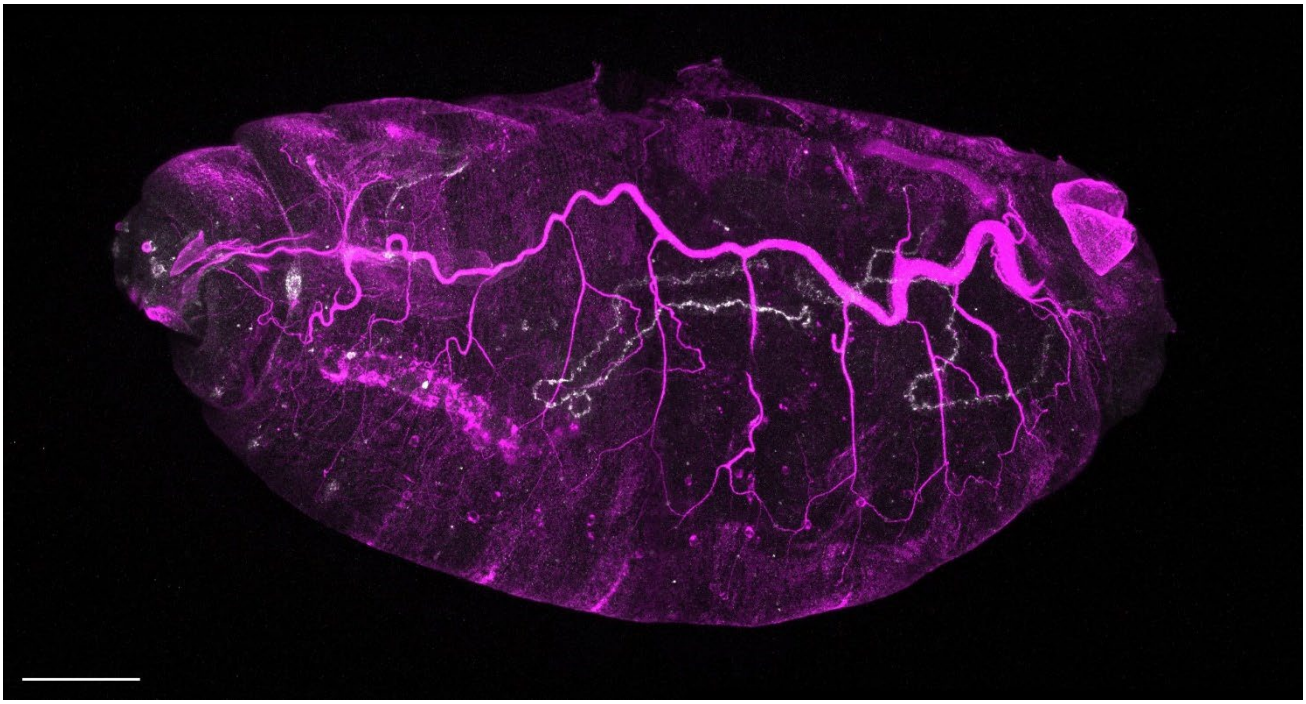
Centro
Andaluz
de Biología
del Desarrollo

Annual Report 2024



EXCELENCIA MARÍA DE MAEZTU

AGENCIA ESTATAL DE INVESTIGACIÓN

Junta de Andalucía

UNIVERSIDAD PABLO DE OLAVIDE SEVILLA

CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

CABD Annual Report 2024

Content

CABD Activities 2024 4
CABD Facts & Figures 2024 6
Vertebrate development and evolution 7
Sy(stem)s Developmental Biology 8
Coordination of stem cells, physiology and behaviour 9
Computational Biology and Bioinformatics 10
Development of the Hemato-Vascular System and Stem Cells 11
Mechanisms of Gene Regulation and Bacterial Biofilm Development 12
Genetic regulation of ageing in *Caenorhabditis elegans* 13
Mechanisms regulating cell shape changes and cell migration 14
Cell fate determination in early mammalian development 15
Programmed cell death during development and disease 16
Cell Division Control 17
Global regulation of gene expression and metabolism of environmental bacteria: *Genetic and species rearrangements in microbial consortia impact biodegradation potential* 18
Developmental Control 19
CRISPR-Cas in vivo optimizations to understand early vertebrate development and human diseases 20
Molecular Evolution of Microbial Interactions Lab (MolEMI Lab) 21
Regulatory mechanisms of gene expression in pollutant-degrading bacteria Bioremediation 22
Mitotic spindle nucleation and coordination with the cell cycle 23
Gene regulation and evolution 24
Mitochondrial signalling control in the regulation of ageing 25
Fungal and yeast biotechnology 26
Epigenetic regulation of virulence in plant pathogenic fungi 26
Developmental Epigenomics 27
Self-Organization of Biological Systems 28
Genetics and functional genomics of genes with small open reading frames 29
Regulation of coenzyme Q synthesis and its implications for mitochondrial health 30
Morphogenesis and mechanosensitive gene regulation 31
Bioinformatics for biological sequence analysis 32
Epigenomics in disease and aging 33

Nuclear Dynamics in Cell and Developmental Biology 34
3D Genomics 35
Molecular Embryology: Functional genomics, mouse transgenesis and myogenesis 36
Stem cell behaviour in their niches and epithelial morphogenesis 37
Nuclear Architecture and Dynamics 38
Control of epithelial morphogenesis in vertebrates 39
Biotechnology of industrial and artisan fermentations. Competitivity factors in microbial communities and their application. 40
Evolution of organogenetic gene networks 41
Bacterial infections: new targets and new prevention and treatment strategies 42
Obtaining New Antimicrobial Compounds and New Antibiotic Resistance Genes Through a Metagenomic Approach 43

Cover: *Drosophila melanogaster* embryo. Visualization of secreted chitin. Labelling is observed in the cephalopharyngeal skeleton and other chitinous structures such as the tracheae (Author: Daniel Barcenilla Merino).

CABD Activities 2024

The Andalusian Centre for Developmental Biology (*Centro Andaluz de Biología del Desarrollo*, CABD) is devoted to investigating the architecture and function of living organisms. Since its foundation in 2003, the CABD has grown steadily and is widely recognised as a centre of scientific excellence. The success of the centre is reflected by two consecutive *María de Maeztu* (MdM) Unit of Excellence awards from the Spanish *Agencia Estatal de Investigación* (2017-2021, 2022-2025). The centre hosts 28 established groups and 4 emerging groups that investigate fundamental aspects of development, genetics, cell biology and microbiology. Many of the processes studied at the CABD are relevant to human health, and several CABD groups collaborate closely with clinicians and patient associations to explore biomedical perspectives.

One group leader (internal promotion) and one junior group leader (external recruitment to incorporate in 2025) were appointed in 2024. New incorporations of pre- and postdoctoral researchers, as well as staff to scientific platforms, service facilities and administration raised the CABD personnel to 270 by the end of 2024. This number includes 48 undergrad students (mainly B.Sc., M.Ms. and FP students) and visitors, reflecting the extensive CABD efforts in training of early career scientists. The personnel are contracted by the Spanish National Research Council (CSIC) and the Pablo de Olavide University (UPO) in roughly equal numbers whereas institutional support is also provided by the Andalusian Regional Government (Junta de Andalucía). The academic activities of CABD personnel in 2024 included a significant number of lectures, practical classes, planning as well as supervision of students at B.Sc., M.Sc., and Ph.D. level: 89 theses were successfully defended at the CABD in 2024.

Funding for 24 new research projects was obtained through competitive calls from regional, national, and international agencies, worth a total of ~5.5 million euros. CABD researchers were also involved in multiple contracts to foster collaborations between public and private entities through for instance the newly started spinoff company ONESTX. The scientific productivity of the CABD in terms of publications amounted to 92 peer-reviewed articles and book chapters and ~4,900 citations during 2024, mostly with open access in leading journals and with the participation of numerous scientists from international institutions. This reflects the open and collaborative spirit of CABD researchers, which is also evident from the many contributions to congresses and the organization of large scientific events, such as the symposium “New Mathematical Approaches in Developmental Biology” celebrated at the 9th European Conference in Mathematics in Seville in 2024. Moreover, the CABD is very active in science dissemination with tailored events for a variety of audiences across age and educational backgrounds.

2024 was the third year of the CABD’s 4-year strategic plan “*Decision Making in Cell Collectives across Scales*” (DMC3). The overarching objective of DMC3 is to understand how cell collectives have evolved and how they are organized to generate and maintain complex living organisms. To achieve a complete picture, the objective is approached at cellular, organismal, and ecological level using a variety of computational, genomics, proteomics and imaging techniques. The strategic plan is organized around five central questions and from the individual group reports included in the following pages, it is evident that many interesting answers are being discovered for each of them. Some CABD groups host associated scientists with independent projects, bringing the number of individual reports to 39.

One of the central questions deals with the relationship that regulatory and structural changes in the genome have with evolutionary innovations (“Q1”). This has been addressed in several studies in 2024. For instance, by comparing 3-dimensional genome structure in neural retina and retinal pigment epithelium, a joint effort between two CABD groups and international collaborators identified cis-regulatory elements implicated in retinal disease. Another study compared gene promoters involved in limb development in mouse and pigs and found evidence for evolutionary rewiring in gene regulatory networks. Single-cell technologies were used to determine gene regulation in variety of biological processes, such as in embryoid bodies, bat wing development and mammalian sex determination. Differential use of regulatory elements was also demonstrated in flagellated bacteria where they control the switch between biofilm and dispersal live styles. Furthermore, comprehensive analyses of microbial consortia were carried out in ambitious projects that aim to develop bioremediation protocols to overcome environmental challenges and identify new antimicrobial compounds.

Another central question relates to the coordinated use of gene regulatory networks between neighbouring cells and organs, and how cells communicate their states across space and time (“Q2”). This is crucial to control, for example, organ size. Studies in the insects *Drosophila* and *Cloeon* revealed new mechanisms that ensure minimal variation both in normal development and during regeneration. *Drosophila* was also used to demonstrate how aging affects specific cell populations in the germline stem cell niche and how gene networks are rewired to control signalling pathways in different organs. Studies in zebrafish have identified novel factors involved in maternal-to-zygotic transition in early embryos whereas a mouse model for hypoxic-ischemic brain injury in neonates was used to investigate transcriptional changes in blood cells by single-cell transcriptomics.

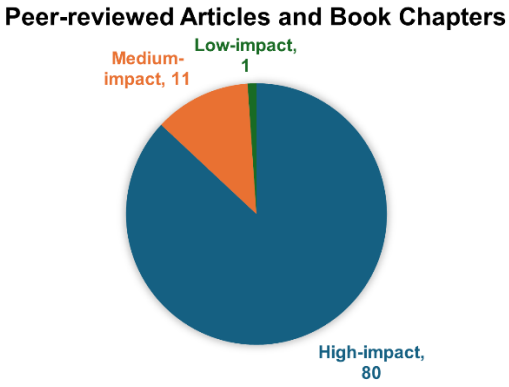
The capacity of cells to sense and respond to changes in their environment represents the third central question: which are the underlying mechanisms and what role do they play in cell collectives’ behaviour (“Q3”)? An innovative project in *Drosophila* described how males sense the presence of females and respond by secreting hormones and growth factors from the brain and muscle, respectively, to increase stem cell proliferation in the testis. Other CABD researchers used *Drosophila* to study the evolution and function of small open reading frames as well as the contribution of the extracellular matrix to epithelial morphogenesis and homeostasis. The interplay between either budding yeast and totiviruses or corn and the fungus *Ustilago maydis* are other examples of how cells modulate their behaviour in response to challenges, in these cases between species. For instance, the Row proteins were discovered as a novel *Ustilago* protein family required during the infection process. Another study focused on the role of histidine kinases in controlling transcriptional responses in the bacterium *Sphingopyxis granuli* TFA upon exposure to environmental stresses. Stress also affects protein folding and a new study in fission yeast revealed that the proteasome assembly chaperone Ump1 is required to maintain proteostasis and suggested that Ump1 deficiency may play a role in aging. In humans, cancer cells experience changes in oxygen and nutrient availability during tumorigenesis, which induces both epigenome and transcriptome remodelling as reported in collaboration with an international team of researchers.

Spatial organization of the genome within the cell nucleus and its physical properties are the theme of the fourth question in the CABD strategic plan (“Q4”). A novel project uses live microscopy in both fission yeast and flies to examine the role of cohesin in chromatin structure during cell proliferation and differentiation. The relevance of chromatin organisation was also addressed in the context of aging through the establishment of a novel *C. elegans* progeria model, which enabled the identification of potential modifiers of age-induced damage. Although the genome of eukaryotic cells is enclosed by the nuclear envelope, it perceives physical stimuli through multiple mechanosensing pathways. One of these involves the YAP protein, which has been the focus of new studies in both zebrafish and mouse embryos.

The fifth question relates to energy constrains and metabolic states and the role they play in tissue morphogenesis, aging and disease (“Q5”). Several CABD groups focus on the biogenesis and function of mitochondria, an organelle that is central to these processes, using a variety of models, including yeast, nematodes, flies, fish, mice and organoids as well as cells derived from human patients. In 2024, these studies identified a new link between mitochondrial function, metabolic flux and the transcriptional coregulator SIN3. They also uncovered that prenatal administration of coenzyme Q10 alleviate muscle defects caused by mitochondrial impairment in mice. Muscle development was further analysed by perturbations in the myogenic regulatory factors Myrf5 and Mrf4. CABD researchers also work closely with patient associations to study samples from individual patients with the potential to develop personalised treatments. Finally, efforts are also devoted to understanding the evolution of microbiota within mammalian hosts and how this may affect human health.

Computational tools are increasingly important in biological research and several CABD groups are involved in their development and implementation. For instance, comparing thousands of bacterial genomes revealed a sophisticated warfare between two bacterial viruses, with potential implications for genome editing purposes and for the use of bacteriophages to combat infections. In another study, *in silico* pharmacokinetic evaluation of >7,000 compounds was performed to accelerate the discovery of potential drugs for the treatment of *Acinetobacter baumannii* infections. Finally, researchers at the CABD developed in 2024 powerful protein language models to automate functional annotation, thereby overcoming an important obstacle to extracting information from the rapidly growing genome databases.

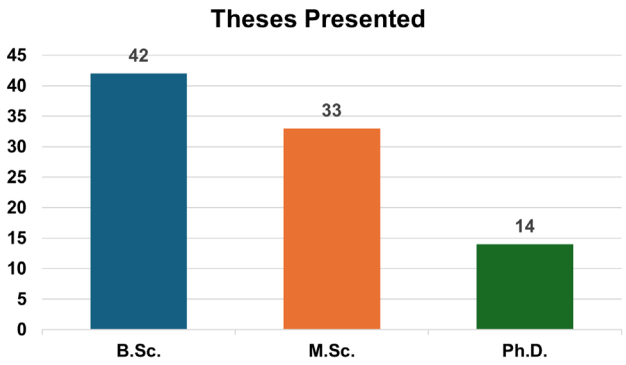
CABD Facts & Figures 2024



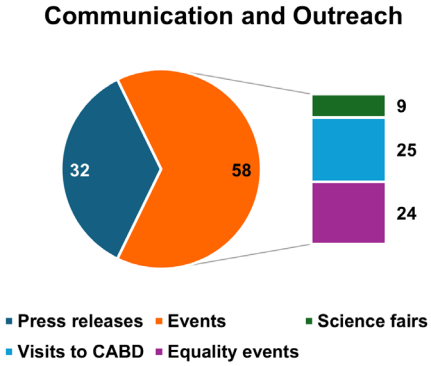
We published 92 peer-reviewed articles and book chapters in 2024. Of these, 80 (87%) were classified as high-impact by the CSIC, 11 (12%) as medium-impact and 1 (1%) as low-impact. Co-authors from foreign institutions contributed to 56 (61%) of the publications.



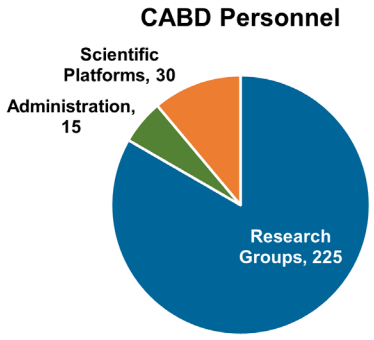
The CABD obtained 24 grants from competitive funding schemes: 17 from the Spanish Government, 1 from the European Commission, 1 from the Human Frontier Science Program and 5 from the University Pablo de Olavide.



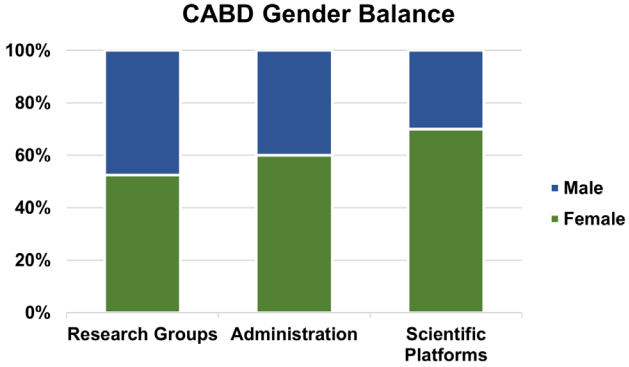
A total of 89 theses were presented in 2024. The majority represented B.Sc. projects (47%), followed by M.Sc. projects (37%) and doctoral theses (16%).



We were in contact with more than 3000 persons across all ages – mainly school and high school students – through 58 outreach events in 2024.



Most CABD personnel are associated to Research Groups (83%), whereas 11% and 6% work in Scientific Platforms and Administration, respectively.



The majority of CABD personnel are women in the three categories: in total 148 females (55%) versus 122 males (45%).

Vertebrate development and evolution

Javier Lopez-Rios and Gretel Nusspaumer

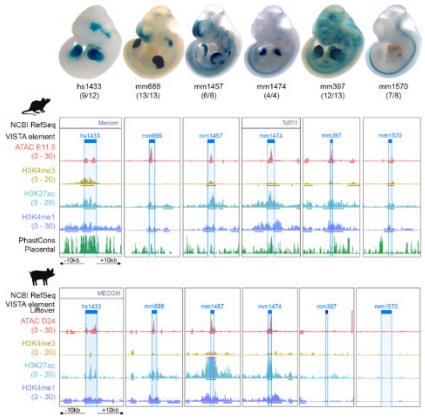
Summary of activities:

Our group is interested in studying the development and evolution of the limb skeleton. This year, we have continued our research on how artiodactyls, particularly pigs, have acquired their highly specialized limb skeletons. As part of these studies, we performed ChIP-seq for various histone marks to profile and categorize the regulatory genome during limb development in mice, which retain the pentadactylous blueprint of tetrapods, and pigs. Comparative analysis of these datasets revealed that while promoters are generally highly conserved at both the sequence and epigenomic levels, there is extensive regulatory divergence in putative enhancers, even among those that are highly conserved. These results support the hypothesis of pervasive rewiring in the gene regulatory networks that control limb morphogenesis. In parallel, we have profiled the transcriptome and epigenome in mouse and pig limb buds using single-cell technology. The resolution of this analysis allowed us to identify several key alterations in morpho-regulatory pathways that are strong candidates for contributing to the anatomical evolution of the distal limb skeleton in artiodactyls. One of the most striking alterations, for example, involves specific expression changes in essential components of the FGF pathway, which we are currently investigating further. This year, we have also expanded our single-cell multiomic approaches to study the etiology of Ewing sarcoma -a rare and aggressive malignant bone and soft tissue tumor that arises early in life- as part of the Andalucía Biotec Salud initiative. Finally, we are continuing our characterization of bone heterogeneity at perinatal stages, with a particular focus on the cells that contribute to establishing an immunosuppressive environment in the bone marrow of neonatal mice. This ongoing research is providing fundamental insights into how regulatory and structural alterations contribute to morphological evolution and human disease.

Two highlights:

A gene desert required for regulatory control of pleiotropic Shox2 expression and embryonic survival. Abassah-Oppong S et al. Nat Commun. 2024 Oct 10;15(1):8793. doi: 10.1038/s41467-024-53009-7.

The cellular landscape of the endochondral bone during the transition to extrauterine life. Rueda AD, Salvador-Martínez I, Sospedra-Arrufat I, Alcaina-Caro A, Fernández-Miñán A, Burgos-Ruiz AM, Cases I, Mohedano A, Tena JJ, Heyn H, Lopez-Rios J, Nusspaumer G. Immunol Cell Biol. 2024 Feb;102(2):131-148. doi: 10.1111/imcb.12718.



Promoters active during mouse and pig limb development are highly constrained, while approximately 40% of *bona fide* enhancers lose their epigenomic signature in the other species. For example, some conserved and validated VISTA elements (top row) exhibit enhancer epigenomic signatures in both species, whereas others (e.g. the last two examples on the right) have lost these characteristics in pig limb buds (Sospedra et al., submitted).

Sy(stem)s Developmental Biology

Elena Camacho Aguilar

Summary of activities:

In November 2023, our lab opened at the CABD to study how cells interpret dynamic signaling to form precise embryonic patterns, focusing on embryonic-extraembryonic interactions in human development. The team now comprises the PI, a research assistant (Andrea), a doctoral researcher (Miguel) and a undergraduate student (Pablo) in co-supervision with M. José Jimenez (U. Sevilla). An MSc student (Irene) completed her thesis in 2024 under joint supervision with Fernando Casares.

In 2024, we established laboratory activities at the CABD, securing material transfer agreements with Rice and Rockefeller for human embryonic and induced pluripotent cell lines (hPSCs and iPSCs). We obtained ethical approval from the Andalusian Ethical Committee (SICEIA) to work with iPSCs and are seeking final permissions for hPSCs from the CSIC Ethics Committee.

With the new cell culture room (opening in August 2024), we cultured iPSCs in both primed and naïve states, verified via immunofluorescence, and are now running differentiation protocols to validate their extended potency. We published an article and submitted a preprint on how human pluripotent stem cells combinatorially interpret BMP4 and WNT, as well as WNT and Nodal signaling: the first (Cell Systems) demonstrates that BMP duration and concentration are not interchangeable due to the combinatorial interpretation with the induced endogenous WNT, while the second (BioRxiv) shows how micropattern confinement changes WNT interpretation via endogenous NODAL.

In 2024, I was awarded a prestigious Ramon y Cajal contract, was invited to speak at 4 conferences and was selected to speak at 4 conferences, including the “From Stem Cell to Human Development 2024” meeting. We collaborate with groups at the University of Seville, the University of Michigan, and the Università degli Studi di Padova to investigate develop new image analysis and modelling methods, patterning, as well as embryonic-extraembryonic interactions in mouse embryos and synthetic models. We co-organized the two-day symposium “New Mathematical Approaches in Developmental Biology” at the 9th European Conference in Mathematics 2024. Outreach activities included talks at IES Ramón Carande, participation in the European Researchers’ Night, and contributing to the ‘New PI Diaries’ blog series.

Two highlights:

Combinatorial interpretation of BMP and WNT controls the decision between primitive streak and extraembryonic fates. Camacho-Aguilar E, Yoon ST, Ortiz-Salazar MA, Du S, Guerra MC, Warmflash A. Cell Syst. 2024 May 15;15(5):445-461.e4. doi: 10.1016/j.cels.2024.04.001.

Endogenous Nodal switches Wnt interpretation from posteriorization to germ layer differentiation in geometrically constrained human pluripotent cells. Ortiz-Salazar MA, Camacho-Aguilar E, Warmflash A.bioRxiv [Preprint]. 2024 Mar 14:2024.03.13.584912. doi: 10.1101/2024.03.13.584912.

Coordination of stem cells, physiology and behaviour

Salvador Herrera

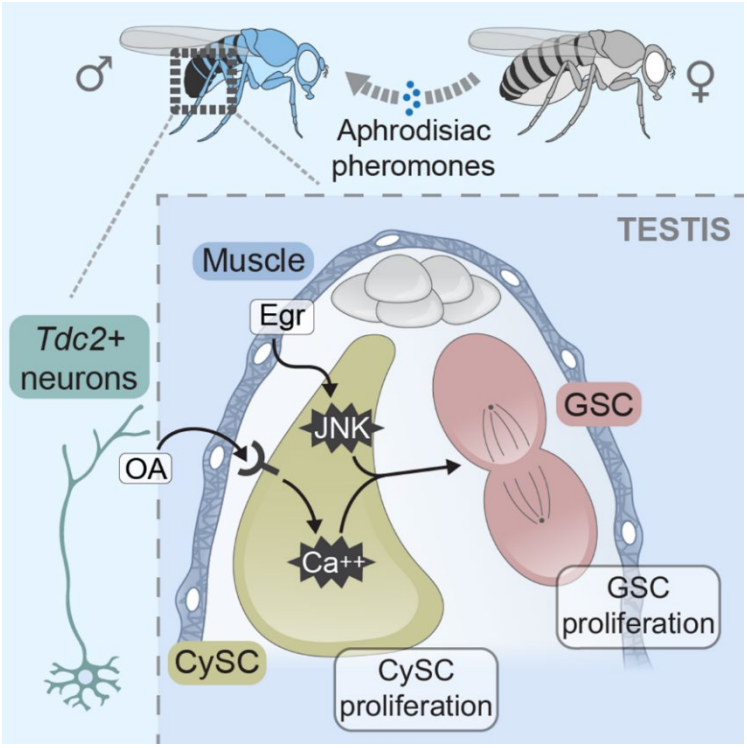
Summary of activities:

During 2024 we have made progress on our research about the effects of social interactions on the stem cell cell biology of the male gonad, using flies as model. We proved that, after sensing the presence of females through pheromones, an activation state is triggered in the two stem cell populations of the testis, increasing their proliferation. This is mediated by inter-organ communication – brain-secreted Octopamine (homologous to the Noradrenalin of vertebrates) and muscle-derived Egr/TNFα. We published these discoveries (Martin-Diaz and Herrera, *Cell Reports*, 2024), proposing that this mechanism coordinates reproductive behaviour and spermatogenesis for the success of male reproduction. The study of this communication between the brain, the gonads and other tissues in response to an external cue (the social environment) is directly related to the question Q3 of the DMC3 strategic plan– how cells respond to their changing environment.

We also started to interrogate how these social environment perception mechanisms change the metabolism and physiological reality of the male, preparing it to cope with the imminent energy demands of reproduction. This research is aligned with the question Q5 of the DMC3 Strategic Plan - the relevance of energy constrains in collective cell decisions.

Highlight:

A stem cell activation state coupling spermatogenesis with social interactions in *Drosophila* males. Martin-Diaz J, Herrera SC. Cell Rep. 2024 Aug 27;43(8):114647. doi: 10.1016/j.celrep.2024.114647.



Model for niche activation induced by female presence.

Computational Biology and Bioinformatics

Ana M Rojas Mendoza

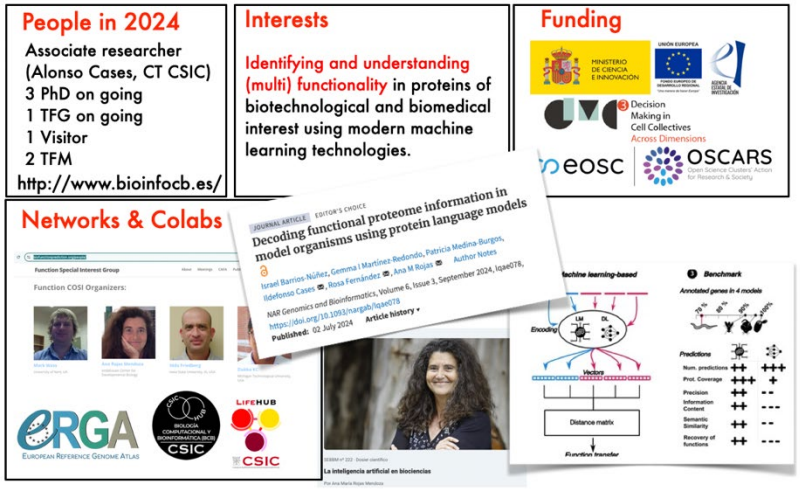
Summary of activities:

Our group has pioneered the large-scale application of protein language models for functional annotation (NARGAB 2024). This innovative work, published as an Editor's Choice, is currently being adopted as the annotation tool by ERGA, the main European biodiversity consortium. This collaboration places us at the core of the annotation effort on a European level. Additionally, we have been invited to contribute to two book chapters, which are set to be published in 2025. The group has secured both national funding (Plan Nacional) and European funding (awarded in 2024) to advance these research lines. Furthermore, we are actively involved in the ENIA Google-USE Chair for Artificial Intelligence as research members. The Principal Investigator (PI) serves on the executive board of Spain's largest computational biology network and the board of the Function Community of Interest of the International Society for Computational Biology. Under our mentorship, we have supervised three undergraduate and master's students, hosted one visiting researcher, and are currently supervising three PhD candidates. Our outreach efforts include participation in public science events such as the Feria de la Ciencia and Ciencia en el Barrio. We have also coordinated a special outreach dossier targeting the broader scientific community, such as the SEBBM special dossier on Artificial Intelligence in Biosciences. In addition to research and outreach, the PI contributes to teaching in three master's programs (USE and UNIA). The group's leading role in integrating Artificial Intelligence into biological research strongly aligns with the objectives of the MdM DMC3 initiative, reinforcing its transversal impact across multiple domains.

Two highlights:

Decoding functional proteome information in model organisms using protein language models. Barrios-Núñez I, Martínez-Redondo GI, Medina-Burgos P, Cases I, Fernández R, Rojas AM. NAR Genom Bioinform. 2024 Jul 2;6(3):lqae078. doi: 10.1093/nargab/lqae078.

Anotación de función en proteínas usando modelos de lenguaje. Rojas Mendoza AM, Cases I, Martinez-Redondo G, Fernández R. 2024. *SEBBM* 222. https://doi.org/10.18567/sebbmrev_222.202412.dc4



Development of the Hemato-Vascular System and Stem Cells

María José Sánchez Sanz

Summary of activities:

Blood cells play a crucial role in tissue damage repair. We initiated a study to characterize changes in blood cells and vascular precursors in response to hypoxic-ischemic brain injury in neonates.

Neonatal hypoxic-ischemic encephalopathy (NHIE) is highly prevalent among newborns. Early detection is crucial for mitigating its devastating effects. This study aims to characterize changes in circulating blood cells in NHIE to identify potential biomarkers of tissue injury.

We compare a neonatal hypoxic-ischemic brain injury mouse model with human blood samples from newborns potentially exposed to NHIE-related damage. In both systems we examine circulating cells using single-cell transcriptomics (scRNA-seq) and validate our findings through flow cytometry and RT-qPCR (Figure 1).

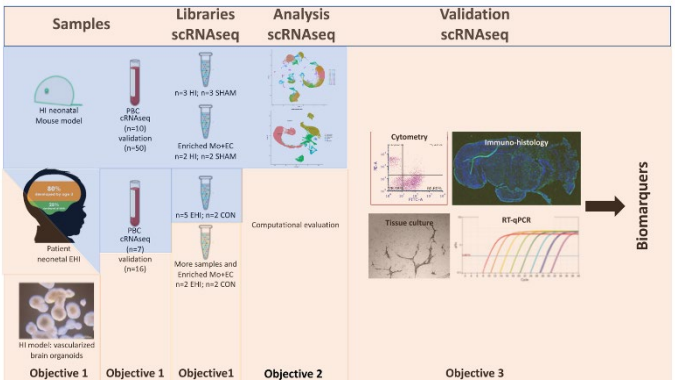
Results from the mouse model reveal decreased transcriptional regulation of inflammatory responses in monocyte, neutrophil, and T lymphocyte during the 'reparative' phase, 48 hours after HI injury. Additionally, we found circulating monocyte subsets with a pro-angiogenic and migratory transcriptional profile. Specifically, Ly6clow Ccr2low monocytes exhibited elevated transcriptional levels of metalloproteases and showed increased expression of genes associated with lipidogenesis and glycogenesis reflecting a reparative signature. These findings suggest that after the acute pro-inflammatory phase (6–24 hours post-injury), reparative mechanisms are driven in monocytes by the activation of a pro-angiogenic program, alongside a coordinated reduction in inflammatory responses across multiple cell populations.

For human samples, five NHIE and two controls samples have been scRNA-seq processed.

This investigation is in alignment with the DMC3 Strategic Plan Q2 & Q3, Cell Collectives at the single-cell Resolution and Stem Cells and Human Development.

Highlight:

scRNAseq transcriptomic analysis of circulating immune and progenitor cells in an experimental model of hypoxic/ischemic neonatal brain damage. Sánchez MJ, Saez Goñi ME, Rangel C, Reyes Corral M, Lao Perez M, Juarez Calvillo C and Ybot-Gonzalez P. 2023. Poster Presentation at the XX Spanish Society for Cell Biology Meeting, Cordoba 13-16 November, Spain.



Circulating Blood and Progenitor cells as Biomarkers of Neonatal Hypoxic/Ischemic Encephalopathy (NHIE). Schematic representation of the experimental design. Blue background: completed experiments; Orange background: ongoing work.

Mechanisms of Gene Regulation and Bacterial Biofilm Development

Fernando Govantes

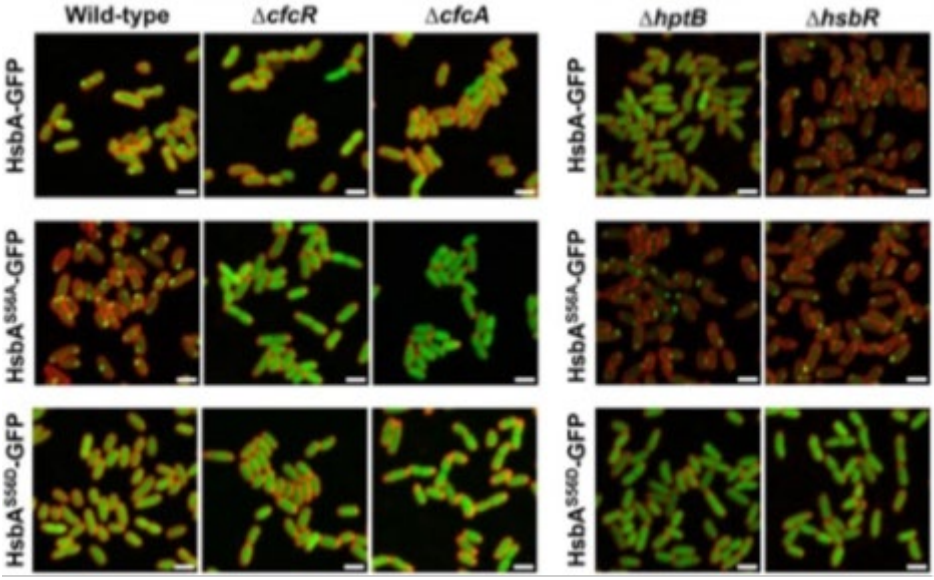
Summary of activities:

We established a molecular basis for the assembly of different numbers of flagella in polarly flagellated bacteria. We completed the characterization of the flagellar transcriptional cascade by establishing a unique role for the two-component system FleS-FleR as backup for the master regulator FleQ. This work establishes mechanisms for cell polarity in bacteria and the self-assembly of the flagellar apparatus (Q3). We characterized a membrane-bound complex involved in the regulation of biofilm formation and dispersal under nutritional stress, highlighting the use of common regulatory elements to achieve opposite results as a function of pathogenic/free living lifestyles (Q1/Q3/Q5). Our collaboration with Alejandro Cuetos at UPO yielded a computer model of nutrient-driven biofilm colony stratification and simulation work on biofilm colony collision interphases and internal pressure profiles in biofilm colonies that emphasize the essential role of the interaction between living cells and their physical environment (Q2). In collaboration with Mª. Trinidad Gallegos (EEZ, CSIC), we lipopolysaccharide as an essential component of the biofilm matrix in the plant pathogen *Pseudomonas syringae*. We also identified two small regulatory RNAs strongly induced in biofilms of this organism. We revealed major discrepancies in biofilm development among members of the genus *Pseudomonas* (Q1/Q3).

Two highlights:

Spatial, temporal and numerical regulation of polar flagella assembly in *Pseudomonas putida*. Pulido-Sánchez M, Leal-Morales A, López-Sánchez A, Cava F, Govantes F. Microbiol Res. 2025 Mar;292:128033. doi: 10.1016/j.micres.2024.128033.

Computer simulation study of nutrient-driven bacterial biofilm stratification. Lobo-Cabrera FJ, Herrero MDR, Govantes F, Cuetos A. J R Soc Interface. 2024 Jun;21(215):20230618. doi: 10.1098/rsif.2023.0618.



Assembly of a membrane-bound complex containing HsbA, CfcR and CfcA as a function of the presence of the three proteins and the phosphorylation state of HsbA.

Genetic regulation of ageing in *Caenorhabditis elegans*

Manuel J. Muñoz

Summary of activities:

As professor at the University Pablo de Olavide I taught 160 hours of classes, divided between the biotechnology degree and the master's degree in health biotechnology. In research, the main dedication has been the fulfillment of the objectives of the project “Regulation of longevity and associated diseases by sulfated steroid hormones” PID2020-120563RB-I00 MICIN (2021-2024). In addition, I have been carrying out coordination tasks as CSO and founder member of the company Olavide Neuron STX, in which we have obtained two grants, one from CDTI (ONESTX-01: New oral medication for treatment of neurodegenerative diseases. SNEO-20222370 2023-2024. Funding: 325,000) and another from the AEI (Alteration of sulfated neurosteroid levels for the diagnosis and therapy of Alzheimer's disease. CPP2022-009908 2023-2026. Public-Private Partnership. Funding: 805,443.47). We have also obtained a new grant entitled “Potencial terapéutico de un inhibidor de sulfatasa de esteroides en la enfermedad de huntington y factores implicados en su efecto antineurodegeneración” 2024-2027 Funding: 957,181.15. We received the third prize of I edition Dissemination Awards of Biomol. Using an invertebrate and a mammal model we have proposed and published a simple intervention for better management of type III galactosemia patients. The model of this genetic disease was established by our group previously.

Two highlights:

Monosaccharides improve symptoms of an animal model for type III galactosemia, through the activation of the insulin pathway. Lucas-Rodríguez P, Brokate-Llanos AM, Hernandez-Curiel JM, Murdoch PDS, Garzón A, Carrión A, Muñoz MJ. Biomed Pharmacother. 2024 Dec;181:117677. doi: 10.1016/j.biopha.2024.117677.

Ribonucleotide reductase inhibition improves the symptoms of a *Caenorhabditis elegans* model of Alzheimer's disease. Brokate-Llanos AM, Sanchez-Ibañez M, Pérez-Jiménez MM, Monje-Moreno JM, Gómez-Marín C, Caro C, Vivar-Rios C, Moreno-Mateos MA, García-Martín ML, Muñoz MJ, Royo JL. G3 (Bethesda). 2024 May 7;14(5):jkae040. doi: 10.1093/g3journal/jkae040.

Mechanisms regulating cell shape changes and cell migration

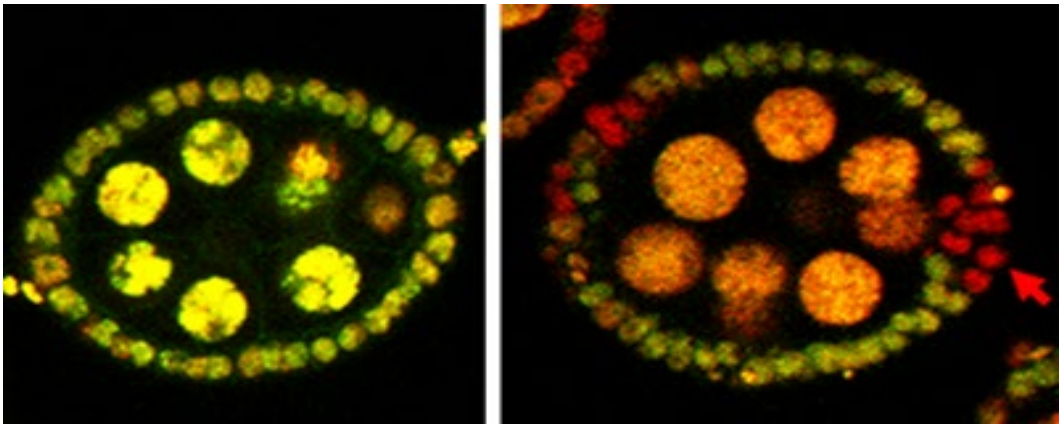
María Dolores Martín Bermudo

Summary of activities:

Our laboratory has 2 main lines of research: 1) uncovering mechanisms regulating cell migration and invasion, and 2) understanding the contribution of cell-extracellular matrix (ECM) interactions and actomyosin contractility to epithelia morphogenesis and homeostasis. We use a multidisciplinary approach combining genetics with transcriptomics, 4D imaging, biophysics and computational exploration. On the role of cell-ECM interactions in epithelia morphogenesis and homeostasis, we have found that forces generated by cell-ECM interactions and actomyosin contractility need to be counterbalanced to preserve the architecture of monolayer epithelia (see illustration below). In fact, we have found that integrins preserve epithelial structures by regulating cellular tension and ECM stiffness during division and rearrangement, a work that has been sent for publication. In addition, we have found that the ECM mediates a crosstalk between tumorigenic epithelial cells and adjacent mesodermal cells, which modulates tumorigenesis. At present, using scRNAseq, we are trying to identify signals regulating this crosstalk

Highlight:

”Cell-BM interactions and epithelia monolayer architecture”. Oral presentation at the EMBO workshop “Molecular and Developmental biology of *Drosophila*” June 2024, Crete, Greece.



Left: normal monolayer epithelium; right: removal of integrins disrupts the monolayer leading to a multilayer epithelium (red arrow).

Cell fate determination in early mammalian development

Bárbara Pernaute

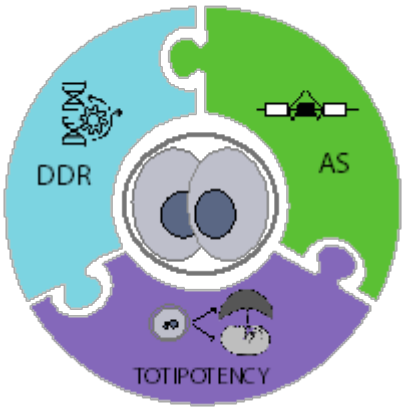
Summary of activities:

Our group studies the mechanisms underlying early development in mammals, focusing on the epigenetic and transcriptomic reprogramming that takes place after fertilization which make the embryo totipotent and unique in its cellular and genomic context. In 2024 I consolidated my recently created group with the incorporation of a master student and a postdoctoral researcher, and the establishment of the resources required to implement our projects, including the generation of KO and reporter mouse embryonic stem cell (mESC) lines and the setup of protocols for mESC reprogramming to totipotency and mouse embryo micromanipulation. This allowed us to progress on two research lines: the analysis of the role of YAP isoforms on embryo pluripotency maintenance, and the modulation of DNA damage responses upon mESC reprogramming to totipotency. In December 2024 I was awarded an ERC Consolidator Grant to investigate, within the embryo, the interplay between DNA damage responses, splicing regulation and reprogramming to totipotency. Collaborations with the groups of Elvan Boke and Thomas Graf at the CRG resulted in a paper published in Cell on proteostasis maintenance in the mammalian oocyte (Zaffagnini et at 2024) and one submitted on the role of CEBPa during the first lineage decision of the mammalian embryo (Wei et al). Finally, I have engaged with numerous outreach activities to bring our research on developmental biology closer to the general public. Among others, I would highlight my participation as invited speaker at the Area de Ciencia of the 2024 Feria del Libro de Granada.

Two highlights:

Mouse oocytes sequester aggregated proteins in degradative super-organelles. Zaffagnini G, Cheng S, Salzer MC, Pernaute B, Duran JM, Irimia M, Schuh M, Böke E. Cell. 2024 Feb 29;187(5):1109-1126.e21. doi: 10.1016/j.cell.2024.01.031.

I was awarded an ERC Consolidator Grant in the 2024 call (EARLYFATE).



The EARLYFATE project funded by an ERC CoG will investigate the interaction between developmental and genome integrity control processes to ensure optimal embryo fitness.

Programmed cell death during development and disease

José Antonio Sánchez Alcázar

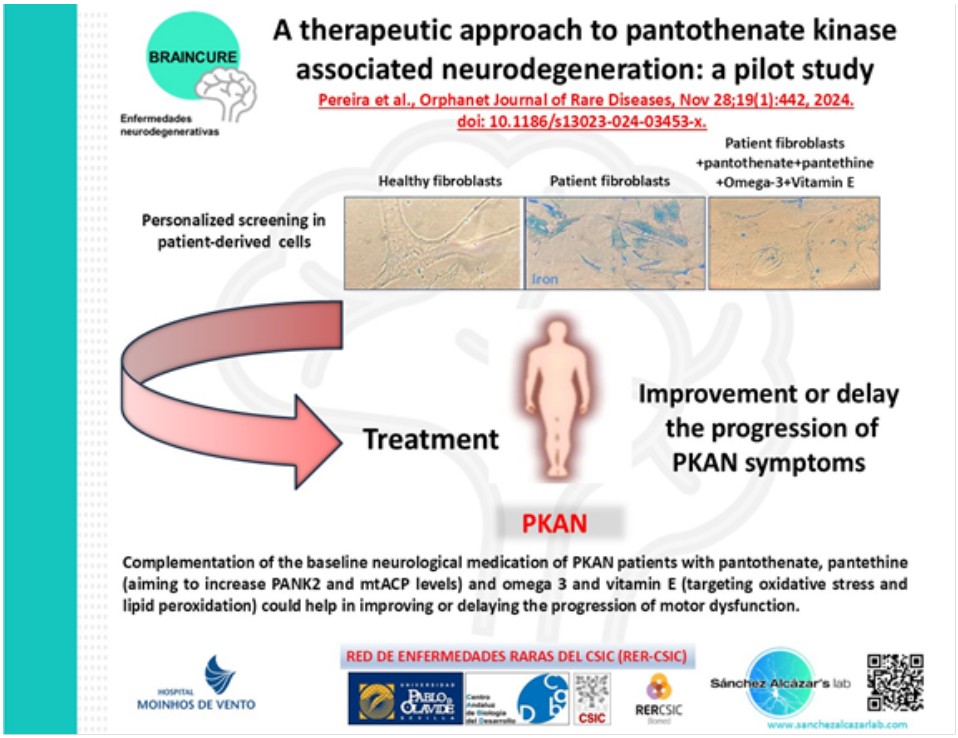
Summary of activities:

The group has continued its work in understanding the pathophysiological mechanisms and the search for therapeutic approaches in rare genetic diseases. It is worth noting that we collaborate in the clinical trials on KAT6A syndrome and mitochondrial diseases that are being carried out at the Virgen Macarena University Hospital in Seville. MdM DMC3 objective: Q5

Two highlights:

A therapeutic approach to pantothenate kinase associated neurodegeneration: a pilot study. Pereira A, Fischinger Moura de Souza C, Álvarez-Córdoba M, Reche-López D, Sánchez-Alcázar JA. Orphanet J Rare Dis. 2024 Nov 28;19(1):442. doi: 10.1186/s13023-024-03453-x.

A Multi-Target Pharmacological Correction of a Lipoyltransferase LIPT1 Gene Mutation in Patient-Derived Cellular Models. Gómez-Fernández D, Romero-González A, Suárez-Rivero JM, Cilleros-Holgado P, Álvarez-Córdoba M, Piñero-Pérez R, Romero-Domínguez JM, Reche-López D, López-Cabrera A, Ibáñez-Mico S, Castro de Oliveira M, Rodríguez-Sacristán A, González-Granero S, García-Verdugo JM, Sánchez-Alcázar JA. Antioxidants (Basel). 2024 Aug 22;13(8):1023. doi: 10.3390/antiox13081023.



Cell Division Control

Juan Jiménez

Summary of activities:

The laboratory develops two main research lines using yeasts as eukaryotic model organisms. The fist, in collaboration with Victor A. Tallada, we have studied the requirement of the EMC insertase in cell cycle progression. Using fission yeast, we demonstrate that the EMC complex facilitates the biogenesis of the sterol transfer protein Lam6/Ltc1 at ER-mitochondria contact sites. Cells that lose EMC function sequester unfolded Lam6/Ltc1 and other proteins at the mitochondrial matrix, leading to surplus ergosterol, cold-sensitive growth and mitochondrial dysfunctions. These results suggest that EMC-assisted biogenesis of Lam6/Ltc1 may provide, through ergosterol homeostasis, optimal membrane fluidity to facilitate biogenesis of other ER-membrane proteins.

In a second research line closely collaborating with Andrés Garzón, we observed that flor yeast, evolutionary adapted budding yeasts that develop a floating biofilm to survive alcoholic stress, are the only wild Saccharomyces yeast free of Totivirus. We constructed Totivirus-infected flor yeast cells to uncover the reason that prevents this infection in wild flor yeasts.

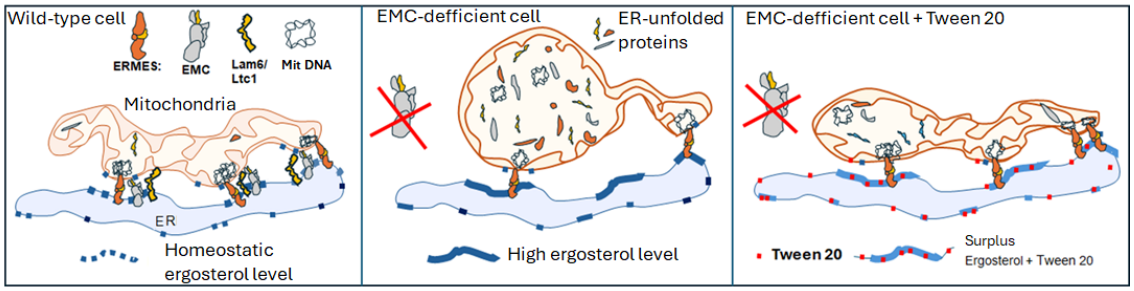
Projects: PGC 2023/00279/001 - Totivirus en el campo, el lagar y el laboratorio. Conocimiento basico y aplicaciones. PIs: A. Garzón and J. Jiménez

Ph D Thesis: Sergio Villa-Consuegra. Coordinación entre el desensamblaje del huso de meiosis I y la segregación de las cromátidas hermanas de meiosis II. 2024. Directors: Juan Jiménez and Victor Alvarez-Tallada

Two highlights:

-Berraquero, M., Tallada, VA and Jimenez, J. EMC regulates cell membrane fluidity to facilitate biogenesis of membrane proteins. Preprint. October 2024. DOI: 10.1101/2024.10.15.618411

-Berraquero, M., Tallada, VA and Jimenez, J. A key role of the EMC complex for mitochondrial respiration and quiescence in fission yeasts. Preprint. December 2024.
DOI: 10.1101/2024.12.16.628658



Altered proteostasis and mitochondrial dysfunction result from surplus ergosterol in EMC-deficient cells.

Global regulation of gene expression and metabolism of environmental bacteria: *Genetic and species rearrangements in microbial consortia impact biodegradation potential*

Inés Canosa and Amando Flores

Summary of activities:

Our main research line focuses on studying the **adaptation of environmental microbial communities** to changing conditions, such as the presence and increasing concentration of emerging contaminants, especially in developed countries. From an evolutionary perspective, soil and water microorganisms are not initially designed to fully assimilate newly created compounds introduced into the environment. However, adapted metabolic pathways capable of degrading these emerging contaminants, such as ibuprofen, naproxen, and some antibiotics, have been identified in many microorganisms from different origins. This suggests that these biodegradation pathways have evolved or been assembled through various mechanisms, reusing or modifying pre-existing metabolic capabilities.

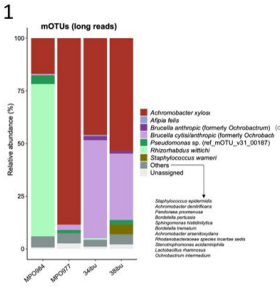
The goal is to identify the genetic processes and mechanisms that enable this adaptation (fig. 1-2). Understanding these processes will help develop strategies to address environmental pollution caused by these compounds. The research focuses on two main strategies: 1) developing methods to efficiently purify contaminated water using natural microbial consortia that can be optimized in the lab, and 2) creating technologies to produce microbial support materials, enhancing biodegradation processes and valorising agricultural waste, leading to positive economic outcomes.

This research advances microbial ecology by exploring microbial consortia, genetic adaptations, and selective environmental stress. It highlights the role of horizontal gene transfer (HGT) and plasmids in adaptation, genomic reorganization, and species dynamics. Additionally, it supports biotechnological applications by informing the design of microbial consortia for bioremediation while enhancing ecological insights into microbial communities and biogeochemical cycles. This topic fits perfectly with the overall objective of the MdM DMC3 *'to improve our understanding of the principles of self-organisation at multiple scales'* from Q1 to Q5.

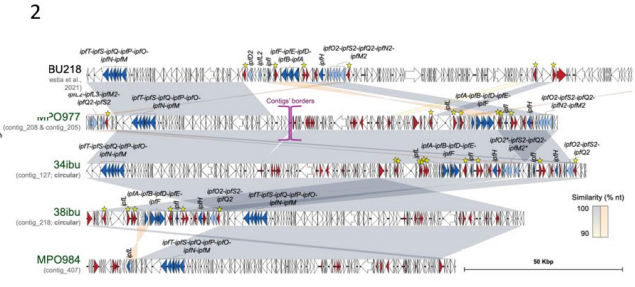
In another research line focused on **Biodiversity**, we participate in a project that examines **the impact of regenerative agriculture** on three plots in Spain (Badajoz, Sevilla, and Córdoba). The study evaluates the carbon sequestration capacity of the treated plots, as well as the effects on microbial and plant biodiversity (fig.3).

Simultaneously, we have contributed over 120 hours of teaching, while also holding management positions such as department director at the university and area head. We are committed to bringing research closer to society and informing the public about the valuable potential of science funding, particularly for the younger generation.

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Developmental Control

Fernando Casares

Summary of activities:

Studying the development of the fly eye **we have discovered a mechanism by which variations in final organ size is minimized**, guaranteeing its optimal functionality. This mechanism operates via a feedback control in which a cellular decision (whether to die or survive) is integrated at a tissue level, thus providing an example of multilevel integration during development (Navarro et al., al 2024). Organ regeneration is a peculiar form of development, and one that is accessible to some organisms and not to others. By working with an insect, the mayfly *Cloeon*, **we have found a number of genes and pathways that are required for organ regeneration**, including the **Neddylation** pathway, which regulates protein stability and degradation (Martín-Blanco et al., 2024). In this way, we have shown how the activation of a specific cellular process is required for the fast growth of a whole regenerating organ. F Casares is the **coordinator** of a large collaborative network, **LifeHUB CSIC** which aims at establishing collaborations among scientists working at different scales to understand the origin, evolution and synthesis of life (https://lifehub.csic.es/), translating the aims of DMC^3 to scientific and technological interactions. The workshop “REWIRING LIFE: The future implications of engineering biology” or the second edition of the course “Principles of Evolution (PoE)” serve as a examples of LifeHUB’s activities.

Two highlights:

Gill regeneration in the mayfly *Cloeon* uncovers new molecular pathways in insect regeneration. Martin-Blanco CA, Navarro P, Esteban-Collado J, Serra F, Almudi I, Casares F. Open Biol. 2024 Nov;14(11):240118. doi: 10.1098/rsob.240118.

Feedback control of organ size precision is mediated by BMP2-regulated apoptosis in the *Drosophila* eye. Navarro T, Iannini A, Neto M, Campoy-Lopez A, Muñoz-García J, Pereira PS, Ares S, Casares F. PLoS Biol. 2024 Jan 30;22(1):e3002450. doi: 10.1371/journal.pbio.3002450.



Driving the expression of the AML1/Runx transcription factor *lozenge* in eye progenitor cells causes the malformation of the eye. This phenotype might be due to *lozenge* maintaining the multipotent state of eye progenitors. If this were the case, this experimental system would allow us to investigate the mechanisms that allow progenitor cells to maintain their plasticity.

CRISPR-Cas in vivo optimizations to understand early vertebrate development and human diseases

Miguel Angel Moreno-Mateos

Summary of activities:

We have completed our CRISPR-Cas RNA-targeting optimizations and applications, consolidating our results in two preprints: Hernandez-Huertas L et al. 2024 under second revision in **Nature Structural & Molecular Biology** and Moreno-Sanchez I, Hernández-Huertas L, Nahón-Cano D et al., 2024, recently accepted in **Nature Communications**). Beyond our lab continues using CRISPR-RfxCas13d to perform functional screenings during early zebrafish embryogenesis and we have uncovered Rfwd3, an E3 ubiquitin ligase that we have identified as a Maternal-to-Zygotic Transition (MZT) regulator. Similarly, we have uncovered new post-transcriptional regulators of MZT in an additional maternal RNA-targeting screening. Finally, we are investigating the role of Wapl, a key factor in cohesin regulation and following from our preliminary findings that knockdown of one of two zebrafish Wapl paralogues, Wapla, disrupts ZGA and epiboly. These and other projects in the lab have been presented in different meetings and conferences such as SEDB 2024 (Sant Feliu de Gixols, Girona) or RiboRed 2024 (Bilbao). Further, we have organized the first meeting of the Andalusian platform of biomodels and resources in genome editing associated with “Plan Complementario de I+D+I en el área de Biotecnología aplicada a la salud of the Government of Andalusia (Antequera, February 15-16, 2024) Beyond the lab has participated in several dissemination activities (Feria de la Ciencia, Noche Europea de los Investigadores, etc). We have also organized the first lab retreat in a Cottage at Puerto de la Torre, Malaga (6-8 November) where we had a set of scientific sessions discussing potential new projects of the lab to be performed over the next years. Finally, the PI of the laboratory has started his permanent position as Científico Titular in January 2024.

Two highlights:

Enhanced RNA-targeting CRISPR-Cas technology in zebrafish. Moreno-Sanchez I et al. bioRxiv [Preprint]. 2024 Oct 9:2024.10.08.617220. doi: 10.1101/2024.10.08.617220. Accepted in Nature Communications (January 2025).

In vivo base editing of a pathogenic Eif2b5 variant improves vanishing white matter phenotypes in mice. Böck D, Revers IM, Bomhof ASJ, Hillen AEJ, Boeijink C, Kissling L, Egli S, Moreno-Mateos MA, van der Knaap MS, van Til NP, Schwank G. Mol Ther. 2024 May 1;32(5):1328-1343. doi: 10.1016/j.ymthe.2024.03.009.



Group picture of our first lab retreat.

Molecular Evolution of Microbial Interactions Lab (MolEMI Lab)

Tanja Dapa

Summary of activities:

In the year 2024 I started executing my first project obtaining by the Spanish national project as a main and only PI. In March 2024, a research technician, Inmaculada Mena Guzman, joined my group and in October 2024 she enrolled into the PhD Programme in Biotechnology, Engineering and Chemical Technology at UPO.

My emerging research group is working on the host-microbe coevolution of the gut microbiota and the mammalian host, pioneering a brand-new field, generated upon the discoveries I made during my last postdoctoral stage. The gut microbiota is a complex microbial community that inhabits the host's intestine and is vital to their health. In my lab we are studying the synergistic coevolution between a key member of the gut microbiota, *Bacteroides thetaiotaomicron*, and its mammalian host. The lab is at the forefront of a new, vibrant, and relevant research field.

In 2024 we established a method to study the evolution of the same bacteria in laboratory conditions, and we purchased two different lines of mice knockouts, which we will use this year. Thanks to this, we can now count on two new mice lines at the Andalusian Center for Development Biology (CABD-UPO-CSIC): mice C57BL/6J-*Rag1*$^{em10Lutzy/J}$ (immunocompromised mice, which lack adaptive immune response) and mice C57BL/6J-*IgA*^{KO} (partially immunocompromised mice, which lack immunoglobulin A).

In 2024, as in the previous year, me and my doctorate student, also contributed to different outreach activities, e.g., I participated as a scientist in the workshops held at the public school to celebrate the International Day of Women and Girls in Science and at visits to the CABD.

Two highlights:

Attendance at the 3rd Polish-Czech Probiotic Conference (Prague, Czech Republic) as an invited Keynote lecture.

Effect of diet on the evolution of gut commensal bacteria. Dapa T, Xavier KB. Gut Microbes. 2024 Jan-Dec;16(1):2369337. doi: 10.1080/19490976.2024.2369337.

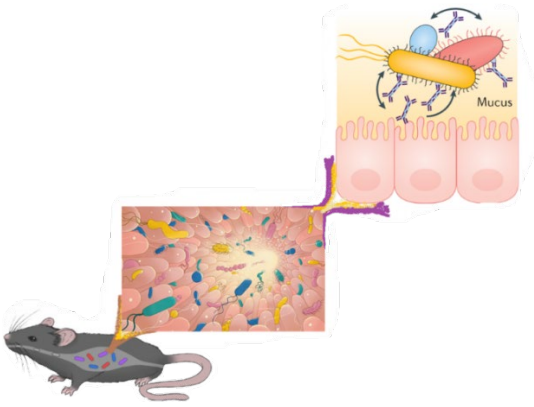


Illustration representing the line of research of my emerging MolEMI Lab – host-microbe interaction withing the mammalian intestine (Illustrations: © Joana C. Carvalho; © PMID: 34862512).

Regulatory mechanisms of gene expression in pollutant-degrading bacteria Bioremediation

Francisca Reyes-Ramírez and Eva María Camacho Fernández

Summary of activities:

My research group focuses on two main areas: the Regulation of gene expression in bacteria and the Bioremediation of plastics.

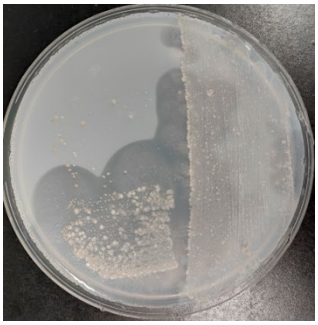
In 2024, we have been investigating the role of two histidine kinases in the signal transduction of various environmental stresses in the tetralin-degrading bacterium *Sphingopyxis granuli* TFA. These Histidine kinases are key components of a global transcriptional response called General Stress Response (GSR), allowing cells to sense and respond to environmental changes by phosphorylating and dephosphorylating anti-anti-sigma factors that control gene expression. Additionally, we have explored the role of small regulatory RNAs (sRNAs) in bacterial stress responses. These sRNAs are essential post-transcriptional regulators that fine-tune gene expression by base-pairing with specific messenger RNAs (mRNAs). Our findings contribute to a deeper understanding of how bacteria regulate stress responses at both the signalling and post-transcriptional levels. These activities are particularly relevant for the question Q3 of MdM: How do cells sense/respond to their changing environment?

Environmental contamination by plastics and microplastics has become a global challenge, posing serious risks to both ecosystems and public health. There is an urgent need to develop new strategies to remove these pollutants, both from already contaminated environments and at their entry points into various ecosystems.

Together with Dr. Eva María Camacho Fernández (Research Associate at CABD), we are executing a project that involves an innovative composting strategy that is done in collaboration with the Chemical Engineering Department at Universidad Pablo de Olavide, and the wastewater treatment plant EMASESA in Seville. This approach incorporates grease and plastic waste into the composting process to create an enriched environment that facilitate the isolation of microorganisms with plastic-degrading capabilities. Additionally, we aim to identify new genes encoding enzymatic activities with potential industrial and environmental applications through Functional Metagenomics.

Two highlights:

We carried out a Technology Transfer Project through a Research and Development Agreement between Universidad Pablo de Olavide and EMASESA to monitor and control physicochemical and microbiological parameters in biofilters during the composting of organic waste.



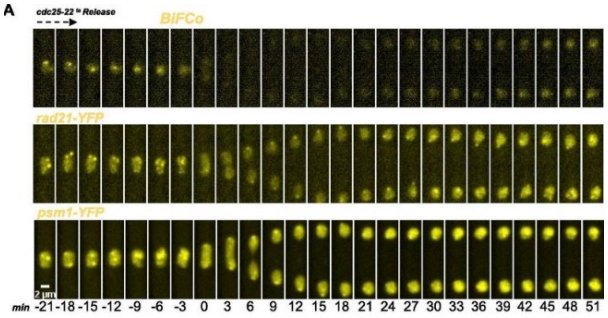
Bacterial growth and detection of polyurethanase activity on agar plate containing polyester-polyurethane Impranil DLN. The zone of clearing produced from the degradation of polyurethane can be observed.

Mitotic spindle nucleation and coordination with the cell cycle

Víctor Álvarez Tallada

Summary of activities:

Cohesin is an essential complex in eukaryotes to keep genome architecture, organising chromatin within the nucleus and regulating gene expression. We have developed a novel strategy as a proof of concept in fission yeast to track the assembled cohesin complex by fluorescence microscopy in living cells but excluding the signal of its individual proteins. This allows the quantification of cohesin loading in living cells in different genetic backgrounds, environment alterations, developmental stages etc. Based on these data and according to institute´s DMC3 Strategic plan, I have established an exciting collaboration with Dr. James Castelli's group (UPO/CSIC collaboration) to implement this tool in *Drosophila* and using it to study the role of cohesin in development, gene regulation and evolutionary conservation of factors necessary for the maintenance of genomic stability that we have identified in our studies in fission yeast. This may be used as a good model to study genetic diseases such as cohesinopathies and cancer. We are sharing a predoc student supported by an FPI grant from Maria de Maeztu program within our institute to bring together the best of both worlds. In addition, we have described a key role for Aurora B kinase required for resetting centrosome´s spindle nucleation function in the transition between meiosis I and II which we have proved essential for equal chromosome segregation in the formation of gametes in meiosis II. This was the subject of Sergio Villa´s PhD, whose thesis was defended last March.





Gene regulation and evolution

Francisca Martínez Real.

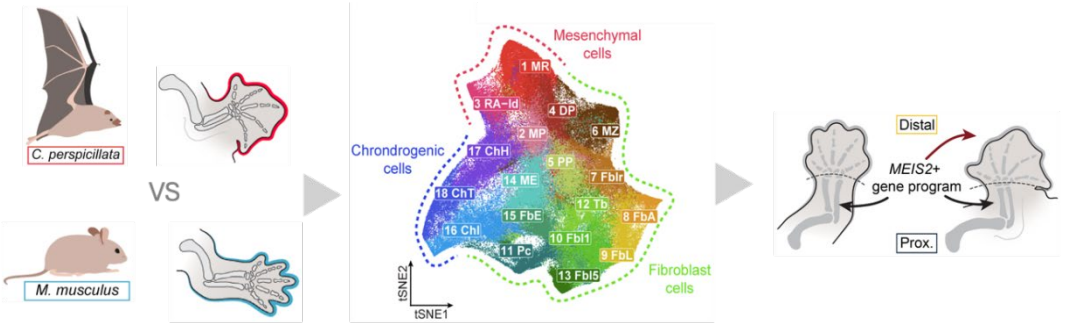
Summary of activities:

In 2024, I established my research group at the CABD after moving from the Max Planck Institute for Molecular Genetics in Berlin, where I had been promoted to group leader less than a year earlier. This transition was made possible by a Ramón y Cajal contract, along with additional funding from the "*Atracción de Talento*" program, which helped set up my lab and recruited a PhD student. Our research focuses on understanding how genomic changes drive the phenotypic diversity across species. We generate and analyze multi-omics data from various organs and species of interest, such as bats and moles, and comparing them to the mammalian reference species, the mouse, to identify genetic variations associated with adaptive traits. Importantly, we validate our findings by generating transgenic mice that replicate these species-specific genomic variations. A key milestone for our lab has been the successful implementation of tetraploid aggregation at the CABD, an advanced transgenic technology we previously used in Germany. Using this method, we genetically modify mouse embryonic stem cells to generate mutant embryos, allowing us to analyze nearly 100% mutant F0 embryos without the need for breeding lines, significantly reducing time, costs, and animal use. This year, I also secured a permanent position as a "*Científico Titular*" and obtained my first project funded by the Spanish Research Agency, along with a PhD contract. In this project, we apply synthetic genomic approaches to investigate how large-scale regulatory changes in the *HoxD* gene cluster contribute to the specialized limb morphology of the Iberian mole, an adaptation to its subterranean, digging lifestyle.

Two highlights:

As part of my collaboration with the lab of Prof. Jef Boeke, pioneer in synthetic genomic approaches, I was invited to the Annual NHGRI Centers of Excellence in Genomic Sciences (CEGS) meeting in San Diego, California, to present as part of his team. My presentation highlighted how the newly developed synthetic genomic technology can be used to study evolutionary processes in *in vivo* transgenic models.

Comparative Single-Cell Analyses Reveal Evolutionary Repurposing of a Conserved Gene Program in Bat Wing Development. Schindler M, Feregrino C, Aldrovandi S, Lo B, Monaco A, Ringel A, Morales A, Zehnder T, Behncke R, Glaser J, Barclay A, Andrey G, Kragesteen B, Hägerling R, Haas S, Vingron M, Ulitsky I, Marti-Renom M, Hechavarria J, Fasel N, Hiller M, Lupiáñez D, Mundlos S, Real FM. 2024. BioRxiv: doi: https://doi.org/10.1101/2024.10.10.617585 (*See illustration below*).



Mitochondrial signalling control in the regulation of ageing

Marta Artal-Sanz and Jesús Fernández Abascal

Summary of activities:

During 2024 the lab initiated a new additional research line focused on the role of the mitochondrial prohibitin (PHB) complex in the aging of the nervous system (led by Jesús Fernandez Abascal, Ramón y Cajal Associated Researcher).

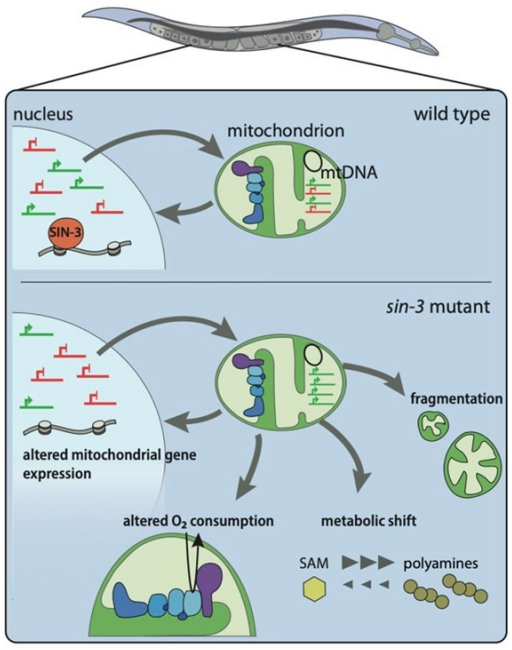
We have followed up on our interest on the regulatory crosstalk between mitochondrial function, metabolism and the epigenetic regulation of gene expression to control cell homeostasis. We uncovered a new stress response mechanism involving a putative histone deubiquitinase. Understanding the molecular bases of this new mechanism is the focus of the PhD Thesis of Sergio Gordillo García (PREP2022-000662). In addition, in a collaborative paper with Francesca Palladino at the Ecole Normale Supérieure de Lyon, we described the role of the transcriptional coregulator SIN-3 in regulating mitochondrial function and metabolism. This is important because SIN3 haploinsufficiency causes intellectual disability/autism syndromes and plays an important role in tumour biology (PMID: 38746662).

Our efforts are relevant to understand the role that energy constrains and metabolic states play in shaping the aging process.

Two highlights:

SIN-3 transcriptional coregulator maintains mitochondrial homeostasis and polyamine flux. Giovannetti M, Rodríguez-Palero MJ, Fabrizio P, Nicolle O, Bedet C, Michaux G, Witting M, Artal-Sanz M*, Palladino F*. iScience. 2024 Apr 22;27(5):109789. doi: 10.1016/j.isci.2024.109789. (*Corresponding authors)

Invited Speaker at the EMBO/FEBS Lecture Course “Mitochondria in life, death and disease”. Lanshut, Germany, 16-20 September 2024.



Graphical Abstract.
SIN-3 transcriptional coregulator maintains mitochondrial homeostasis and polyamine flux.
iScience, 2024
(PMID: 38746662)

Fungal and yeast biotechnology

José Ignacio Ibeas Corcelles

Epigenetic regulation of virulence in plant pathogenic fungi

Ramón Ramos Barrales

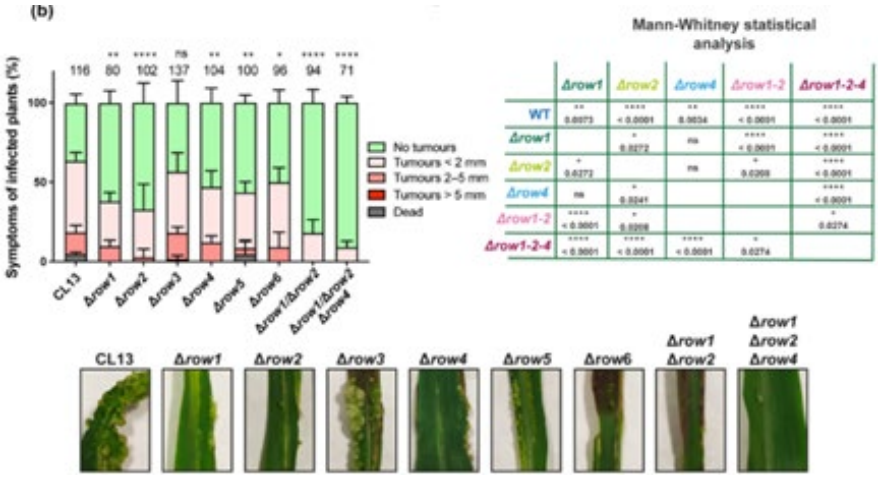
Summary of activities:

In the context of our work with the phytopathogenic fungus *Ustilago maydis*, we have published a paper describing a new family of proteins, Row family, involved in virulence factors secretion relevant for the infection process. When fungal pathogens invade host plants, a complex set of genes involved in virulence are activated and many of the synthetized proteins secreted to the cell wall and to the media. A correct secretion is essential to complete the infection process and we have found Row protein family as required for this secretion. This work has been published in a D1 paper in New Phytologist and a PhD thesis has been presented both during 2024. In addition to our work in virulence factors, we are interested in the regulation of virulence genes through chromatin modification. We are analysing the role for Lem2, a Laminin protein in nuclear movement and virulence gene activation as a response to the fungal/plant contact. In parallel, as part of our work in chromatin modification, we have finished a collaboration with the group of Dr. Sigurd Braun in Germany, where we have performed functional genomics in order to uncover new factors and pathways involved in heterochromatin regulation in the yeast model organism *Schyzosaccharomyces pombe*.

Two highlights:

A systematic quantitative approach comprehensively defines domain-specific functional pathways linked to *Schizosaccharomyces pombe* heterochromatin regulation. Muhammad A, Sarkadi Z, Mazumder A, Ait Saada A, van Emden T, Capella M, Fekete G, Suma Sreechakram VN, Al-Sady B, Lambert SAE, Papp B, Barrales RR, Braun S. Nucleic Acids Res. 2024 Dec 11;52(22):13665-13689. doi: 10.1093/nar/gkae1024.

Row1, a member of a new family of conserved fungal proteins involved in infection, is required for appressoria functionality in *Ustilago maydis*. Pejenaute-Ochoa MD, Tomás-Gallardo L, Ibeas JI, Barrales RR. New Phytol. 2024 Aug;243(3):1101-1122. doi: 10.1111/nph.19798.



Effect on plant infection after deletion of all different Row members.

Developmental Epigenomics

Ozren Bogdanovic

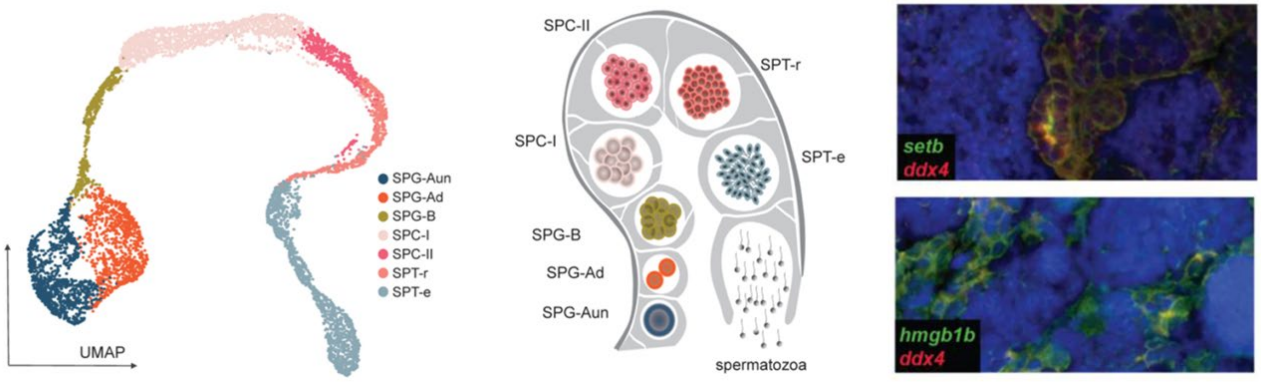
Summary of activities:

Our lab investigates how the epigenome and in particular DNA modifications such as methylation contribute to or drive developmental transitions and disease states. In 2024, we studied how gene-regulatory states are established during spermatogenesis in zebrafish (Burgos Ruiz et al, *bioRxiv*) and examined how key regulatory elements, such as CpG islands, evolved at the invertebrate - vertebrate boundary. We also participated in international collaborations focusing on epigenome and transcriptome remodelling during tumorigenesis (Filipe et al, 2024; *Adv Sci Weinh*), as well as in the identification of novel DNA methylation readers in plants (Cahn et al, 2024; *Genome Res*). Most notably, we were awarded the MSCA Doctoral Network - DANIO-ReCODE Coordinator: O.B. https://danio-recode.eu/), a collaborative endeavour uniting 15 laboratories across 12 EU and UK institutions to unravel the molecular mechanisms underlying vertebrate heart, nervous system, and eye regeneration. Our research aligns with multiple integrative and transversal questions of the DMC3 scheme, including: Q1: What is the relationship that regulatory and structural changes in the genome have with evolutionary innovations? Q2: How are gene regulatory networks harmonized between neighbouring cells, organs, and even neighbouring species? Q3: How do cells sense/respond to their changing environment?

Two highlights:

1) A single-cell multiomics roadmap of zebrafish spermatogenesis reveals regulatory principles of male germline formation. Burgos Ruíz AM, Geng FS, Pujol G, Sanabria E, Brethouwer T, Almuedo-Castillo M, Ruiz-Herrera A, Tena JJ* and Bogdanovic O*****. *bioRxiv* (* corresponding).

2) O Bogdanovic, CABD/CSIC (Coordinator): "*DANIO-ReCODE: DANIO-CODE - the next frontier: Decoding transcription regulation in regeneration by advanced genomics and computational tools".* MSCA Doctoral Network, European Commission, 101169349 (2024 – 2028).



Zebrafish germ cell populations revealed by sc-RNA-seq (left and middle). Double FISH validation of novel spermatogonial (setb) and spermatocyte (hmgb1b) driver genes (right).

Self-Organization of Biological Systems

Luciano Marcon

Summary of activities:

Inspired by Turing´s and Waddington´s legacy, our primary goal is to uncover general principles of self-organization in early embryos. To do so, we integrate Lightsheet microscopy, single cell RNA Sequencing (scRNA seq), high-throughput screenings, and mathematical modelling to understand how complex structures spontaneously form during embryo development.

In 2024, we published a novel Clock and Wavefront Self-Organizing model of mouse somitogenesis [1]. Using our Sevilletor reaction-diffusion framework, we demonstrated that sequential somitogenesis waves of Wnt and Notch signalling can self-organize, independent of global gradients, explaining presomitic mesoderm excitability.

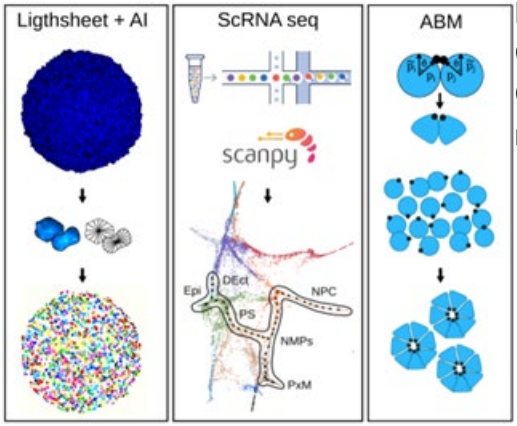
We kept advancing in the study of embryonic axis formation and symmetry-breaking using embryoid bodies (EBs) as an idealized in vitro system. For the first time, we employed artificial intelligence-based segmentation of nuclei in EBs, significantly enhancing our ability to quantify cellular-level gene expression patterns and polarity (Figure, left). This opens a completely new quantitative avenue for the lab to integrate microscopy data directly with single-cell RNA sequencing (Figure, centre), providing unprecedented insights into embryonic self-organization.

Our lab also developed an agent-based computational simulator to investigate how neural rosettes and convergence-extension can spontaneously emerge (Figure, right), modelling polarity-driven interactions and apical constriction in neural morphogenesis.

Finally, we celebrated the successful defences of our first two PhD students, reflecting our commitment to training the next generation of developmental biologists. We continued teaching computational modelling in biological systems within the Master's program MADOBIS, jointly offered by Universidad de Sevilla and Universidad Internacional de Andalucía, participated in outreach activities, and expanded international collaboration through EMBO Short-Term Fellowship exchange for one of our PhD students at IMBA, Vienna.

Highlight:

The Clock and Wavefront Self-Organizing model recreates the dynamics of mouse somitogenesis in vivo and in vitro. Klepstad J, Marcon L. Development. 2024 May 15;151(10):dev202606. doi: 10.1242/dev.202606.



Left: AI-driven segmentation of DAPI in Embryoid bodies. Center: scRNA seq analysis of embryoid bodies. Right: Computational simulation illustrating self-organized neural rosettes formation in agent based models (ABM).

Genetics and functional genomics of genes with small open reading frames

Juan Pablo Couso

Summary of activities:

We published a review of recent discoveries on the function of small ORFs and we proposed a Cis to Trans model for the acquisition of peptide function from cis-non-coding functions through evolution (see Figure). We also showed how 50-60% of smORFs reveal non-overt genetic requirements, sometimes only under stress. This is expected from newly evolved genes. All this is relevant for questions Q1 and Q3 of *MdM DMC3.* We obtained two projects from the Agencia Estatal de Investigación: EUR2022-134063 "Fundamentos de la funcionalizacion de small ORFs" and PID2022-141831NB-I00 "Activacion de ORF pequeñas en el genoma no codificante".

Two highlights:

Cis to trans: small ORF functions emerging through evolution. Baena-Angulo C, Platero AI, Couso JP. Trends Genet. 2025 Feb;41(2):119-131. doi: 10.1016/j.tig.2024.10.012. Epub 2024 Nov 27.

Pervasiveness of Microprotein Function Amongst Drosophila Small Open Reading Frames (SMORFS). Platero AI, Pueyo JI, Bishop SA, Magny EG, Couso JP. Cells. 2024 Dec 18;13(24):2090. doi: 10.3390/cells13242090.

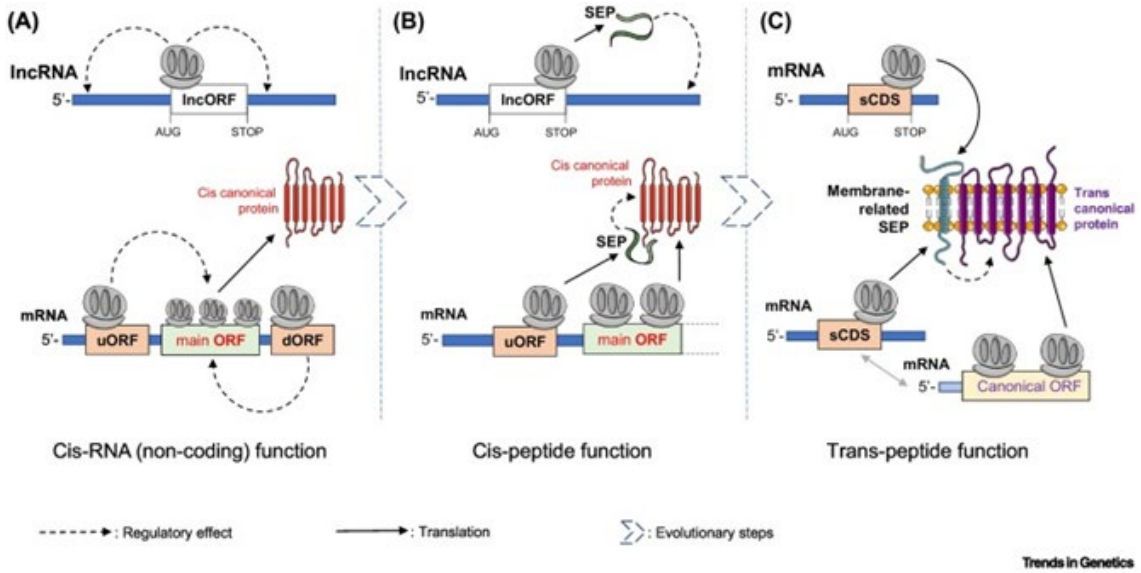


Figure: (A) smORFs in RNAs attract ribosomes during RNA processing, degradation, or translation, influencing their encoding RNAs, either regulating the processing and degradation of their miRNA-containing lncRNAs (top) or as upstream ORFs (uORFs) and downstream ORFs (dORFs) by regulating the translation of canonical ORFs in a mRNA (bottom).This can be modulated by evolution by enhancing or reducing smORF translation.(B) Beyond a threshold of translation, the smORF-expressed peptide (SEP) can also exert a 'cis' function, either on its encoding lncRNA (top) or on the canonical protein (red) cis-encoded in the same mRNA (bottom).(C) If SEP expression and stability are increased, the newly abundant SEP can diffuse across the cell and regulate other trans-protein partners (top), either from proteins paralogous to the earlier cis-partners or simply proteins containing a similar domain (purple). In time, a shorter version of the mRNA may appear with the peptide-producing smORF as the sole ORF, that is a new coding gene or short coding sequence (sCDS). Thus, a 'cis-to-trans path to function' might provide a niche for smORF translation, and then allow the evolution of a beneficial peptide.

Regulation of coenzyme Q synthesis and its implications for mitochondrial health

Carlos Santos Ocaña

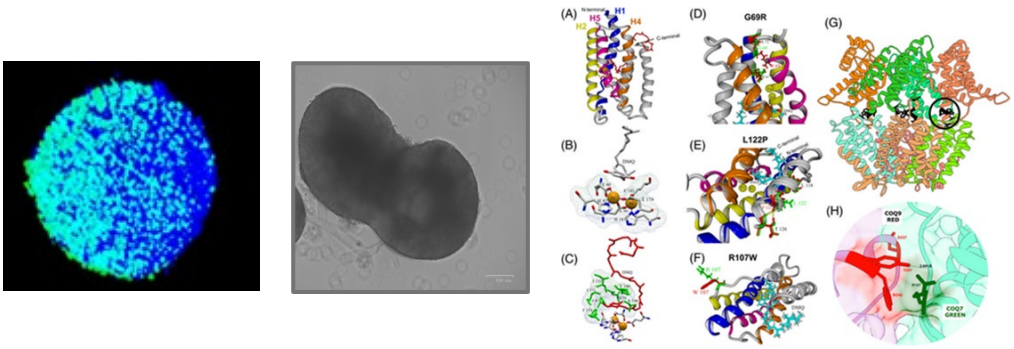
Summary of activities:

Mitochondrial diseases represent a diverse group of conditions characterized by alterations in the structure or function of mitochondria. These diseases are often severe and can lead to significant disability. Our research group focuses on various aspects related to these diseases, including diagnosis, understanding the pathophysiological mechanisms, and exploring potential therapies. We collaborate in diagnosing patients, and an important highlight of our work is the expanded understanding of the phenotype associated with CoQ10 deficiency due to variants in the COQ7 gene. This research has helped identify potential markers for disease severity. Additionally, we have developed various preclinical models, including the generation of embryonic bodies and brain organoids from induced pluripotent stem cells (iPSCs) derived from patients with CoQ10 deficiency. Our studies have also demonstrated that prenatal supplementation with CoQ10 can improve muscle dysfunction in mice caused by variants in the ADCK2 gene. In 2024, our group secured funding from the National Plan to develop a zebrafish model of CoQ10 deficiency, and we received funding from a call from CIBERER to identify a therapy for a mitochondrial disease known as EMPF1. Furthermore, we actively participate in organizing dissemination activities. We hosted a summer course at the University of Pablo Olavide (UPO) in Carmona focusing on diagnosing mitochondrial diseases, as well as a workshop on the characterization of organoids at the Instituto de Investigación en Enfermedades Raras (IIER, ISCIII) in Majadahonda. As professors at UPO, we continue to engage in teaching activities for both undergraduate and postgraduate students.

Two highlights:

Prenatal and progressive coenzyme Q10 administration to mitigate muscle dysfunction in mitochondrial disease. Hernández-Camacho JD, Vicente-García C, Ardila-García L, Padilla-Campos A, López-Lluch G, Santos-Ocaña C, Zammit PS, Carvajal JJ, Navas P, Fernández-Ayala DJM. J Cachexia Sarcopenia Muscle. 2024 Dec;15(6):2402-2416. doi: 10.1002/jcsm.13574.

New variants expand the neurological phenotype of COQ7 deficiency. Fabra MA et al. J Inherit Metab Dis. 2024 Sep;47(5):1047-1068. doi: 10.1002/jimd.12776.



Left: EB from COQ7 patient. **Center:** Brain organoid from COQ7 patient. **Right:** Structural changes in COQ7 protein produced by variants identified in COQ7 patients.

Morphogenesis and mechanosensitive gene regulation

Maria Almuedo Castillo

Summary of activities:

In 2024 we have been developing the use of a DNA and RNA fixative prior to cell dissociation to uncover the set of gene-regulatory sequences related to cell-cell and cell-ECM contacts, as well to cell and nucleus geometries. We also set up a workstation for making hydrogels with different mechanical properties and continue developing techniques on zebrafish ESCs culture and organoid generation.

Finally, we have been developing other projects on the relevance of cell geometries in gene regulation in two biological contexts: zebrafish gastrulation and eye formation. These questions are in line with Q2, Q3 and Q4 of the MdM DCM3.

Collaborations: we have been awarded two international collaborative projects. The Early Career grant from the Human Frontier Science Program and the DanioReCode Doctoral Network from the Marie Sklodovska-Curie Actions.

Teaching & training: I am a teacher of the “Regeneración tisular” class of the UPO Máster of Biotecnología Sanitaria.

Bioinformatics for biological sequence analysis

Antonio J. Pérez Pulido

Summary of activities:

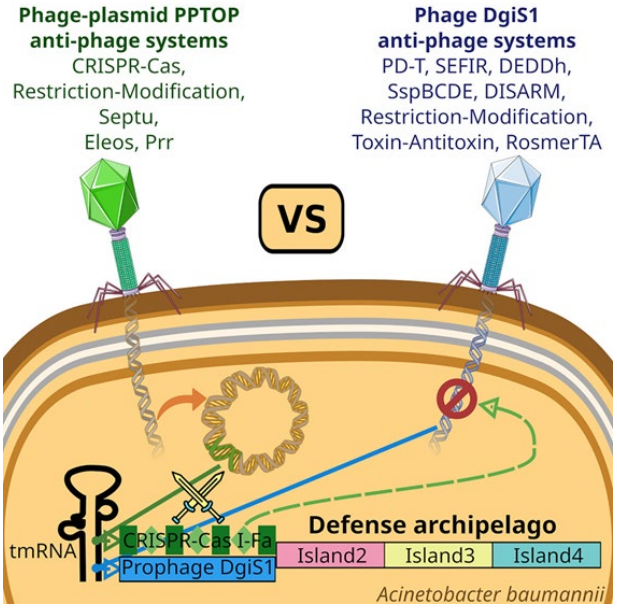
Our group analyses pangenomes from thousands of bacterial genomes to search for gene markers related to bacterial-phage interactions. This year we have discovered the fight between two phages in the genome of the bacterium *Acinetobacter baumannii*, and more specifically in a defence archipelago in which the bacterium accumulates antiviral systems. One of the viruses, which we have named PPTOP (Phage-Plasmid Terminator of Other Phages), carries, among others, a CRISPR-Cas system. This system appears to be specialized to recognize and prevent infection of the bacterium by a second virus, which we have named DgiS1 (prophage Genomic Dominant Island in SsrA 1). In turn, this second virus defends itself against PPTOP by selecting changes in the protospacers and PAM sequences of its genome, which would make it resistant to the CRISPR-Cas system.

In addition, this year we have strengthened our collaboration with Dr. Ronan McCarthy's group at Brunel University London, obtaining shared funding and publishing a collaborative article in which we show cyclic AMP as a global virulence regulator governing inter- and intrabacterial signalling in the same bacteria.

Two highlights:

Biological warfare between two bacterial viruses in a defence archipelago sheds light on the spread of CRISPR-Cas systems. Rubio A, Garzón A, Moreno-Rodríguez A, Pérez-Pulido AJ. Cell Rep. 2024 Dec 24;43(12):115085. doi: 10.1016/j.celrep.2024.115085.

Cyclic AMP is a global virulence regulator governing inter and intrabacterial signalling in Acinetobacter baumannii. Harkova LG, de Dios R, Rubio-Valle A, Pérez-Pulido AJ, McCarthy RR. PLoS Pathog. 2024 Sep 6;20(9):e1012529. doi: 10.1371/journal.ppat.1012529.



Warfare between PPTOP and DgiS1 bacteriophages in the bacterium *Acinetobacter baumannii*. Both carry different antiphage defence systems to probably fight each other, highlighting a CRISPR-Cas I-Fa system in PPTOP, specialized in targeting DgiS1. All this means that in an archipelago of defence of the bacterial genome, either the DgiS1 prophage or the CRISPR-Cas system appears in an exclusive way.

Epigenomics in disease and aging

Juan J. Tena and Martin Franke

Summary of activities:

Members of our laboratory have participated in 13 publications during 2024, 4 of them as corresponding authors. We have also contributed to several projects in collaboration with national and international groups, like the study of dorsoventral pattern of the vertebrate limb, with Prof. Marian Ros, or the study of retinal hereditary diseases for the International Training Network ProgRet (which produced a publication in the journal Genome Biology during 2024, with the PI as co-corresponding author) These two project are particularly interesting for the objectives of MdM DMC3.

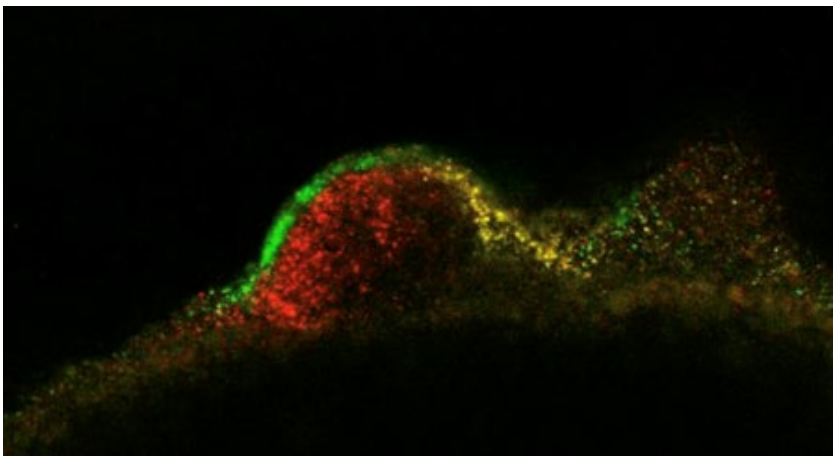
We carried out some teaching activities: a Doctorate course, “Introduction to R programming language”, and different Master courses. Finally, we also participated in several outreach activities, like ‘Ciencia en el cole’ and ‘Sábados científicos en familia”.

Two highlights:

Hemichordate cis-regulatory genomics and the gene expression dynamics of deuterostomes. Pérez-Posada A, Lin CY, Fan TP, Lin CY, Chen YC, Gómez-Skarmeta JL, Yu JK, Su YH*, Tena JJ*. Nat Ecol Evol. 2024 Dec;8(12):2213-2227. doi: 10.1038/s41559-024-02562-x.

Comparative 3D genome analysis between neural retina and retinal pigment epithelium reveals differential cis-regulatory interactions at retinal disease loci. D'haene E, López-Soriano V, Martínez-García PM, Kalayanamontri S, Rey AD, Sousa-Ortega A, Naranjo S, Van de Sompele S, Vantomme L, Mahieu Q, Vergult S, Neto A, Gómez-Skarmeta JL, Martínez-Morales JR*, Bauwens M*, Tena JJ*, De Baere E*. Genome Biol. 2024 May 17;25(1):123. doi: 10.1186/s13059-024-03250-6.

* Corresponding authors



HCR of a 36hpf zebrafish finbud, showing markers for dorso-ventral axis: Lmx1bb in red, in the dorsal mesoderm, Wnt7a in green and En1a in yellow, in dorsal and ventral ectoderm, respectively.

Nuclear Dynamics in Cell and Developmental Biology

Peter Askjaer

Summary of activities:

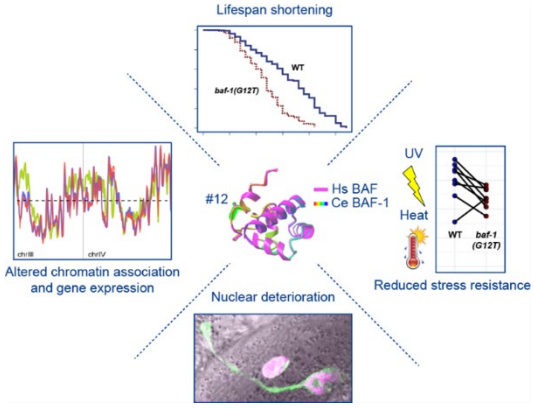
Our group focuses on the biogenesis of the nuclear envelope and the role of nuclear envelope proteins in chromatin organization and gene expression (key processes highlighted in the MdM DMC3 strategic plan). Alterations in the nuclear envelope are linked to a variety of rare diseases termed laminopathies. A single amino acid substitution at position 12 (A12T) of the human nuclear envelope protein BAF (Barrier to Autointegration Factor) causes Néstor-Guillermo Progeria Syndrome (NGPS). This premature ageing condition leads to growth retardation and severe skeletal defects, but the underlying mechanisms are unknown. In 2024, we reported a novel *in vivo* model for NGPS by modifying the *baf-1* locus in *C. elegans* to mimic the human NGPS mutation. These *baf-1(G12T)* mutant worms displayed multiple phenotypes related to fertility, lifespan, and stress resistance. Importantly, nuclear morphology deteriorated faster during aging in *baf-1(G12T)* compared to wild-type animals, recapitulating an important hallmark of cells from progeria patients. Tissue-specific chromatin binding and transcriptome analyses showed reduced BAF-1 association in most genes deregulated by the *baf-1(G12T)* mutation, suggesting that altered BAF chromatin association induces NGPS phenotypes via altered gene expression.

During cell division, the nuclear envelope undergoes a cycle of disassembly and reassembly, which involves extensive rearrangements of protein-protein and protein-lipid contacts. Together with the group of Dr Julien Dumont at the Institut Jacques Monod in Paris, we reported the identification of a unique lipid bilayer that surrounds chromosomes during segregation of meiotic chromosomes in C. elegans oocytes. In 2024 we also developed a novel technique to evaluate protein-protein contacts *in vivo* together with Dr Antonio Miranda at the IBiS in Seville.

Two highlights:

A human progeria-associated BAF-1 mutation modulates gene expression and accelerates aging in *C. elegans*. Romero-Bueno R, Fragoso-Luna A, Ayuso C, Mellmann N, Kavsek A, Riedel CG, Ward JD, Askjaer P. EMBO J. 2024 Nov;43(22):5718-5746. doi: 10.1038/s44318-024-00261-8.

An interkinetic envelope surrounds chromosomes between meiosis I and II in *C. elegans* oocytes. El Mossadeq L, Bellutti L, Le Borgne R, Canman JC, Pintard L, Verbavatz JM, Askjaer P, Dumont J. J Cell Biol. 2025 Mar 3;224(3):e202403125. doi: 10.1083/jcb.202403125. Epub 2024 Dec 26.



Barrier to autointegration factor (BAF) is an essential nuclear envelope protein involved in chromatin regulation and nuclear integrity. We established a *C. elegans* model to understand how a single amino acid substitution in human BAF causes Néstor-Guillermo Progeria Syndrome.

3D Genomics

Darío Jesús Lupiáñez García

Summary of activities:

In 2024, we continued our efforts in understanding gene regulation in the context of a 3D genome. Supported by the European Research Council (ERC), we are investigating how vertebrate sex determination can be triggered by various genomic or environmental cues. A project funded by the Agencia Estatal de Investigación (AEI) focus on studying enhancer-promoter communication mechanisms.

Among other achievements, this year featured the publication of our study in collaboration with Rafael Jimenez and Paco Barrionuevo's group (University of Granada). There, we discovered a novel role for microRNAs in orchestrating mammalian sex determination, by controlling the expression of the mammalian sex-determining gene *Sry* (Hurtado et al., *Nature Communications* 2024). Recently, the Spanish Society of Developmental Biology (SEBD) recognized the previous achievements of our group through the Jose Luis Gómez Skarmeta Award. During this period, I also joined the board of directors of the SEBD and continued performing with similar activities at the Sociedad Española de Genética (SEG). We participated in national networks such as the Conexion Genoma from CSIC and the AEI-funded Regulatory Genomics Network (R2G), and international ones, like the "Spatial Genome Architecture in Development and Disease" from the German Research Agency (DFG). The members of our group also engaged in numerous dissemination activities. A prominent example is a series of interviews that contributed to a book authored by Russ Hodge and Kat Menschick, where an entire chapter was dedicated to our research.

Two highlights:

1. **"Lebenkünstler"** by Russ Hodge and Kat Menschick. Editorial Galiani, Berlin. https://www.galiani.de/verlag/rights/book/kat-menschik-kat-menschik-lebenskuenstler-9783869713090
2. **"Jose Luis Gómez-Skarmeta" Award to scientific excellence**, by the Spanish Society of Developmental Biology (SEBD). https://sebd.es/wp-content/uploads/2024/07/resolucion-premio-jose-luis-gomez-skarmeta-2024-1.pdf



"Jose Luis Gómez-Skarmeta" Award by the SEBD

Molecular Embryology: Functional genomics, mouse transgenesis and myogenesis

Jaime J. Carvajal García-Valdecasas and Cristina Vicente García

Summary of activities:

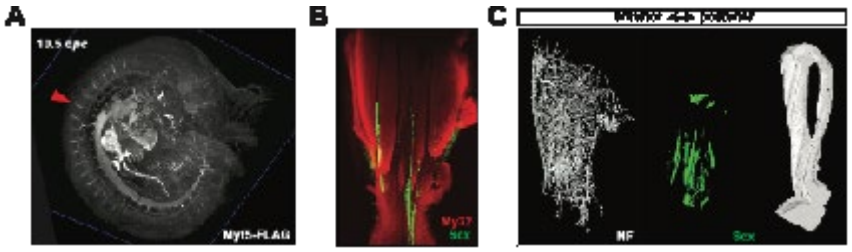
The main interest in our group is to understand how skeletal muscle develops, and how postnatal homeostasis is disturbed upon different insults or during ageing. In particular, we have been studying the role of the myogenic regulatory factors Myf5 and Mrf4 in these processes for decades. Last year, we started to investigate these issues taking into account the influence skeletal muscle specific defects could have in the co-development of related organs such as bones or tendons. We have found that the absence of Mrf4 leads to unexpected alterations in the morphology of limb long bones, underlining the importance of performing studies from an integrative perspective. Furthermore, in 2024 we optimized the application of several cutting-edge methods, including super-resolution microscopy and light-sheet microscopy, to investigate skeletal muscle biology from multiple scales: from the nano- to the mesoscale levels. In addition, we have deepened our understanding in the mechanisms that control skeletal muscle heterogeneity showing that muscles use differential splicing to tailor their mechanical properties to better suit their specific contractile requirements. Out latest findings were presented in the *Skeletal Muscle Development, Metabolism, and Repair during Homeostasis and Disease* EMBO Workshop that was held in October in Sicily, where we were awarded with a “Best poster presentation” prize for one of our posters.

Alongside his duties as group leader, Dr. Carvajal holds the position of Deputy Vice-President for Scientific Programs (VAPC) at CSIC from 2022. In parallel, Dr. Vicente teaches R programming in the Diploma in Bioinformatic Analysis as well as occasional classes in the Master’s Degree in Health Biotechnology at University Pablo de Olavide.

Two highlights:

In 2024, Dr. Carvajal and Dr. Vicente were awarded funding from the *Agencia Estatal de Investigación* for the first time as co-IPs. In our new project, which starts in 2025, we will delve into the molecular causes of muscle diversity, further exploring the contribution of factors such as sex, age and the role of the myogenic regulatory factors.

Prenatal and progressive coenzyme Q10 administration to mitigate muscle dysfunction in mitochondrial disease. Hernández-Camacho JD et al. J Cachexia Sarcopenia Muscle. 2024 Dec;15(6):2402-2416. doi: 10.1002/jcsm.13574.



Light-sheet microscopy imaging. Myf5 protein expression in the myotome at 10.5 days *post coitum* (**A**). Whole-mount immunostaining of muscles (red) and tendons (green) (**B**), and 3D reconstruction of nerves, tendons and bones (**C**) of a WT neonatal hindlimb. My32, myosin heavy chain; Scx, transgenic line ScxGFP; NF, neurofilament-L.

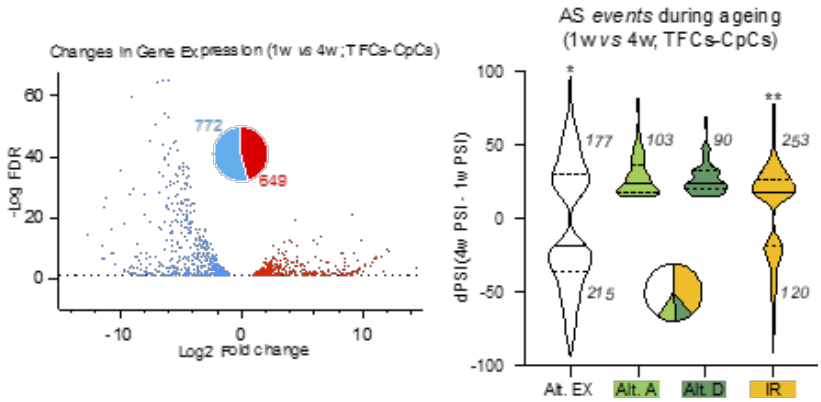
Stem cell behaviour in their niches and epithelial morphogenesis

Acaimo González Reyes

Summary of activities:

This year we have completed two research lines that have taken a great deal of effort, resources and energy to finish. One of them deals with the effect of ageing in a well-defined stem cell niche, that of the ovary of the fruit fly. It is known that gene expression (GE) and alternative splicing (AS) contribute to the formation of new interaction networks with potentially significant cellular functions. We investigated ageing in the *Drosophila* female germline stem cell (GSC) niche and described functional changes in both GE and AS. The GSC niche comprises three types of support cells, whose ageing transcriptomes revealed differential GE and AS variations related to cell adhesion, cytoskeleton and neural signalling. Because each population showed distinctive GE and AS changes, niche cell types possess unique ageing signatures. Groups of genes displayed changes in both GE and AS, revealing a coordinated regulation of both during niche ageing (*Nature Communications*, in press).

Gonad development is a pre-requisite for gametogenesis and reproduction. During female gonad formation in *Drosophila*, the EGF receptor (EGFR) signalling pathway ensures the correct number of primordial germ cells (PGCs) populate the larval gonad. The gene pointed (*pnt*), which acts downstream of the EGFR receptor and belongs to the ETS transcription factor family, has a previously unknown function in gonadogenesis. We found that *pnt* was expressed in female larval gonads and later in the adult ovarian germline niche and that it was required for proper gametogenesis. Loss of *pnt* function, similar to the EGFR, induced PGC overproliferation. Conversely, we isolated a novel loss-of-function allele, pnt^{aga}, which resulted in agametic gonads and ovaries. Molecular characterization of pnt^{aga} revealed reduced expression levels of the different *pnt* isoforms, unveiling a complex autoregulatory network involving the three Pnt proteins (in second revision in *PLoS Genetics*).



Changes in GE — represented as log2 fold change at 4 weeks/1 week versus the -log10 false discovery rate (FDR) — identified 649 genes upregulated (red) and 772 (blue) genes downregulated in niche cells (linear fold change <0.5 or >2; FDR <10%). Graph showing the distribution of the 958 changes (affecting 673 genes) in AS events during niche ageing identified in our studies (Alt. E: alternative exons refer to skipped and/or mutually exclusive exons; Alt. A or D: alternative acceptor or donor splice sites correspond to variations in 3' and/or 5' splice-site selections; IR: intron retentions; medians are shown as horizontal lines in the boxes; dotted lines represent first and third quartiles). Changes are represented as variations in percent splicing inclusion (dPSI=4w PSI – 1w PSI). Only events with an absolute dPSI>15 were considered.

Nuclear Architecture and Dynamics

Rafael R. Daga and Silvia Salas Pino

Summary of activities:

Our group has published 3 papers in 2024. We have presented our work as a selected talk at the 10th Proteasome & Autophagy International Congress celebrated in Paris. In addition, one of our PhD students defended his thesis. The lab has participated in outreach activities and, as university professors, we have actively participated in the teaching associated with the Genetic Area. Our research line is related to MdM Q3, as we are studying proteasome response and proteome rearrangement and the dynamic formation of bio-condensates as cellular responses to folding stress.

Ongoing Project:Title: Control of proteostasis during unperturbed cell cycle and under proteotoxic stress. Ministerio de Ciencia e Innovación Ref.:PID2021-128408OB-I00. Budget: 217.000€. Period: 1/9/2022-31/08/25.

Collaborations: Mark Hochstrasser (Yale University, USA), Alfonso Fernández (IBFG, Salamanca), Elena Hidalgo (U. Pompeu Fabra, Barcelona), Fred Chang (UCSF, USA). Kathy Gould (Vanderbilt U., USA), Alejandro Díaz Moscoso (IIQ, Seville), Manuel Bernal (Málaga U.), Ana Rojas (CABD), Antonio Pérez Pulido (CABD).

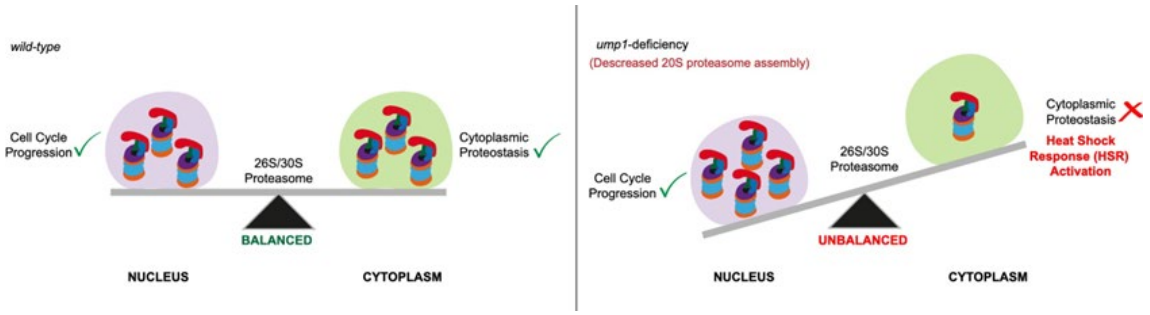
Doctoral Thesis: PhD: Gabriel Ruiz Romero. Title: Proteasome response to folding stress in fission yeast. (*Internacional mention*). TFMs &TFGs. 1 TFM and 2 TFGs directed.

Teaching: Genética molecular y *Molecular genetics* (Grado Biotecnología), Genética humana (Grado nutrición humana y dietética), Estrategias nuevos fármacos, Bases celulares y moleculares del cáncer, Métodos prácticos en el estudio de patologías, Modelos animales en biotecnología (Máster).

Two highlights:

Limiting 20S proteasome assembly leads to unbalanced nucleo-cytoplasmic distribution of 26S/30S proteasomes and chronic proteotoxicity. Ruiz-Romero G, Berdún MD, Hochstrasser M, Salas-Pino S, Daga RR. iScience. 2024 Oct 4;27(11):111095. doi: 10.1016/j.isci.2024.111095.

MAPK-dependent control of mitotic progression in *S. pombe*. Iglesias-Romero AB, Soto T, Flor-Parra I, Salas-Pino S, Ruiz-Romero G, Gould KL, Cansado J, Daga RR. BMC Biol. 2024 Mar 25;22(1):71. doi: 10.1186/s12915-024-01865-6.



Schematic representation of proteasome distribution between the nucleus and the cytoplasm in wt vs ump1 deficiency. Inactivation of the 20S proteasome assembly chaperone Ump1 results in a drop in proteasome particles, which preferentially accumulate in the nucleus. This condition allows cell cycle progression but results in deficient cytoplasmic proteostasis that eventually leads to the accumulation of insoluble aggregates, thus mimicking an aged cell.

Control of epithelial morphogenesis in vertebrates

Juan Ramón Martínez Morales

Summary of activities:

2024 has been a transition year in the lab at very different levels. Thus, instead of highlighting published articles, I would like to emphasize two awarded grants, which will set the bases of the two main research lines in the laboratory.

a) **ProgRet-ITN**. In collaboration with Juan Tena, we participate in a European Training network aimed to understand, diagnose and treat inherited retinal diseases: Reference H2020-MSCA-ITN-2022 (ID:101120562). Coordinator: Elfride de Baere (U. Ghent). 1/2024-12/2027. Total Budget: 3.776.714,28. Partner 5 budget 250.904,88 €. IP Juan R Martinez-Morales.

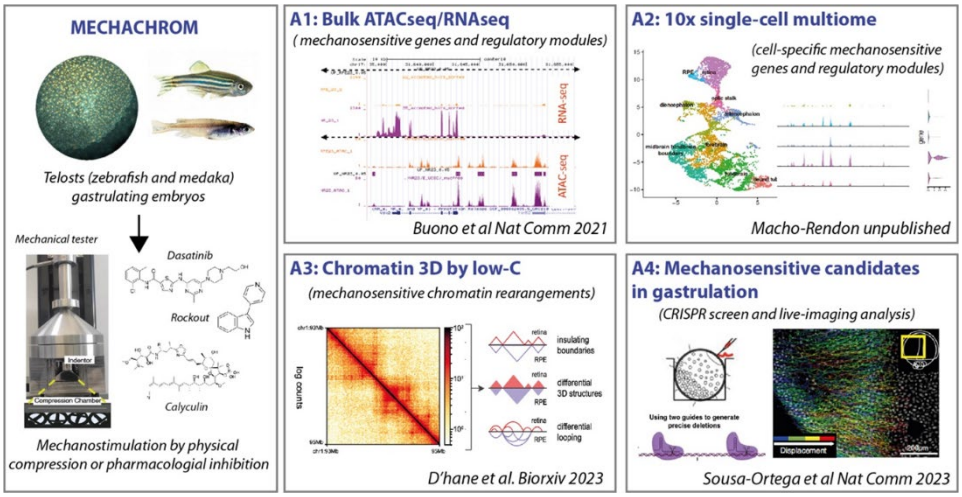
The objectives of this network tackle questions overlapping with the interests of the Maria de Maeztu (MdM) project. Particularly as to how regulatory and structural changes in the genome relate to pathological variants responsible for genetic diseases (Q1). The ongoing collaboration with Elfride de Baere and Juan Tena has already result in a high impact publication (D'Haene, López-Soriano and Martínez-Garcia et al 2024, Genome Biol)

b) **MechaChrom**. Mechanical regulation of chromatin and transcription during tissue morphogenesis. MINECO. REF: PID2023-152286NB-I00. 9-2024/8-2027. Budget: 425.000,00 € + Fellowship (PIF). PI: Martinez-Morales JR. This proposal tackles a basic question at the interface between cell and developmental biology: as to how mechanical information is interpreted in the context of developmental programs in the embryo. This is precisely one of the main questions (Q3) intended in the MdM proposal.

Highlight:

Comparative 3D genome analysis between neural retina and retinal pigment epithelium reveals differential cis-regulatory interactions at retinal disease loci. D'haene E, López-Soriano V, Martínez-García PM, Kalayanamontri S, Rey AD, Sousa-Ortega A, Naranjo S, Van de Sompele S, Vantomme L, Mahieu Q, Vergult S, Neto A, Gómez-Skarmeta JL, Martínez-Morales JR*, Bauwens M*, Tena JJ*, De Baere E*. Genome Biol. 2024 May 17;25(1):123. doi: 10.1186/s13059-024-03250-6.

* Corresponding authors



Graphical abstract depicting the objectives of the awarded grant MechaChrom.

Biotechnology of industrial and artisan fermentations. Competitivity factors in microbial communities and their application.

Andres Garzon

Summary of activities:

In 2024, our research group has made progress in the implementation of our project from the Knowledge Generation Program, which focuses on the role of yeast killer elements in intraspecific competition interactions across various yeast life stages (natural environment, fermentations, and biofilm formation). We have a PhD student conducting research on this topic.

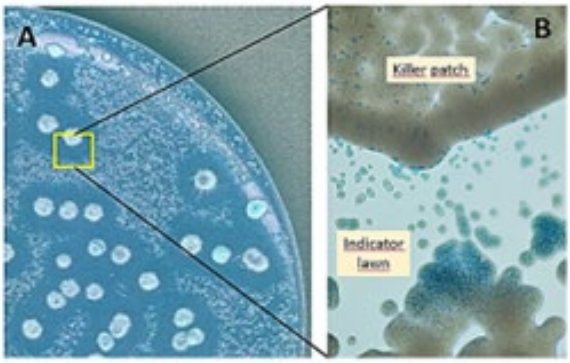
We maintain our applied science focus through ongoing collaborations with two companies, Bodegas Gonzalez Palacios and Cultivando Empleo. Additionally, we have developed a knowledge transfer project within the UPO's intramural research program. We have applied for a Junta de Andalucia applied research grant. We have organized a summer university course where we have explained our scientific applied science projects.

We continue to sustain productive collaborations with the research groups of Juan Jimenez, Antonio Pérez-Pulido, Manuel Muñoz, and the zoologist Antonio Ruiz-García. These collaborations have resulted in four publications, one manuscript submitted for review, and a patent application in progress.

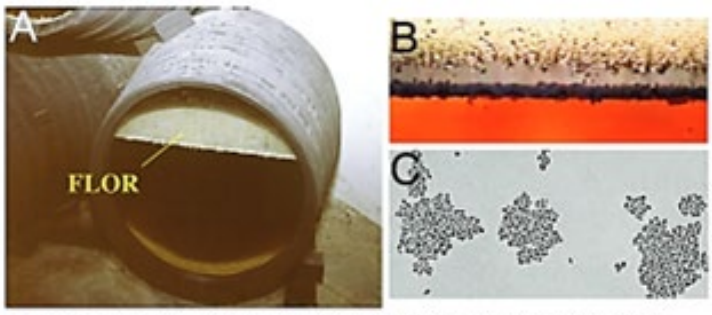
Two highlights:

Biological warfare between two bacterial viruses in a defense archipelago sheds light on the spread of CRISPR-Cas systems. Rubio A, Garzón A, Moreno-Rodríguez A, Pérez-Pulido AJ. Cell Rep. 2024 Dec 24;43(12):115085. doi: 10.1016/j.celrep.2024.115085

Monosaccharides improve symptoms of an animal model for type III galactosemia, through the activation of the insulin pathway. Lucas-Rodríguez P, Brokate-Llanos AM, Hernandez-Curiel JM, Murdoch PDS, Garzón A, Carrión A, Muñoz MJ. Biomed Pharmacother. 2024 Dec;181:117677. doi: 10.1016/j.biopha.2024.117677.



L-A/M-mediated killer effect. A) MBA plate killer phenotype assay. Replicate colonies of a killer strain on a sensitive background. The presence of methylene blue, a dye that stains dead cells, helps to confirm the effect of the toxin on the sensitive strain. B) Enlargement of the zone of inhibition where the effect of the toxin can be seen reducing the growth of nearby colonies and producing lethality in colonies further away up to a certain distance from the producer strain.



S. cerevisiae "flor yeasts" are naturally able to grow on the sherry wine surface. (A) Oak barrels filled to ¾ with sherry wine spontaneously form self-supporting yeast biofilm on the wine surface, a velum known as "floc."(B) This thick biofilm floats and allows flor yeast to gain direct access to oxygen. (C) Cells in this buoyant biofilm are strongly aggregated, showing a flocculation phenotype.

Evolution of organogenetic gene networks

James C-G Hombría and Sol Sotillos

Summary of activities:

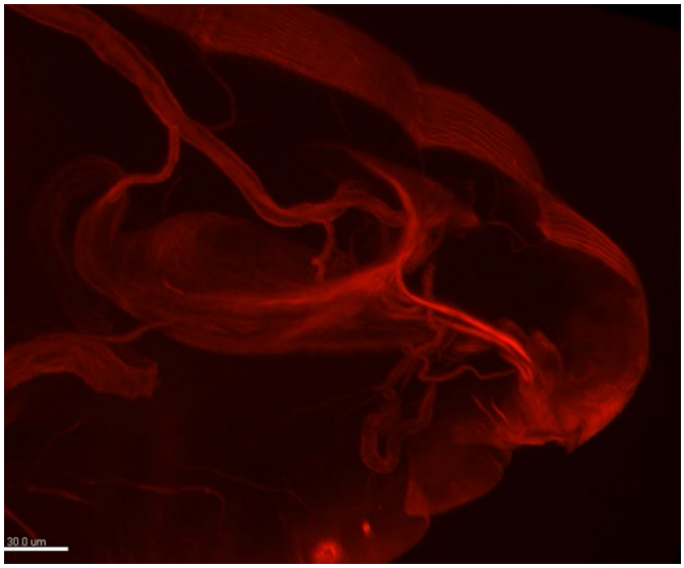
Focusing on the formation of various *Drosophila* organs, we are analysing how their complex organogenetic gene networks change during evolution. We study how new organs originate by the divergence of their gene network, as well as how the sudden cooption of a gene network into a different organ affects its evolution.

Divergence: We have found that the insect cephalic endoskeleton, the tentorium, required for the insertion of the masticatory muscles, originates at the intercalary, maxillary and labial segments from primordia located at homologous positions to those that form the tracheae in more posterior segments. These cells constitute a *homology group* that also contributes in the head to the formation of the glands regulating moulting and metamorphosis (the *corpora allata* and prothoracic glands). Despite their functional divergence all four organs are specified using similar upstream regulators, that include the Hox genes and the JAK-STAT signalling pathway, and can be transformed into each other, uncovering an amazing case of plasticity during evolution.

Cooption: We are analysing how the gene network required for the formation of the external respiratory organs of the larva (the posterior spiracles) has been recently recruited (coopted) to the testis in the Drosophilids. We discovered that the coopted gene network activates in the testis a gene, *ripley,* that has originated *de novo* from an intergenic region and which uses its neighboring gene *cis*-regulatory elements to be expressed. Ripley is not required for the function of the larval spiracles, but is required for the liberation of the mature sperms from the head cyst cells holding them, which function as the *Drosophila* equivalent of the human Sertoli cells. In *ripley* mutant males the sperm tails degrade and the animal becomes sterile.

These two models provide complementary systems to understand how the modulation of complex gene networks during evolution give rise to new functions.

Head of a late *Drosophila* embryo stained with a chitin binding probe to label the cuticle of the cephalopharyngeal skeleton and the tracheae**.**



Bacterial infections: new targets and new prevention and treatment strategies

Younes Smani

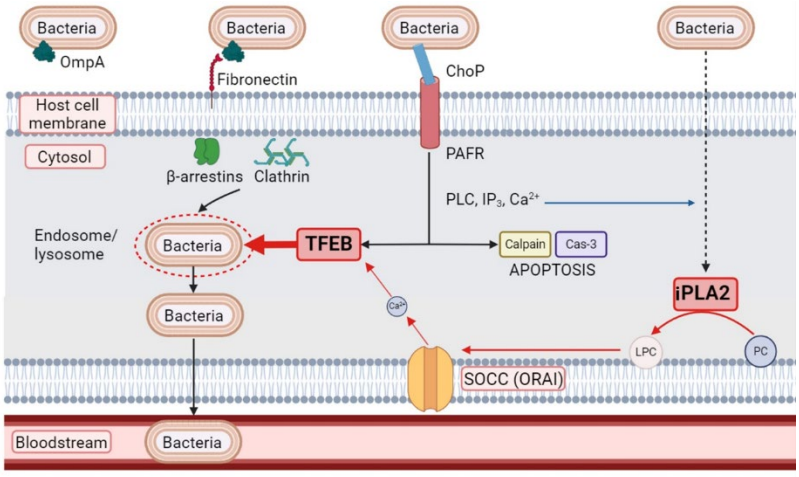
Summary of activities:

During 2024, we initiated a new cellular infection model to enhance our understanding of host-pathogen interactions from *in vitro* to *ex vivo* and *in vivo* models, in alignment with the DMC3 Strategic Plan. Currently, we are generating pulmonary organoids, supported by ProyExcel_00116 funding. Concurrently, we are investigating the roles of OMPs, TFEB, and PLA2 in *Acinetobacter baumannii* intracellular trafficking withing host cells (PPI A3, UPO). We have deciphered the roles of iPLA2 and TFEB in *A. baumannii* invasion and intracellular dynamics, contributing to a deeper understanding of its intracellular lifestyle and its strategies for evading the immune system's defences.
Furthermore, it is well known that biological systems exhibit plasticity throughout evolution and development, enabling adaptation to changing environments while balancing robustness against fragility to ensure survival and functionality. Currently, we are addressing one key question. What are the dynamic omics mechanisms and signalling pathways that *A. baumannii* utilizes to adapt in a climate change and resist host-mediated killing? (DMC3 Strategic Plan Q1 & Q3)
Additionally, we have extended the patent for thiophene derivatives as antimicrobial agents [PCT202330628] and published the data in Frontiers in Pharmacology [PMID: 39228527].
We have also contributed to peer-review activities for international and national agencies (EUREKA, ESCMID, Poland National Science Centre, Sapienza University, ANEP, ISCIII Comunidad de Madrid, and Cidi Salud), as well as scientific journals (Nat Com, JAC and AAC) and international congresses (ECCMID), and we are members of the Conexion-CSIC Resistencia antimicrobianos.

Two highlights:

Seguin-Devaux C, Mestrovic T, Arts JJ, Sen Karaman D, Nativi C, Reichmann D, Sahariah P, Smani Y, Rijo P, Mori M; COST Action CA21145 EURESTOP. Solving the antibacterial resistance in Europe: The multipronged approach of the COST Action CA21145 EURESTOP. Drug Resist Updat. 2024 May;74:101069. doi: 10.1016/j.drup.2024.101069.

Antibiotic discovery with artificial intelligence for the treatment of Acinetobacter baumannii infections. Boulaamane Y, Molina Panadero I, Hmadcha A, Atalaya Rey C, Baammi S, El Allali A, Maurady A, Smani Y. mSystems. 2024 Jun 18;9(6):e0032524. doi: 10.1128/msystems.00325-24.



Cas-3: caspase-3; **PAFR**: platelet activating-factor receptor; **SOCC**: store operated channel **ChoP**: phosphorylcholine; **PLC**: phospholipase C; **IP_3**: inositol 3-phosphate

Suggested mechanism for TFEB activation dependent on iPLA2.

Obtaining New Antimicrobial Compounds and New Antibiotic Resistance Genes Through a Metagenomic Approach

Amando Flores and Eva Camacho

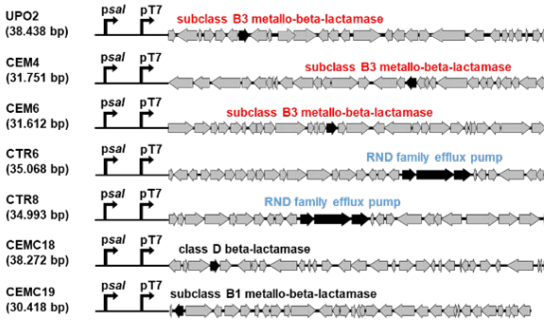
Summary of activities:

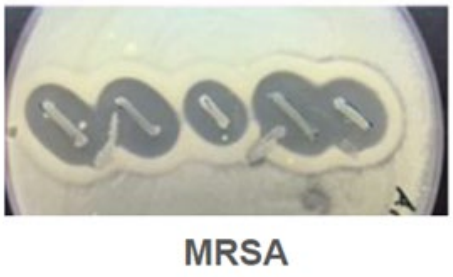
It is estimated that over 99% of microbial taxa within the Earth's microbiome remain undiscovered. Techniques like metagenomics offer access to this untapped reservoir of genetic information. At present, multidrug-resistant pathogens represent one of the most significant public health threats of the 21st century. In response to the growing warning of multidrug-resistant bacteria, in our laboratory, we use functional metagenomics to find novel solutions to this challenge. Given that most clinical antibiotics ultimately originate from natural sources, that most of the resistance genes found in pathogens come from bacteria in the environment—and considering the vast microbial diversity that remains inaccessible due to the inability to culture many microorganisms in the laboratory—searching nature for genetic determinants encoding novel antimicrobials is a promising strategy. Using functional metagenomics, we have developed improved vectors and bacterial host strains to perform functional screens and have constructed diverse metagenomic libraries from various environmental sources.

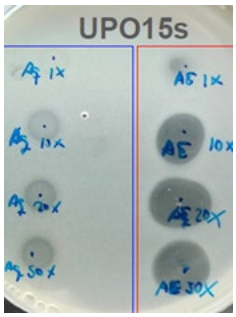
Through this approach, we have identified several novel genes associated with antibiotic resistance and antimicrobial synthesis. Notably, we have isolated clones producing new antimicrobial compounds that are effective against multidrug-resistant pathogens, including MRSA. We are currently working to purify and characterize these compounds for potential antibiotic applications. Additionally, we have identified clones in our libraries containing previously uncharacterized resistance genes to last-resort antibiotics like carbapenems. These genes could cause future risks if transferred to pathogenic bacteria. While these resistance mechanisms are not yet a clinical threat, we are actively investigating their activity to better understand and potentially mitigate their impact.

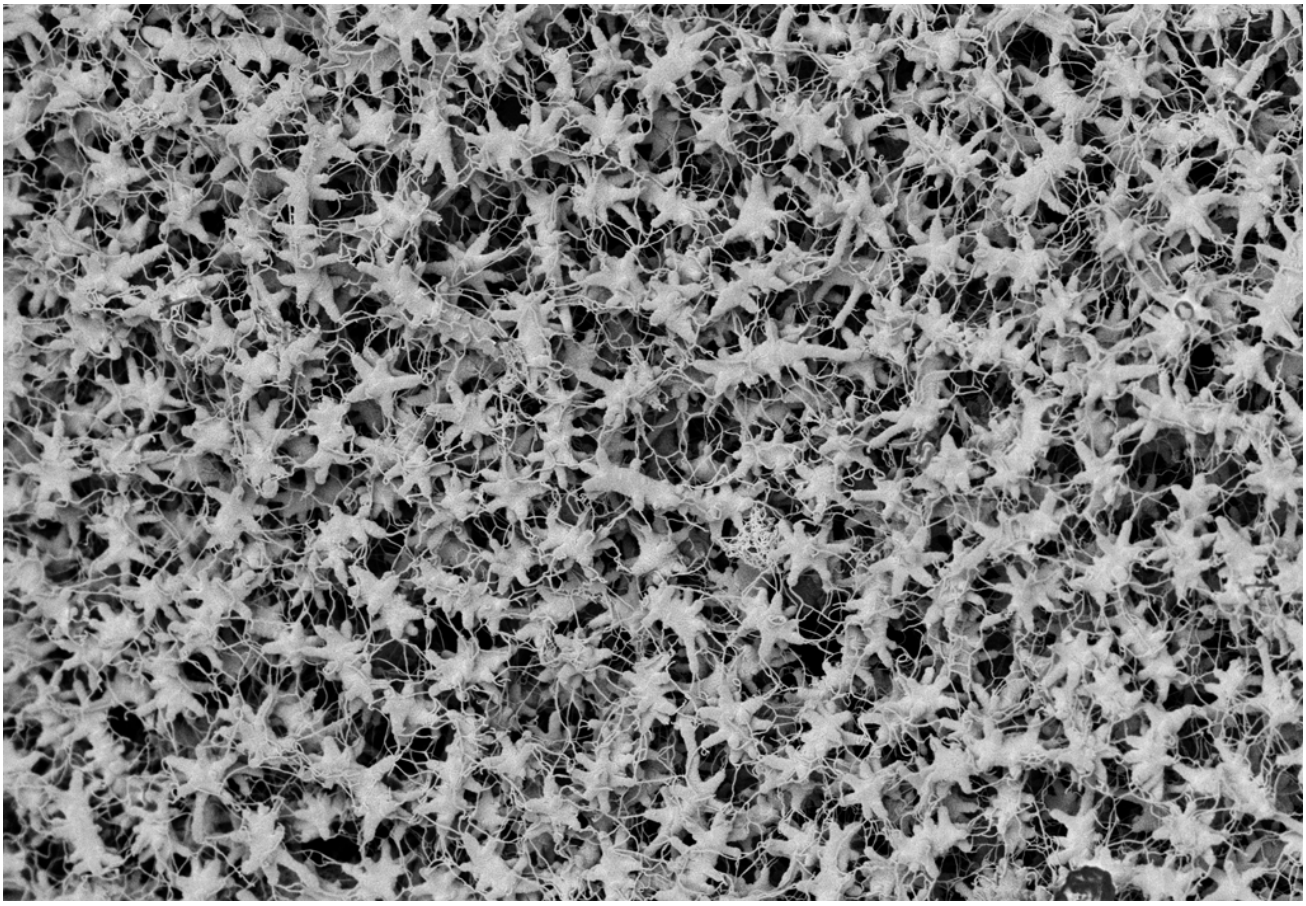
On the other hand, we have also developed genetic tools to transfer the constructed metagenomic libraries and express environmental DNA in bacterial genera other than *E. coli*, such as *Acinetobacter* and *Pseudomonas*, which include some pathogenic strains. This has enabled us to identify new genes that confer resistance to last-resort antibiotics in these bacteria.

In addition, we are both professors at UPO, where we teach over 100 hours of classes, supervise undergraduate and master's theses, and hold management positions within the university.









Microcosmic mesh of *Verrucomicrobium spinosum* prosthecae. Underneath the gaze of electron microscopy lies a network of prosthecae, the extensions of *Verrucomicrobium spinosum*, connected through delicate fimbriae. This image reveals the architecture of microbial morphology, a testament to nature's elegant design at the microscopic scale (Author: Valentina Henriques).

Centro Andaluz de Biología del Desarrollo

EXCELENCIA MARÍA DE MAEZTU

AGENCIA ESTATAL DE INVESTIGACIÓN

Junta de Andalucía

UNIVERSIDAD PABLO DE OLAVIDE SEVILLA

CSIC

CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS