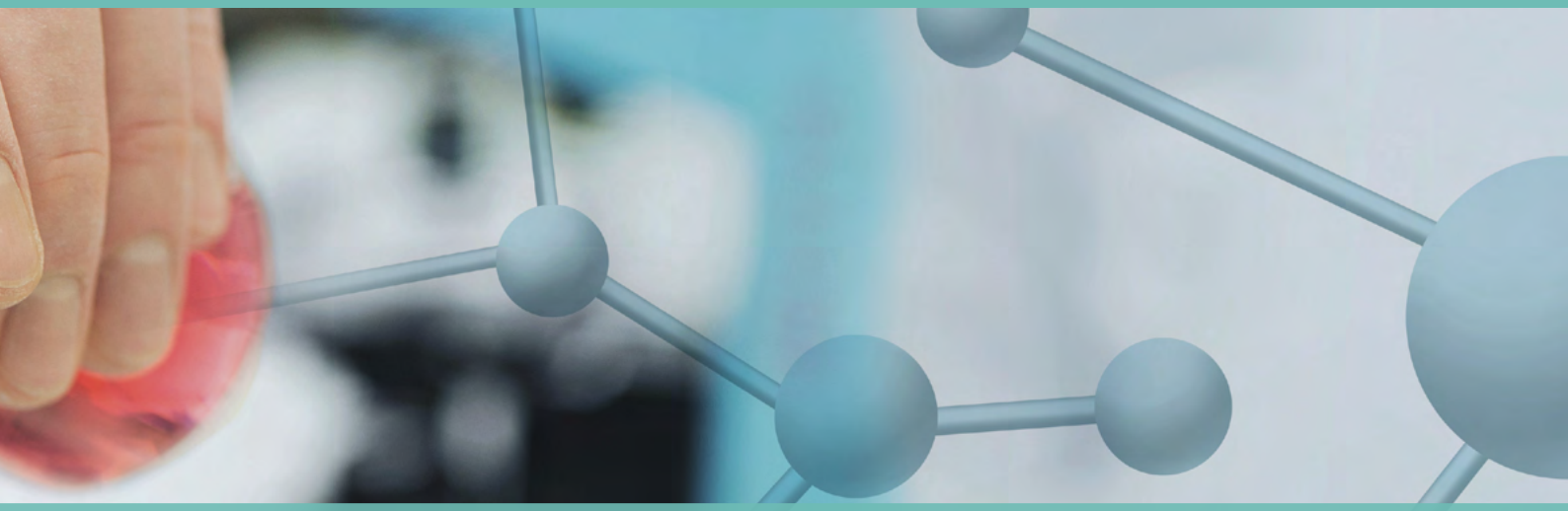
Extracellular Polymeric Substances: Still Promising Antivirals (Viruses. 2022 Jun 19;14(6):1337. doi: 10.3390/v14061337)

Awards

» 2021: I CSIC-BBVA Foundation Award for Scientific Communication.

» 2021: Specific mention in the VI "Transfiere" Journalism Award.

» 2021: Second prize in the VII Roche Institute Foundation Journalism Award in Personalized Precision Medicine.



Scientific Facilities

ANIMAL FACILITY

Group Members

Technical director:
Elena Hevia (2021)
Beatriz Barrocal López (2022)

Scientific supervisor:
Dr. César Cobaleda

Animal health veterinary advisor and Animal welfare supervisor:
Beatriz Barrocal López

Veterinarian Support:
Blanca Estévez Santiago

Technical personnel:
Laura López Martínez
José Ignacio Herrero Lahuerta
Beatriz García Martínez
José Mª Sedano Torres
Marta González Mella
Virginia González de la Torre
Blanca Sánchez Novillo
Marisol López Martín
Isidro García Gómez
Amanda Abad Serrano (Vivotecnia)

Administrative support:
Miguel A. Bordallo Martín-Fontecha

Animal keepers:
Alfonso Gutiérrez García
Staff from Vivotecnia

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

Description

It is a modern facility, designed and equipped with the latest advances in this type of facilities and its purpose is the production and maintenance of rodents and aquatic species (zebrafish, medakas and xenopus) under optimum conditions for use in the projects developed in the Center.

The installation also includes:

- SPF (Specific Pathogen Free) zone where the lines of genetically modified mice are produced.
- Conventional or experimental zone.
- Zones with Biocontainment Level 2 (BSL2), for mice
- Zone with Biocontainment (BSL3), for mice.
- Zone for the maintenance of aquatic animals (zebra fish, medaka fish and xenopus frog)
- Laboratories / Operating Rooms / Behavior Rooms/ Quarantines.

On a regular basis, seminars are organized on subjects related to laboratory animals for the continuous training of both users and staff.

Our goal is good health and animal welfare.

Services

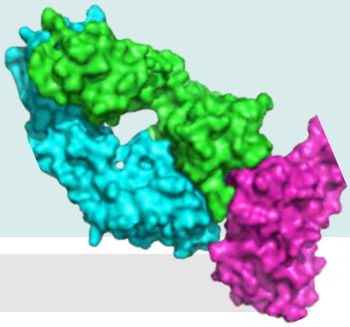
- Rederivation of genetically modified lines of mice for introduction into the barrier zone
- Reproductive Engineering techinques in mice
- Maintenance and production of mice under SPF, BSL2, BSL3 and conventional conditions.
- Maintenance and production of zebrafish and medakas
- Maintenance of Xenopus frogs.
- Management of colonies of transgenic mice.
- Production of pregnant females of rodents with known date.
- Various types of administrations and extractions.
- Support and technical advice to researchers.
- Quarterly animal health checks externally, following the recommendations of FELASA.
- Training of animal staff and users Services Offered

BIOINFORMATICS FACILITY

Group Members

Technical Director:
David Abia Holgado

Scientific Supervisor:
Ugo Bastolla Bufalini



https://tinyurl.com/bioinfcbm
http://ub.cbm.uam.es

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.

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.

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

ELECTRON MICROSCOPY FACILITY (EMF)

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

Group Members

Technical director:
Dra. María Teresa Rejas

Scientific supervisor:
Dr. Germán Andrés Hernández

Personnel:
Milagros Guerra Rodríguez
Dra.Tania Matamoros Grande (until December 2021)
Dra. María José Rodríguez Gómez (since August 2022)

Description

The electron microscopy facility (EMF) provides scientific and thecnical support to research teams interested in using transmission EM to analyze macromolecular assembles, virus, bacteria, eukaryotic cells, multicelular specimens and tissues. Available methods for sample processing include conventional techniques and cryo-techniques such as freeze-substitution, cryosectioning and freeze-fracture/freeze-etching. We also offer immunoelectronmicroscopy methods including correlative light-electron microscopy. The EMF equipment includes two transmission electron microscopes of 100kV and 120kV equiped with 4k x 4k CMOS cameras, an inverted fluorescence microscope and the following instruments for sample processing: a plunge-freezing unit, an automatic freeze-substitution system, a conventional ultramicrotome, a cryoyltramicrotome and a freeze-fracture/freeze etching unit. Over the year 2021 the EMF has carried out work for 24 internal and 10 external to CBMSO research groups and over the year 2022 for 26 internal groups and 17 external groups. During the 2021-2022 period the sevice has contributed to at least 26 publications.

Services

- Technical and scientific supervision on experimental design and data analysis.
- User assistance and training.
- Negative staining of macromolecular complexes, extracelular 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´s cryosectioning.
- Immunolectron microscopy with gold conjugates.
- Correlative light-electron microscopy.
- Freeze-fracture, freeze-etching and Pt-C replication.
- Electron microscopy of nucleic acids.

List of publications

- Gutiérrez-Álvarez, J; Honrubia, JM; Sanz-Bravo; González-Miranda, E; Fernández-Delgado, R; Rejas, MT; Zúñiga, S; Sola, I; Enjuanes, L. (2021) Proc Natl Acad Sci USA 118(43), doi: 10.1073/pnas.2111075118.
- Cacho-Navas C, Reglero-Real N, Colás-Algora N, Barroso S, de Rivas G, Stamatakis K, Feito J, Andrés G, Fresno M, Kremer L, Correas I, Alonso MA, Millán J.(2022) Cell Mol Life Sci. 79(1):61. doi: 10.1007/s00018-021-04095-z.
- Ávila-Pérez G, Rejas MT, Chichón FJ, Guerra M, Fernández JJ, Rodríguez D. (2022) Mol Microbiol.117, 837-850. doi: 10.1111/mmi.14875.
- Morel E, Herranz-Jusdado JG, Simón R, Abós B, Perdiguero P, Martín-Martín A, Andrés G, Muñoz-Atienza E, Guerra Rodríguez M, Díaz-Rosales P, Tafalla C. (2022) iScience. 26(1):105854. doi: 10.1016/j.isci.2022.105854.
- 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(1): doi:10.1371/journal.ppat.1011136.

FERMENTATION FACILITY

Group Members

Technical Director:
Dionisio Ureña Rodríguez

Personnel:
María Isabel Carrascal Blanco

Scientific Supervisor:
José Berenguer Carlos

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

Description

The Fermentation facility plays two main roles on the CBMSO scientific activities, one focused on the growth of natural and recombinant microorganisms, and the other centered on the production of biological consumables. At the level of microorganism growth, the Fermentation Facility provides advice on the appropriate bacterial strains, plasmid expression systems, and conditions for the overproduction of recombinant proteins and the scale up of the production process from Erlenmeyer to larger fermenters, allowing also the isotopic labeling of the expressed proteins when required for structural analysis. The facility also allows for the cultivation of a great variety of non-recombinant microorganisms in large volumes. In all cases, the growth in fermenters is monitored for the main parameters (temperature, stirring, pH, oxygen concentration, foam and biomass, in compliance with cGMP rules (current Good Manufacturing Practice). On the other hand, the Fermentation Facility provides ready-to-use competent preparations of different strains of *E.coli* suitable for gene cloning or expression of recombinant proteins. It also produces DNA size markers for the internal use by most of the research groups in the CBMSO.

Services

- Cultures of microorganisms in 4, 10 or 30 liters reactors.
- Improvement and production of recombinant proteins in cultures of bacteria, yeast or filamentous fungi.
- Scale up for overproduction in cultures from Erlenmeyer to high capacity fermenter.
- Isotope labeling of expressed proteins in *Escherichia coli* for structural analysis.
- Ready-to-use *Escherichia coli* competent cells.
- DNA molecular weight markers
- Disruption of cell cultures by French Press

FLOW CYTOMETRY FACILITY

Group Members

Technical Director:
Berta Raposo Ponce

Flow Cytometry Scientific Supervisor:
María Luisa Toribio

Cell Metabolism Scientific Supervisor:
Núria Martínez

Personnel:
Silvia Andrade Calvo
Raquel Nieto Pintado

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

Description

The flow cytometry facility provides access to state-of-the-art multiparametric flow cytometry, spectral flow cytometry and cell sorting. Available flow cytometry techniques range from classic cell immunophenotype, cell cycle and apoptosis determinations, or quantifications of cytokine production, cell signaling and metabolic pathways, to analyses of prokaryote organisms, dissociated discs of *Drosophila* larvae, or protoplasts and various plant cell types. 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 protein-protein 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 two analogical cytometers with two lasers and four fluorescence detectors (FACScalibur), three digital cytometers, one with two lasers and six fluorescence detectors (FACSCanto A), and two with three lasers and eight fluorescence detectors (FACSCanto II), one equipped with High Throughput Sampler. Spectral flow cytometry equipment includes a Cytek cytometer (AURORA) equipped with 4 lasers (V/B/YG/R), an analysis station equipped with a Spectroflo license, and a next-generation software (OMIQ) containing the most commonly used algorithms for multidimensional data analysis. The facility also includes two cell sorters, a FACSVantage SE with three lasers and six fluorescence detectors and a FACS Aria Fusion integrated inside a Class II biosafety cabinet, equipped with four lasers and sixteen fluorescence detectors.

For cell metabolism analysis, the facility is equipped with a Seahorse metabolic analyser, that enables the detection of discrete changes in cellular bioenergetics in real-time using living cells in a 96-well platform, and with a multiwell motorized microscope (Cytation) that allows for normalization of seahorse assays.

Personnel support includes

- Training and advice on instrument operation.
- Advice on new experimental designs and adaptive setups.
- Training on post-acquisition data analysis using FlowJo, FACSDiva, Spectrofo and OMIQ softwares.
- Equipment operation and maintenance.
- Availability to commonly used reagents.

GENOMICS AND NEXT-GENERATION SEQUENCING FACILITY (GENGS)

Group Members

Scientific supervisor:
Begoña Aguado Orea, PhD

Technical director Genomics section:
Fernando Carrasco Ramiro (until Nov 2022)
Laura Tabera Moreno (from Nov 2022)

Technical director NGS section:
Fernando Carrasco Ramiro (until Nov 2022)
Sandra González de la Fuente (from Nov 2022)

Personnel:
María José López Sánchez (until Nov 2021)
Sandra Gonzalo Flores (until Jan 2022)
Adrián Gómez Repollés (until Jan 2022)
Eva Sacristán Horcajada (until July 2022)
Manuel Belda Ávila (until Nov 2022)
Paula Martínez García (from May 2022)
Iván Lorca Alonso (from June 2022)
Gabriela Atencia Cibreiro (from June 2022)
María Santos Galindo (from Sept 2022)

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

Description

The Genomics and Next-Generation Sequencing (NGS) Facility (GENGS) is responsible for the implementation and development of genomic and NGS technologies and provides comprehensive experimental and technical advice on these methodologies to research groups. In particular, the Genomics section is specialised in PCR, RT-PCR, qPCR, RT-qPCR and, more recently, digital PCR experiments. It offers technical advice, support, experimental design, performance and data analysis of these technologies. The NGS section is specialised in tailored computational analysis of data obtained from NGS technologies (genomics, transcriptomics, single cell-omics, metagenomics, amplicons, etc.), proteomics, metabolomics and any other omic technologies. In addition, it offers advice on experimental design, mediates between diverse NGS platforms and users, and monitors the development of the projects.

The GENGS facility helps on grant proposals, organises specialised seminars, meetings and courses (personalised and general) and is involved in different teaching programs (from school to university). It also contributes to different science dissemination activities through the CBM. The facility has actively contributed to SARS-CoV-2 research projects, such as the "Centinela" led by Dr Margarita del Val, and the "Air-COVID" directed by Dr Antonio Alcamí, among others. The facility has collaborated with the CSIC Health Surveillance Unit, performing RT-qPCR COVID tests for the Comunidad de Madrid CSIC personnel. The facility is an authorised recognised lab for those tests. In addition, it has collaborated with Dr Iñaki Comas (IBV, Valencia) to sequence and assign COVID lineages of positive samples.

Courses

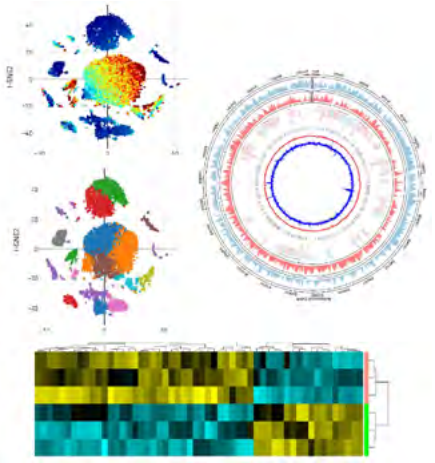
- NGS data analysis course. Gabinete formación CSIC 2021-2022
- Personalised NGS (data analysis) and PCR (Technical and data analysis) courses 2021-2022

Services

- Advice on grant proposals
- Experimental design, performance and data analysis.
- Specialised personalised courses.

Genomics Section

- Nucleic acids extraction
- Nucleic acids quantification
- RNA integrity
- Primer design
- Reverse transcription (RT)
- PCR, RT-PCR, qPCR, RT-qPCR
- Droplet Digital PCR (ddPCR)
- qPCR data analysis software. GenEx professional (Multid Analyses AB)



NGS Section

- Mediation with external sequencing platforms and follow-up of projects. Access to different technologies: Illumina, PacBio and Oxford Nanopore.
- NGS data computational analysis (DNA-seq, RNA-seq, ChIP-seq, single cell, metagenomics, amplicons, among others)
- Tailored software development.
- Statistical data analysis
- Microarray analysis
- Ingenuity Pathway Analysis (IPA) software.

List of publications

- Sacristán-Horcajada E, González-de la Fuente S, Peiró-Pastor R, Carrasco-Ramiro F, Amils R, Requena JM, Berenguer J, Aguado B. ARAMIS: From systematic errors of NGS long reads to accurate assemblies. Brief Bioinform. 2021 Nov 5;22(6):bbab170. doi: 10.1093/bib/bbab170. PMID: 34013348; PMCID: PMC8574707.
- Camacho E, González-de la Fuente S, Solana JC, Rastrojo A, Carrasco-Ramiro F, Requena JM, Aguado B. Gene Annotation and Transcriptome Delineation on a De Novo Genome Assembly for the Reference *Leishmania* major Friedlin Strain. Genes (Basel). 2021 Aug 29;12(9):1359. doi: 10.3390/genes12091359. PMID: 34573340; PMCID: PMC8468144.

OPTICAL AND CONFOCAL MICROSCOPY CORE FACILITY (SMOC)

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

Group Members

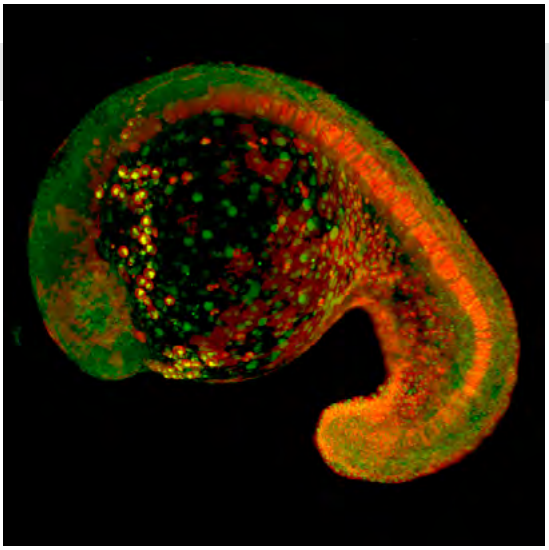
Scientific supervisor:
Dr. Javier Díez Guerra

Technical director:
Ángeles Muñoz Alcalá
(until November 2022)

Personnel:
Teresa Villalba Villacorta
Carmen Sánchez Jiménez
Carlos Gallego García
Francisco José Vega Sabugo
Elena Calvo Cazalilla

Description

SMOC manages and maintains advanced light microscopy equipment in the CBMSO, featuring seven systems of laser scanning confocal microscopy -one includes super-resolution by stimulated emission depletion (STED) and fluorescence lifetime imaging microscopy (FLIM)-, a spinning disk microscope and four wide-field fluorescence microscopes. SMOC has two workstations for image analysis, advanced deconvolution and rendering, a vibratome and a stereomicroscope, and distributes common consumables. The main tasks aim to inform and train users, organize seminars and practical sessions, and take feedback from them to configure systems and techniques of future incorporation. Administrative tasks include the management of the scheduling and online booking of systems, and user invoicing supported by PPMS. SMOC works to get external funds for new equipment and human resources, prepares annual budgets to cover running costs, and deals with suppliers. SMOC maintains since 2009 a quality management system ISO 9001:2015 (AENOR), which is subject to annual accreditation and certification controls. SMOC belongs to the Spanish Network of Advanced Optical Microscopy (REMOA), the "Red de Laboratorios e Infraestructuras (RedLab)" and the "Plataforma de Microscopía para Biociencias" managed by "Comunidad de Madrid" (laboratory nº216).



Services

- Controlled access and use of the microscopy equipment.
- Management of online reservations and invoicing.
- User assistance and training.
- Preventive and corrective maintenance to ensure optimum equipment performance.
- Organization of seminars and development of tutorials and practical guides.
- Maintenance and update of the facility web page (http://www.cbm.uam.es/confocal).
- Stock and distribution of common consumables for light microscopy applications.
- Forecast future needs of the Institute in microscopy through user surveys.

PROTEOMICS AND PROTEIN CHEMISTRY CORE FACILITY

Group Members

Technical director:
Dra. Anabel Marina Ramírez (until Aug 2022)
Dr. Carlos García García (since Sep 2022)

Scientific supervisor:
Dr. José Manuel Cuezva

Personnel:
Dr. Carlos García García
Dra. Esperanza Morato
Nuria Sánchez López (until Dec 2021)
Dra. Tamara Rosell García (since Nov 2022)

www.cbm.uam.es/servicios/proteomica.htm
www.proteored.org.

Description

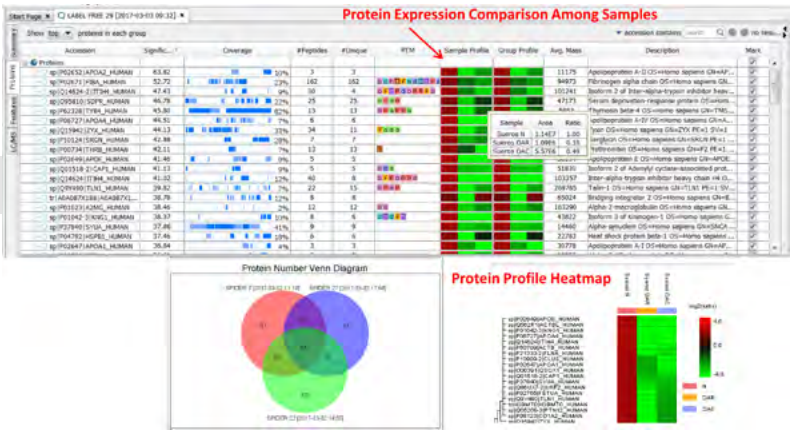
In 1993 the Protein Chemistry Facility was created in the CBMSO. The trajectory of the Proteomics Facility has ever since paralleled the evolution of the scientific community in the study of proteins and its objectives are to provide technical support and advice to researchers on the appropriate methods of sample preparation and design of the appropriate work-flow for each objective. The laboratory is equipped with systems to run electrophoresis and two mass spectrometers: a LTQ-VELOS (2D-Ion Trap in tandem) and a LTQ-ORBITRAP-VELOS-PRO (2D-Ion Trap in tandem coupled to Orbitrap analyzer) both from Thermo-Scientific. Our service has been used for numerous research projects in a broad range of scientific fields.

List of publications

- Terrile, M. D., Tebez, N. M., Colman, S. L., Mateos, J. L., Morato-Lopez, E., Sanchez-Lopez,N., Izquierdo-Alvarez, A., Marina, A., Calderón Villalobos, L. I. A., Estelle, M., Martínez-Ruiz, A., Fiol, D. F., Casalongué, C. A. and Iglesias, M. J. (2021) S-Nitrosation of E3 Ubiquitin Ligase Complex components regulates hormonal signaling in Arabidopsis. Front. Plant Sci. 2021; 12: 794582. doi: 10.3389/fpls.2021.794582.
- Suárez, H., Andreu, Z., Mazzeo, C., Toribio, V., Pérez-Rivera, A. E., López-Martín, S., García-Silva, S., Hurtado, B., Morato, E., Peláez, L., Arribas, E. A., Tolentino-Cortez, T., Barreda-Gómez, G., Marina, A. I., Peinado, H. and Yáñez-Mó, M. (2021) CD9 inhibition reveals a functional connection of extracellular vesicle secretion with mitophagy in melanoma cells. J. Extracell. Vesicles. 2021 May;10 (7): e12082. doi: 10.1002/jev2.12082.
- Briso-Montiano, Á., Vilas, A., Richard, E., Ruiz-Sala, P., Morato, E., Desviat, L. R., Ugarte, M., Rodríguez-Pombo, P. and Pérez, B. (2022) Hepatocyte-like cells differentiated from methylmalonic aciduria cblB type induced pluripotent stem cells: A platform for the evaluation of pharmacochaperoning. Biochim. Biophys. Acta Mol. Basis Dis. 2022 Sep 1;1868(9):166433. doi: 10.1016/j.bbadis.2022.166433.
- Verdes, A., Taboada, S., Hamilton, B. R., Undheim, E. A. B., Sonoda, G. G., Andrade, S. C. S., Morato, E., Marina, A. I., Cárdenas, C. A., Riesgo, A. (2022) Evolution, expression patterns, and distribution of novel ribbon worm predatory and defensive toxins. Mol. Biol. Evol. 2022 May 3;39(5): msac096. doi: 10.1093/molbev/msac096.
- San Francisco, J., Astudillo, C., Vega, JL., Catalán, A., Gutierrez, B., Araya, J., Zeilberger, A., Marina, A., García, C., Sanchez, N., Osuna, A., Vilches, S., Ramírez, M., Macedo J., Feijoli, V., Palmisano, G., González, J. (2022) Trypanosoma cruzi pathogenecity depends on virulence factor expression and upregulation of bioenergetics and biosynthetic pathways. Virulence. 2022, 13(1): 1827-1848. doi: 10.1080/21505594.2022.2132776.

Services offered

- SDS-PAGE electrophoresis.
- Gel staining.
- Sample preparation (precipitation, digestion, desalting).
- Protein Identification by LC-MS/MS (ESI-LTQ-VELOS/ORBITRAP).
- Protein and proteome characterization (analysis of post-traslational modifications).
- Relative quantification of differences in protein abundance by isobaric methods (TMT, iTRAQ, SILAC) and Label Free methods.
- *De novo sequencing.*



Training courses and seminars

- "X Curso de Proteómica Cuantitativa" CNB, Madrid.
- "Decrypting Liquid Chromatography Variation-Achieving Reproducibility in results-EUR", eLearning Phenomenex course, on line, 2021.
- "Special Webinar: Cold Sticky Blood, Hair, Saliva Pretreatment & Cleanup-EUR", eLearning Phenomenex course, on line, 2021.
- "Method Development Strategies for Peptide Analysis-EUR", eLearning Phenomenex course, on line, 2022.
- "Using Core Shell Particle Technology to improve Micro and Nano LC Separations - EUR", eLearning Phenomenex course, on line, 2022.
- "Consejos y trucos para solucionar problemas de LC-ES" eLearning Phenomenex course, on line, 2022.
- Participation in national and international meetings on proteomics
- "MYSP: Meeting for Young Scientists in Proteomics of FPS, SEProt and redeProCura", on-line, 2021.
- "2nd Joint Meeting of Spanish, French and Portuguese Proteomics Societies", Vilamoura, Portugal, 2022.

CBM-CNB TRANSGENESIS FACILITY

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

Group Members

Scientific Supervisor and Technical Director:
Dra. Mª Belén Pintado Sanjuanbenito (CNB)

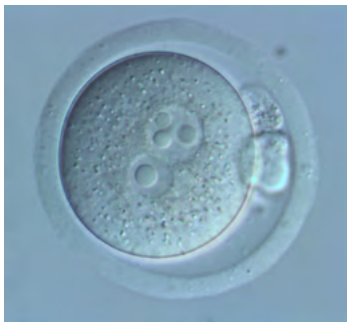
Personnel:
Verónica Domínguez Plaza (ES cell and CRISPR/Cas9 based models) CBMSO
Mª José Palacios Barea (2021) (embryo collection and transfer, colony management)
Mélani Margullón Cardoso (2021-2022) Embryo micromanipulation colony management

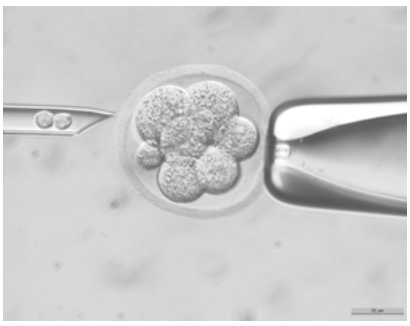
Description

The Transgenesis Service is a joint scientific service shared by CBMSO and CNB that provides support to research groups in the creation, interchange and management of genetically modified mouse models. The service covers all the required steps: from founder generation to breeding and management of lines. Models can be generated with traditional technology, transgenesis or ES cell derived gene targeting, and also with CRISPR/Cas9 based Genome Edition with embryo microinjection or electroporation. The Transgenesis Service offers technical and scientific support complementing the expertise of our customers, advising on the best strategy to obtain the desired model. The service counts with two fully equipped microinjection settings, one electroporator specifically designed for embryo edition, a standard molecular biology laboratory and a laboratory for ES cells. With full access to the animal facilities of CBMSO and CNB, we deliver the newly created animal models in the barrier of both centers. The service is integrated in the scientific-technological platform INNOTEK (UAM+CSIC). The service is also involved in the production and characterization of two new mouse models for the study of COVID-19 The general activity of the service is complemented with the organization of specific workshops and the participation in specialization courses and master programs.

Services

- Rederivation of genetically modified mouse lines from external animal facilities
- Technical and scientific support in the design of target vectors, transgenes and guides for genome edition
- Pronuclear microinjection of plasmidic, BAC and YAC DNA
- Gene edition based on CRISPR/Cas9 technology, from guides design and validation to embryo microinjection and electroporation
- Generation of KO and KI models based on ES cell lines,
- Derivation of new murine ES cell lines
- Support in establishment and management of genetically altered mouse lines
- Specific training in collection, handling and culture of mouse embryos in preimplantational stages





List of publications

- Hortal AM, Oeste CL, Cifuentes C, Alcoceba M, Fernández-Pisonero I, Clavaín L, Tercero R, Mendoza P, Domínguez V, García-Flores M, Pintado B, Abia D, García-Macías C, Navarro-Bailón A, Bustelo XR, González M, Alarcón B. Overexpression of wild type RRAS2, without oncogenic mutations, drives chronic lymphocytic leukemia. Mol Cancer. 2022 Feb 4;21(1):35. doi: 10.1186/s12943-022-01496-x. PMID: 35120522.
- Raboso-Gallego J, Casado-García A, Jiang X, Isidro-Hernández M, Gentles AJ, Zhao S, Natkunam Y, Blanco O, Domínguez V, Pintado B, Alonso-López D, De Las Rivas J, Vicente-Dueñas C, Lossos IS, Sanchez-Garcia I. Conditional expression of HGAL leads to the development of diffuse large B-cell lymphoma in mice. Blood. 2021 Apr 1;137(13):1741-1753. doi: 10.1182/blood.2020004996. PMID: 33024996

DROSOPHILA TRANSGENESIS FACILITY

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

Group Members

Technical Directors:
Mar Casado García
Nuria Esteban Delgado

Scientific Supervisor:
Dr 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.

Services

» 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-attP-22A, 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, Vasa-cas9 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.

VIRAL VECTORS FACILITY

Group Members

Technical Director:
Mercedes Dávila Cerrato

Scientific Supervisor:
Jaime Millán Martínez

Personnel:
Marta Fierro Fernández

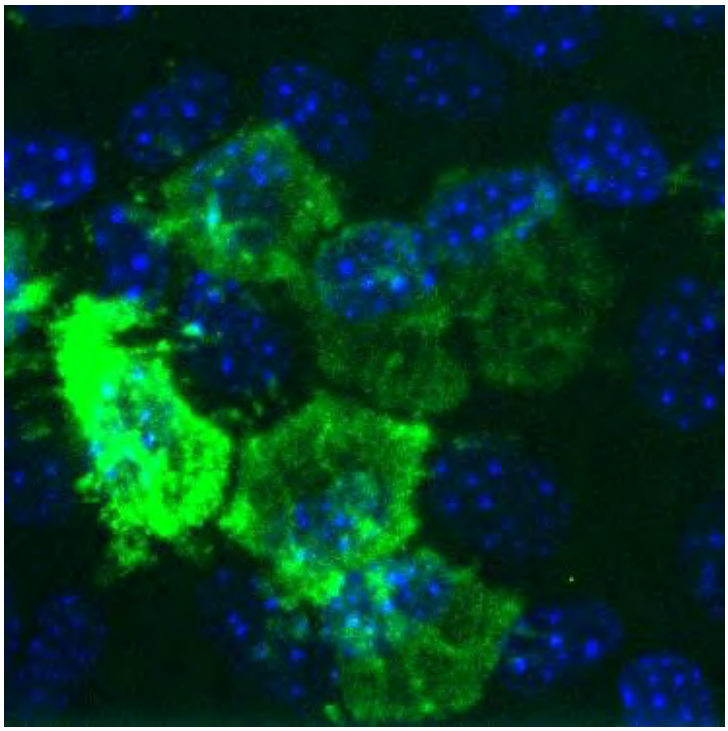
http://www.cbm.uam.es/viralvectors

Description

The CBMSO Viral Vector Facility is designed to make high quantity and quality lentiviruses for non-profit research purposes. The aim of the unit is the production, purification and titration of lentiviral vectors for *in vitro* and *in vivo* experiments.

Services

» We provide lentiviral vectors packaging services including small-scale viral packaging for *in vitro* experimentation and large-scale packaging required for successful *in vivo* studies. We perform the determination of the functional titer of fluorescent lentiviral particles by flow citometry. Aliquots of lentivirus containing the GFP reporter gene for preliminary experiments are available in the unit. We also offer assistance in the selection and design of lentiviral vectors for basic and pre-clinical research.



Murine corneal endothelial cells infected with lentiviral vectors (Green). Scale bar: 20 µm.


CBM
Severo Ochoa

General Services

ADMINISTRATION

Jaime García Martín-Delgado
David Arjona Lara
Carmen Arroyo Martín
Montserrat Barbero Maturana
Francisco Jiménez Condes
Miguel Pérez Pulido
Sonia Rubio Lago
Rosa Sánchez Caballero
Gregorio Javier Sánchez García
Belén Villar Pérez
Montserrat de la Fuente Chivato
Lidia Barbero Jiménez

CELL CULTURE, WASHING AND STERILIZATION

Mercedes Dávila Cerrato
Mª Ángeles Blanco Ferreras
María Cazorla Plaza
Antonia Cerrato Gómez
Marta Fierro Fernández
Anunciación Gaceo Esteban
Miriam García Carrascal
Lorena García Murillo
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F. Borja Mirasol Burgos
Ana Mª Pérez Colmenar
Juan Antonio Rebelles Vicente

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Jessica Martínez Santos
María Paz Yubero Martín

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Ángel J. Gonzalo Gonzalo
María Peña Pérez García
Eugenio Barrientos Ruiz
Adrián Contreras

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Mª del Carmen López Vara

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Antonia Condes Cano

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Volga del Castillo Domingo

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José Antonio Pérez Gracia

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Iris Esparza Collado

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Germán Lerma Rodrigo

LIBRARY

Josefa González-Nicolás Garrido
Marisa Gorines

SAFETY AND OCCUPATIONAL RISK PREVENTION

Fernando García Muñoz

INSTRUMENTS AND EQUIPMENT

Francisco Gutiérrez de la Cruz
Juan A. Delgado Rodríguez
Fernando Muñoz Maqueda

MAINTENANCE

Jorge Ignacio Ocampo Pérez
Pedro Pablo Cordón Polanco
César Martos Valladares
José María Sanz Mejías
Miguel Ángel Ollero Serrano

INSTITUTIONAL RELATIONS

Almudena Hernando Bellido
Carmen Hermoso Crispín


NH2 CYTOSINE
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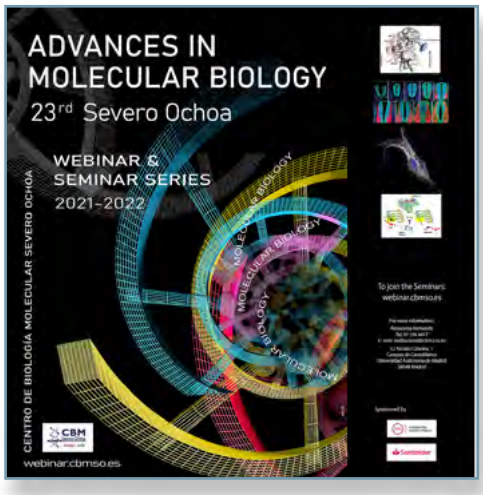
Seminars and Lectures


ADVANCES IN
MOLECULAR BIOLOGY
23rd Severo Ochoa
WEBINAR &
SEMINAR SERIES
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CENTRO DE BIOLOGÍA MOLECULAR SEVERO OCHOA
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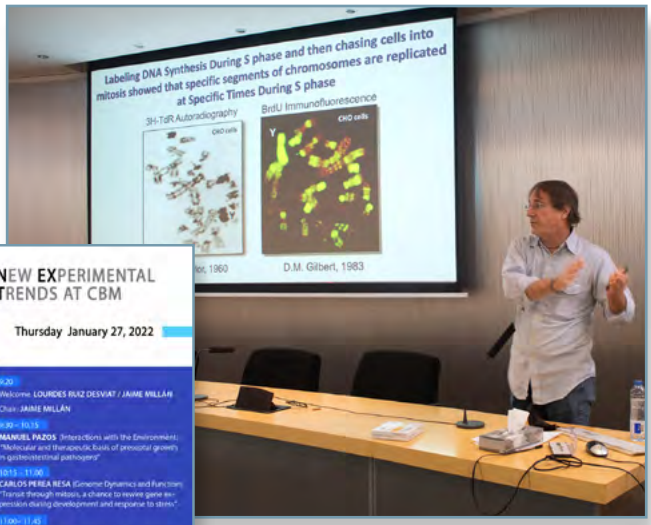
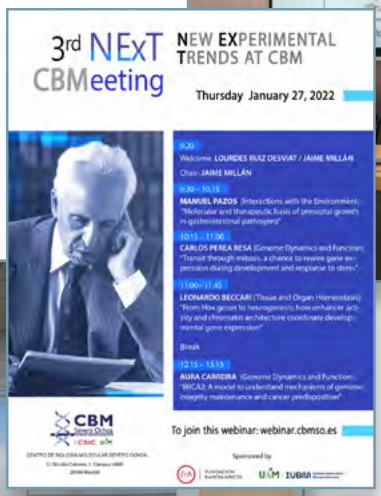
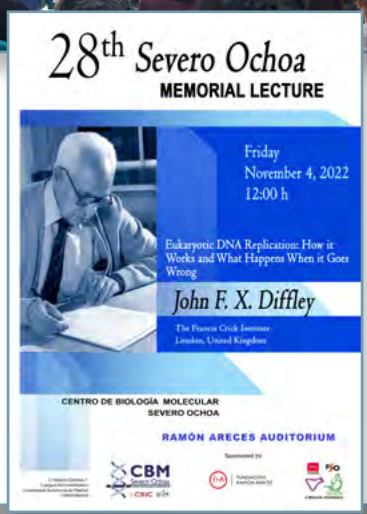

Labeling DNA Synthesis During S phase and then chasing cells into mitosis showed that specific segments of chromosomes are replicated at Specific Times During S phase
3H-TdR Autoradiography
BrdU Immunofluorescence
D.M. Gilbert, 1983


3rd NExT CBMeeting
NEW EXPERIMENTAL TRENDS AT CBM
Thursday January 27, 2022
To join this webinar: webinar.cbmso.es


28th Severo Ochoa
MEMORIAL LECTURE
Friday
November 4, 2022
12:00 h
Eukaryotic DNA Replication: How it Works and What Happens When it Goes Wrong
John F. X. Diffley
The Francis Crick Institute
London, United Kingdom
CENTRO DE BIOLOGÍA MOLECULAR
SEVERO OCHOA
RAMÓN ARECES AUDITORIUM


SPECIAL SEMINAR
CENTRO DE BIOLOGÍA MOLECULAR SEVERO OCHOA
Multicellular coordination in context
Takashi Hiiragi
Hubrecht Institute, Utrecht, Netherlands
Tuesday November 8, 2022
15:00h
RAMON ARECES AUDITORIUM
Host:
Miguel Manzanares
mmanzanares@cbm.csic.es
Ext. 4475
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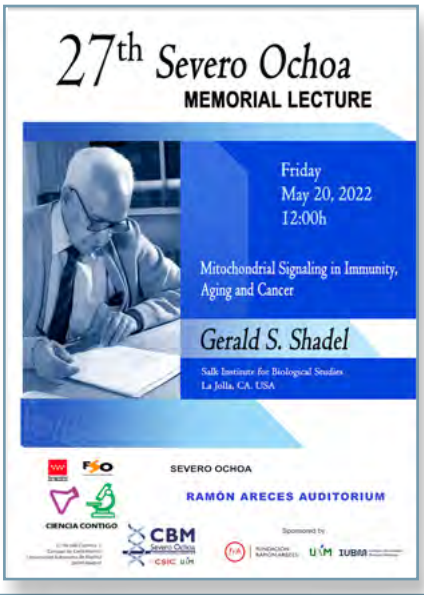
27th Severo Ochoa
MEMORIAL LECTURE
Friday
May 20, 2022
12:00h
Mitochondrial Signaling in Immunity, Aging and Cancer
Gerald S. Shadel
Salk Institute for Biological Studies
La Jolla, CA. USA
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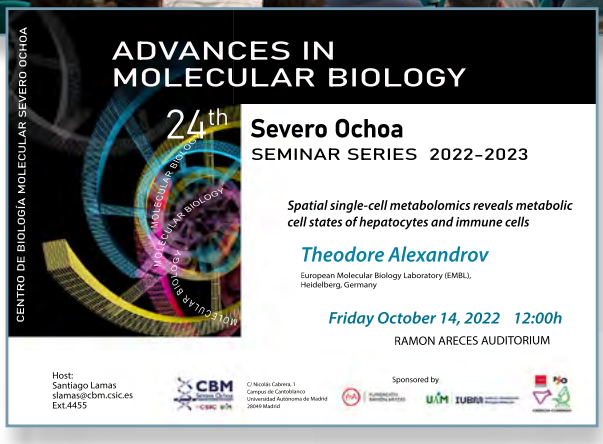
CENTRO DE BIOLOGÍA MOLECULAR SEVERO OCHOA
ADVANCES IN MOLECULAR BIOLOGY
24th
Severo Ochoa
SEMINAR SERIES 2022-2023
Spatial single-cell metabolomics reveals metabolic cell states of hepatocytes and immune cells
Theodore Alexandrov
European Molecular Biology Laboratory (EMBL), Heidelberg, Germany
Friday October 14, 2022 12:00h
RAMON ARECES AUDITORIUM
Host:
Santiago Lamas
slamas@cbm.csic.es
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2nd NExT
CBMSO meeting
NEW EXPERIMENTAL TRENDS AT CBMSO
Friday, February 19th, 2021
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DOCTORAL THESES 2021-2022

2021

DATE	DOCTORAL STUDENT	PROGRAM	DIRECTOR	TITLE
25/02/2021	JORGE VAL CALVO	INTERACCIONES CON EL ENTORNO	WILFRIED J.J. MEIJER	The pLS20 family of plasmids and regulation of their establishment genes
05/03/2021	MARIA MARGARIDA MARTINS NEVES	PROCESOS FISIOLOGICOS Y PATOLOGICOS	FEDERICO MAYOR/ PETRONILA PENELA	Molecular mechanisms underlying the role of GRK2 in Breast Cancer progression
09/03/2021	CARLA SANCHEZ CASTILLO	PROCESOS FISIOLOGICOS Y PATOLOGICOS	JOSE ANTONIO ESTEBAN GARCÍA	Role of Class IA PI3K isoforms in synaptic plasticity and cognitive function
15/03/2021	CARLOS FERNANDEZ LINARES	INTERACCIONES CON EL ENTORNO	ANTONIO ALCAMI/ ALBERTO RASTROJO	Análisis Bioinformático de Metagenomas de Virus Polares
17/03/2021	ALVARO CABALLERO LOMBRAÑA	PROCESOS FISIOLOGICOS Y PATOLOGICOS	CATALINA RIBAS/ FEDERICO MAYOR	Nuevo interactoma de Gq y su implicación en la homeostasis vascular
18/03/2021	INES SÁNCHEZ GÓMEZ	INTERACCIONES CON EL ENTORNO	MANUEL FRESNO/ NURIA GIRONÉS	The Role of the Transcription Factor TCFL5 in the Physiopathology of B Lymphocytes
23/03/2021	SARA AHMED DE PRADO	HOMEOSTASIS Y TEJIDOS DE ÓRGANOS	ANTONIO BAONZA	Estudio de los mecanismos que regulan la re-especificación celular y la pérdida de la capacidad proliferativa en respuesta al daño en el disco imaginal de ala de *Drosophila melanogaster*
14/04/2021	PETER ELIAS KIDIBULE	INTERACCIONES CON EL ENTORNO	MARIA FERNANDEZ LOBATO	Utilización de materiales quitinolíticos para la obtención de oligosacáridos bioactivos. Caracterización de biocatalizadores y productos
15/04/2021	MARIO LEDESMA TERRÓN	HOMEOSTASIS Y TEJIDOS DE ÓRGANOS	DAVID MÍGUEZ	A systems biology study to understand the dynamics of progenitor cells populations in the tissue development
16/04/2021	DOLORES PINIELLA	PROCESOS FISIOLOGICOS Y PATOLOGICOS	FRANCISCO ZAFRA/ CECILIO GIMÉNEZ	Identificación de nuevas proteínas que interaccionan con los transportadores de glutamato y dopamina (GLT-1 y DAT) mediante alteraciones en sus respectivos entornos
28/04/2021	GABRIELA GRISEL DESDÍN MICÓ	HOMEOSTASIS Y TEJIDOS DE ÓRGANOS	MARIA MITTELBRUNN	Mitochondrial T cell failure induces multimorbidity and aging

2021

DATE	DOCTORAL STUDENT	PROGRAM	DIRECTOR	TITLE
30/04/2021	SANDRA BOSCH REÑÉ	INTERACCIONES CON EL ENTORNO	AURELIO HIDALGO	Directed evolution and rational design approaches for the thermostabilization of an industrially relevant aldolase
21/05/2021	CARLOS GALLEGO GARCIA	INTERACCIONES CON EL ENTORNO	JOSE M. ALMENDRAL	Terapia de Glioblastoma con el Parvovirus MVM: implicación de p53 y su modulación por quimioterapia genotóxica
09/06/2021	ADOLFO JAVIER MOLEJÓN GARCÍA	PROCESOS FISIOLOGICOS Y PATOLOGICOS	PETRONILA PENELA	G Protein Coupled Receptor Kinase (GRK)-2: Involvement in DNA Damage Response and Functional Repercussions in Senescence
10/09/2021	IRENE BRAVO ALONSO	PROCESOS FISIOLOGICOS Y PATOLOGICOS	PILAR RODRÍGUEZ/ BELÉN PÉREZ	Caracterización genética de la Acidosis Láctica Congénita: Un ejemplo de análisis integral de una enfermedad rara
15/09/2021	ANGEL GARCÍA HORSTMANN	PROCESOS FISIOLOGICOS Y PATOLOGICOS	ADRIAN GARCIA/ MARÍA FERNÁNDEZ LOBATO	Estudio de microorganismos halófilos moderados productores de exopolisacáridos pertenecientes a salinas de interior en Castilla-La Mancha
28/09/2021	ANA LAURA BARRIOS MUÑOZ	PROCESOS FISIOLOGICOS Y PATOLOGICOS	EVA PORLAN	Plk1: un nuevo regulador de la neurogénesis adulta
18/10/2021	SERGIO RIVAS MUÑOZ	PROCESOS FISIOLOGICOS Y PATOLOGICOS	FRANCISCO WANDOSELL/ INÉS MARÍA ANTÓN	Análisis de la vía protumoral WIP-YAP/TAZ mediante proteómica y transcriptómica diferencial
04/11/2021	CARLOS REY SERRA	PROCESOS FISIOLOGICOS Y PATOLOGICOS	SANTIAGO LAMAS	Impact of the circadian regulation on the metabolic basis ofImpact of the circadian regulation on the metabolic basis ofkidney fibrosis
19/11/2021	ZORAN MERDZO	INTERACCIONES CON EL ENTORNO	MARIA FERNANDEZ LOBATO	Estudio de la α-glucosidasa de *Schwanniomyces occidentalis* para la síntesis de isomaltooligosacáridos y otros compuestos glucosilados
19/11/2021	TANIA CALVO LÓPEZ	INTERACCIONES CON EL ENTORNO	JOSÉ MARÍA ALMENDRAL	Ingeniería de dominios funcionales de la cápsida del Parvovirus MVM con péptidos que bloquean VEGF: efectos en ensamblaje, inmunogenicidad y tropismo

DOCTORAL THESES 2021-2022

2022

DATE	DOCTORAL STUDENT	PROGRAM	DIRECTOR	TITLE
10/01/2022	JOSÉ MANUEL MARTÍNEZ LOZANO	INTERACCIONES CON EL ENTORNO	RICARDO AMILS	Ecología microbiana en el Salar de Uyuni (Bolivia) como factor limitante para la vida
20/01/2022	BEATRIZ CARDEÑES	HOMEOSTASIS Y TEJIDOS DE ÓRGANOS	CARLOS CABAÑAS	Implicación de la integrina α5β1, ADAM17 y CD9 en adhesión celular y en la unión y captación de exosomas tumorales por células receptoras
27/01/2022	ANGEL BAGO PLAZA	INTERACCIONES CON EL ENTORNO	JUAN MANUEL SERRADOR/MIGUEL A. IÑIGUEZ	Acciones de los nitro-ácidos grasos sobre la activación de los linfocitos T
11/02/2022	CRISTINA SANCHEZ GONZALEZ	PROCESOS FISIOLOGICOS Y PATOLOGICOS	LAURA FORMENTINI	Papel de la bioenergética mitocondrial en el metabolismo del músculo esquelético en patología y ejercicio
07/04/2022	CÉSAR GAGO CÓRDOBA	INTERACCIONES CON EL ENTORNO	WILFRIED J.J. MEIJER	The initial steps of the pLS20 conjugation process: insights into the mechanisms of recipient cell selection and attachment by donor cells
29/04/2022	IZARNE MEDINA AZPIAZU	HOMEOSTASIS Y TEJIDOS DE ÓRGANOS	GINÉS MORATA	Tumorogénesis y competición celular en células con falta de función del gen polyhomeotic en *Drosophila melanogaster*
29/04/2022	LAURA VALLÉS SAIZ	PROCESOS FISIOLOGICOS Y PATOLOGICOS	FÉLIX HERNÁNDEZ	Nuevas funciones para la proteína tau en el Sistema Nervioso Central y en tejidos periféricos
29/04/2022	CARLOS DAVID ORDÓÑEZ CENCERRADO	DINAMICA Y FUNCION DEL GENOMA	MARGARITA SALAS/ MODESTO REDREJO	Novel activities and new members of B-family DNA polymerases with applications in biotechnology
06/05/2022	ARMANDO RUBIO RAMOS	HOMEOSTASIS Y TEJIDOS DE ÓRGANOS	MIGUEL ANGEL ALONSO LEBRERO	La proteína MALL: identificación en los cuerpos PML y su implicación en la organización nuclear
19/05/2022	ESTHER CAMACHO CANO	DINAMICA Y FUNCION DEL GENOMA	JOSÉ MARÍA REQUENA/BEGOÑA AGUADO	Estudios genómicos y transcriptómicos en *Leishmania*
20/05/2022	LETICIA LABAT DE HOZ	HOMEOSTASIS Y TEJIDOS DE ÓRGANOS	MIGUEL ANGEL ALONSO LEBRERO	Regulación de la formina INF2 normal y patogénica: papel del extremo amino terminal
27/05/2022	NATALIA COLÁS ALGORA	HOMEOSTASIS Y TEJIDOS DE ÓRGANOS	JAIME MILLÁN	Estudio de la regulación de la barrera endotelial por las Rho GTPasas de la subfamilia RhoA

2022

DATE	DOCTORAL STUDENT	PROGRAM	DIRECTOR	TITLE
31/05/2022	JORGE BRAVO VILLANUEVA	INTERACCIONES CON EL ENTORNO	AURELIO HIDALGO	Development of a droplet-based, “vectorless” system for in vitro, ultrahigh-throughput functional metagenomics
07/06/2022	CLARA ECHEVARRIA ZOMEÑO	DINAMICA Y FUNCION DEL GENOMA	CRISTANTO GUTIÉRREZ / BÉNÉDICTE DESVOYES	Coordination of cell proliferation with developmental programs in Arabidopsis
07/06/2022	DIEGO MARTÍN GARCÍA GONZÁLEZ	INTERACCIONES CON EL ENTORNO	MARÍA FERNÁNDEZ LOBATO	Estudio de la α-glucosidasa de la levadura Metschnikowia reukaufii y su empleo en procesos biocatalíticos para la producción de oligosacáridos bioactivos de la miel
24/06/2022	LAURA TORRESANO CICUÉNDEZ	PROCESOS FISIOLOGICOS Y PATOLOGICOS	JOSÉ MANUEL CUEZVA	El proteoma del metabolismo en cáncer de pulmón: nuevas dianas terapéuticas
01/07/2022	SARA PICÓ DEL PINO	PROCESOS FISIOLOGICOS Y PATOLOGICOS	JOSÉ JAVIER LUCAS	Estudio de la Enfermedad de Huntington como una deficiencia de tiamina asociada a SLC19A3 y sus implicaciones terapéuticas
06/07/2022	JUAN CRUZ HERRERO MARTÍN	PROCESOS FISIOLOGICOS Y PATOLOGICOS	LAURA FORMENTINI	Papel de las deshidrogenasas FAD dependientes en la fisiopatología del músculo esquelético
13/07/2022	SANTOS DOMINGUEZ ZOTES	INTERACCIONES CON EL ENTORNO	MAURICIO GARCIA MATEU/ALEJANDRO VALBUENA	Modulación de las propiedades mecánicas y dinámicas del entramado proteico que forma la cápsida del virus de la inmunodeficiencia humana
18/07/2022	CARLOS CAMACHO DE LA MACORRA	HOMEOSTASIS Y TEJIDOS DE ÓRGANOS	PAOLA BOVOLENTA/ MARCOS CARDOZO	Role of the transcriptional cofactors Vestigial-like protein 4 (VGLL4) and Yes-associated protein 1 (YAP1) in zebrafish morphogenesis
22/09/2022	ANTONIO LAHERA	DINAMICA Y FUNCION DEL GENOMA	JOSÉ FERNANDEZ PIQUERAS/MARÍA VILLA MORALES	La desregulación de STAT5 en las neoplasias linfoblásticas de células T: bases moleculares subyacentes y posibles líneas de tratamiento
07/10/2022	BEATRIZ SOTO HUELIN	PROCESOS FISIOLOGICOS Y PATOLOGICOS	Mª DOLORES LEDESMA	Aproximaciones terapéuticas para tratar la patología cerebral en la enfermedad de Niemann Pick tipo A

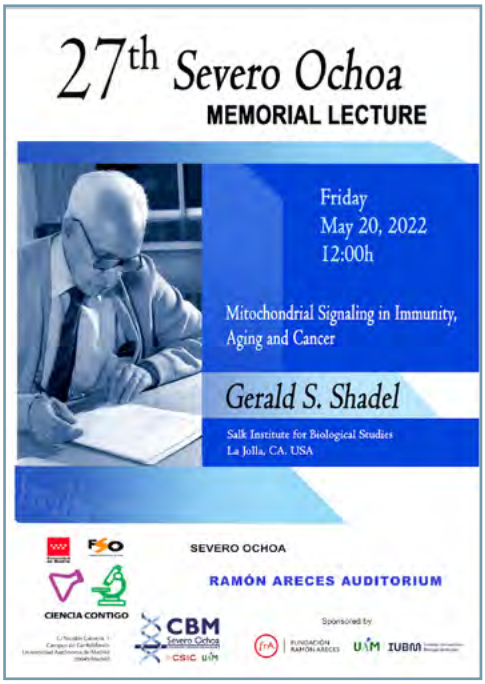
DOCTORAL THESES 2021-2022

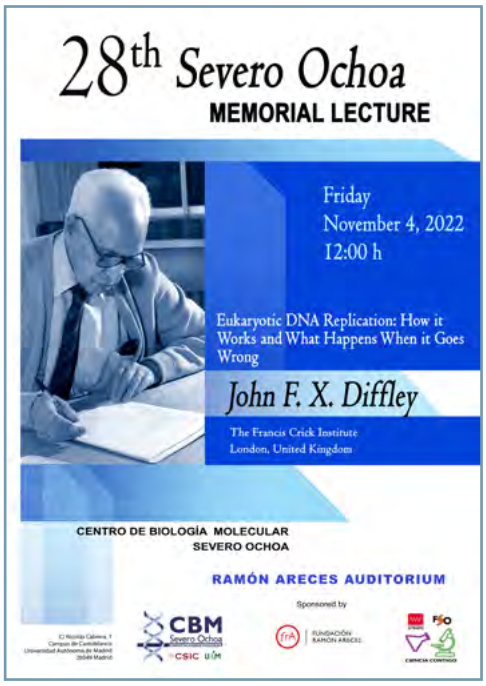
2022

DATE	DOCTORAL STUDENT	PROGRAM	DIRECTOR	TITLE
10/11/2022	JULIA TERREROS RONCAL	PROCESOS FISIOLOGICOS Y PATOLOGICOS	MARIA LLORENS	Neurogénesis hipocampal adulta en sujetos neurológicamente sanos y pacientes con enfermedades neurodegenerativas
11/11/2022	MIREYA RUIZ LOSADA	HOMEOSTASIS Y TEJIDOS DE ÓRGANOS	CARLOS ESTELLA	Coordinación entre la progresión del ciclo celular y la actividad proapoptótica de p53 en respuesta al daño en el ADN
18/11/2022	ALVARO CASADOMÉ PERALES	PROCESOS FISIOLOGICOS Y PATOLOGICOS	CARLOS G. DOTTI	Cambios en la composición de las vesículas extracelulares de la corteza cerebral del ratón durante el envejecimiento: enfoque en la Ceramida sintasa 2
02/12/2022	SAMARA MARTÍN ALONSO	INTERACCIONES CON EL ENTORNO	LUIS MENÉNDEZ	Molecular determinants of strand displacement polymerization by HIV reverse transcriptases
16/12/2022	ANA TOLEDANO ZARAGOZA	PROCESOS FISIOLOGICOS Y PATOLOGICOS	MARIA DOLORES LEDESMA	Alteraciones de mGluR5 y el sistema endocannabinoide en la patología psiquiátrica de Niemann Pick Tipo C.
16/12/2022	RAQUEL GARCÍA BELMONTE	INTERACCIONES CON EL ENTORNO	YOLANDA REVILLA/ DANIEL PÉREZ	El Virus de la Peste Porcina Africana modula la vía de señalización cGAS-STING a través del factor de virulencia MGF505-2R
16/12/2022	SONIA DOMINGUEZ ZORITA	PROCESOS FISIOLOGICOS Y PATOLOGICOS	JOSÉ MANUEL CUEZVA	Implicaciones fisiopatológicas de la ATP sintasa mitocondrial en la homeostasia del colon

MEMORIAL LECTURES 2021-2022

DATE	NAME	CENTER	TITLE
20/05/2022	27 LECCIÓN CONMEMORATIVA SEVERO OCHOA	Gerald S. Shadel (Salk Institute for Biological Studies, La HOLLA, CA, USA)	Mitochondrial Signaling in Immunity, Aging and Cancer
04/11/2022	28 LECCIÓN CONMEMORATIVA SEVERO OCHOA	John F. X. Diffley (The Francis Crick Institute, London, UK)	Eukaryotic DNA Replication: How it Works and What Happens When it Goes Wrong





SPECIAL SEMINARS 2021-2022

2021

DATE	NAME	CENTER	TITLE
28/05/2021	Francisco Verdeguer	Universidad de Zurich. The Switzerland	Transcriptional regulation of energy homeostasis
23/09/2021	Michael N. Sack.	Cardiovascular Branch, National Heart, Lung and Blood Institute, NIH (Bethesda, Maryland, USA	Identifying fasting and NAD+-dependent regulatory pathways modulating innate and adaptive inmunity
30/09/2021	Laura Molina García	Dept. Cell and Developmental Biology. University College London, London, UK	From bacterial amyloids to neural circuits and beyond
4/10/2021	Adolfo García Sastre	Icahn School of Medicine at Mout Sinai, New York, USA	New vaccine strategies for influenza and COVID-19
05/10/2021	Ana Fernández Sesma	Icahn School of Medicine at Mout Sinai, New York, USA	Modulation of human immune responses by RNA viruses
25/11/2021	Josep Sardayés Cayuela	Centre de Recerca Matemàtica, Barcelona, Spain	Mathematical approaches to RNA virus dynamics and evolution

2022

DATE	NAME	CENTER	TITLE
24/05/2022	Guillermo Oliver	Northwestern University, Evanston, IL, USA	The lymphatic vasculature: developmental mechanisms and novel functional roles
06/06/2022	Lieven de Veylder	VIB center of Plant Systems Biology, Ghent, Belgium	Cell death activated root stem cell regeneration
10/06/2022	Alejo Rodríguez	IRB Barcelona, Spain	Clonal Determinants of Stem Cell Heterogeneity
16/06/2022	Julia Liu	University of Minnesota Medical School, USA	Consequences of mitochondrial calcium dysregulation *in vivo*
05/07/2022	Asier Echarri	Centro Nacional de Investigaciones Cardiovasculares, Madrid. Spain	Mechanotransduction at a glance: From the plasma membrane to the nucleus and beyondClonal Determinants of Stem Cell Heterogeneity
26/09/2022	Estela Area Gómez	Centro Investigaciones Biológicas "Margarita Salas". Madrid, Spain	The role of MAM in the regulation of cholesterol:The role of MAM in the regulation of cholesterol:Relevance for age-related disorder
11/10/2022	Esteban Hoijman	Biomedical Research Institute (IDIBELL). Barcelona. Spain	Surveillance of stem cells in early embryos: quantitative live imaging of epithelial phagocytosis
19/10/2022	Rafael Radi	Centro de Investigaciones Biomédicas (CEINBIO), Universidad de la República. Montevideo, Uruguay	The role of metabolic carbon dioxide in the modulation of cellular redox signaling and stress
03/11/2022	Gabriel Arismendi Morllo	Instituto de Investigaciones Biológicas, Univ. Zulia, Maracaibo, Venezuela	Mitocondria y cáncer: ¿una puerta para terapias no tóxicas?
08/11/2022	Takashi Hiiragi	Hubrecht Institute, Utrecht, Netherlands	Multicellular coordination in context
15/11/2022	María Alieva Krasheninnikova	Prinses Maxima Center for Pediatric Oncology, Utrecht, the Netherlands	Depicting the behavioral-transcriptomic landscape of engineered T cells to understand and improve cancer immunotherapy
13/12/2022	Alain Juan de Solis	Max Planck Institute for Metabolism Research. Germany	Coordinated roles of hypothalamic neurocircuits in the control of metabolism

SEVERO OCHOA SEMINARS CYCLE 2021-2022

2021

DATE	NAME	CENTER	TITLE
15/01/2021	Annemiek van Spriel	Radbound UMC, Nijmegen, The Netherlands	Tetraspanins: molecular organisers of the immune cell surface
12/02/2021	Katalin Susztak	Perelman School of Medicine, University of Pennsylvania. Philadelphia, Pennsylvania, USA	Tubular Cell Metabolic Reprogramming in Renal Fibrogenesis"
26/02/2021	Volker Müller	Johann Wolfgang Goethe-Universität Frankfurt am Main, Germany	An ancient pathway for CO2 reduction: from the origin of life to modern biotechnology
12/03/2021	Íñigo Martincorena	Wellcome Sanger Institute, Hinxton, Cambridgeshire UK	Somatic mutation and clonal expansion in normal tissues
09/04/2021	Fátima Gebauer	Gene regulation, stem cells, and cancer. Centre for Geneomic Regulation, Barcelona	RNA binding proteins in cancer progression
22/10/2021	Jonas Frisen	Department of cell and molecular biology. Karolinska Institu. Sweden	New cells in old brains
12/11/2021	Lluis Montoliú	Centro Nacional de Biotecnología (CNB) Madrid, España	Los retos de la experimentación animal hoy en día
23/11/2021	David M. Gilbert	Biological Sciencie, Florida State University. USA	Regulation OfReplication Timing And Chromosome Architecture
26/11/2021	Cristian Frezza	MRC Cancer Unit, University of Cambridge. Cambridge, UK	Fumarate hydratase loss as a paradigm of oncometabolism
03/12/2021	Magdalena Goetz	Ludwig-Maximilians-Universität München, BioMedical Center– BMC, Germany	Novel mechanisms of neurogenesis and neural reapair

2022

DATE	NAME	CENTER	TITLE
04/02/2022	Thorsten Hoppe	Institute of Genetic. CECAD Research Center, University of Cologne, Cologne, Germany	Ubiquitin Tips the Balance: Coordination of Protein Homeostasis & Aging
25/02/2022	Angel Barco	Instituto de Neurociencias, San Juan de Alicante, Alicante	Epigenetic mechanisms in neuronal plasticity & intellectual disability
18/03/2022	Pura Muñoz-Cánoves	Centro Nacional de Investigaciones Cardiovasculares, CNIC. Madrid, Spain	New approaches to enhance regeneration of aged skeletal muscle
25/03/2022	Nicolás Manel	Institut Curie, PSL Research University, Paris, France	Intracellular DNA sensors: from antiviral immunity to cancer immunotherapy and aging
01/04/2022	Luis Angel Fernández Herrero	Centro Nacional de Biotecnología (CNB) Madrid, Spain	Synthetic biology of *E. coli* for cancer therapy and antibody generation
22/04/2022	Philippe Pasero	Institute of Human Genetics, CNRS and University of Montpellier, France	Cellular responses to replication stress: from stalled forks to cytosolic DNA and beyond
06/05/2022	Ingrid Lohmann	Centre for Organismal Studies (COS), Heidelberg University, Heidelberg, Germany	Hox Transcription Factors in *Drosophila* Development
13/05/2022	Silvia Paracchini	School of Medicine, University of St Andrews, St. Andrews, UK	Multidisciplinary approaches to dissect the link between handedness, brain asymmetries and neurodevelopmental disorders
16/09/2022	Iñaki Comas	Instituto de Biomedicina de Valencia. Valencia. Spain	Genomic approaches for global health pathogens:Genomic approaches for global health pathogens:from biology to infection control
14/10/2022	Theodore Alexandrov	European Molecular Biology Laboratory (EMBL), Heidelberg, Germany	Spatial single-cell metabolomics reveals metabolic cell states of hepatocytes and immune cells
11/11/2022	Almudena Ramiro	Centro Nacional de Investigaciones Cardiovasculares CNIC, Madrid, Spain	Antibodies meet the atheroma plaque: a match made in heaven?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SCIENTIFIC REPORT CBM 2021-2022

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