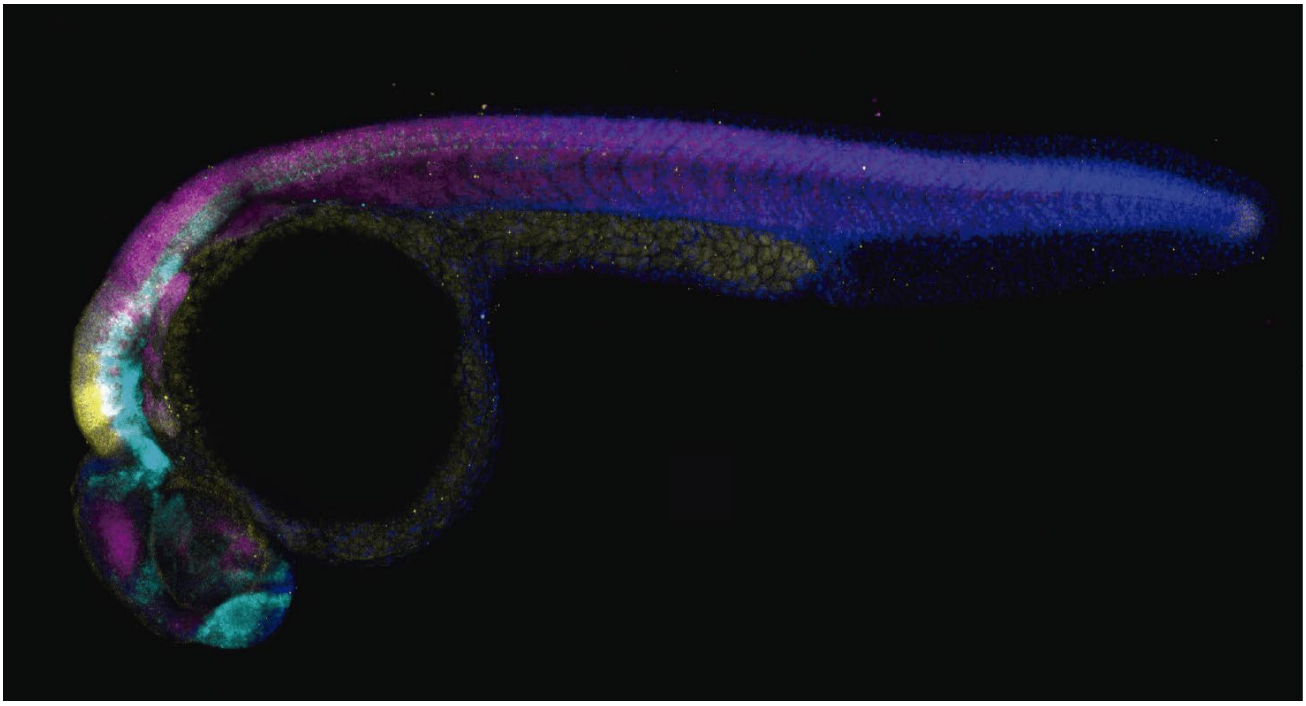
Centro
Andaluz
de Biología
del Desarrollo

Annual Report 2023



Zebrafish embryo at the 24 hpf stage is illustrated using the Highly Multiplexed Chromogenic RNA (HCR) technique. In this image, the elavl3 gene is highlighted in cyan, znf503 in magenta, znf703 in yellow, and cell nuclei are marked in blue with DAPI staining (Marta Oñate).

Junta de Andalucía

Universidad Pablo de Olavide Sevilla

CSIC
Consejo Superior de Investigaciones Científicas

CABD Annual Report 2023

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CABD Activities 2023

The CABD celebrated its 20th anniversary during the days June 5-7, 2023. The three days were packed with scientific and institutional events and marked a moment of reflection about the past, present and future of the institute. 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 Unit of Excellence awards from the Spanish *Agencia Estatal de Investigación* (2017-2021, 2022-2025). The centre hosts 28 established groups and 5 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 organizations to explore their biomedical perspective.

Six group leaders and five junior group leaders were appointed in 2023. The majority were through internal promotions whereas others arrived from positions in Houston, Berlin and Barcelona. New incorporations of pre- and postdoctoral researchers, as well as staff to scientific platforms, service facilities and administration raised the CABD personnel to 234 by the end of 2023. 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 2023 include more than 220 teaching credits and supervision of students at B.Sc., M.Sc., and Ph.D. level: 71 theses were successfully defended at the CABD in 2023.

Funding for 29 new research projects was obtained through competitive calls from regional, national, and international agencies, worth a total of 7.7 million euros. Moreover, two new contracts on scientific collaborations with companies were initiated in 2023. The scientific productivity of the CABD in terms of publications amounted to almost 4,800 citations, 69 peer-reviewed articles and 5 book chapters during 2023, mostly with open access in leading journals and with the participation of 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 two EMBO Workshops celebrated in Seville in 2023. Finally, the CABD is very active in science dissemination with tailored events for a variety of audiences across age and educational backgrounds.

2023 was the second 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 36.

One of the central questions deals with the relationship that regulatory and structural changes in the genome have with evolutionary innovations. This has been addressed in several studies in 2023. For instance, by comparing regulatory genomic regions across species, a joint effort between four CABD groups and multiple international laboratories revealed how the wing-like fins of skates evolved more than 200 million years ago. Another study in *Drosophila melanogaster* found that recruitment of whole gene networks by cooption into different organs can make immediate use of sophisticated regulatory chains that would need thousands of years to evolve *de novo*. The relevance of genome architecture has also been studied in the context of ecological relationships within microbial communities where the differential use of conserved regulatory elements in the genus *Pseudomonas* impinges on the alternation between biofilm and dispersal live styles. Moreover, the compositions of microbial consortia selected for their capacity to degrade specific pollutants were investigated to optimise their efficiencies and to determine the evolution of species within the consortia.

Another central question related to the coordinated use of gene regulatory networks between neighbouring cells and organs, and how cells communicate their states across space and time. This is crucial to control, for example, organ size. Analysis of *Drosophila* development uncovered a feedback control mechanism where cell signalling and apoptosis sustain robust eye size. Eye development was also studied in zebrafish, which led to the conclusion that redundancy of Visual System Homeobox genes (VSX) is an important mechanism to ensure integrity of the retinal specification pathway. Muscle development is another example of how complex gene regulatory networks influence cells both autonomously and non-autonomously: CABD researchers

demonstrated that knockout of the myogenic factor Mrf4 affects not only skeletal muscle where it is normally expressed, but also bone and heart.

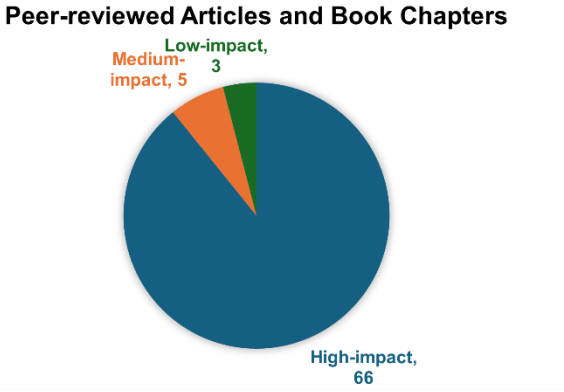
The capacity of cells to sense and respond to changes in their environment represents the third central question: which are the underlying mechanisms and what role do they play in cell collectives' behaviour? Budding yeast form different types of communities, such as flocculation, filamentation or biofilm in response to their environment. These forms of multicellular organization require mechanisms of communication and a balance between cooperation and competition. A novel CABD project aims to characterize a dsRNA virus as a modulator of cellular interactions within these communities. The decision to enter meiosis is among the key responses of yeast to changes in the environment. In 2023, CABD researchers found that chromosome segregation in meiosis requires the re-localization of Aurora B kinase from kinetochores to the spindle midzone to reset the spindle microtubule nucleation function of centrosomes. Within animals, the extracellular matrix (ECM) connects cells and transduces mechanical stimuli between them. By studying the behaviour of follicle stem cells of the *Drosophila* ovary, the ECM was found to regulate cell proliferation through the Notch/Delta signalling pathway. Another study in *Drosophila* showed how an external cue in form of pheromones triggers physiological reactions in multiple organs to produce a courtship behavioural response. Sensing changes in the environment is also essential to respond properly to injuries. This is currently being investigated in models of ischemic damage to the neonatal brain and host-pathogens interactions upon infection with *Acinetobacter baumannii*. Plant pathogens also integrate information from their environment as exemplified with the observation by CABD researchers that the fungus *Ustilago maydis* uses the sirtuin Sir2 to coordinate gene expression during infection of corn. Other host-microbe interactions can be beneficial as in the case of *Bacteroides thetaiotaomicron*, which is considered a symbiotic bacterium in the human gut and represents a new research focus at the CABD. Finally, the capacity of the bacterium *Sphingopyxis granuli* TFA to withstand environmental stresses through changes in transcriptional programs is being scrutinized and has potential implications for bioremediation.

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. To understand the origins of membrane compartments in eukaryotic cells, including the cell nucleus, CABD researchers study the evolution of membrane coat-like proteins in *Gemmata obscuriglobus* and related bacteria. The acquisition of membranes to enclose the nuclear genome led to the evolution of nuclear pores and LINC complexes to enable regulated communication between the nucleus and the surrounding cytoplasm, organelles, and cytoskeleton. CABD groups found that temperature stress and aging induce nuclear pore protein condensates in yeast and nematodes and a CABD computational group recently joined efforts to investigate this phenomenon in further detail. Likewise, a new project to study the effects of mechanical stimuli on nuclear geometry and on the Yap transcription co-factor in zebrafish stem cells was initiated in 2023. Yap also functions in mammals during stem cell differentiation, and the possibility to explore this function in cell reprogramming is currently being tested.

The fifth question relates to energy constrains and metabolic states and the role they play in tissue morphogenesis, aging and disease. 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 2023, these studies identified new mechanisms that link the mitochondrial unfolded protein response to organization of the nuclear genome through the heterochromatin protein 1 family and new therapeutic opportunities for mitochondrial diseases. Another example is the discovery that mutations in *GEMIN5* lead to defective mitochondria, reduced CoQ10 synthesis, and neurodegeneration. Importantly, disease symptoms in flies and humans can be alleviated by administering CoQ_{10}. Combining nematode and mouse models of neurodegeneration led recently other CABD researchers to identify a drug with potential to treat Alzheimer's disease and significant financial support was obtained in 2023 to develop this towards clinical testing.

Scientific discoveries are often linked to technological advances. Several CABD groups devote significant efforts to the design and implementation of novel technologies for the benefit of their research lines and for the broader scientific community. Examples include optimization of CRISPR-Cas systems for genome editing and mRNA knockdown in multiple model systems. Another example is the bioinformatics tool ASACO (Automatic and Serial Analysis of gene CO-expression based on transcriptomics data) that was developed to facilitate the identification of potential drug targets in the human genome. Finally, establishment of single-cell omics protocols by CABD groups enabled in 2023 the detailed analyses of chromatin structure in zebrafish embryos and transcriptomic landscapes underlying self-organization in mouse embryoids.

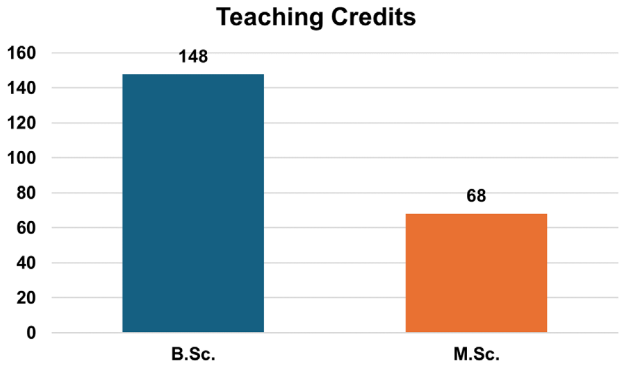
CABD Facts & Figures 2023



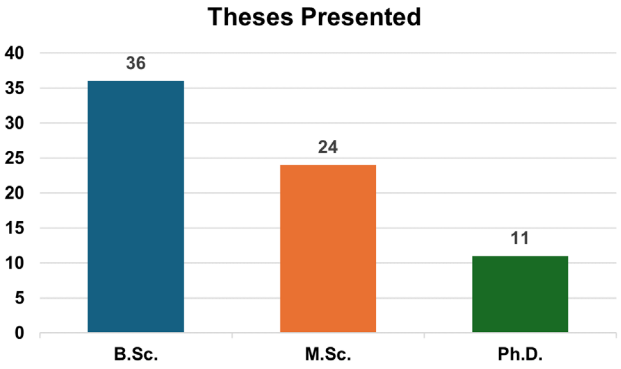
We published 74 peer-reviewed articles and book chapters in 2023. Of these, 66 (89%) were classified as high-impact by the CSIC, 5 (7%) as medium-impact and 3 (4%) as low-impact. Co-authors from foreign institutions contributed to 33 (45%) of the publications.



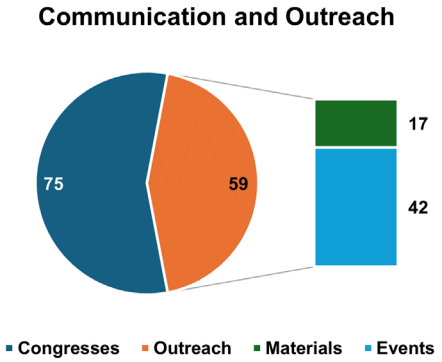
The CABD obtained 29 grants from competitive funding schemes: 20 from the Spanish Government, 5 from the Andalusian Regional Government, 2 from the European Commission and 2 from the University Pablo de Olavide.



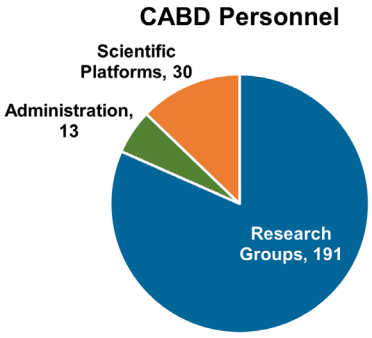
Our teaching activities amounted to 216 credits in 2023. Approximately two thirds corresponded to B.Sc. degrees and one third to M.Sc. degrees.



A total of 71 theses were presented in 2023. The majority represented B.Sc. projects (51%), followed by M.Sc. projects (34%) and doctoral theses (15%).



We presented our research with 75 contributions at scientific congresses and 59 outreach activities to the public: 42 outreach events (science fairs, visits to schools, etc.) and 17 dissemination materials (newspaper articles, radio interviews, blogs, etc.).



Most CABD personnel are associated to Research Groups (82%), whereas 13% and 6% work in Scientific Platforms and Administration, respectively.

Control of Epithelial Morphogenesis in Vertebrates

Juan Ramón Martínez Morales

Summary of activities:

Highlights of 2023 include two relevant publications and a new funded project. By analysing the phenotype of yap1/yap1b double mutants in medaka fish, (*O. latipes*) we showed that Yap proteins are engaged in a mechano-regulatory feedback loop that controls the migratory behaviour of precursors cells during gastrulation (Sousa-Ortega & Vázquez-Marin et al. Nat Comm 2023). This article is the group's entry point into the mechanobiology field and **is particularly relevant to understand how mechanical information is incorporated into tissue self-organization.**

In our Elife article by Letelier & Buono et al we analysed the role of vsx genes in the specification and differentiation of the zebrafish (*D. rerio*) retina. We showed that *Vsx* genes play a conserved role bipolar cells differentiation and uncover an unexpected degree of redundancy in the early specification program. This article is in line with a main objective in the team, to characterize retinal gene regulatory networks, and pertains a main question in MdM DMC3: **as to how gene regulatory robustness is achieved.**

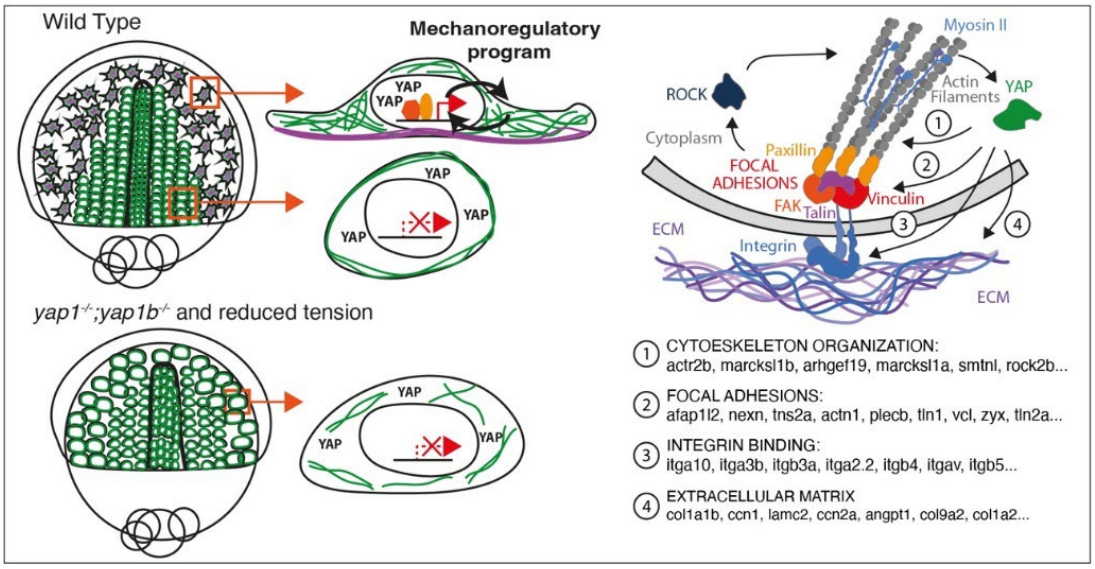
Our HORIZON-MSCA-2022-DN-01 ITN Proposal, ProgRET: "a training network foused in understanding, diagnosing and treating autosomal dominant inherited retinal disease (adIRD)", was successfully funded. Our group participates together with Juan Tena's team as a CSIC partner in this proposal coordinated by Elfride de Baere (Ghent U.). Our sub-project aims to understand at a transcriptional and epigenetic levels the regulatory interaction between aging and retinal dystrophies using killifish (*N. furzeri*). As the project aims to understand the impact on IRDs of regulatory and structural changes in the genome, **it is framed within one of the main questions of the MdM DMC3 proposal.**

Selected references:

Letelier J, Buono L, Almuedo-Castillo M, Zang J, Mounieres C, González-Díaz S, Polvillo R, Sanabria-Reinoso E, Corbacho J, Sousa-Ortega A, Diez Del Corral R, Neuhauss SCF, Martínez-Morales JR. Mutation of *vsx* genes in zebrafish highlights the robustness of the retinal specification network. Elife. 2023 May 25;12:e85594. doi: 10.7554/eLife.85594.

Sousa-Ortega A, Vázquez-Marín J, Sanabria-Reinoso E, Corbacho J, Polvillo R, Campoy-López A, Buono L, Loosli F, Almuedo-Castillo M, Martínez-Morales JR. A Yap-dependent mechanoregulatory program sustains cell migration for embryo axis assembly. Nat Commun. 2023 May 16;14(1):2804. doi: 10.1038/s41467-023-38482-w.

A Yap-dependent mechanoregulatory program sustains cell migration for embryo axis assembly



Mitotic Spindle Nucleation and Coordination with the Cell Cycle

Víctor Álvarez Tallada

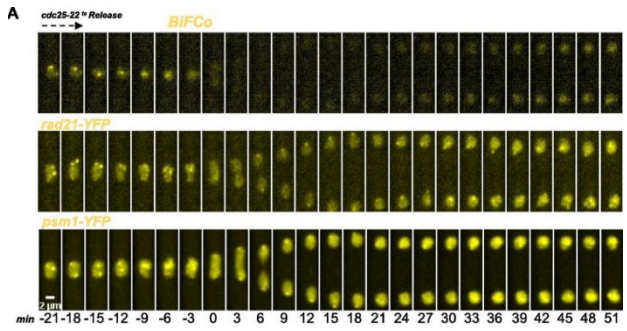
Summary of activities:

Cohesin is an essential complex in eukaryotes to keep genome architecture, organising chromatin within the nucleus and regulating gene expression. In 2023 we have published 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, in a second publication, 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 year we have also celebrated one of our students doctorate whose work is shedding light on exciting ɣ-Tubulin Ring Complex remodelling in mitotic prophase. The whole lab has also taken our research out of the CABD to the Science fair and the European researchers' night in Seville that we enjoyed very much.

Selected references:

Villa-Consuegra S, Tallada VA*, Jimenez J*. Aurora B kinase erases monopolar microtubule-kinetochore arrays at the meiosis I-II transition. iScience. 2023 Oct 27;26(11):108339. doi: 10.1016/j.isci.2023.108339. *corresponding authors.

González-Martín E, Jiménez J*, Tallada VA*. BiFCo: visualizing cohesin assembly/disassembly cycle in living cells. Life Sci Alliance. 2023 May 9;6(7):e202301945. doi: 10.26508/lsa.202301945. *corresponding authors.





Cell Cycle Control

Juan Jiménez

Summary of activities:

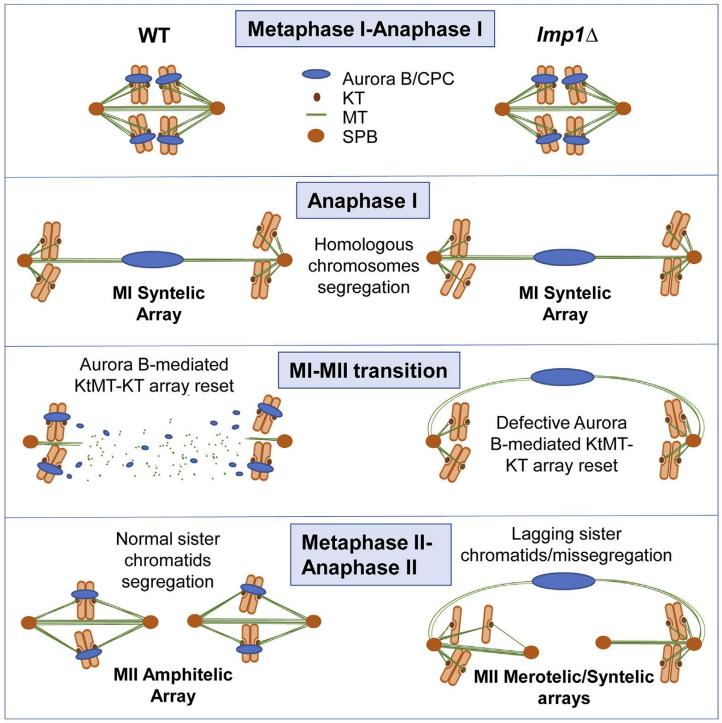
During the last years, the group has focus on mechanisms coordinating spindle disassembly and chromosome segregation during meiosis. Meiosis faithful chromosome segregation requires monopolar spindle microtubule-kinetochore arrays in MI to segregate homologous chromosomes, but bipolar in MII to segregate sister chromatids. We found that the universal Aurora B kinase localizes to kinetochores in metaphase I and in the mid-spindle during anaphase I, as in mitosis; but in the absence of an intervening S phase, the importin α Imp1 propitiates its release from the spindle midzone to re-localize at kinetochores during meiotic interkinesis. We have shown that "error-correction" activity of kinetochore re-localized Aurora B becomes essential to erase monopolar arrangements from anaphase I, a prerequisite to satisfy the spindle assembly checkpoint (SAC) and to generate proper bipolar arrays at the onset of MII. This microtubule-kinetochore resetting activity of Aurora B at the MI-MII transition is required to prevent chromosome missegregation in meiosis II, a type of error often associated with birth defects and infertility in humans.

As part of our work, we carry out the genetics analysis of industrial yeasts. In collaboration with Bodegas Salado, the combinatorial diversity found in natural yeasts demonstrates the existence of a sexually active population of *S. cerevisiae* yeasts, an extremely useful discovery to preserve wine making under environmental global changes.

Selected references:

Villa-Consuegra S, Tallada VA*, Jimenez J*. Aurora B kinase erases monopolar microtubule-kinetochore arrays at the meiosis I-II transition. iScience. 2023 Oct 27;26(11):108339. doi: 10.1016/j.isci.2023.108339. *corresponding authors.

González-Martín E, Jiménez J*, Tallada VA*. BiFCo: visualizing cohesin assembly/disassembly cycle in living cells. Life Sci Alliance. 2023 May 9;6(7):e202301945. doi: 10.26508/lsa.202301945. *corresponding authors.



From Villa-Consuegra et al., 2023.

Programmed Cell Death during Development and Disease

José Antonio Sánchez Alcázar

Summary of activities:

The patient's own derived cells can be used for pathophysiological studies and personalized drug screenings in rare genetic diseases. In order for precision medicine to be successful at the therapeutic level, in addition to the information provided from genomics, pharmacogenomics, metabolomics and proteomics, our proposal argues that it is also necessary to know the cellular response, and therefore the behaviour of particular mutations in vitro, to various therapeutic options. Precision medicine is based on the assumption that different mutations and marked inter-individual genetic variations can contribute significantly to drug response. The goal of personalized medicine is to maximize the likelihood of therapeutic efficacy for an individual patient.

Our group has developed four platforms for the performance of precision or personalized medicine in diseases in four major groups of rare diseases: the BrainCure platform for rare neurodegenerative diseases associated with brain iron accumulation; the MitoCure platform for mitochondrial diseases; the MyoCure platform for congenital myopathies; and the CancerCure platform for genetic mutations with increase susceptibility to cancer.

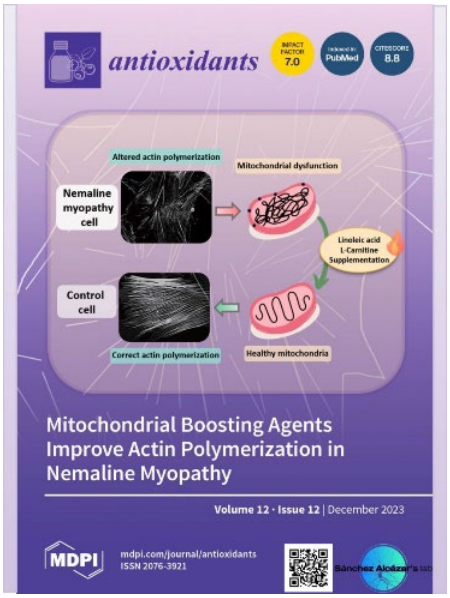
During 2023 we produced 8 publications (7 in Q1, 1 in Q2) and our work was cited 1,262 times. Two PhD theses were defended, and we promoted two clinical trials in mitochondrial diseases and KAT6A syndrome at Virgen Macarena Hospital (Seville). We collaborated with Dr. Ana Maria Cuervo (Albert Einstein College of Medicine, NY, USA) and Dr. José Manuel García Verdugo (Universitat de València, Spain).

In summary, we discovered an interplay of lipid peroxidation and iron accumulation in neurodegenerative diseases, a protective role of mitochondrial unfolded protein response in mitochondrial diseases, an essential role of actin filaments in mitochondrial function and alternative therapeutic approaches for genetic neurodegenerative, mitochondrial and muscular diseases.

Selected references:

Piñero-Pérez R, López-Cabrera A, Álvarez-Córdoba M, Cilleros-Holgado P, Talaverón-Rey M, Suárez-Carrillo A, Munuera-Cabeza M, Gómez-Fernández D, Reche-López D, Romero-González A, Romero-Domínguez JM, de Pablos RM, Sánchez-Alcázar JA. Actin Polymerization Defects Induce Mitochondrial Dysfunction in Cellular Models of Nemaline Myopathies. Antioxidants (Basel). 2023 Nov 21;12(12):2023. doi: 10.3390/antiox12122023.

Suárez-Carrillo A, Álvarez-Córdoba M, Romero-González A, Talaverón-Rey M, Povea-Cabello S, Cilleros-Holgado P, Piñero-Pérez R, Reche-López D, Gómez-Fernández D, Romero-Domínguez JM, Munuera-Cabeza M, Díaz A, González-Granero S, García-Verdugo JM, Sánchez-Alcázar JA. Antioxidants Prevent Iron Accumulation and Lipid Peroxidation, but Do Not Correct Autophagy Dysfunction or Mitochondrial Bioenergetics in Cellular Models of BPAN. Int J Mol Sci. 2023 Sep 26;24(19):14576. doi: 10.3390/ijms241914576.



Stem Cell Behaviour in their Niches and Epithelial Morphogenesis

Acaimo González-Reyes

Summary of activities:

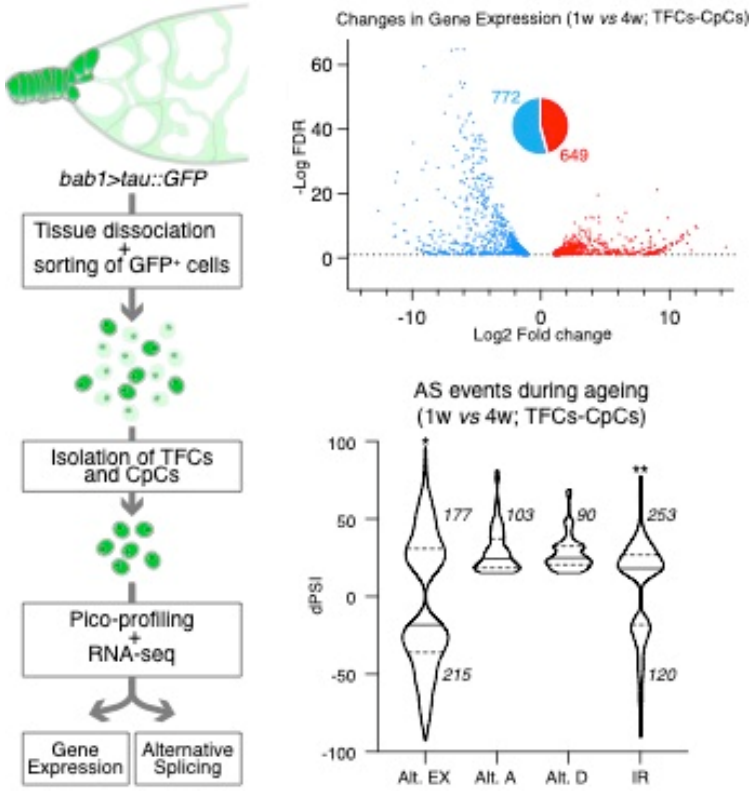
During 2023 we have continued our research on the genetic, molecular and cellular characterisation of a stem cell niche. We have focused on the functions that alternative splicing has during the ageing of the niche support cells, utilising the *Drosophila* ovarian niche as our model system. We have paid particular attention to the Smu1 gene and to the activity of ion channels and Calcium metabolism in niche homeostasis. This work is being prepared for submission and it is directly related to one of the main objectives of the DMC3 Strategic plan, namely to determine how cells sense and respond to their changing environment.

In collaboration with Dr. Martín-Bermudo, we have spent some effort trying to decipher how integrins control epithelial morphogenesis and architecture. We have published a work (Rincón-Ortega et al, Frontiers in Cell + Dev. Biol. 2023) in which we describe the interconnection between integrins and the Notch pathway for proper follicular epithelium organisation. At present, we are using live imaging to determine the origin of the epithelial hyperplasia typical of epithelia lacking integrin function. This line of research deals with aspects of mechanical cues and their role both during morphogenesis and in cell collective's behaviour as prioritised in our María de Maeztu plan.

Selected references:

Rincón-Ortega L, Valencia-Expósito A, Kabanova A, González-Reyes A*, Martin-Bermudo MD*. Integrins control epithelial stem cell proliferation in the Drosophila ovary by modulating the Notch pathway. Front Cell Dev Biol. 2023 Feb 28;11:1114458. doi: 10.3389/fcell.2023.1114458. * corresponding authors.

Rosales-Nieves AE, Marín-Menguiano M, Campoy-Lopez A, González-Reyes A. Visualization and Quantification of Drosophila Larval Ovaries. Methods Mol Biol. 2023;2626:37-47. doi: 10.1007/978-1-0716-2970-3_2.



Evolution of Organogenetic Gene Networks

James C.-G. Hombría (Group Leader) and Sol Sotillos (Associated Investigator)

Summary of activities:

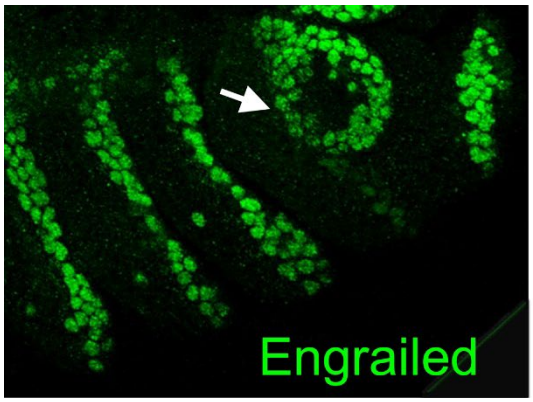
We have analysed how developmental gene networks evolve by *cooption* (the repurposing of a previously active organogenetic gene network into another organ) and by *divergence*.

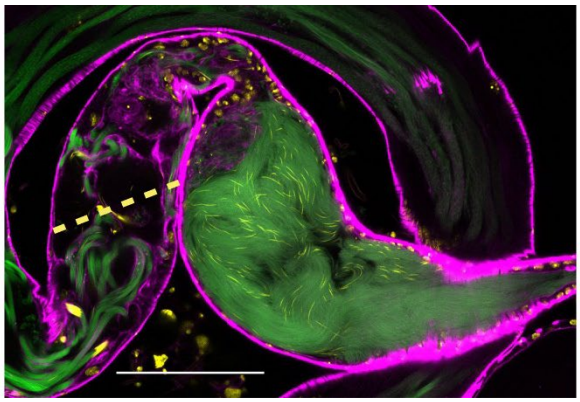
Cooption: We found that the gene network forming the external respiratory organs of the fly larva (the posterior spiracles) has been recruited during recent *Drosophila* evolution to the adult testis mesodermal cells protecting the germ cells during spermatogenesis where is required for the liberation of the mature sperms. Surprisingly, we found that *engrailed*, a posterior spiracle network gene that plays a fundamental role sperm liberation, is irrelevant for the spiracle formation despite being regulated in a very precise manner during organogenesis. Thus, in cases of coopted gene networks, evolutionary modifications selected because of their function in one organ will be mirrored in all *interlocked* organs sharing the same gene network, resulting in sophisticated gene expression patterns without any relevant function in the associated organs. These results could explain the appearance of apparently capricious morphological patterns defying an explanation for their selection during evolution.

Divergence: We are analysing how gene networks used by metamerically repeated organs change during evolution giving rise to specialised variations in some of the repeated organs. We have identified that the insect's *tentorium*, a cephalic conserved structure required for the muscle attachment to the mouth endoskeleton, evolved from a primitive metameric primordium that also gave rise to the trunk respiratory tracheal tubes. This system serves as a model to understand how gene network divergence gives rise to morphologically and functionally different structures.

Selected reference:

Molina-Gil S, Sotillos S, Espinosa-Vázquez JM, Almudi I, Hombría JC. Interlocking of co-opted developmental gene networks in Drosophila and the evolution of pre-adaptive novelty. Nat Commun. 2023 Sep 15;14(1):5730. doi: 10.1038/s41467-023-41414-3.





Bioinformatics for Biological Sequence Analysis

Antonio J. Pérez Pulido

Summary of activities:

Our group works with pangenomes from thousands of bacterial genomes to search for gene markers related to bacterial-phage interaction. This year we have reduced the dark matter of CRISPR-Cas systems, which has allowed us to establish a new theory that we call the membranome-phage-CRISPR triad. It suggests that these antiphage defense systems, which appear in only 40% of genomes, are maintained because the bacterium also has certain accessory genes of interest encoding membrane proteins, which in turn are phage receptors. In contrast, when the bacterium does not possess the phage entry receptor, it does not need to have a CRISPR-Cas system.

On the other hand, this year we have developed a new bioinformatics tool, ASACO (Automatic and serial analysis of gene co-expression based on transcriptomics data; http://www.bioinfocabd.upo.es/asaco/). This allows us to search for human gene modifiers, which could be targets for drugs against diseases. Using ASACO, we have been able to find a modifier and a potential drug for Spinal Muscular Atrophy, in a collaboration that has included assays in *C. elegans* with colleagues from Dr. Manuel Muñoz's group (CABD) and assays with patient cell cultures with Dr. Rosa Soler's group (IRB Lleida).

Selected references:

Rubio A, de Toro M, Pérez-Pulido AJ. The most exposed regions of SARS-CoV-2 structural proteins are subject to strong positive selection and gene overlap may locally modify this behavior. mSystems. 2024 Jan 23;9(1):e0071323. doi: 10.1128/msystems.00713-23.

Moral-Turón C, Asencio-Cortés G, Rodriguez-Diaz F, Rubio A, Navarro AG, Brokate-Llanos AM, Garzón A, Muñoz MJ, Pérez-Pulido AJ. ASACO: Automatic and Serial Analysis of CO-expression to discover gene modifiers with potential use in drug repurposing. Brief Funct Genomics. 2024 Feb 29:elae006. doi: 10.1093/bfgp/elae006.



Nuclear Dynamics in Cell and Developmental Biology

Peter Askjaer

Summary of activities:

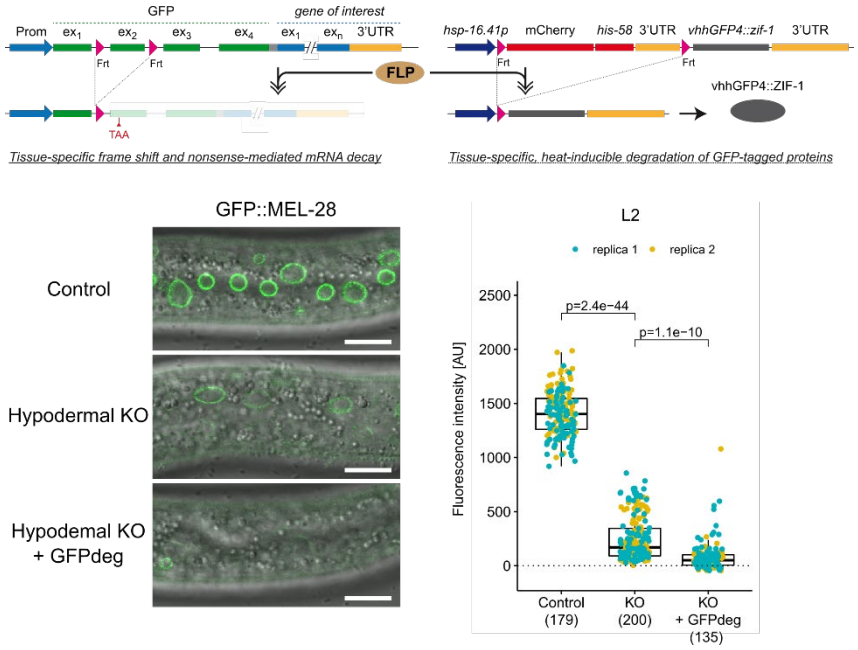
Our group focuses on the biogenesis of the nuclear envelope and the role of nuclear envelope proteins in chromatin organization and gene expression (key processes highlighted in the MdM DMC3 strategic plan). Nuclear pore complexes are macromolecular assemblies of several hundreds of proteins that regulate transport across the nuclear envelope. During 2023 we collaborated with the laboratories of Dr Lionel Pintard and Dr Geraldine Seydoux to determine mechanism underlying nuclear pore complex disassembly at mitotic entry and the dynamics of nuclear pore proteins in interphase. We showed that nuclear pore proteins form cytoplasmic condensates in a stress and age-dependent manner and that several of the proteins are targets of Polo-like kinase 1 in mitosis. Surprisingly, we found that overexpression of a single nuclear pore protein in neurons is sufficient to cause paralysis.

Together with the group of Dr Marta Artal at the CABD, we reported the first map of binding sites of the two heterochromatin protein 1 homologs in *Caenorhabditis elegans*, HPL-1 and HPL-2. HPL-2 binding correlated positively with methylated forms of H3K9 and H3K27, whereas HPL-1 showed a significant intersection with the BLMP-1/PRDM1 transcription factor. Our work suggested both shared and singular properties of the two proteins in control of gene expression. To test this hypothesis, we have developed novel tools for tissue-specific transcriptomic profiling in *C. elegans* and will employ these techniques in our future studies.

Selected references:

Thomas L, Taleb Ismail B, Askjaer P, Seydoux G. Nucleoporin foci are stress-sensitive condensates dispensable for C. elegans nuclear pore assembly. EMBO J. 2023 Jul 3;42(13):e112987. doi: 10.15252/embj.2022112987.

Fragoso-Luna A, Romero-Bueno R, Eibl M, Ayuso C, Muñoz-Jiménez C, Benes V, Cases I, Askjaer P. Expanded FLP toolbox for spatiotemporal protein degradation and transcriptomic profiling in Caenorhabditis elegans. Genetics. 2023 Jan 12;223(1):iyac166. doi: 10.1093/genetics/iyac166.



We developed a tissue-specific recombination system to simultaneously knockout expression and degrade GFP-tagged proteins, here exemplified with depletion of GFP::MEL-28 in the skin of *C. elegans* (Fragoso-Luna et al. 2023).

Computational Biology and Bioinformatics

Ana M Rojas Mendoza

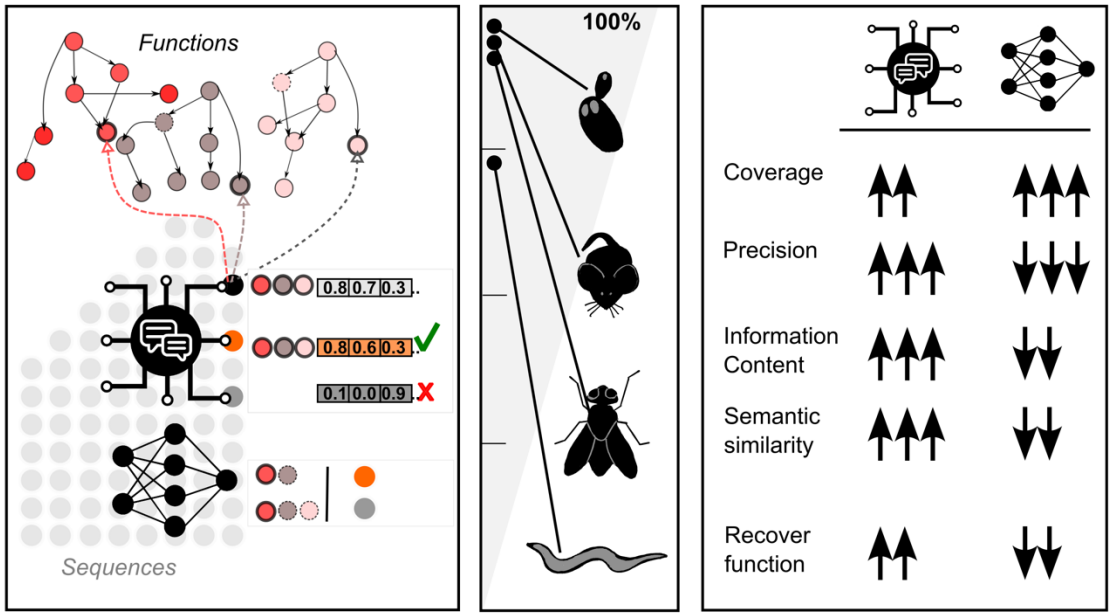
Summary of activities:

The group has contributed to the main conference in Computational Biology (ISMB2023) with a poster and an oral contribution, and to the EMBO meeting, Evolution of animal Genomes, where we have shown how to use AI-based methods for function annotation. The PI has been also invited as plenary speaker, and also invited to describe the challenges of AI within a devoted meeting in Madrid, regarding AI. The PI has been elected Track Chair of the Function Community of Interest (COSI) within the International Society for Computational Biology. The PI is a member of the awarded Google Spain-Univ of Seville Chair in Artificial Intelligence as a Biology domain expert. The group has settled the foundations of the use of AI based methods to approach function annotation of proteins. The group has increased with a new technician, we have been awarded a MdM joint FPI with Silvia Salas's group, and we are very active in the field of method development, that we have created in order to answer questions related to the evolution of traits in particular organisms (see regeneration processes in *C. dipterum*). We have created an annotation pipeline, FANTASIA, to annotate non-model genomes using the AI-based embedding space to understand the functional repertoire of these organisms (https://github.com/MetazoaPhylogenomicsLab/FANTASIA). The PI has contributed to outreach with several talks in high-school institutions, as well as talks towards equality in the program "Ciencia en el Barrio".

Selected references:

Martínez-Redondo GI, Barrios-Núñez I, Vázquez-Valls M, Rojas AM, Fernández R. Illuminating the functional landscape of the dark proteome across the Animal Tree of Life through natural language processing models. bioRxiv doi: 10.1101/2024.02.28.582465.

Barrios-Núñez I, Martínez-Redondo GI, Medina-Burgos P, Cases I, Fernández R, Rojas AM. Decoding proteome functional information in model organisms using protein language models. bioRxiv doi: 10.1101/2024.02.14.580341.



This figure represents the benchmark of AI-based methods in model organisms. Some sequences have functions assigned. The annotation status of model organisms is variable, being the worm the less annotated. Our computational analyses indicate that Language models are better alternatives for genome annotation.

Developmental Epigenomics

Ozren Bogdanovic

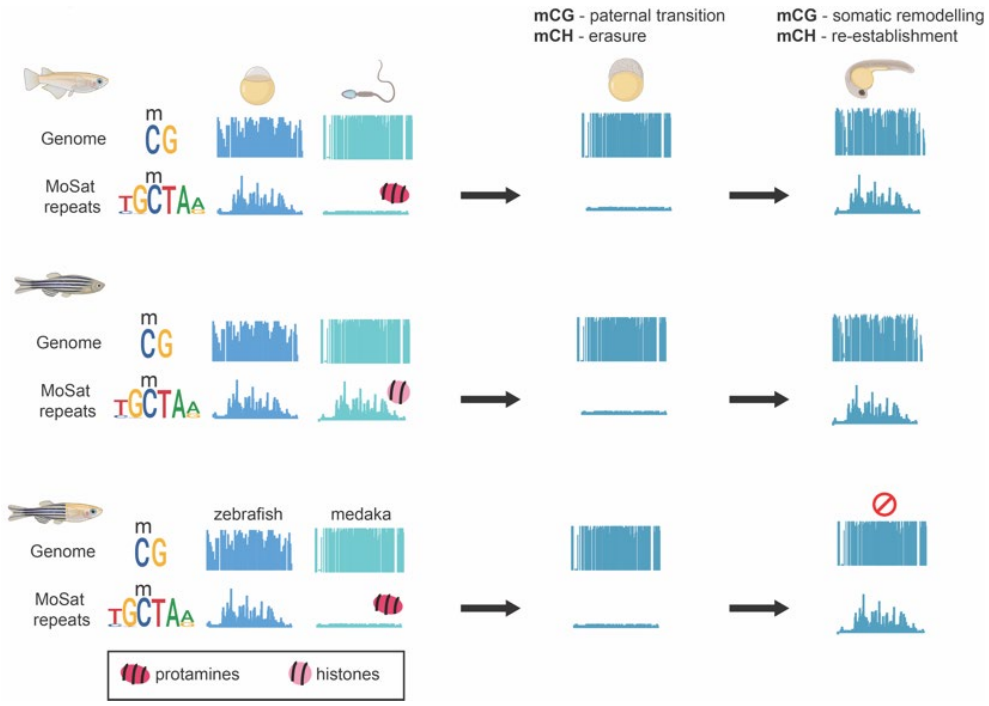
Summary of activities:

In 2023, capitalizing on latest next generation and single molecule sequencing technologies, our lab has provided major insights into how the epigenome participates in the regulation of embryonic development of diverse vertebrate organisms. We have demonstrated that cytosine methylation, a frequent and highly abundant form of DNA modification is reprogrammed during teleost and lamprey embryogenesis. Specifically, we have shown that prior to the activation of the zygotic genome, DNA methylation is reprogrammed in a maternal to paternal manner, without exhibiting complete erasure and re-establishment, as observed in mammals. Therefore, these findings suggest that non-mammalian vertebrates may have a higher propensity for transgenerational epigenetic inheritance when compared to mammals, as methylation acquired by the parental gametes is not erased in neither the embryo nor the developing germline of the next generation. This could mean that non-mammalian vertebrate populations could be more susceptible to factors that can influence gametic methylomes, such as environmental changes or toxins.

Selected references:

Ross SE, Vázquez-Marín J, Gert KRB, González-Rajal Á, Dinger ME, Pauli A, Martínez-Morales JR, Bogdanovic O. Evolutionary conservation of embryonic DNA methylome remodelling in distantly related teleost species. Nucleic Acids Res. 2023 Oct 13;51(18):9658-9671. doi: 10.1093/nar/gkad695.

Angeloni A, Fissette S, Kaya D, Hammond JM, Gamaarachchi H, Deveson IW, Klose RJ, Li W, Zhang X, Bogdanovic O. Extensive DNA methylome rearrangement during early lamprey embryogenesis. Nat Commun. 2024 Mar 4;15(1):1977. doi: 10.1038/s41467-024-46085-2.



3D Genomics

Darío Jesús Lupiáñez García

Summary of activities:

In 2023, our laboratory relocated from the Berlin Institute for Medical Systems Biology / Max Delbrück Center to the Centro Andaluz de Biología del Desarrollo (CABD) in Sevilla. Despite this significant move, we continued with our research on the influence of 3D gene regulation on phenotype evolution. Through collaboration with international colleagues, including those from CABD, we uncover molecular mechanisms underlying the evolution of wing-like fins in skates (Marlétaz et al., *Nature* 2023).

Our laboratory is also interested in the propagation of developmental signals from restricted cell populations, to transform organs and embryos. In collaboration with Rafael Jimenez and Paco Barrionuevo's group (University of Granada), we identified a novel role for microRNAs in orchestrating mammalian sex determination, by controlling the expression of the mammalian sex-determining gene *Sry* (Hurtado et al., *BioRxiv* 2023).

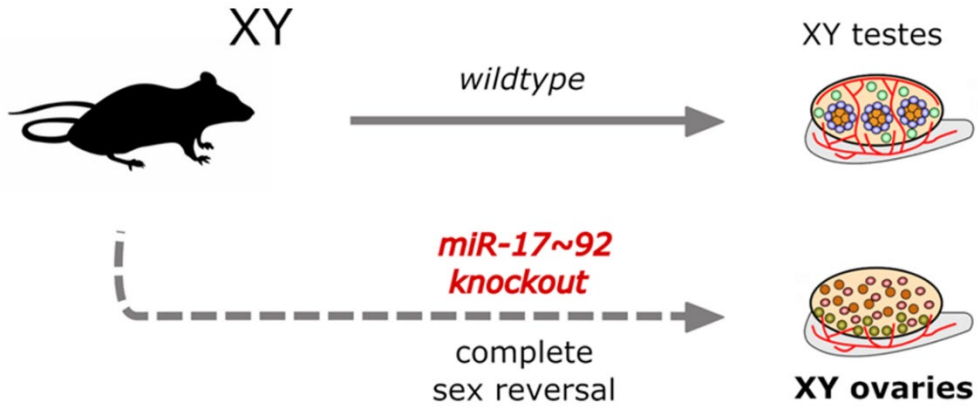
Looking ahead, our efforts will center on understanding 3D gene regulation mechanisms in development and evolution. Supported by the European Research Council (ERC), we are investigating how vertebrate sex determination can be triggered by various genomic or environmental cues. A project recently funded by the Agencia Estatal de Investigación (AEI) focus on studying enhancer-promoter communication mechanisms.

Additionally, in 2023, I engaged extensively with the Spanish scientific community. This included the organization of an EMBO workshop on "The Evolution of Animal Genomes" in Sevilla, joining the board of directors of the Sociedad Española de Genética (SEG), and participating in national networks such as the Conexion Genoma from CSIC and the AEI-funded Regulatory Genomics Network (R2G).

Selected references:

Marlétaz F, de la Calle-Mustienes E, Acemel RD, Paliou C, Naranjo S, Martínez-García PM, Cases I, Sleight VA, Hirschberger C, Marcet-Houben M, Navon D, Andrescavage A, Skvortsova K, Duckett PE, González-Rajal Á, Bogdanovic O, Gibcus JH, Yang L, Gallardo-Fuentes L, Sospedra I, Lopez-Rios J, Darbellay F, Visel A, Dekker J, Shubin N, Gabaldón T, Nakamura T*, Tena JJ*, Lupiáñez DG*, Rokhsar DS*, Gómez-Skarmeta JL*. The little skate genome and the evolutionary emergence of wing-like fins. Nature. 2023 Apr;616(7957):495-503. doi: 10.1038/s41586-023-05868-1. Epub 2023 Apr 12. *corresponding and co-last authors.

Hurtado A, Mota-Gómez I, Lao M, Real FM, Jedamzick J, Burgos M, Lupiáñez DG*, Jiménez R*, Barrionuevo FJ* (2023) Complete male-to-female sex reversal in XY mice lacking the miR-17~92 cluster. bioRxiv doi: 10.1101/2023.03.22.533123. *corresponding and co-last authors.



Complete male-to-female sex reversal in XY mice lacking the miR-17~92 cluster. XY mice lacking the microRNA *miR-17~92 cluster* develop ovaries that are indistinguishable from XX wild type mice. (Hurtado et al., *BioRxiv* 2023).

Molecular Evolution of Microbial Interactions Lab

Tanja Dapa

Summary of activities:

In the year 2023 I started my independent group at the Andalusian Center for Development Biology (CABD-UPO-CSIC) as a Junior Group Leader. I secured funds by obtaining a Spanish national project titled as a main and only PI.

In November 2023 I started a Beatriz Galindo Senior Distinguished Researcher Fellowship, and I was further awarded with a prestigious Ramon y Cajal contract, which I declined in favor of Beatriz Galindo Fellowship.

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. The main objective of the group is to reveal how the mammalian host immune system promotes and maintains a stable community of symbiotic bacteria in the gut and to identify bacterial and host factors important for the interaction.

In 2023 I 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 La Caixa Speed dating during the European Researchers Night.

Two highlights:

Attendance of the 11th annual Gut Microbiota for Health World Summit 2023 (Prague, Czech republic) as and invited speaker.

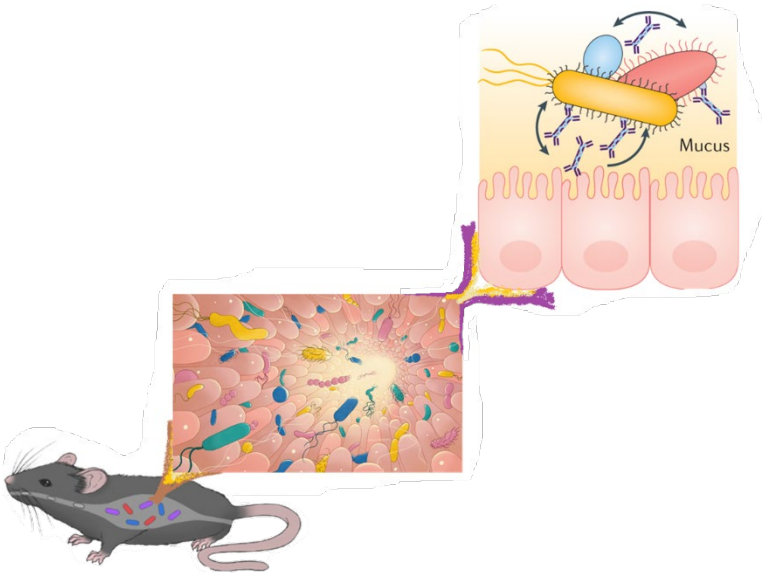


Illustration representing the line of research of my emerging MolEMI Lab – host-microbe interaction withing the mammalian intestine (Illustrations: © Joana C. Carvalho; © PMID: 34862512).

CRISPR-Cas *in vivo* Optimizations to Understand Early Vertebrate Development and Human Diseases

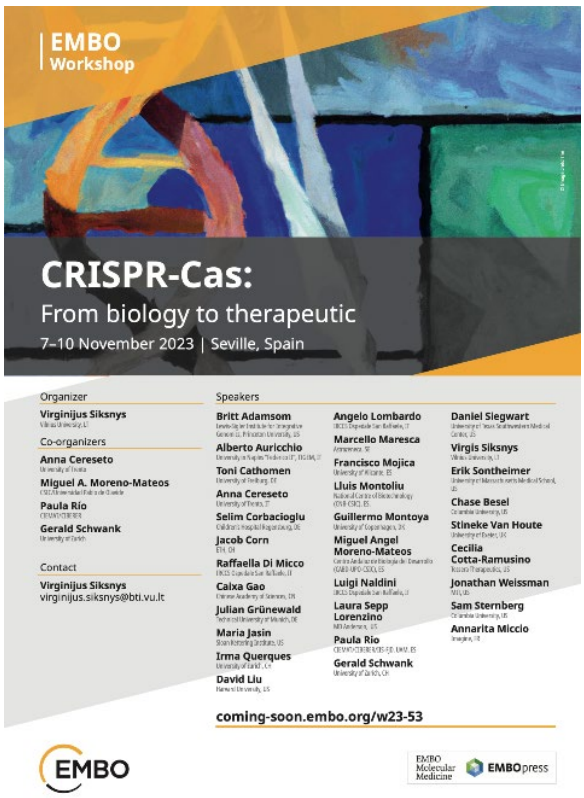
Miguel Angel Moreno-Mateos

Summary of activities:

We have been able to optimize and efficiently apply novel CRISPR-Cas RNA-targeting systems to uncover new regulators of the maternal to zygotic transition (MZT) in teleosts. While transcriptional factors and chromatin remodelers have been more studied in this biological context during early development other modes of regulation are much less known. Thus, performing a comprehensive maternal RNA knockdown screening, we have uncovered novel posttranslational and posttranscriptional regulatory factors involved in MZT. In particular for one of these factors, BCKDK, is involved in the regulation of MZT collaborating in the activation of the genome after fertilization. Indeed, we have uncovered one of BCKDK targets, PHF10 that is part of pBAF complex whose loss-of-function is also affecting to the transcriptional competence and genome organization of the zygotic genome in zebrafish and medaka. Therefore, these results help to address fundamental questions related to gene regulatory networks and structural changes in the genome affecting the first steps of embryogenesis in teleosts. Part of these results are now about to be submitted in two papers (one about the screening and other about CRISPR-Cas RNA-targeting optimizations *in vivo*). These and other projects in the lab have been presented in an EMBO workshop I co-organized past November and other important meetings in our field (i.e. Zebrafish European meeting in Krakow, Poland). In addition, we have initiated a collaboration with a company (ZIP solutions) to enhance precise genome editing using zebrafish as *in vivo* model. The lab has been funded with two new grants: Ayudas para incentivar la consolidación Iinvestigadora (192K) and Planes Complementarios para biotecnogía de la salud (203K). Finally, the lab has participated in different outreach activities such as Pint of Science, Feria de la Ciencia, etc.

2023 highlight:

Organisation of the EMBO Workshop “CRISPR-Cas: From biology to therapeutic applications”. An interdisciplinary meeting with presentations on bacterial defense mechanisms, structural biology, functional genomics and therapeutic applications. https://meetings.embo.org/event/23-crispr



Genetic Regulation of Aging in *Caenorhabditis elegans*

Manuel J. Muñoz

Summary of activities:

As professor at the University 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 01/09/2023-01/09/2026. Public-Private Partnership (AEI). Funding: 805443,47). We received the first prize of XV edition UPOemprende, transfer category.

In other transfer activities we obtained a research contract to evaluate the possibility of producing human proteins in C. elegans, with the company OneChain. This work, if successful, would open up the possibility of generating human active proteins in a simpler and much cheaper way than is currently done. As director of the certified service of drug assays in C. elegans, the company Ingredalia has requested our services, which has been successfully carried out and has allowed the planning of new services to this company.

The group has published three articles as a result of collaborations. In addition, it has participated in transfer fairs such as the transfiere forum in Malaga, Al Andalus Innovation Venture and Alhambra Ventura and in two international congresses.

Selected references:

Calderón-Olvera RM, Nuñez NO, González-Mancebo D, Monje-Moreno JM, Muñoz MJ, Gómez-González E, Arroyo E, Torres-Herrero B, de la Fuente JM, Ocaña M. Europium doped-double sodium bismuth molygdate nanoparticles as contrast agents dor luminescence bioimagin and X-ray computed tomography. Inorg. Chem. Front., 2023,10, 3202-3212.

Hamdi A, Viera-Alcaide I, Guillén-Bejarano R, Rodríguez-Arcos R, Muñoz MJ, Monje Moreno JM, Jiménez-Araujo A. Asparagus Fructans as Emerging Prebiotics. Foods. 2022 Dec 23;12(1):81. doi: 10.3390/foods12010081.

Development of the Hemato-Vascular System and Stem Cells

María José Sánchez Sanz

Summary of activities:

Our group aims to understand the coordination of activity between hematopoietic cells and vascular endothelial cells throughout the development, physiology and pathology of the vasculature.

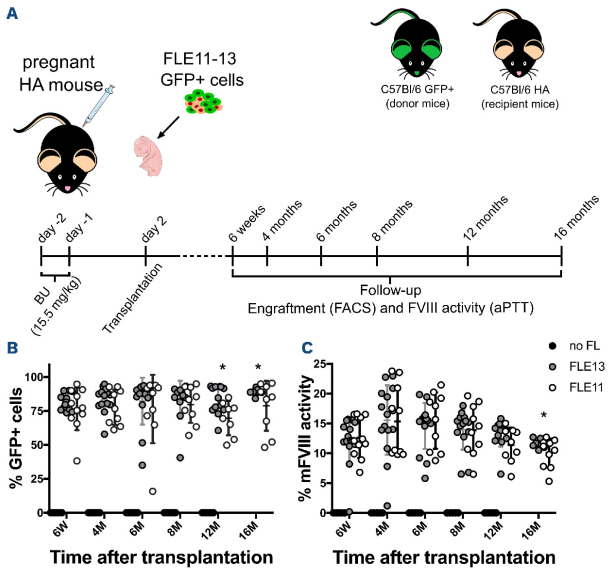
The group has made relevant contributions to the functional characterization of embryonic endothelial and hematopoietic progenitor cells in mice (Cañete et al, Stem Cells, 2017; Serrano et al, Thromb.&Haemos, 2018). Recently, we uncovered that transplanted fetal liver (FL) progenitor cells into neonatal mice with haemophilia A, mutant for the coagulation factor VIII (FVIII), robustly reconstituted blood and endothelium. Engrafted donor cells rescued the FVIII deficiency for life, even without recipient conditioning, providing new insights for cell therapy strategies to treat haemophilia A in neonates (Merlin et al, Haematologica, 2023; De Pablo-Moreno et al, Int.J.Mol, 2022) (Collaborations A Follenzi, UPO,Italy; A Liras, UCM, Madrid).

Presently, our focus lies in comprehending the self-organization principles and coordinated role of hematopoietic and endothelial cell's reparative responses following ischemic damage to the neonatal brain. This involves characterizing transcriptional changes at the single-cell resolution (scRNAseq) within circulating immune and progenitor cell subsets (Collaboration P Ybot-González, IBIS, Seville).

Progress on the bioinformatic analysis data was presented at the XX Congress of the Spanish Society of Cellular Biology in Cordoba, 2023 and the Workshop on Haematopoiesis and Haematological Malignancies, Cambridge International Seminar, 2023. Also, student Carlos Rangel presented his Master´s Thesis titled "Single Cell Transcriptomics of Peripheral Blood Neutrophils in a Neonatal Mouse Model of Stroke", Analysis of Omics Data and Systems Biology, Universidad de Sevilla and UNIA.

Selected reference:

Merlin S, Akula S, Cottonaro A, Garcia-Leal T, Serrano LJ, Borroni E, Kalandadze V, Galiano R, Borsotti C, Liras A, Sanchez MJ, Follenzi A. Therapeutic potential of fetal liver cell transplantation in hemophilia A mice. Haematologica. 2023 Jun 1;108(6):1544-1554. doi: 10.3324/haematol.2022.282001.



Engraftment of fetal liver cells into newborn hemophilia A mice. (A) Hemophila A (HA) pregnant mice received busulfan (BU) prior to birth. HA newborn mice were transplanted with fetal liver (FL) cells from GFP+ mice. (B) Engraftment of transplanted GFP+ cells, was evaluated by flow cytometry on peripheral blood at different time points. GFP+ cells were detectable in all transplanted mice (n=10-11). (C) FVIII activity in plasma of transplanted mice was evaluated by activated partial thromboplastin time (aPTT) assay. FVIII activity was detectable up to 16 months in all FL transplanted mice.

Epigenomics in Disease and Aging

Juan J. Tena

Summary of activities:

In 2023, our lab achieved important milestones, marking a pivotal moment in my independent research career. Our collaborative efforts resulted in a key publication on the evolutionary drivers of the striking shape of pectoral fins in the little skate, *Leucoraja erinacea*, in *Nature*. This work, with Christina Paliou, postdoctoral researcher from our group, as one of the principal authors, highlights the importance of the regulatory genome in organ and body shape evolution.

Additionally, we completed another study involving a former postdoc from our lab, Alberto Pérez-Posada, investigating transcriptomic and gene regulatory networks in the hemichordate *Ptychodera flava* to illuminate the evolutionary history of deuterostome embryonic development. This study was pre-printed in BioRxiv and is currently under review for publication in Nature Ecology and Evolution.

Another important landmark was the successful defence of the first PhD project under my supervision, conducted by Alejandro Gil-Gálvez, focused on the fast evolution of different populations of the Mexican tetra, *Astyanax mexicanus*.

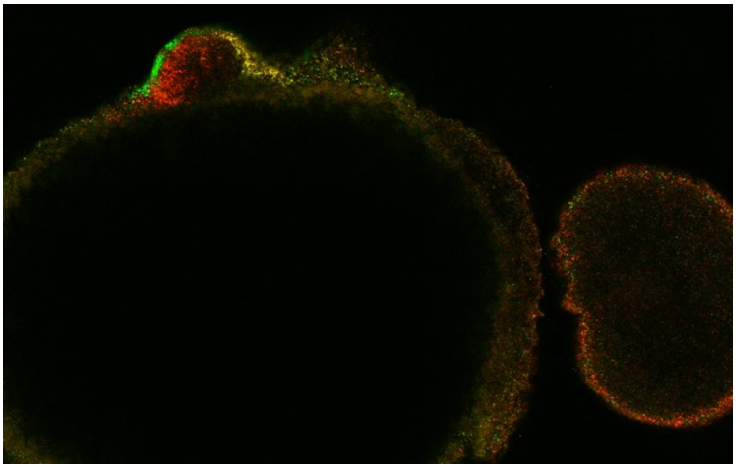
For our group, collaborations with other laboratories have always been of great importance. During last year, in collaboration with Prof. Marian Ros's laboratory in Cantabria, Spain, we initiated a project exploring genetic networks involved in the development of the dorsoventral axis of vertebrate limbs.

Finally, in 2023, our group received a National Grant from the Spanish Government. This project uses killifish, a small fish with a very short lifespan, as a model system to investigate the impact of chromatin's three-dimensional structure on the aging process across various organs.

Two highlights:

Marlétaz F, de la Calle-Mustienes E, Acemel RD, Paliou C, Naranjo S, Martínez-García PM, Cases I, Sleight VA, Hirschberger C, Marcet-Houben M, Navon D, Andrescavage A, Skvortsova K, Duckett PE, González-Rajal Á, Bogdanovic O, Gibcus JH, Yang L, Gallardo-Fuentes L, Sospedra I, Lopez-Rios J, Darbellay F, Visel A, Dekker J, Shubin N, Gabaldón T, Nakamura T*, Tena JJ*, Lupiáñez DG*, Rokhsar DS*, Gómez-Skarmeta JL*. The little skate genome and the evolutionary emergence of wing-like fins. Nature. 2023 Apr;616(7957):495-503. doi: 10.1038/s41586-023-05868-1. Epub 2023 Apr 12. *corresponding and co-last authors.

Thesis project defended by Alejandro Gil-Gálvez: “The role of Cis-Regulatory elements during evolution at different evolutionary time points.”



HCR technique on a zebrafish embryo showing three different genes involved in the dorsoventral patterning of the developing pectoral fin.

Bacterial Infections: New Targets and New Prevention and Treatment Strategies

Younes Smani

Summary of activities:

During 2023, we initiated a new cellular model of infections to enhance our understanding of host-pathogens interactions from *in vitro* to *ex vivo* and *in vivo* models, aligning with the DMC3 Strategic Plan. We're currently generating pulmonary organoids from IPSCs, supported by ProyExcel_00116 funding. Concurrently, we're examining OMPs, TFEB, and PLA2 in *Acinetobacter baumannii* intracellular trafficking into the host cells (PPI A3, UPO).

Furthermore, we validated the concept of ESRI (Extended Spectrum Resistance to β-lactams/β-lactamase inhibitors [BL/BLI]) [PMID:31613964] in patients with intraabdominal infections caused by *Escherichia coli*, revealing the significance of BL/BLI resistance evolution for treatment efficacy and preventing resistance spread [PMID:37442373]. These insights were awarded the prize “Second-best published manuscript in the Medicine Faculty (Seville University)” and integrated into Lidia Gálvez's international thesis defended in 2023.

We also patented data on thiophen derivatives as antimicrobial agents [P202330628] and obtained AEI funding to advance their development [PID2022-136357OB-I00]. Collaborations with Rouen University-France, Kremlin-Bicêtre-France, Milan University-Italy, and CIB-CSIC facilitated these discoveries.

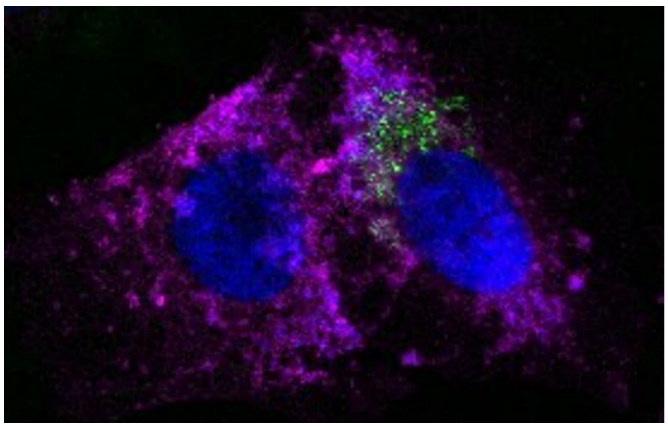
Additionally, we contributed to reviewing activities for international and national agencies (JPIAMR, EUREKA, ESCMID, Poland National Science Centre, Naples University, ANEP, Comunidad de Madrid), as well as scientific journals (mSystems, JAC, AAC, Microbiology Spectrum) and international congresses (ECCMID).

In terms of management and organization, the group leader was appointed Director of the CABD's Cellular Biology and Biotechnology Department, Quality Manager for the UPO Master's in Health Biotechnology, Quality Representative in the UPO Postgraduate Health Area, and WG2 leader of European Network “EURESTOP”.

Selected reference:

Gálvez-Benítez L, de la Rosa JMO, Rodriguez-Villodres A, Casimiro-Soriguer CS, Molina-Panadero I, Alvarez-Marin R, Bonnin RA, Naas T, Pachón J, Cisneros JM, Lepe JA, Smani Y. Role of blaTEM and OmpC in the piperacillin-tazobactam resistance evolution by E. coli in patients with complicated intra-abdominal infection. J Infect. 2023 Sep;87(3):220-229. doi: 10.1016/j.jinf.2023.07.005.

Boulaamane Y, Molina Panadero I, Hmadcha A, Atalaya Rey C, Baammi S, El Allali S, Maurady A, Smani Y*. Antibiotic discovery with artificial intelligence for the treatment of Acinetobacter baumannii infections. Research Square 10.21203/rs.3.rs-3664762/v1, Accepted for publication in mSystems in March 2024.



Acinetobacter baumannii (in green), stained with antibodies targeting its outer membrane protein A (OmpA), adheres to and invades a human lung epithelial cell (in magenta), stained with antibodies targeting one of its surface proteins (fibronectin). The cell nucleus (in blue) is stained with DAPI.

Biotechnology of Industrial and Artisan Fermentations. Competitivity Factors in Microbial Communities and their Application

Andres Garzon

Summary of activities:

During 2023 our group has initiated a change of orientation maintaining our vocation of applied science and technology transfer, but also adopting a more Basic Science approach, oriented, as far as possible, to the goals of the MdM program. As a consequence, we have been awarded a project from the Knowledge Generation Program focused on the role of yeast killer elements in intraspecific competition interactions along the different yeast life stages (natural environment, fermentations and biofilm formation). We have initiated a doctoral thesis on this topic.

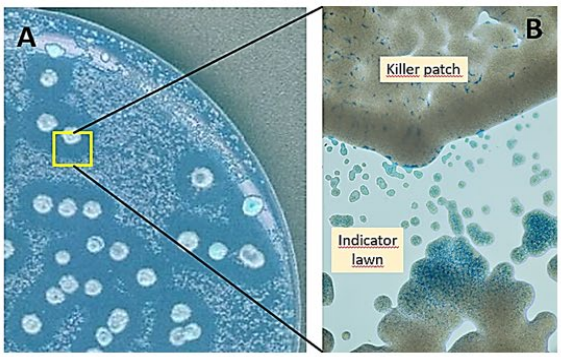
We maintain our applied science vocation by initiating collaborations with two companies, Bodegas Gonzalez Palacios and Cultivando Empleo. We have obtained a knowledge transfer project from the UPO's intramural research program.

We continue to maintain fruitful collaborations with the groups of Juan Jimenez, Antonio Pérez-Pulido and Manuel Muñoz with one high impact publication, two manuscripts sent to review and a patent application in progress.

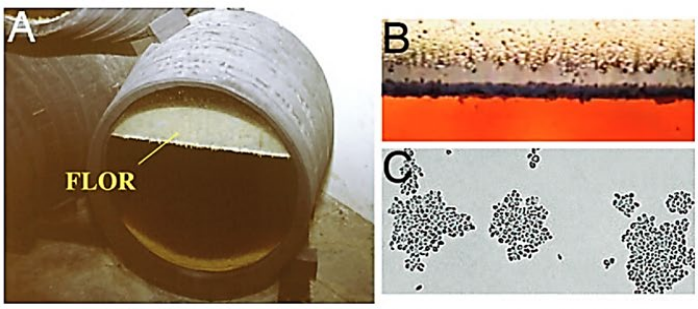
Two highlights:

Rubio A, Sprang M, Garzón A, Moreno-Rodriguez A, Pachón-Ibáñez ME, Pachón J, Andrade-Navarro MA, Pérez-Pulido AJ. Analysis of bacterial pangenomes reduces CRISPR dark matter and reveals strong association between membranome and CRISPR-Cas systems. Sci Adv. 2023 Mar 24;9(12):eadd8911. doi: 10.1126/sciadv.add8911.

We were awarded funding from the Agencia Estatal de Investigación for our project *"Totivirus en el campo, el lagar y el laboratorio. Conocimiento básico y aplicaciones"* (PID2022-141222NB-I00).



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 "flor."(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.

Vertebrate Development and Evolution

Javier Lopez-Rios (Group Leader) and Gretel Nusspaumer (Associated Investigator)

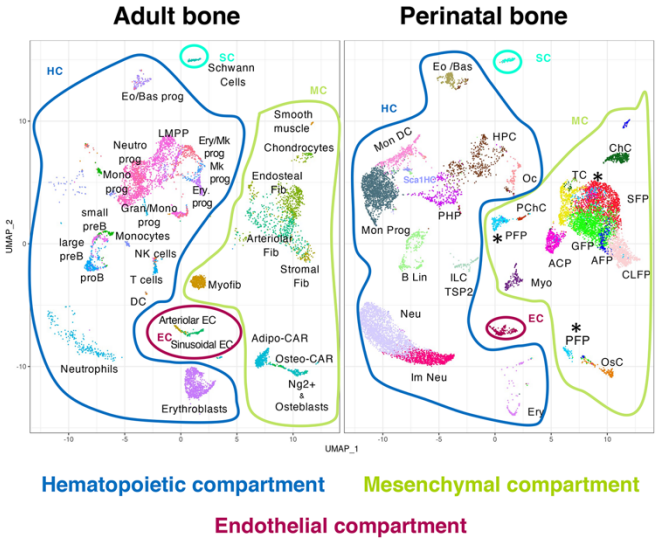
Summary of activities:

Our group studies the morpho-regulatory programs that shape the limb skeleton during embryonic and postnatal development, which a particular interest in evolution. Artiodactyls, such as pigs, exhibit limb skeletons that have evolutionarily diverged from the pentadactyl tetrapod blueprint seen in mice and humans, having acquired symmetrical handplates with fewer and smaller digits that support unguligrade locomotion. Using single cell genomics and epigenomics single-cell approaches in mouse an pig limb buds, we have identified homologous cell populations in limb buds of both species and extracted cluster-specific expression and regulatory profiles. This ongoing analysis aims to define the topology of gene regulatory networks within specific mesenchymal populations of both species and identify regulatory alterations that may have contributed to the evolutionary diversification of limb skeletons in artiodactyls. In a related project, we have genetically dissected the *Ptch1* genomic landscape and identified that several enhancers with overlapping activities control *Ptch1* expression in the mouse limb buds. Notably, the most ancient enhancer, active even in the paired fins of fish, has undergone functional degeneration in various artiodactyl lineages. Our group has also contributed to collaborative studies on other aspects of morphological evolution and regulatory robustness. Furthermore, we have also employed single-cell technologies to study the ontogeny and dynamics of early mesenchymal progenitor populations in endochondral bone throughout development, with a particular emphasis on the differences between perinatal and adult bone and the alterations that take place at birth. Our group is very active in public outreach, including visits to secondary schools and participation in science fairs. In late 2023, the IP was appointed associate professor of Embryology at the Loyola University School of Health Sciences.

Selected references:

Rajderkar S, Barozzi I, Zhu Y, Hu R, Zhang Y, Li B, Alcaina Caro A, Fukuda-Yuzawa Y, Kelman G, Akeza A, Blow MJ, Pham Q, Harrington AN, Godoy J, Meky EM, von Maydell K, Hunter RD, Akiyama JA, Novak CS, Plajzer-Frick I, Afzal V, Tran S, Lopez-Rios J, Talkowski ME, Lloyd KCK, Ren B, Dickel DE, Visel A, Pennacchio LA. Topologically associating domain boundaries are required for normal genome function. Commun Biol. 2023 Apr 20;6(1):435. doi: 10.1038/s42003-023-04819-w.

Marlétaz F, de la Calle-Mustienes E, Acemel RD, Paliou C, Naranjo S, Martínez-García PM, Cases I, Sleight VA, Hirschberger C, Marcet-Houben M, Navon D, Andrescavage A, Skvortsova K, Duckett PE, González-Rajal Á, Bogdanovic O, Gibcus JH, Yang L, Gallardo-Fuentes L, Sospedra I, Lopez-Rios J, Darbellay F, Visel A, Dekker J, Shubin N, Gabaldón T, Nakamura T, Tena JJ, Lupiáñez DG, Rokhsar DS, Gómez-Skarmeta JL. The little skate genome and the evolutionary emergence of wing-like fins. Nature. 2023 Apr;616(7957):495-503. doi: 10.1038/s41586-023-05868-1.



The mesenchymal and hematopoietic compartments of mouse endochondral bone exhibit significant differences between adult and perinatal stages, as revealed by scRNA-seq analysis.

Nuclear Architecture and Dynamics

Rafael Rodríguez Daga (Group Leader) and Silvia Salas Pino (Associated Investigator)

Summary of activities:

During 2023 our group has done important progress in understanding the cellular response to thiol stress. This is a breakthrough study that has been presented in international meetings and will be completed and submitted for publication during 2024. During 2023 our group has completed a collaboration with the group of Dr. Elena Hidalgo at Pompeu Fabra University (Barcelona) to follow up a previous study of reversible protein aggregation. Our group has also maintained an active collaboration with Dr. Mark Hoschtrasser (Yale University, USA) to study the role of the proteasome in heat stress, a condition that leads to massive protein unfolding and in which the proteosome in charge of the elimination of terminally unfolded proteins to prevent their deleterious aggregation. We are currently preparing a manuscript for publication. We have also completed a collaboration with Dra. Kathy Gould (Vandervilt University (Tenessi, USA) that resulted in a publication in early 2024. Finally, we have also maintained a close collaboration with Dr Fred Chang (UCSF, USA). We are co-directing an international thesis to study control of nuclear size that will be completed during 2024.

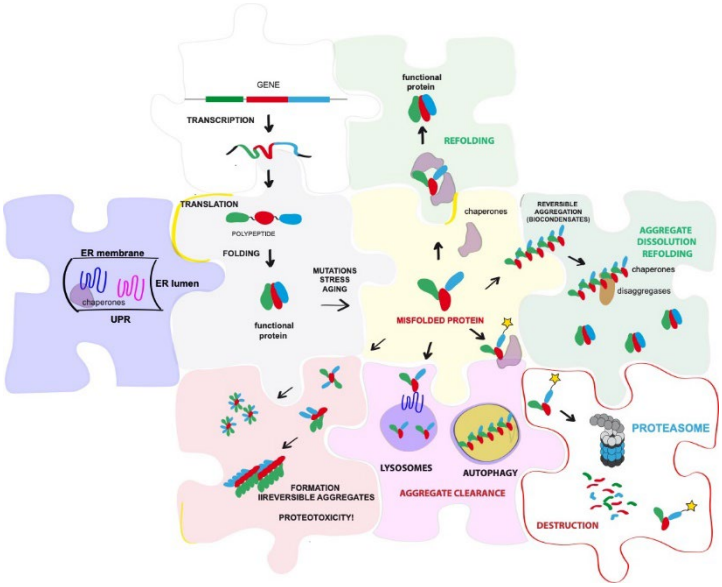
In addition, Silvia Salas Pino has stablished a collaboration with Dra. Ana Rojas (CSIC-CABD) while Rafael R. Daga has stablished a collaboration with Dr. Peter Askjaer (CSIC-CABD). Both collaborations have been supported by the Maria de Maeztu through FPI grants following an internal selective process. In both collaborations we aim to further explore principle of biocondensate formation and to explore moonlighting functions of nucleoporin across species.

Supported by the OTRI (UPO), we have also created an UPOtec service entitled: Systematic evaluation of drugs or bioactive compounds that prevent or reverse the formation of protein aggregates. (https://www.upo.es/upotec/catalogo/salud/evaluacion-sistematica-de-farmacos-o-compuestos-bi/). We also offer a tool for in vitro measurement of proteasome activity which can be used for a drug screen.

We have participated in several outreach activities and maintained our teaching activity on Biotechnology, and Human Nutrition degrees as well in Master at the University Pablo de Olavide. We teach subjects such as Molecular Genetics, Human Genetics, Genetic engineering, Development of new drugs or Cellular and Molecular Bases of Cancer.

Selected reference:

Boronat S, Cabrera M, Vega M, Alcalá J, Salas-Pino S, Daga RR, Ayté J, Hidalgo E. Formation of Transient Protein Aggregate-like Centers Is a General Strategy Postponing Degradation of Misfolded Intermediates. Int J Mol Sci. 2023 Jul 7;24(13):11202. doi: 10.3390/ijms241311202.



Schematic representation of several mechanisms involved in proteostasis. Our lab is currently studying principles of reversible protein aggregation, the role of the proteasome in the clearance of misfolded proteins, and new proteostatic mechanisms operating at ER, the site of synthesis of secretory and transmembrane proteins.

Regulatory Mechanisms of Gene Expression in Pollutant-degrading Bacteria. Bioremediation

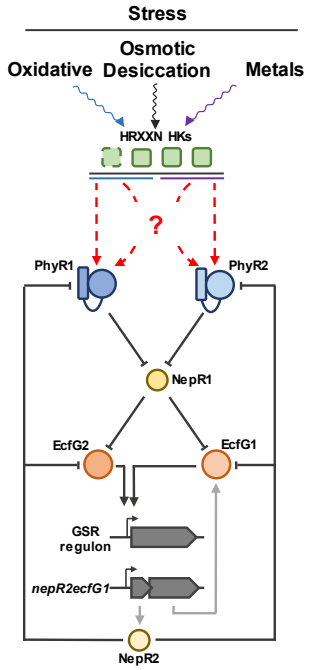
Francisca Reyes-Ramírez

Summary of activities:

My research group is interested in knowing how bacteria of environmental interest respond and protect themselves against various environmental stresses. In *Sphingopyxis granuli* TFA, a bacterium with an important role in Bioremediation, this involves a global transcriptional response called the **General Stress Response (GSR)**. This adaptive response prepares the cell to deal with a range of adverse conditions, which would otherwise not support cell growth and survival. We want to know how different signalling inputs are perceived and integrated to regulate GSR expression. Our current efforts are focused on mutating several histidine kinases to understand their specialisation in sensing and transmitting different stresses. In addition, we want to know what role Hfq plays in stress regulation, as mutants in this RNA chaperone are more sensitive to different stresses. This implies an additional layer of regulation at the post-transcriptional level.

In collaboration with Dr. Eva María Camacho Fernández we are also interested in **Bioremediation** of contaminants. We are using **Functional Metagenomics** to search genes encoding enzymatic activities of industrial and environmental interest. This includes enzymes for the utilization of lignocellulosic wastes and for degradation of plastics. For this, we are collaborating with chemical engineers at the University Pablo de Olavide and with the EMASESA's wastewater treatment plant station Copero, in Seville. We have built composting piles with different composition of sludge, pruning and roughing, which it must be enriched in degrading microorganisms of lignocellulose and plastics, to construct a metagenomic library and to monitor microbial communities during the composting process.

A



B



A: **The GSR regulatory pathway in *Sphingopyxis granuli* TFA.** Core GSR regulators: PhyR1-PhyR2 anti-anti-sigma factors, NepR1-NepR2 anti-sigma factors, EcfG1-EcfG2 sigma factors. Questions: how the stress signals are sensed through different kinases and how this information is integrated through PhyR1 and/or PhyR2 to adjust the pathway output.

B: Preparation of the **compost pile**, source of metagenomes.

Developmental Control

Fernando Casares

Summary of activities:

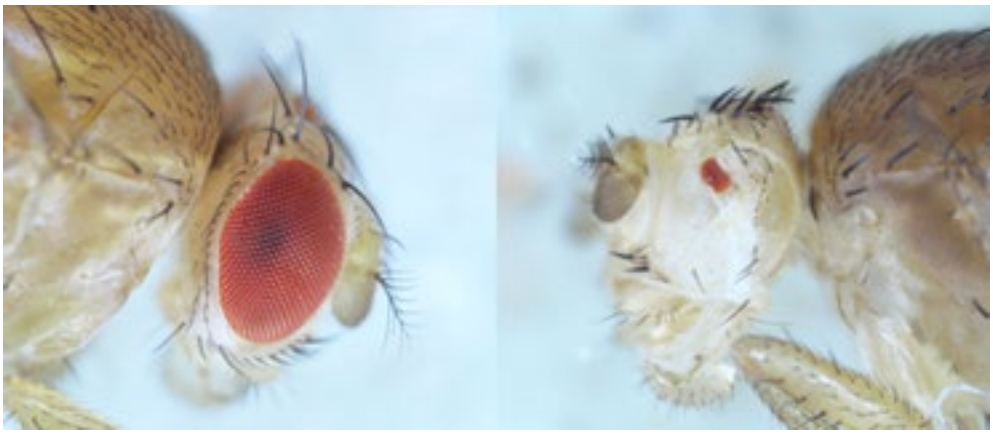
The Developmental Control group aims at understanding the basis of two apparently contradictory facts of animals: the amazing robustness of development (developmental stability), which ensures the consistent generation of functional organisms across generations, and the capacity of organisms to vary (developmental evolution) across much larger time scales. During year 2023 the group has (1) leaned that the gene regulatory network governing eye development is deeply conserved within flies, (2) discovered a mechanism of feedback control that involves the regulation of apoptosis which ensures the precision of eye size, and (3) established a new regeneration paradigm in insects with which we have found novel mechanisms required for organ regeneration which are likely conserved across most of the insect tree of life. This research falls within the broader scope of our María de Maeztu Project “Decision Making in Cell Collectives Across Scales” and specifically within those aiming at understanding the relationship that regulatory and structural changes in the genome have with evolutionary innovations, and how gene regulatory networks are harmonized between neighbouring cells through cell communication.

In addition, F. Casares, acting as Coordinator of the large CSIC collaborative consortium **LifeHub.CSIC** (https://lifehub.csic.es/), has contributed to steer a number of scientific activities seeking to foster multidisciplinary thinking to tackle frontier problems on the origin, evolution, diversification and synthesis of life.

Selected reference:

The main work from the group on feedback mechanisms controlling organ size robustness was published as a Preprint in 2023 (https://doi.org/10.1101/2023.03.02.530805), but only appeared as a peer-reviewed article in early 2024:

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



If during *Drosophila* development (left) the BMP signalling pathway is attenuated, the resulting eyes are dramatically reduced. This signalling is also used to ensure organ size constancy. (F. Casares/ CABD).

Fungal and Yeast Biotechnology

Jose I Ibeas Corcelles

Summary of activities:

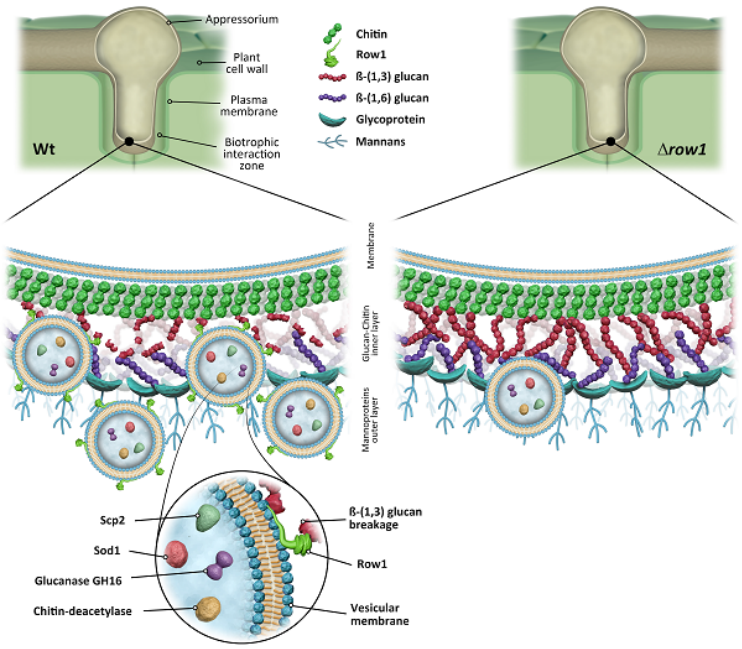
During the last years we have been studding the role for chromatin modification in the regulation of the expression for genes involved in plant infection. We performed a systematic study of sirtuins in the maize pathogen *Ustilago maydis*, finding Sir2 to be involved in the dimorphic switch from yeast cell to filament and pathogenic development. Transcriptomic analysis revealed that Sir2 represses genes that are expressed during biotrophism development. (Navarrete et al, 2023). Moreover, since 2008, we have been working to demonstrate the role for protein glycosylation during plant infection. Up today we have established that N- and O-glycosylation are essential for *U. maydis* infection, and we have identified some proteins that have to be properly glycosylated to complete plant infection. Among them Row1 was the last described for which we have found to belongs to a protein family involved in cell wall formation, determining cell wall structure and protein secretion thus influencing appressoria formation and plant penetration. (Under last phase of review in New Phytologist).

Our industrial project is related to the identification of new yeast for beer production. We have identified and characterize more than 4000 yeast and selected five that are on their way to be assayed in industrial traits in collaboration with craft breweries.

During this year a Doctoral Thesis with international recognise have been presented by our group and three Master Degree Thesis related to beer microbiology.

Selected reference:

Navarrete B, Ibeas JI*, Barrales RR*. Systematic characterization of Ustilago maydis sirtuins shows Sir2 as a modulator of pathogenic gene expression. Front Microbiol. 2023 Apr 11;14:1157990. doi: 10.3389/fmicb.2023.
* corresponding authors.



Row1, an Ustilago undescribed protein involved in the cell wall remodelling, affects morphological changes and protein secretion during plant infection.

Mechanisms Regulating Cell Shape Changes and Cell Migration

Maria Dolores Martin Bermudo

Summary of activities:

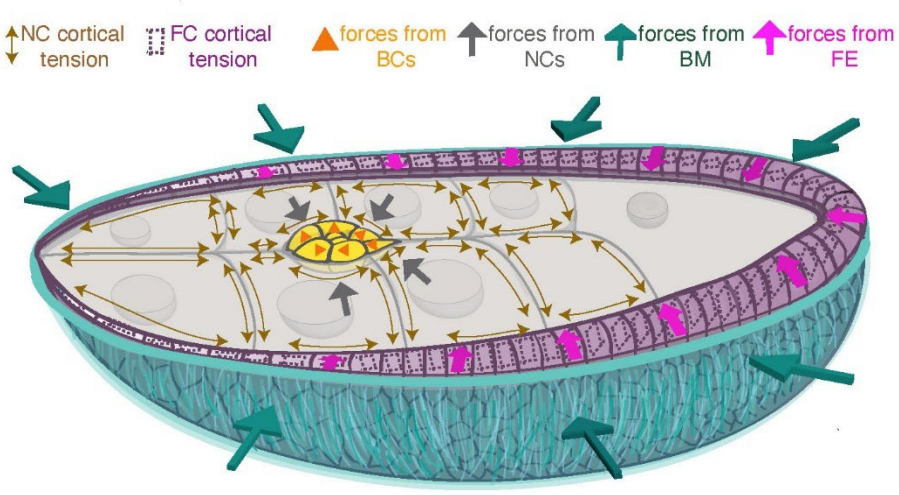
Our laboratory has 2 main lines of research: 1) uncovering mechanisms regulating cell migration and invasion, and 2) understanding the contribution of cell-extracellular matrix (ECM) interactions and actomyosin contractility to epithelia morphogenesis and homeostasis. We use a multidisciplinary approach combining genetics with transcriptomics, 4D imaging, biophysics and computational exploration. Regarding cell migration, in this last year, we have shown that besides its conventional role as substratum, the ECM also regulates cell migration by imposing mechanical forces over the 3D environment cells move through (Molina et al. PLoS Biol.2023). Concerning cell invasion, we have identified new regulators of EGFR/Ras tumorigenic capacity, including the Tensin family of proteins (Martínez-Abarca et al. Genes, 2023) and integrins (Martínez-Abarca et al. Cancer, 2023).

In relation to the role of cell-ECM interactions and actomyosin contractility on epithelia morphogenesis and homeostasis, we have found that cell-ECM interactions control epithelial SC proliferation by modulating Notch (Rincón-Ortega et al. Front. Cell and Dev. Biol.,2023). We have also found that integrins preserve epithelial structures by regulating cell tension during division and rearrangement.

Selected references:

Molina López E, Kabanova A, Winkel A, Franze K, Palacios IM, Martín-Bermudo MD. Constriction imposed by basement membrane regulates developmental cell migration. PLoS Biol. 2023 Jun 28;21(6):e3002172. doi: 10.1371/journal.pbio.3002172.

Martínez-Abarca Millán A, Martín-Bermudo MD. Integrins Can Act as Suppressors of Ras-Mediated Oncogenesis in the Drosophila Wing Disc Epithelium. Cancers (Basel). 2023 Nov 15;15(22):5432. doi: 10.3390/cancers15225432.



External forces from the BM and the FE influence NC membrane tension, which in turn regulates BC migration speed and mode.

Mitochondrial Signalling Crosstalk in the Regulation of Ageing

Marta Artal Sanz

Summary of activities:

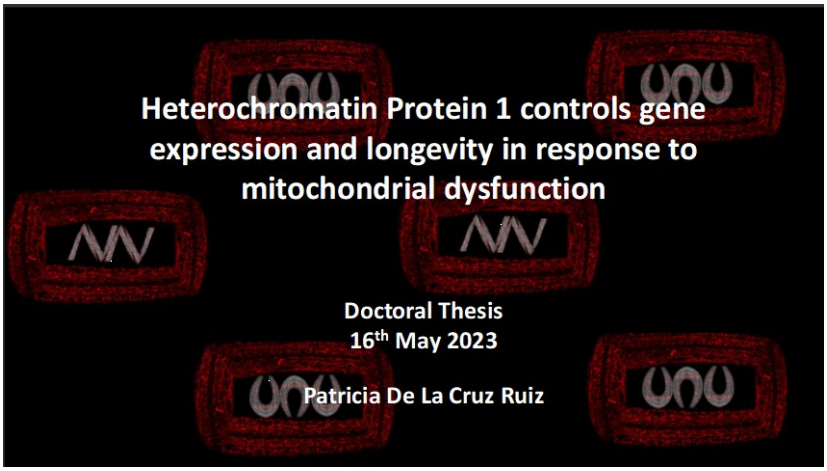
In collaboration with Peter Askjaer, at the CABD, we identified heterochromatin protein 1 (HP1) family members HPL-1 and HPL-2, as important regulators of mitochondrial function and longevity. These efforts resulted in a PhD Thesis and two selected talks at international prestigious meetings. In addition, we made important advances in our understanding of common and differential roles of HP1 proteins in different tissues of the nematode (PMID: 37119802).

We collaborated with Francesca Palladino at the Ecole Normale Supérieure de Lyon, to understand how the transcriptional coregulator SIN-3 influences mitochondrial function and metabolism in the context of a whole organism. This study is important because SIN3 haploinsufficiency causes intellectual disability/autism syndromes and plays an important role in tumour biology (accepted in iScience).

These studies address a central question in cell physiology, that is the regulatory crosstalk between mitochondrial function, metabolism and the epigenetic regulation of gene expression to control cell homeostasis. This is relevant to understand ageing and disease.

Selected reference:

de la Cruz-Ruiz P, Rodríguez-Palero MJ, Askjaer P, Artal-Sanz M. Tissue-specific chromatin-binding patterns of Caenorhabditis elegans heterochromatin proteins HPL-1 and HPL-2 reveal differential roles in the regulation of gene expression. Genetics. 2023 Jul 6;224(3):iyad081. doi: 10.1093/genetics/iyad081.



PhD Thesis Defended by: Patricia De La Cruz Ruiz

Co-supervised by: Peter Askjaer and Marta Artal Sanz.

Cis-regulatory Landscape Dynamics in Development and Disease

Martin Franke

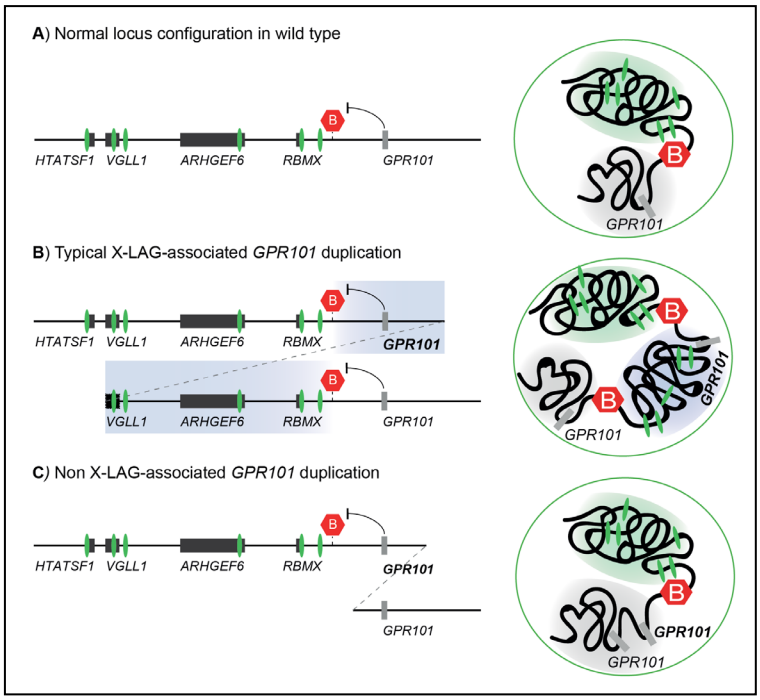
Summary of activities:

In 2023, I undertook a diverse range of research activities to advance my independent research agenda at the CABD. Firstly, to consolidate my future research towards a deeper comprehension of gene regulatory mechanisms during embryonic development, I formalized a significant research project and applied for the ERC starting grant, call 2024. Secondly, I expanded my international network of clinical collaborators to translate novel gene regulatory concepts into clinical genetics. Specifically, through the analysis of cells from patients with X-linked acrogigantism (X-LAG), a rare disease characterized by aggressive growth hormone-secreting pituitary tumors, we utilized chromosome conformation capture technologies (4C/HiC-seq) to investigate the impact of X-LAG associated duplications on genome organization and gene regulation. Our findings facilitated the refinement of the genetic diagnosis of X-LAG, enabling differentiation between pathogenic and non-pathogenic duplications (refer to illustration). This ultimately aided in excluding a presumptive X-LAG diagnosis in an infant, thereby obviating the need for intensive clinical follow-up (manuscript in preparation as corresponding author). Thirdly, I established experimental protocols to explore the genome-wide loss of chromatin structure on gene expression during zebrafish embryonic development at single-cell resolution.
Collectively, by utilizing 4C/HiC-seq as a clinical tool to assess the impact of structural changes in the genetic disease X-LAG and by investigating the functional relationship between genome structure and gene expression during embryonic development, I aim to elucidate gene regulatory mechanisms during development and disease, aligning with the objectives of the DMC3 strategic plan.

Two highlights:

On May 25, 2023, I presented my research as oral presentation from selected abstracts at the EMBO Workshop – “Systems biology: Linking chromatin and epigenetics to disease and development”, in Alexandroupoli, Greece (Title: “The role of the genome organizer CTCF in cell-specific gene expression and differentiation”).

On March 15, 2023, I collaborated with St. Mary’s High School in Sevilla to organize a workshop aimed at sharing my research and research journey to promote science to young students (Title: My research path studying non-coding mutations).



The impact of pathogenic and non-pathogenic duplications at the X-LAG locus on chromatin organisation and gene expression. (A) Under normal conditions, *GPR101* is separated by a TAD boundary (red hexagon) from pituitary enhancers (green ovals). (B) Genomic duplications associated with X-LAG, spanning the TAD boundary, lead to the formation of a neo-TAD (blue). This promotes ectopic chromatin interactions between *GPR101* and pituitary enhancers, consequently causing *GPR101* misexpression and gigantism in X-LAG. (C) Duplications of *GPR101* that "preserve" the TAD boundary do not generate a neo-TAD. As a result, the additional copy of *GPR101* remains segregated from pituitary enhancers. Note that duplication size and position are indicated by overlap in the schematic representation. Unpublished work. TAD, topologically associating domain.

Coenzyme Q Synthesis in *Saccharomyces cerevisiae* and its Implication in the Mitochondrial Health, Lifespan, and Ageing

Carlos Santos Ocaña

Summary of activities:

This year, we continued our work on diagnosing mitochondrial diseases using animal and cell models for functional validation studies. This approach helps us understand the disease's molecular mechanisms at the cellular level. However, it can be limited when considering the disease in a multicellular structure. Therefore, we obtained human brain cortical organoids from induced stem cells from patients with mitochondrial diseases. These advanced cellular structures will enable us to analyze how metabolic and bioenergetic changes in patients produce the disease.

Our research on the relationship between bioenergetics and development has also provided valuable information. We published a study on how mutations in the *GEMIN5* gene, which encodes a protein involved in the regulation of transcription and translation, cause mitochondrial dysfunction and a CoQ_{10} deficit associated with neurodevelopmental alterations. We found that CoQ_{10} supplementation recovered disease symptoms in both flies and humans.

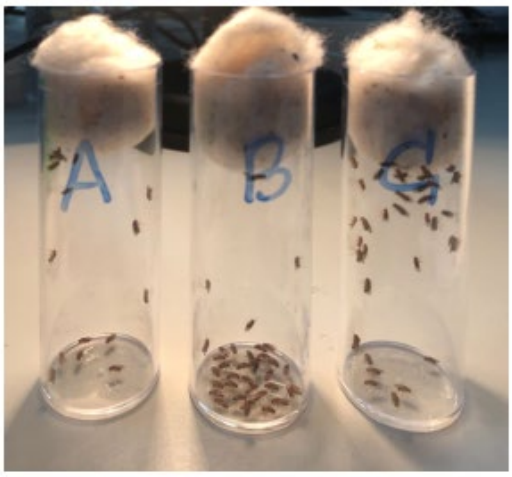
Another significant milestone was our group's participation in demonstrating the catalytic function of the COQ4 protein as a decarboxylase directly involved in synthesizing the CoQ_{10} coenzyme, the last reaction in this pathway that remains unveiled.

This year, we renewed the FIS project of the Instituto de Salud Carlos III and obtained an ACCI project from CIBERER to work on mitochondrial disease therapy. Additionally, we have maintained our teaching activities at the Universidad Pablo de Olavide with excellent assessment.

Selected references:

Cascajo-Almenara MV, Juliá-Palacios N, Urreizti R, Sánchez-Cuesta A, Fernández-Ayala DM, García-Díaz E, Oliva C, O Callaghan MDM, Paredes-Fuentes AJ, Moreno-Lozano PJ, Muchart J, Nascimento A, Ortez CI, Natera-de Benito D, Pineda M, Rivera N, Fortuna TR, Rajan DS, Navas P, Salviati L, Palau F, Yubero D, García-Cazorla A, Pandey UB, Santos-Ocaña C, Artuch R. Mutations of GEMIN5 are associated with coenzyme Q10 deficiency: long-term follow-up after treatment. Eur J Hum Genet. 2024 Feb 5. doi: 10.1038/s41431-023-01526-2.

Pelosi L, Morbiato L, Burgardt A, Tonello F, Bartlett AK, Guerra RM, Ferizhendi KK, Desbats MA, Rascalou B, Marchi M, Vázquez-Fonseca L, Agosto C, Zanotti G, Roger-Margueritat M, Alcázar-Fabra M, García-Corzo L, Sánchez-Cuesta A, Navas P, Brea-Calvo G, Trevisson E, Wendisch VF, Pagliarini DJ, Salviati L, Pierrel F. COQ4 is required for the oxidative decarboxylation of the C1 carbon of coenzyme Q in eukaryotic cells. Mol Cell. 2024 Mar 7;84(5):981-989.e7. doi: 10.1016/j.molcel.2024.01.003.



***D. melanogaster* model for the study of *GEMIN5* gene variants identified in patients with CoQ_{10} deficiency.**

Flies in tube B, where the GEMIN5 gene has been silenced, show an inability to climb up the tube walls compared to controls in tube A. When the food of the silenced flies in tube C is supplemented with CoQ_{10}, the flies recover CoQ levels and their ability to climb the tube wall.

Epigenetic Regulation of Virulence in Plant Pathogenic Fungi

Ramón Ramos Barrales

Summary of activities:

Pathogenic fungi need to change their growth pattern depending on the stage of infection they are in (yeast-like, filamentous, specialized penetration structures, spores, etc.). This constant behavioural shift during infection is highly regulated. In our laboratory, we are interested in the mechanisms employed by pathogenic fungi to control these changes. Specifically, we are studying the epigenetic mechanisms controlling pathogenesis, using the model organism *Ustilago maydis*, a corn pathogen. In 2023, we have published a paper describing how this fungus uses the sirtuin Sir2 to repress a group of virulence genes that express in a specific moment of the infection process. However, we have observed that this sirtuin is not controlling heterochromatin formation. This work has been part of a PhD thesis defended in 2023. As *U. maydis* has no other enzymes typically involved in heterochromatin establishment, as H3K9 or H3K27 methyltransferases, we have aimed to find the new mechanism this organism uses to produce heterochromatin. We have found that other histone methyltransferase is involved in this process and are currently studying the mechanism of action. In a parallel project, we are interested in studying the role of the nuclear structure on the virulence process. In 2023, we have found that the role in telomere placement on the nuclear periphery of a lamina associated protein homologue of *U. maydis* is essential for this fungus to penetrate the plant.

Selected reference:

Navarrete B, Ibeas JI*, Barrales RR*. Systematic characterization of Ustilago maydis sirtuins shows Sir2 as a modulator of pathogenic gene expression. Front Microbiol. 2023 Apr 11;14:1157990. doi: 10.3389/fmicb.2023.
* corresponding authors.

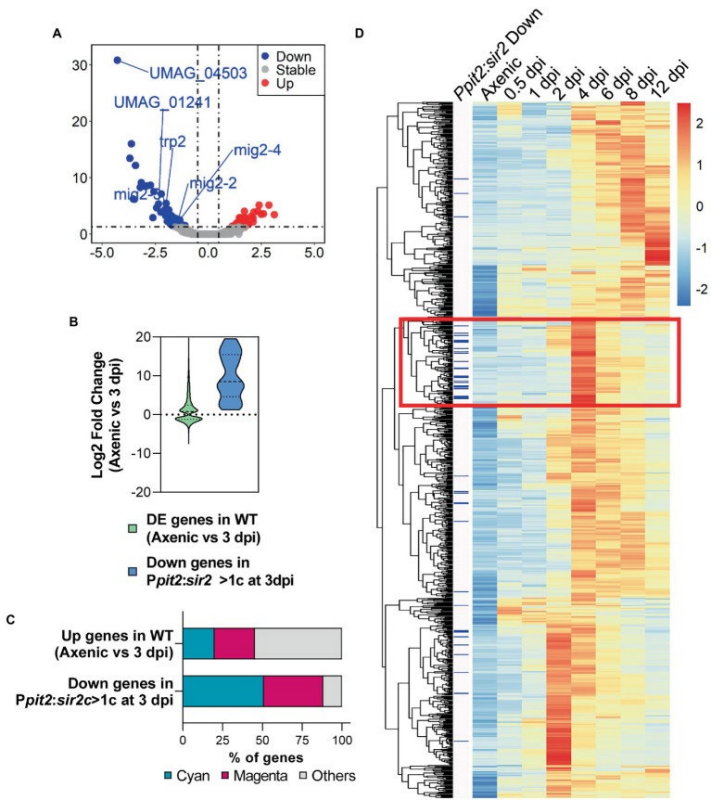


Figure from the paper where we describe the role of Sir2 during pathogenesis. In the figure is shown how the overexpression of Sir2 during infection avoids the expression of a group of genes induced at day 4 post infection.

Microbiology's Platypus

Damien Devos (Group Leader) and Elena Rivas-Marín (Associated Investigator)

Summary of activities:

Bacteria from the PVC (*Planctomycetota*, *Verrucomicrobiota*, *Chlamydiota*) superphylum are key species in evolutionary innovations. They are thought to be related to the process of eukaryogenesis, the making of our own cell type. In addition, these bacteria are a perfect starting point to reveal the biodiversity of non-model organisms, those that are outside of our Petri dishes. Thus, the need to characterize them to identify the genetic determinants behind this diversity.

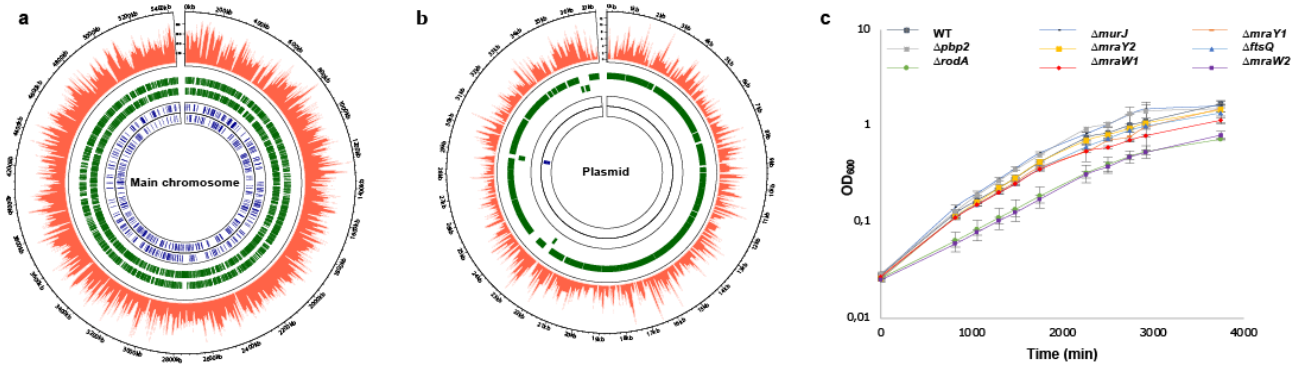
By readdressing the presence of membrane coat proteins in the Planctomycete *Gemmata obscuriglobus*, we strengthened previous structure predictions and also detected new putative membrane coat-like (MCL) proteins. However, no significant hits were found in *Archaea*, including Asgard archaea. More than ten years after their original detection, PVC bacteria are still uniquely linked to eukaryotes through the structure of the MCL proteins sustaining their endomembrane system.

Collaboration with the Universidad Autónoma de México allowed us to evaluate the conservation of proteins in the bacterial superphylum PVC. We reported a genome wide analysis of essential gene content in the Planctomycete *Planctopirus limnophila*. This report showed that certain genes involved in peptidoglycan synthesis or cell division, which are essential in most bacteria, are not essential for growth under laboratory conditions in this species. Our results provide insights into the divergent molecular and cellular biology of Planctomycetes.

Selected references:

Rivas-Marin E, Moyano-Palazuelo D, Henriques V, Merino E, Devos DP. Essential gene complement of Planctopirus limnophila from the bacterial phylum Planctomycetes. Nat Commun. 2023 Nov 9;14(1):7224. doi: 10.1038/s41467-023-43096-3.

Ferrelli ML, Pidre ML, García-Domínguez R, Alberca LN, Del Saz-Navarro D, Santana-Molina C, Devos DP. Prokaryotic membrane coat - like proteins: An update. J Struct Biol. 2023 Sep;215(3):107987. doi: 10.1016/j.jsb.2023.107987.



Genome and plasmid-wide transposon insertion sites of *P. limnophila* transposon library. a) and b) represent the main chromosome and the plasmid, c) Growth curve of *P. limnophila* cell division mutants

Coordination of Stem Cells, Physiology and Behaviour

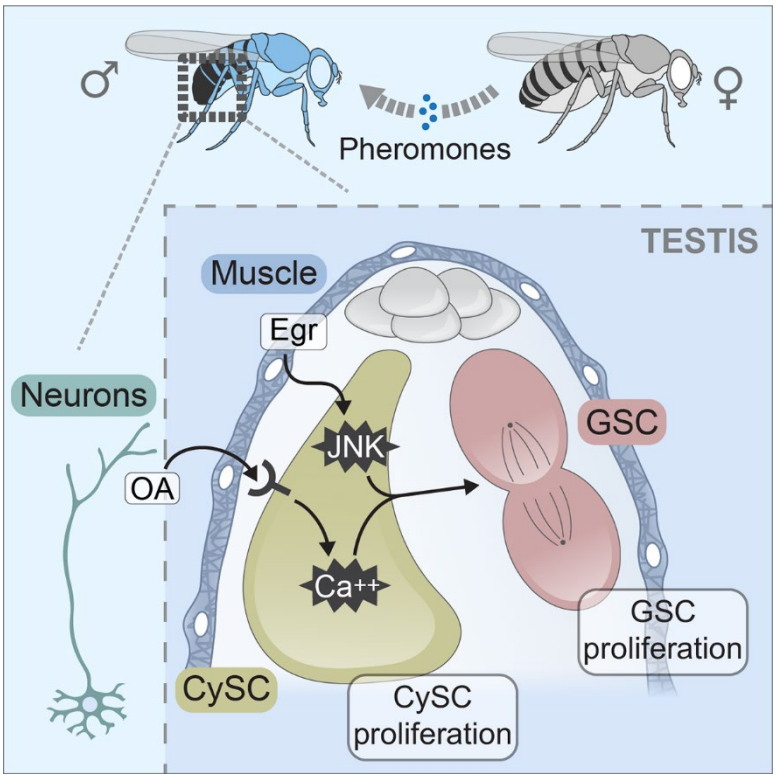
Salvador Herrera

Summary of activities:

We made notorious advances on our research about how the social interactions that the male animals experience can affect the stem cell biology of the gonads and the physiology of the entire organism, using flies as model organism. 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. These social inputs are transduced to the testis by an inter-organ communication network implicating signals released from the brain and the muscle – Octopamine, homologous to the Noradrenalin of vertebrates, and Egr/TNFα respectively. We propose that this mechanism coordinates reproductive behaviour and spermatogenesis for the success of male reproduction. This work is currently under review on *Cell Reports*. The study of this communication between the brain, the gonads and other tissues in response to an external cue (the social environment) relates to main DMC3 objectives. We suspect that this testis activation is part of a previously undescribed systemic response - a pre-mating response of male animals, aimed to cope with the imminent demands of reproduction. Our foundational discoveries enable us now to study potentially novel physiological regulations, as metabolic changes and energy stores mobilization during male reproduction, topics connected to the DMC3 objective to describe the relevance of energy constrains in collective cell decisions. Additionally, during 2023 we published a chapter on *Springer Protocols*.

Selected reference:

Herrera SC, Bach EA. Spermatogonial Dedifferentiation into Germline Stem Cells in Drosophila Testes. Methods Mol Biol. 2023;2677:139-149. doi: 10.1007/978-1-0716-3259-8_8.



Mechanisms of Gene Regulation and Bacterial Biofilm Development

Fernando Govantes

Summary of activities:

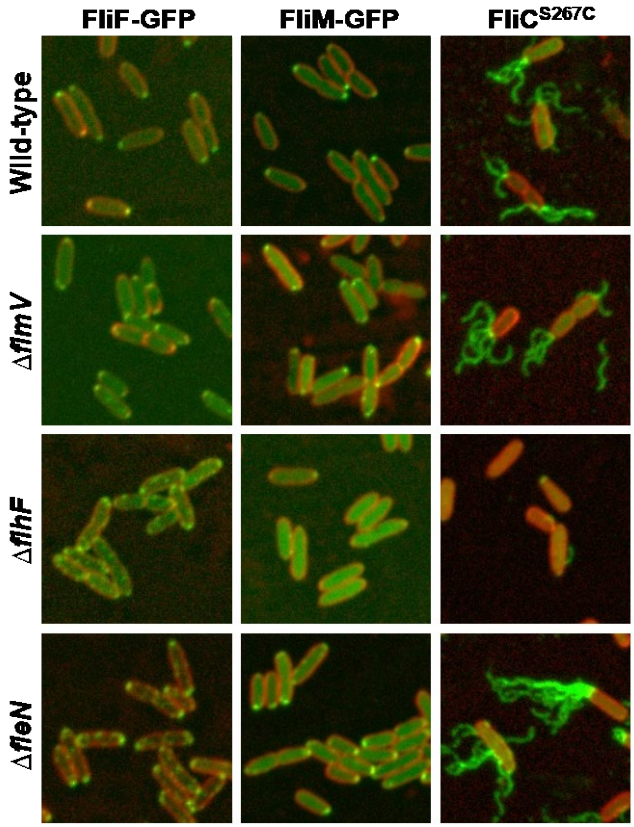
During the year 2023 the main achievements of our group are the following:

- We have solved the mechanisms by which *Pseudomonas putida* regulates the timing, location and number of flagella assembled, including a collaboration woth Felipe Cava (University of Umeå, Sweden)
- We have established the role of the anti-σ factor antagonist protein HsbR in repressing biofilm formation in stationary phase and characterized the involvement of HsbR phosphorylation and its interaction with diguanylate cyclase CfcR in this process.
- We have continued our collaboration with Mª. Trinidad Gallegos (EEZ, CSIC), and solved the regulatory mechanism of cellulose biosynthesis in *Pseudomonas syringae* by the coordinate action of FleQ, FleN, AmrZ and c-di-GMP.
- We have continued our collaboration with our fellow UPO professor Alejandro Cuetos of the Chemical Physics Area, the highlights being new computer models of biofilm colony development in 2D and 3D including the responses to nutrient deprivation, the outcome of colony collisions and the responses to pressure buildup in the central section of the colonies.
- We have reinitiated an old collaboration with Isabel Usón (Institut de Biologia Molecular de Barcelona) on the topic of the development of new methods to improve AlphaFold predictions of multimeric protein structures using LysR-type regulators as a model.

Selected references:

Martínez-Rodríguez L, López-Sánchez A, García-Alcaide A, Govantes F, Gallegos MT. FleQ, FleN and c-di-GMP coordinately regulate cellulose production in Pseudomonas syringae pv. tomato DC3000. Front Mol Biosci. 2023 Mar 27;10:1155579. doi: 10.3389/fmolb.2023.1155579.

Pulido-Sánchez M, Leal-Morales A, López-Sánchez A, Cava F, Govantes F. The regulation of polar flagella assembly in *Pseudomonas putida*. BioRxiv 2023.12.15.571843. doi: 10.1101/2023.12.15.571843.



The impact of regulatory mutations of flagellar assembly. Fluorescence images of wild-type, Δ*fimV*, Δ*flhF* and Δ*fleN* cells expressing FliF-GFP (MS-ring), FliM-GFP (C-ring) or FliC^{S267C} (filament). FM^{TM} 4-64 was used as membrane stain (red).

Cell Fate Determination in Early Mammalian Development

Bárbara Pernaute

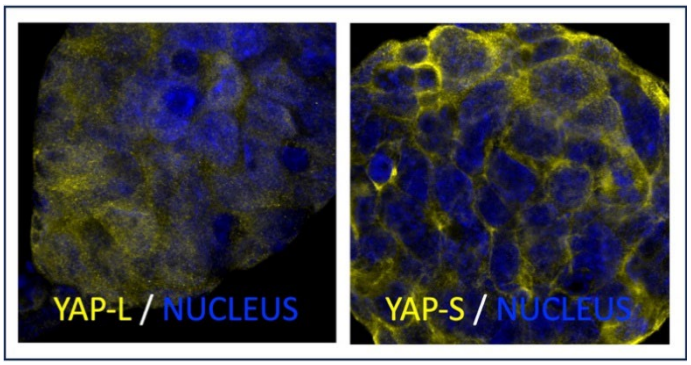
Summary of activities:

During 2023 I joined the CABD and began to establish my group, with a focus on the study of the first critical steps of development in mammals in which totipotency, the capacity to give rise to all embryonic and extraembryonic lineages, is first acquired and then quickly lost. Scientific highlights of 2023 include the completion of the experimental part of a project on YAP isoform function during stem cell differentiation (manuscript now in preparation), that led to the identification of a potential role for YAP in stem cell reprogramming to totiponecy, opening a research line that we are now pursuing. The obtention of a Plan Estatal project in 2023, the first grant for the lab, is allowing us to establish and develop our main research line to investigate how the mechanisms that control genome integrity adapt to the genomic changes occuring during early mammalian development. In addition, during 2023 we completed collaborations with the groups of Elvan Böke (CRG, Barcelona), studying proteostasis in mammalian oocytes (accepted in Cell in 2023) and Brad Cobb (RVC London), studying miRNA mediated regulation of the stress response in embryonic stem cells (published in Cells in 2023). Additional activities include the co-organization of the XXXIV Jornada de Biologia del Desarrollo of the Sociedad Catalana de Biología: DevBioCat synergy meeting 2023, in which we gave a special focus on the recent advances on *in vitro* production of “synthetic” mammalian embryos from stem cells.

Selected highlights:

- Joining the CABD as Junior Group Leader and establishing the group.

- Obtaining the first grant for the group, a Plan Estatal project.



Immunostaining showing distinct localization of YAP isoforms differing in the inclusion of exon 6 in mouse embryonic stem cells

Morphogenesis and Mechanosensitive Gene Regulation

María Almuedo Castillo

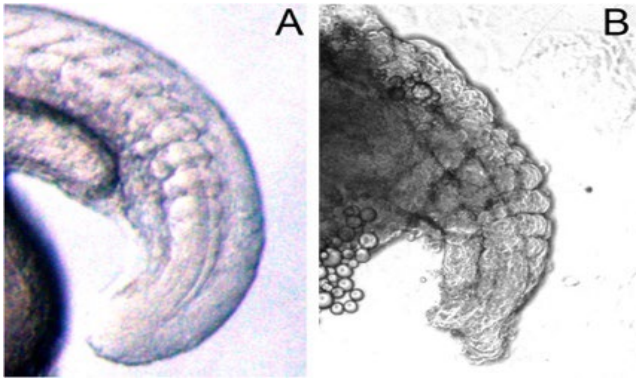
Summary of activities:

In 2023 I consolidated my independent group as a Ramón y Cajal Principal Investigator, where I ranked first in the young researchers call, and with the “Proyecto de Consolidación Investigadora”. My new team aims to understand how cell mechanosensation is sensed and propagated through gene regulatory adaptations during embryogenesis. This proposal catalyzed with our recent funding that the Yap transcriptional program is specifically activated in cells with a particular stretched nuclear geometry. We are currently setting up novel technologies that will allow us to link cellular and nuclear geometries and mechanical perturbations to specific gene regulatory adaptations, such as DNA and RNA fixation protocols prior to cell dissociation, culture of truly naïve zebrafish stem cells in different matrices and zebrafish organoids generation. Moreover, *i)* I established solid international collaborations with researchers from UCL, Biofisika Bilbao and UCSD, *ii)* my first PhD candidate, Ana Sousa, defended brightly her thesis in June 2023 and I began to co-supervise other 2 PhD thesis (Juan Aperador and Maria José López), *iii)* I continued with my commitment with knowledge sharing and mentoring younger scientists, science outreach and the promotion of women in academia (practical epigenetic course at UFPA, Brasil, and talks at 11F and 8M), and *iv)* presented my research with one selected (Advances in Biomedical Research V – MedILS) and two invited talks (Epigenetics meeting in UPFA and at the CSBMB Seminars in Zagreb).

Selected references:

Sousa-Ortega A, Vázquez-Marín J, Sanabria-Reinoso E, Corbacho J, Polvillo R, Campoy-López A, Buono L, Loosli F, Almuedo-Castillo M*, Martínez-Morales JR*. A Yap-dependent mechanoregulatory program sustains cell migration for embryo axis assembly. Nat Commun. 2023 May 16;14(1):2804. doi: 10.1038/s41467-023-38482-w. * corresponding authors.

Letelier J, Buono L, Almuedo-Castillo M, Zang J, Mounieres C, González-Díaz S, Polvillo R, Sanabria-Reinoso E, Corbacho J, Sousa-Ortega A, Diez Del Corral R, Neuhauss SCF, Martínez-Morales JR. Mutation of *vsx* genes in zebrafish highlights the robustness of the retinal specification network. Elife. 2023 May 25;12:e85594. doi: 10.7554/eLife.85594.



*Posterior somites in zebrafish **(A)** 18hpf embryos and **(B)** somite organoids.*

Self-Organization of Biological Systems

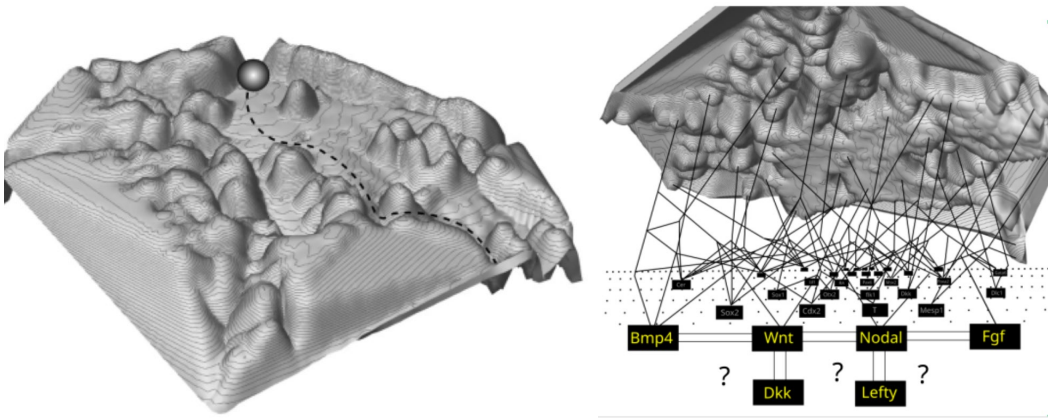
Luciano Marcon

Summary of activities:

This has been an exciting year for the Self-organization of Biological Systems Laboratory. The lab has made significant progress towards understanding how epiblast cells communicate and self-organize to shape the embryo's body plan when cultured as embryoids. A key achievement of the group was a groundbreaking single-cell analysis of embryoids, which provided a detailed transcriptomic landscape reminiscent of C.H. Waddington's Epigenetic Landscape, offering insights into the spatiotemporal dynamics of epiblast cell differentiation into germ layers. The lab has also developed two innovative models to elucidate the self-organization of the embryo's main axis. Through experimental perturbation and live imaging, the group has constructed a model explaining anterior-posterior axis formation, while developing a new set of mathematical equations named Sevillator has revealed how genetic oscillations coordinate the segmentation of the anterior-posterior axis. The lab has shared its insights into embryonic self-organization and modelling through various channels. Dr. Marcon taught two modules on these topics within an official Master at the University of Seville and the Universidad Internacional de Andalucía. The other researchers in the lab engaged in outreach talks at primary and secondary schools. Finally, the lab has communicated its science to the public through art, with a captivating collage of self-organizing embryoids showcased by our PhD student Rosana Caceres, awarded with the possibility to be exhibited in Casa de la Ciencia (Madrid). We have also given talks at primary schools on the 11F day, promoting inclusivity and diversity in science.

Selected reference:

Klepstad J, Marcon L. A Clock and Wavefront Self-Organizing Model Recreates the Dynamics of Mouse Somitogenesis in-vivo and in-vitro. Development, in press; bioRxiv https://doi.org/10.1101/2023.01.18.524516.



Experimental Epigenetic Self-Organizing Landscape of the Mouse Epiblast

Global Regulation of Gene Expression and Metabolism of Environmental Bacteria

Inés Canosa

Summary of activities:

In recent years, two new lines of research with a strong environmental focus have been launched in the group. These involve the application of knowledge to bioremediation strategies for pharmaceuticals spilled into the environment and studies of biodiversity through the application of certain techniques in agriculture. The researcher is PI of two competitive research projects in each of these areas. The aim of the first project is to improve knowledge of the interactions between the micro-organisms that are part of a bacterial community and the transfer of genetic information between the components of the consortium. The evolution of the composition and diversity of consortium members is studied in order to optimise a common task, such as the biodegradation of drugs like ibuprofen, naproxen or some new-generation antibiotics, released into the environment due to their widespread use in modern society. In this field, we collaborate with the Chemical Engineering, Physical Chemistry and Ecology departments of the UPO to implement techniques for disinfecting and decontaminating local rivers, through the development of matrices that favour these processes. Currently, and despite the recent implementation of this line of research, we have 3 manuscripts in preparation with the first results of this research.

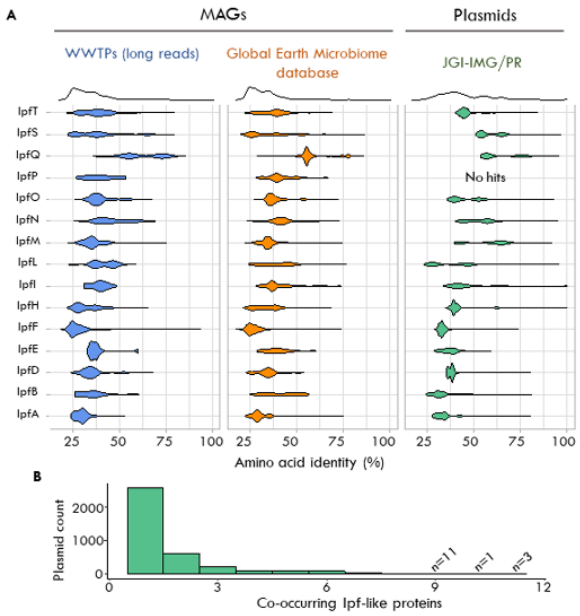
As university lecturer, I taught 160 hours teaching in the degrees of Biotechnology, Human Nutrition and Dietetics, and Environmental Sciences at the University Pablo de Olavide, as well as in doctoral programmes and supervising master's theses. My institutional commitments also include helping to disseminate Scientific research to secondary schools.

For the last 12 years, I been involved in the management of the University as Secretary and Head of the Department of Molecular Biology and Biochemical Engineering. I am also member of several institutional committees of the University related to quality management, of the University Claustro and of the Covid Commission for the implementation of strategies for the prevention of occupational risks at the UPO.

Selected references:

Saati-Santamaría Z, Navarro-Gómez P, Martínez-Mancebo JA, Juárez-Mugarza M, Flores A, Canosa I. Genetic information exchange in microbial consortia determines the ability to biodegrade ibuprofen. 2024. *Submitted to ISME J.*

Monteagudo-Cascales E, Santero E, Canosa I. The Regulatory Hierarchy Following Signal Integration by the CbrAB Two-Component System: Diversity of Responses and Functions. Genes (Basel). 2022 Feb 18;13(2):375. doi: 10.3390/genes13020375.



Distribution of sequence similarity search hits of Ipf proteins in Metagenome-Assembled Genomes (MAGs) recovered from metagenomes.

Molecular Embryology

Jaime J. Carvajal García-Valdecasas (Group Leader) and Cristina Vicente García (Associated Researcher)

Summary of activities:

Our group explores the genetic mechanisms of skeletal muscle (SkM) formation during mammalian development and muscle homeostasis in the adult. Over the past years our research has been focused on the function of the myogenic regulatory factor Mrf4, expressed both during development and in the adult. We have generated a knockout strain and started an in-depth analysis of the postnatal phenotype. Even if Mrf4 is only expressed in skeletal muscle, there are alterations in bones, adipose tissue, or the heart.

During 2003 we have shown that skeletal muscles have specific transcriptional programs not dependent on developmental origin; among the analysed 10 muscle groups, *digastricus* and *soleus* are the most divergent. In *soleus*, structural differences (i.e., the expression of slow type I myosin heavy chain) explain this divergence, while the expression of several kallikrein genes distinguish the *digastricus*.

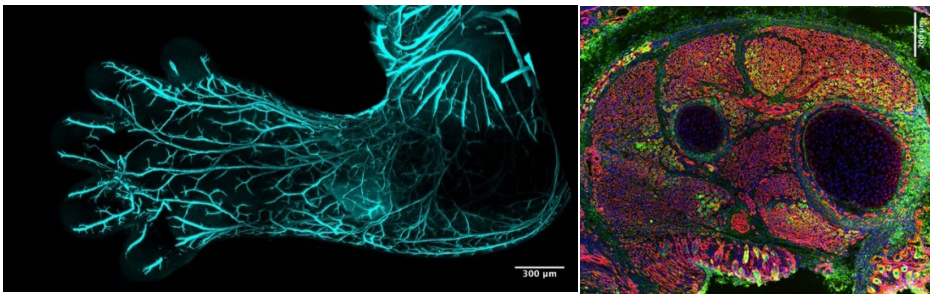
As expected, the transcriptomes of different SkMs have sex-specific signatures; the number of differentially expressed genes between sexes peaks at the adult stage, with young and old SkMs being remarkably more similar. Many differentially expressed genes in wild type do not show sex-specific differences in the absence of Mrf4, suggesting a role of Mrf4 integrating hormone-related inputs. Methylome analyses in the Mrf4-KO shows that samples cluster by genotype and not by muscle type (having shown that SkMs are transcriptionally different) and functional enrichment analysis of genes associated with differentially methylated region in *soleus* highlight processes that we know altered in the absence of Mrf4.

Finally, and in collaboration with Darío Lupiañez at CABD, we have generated a new FLAG-tagged allele for Mrf4, which will be used to identify the targets of this transcription factor.

Selected references:

Navarro-Martínez A, Vicente-García C, Carvajal JJ. NMJ-related diseases beyond the congenital myasthenic syndromes. Front Cell Dev Biol. 2023 Aug 4;11:1216726. doi: 10.3389/fcell.2023.1216726.

Muñoz-Gallardo MDM, Garcia-Padilla C, Vicente-Garcia C, Carvajal J, Arenega A, Franco D. miR-195b is required for proper cellular homeostasis in the elderly. Sci Rep. 2024 Jan 8;14(1):810. doi: 10.1038/s41598-024-51256-8.



Sy(stem)s Developmental Biology

Elena Camacho Aguilar

Summary of activities:

In November 2023, Dr. Camacho-Aguilar started her independent position as a Maria de Maeztu Junior Group Leader, establishing the Sy(stem)s Developmental Biology Lab at the CABD. Our lab aims to understand how cells interact and interpret dynamic signalling in space and time to create precise patterns during embryonic development, with a focus on the role of embryonic-extraembryonic interactions in human embryonic development.

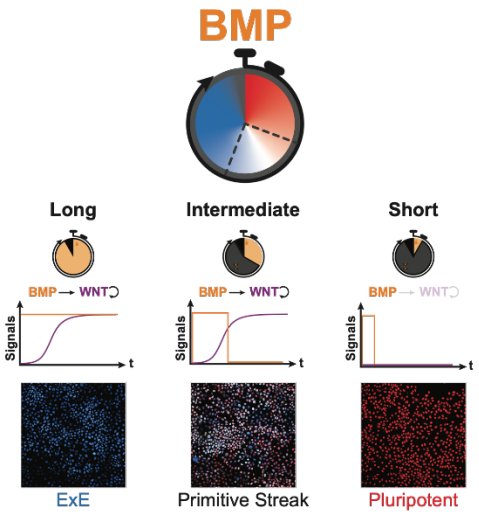
As gastrulation is a key stage in human development in which cells are exposed to changing concentrations of morphogens over time, in a recent study, we aimed to understand how human pluripotent stem cells interpret the duration and the concentration of the gastrulation-inducing signal BMP4. We discovered that BMP duration and concentration are not interchangeable, and we show how this is due to a combinatorial interpretation of BMP4 and endogenously induced Wnt signalling. This work has been accepted in Cell Systems, and has been presented at four conferences and workshops, including the 'Dynamics of Self-Organization in Animal and Plant Development' meeting at the Kavli Institute of Theoretical Physics, Santa Barbara, USA.

We have also established collaborations with the groups of Fco. Jesús Castro-Jimenez, in the Mathematics Department at the University of Seville, and the group of Gianluca Amadei, at the Università degli Studi di Padova, with whom we are investigating how embryonic-extraembryonic interactions play a role in the development of the mouse embryo and synthetic mouse embryo models. The analysis of the data provided by the Amadei Lab is the focus of the master's thesis of Irene Carrero Castro, in co-supervision with Fernando Casares.

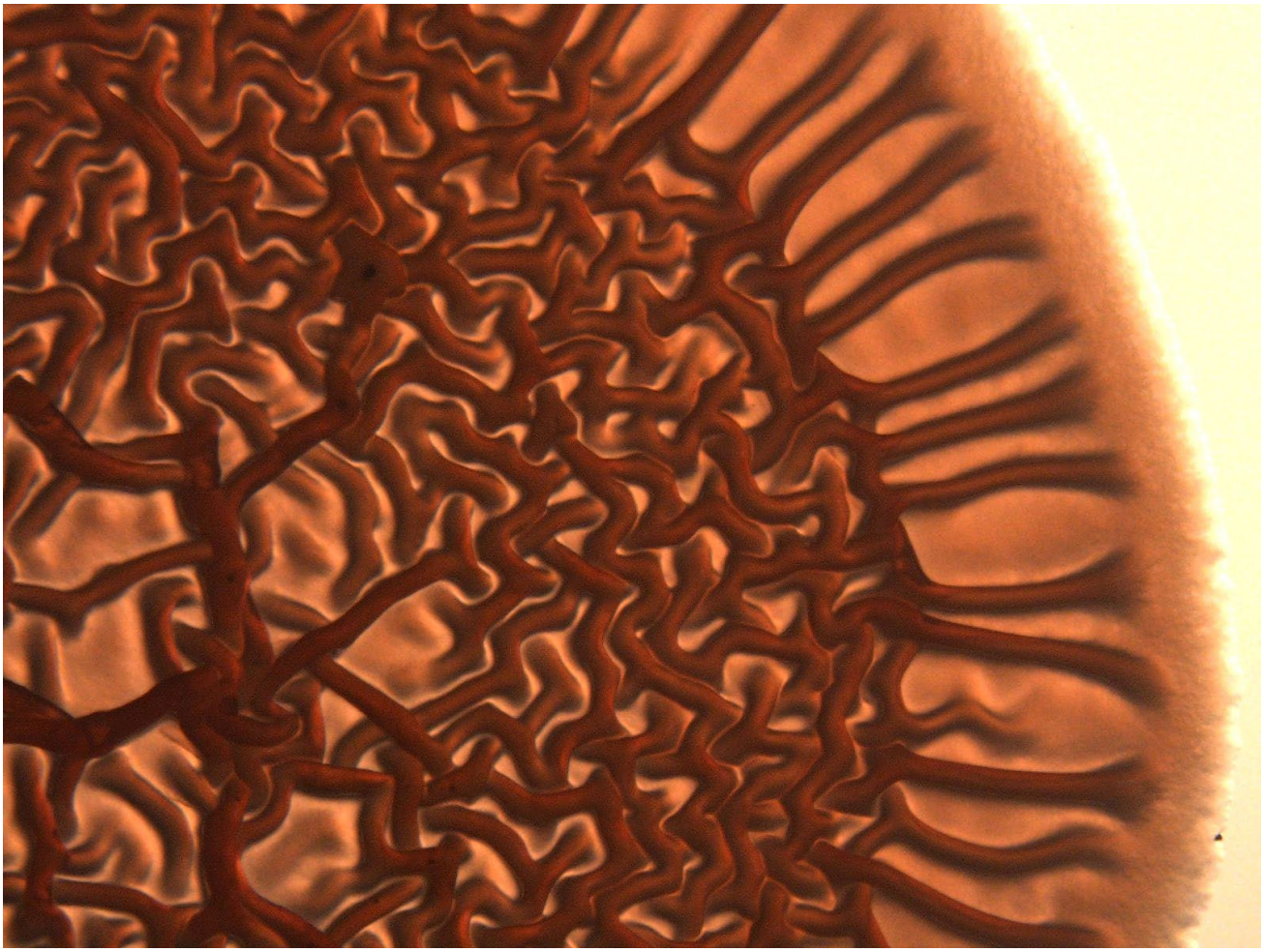
With regard to outreach activities, we are contributing to the 'New PI Diaries' blog series organized by The Node, where we posted one blog post about the start of our lab at the CABD.

Selected reference:

Camacho-Aguilar E*, Yoon ST, Ortiz-Salazar MA, Du S, Guerra MC, Warmflash, A*. Combinatorial interpretation of BMP and WNT allows BMP to act as a morphogen in time but not in concentration. Cell Systems, in press; bioRxiv doi:10.1101/2022.11.11.516212.



A "French flag" in time. Short, intermediate, and long durations of the BMP4 signal induce pluripotent, primitive streak, or extraembryonic fates due to an interplay with bistable endogenous WNT signaling.



Biofilm matrix: Rongo Red staining of a *Pseudomonas putida* colony. Congo Red stains the cellulose-like polysaccharides of the extracellular matrix (Marta Pulido Sanchez).

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