

scientific report  
**2016/2017**



**cabimer**   
ANDALUSIAN CENTER FOR MOLECULAR BIOLOGY  
AND REGENERATIVE MEDICINE







scientific report  
2016/2017

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## WELCOME

### Andres Aguilera

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DIRECTOR

It is my pleasure to present the scientific report of CABIMER (Centro Andaluz de Biología Molecular y Medicina Regenerativa / Andalusian Centre of Molecular Biology and Regenerative Medicine) for the period 2016 to 2017. As a groundbreaking multidisciplinary biomedical research center in Andalusia, CABIMER draws together basic and applied research with the aim of transforming the results of the scientific work into direct improvements for citizens' health and quality of life. CABIMER provides a rich intellectual environment to support individual researchers and to foster collaborations among faculty members, postdoctoral fellows, graduate students, technicians, visiting scientists and trainees. A large number of international scientists working at the Centre contribute to a stimulating and international atmosphere, and international seminars take place in the Center on a regular frequency all year round, improving the recognition and visibility of its research and researchers.

During this 2-year period there has been a real consolidation of CABIMER activities and infrastructural facilities to support the science undertaken by the 19 Principal Investigators (PI's) and their research group members. In 2016, CABIMER's 10th Anniversary celebration took place and was attended by different personalities from different Institutions and Public Organisms, and a New Agreement was also reached between the 4 partner Institutions to guarantee CABIMER's correct functioning. In 2017, CABIMER was successfully evaluated by the Scientific Advisory Board formed by prestigious European Scientists and the interaction and collaborations between the research groups of the Center has been stimulated with the celebration of an internal scientific workshop and a retreat for the young investigators, among other events. Some highlights of the past 2 years are the success of CABIMER researchers

in obtaining funding from competitive calls from national and international Agencies, a significant improvement of the quality of its publications and grant incomes, or the number and quality of PhD students and postdoctoral researchers, which has led to the defense of 25 PhD theses during this 2-year period, among other achievements.

In the 2016-17 period CABIMER has updated and acquired new equipment for its 10 fully functional core services including the nationally accredited GMP facility, the state of the art Biological Research Unit with a special unit for the generation of genetically modified mice, the Genomic platform for the use of external and internal services, the advanced Imaging unit, as well as Histology and Model organism services to support the different research activities of the Center using the most modern molecular and cellular technologies.

CABIMER is successfully increasing its reputation as an International Research Center of Excellence and a major centre of biomedical research in Spain. To accomplish these goals and improve its capabilities in the next future CABIMER has drawn up a Strategic Plan for the next 4 years (2018-2021) that aim at expanding the number of research groups and research lines in the Center, with special emphasis on young researchers. We are proud of the effort and dedication of all our PI's and researchers, as well as the support staff who have all contributed to the success of CABIMER as a referent in Molecular Biology and Biomedical research in Spain, with an increasing international visibility and strength. We still have a long way to go and many objectives to accomplish, but many new exciting discoveries lie ahead of us. I hope the information summarized in our Scientific Report conveys this ambition.





Cabimer by  
the Manager

**Pilar Cebolla**

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MANAGER

Welcome to CABIMER' Scientific Report for the period 2016 to 2017 that gathers all the research implemented at the Center and supported by the partnership of the Spanish National Research Council (CSIC), the University of Seville, the University "Pablo de Olavide", the Andalusian Regional Ministry of Health and the Andalusian Regional Ministry of Economy and Knowledge.

An annual running budget on average of 2.2M€ in this period, ensures our scientific community to keep their effort on research providing managing and technical services through the 10 Core Services Unit of CABIMER.

The Core Services Units, with technicians highly specialised in Genomics, Biological Resources, Cell Culture, Citometry & Sorter, Microscopy, GMP, Histology, Model Organisms, Biological Safety and Washing and Sterilization provide competitive services to our 19 Research Groups and external users.

The Management & Services Department is made up of an experienced team divided into 8 areas: projects management, accounting and economic control, purchasing, HHRR, safety, maintenance, storehouse and TI.

At the end of 2017, 183 persons worked at CABIMER, including 19 PIs, 13 senior researchers with stable positions, and a total of 71 PhDs researchers. In addition to the 183 workers, CABIMER has Master and last-year Bachelor students in practice, which usually constitute a mass of ~15 persons distributed among the different groups.

CABIMER is proud of promoting the career of young technicians and researchers and it has become a real training Center in a research environment that fosters the gender's equality. In December 2017, women represented the 63% of a total of 183 professionals.

Cabimer's ability to attract talent is demonstrated not only by the number of PhD Thesis but also for the origin of the students and young researchers that come from Spain and different countries around the world.

With an average of 50-60 projects per year developed in our Centre, numerous collaborations with high-level biotechnological companies and international stakeholders have been taken.

During this period the CABIMER has obtained resources for a total amount of 26.20MM€ including high-competitive grants from international institutions such as the European Research Council and the H2020 Programme from EU.

CABIMER' Strategic Plan for the next four years pursues its main objective: to improve the citizens' health and quality of life.



## Description of Research Activities

### Genome Biology

The Department of Genome Biology is focused on the study of all those processes related to the function and structure of eukaryotic genomes in non-pathological conditions, as well as the alterations of these processes that determine the appearance of human diseases, specially cancer and genetic diseases. We can group these processes into three major areas of research: 1) Genome expression, including studies on gene transcription, processing and transport of RNA and its regulation; 2) Genome structure, including structure of chromatin and nuclear architecture and 3) Dynamics and instability of the genome, including the processes of replication, recombination, and DNA repair, chromosome segregation and inheritance. Along with these lines of research, the department investigates in epigenetics, an increasingly transversal area that deals with the molecular mechanisms of the memory of chromatin states. The research lines of 6 groups of the Center are included in this area.

### Cell Dynamics and Signalling

The activity of this department is devoted to study the mechanisms of signal transduction and basic physiological processes with emphasis on degeneration, cell death and cancer. The different areas of research cover: Cell signaling, Cell polarity, adhesion and motility, Cell cycle control, Cell differentiation signaling, Cell signaling in immune regulation. Research in the area of Cell Signaling and dynamics is devoted to the study of mechanisms and proteins that control cell behavior, both on an individual level and in the context of the organ and tissue of which it forms a part with the aim of advancing in the knowledge of neoplastic, autoimmune and degenerative pathologies. In line with the most important international research centers, CABIMERs includes as a key research focus the study of mechanisms of cell signaling and the transmission of biochemical signals from outside the cell to the nucleus. It is complementary to the Genome Biology Area of Research, so that it focused on the impact that genome alterations have on cell dynamics, traffic and signaling as a possible molecular mechanism to explain disease, with a particular focus on the deficiencies of cell division processes, cell proliferation and differentiation or apoptosis as responsible of diseases, including cancer. This Department is former with 5 active groups.

### Regeneration and Cell Therapy

This department applies the results of molecular, cellular and clinical bases of new treatments for diabetes and metabolic and neurodegenerative diseases. The different areas of research are: Advanced therapy medicinal products (ATMP) for Diabetes Mellitus and neurodegenerative diseases Pancreatic Islet function, survival and regeneration Nutri-genomics, nutrigenetics and metabolic diseases Stem cell properties and differentiation Drug development, pre-clinical studies and clinical Trials Celular and Molecular Neuroimmunology The research activity in Stem Cells and Cellular reprogramming is focused on the study of stem cells, in particular self-renewal, differentiation as well as their ability to colonize and repopulate tissues, together with drugable and expandable adult progenitors are the biological basis for regenerative medicine. Great importance is given to stem cells (embryo, adult and fetal) and their capacity to develop into cells of whatever type of tissue, as well as its capacity to be used for other therapeutic applications such as toxicological and pharmacological assays as well as for the study of the first stages in the appearance of specific and genetic diseases. 8 groups work in research lines that belong to this Area of Research.





## Epigenetics and Gene Expression

**Dr. José C. Reyes**

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HEAD OF THE GENOME BIOLOGY DEPARTMENT



## Genome Biology Department

### Group Members updated

#### PhD student

Jose A. Guerrero Martínez

#### Technicians

Laura Basurto

Elena Gómez-Marín

### Academic Background of PI

- 1990: Degree. University of Seville, B.Sc. in Biology
- 1994: PhD. University of Seville, Biology

### Current Position

- Since 2009: Scientific Researcher, National Council for Research, CSIC/CABIMER
- Since July 2016: Vice-Director of CABIMER, Seville, Spain
- Since July 2016: Chair of the Department of Genome Biology of CABIMER, Seville, Spain

### Positions Held

- 2006-2009: Research Scientist CSIC/CABIMER, Seville
- 2000-2006: Research Scientist CSIC/ Instituto de Bioquímica Vegetal y Fotosíntesis (IBVF), Seville
- 2002-2006: Vice-Director of Instituto de Bioquímica Vegetal y Fotosíntesis (IBVF), Seville, Spain.
- 1995-1998: Postdoctoral Research Investigator Institute Pasteur. Paris, France

### Research Activity

#### OVERVIEW

Development and cell differentiation are the consequence of a precise choreography of genes whose expression is controlled in a temporal and spatial manner. Alterations in the process of gene expression are the origin of many congenital malformations and diseases, including cancer. Chromatin – the supramolecular complex formed by DNA and histone proteins – plays a fundamental role in gene expression. The main goal of our group is to understand how chromatin of regulatory elements and gene bodies change during transcription, how these changes are regulated and inherited and what protein factors are responsible for them. We specially investigate how alterations of these chromatin mechanisms are implicated in human disease, particularly in cancer.

#### RESEARCH HIGHLIGHTS

##### Epigenetic changes during the Epithelial to Mesenchymal Transition.

Epithelial and mesenchymal cellular phenotypes are the edges of a spectrum of states that can be transitory or stable. The epithelial to mesenchymal transition (EMT) (Figure 1) and its reversion (MET) have attracted considerable interest due to the fact that they seem to be related to tumor cells dissemination and metastasis formation. In our group we investigate epigenetic changes that occurs during EMT and MET and the chromatin factors implicated. We have shown that the protein HMG20A, associated to the LSD1/CoREST histone demethylase complex, is involved in repression of epithelial genes during this process (Rivero et al., 2015). During the period 2016-2017 we have studied the role of HMG20A, and other chromatin factors identified in a genetic screen, in EMT.



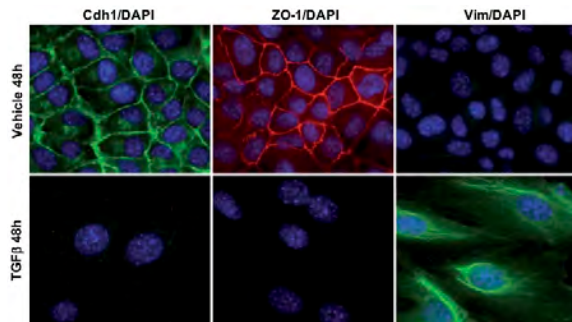


Figure 1

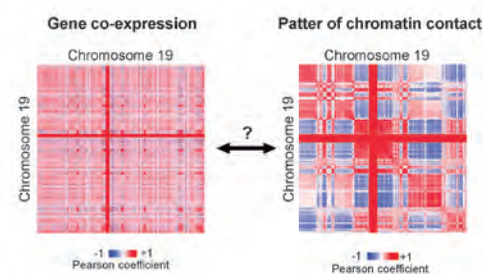


Figure 2

### CHD8 a chromatin remodeler involved in autism.

CHD8 is an ATPase of the SNF2 family involved in chromatin remodelling. Truncating mutations in the human CHD8 gene cause a specific type of autism. Alterations in the levels of CHD8 have also been associated with cancer. We have shown that CHD8 binds and regulates active promoters and enhancers (Ceballos-Chávez et al. 2015). During the period 2016-2017 we have investigated the mechanism by which CHD8 regulates transcription.

### Co-expression domains and relationship with the 3D chromatin nuclear organization.

We have also investigated the relationship between 3D chromatin contacts and gene expression through computational methods. The study of co-transcriptional networks evidenced that co-expressed genes tend to be grouped in the genome. We call this group of collinear genes CO-expression Domains (CODs). Analysis of the relationship between CODs and chromatin 3D organization using Hi-C contact data, demonstrated that genes inside CODs present similar patterns of chromatin contacts (Figure 2) (Soler-Oliva et al., 2017).

### Publication Highlights

- Soler-Oliva M.E., Guerrero-Martínez J.A., Bachetti V., Reyes J.C. 2017. Analysis of the relationship between co-expression domains and chromatin 3D organization. *PLoS Computational Biology*. 13(9):e1005708
- Jimeno-Gonzalez S., Payán L., Muñoz-Cabello A.M., Guijo M., Gutierrez G., Prado F., Reyes J.C. 2015. Defective histone supply causes changes in RNA polymerase II elongation rate and co-transcriptional pre-mRNA splicing. *Proc Natl Acad Sci USA*. 112(48):14840-14845
- Ceballos-Chávez M., Subtil-Rodríguez A., Giannopoulou E.G., Soronellas D., Elemento O., Beato M., Reyes J. C. 2015. The chromatin remodeler CHD8 is required for activation of progesterone receptor-dependent enhancers. *PLoS Genet*. 11(4):e1005174
- Rivero S., Ceballos-Chávez M., Bhattacharya S.S., Reyes J.C. 2015. HMG20A is required for SNAI1-mediated Epithelial to Mesenchymal Transition. *Oncogene*. 34(41):5264-76
- Ceballos-Chávez M., Rivero S., García-Gutiérrez P., Rodríguez-Paredes M., García-Domínguez M., Bhattacharya S. S., Reyes J. C. 2012. Control of neuronal differentiation by sumoylation of BRAF35, a subunit of the LSD1-CoREST histone demethylase complex. *Proc Natl Acad Sci USA*. 109(21):8085-8090

### Grants

2015-2017: BFU2014-53543-P. Ministerio de Economía y Competitividad



## Genome Instability and Cancer

**Dr. Andrés Aguilera**

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GROUP LEADER



## Genome Biology Department

### Group Members updated

#### Senior Researchers

Rosa Luna  
Hélène Gaillard  
Tatiana García-Muse  
Ana G. Rondón

#### Postdoctorals

Sonia Barroso  
Aleix Bayona Feliu  
María García-Rubio  
Belén Gómez González  
Sergio Muñoz Sánchez  
Lola Pérez de Camino Cantos  
Sonia Cristina Pinela da Silva  
Emanuela Tumini

#### PhD Students

Francisco García Benitez  
Cristina Guillén Mendoza  
Juan Carlos Martínez Cañas  
José Antonio Mérida Cerro  
Pedro Ortega Moreno  
Carmen Pérez Calero  
Marta San Martín Alonso  
María Eugenia Soler Oliva

#### Technicians

Victor Manuel González  
Basallote  
Esther Marchena Cruz  
Daniel Salas Lloret  
José Javier Marqueta Gracia

#### Administrative Assistant

Zoë Cooper

### Academic Background of PI

- 1979: Degree, University of Seville, B.Sc. in Biology
- 1983: PhD, University of Seville, PhD Thesis in Biology

### Current Position

- 2004: Professor of Genetics, University of Seville, Spain
- Since April 2016: Director of CABIMER, Seville, Spain

### Positions Held

- Since July 2006: Chair of the Department of Molecular Biology of CABIMER, Seville, Spain
- April 2007-July 2008 – Sept. 2010-Dec.2012: Vice-Director of CABIMER, Seville, Spain
- 1990-2004: Associate Professor of Genetics, University of Seville, Spain
- 1986-1990: Research Associate, NYU Medical Centre, New York, NY, USA
- 1984-1986: Postdoctoral Fellow F. Juan March, DAAD & EMBO. Tech. Universität Darmstadt, Germany

### Research Activity

#### OVERVIEW

The key role of genome instability in tumorigenesis and a number of rare cancer-prone genetic diseases, as well as its potential risks in stem cell-based therapies, has made it a major subject in basic biological research, cancer biology and biomedicine. Our research is focused on the factors and mechanisms responsible for genome instability associated with replication stress and replication-born DNA breaks, including that caused by transcription-replication conflicts and R-loop accumulation. Our ultimate goals are: 1) to identify the main determinants of replication failures that lead to the stalling or collapse of the replication fork and to DNA breaks; 2) to understand how a replication-born DNA break is repaired to allow replication restart and prevent chromosome rearrangements and genome instability; and 3) to evaluate the implication of such determinants and processes in the origin of cancer and its potential use in cancer therapy. Our research is performed in human cells and the model organism *Saccharomyces cerevisiae*, with specific incursions in *Caenorhabditis elegans*.

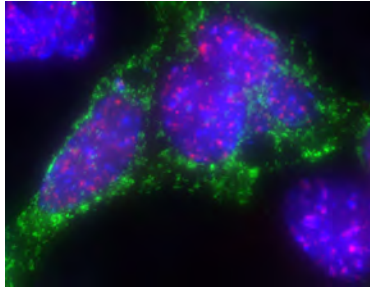
#### RESEARCH HIGHLIGHTS

Our work has focused on the identification of new factors and mechanisms responsible for genome instability in eukaryotic genomes, as a hallmark of cancer. The main highlights can be summarized as follows:

##### 1. Histone modifications as a determinant of R loop genome instability

To explore how RNA-DNA hybrids could modulate chromatin and genome integrity we screened a *Saccharomyces cerevisiae* library of histone H3 and H4 point mutants and found R loop-accumulating mutants that do not lead to genome instability by themselves, but only when human activation-induced cytidine deaminase (AID) is overexpressed. Contrary to mutants causing R loop-mediated instability, these histone mutants do not accumulate H3 serine-10 phosphate (H3S10-P). We propose a two-step mechanism in which, first, an altered chromatin facilitates R loops, and second, chromatin is modified as a requisite for compromising genome integrity.





*γ-H2AX foci (red) in HeLa cells treated with inter-strand crosslinking agent mitomycin C and overexpressing RNaseH1 (green). Cell nuclei are stained in blue*

## 2. mRNP biogenesis and Genome Instability

From a search of new human proteins interacting with THO, a complex that links mRNP biogenesis with transcription-associated genome instability, we found that human THO interacts with the Sin3A histone deacetylase complex to suppress co-transcriptional R loops. Our data provide a physical and functional crosstalk between RNA-binding factors and chromatin modifiers with a major role in preventing RNA-mediated genome instability. In addition, we have also found that a proper stoichiometry of Yra1, a yeast essential nuclear export factor, is required to maintain genome integrity and telomere homeostasis, suggesting that the cellular imbalance between transcribed RNA and specific RNA-binding factors may become a major cause of genome instability mediated by co-transcriptional replication impairment.

## 3.- Physical proximity of chromatin to nuclear pores prevents R loop mediated genome stability

After a screening of 400 *Saccharomyces cerevisiae* selected strains deleted in nuclear genes we found that loss of Mlp1 and/or Mlp2, nuclear basket proteins, leads to R loop accumulation, genome instability, and replication impairment, phenotypes that can be reverted by RNase H1 overexpression. Consistent with the Mlp1/2 role in gene gating to nuclear pores, artificial tethering to the nuclear periphery of a transcribed locus suppressed R loops in *mlp1Δ* cells, indicating that proximity of transcribed chromatin to the nuclear pore helps restrain pathological R loops.

## 4. Repair of replication-born DNA breaks and replication restart

Using a molecular assay, developed by us, to detect recombination events between sister chromatids (SCE) as the mechanism of repair of replication-born double-strand breaks (DSBs), we have shown that cells lacking the replisome component Rrm3 helicase are defective in DSB repair, which indicates that Rrm3 recruitment to replication-born DSBs is crucial for viability, uncovering a new role for Rrm3 in the repair of broken replication forks.

## 5. RNA-DNA hybrids metabolism and helicases.

We have provided evidence that helicase Mph1, the yeast homolog of Fanconi anemia protein M (FANCM), is required for cell viability in the absence of RNase H enzymes. The integrity of the Mph1 helicase domain is crucial to prevent the accumulation of RNA-DNA hybrids and RNA-DNA hybrid-dependent DNA damage. Our data support a model, where Mph1's helicase activity plays a crucial role in responding to persistent RNA-DNA hybrids. These findings could be important in the context of diseases associated with faulty RNA-DNA hybrid processing.

## Publication Highlights

- Bathia V., Barroso S., García-Rubio M., Tumini E., Herrera-Moyano E., Aguilera A., 2014. BRCA2 prevents R-loop accumulation and associates with TREX-2 mRNA export factor PCID2. *Nature*. 511(7509):362-365
- Herrera-Moyano E., Mergui X., García-Rubio M., Barroso S., Aguilera A. 2014. The yeast and human FACT chromatin reorganizing complexes solve R-loop-mediated transcription–replication conflicts. *Genes Dev.* 28:735-748
- García-Pichardo D., Cañas J.C., García-Rubio M.L., Gomez-Gonzalez B., Rondon A.G., Aguilera A. 2016. Histone Mutants Separate R Loop Formation from Genome Instability Induction. *Mol. Cell.* 66: 597-609
- Salas-Armenteros I., Pérez-Calero C., Bayona-Feliu A., Tumini E., Luna R., Aguilera A. 2017. Human THO-Sin3A interaction reveals new mechanisms to prevent R-loops that cause genome instability. *EMBO Journal.* 36(23):3532-3547
- García-Benítez F., Gaillard H., Aguilera A. 2017. Physical proximity of chromatin to nuclear pores prevents harmful R loop accumulation contributing to maintain genome stability. *PNAS.* 114(41):10942-10947

## Grants

2017-2019: BFU2016-81796-REDC. Redes de Excelencia. Ministerio de Economía y Competitividad  
 2017-2019: BFU2016-75058-P Ministerio de Economía y Competitividad  
 2016-2019: RTC-2016-4611-1 Ministerio de Economía y Competitividad  
 2015-2020: ERC2014-0015 Advanced. TARLOOP. European Research Council  
 2015-2017: 15/0098. Worldwide Cancer Research (formerly known as AICR)  
 2014-2019: P12/BIO-1238. Proyecto de Excelencia, Junta de Andalucía  
 2014-2016: BFU2014-51672-REDC. Ministerio de Economía y Competitividad  
 2014-2016: BFU2013-42918-P. Ministerio de Economía y Competitividad  
 2014-2017: PharmaMar  
 2017-2018: PharmaMar  
 2013-present: VEC – 001/2014 FVEC-FPS. Fundación Vencer el Cáncer





## Chromatin Integrity and Function

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GROUP LEADER



## Genome Biology Department

### Group Members updated

#### Staff Scientist

Macarena Morillo Huesca

#### Postdoctoral

Douglas Maya Miles

#### PhD Students

María Isabel Cano Linares

Marta Barrientos Moreno

Aurora Yañez Vilches

#### Technician

Elena Gomez Marín

### Academic Background of PI

- 1992: Degree. University of Seville, B. Sc. in Biology
- 1996: PhD. University of Seville, Molecular and Cellular Biology

### Current Position

- Since 2006: Research Scientist CSIC/CABIMER

### Positions Held

- 1997-1999: Postdoctoral fellow. IMT Institute, Philipp University-Marburg, Germany
- 2000-2005: Postdoctoral fellow/Ramón y Cajal Researcher. Biology School, University of Seville

### Research Activity

#### OVERVIEW

Faithful replication of the complete genome is essential for preventing any loss of genetic information. However, this is not an easy task; in fact, the genetic instability that accompanies tumor progression during early stages is associated with replicative stress. Using the yeast *Saccharomyces cerevisiae* as living model, and a wide repertoire of techniques in genetic, biochemistry, genomic, and molecular and cellular biology, we are focused on two different aspects associated with replication dynamics that have a direct impact on genome integrity and cell cycle progression: chromatin assembly and tolerance to replicative DNA damage. Our main goal is to get a deeper insight into their mechanistic and regulation, as well as in the consequences for cell fitness of mutations in the genes encoding their components.

#### RESEARCH HIGHLIGHTS

The assembly of the newly replicated DNA into nucleosomes to form the chromatin fiber is a highly regulated process where genetic and physical interactions between histone chaperones, chromatin assembly factors and components of the replisome ensure a rapid and correct deposition of histones behind the advancing fork. We have established the importance of chromatin assembly on the maintenance of replication fork stability, cell cycle progression and genome integrity. Our current work have revealed a role for the cohesin complex in the establishment of the primary structure of chromatin, which in turn may affect the fate and stability of replication forks. A second line of research has focused on the relevance of a scheduled reduction in the pool of histones during senescence. We have demonstrated that this reduction of histones protects telomeres from fusions by facilitating their recombinational processing and preventing the activity of NHEJ activities. Finally, our results on the chromatin remodeling complex SWR1 have uncovered a role for the hub actin cables/nuclear envelope/chromatin in the correct dynamics of the recombinational repair centers.

A major source of genetic instability is associated with the encounter of the replication fork



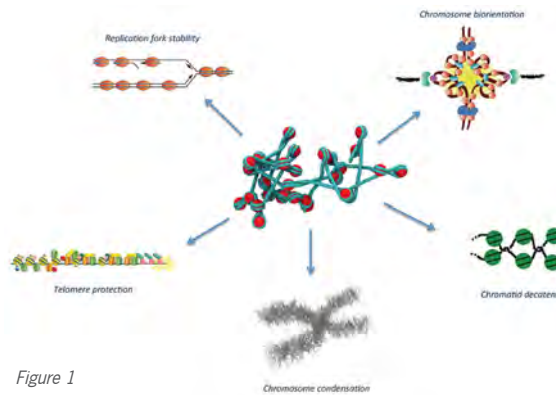


Figure 1

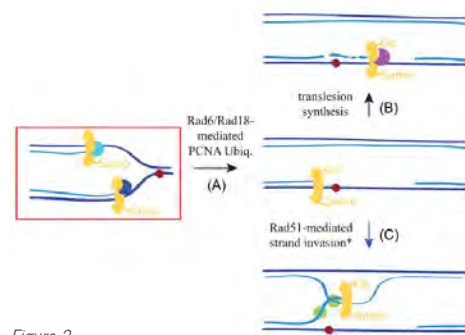


Figure 2

with DNA adducts that hinder its advance. In this case, replication fork stability and genome integrity are maintained by a number of error-free and error-prone mechanisms that help the fork to pass through the lesions and to fill in the gaps of single-stranded DNA (ssDNA) generated during the process of fork blockage and lesion bypass. Consequently, this DNA damage tolerance (DDT) response is essential for cell cycle progression, genome integrity, and cancer avoidance. A major research line in our lab is aimed at understanding the coordination of these mechanisms during the cell cycle, paying particular attention to the role of homologous recombination (HR) proteins as master players of the error-free pathways. We have reported a specific cell cycle regulation for HR proteins during DDT that prevent interferences between replication and repair. We have demonstrated and characterized physical interactions between the recombination proteins Rad51 and Rad52 with the replicative helicase Mcm2-7; their functional characterization suggests a role for these interactions in the decision of tolerating the DNA damage lesions by HR or translesion synthesis (TLS). Other lines of research in our lab further support this unexpected connection between HR and TLS.

### Publication Highlights

- Murillo-Pineda M., Cabello-Lobato M.J., Clemente-Ruiz M., Monje-Casas F., Prado F. 2014. Defective histone supply causes condensin-dependent chromatin alterations, SAC activation and chromosome decatenation impairment. *Nucleic Acid Research*. 42:12469-12482
- Gonzalez-Prieto R., Muñoz-Cabello A., Cabello-Lobato María J., Prado F. 2013. Rad51 replication fork recruitment is required for DNA damage tolerance. *EMBO Journal*. 32:1307-1321
- Clemente-Ruiz M., González-Prieto R., Prado F. 2011. Histone H3K56 acetylation, CAF1 and Rtt106 coordinate nucleosome assembly and stability of advancing replication forks. *PLoS Genetics*. 7 (11) e1002376
- Morillo-Huesca M., Clemente-Ruiz M., Andújar E., Prado F. 2010. In the Absence of H2A.Z the SWR1 Histone Replacement Complex Causes Genetic Instability and Genome-Wide Transcription Misregulation. *PLoS One*. e12143
- Clemente-Ruiz M., Prado F. 2009 Chromatin assembly controls replication fork stability. *EMBO reports*. 10:790-796

### Grants

2015-2018: BFU2015-63698-P. Ministerio de Economía y Competitividad

2016-2018: BFU2015-69142-REDT. Ministerio de Ciencia e Innovación

2014-2016: P12-CTS-2270. Junta de Andalucía "Proyectos de Excelencia"





## Mitochondrial Plasticity and Replication

**Dr. Ralf E. Wellinger**

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GROUP LEADER



### Group Members updated

#### Research Associates

Elisabet Fernández García  
Mónica Venegas Calerón

#### PhD Student

Hayat Heluani Gahete

#### Technicians

Elena Sánchez Martín  
Almudena Sánchez García

### Academic Background of PI

- 1992 Degree: Diplom Biologe Universität Kaiserslautern (D)
- 1996 PhD: Dr. sc. Nat. Eidgenössische Technische Hochschule Zürich (ETH, CH)

### Current Position

- Since 2009 Associate Professor at the Department of Genetics,  
University of Seville - CABIMER

### Positions Held

- 1996-2001: Postdoctoral fellow at the ETH Zürich
- 2001-2003: Marie-Curie fellow at the University of Seville
- 2003-2007: Ramón y Cajal fellow at the University of Seville-CABIMER
- 2007-2009: Assistant Professor at the University of Seville-CABIMER

### Research Activity

#### OVERVIEW

The genetic material is constantly exposed to endogenous or exogenous stress including telomeric and nucleolar, ribosomal DNA. Telomeres and ribosomal DNA are hot-spots of DNA damage causing genome instability, disease and premature aging.

We are interested in the factors and molecular pathways that lead to genome instability in telomeric and nucleolar DNA. For example, excess of intracellular manganese inhibits the RNaseH activity of telomerase, while the lack of RNaseH1 and H2 activities lead to persistent RNA:DNA hybrids (R-loops) that prime transcription-initiated replication (TIR) in the budding yeast ribosomal DNA locus. These R-loop-mediated replication events potentially contribute to copy number variation, particularly relevant to carcinogenesis. Therefore, we study the genetic and molecular factors that contribute Mn-homeostasis and TIR in order to gain better insight into aging and human disease.

#### RESEARCH HIGHLIGHTS

**Origin-independent replication by transcription initiated replication (TIR):** Understanding the process of DNA replication is fundamentally important because of its contribution to genome instability. DNA replication initiates at defined replication origins along eukaryotic chromosomes, ensuring complete genome duplication within a single S-phase. A key feature of replication origins is their ability to control the onset of DNA synthesis mediated by DNA polymerase- $\alpha$  and its intrinsic RNA primase activity. Our recent work describes a novel origin-independent replication process that, similar to the initiation of mitochondrial DNA replication, is mediated by transcription.



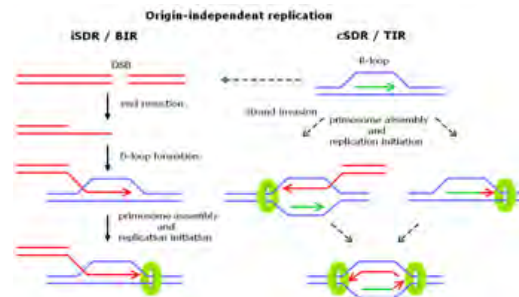
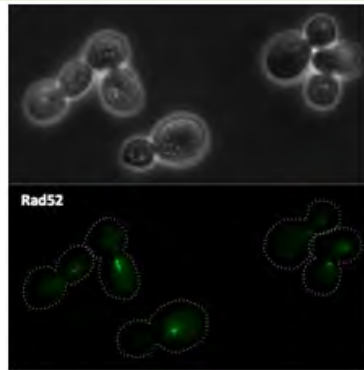


Figure 1: Rad52 foci formation indicative of DNA breakage (DSB) in budding yeast mutants (left) and a scheme of molecular events that are possibly connected to origin-independent replication (right, see Ravoityté and Wellinger, *Genes* 2017).

We found that in the absence of RNaseH and Top1, RNA polymerase I transcription constraints lead to persistent RNA:DNA hybrids (R-loops) that prime replication in the ribosomal DNA locus. Since the concomitant loss of RNaseH and Top1 activities is lethal, we screened for suppressors of this phenotype. Cells become viable in the absence of the Pif1 helicase suggesting that break-induced replication (BIR) could cause toxic replication intermediates that impair cell viability. We are currently investigating the role other suppressors that link R-loop dependent genome instability to cell cycle regulation.

#### Manganese homeostasis and stress response:

The yeast ATP2C1 orthologue PMR1 codes for a Mn(2+)/Ca(2+) transporter that is crucial for cis-Golgi manganese supply. Regulation of intracellular ion homeostasis is essential for eukaryotic cell physiology. An example is provided by loss of ATP2C1 function, which leads to skin ulceration, improper keratinocyte adhesion, and cancer formation in Hailey-Hailey patients. We studied the impact of Mn(2+) on cell cycle progression and show that an excess of cytosolic Mn(2+) bypasses the need for S-phase cell cycle checkpoints and predisposes to genomic instability. Interestingly, manganese was found to inhibit telomerase activity leading to short telomeres in *pmr1Δ* mutants. Moreover, replicative life span of *pmr1Δ* mutants is dramatically shortened. Thus, we our current aim is to understand how impaired Mn-homeostasis triggers cellular stress responses and premature aging.

#### Publication Highlights

- Ravoityté B., Wellinger R.E. Non-canonical replication initiation: You're fired!. 2017. *Genes* (Basel). 8(2):54
- Guintini L., Tremblay M., Toussaint M., D'Amours A., Wellinger R.E., Wellinger R.J., Conconi A. Repair of UV-induced DNA lesions in natural *Saccharomyces cerevisiae* telomeres is moderated by Sir2 and Sir3, and inhibited by yKu-Sir4 interaction. 2017. *Nucleic Acids Res.* 45(8):4577-4589
- Cid Martín J.J., Assali M., Fernández-García E., Wellinger R.E., Fernández I., Khiar N. Tuning of glyconanomaterial shape and size for selective bacterial cell agglutination. 2016. *Journal of Materials Chemistry B*. 4: 2028-2037
- Stuckey R., García-Rodríguez N., Aguilera A., Wellinger R.E. 2015. Role for RNA:DNA hybrids in origin-independent replication priming in a eukaryotic system. *Proc Natl Acad Sci U S A*. 112(18):5779-84
- García-Rodríguez N., Manzano-López J., Muñoz-Bravo M., Fernández-García E., Muñoz M., Wellinger R.E. 2015. Manganese Redistribution by Calcium-stimulated Vesicle Trafficking Bypasses the Need for P-type ATPase Function. *J Biol Chem*. 290(15):9335-47

#### Grants

- 2015-2018: BFU2015-69183-P. Ministerio de Ciencia e Innovación
- 2013-2017: P08-CTS-04297. Consejería de Economía, Innovación y Ciencia, Junta de Andalucía





## DNA damage response

**Dr. Felipe Cortés-Ledesma**

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GROUP LEADER



### Group Members updated

#### Senior Researchers

José F. Ruiz Pérez  
Silvia Jimeno González

#### Postdocs

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Pedro Martínez García  
Marta Muñoz Barrera  
María Domínguez Sánchez

#### PhD Students

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Andrés Herrero Ruiz  
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#### Technicians

Irene Delgado Sainz  
Cristina Bernal Lozano

#### Administrative Assistant

Rosana Herrera Palau

### Academic Background of PI

- 2003: PhD. University of Córdoba, Biochemistry
- 2006: PhD. University of Seville, PhD in Molecular and Cellular Biology

### Current Position

- Since 2014: Research Associate Professor, CSIC /CABIMER

### Positions Held

- 2006-2007: Postdoctoral Researcher. University of Seville - CABIMER
- 2007-2008: EMBO long-term Fellow. Genome Damage and Stability Centre, University of Sussex (UK)
- 2008-2010: Marie Curie Fellow. Genome Damage and Stability Centre, University of Sussex (UK)
- 2010-2014: Ramón y Cajal Fellow. University of Seville - CABIMER

### Research Activity

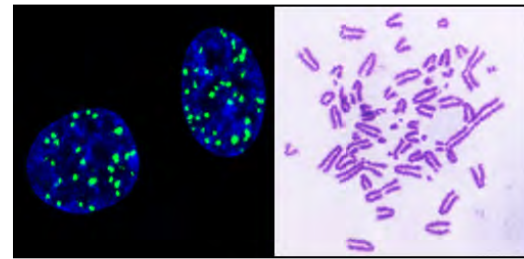
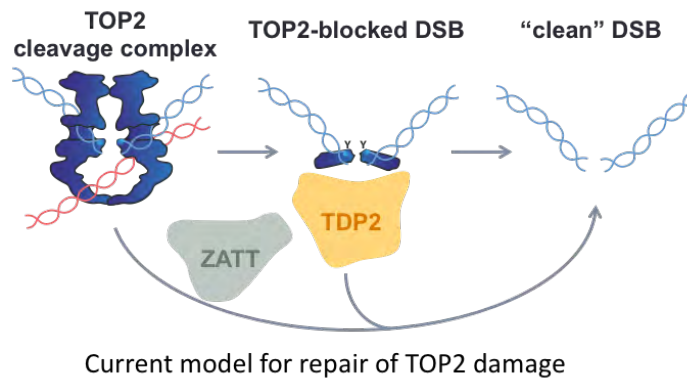
#### OVERVIEW

The DNA damage response (DDR) ensures efficient and accurate repair of DNA breaks, coordinating it with important cellular functions such as cell cycle progression, chromatin dynamics and gene expression. Deficiencies in the DDR can compromise cell survival, genome integrity and tissue homeostasis, with the consequent implications for human health. This is exemplified by a range of human genetic syndromes with characteristic developmental, degenerative and/or cancer predisposition problems. We apply a comprehensive approach, covering from the detailed molecular analysis of the process to the pathological implications in patients and animal models, in order to understand how DNA breaks are signalled and repaired, and how, if inefficient or aberrant, these processes can impact on human health.

#### RESEARCH HIGHLIGHTS

Our research these years has specifically focused on DNA double-strand breaks (DSBs) arising by the aberrant action of DNA topoisomerase II (TOP2), which, not only constitute an important source of spontaneous DNA damage, but also underlies the clinical efficacy of widely used anticancer agents known as topoisomerase “poisons” (epipodophyllotoxins, anthracyclines, ...). These breaks are characteristic in that the topoisomerase remains covalently bound to DNA termini blocking them for subsequent repair. Tyrosyl DNA phosphodiesterase 2 (TDP2) has the unique capacity to specifically cleave the bond between protein and DNA, eliminating these protein-adducts, and facilitating thus the repair of TOP2-induced DSBs. This activity is important for cell survival and maintaining genome integrity, having important implications in the context of cancer and neurological disease.





Recently, we have identified a novel component in the cellular response to TOP2-induced DNA breaks, ZATT (aka ZNF451), an unconventional SUMO E3 ligase with yet poorly defined substrates and functions. ZATT sumoylates and remodels TOP2-blocked lesions, favouring accessibility to the tyrosyl-phosphodiester bond. These modifications, in turn, promote the recruitment of TDP2 by novel SUMO-specific interactions, and facilitate its action in the removal of full-length TOP2 from DNA ends. These results change our concept of how cells deal with TOP2 lesions, which were previously thought to require proteolytic processing previous to the action of TDP2.

In addition, we have obtained an important body of evidence suggesting that TDP2 can be involved in other features of TOP2 biology, independently of its role in DSB repair, and which provide very interesting clues regarding the regulation of TOP2 functions in genome organization, expression and stability, and which we are currently exploring in detail.

### Publication Highlights

- Schellenberg M.J.\*\*, Lieberman J.A.\*\*, Herrero-Ruiz A., Butler L.R., Williams J.G., Muñoz-Cabello A.M., Mueller G.A., London R.E., Cortés-Ledesma F., Williams R.S.\* .2017. ZATT (ZNF451)-mediated resolution of topoisomerase 2 DNA-protein cross-links. *Science*. 357:1412-1416 (\*corresponding authors; \*\*first authorship)
- Sastre-Moreno G., Pryor J.M., Moreno-Oñate M., Herrero-Ruiz A.M., Cortés-Ledesma F., Blanco L., Ramsden D.A., Ruiz J.F. 2017. Regulation of human pol $\lambda$  by ATM-mediated phosphorylation during non-homologous end joining. *DNA Repair*. 51:31-45
- Álvarez-Quilón A., Serrano-Benítez A., Lieberman J.A., Quintero C., Sánchez-Gutiérrez D., Escudero L.M., Cortés-Ledesma F. 2014. ATM specifically mediates repair of double-strand breaks with blocked DNA ends. *Nature Communications*. 5:3347
- Gomez-Herreros F., Schuurs-Hoeijmakers J.H.M., McCormack M., Grealley M.T., Rulten S., Romero-Granados R., Counihan T.J., Chaila E., Conroy J., Ennis S., Heinzen E., Goldstein D.B., Delanty N., Cortés-Ledesma F., de Brouwer A.P.M., Cavalleri G.L., El Khamisy S.F., de Vries B.B.A., Caldecott K.W. 2014. TDP2 protects transcription from abortive Topoisomerase 2 activity and maintains normal neural function. *Nature Genetics*. 46:516-521
- Gómez-Herreros F., Romero-Granados R., Zeng Z., Álvarez-Quilón A., Quintero C., Ju L., Umans L., Vermeire L., Hyulebroeck D., Caldecott K.W., Cortés-Ledesma F. 2013. TDP2-dependent non-homologous end-joining protects against topoisomerase II-induced DNA breaks and genome instability in cells and in vivo. *PLoS Genetics*. 9:e1003370

### Grants

2015-2020: ERC-CoG-2014-647359. Consejo Superior de Investigaciones Científicas  
 2015-2017: SAF2014-55532-R. Consejo Superior de Investigaciones Científicas  
 2015-2017: Fundación Ramón Areces





## DNA double strand breaks repair and human disease

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GROUP LEADER



### Group Members updated

**Senior Researcher**  
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Fernando Mejías Navarro  
María Rosario Prados Carvajal

**Technicians**  
Javier Ramón Pasías  
Guillermo Rodríguez Real

**Master Student**  
Blanca Lago Solis

### Academic Background of PI

- 1998: Degree. University of Seville, B.Sc. in Biology
- 2004: PhD. University of Seville, Ph.D. Thesis in Molecular and Cellular Biology

### Current Position

- Research Scientist CABIMER
- Associate Professor of the University of Seville

### Positions Held

- 2004-2010: The Gurdon Institute for Cancer Research and Developmental Biology, University of Cambridge, UK
- 2010-2015: Ramón y Cajal, University of Seville/ CABIMER
- 2015-2016: Profesor Contratado Doctor, University of Seville/CABIMER

### Research Activity

#### OVERVIEW

Double strand breaks (DSBs) repair is essential for normal development. Lack of DSBs repair leads to cell death, but mutations that hamper this process cause genetically inherited syndromes and cancer predisposition. There are two ways to repair DSBs. Non-processed breaks can be simply rejoined by the non-homologous end-joining pathway (NHEJ). However, sometimes they are processed to create RPA-coated single stranded DNA, effectively blocking NHEJ and triggering a more complex repair pathway called homologous recombination (HR). Mutations in NHEJ or HR components correlate with several inherited human syndromes or cancer predisposition. Interesting, defects in the repair of DSBs are also commonly exploited for the treatment of cancer. In our laboratory, we are studying how the formation of ssDNA is regulated. This key step controls both the switch between NHEJ and HR. Using a comprehensive list of cellular and molecular biology techniques, we are gaining further insight in this regulatory network.

#### RESEARCH HIGHLIGHTS

The 2016-2017 period correspond with the consolidation of the lab as a referent in the field at national and international level. Using a combination of genetic screens and molecular biology we have expanded our knowledge on how cells exert the decision between different repair pathways when faced with broken chromosomes. In addition to uncover new, key elements of such regulatory network, we have advanced on the understanding of the regulation of previously known factors.

Not only we have been able to publish papers in mid and high tier journals, but we have created a solid international collaboration network. Moreover, the PI has been granted with the admission in the prestigious Young Investigator Program of EMBO and received several research awards.



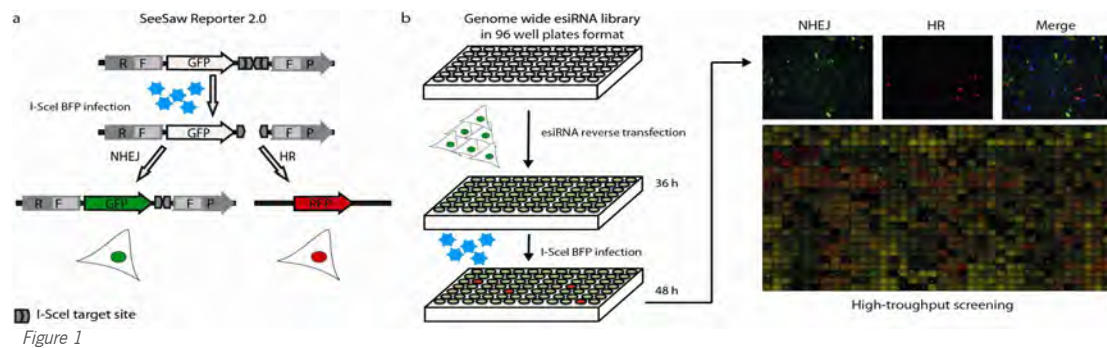


Figure 1

## Publication Highlights

- Gavish-Izakson M., Velpula B.B., Elkon R., Prados-Carvajal R., Barnabas G.D., Ugalde A.P., Agami R., Geiger T., Huertas P., Ziv Y., Shiloh Y. 2018. Nuclear poly(A)-binding protein 1 is an ATM target and essential for DNA double-strand break repair. *Nucleic Acids Res.* 46(2):730-747
- Soria-Bretones I., Cepeda-García C., Checa-Rodríguez C., Heyer V., Reina-San-Martin B., Soutoglou E., Huertas P. 2017. DNA end resection requires constitutive sumoylation of CtIP by CBX4. *Nat Commun.* 8(1):113
- Rabinowicz N., Mangala L.S., Brown K.R., Checa-Rodríguez C., Castiel A., Moskovich O., Zarfati G., Trakhtenbrot L., Levy-Barda A., Jiang D., Rodríguez-Aguayo C., Pradeep S., van Praag Y., Lopez-Berestein G., David A., Novikov I., Huertas P., Rottapel R., Sood A.K., Izraeli S. 2017. Targeting the centriolar replication factor STIL synergizes with DNA damaging agents for treatment of ovarian cancer. *Oncotarget.* 8(16):27380-27392
- Gómez-Cabello D., Checa-Rodríguez C., Abad M., Serrano M., Huertas P. CtIP-Specific Roles during Cell Reprogramming Have Long-Term Consequences in the Survival and Fitness of Induced Pluripotent Stem Cells. 2017. *Stem Cell Reports.* 8(2):432-445
- López-Saavedra A., Gómez-Cabello D., Domínguez-Sánchez M.S., Mejías-Navarro F., Fernández-Ávila M.J., Dinant C., Martínez-Macias M.I., Bartek J., Huertas P. 2016. A genome-wide screening uncovers the role of CCAR2 as an antagonist of DNA end resection. *Nat Commun.* 7:12364

## Grants

2017-2019: SAF2016-74855-P. Ministerio de Economía y Competitividad  
 2014-2017: Fundación Vencer el Cáncer  
 2014-2017: P12-BIO-515 Junta de Andalucía  
 2012-2016: ERC StG -European Research Council





## Membrane Traffic and Cytoskeleton in Cell Dynamics

**Dr. Rosa M. Rios**

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HEAD OF THE CELL DYNAMICS AND  
SIGNALLING DEPARTMENT



### Group Members updated

#### Postdoctorals

Laura Martínez  
María P. Gavilán  
Elena Gavilán  
Eduardo Ródenas

#### PhD Students

Pablo Gandolfo Dominguez  
Jesús Roca

#### Technician

Loida Pérez García

### Academic Background of PI

- 1984: Degree. University of Seville, B.Sc. in Biology
- 1988: PhD. University of Seville

### Current Position

- 2006 - Present: Scientific Researcher, Spanish Research Council CSIC/CABIMER

### Positions Held

- 1989-1990: Postdoctoral fellow MEC-EU CGM, CNRS, Gif-sur-Yvette, France
- 1991-1993: Postdoctoral Fellow MEC University of Seville
- 1994: Postdoctoral Fellow U. Seville University of Seville
- 1995: CNRS Investigator Institut Curie, CNRS, France
- 1994-2002: Associated Professor. University of Seville
- 2002-2006: Ramón y Cajal Investigator. University of Seville
- 2006-2011: Research Scientist Spanish Research Council, CSIC
- 2010-2011: Sabbatical leave CRI-CRUK Cambridge, UK

### Research Activity

#### OVERVIEW

Proper organization of microtubule (MT) arrays, as well as the number of MTs, are essential for cellular functions. Although the centrosome generally serves as the primary microtubule-organizing center (MTOC) in animal cells, MTs are also nucleated at the Golgi Apparatus (GA) in a wide range of mammalian cell types as we reported several years ago (Rivero et al., EMBO J, 2009). The activity and subcellular positioning of both MTOCs define the final geometry of the MT array. It is known that centrosomal MT nucleation is modulated during cell division or cell differentiation. These functional changes are usually linked to variations in pericentriolar material (PCM) levels and/or shedding of some PCM components. Interestingly, an increasing body of data indicates that MT nucleation activity of the GA is coordinately regulated with centrosome activity.

The main focus of my lab is to understand how MT nucleation activities of the centrosome and the GA are regulated along the cell cycle or during cell differentiation, and how this regulation impacts MT array geometry in order to be adapted to each physiological condition.

#### RESEARCH HIGHLIGHTS

MT nucleation primarily relies on  $\gamma$ -tubulin and its associated proteins (GCP2-GCP6) that, in higher eukaryotes, form ring-shaped complexes known as  $\gamma$ -TuRCs. Efficient  $\gamma$ -TuRC-mediated MT nucleation depends on additional regulatory factors. Four mammalian proteins, pericentrin (PCNT) and its paralog AKAP450, and CDK5Rap2 (Cep215) and its paralog myomegalin, contain conserved, yet degenerate, motifs for  $\gamma$ -tubulin binding [18]. All of them are considered  $\gamma$ -TuRC receptors, and it is widely assumed that they play a role in MT nucleation. Interestingly, all the four proteins are shared by the centrosome and the GA in different cellular contexts. To gain further insights into a possible crosstalk between the two organelles, we developed CRISPR/Cas9-based single and double KO cell lines of PCNT, AKAP450 and CDK5Rap2. We also used the recently described PLK4 inhibitor centrinone, which reversibly blocks the duplication of centrioles, in order to manipulate the number of centrosomes. Centrinone treatment generates

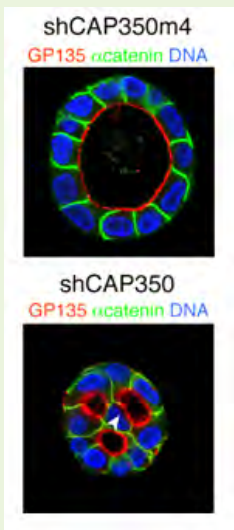
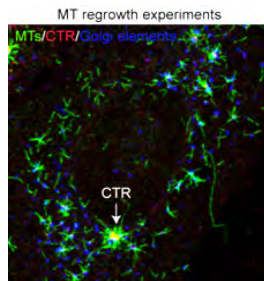
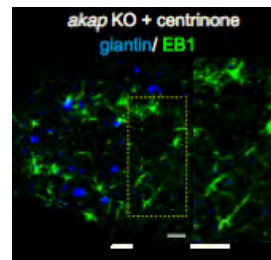


Figure 3: Defective cystogenesis in MDCKII cells lacking Junctional CAP350





*Figure 1: In most primary mammalian cell lines MT nucleation takes place at both the CTR and the GA*



*Figure 2: 3 min MT regrowth*

cells lacking centrosomes while removal of the drug triggers a wave of centriole over-duplication resulting in cells containing higher number of centrosomes.

By using these tools, we have been able to specifically modulate MT nucleation from either the centrosome or the GA and to analyze the consequences on MT network organization. We found that, contrarily to the most extended view, canonical  $\gamma$ -TuRC binding proteins are not essential for MT nucleation at the centrosome whereas they control MT nucleation at the GA. The absence of centrosomes strongly stimulated Golgi-based MT nucleation whereas amplification of the centrosome number inhibited it. Switching off MT nucleation at the Golgi did not affect centrosomal activity. Finally, inhibition of Golgi MT nucleation activity in centrosome-free cells (centrinone-treated AKAP450 KO cells) triggered the formation of numerous acentriolar cytoplasmic structures that actively nucleated MTs. Strikingly, when all MT nucleation was inhibited, individual MTs formed throughout the cytoplasm suggesting that MT assembly is a default property of mammalian cytoplasm. This data also supports that MT nucleation is a hierarchically regulated process with the centrosome located at the apex.

Fernando R. Balestra joined my lab in 2015 with a 2-years Marie Curie International European Fellowship contract. He searched for regulators of centrosome position by re-analyzing a collection of images from a siRNA functional screen in HeLa cells performed during his previous postdoctoral stay in Pierre Gonczy's lab (FR Balestra et al., Dev Cell 2013). He screened for candidates in which the centrosome was frequently positioned more than 3 microns away from the nuclear surface. He identified 52 genes potentially involved in centrosome positioning and validated a subgroup of these candidates. We selected for further characterization a group of genes that corresponds to proteins located to centriolar subdistal appendages including Ninein-like protein, CEP170 and CEP170B proteins. We uncover that centrosomal mislocalization by knock down of subdistal appendages proteins lead to nucleus mislocalization and migration defects (FR Balestra, F Arias, P Strnad, P Gonczy and RM Rios. A functional genomic screen identifies CEP170 as a centrosome and nucleus position regulator. In preparation).

During mitosis, astral MTs connect centrosomes at spindle poles with the cell cortex thus controlling spindle orientation. We recently discovered that CAP350, a MT-binding centrosomal protein, also localizes to cell-cell contacts by interacting with alpha-catenin. We reported that CAP350 organizes the apico-basal MT array characteristic of polarized epithelial cells allowing them to acquire a columnar morphology (Gavilan et al PLoS Biol. 2015). MDCKII kidney epithelial cells grown in matrigel develop tridimensional structures known as cysts that are composed by a single cell layer surrounding a central lumen. Proper cystogenesis depends on both plasma membrane polarization and mitotic spindle orientation. Knocking-down CAP350 by shRNA led to defective cystogenesis. We found that membrane polarity markers were well-localized in CAP350 depleted cysts. However, while in normal cysts mitotic spindle axis was found parallel to the basal surface thus allowing the cysts to grow properly, in cysts lacking CAP350 mitotic spindles appeared randomly oriented. We have found that CAP350 loss leads to spindle misorientation by interfering with the stability of astral MTs.

M Paz Gavilan was awarded in 2016 with a postdoctoral fellowship from the AECC to study the role of the Wnt/beta catenin signaling pathway in mitotic spindle orientation using colon cancer models. Wnt signaling promotes the proliferation of colon crypt cells and its deregulation is the main risk factor for colon cancer. We have analyzed Wnt proteins (beta-catenin, APC and axin) dynamics at the centrosome in transformed colon cell lines as well as spindle mitotic orientation in 3D models of colon cancer including organoids generated from cancer cell isolated from intestinal crypts of colon cancer patients.

## Publication Highlights

- Gavilan M.P.\*, Gandolfo P.\*, Balestra F.R., Arias F., Bornens M.Rios R.M. The Centrosome Controls the Number and Spatial Distribution of Microtubules by Negatively Regulating Alternative MTOCs. September 2017. BioRxiv. Ref 153304. doi: <https://doi.org/10.1101/153304> (under review)
- Gavilan M.P., Arjona M., Zurbano A., Formstecher E., Martinez-Morales J.R., Bornens M., Rios R.M. 2015. Alpha-catenin dependent recruitment of the centrosomal protein CAP350 to adherens junctions allows epithelial cells to acquire a columnar shape. PLoS Biology. 13(3):e1002087
- Rios R.M. 2014. The Centrosome-Golgi nexus. Phil Soc Trans B. (invited review) 5;369 (1650). pii: 20130462

## Grants

- 2016-2018: BFU2015-65747-P. Plan Nacional. MINECO
- 2014-2018: CTS 2071. Proyecto de Excelencia. Junta de Andalucía
- 2017-2018: BFU2016-81912-REDC Consolider Network. MINECO





## Cell Death Signalling

### Dr. Abelardo López Rivas

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GROUP LEADER



## Cell Dynamics and Signalling

### Group Members updated

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#### Postdoctoral

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Rocío Mora Molina

#### Technicians

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Inés Casares Ruiz

### Academic Background of PI

- 1976: Degree. University of Seville, B.Sc. in Biology
- 1980: PhD. University of Madrid, Doctor in Sciences

### Current Position

- Since 2006, Full Professor CSIC, Andalusian Center for Molecular Biology and Regenerative Medicine-CABIMER, Seville, Spain

### Positions Held

- 1980-1984: Postdoctoral Fellow, Imperial Cancer Research Fund, London, England
- 1984-1987: Postdoctoral Fellow, Molecular Biology Center Severo Ochoa, CSIC UAM, Madrid, Spain
- 1987-1990: Tenure Scientist CSIC, Institute of Biomedical Research Alberto Sols, Madrid, Spain
- 1990-1992: Research Scientist CSIC, Institute of Biomedical Research Alberto Sols, Madrid, Spain
- 1992-2003: Research Scientist CSIC, Institute of Parasitology and Biomedicine CSIC, Granada, Spain
- 2003-2004: Full Professor CSIC, Institute of Parasitology and Biomedicine CSIC, Granada, Spain
- 2004-2006: Full Professor CSIC, Andalusian Center for Developmental Biology, Seville, Spain

### Research Activity

#### OVERVIEW

High growth rate of cancer cells along with the poor vascularization of tumors result in stressful conditions in the tumor microenvironment, including low oxygen supply, lack of nutrients and pH changes, leading to metabolic stress. To survive this hostile environment a number of intracellular signaling pathways are activated in tumor cells to restore homeostasis, facilitating tumor growth. However, if the stress is prolonged or there is excessive stimulation of these signaling pathways, irreversible activation of the apoptotic machinery and thereby cell death will take place. A major goal of our research is to decipher the role of the TRAIL system and the apoptosis inhibitor FLIP in the decision between an adaptive response and cell death by apoptosis after metabolic stress generated during tumor growth. Within this general objective, we aim at defining the impact of extracellular matrix composition and stiffness in the response of tumor cells to metabolic stress.

#### RESEARCH HIGHLIGHTS

Truncated or mutant isoforms of the HER2/ERBB2 receptor tyrosine kinase found in a number of breast tumors show increased oncogenicity compared to the wild-type receptor. Transformation of human breast epithelial cells by the p95HER2/611CTF oncogene markedly sensitizes these cells to metabolic stress induced by the simultaneous inhibition of glucose and glutamine metabolism. In p95HER2/611CTF-transformed cells, metabolic stress activates a TNF related apoptosis-inducing ligand (TRAIL)-R and caspase-8-dependent apoptotic process that requires prior down-regulation of cellular FLICE-like inhibitor protein (c-FLIP) levels. Importantly, sustained mTOR activation is involved in FLIP down-regulation and apoptosis induced by metabolic stress. In vivo experiments in immunodeficient mice demonstrate a requirement for caspase-8 in restraining primary tumor growth of xenografts with p95HER2/611CTF-transformed cells. Collectively, these data define a critical role of the extrinsic pathway of apoptosis in the control of tumor initiation by microenvironmental cues.



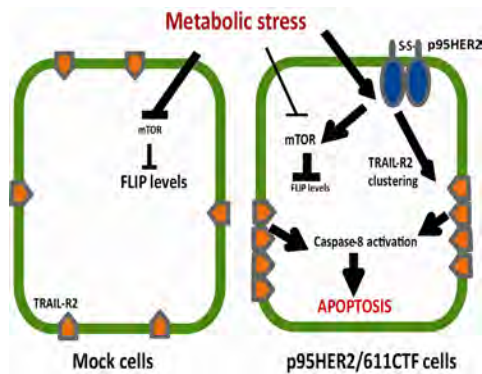


Figure 1: Schematic overview of the differential response to metabolic stress in normal and p95HER2/611CTF-transformed cells

Recent evidences indicate that triple-negative breast cancer (TNBC) cells with a mesenchymal phenotype show a basal activation of the unfolded protein response (UPR) that increases their sensitivity to endoplasmic reticulum (ER) stress although the underlying cell death mechanism remained largely unexplored. We have demonstrated that both caspase-8-dependent and -independent apoptotic mechanisms are activated in TNBC cells undergoing sustained endoplasmic reticulum (ER) stress. Activation of the extrinsic apoptotic pathway by ER stress involves ATF4-dependent up-regulation of tumor necrosis factor-related apoptosis-inducing ligand receptor 2 (TRAIL-R2/DR5). In addition, accumulation of BH3-only protein Noxa at the mitochondria further contributes to apoptosis following ER stress in TNBC cells. Accordingly, simultaneous abrogation of both extrinsic and intrinsic apoptotic pathways is required to inhibit ER stress-induced apoptosis in these cells. Importantly, persistent FLICE-inhibitory protein (FLIP) expression plays an adaptive role to prevent early activation of the extrinsic pathway of apoptosis upon ER stress. Overall, our data show that ER stress induces cell death through a pleiotropic mechanism in TNBC cells and suggest that targeting FLIP expression may be an effective approach to sensitize these tumor cells to ER stress-inducing agents.

Glutamine plays an important role in the metabolism of tumor cells through its contribution to redox homeostasis, bioenergetics, synthesis of macromolecules and signaling. Triple-negative breast cancers (TNBC) are highly metastatic and associated with poor prognosis. TNBC cells show a marked dependence on extracellular glutamine for growth. We have demonstrated that TNBC cells are markedly sensitized to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis upon glutamine deprivation. Up-regulation of pro-apoptotic TRAIL receptor 2 (TRAIL-R2/DR5) and down-regulation of FLICE-inhibitory protein (FLIP) are observed in glutamine-deprived TNBC cells. Activation of the amino acid-sensing kinase general control nonderepressible 2 (GCN2) upon glutamine deprivation is responsible for TRAIL-R2 up-regulation through a signaling pathway involving ATF4 and CHOP transcription factors. In contrast, FLIP down-regulation in glutamine-deprived TNBC occurs by a GCN2-independent mechanism. Importantly, silencing cFLIP expression by RNA interference results in a marked sensitization of TNBC cells to TRAIL-induced apoptosis. In addition, pharmacological or genetic inhibition of transaminases increases TRAIL-R2 expression and down-regulates FLIP levels, sensitizing TNBC cells to TRAIL. Interestingly, treatment with L-asparaginase markedly sensitizes TNBC cells to TRAIL through its glutaminase activity. Overall, our findings suggest that targeting the glutamine addiction phenotype of TNBC can be regarded as a potential antitumoral target in combination with agonists of proapoptotic TRAIL receptors.

## Publication Highlights

- Cano-González A., Mauro-Lizcano M., Iglesias-Serret D., Gil J., López-Rivas A. 2017. Involvement of both caspase-8 and Noxa-activated pathways in ER stress-induced apoptosis in triple-negative breast tumor cells. *Cell Death and Disease*. 9(2):134
- Martín-Pérez R., Yebes R., Mora-Molina R., Cano-González A., Arribas J., Mazzone M., López-Rivas A., Palacios C. 2017. Oncogenic p95Her2/611CTF primes human breast epithelial cells for metabolic stress-induced activation of TRAIL-R/Caspase-8-dependent apoptosis. *Oncotarget*. 8:93688-93703
- Cano-González A., López-Rivas A. 2016. Opposing roles of TGF- $\beta$  and EGF in the regulation of TRAIL-induced apoptosis in human breast epithelial cells. *BBA Mol Cell Res*. 1863:2104-2114
- Sánchez-Pérez T., Medema R.H., López-Rivas A. 2015. Delaying mitotic exit down-regulates FLIP expression and strongly sensitizes tumor cells to TRAIL. *Oncogene*. 34:661-669
- Martín-Pérez R., Palacios\* C., Yebes\* R., Cano-González A., Iglesias-Serret D., Gil J., Reginato M.J., López-Rivas A. (\*equal contribution). 2014. Activated ERBB2/HER2 licenses sensitivity to apoptosis upon endoplasmic reticulum stress through a PERK-dependent pathway. *Cancer Research*. 74:1766-1777

## Grants

2013-2017: RD12/0036/0026. Instituto de Salud Carlos III

2014-2018: BIO 778. Proyecto de Excelencia de la Junta de Andalucía

2016-2018: SAF2015-64383-P, Ministerio de Economía y Competitividad

2017-2018: CB16/12/00421, Centro de Investigación Biomédica en Red (CIBERONC). Instituto de Salud Carlos III

2016-2017: BFU2015-71869-REDT. Red de Excelencia para el estudio de la Autofagia. Ministerio de Economía y Competitividad





## Cell Cycle and Oncogenesis

**Dr. José A. Pintor Toro**

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GROUP LEADER



### Group Members updated

#### PhD Student

Cristina Rodríguez Mateo

#### Technician

Belén Torres Agrela

### Academic Background of PI

- 1976: Degree. University of Seville, B.Sc. in Biology
- 1980: PhD. University of Madrid, PhD in Sciences

### Current Position

- Since 1986: Professor CSIC, Andalusian Center for Molecular Biology and Regenerative Medicine-CABIMER, Seville, Spain

### Positions Held

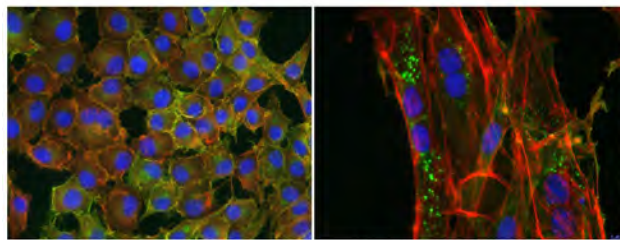
- 1984-1986: Assistant Professor of Biochemistry. Polytechnic University. Madrid, Spain
- 1986-2006: Research Scientist. IRNASE. CSIC. Seville, Spain
- Since 2006: Full Professor. CABIMER/CSIC. Seville, Spain

### Research Activity

#### OVERVIEW

transcription factors and miRNAs have been identified as regulatory molecules at the early stages of the epithelial-mesenchymal transition (EMT). Using NMuMG mouse cells that show a very clear epithelial-mesenchymal transition after treatment with TGF- $\beta$ , we have identified, through transcriptomic analysis, lncRNAs that transcriptionally regulate early EMT, and also lncRNAs, identified by the analysis of RNA-Argonata2 complexes (RNA-AGO2), that regulate post-transcriptionally this process. We are studying the mechanism of action of two new lncRNAs, lnc-Nr6a1 and lnc-Klhl33, that are induced during the first two hours of the onset of EMT. They are associated with chromatin and their regulation is independent of de novo synthesis of proteins. We will study the effects upon the cellular phenotype and the transcriptomic profiles of both their expression and depletion, also the genome regions that interact with these lncRNAs and, given the conservation in the human genome, their expression in other EMT models and in human tumors. On the other hand, the RNAs post-transcriptionally most affected in the early EMT are the lnc-RNA Malat1 and the mRNA Serpine1, which were identified by iCLIP technique. Malat1 interacts throughout its sequence with the AGO2 protein, the catalytic component of the RISC silencing complex, and the number of Malat1-AGO2 complexes decreases rapidly upon initiation of EMT, suggesting a possible role of Malat1 as a reservoir of numerous and different miRNAs, which would be released at the onset of EMT, blocking the translation of specific epithelial mRNAs. The study of the Serpine1 gene is of great interest; on the one hand, this gene is transcriptionally regulated, increasing rapidly its transcript levels at the onset of EMT; but, on the other hand, it is simultaneously post-transcriptionally regulated, interacting with AGO2, blocking paradoxically its translation. Serpine1 mRNA shows specific interaction sites with AGO2, which correspond to sites recognized by certain miRNAs; we want to demonstrate that these





Control cells

Lnc-Riken6-depleted cells

***Lnc-Riken6-depleted cells are binucleated cells  
and show a mesenchymal phenotype***

miRNAs would be sequestered by the Serpine1 mRNA, functionally unblocking other transcripts important for the epithelial-mesenchymal transition. This paradoxical would mean that the early regulation of EMT by Serpine1 would be carried out exclusively by the mRNA molecule regardless of its coding function. This raises the possibility that Serpine1 mRNA could be a promising new therapeutic target instead its encoded protein. We propose to determine the Serpine1 mRNA-interacting miRNAs and the affected transcripts, the phenotypic and transcriptional effects of Serpine1 depletion and overexpression of different mutated constructs of this gene. Also the expression of Serpine1 and the sequestered miRNAs will be analyzed in other EMT models and in tumors with different degrees of invasiveness. It should be stressed that in the EMT process, we have identified other coding RNAs that, like Serpine1, are regulated early transcriptional and post-transcriptionally, thus questioning some of the current therapeutic strategies.

## RESEARCH HIGHLIGHTS

It has been generated a new platform for exploring those small RNAs that act as tumour suppressors. This new approach for generating lentiviral libraries is based on microRNA depletion by sequestration using small RNAs sponges. For this purpose, we used standard procedures for the cloning of small RNAs followed by an amplification step using phi29 DNA polymerase to obtain several targets for a particular small RNA for each sponge.

A TGF- $\beta$ -downregulated long non-coding RNA, Inc-Spy1, has been identified as an immediate early regulator of EMT. Knockdown of Inc-Spy1 promotes a mesenchymal-like phenotype and results in increased cell migration and invasion. Lnc-Spy1 depletion preferentially affects the expression of TGF- $\beta$ -regulated gene targets. Also, Inc-Spy1 associates with U2AF65 and PUF60 splicing factors, suggesting a role in alternative splicing. Depletion of Inc-Spy1 induced, as TGF- $\beta$ , isoform switching of fibroblast growth factor receptors, resulting in FGF-2-sensitive cells. Taken together, these results show that Inc-Spy1 is an early mediator of TGF- $\beta$  signalling and reveal different roles for a lncRNA in modulating transcriptional and post-transcriptional gene expression

By transcriptome and Argonaute iCLIP analysis we suggest that some protein-coding genes differentially expressed in EMT might contribute to this process acting both as protein-coding genes and non-coding RNAs. Such dual-function regulation of gene expression networks would reflect a new complexity level of regulation by the coding genome

## Publication Highlights

- Rodríguez-Mateo C., Torres B., Gutiérrez G., Pintor-Toro J.A. 2017. Downregulation of Lnc-Spy1 mediates TGF- $\beta$ -induced epithelial-mesenchymal transition by transcriptional and posttranscriptional regulatory mechanisms. *Cell Death Differ.* 24(5):785-797
- Méndez-Vidal C., del Mar Gámez-Del Estal M., Moreno-Mateos M.A., Espina-Zambrano Á.G., Torres-Agrela B., Pintor-Toro J.A. 2013. PTTG2 silencing results in induction of epithelial-to-mesenchymal transition and apoptosis. *Cell Death Dis.* 4:e530
- Moreno-Mateos M.A., Barragán V., Torres B., Méndez-Vidal C., Mudduluru G., Allgayer H., Pintor-Toro J. A. 2013. Novel small RNA expression libraries uncover hsa-miR-30b and hsa-miR-30c as key factors in anoikis resistance. *RNA.* 19:1711-1725
- Muñoz-Mayor A., Pineda B., García-Abellán J.O., Antón T., García-Sogo B., Sanchez-Bel P., Flores F.B., Atarés A., Angosto T., Pintor-Toro J. A., Moreno V., Bolarin M.C. 2012. Overexpression of dehydrin tas14 gene improves the osmotic stress imposed by drought and salinity in tomato. *J Plant Physiol.* 169:459-468
- Moreno-Mateos M. A., Espina Á.G., Torres B., Gámez del Estal M.M., Romero-Franco A., Ríos R.M., Pintor-Toro J.A. 2011. PTTG1/securin modulates microtubule nucleation and cell migration. *Mol Biol Cell.* 22:4302-4311

## Grants

2015-2017: SAF2014-57632-P. Ministerio de Ciencia e Innovación





Cell Division  
Control

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GROUP LEADER



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Ana Isabel de los Santos Velázquez

#### Technician

José Carlos Blanco Mira

### Academic Background of PI

- 1998: Bachelor's Degree in Science (Biochemistry). University of Córdoba (Spain)
- 2003: Ph.D. in Biochemistry. University of Córdoba (Spain)

### Current Position

Staff Scientist. Spanish National Research Council (CSIC)

### Positions Held

- 2016-present: Staff Scientist. Spanish National Research Council (CSIC)
- 2013-2016: Researcher. University of Seville (Spain)
- 2008-2013: "Ramón y Cajal" Research Fellow. University of Seville (Spain)
- 2003-2008: Postdoctoral Research Fellow. MIT (USA)

### Research Activity

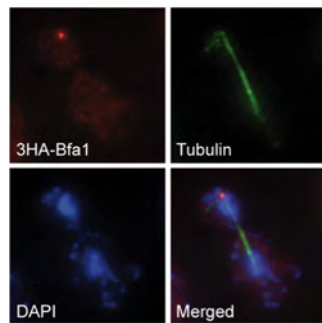
#### OVERVIEW

The research in our group aims to better understand how cells divide and how the proper distribution of the genome is monitored during the process of cell division in order to maintain a correct cellular ploidy. We are particularly interested in unveiling the signalling pathways that orchestrate cell cycle progression, as well as in deciphering the molecular mechanisms by which the main cellular checkpoints regulate cell division to ensure the fidelity of chromosome segregation. Problems with the distribution of the genetic material during mitosis can give rise to aneuploidy, an alteration of the normal number of chromosomes in the cell that is a hallmark of cancer and a number of different genetic diseases. A deeper knowledge about the regulation of cell division is therefore essential to shed light on the mechanisms that underlay these diseases.

#### RESEARCH HIGHLIGHTS

The cells have developed a number of surveillance mechanisms to verify the integrity of the genetic material and its proper distribution during cell division, such as the DNA damage checkpoint (DDC), which detects lesions in the DNA and promotes their repair, or the spindle assembly checkpoint (SAC), which ensures that every chromosome has been attached to the mitotic spindle. In asymmetric cell divisions, additional surveillance mechanisms also control the orientation of the spindle. This was first found in the yeast *Saccharomyces cerevisiae*. In this organism, the spindle position checkpoint (SPOC) avoids that cells with a misaligned spindle exit from mitosis, thereby ensuring an equal partitioning of the chromosomes between the mother and the newly-generated daughter cell. Remarkably, in *S. cerevisiae* cells, and although the DDC, the SAC and the SPOC are triggered by different signals and in different stages of the cell cycle, all these checkpoints promote the inhibition of mitotic exit, which highlights the importance of the regulation of this cell cycle transition. In this organism, exit from mitosis is facilitated by the Mitotic Exit Network (MEN), a signalling pathway that is initiated by the Tem1 GTPase. MEN signalling is blocked by the DDC, the SAC and the SPOC through the activation of Bfa1/Bub2, two proteins that together inhibit Tem1 activity. Once that the previous mitotic checkpoints are satisfied, activation of the MEN during anaphase determines the release of the Cdc14 phosphatase from the nucleolus, where it is normally sequestered, which promotes mitotic spindle disassembly, chromosome de-condensation and cytokinesis through the inhibition of mitotic CDK activity.





**Figure 1:**  
Asymmetric localization of Bfa1 during anaphase.  
Immunofluorescence image displaying the localization of a 3HA-tagged version of the MEN inhibitor Bfa1 to the spindle pole body that enters the daughter cell during anaphase (3HA-Bfa1, in red). The spindle (tubulin, in green) and the nucleus (DAPI, in blue), as well as a merged image, are also shown as a reference.

A main focus of our lab is to understand how mitotic exit is regulated, both during a normal cell cycle and after the activation of the mitotic checkpoints. During the 2016-2017 period, we have made important contributions to shed light on this aspect of the regulation of cell division. As such, we have recently demonstrated that a precise temporal control of the compaction of the nucleolus is essential in order to ensure the equal distribution of chromosomes during mitosis without interfering with cell cycle progression. Besides its critical role during ribosome biogenesis, the nucleolus plays pivotal functions in many other key cellular processes, being the nucleolar sequestration of the Cdc14 phosphatase a paradigmatic example. The peculiar structure of the nucleolus, however, has also its drawbacks. The repetitive nature of the rDNA gives rise to cohesion-independent linkages whose resolution in budding yeast requires the Cdc14-dependent inhibition of rRNA transcription, which facilitates condensin accessibility to this locus. Thus, the rDNA condenses and segregates later than most other yeast genomic regions. We have recently shown that the defective function of a small nucleolar ribonucleoprotein particle (snoRNP) assembly factor facilitates condensin accessibility to the rDNA and induces nucleolar hyper-condensation. Interestingly, this increased compaction of the nucleolus interferes with the proper release of Cdc14 from this organelle. This observation provides an explanation for the delayed rDNA condensation in budding yeast, which is necessary to efficiently coordinate timely Cdc14 release and mitotic exit with nucleolar compaction and segregation. Finally, our work further suggests that cells could use the degree of nucleolar compaction as a way to restrain cell cycle progression under adverse growth conditions, such as starvation.

Our group is also extremely interested in the analysis of the mechanisms by which the cell generates asymmetry during cell division. In higher eukaryotes, the stereotypical example of asymmetric cell divisions are stem cells. The asymmetric division of stem cells is a key mechanism to sustain their correct number in the different tissues. A reduction in the population of stem cells causes tissue disorganization, degeneration and aging. Conversely, an excessive stem cell number determines tissue hyperplasia and can contribute to the development of cancer. New insights into the mechanisms that regulate asymmetric cell divisions are thus extremely important to understand the origin of these problems. Remarkably, *S. cerevisiae* is an ideal model where to study how asymmetry can be established during cell division. In this organism, due to its particular pattern of division by budding, each cell division is intrinsically asymmetric, and the newly generated daughter cell differs from its mother in size, cellular composition, and replicative life span. Asymmetric cell divisions require the polarization of the cell along a predetermined axis, followed by the orientation of the mitotic spindle along this polarity axis and perpendicular to the division plane, this way ensuring the asymmetric distribution of polarization factors between the mother and the newly-generated cell. Interestingly, the mitotic spindle is, in fact, an asymmetric structure in nature, especially at the level of the microtubule-organizing centers (MTOCs), from which the microtubules that constitute the spindle emanate. In our laboratory, and during the 2016-2017 period, we have evaluated the role of spindle-associated asymmetries in the regulation of cell cycle progression, as well as the consequences that the problems during the establishment of these asymmetries determine on cell division, differentiation or aging. As such, we have identified a new factor that is necessary for the establishment of the non-random inheritance pattern of MTOCs between the mother and the daughter cell in budding yeast. Furthermore, and excitingly, we have also generated a system to evaluate the functional consequences of the reversal of this pre-established asymmetric MTOC inheritance pattern. Unveiling the functional significance of the differential MTOC distribution patterns in cell division could help us in the future to better understand pathologies that may arise as a result of problems with the establishment of this asymmetry.

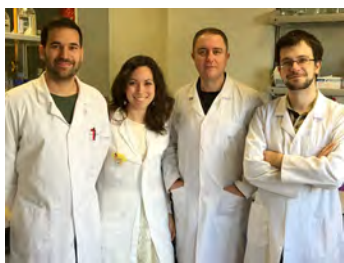
### Publication Highlights

- de los Santos-Velázquez A.I., de Oya I.G., Manzano-López J., Monje-Casas F. 2017. Late rDNA condensation ensures timely Cdc14 release and coordination of mitotic exit signaling with nucleolar segregation. *Current Biology*. 27(21):3248-3263
- Muñoz-Barrera M., Aguilar I., Monje-Casas F. 2015. Dispensability of the SAC depends on the time window required by Aurora B to ensure chromosome biorientation. *PLoS One* (2015). 10(12):e0144972
- Muñoz-Barrera M., Monje-Casas F. 2014. Increased Aurora B activity causes continuous disruption of kinetochore-microtubule attachments and spindle instability. *Proc Natl Acad Sci USA*. 111(38):E3996-E4005
- Valerio-Santiago M., de los Santos-Velázquez A.I. Monje-Casas F. 2013. Inhibition of the Mitotic Exit Network in response to damaged telomeres. *PLoS Genetics*. 9(10):e1003859
- Valerio-Santiago M., Monje-Casas F. 2011. Tem1 localization to the spindle pole bodies is essential for mitotic exit and impairs spindle checkpoint function. *The Journal of Cell Biology*. 192(4):599-614

### Grants

2017-2019: BFU2016-76642-P. Ministerio de Economía y Competitividad  
2014-2016: BFU2013-43718-P. Ministerio de Economía y Competitividad





## Cell Differentiation Laboratory

**Dr. Mario García-Domínguez**

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GROUP LEADER



### Group Members updated

#### PhD Students

Francisco de Asís Gallardo Chamizo

Noelia Luna Peláez

Juan Francisco Correa Vázquez

#### Postdoc

María Ceballos Chávez

### Academic Background of PI

- 1993: Degree. University of Seville, B.Sc. in Biology
- 1999: PhD. University of Seville, Biology

### Current Position

- Since 2009: Research Scientist CSIC/Cabimer, Seville, Spain

### Positions Held

- 2003-2008: Ramón y Cajal Investigator, IBVF and Cabimer, Seville, Spain
- 2001-2003: Marie Curie postdoctoral fellow, Ecole Normale Supérieure, Paris, France
- 1999-2001: FEBS postdoctoral fellow, Ecole Normale Supérieure, Paris, France
- 1994-1997: PhD student, FPU fellowship, IBVF, Seville, Spain

### Research Activity

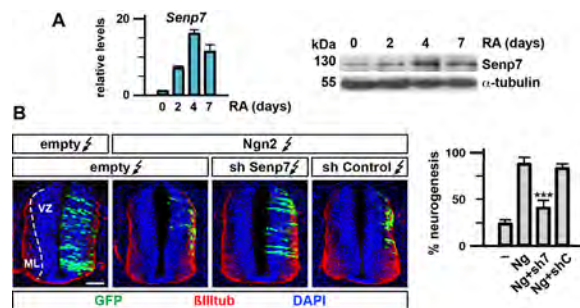
#### OVERVIEW

Main objective of our research consists in deciphering the mechanisms involved in neuronal differentiation, especially in the transition from proliferation to differentiation. In the central nervous system, neuronal progenitors exit the cell cycle to differentiate into neurons along development. In this context, we investigate two regulatory systems. One is concerning the cell cycle-associated chromatin adaptors from the BET family, and the other is related to the post-translational modification of proteins by covalent attachment of the Sumo polypeptide. BET proteins (Brd2, Brd3, Brd4 and Brdt in mammals) are bromodomain-containing proteins able to recognize acetylated histones in the chromatin. They are transcriptional activators classically associated to proliferation, although recent reports suggest also a role in differentiation. Sumo attachment to proteins is essential in eukaryotes and is involved in regulating many cellular processes, especially transcription.

#### RESEARCH HIGHLIGHTS

Sumo polypeptide is quite similar to the Ubiquitin and its attachment to proteins has a severe impact in properties of these. The conjugating enzyme Ubc9 is the responsible of the transfer to target proteins, while Sumo ligases by enhancing sumoylation of specific targets and Sumo proteases by excising Sumo from targets are the main regulators of the process. In this context, we have observed that the Sumo protease Senp7 is strongly induced under neuronal differentiation conditions, being required for proper progression of neurogenesis (Figure 1). Different results indicate that sumoylation is involved in development of the nervous system. However, very little is known about the involvement of Sumo in the initial steps of neurogenesis. Thus, we recently performed a SILAC-based proteomic approach to identify tens of proteins differentially





**Figure 1.** The Sumo protease Senp7 is induced and required during neurogenesis. **A)** Senp7 is induced at day 4 of neuronal differentiation with retinoic acid (RA), at mRNA (left) and protein (right) levels (tubulin: loading control). **B)** Electroporation of embryo spinal cord with the neurogenic factor Neurogenin 2 (Ngn2, Ng) leads to margin localization and  $\beta$ III-Tubulin expression (red) of electroporated cells (green), indicative of differentiation. Downregulation of Senp7 by shRNA (sh7) abrogates differentiation, compared with control shRNA (shC) (DAPI: nuclei, \*\*\*  $p < 0.001$ , Student t-test sh7 vs. shC). Scale bar 50  $\mu$ m.

sumoylated under proliferation versus neuronal differentiation conditions. Therefore, the molecular analysis and functional characterization of these Sumo modifications in the transition from proliferation to neuronal differentiation will contribute to depict the landscape of Sumo contribution to the control of neurogenesis. Besides this, it was recently reported that enhancing sumoylation reduces cell death after ischemia. In this sense, we have also initiated a proteomic approach to identify changes in protein sumoylation after simulated ischemia conditions in vitro, what will contribute to select putative targets for therapeutic intervention.

Overexpression of BET proteins is associated to many types of cancers, and antagonizing its binding to the chromatin by drugs mimicking acetylated histones have been successfully used to alleviate a variety of cancers in animal models. Therefore, there is a great interest in unravel the mechanisms accounting for BET protein association to the chromatin. Although BET proteins have been unambiguously involved in cell cycle progression and their aberrant expression clearly associates with cancer, several observations strongly suggest the involvement of BET proteins in differentiation processes as well. Brd2 is the family member more clearly implicated in differentiation. Though we have previously reported that induction of Pleiotrophin during neuronal differentiation enhances neurogenesis by antagonizing Brd2, others and we predict active roles for Brd2 during differentiation processes. BET proteins are linked to active chromatin, as they associate to acetylated histones. Thus, it is reasonable to assume that they associate with cell cycle genes during proliferation while they associate with a different subset of active genes during differentiation. To shed light on these aspects we investigate Brd2 distribution along the genome under proliferation and neuronal differentiation conditions, to compare it with relevant histone modifications. In addition, we identified a variety of Brd2 partners from a two-hybrid screening, which are being analyzed in the context of Brd2 function and genome association under proliferation and differentiation conditions. One of the identified Brd2 partners, the Lys transcription factor, participates in recruitment of Brd2 to the chromatin assuring proper downregulation timing of key pluripotency factors following induction of differentiation.

## Publication Highlights

- Juárez-Vicente F., Luna-Peláez N., García-Domínguez M. 2016. The Sumo protease Senp7 is required for proper neuronal differentiation. *Biochim Biophys Acta*. 1863:1490-1498
- de Frutos C.A., Bouvier G., Arai Y., Thion M.S., Lokmane L., Keita M., García-Domínguez M., Charnay P., Hirata T., Riethmacher D., Grove E.A., Tissir F., Casado M., Pierani A., Garel S. 2016. Reallocation of olfactory Cajal-Retzius cells shapes neocortex architecture. *Neuron*. 92: 435-448
- García-Gutiérrez P., Juárez-Vicente F., Wolgemuth D.J., García-Domínguez M. 2014. Pleiotrophin antagonizes Bromodomain-containing protein 2 (Brd2) during neuronal differentiation. *J Cell Sci*. 127:2554-2564
- Ceballos-Chávez M., Rivero S., García-Gutiérrez P., Rodríguez-Paredes M., García-Domínguez M., Bhattacharya S.S., Reyes J.C. 2012. Control of neuronal differentiation by Braf35 sumoylation. *Proc Natl Acad Sci USA*. 109: 8085-8090
- García-Gutiérrez P., Mundi M., García-Domínguez M. 2012. Association of bromodomain BET proteins to the chromatin requires dimerization through the conserved motif B. *J Cell Sci*. 125:3671–3680

## Grants

2016-2018: BFU2015-64721-P/BFI. Ministerio de Economía y Competitividad

2014-2017: P12-CTS-2064. Proyectos de Excelencia. Consejería de Economía, Innovación, Ciencia y Empleo, Junta de Andalucía





## Cellular Therapy of the Diabetes Mellitus and its Complications

**Dr. Bernat Soria**

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HEAD OF THE REGENERATION AND  
CELL THERAPY DEPARTMENT



### Group Members updated

**Senior Researcher**  
Karim Hmadcha

**Postdoctorals**  
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Christian Lachaud  
Emilio Javier López  
Natalia Escacena

**PhD Student**  
Mehrdad Vakilian

**GMP Technicians**  
Victoria Eugenia Jimenez  
Maria Gálvez

### Academic Background of PI

- 1974: Degree. University of Valencia, Medical Doctor
- 1978: PhD. University of Valencia

### Current Position

- 2017: Chairman of the new Department of Regeneration and Advanced Therapies of CABIMER and Scientific Coordinator of GMP-Facilities

### Positions Held

- 1982-1984: Adjunct Professor of Biochemistry and Physiology, School of Medicine, Valencia University, Valencia
- 1984-1985: Adjunct Professor of Biochemistry and Physiology, School of Medicine, Alicante University, Valencia
- 1986-2005: Full Professor of Physiology, Valencia
- 2005-2007: Director of CABIMER, Seville, Spain
- 2005-2007: Extraordinary Professor of Regenerative Medicine, Pablo de Olavide University, Seville, Spain
- 1979-1982: Former positions at Max Planck Institut (Göttingen)
- 1979-1980: Senior Research Associate Dept. of Biophysics, School of Biological Sciences, University of East Anglia, Norwich

### Research Activity

#### OVERVIEW

The "Cellular Therapy of the Diabetes Mellitus and its Complications" started his activity in CABIMER in January 2006, with a special interests in: i) understanding the molecular and cellular basis of pluripotency and differentiation of stem cells, ii) designing new protocols to expand and differentiate stem cells and iii) promoting clinical trials under European Regulations. During this period this group has published more than 60 papers, filed 10 patents (3 of them licensed) and promoted 4 Clinical Trials. In addition to Basic and Clinical Research the group offers technical and scientific support to boost several clinical trials through the GMP unit of CABIMER is engaged in the scale-up of human cells considered as Advanced Therapy Medicinal Products. The Mission of the group consists in improving Stem Cell basic and preclinical research on stem cell biology to foster breakthroughs in Advanced Therapies and Regenerative Medicine.



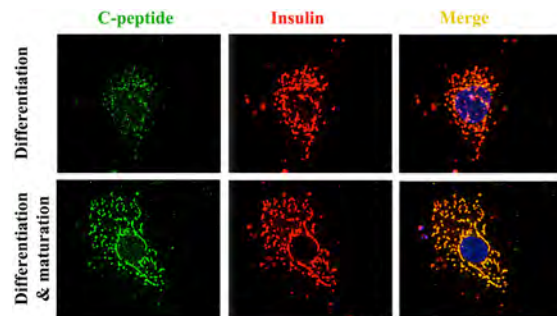


Figure 1. Effect of late maturation on hESCs-derived insulin-secreting cells (Soria B. et al. *Expt Opin. Biol. Ther.* 2015)

## RESEARCH HIGHLIGHTS

**1. Insulin producing cells from human embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC):** **A.** Describing and patenting a new xeno-free and human components free culture media which may be used both in embryonic and adult stem cells (Patent EP 15382417.2 (05-08-2015) ); **B.** New protocols to obtain mature beta cells from embryonic and iPSC cells (Stem Cells Int. 2014:379678. PLoS One. 2015 Mar 16;10(3):e0119904, Patent PCT/EP2015/070501 (08-09-2015) we have committed ourselves to have a CABIMER-protocol to obtain mature clinical grade pancreatic beta cells that may be used in clinical trials (P2011070165 (20-11-2012)); **C.** Pancreatic islet physiology: Diabetes. 2013 Jan;62(1):18-21; Islets. 2014;6(5-6):e995997

**2. Basic Research on Stem Cells: Embryonic and induced Pluripotent Stem cells, Adult Progenitors, Mesenchymal Stromal Cells, Liver Stellate cells and Mesothelial Cells.** Most of the effort has been devoted to keep human embryonic stem cell lines, derive new cell lines and generate protocols that provide mature and differentiated beta cells. **A.** New human embryonic stem cell lines have been derived from healthy and monogenetic disease embryos and the molecular basis of pluripotency explored (Cell Death Dis. 2013 Apr 4;4:e570); **B.** Role of Ionic channels in embryonic stem cell membranes J Membr Biol. 2015, 45(8): 2324-34; J Membr Biol. 2013 Mar;246(3):215-30; **C.** Lessons from islet development: i. In collaboration with the group of Dr Gauthier an expandable Pax-4 positive population has been described (Sci Rep 5: 15672, 2015) or ii. In collaboration with Dr Rojas it was described the role of GATA4 and GATA6 in beta cell development. (J Clin Invest 122(10): 3504-3515, 2012; Cell Mol Life Sci. 2014 71(13):2383-402) and more recently the tentative role of GATA 6 in type 2 diabetes is being explored; **D.** Liver Stellate cells. A close correlation between liver stellate cells GATA-decrease and liver fibrosis has been described (Hepatology 59(6): 2358-70, 2014; Patent: P201330636 (30-04-2013); **E.** Mesothelial cells. We have explored whether mesothelial cells may produce vessels (PLoS One. 2013;8(2):e55181; Cell Death Dis. 5: e1304, 2014) or corneal endothelium (Invest Ophthalmol Vis Sci. 55(9): 5967-78, 2014, Patent EP 13382213.0 (05-06-2013). PCT/EP2014/061746(01/08/2014)

**3. GMP Regulations, Clinical Research and Cell therapy.** **A.** Molecular basis of adverse reactions: describing the safety and efficacy pattern in the use of mesenchymal cells (Diabetes 62(12): 4266-9, 2013; Patent EP 13382315.3 (01-08-2013) PCT/EP2014/066600 (01-08-2014); **B.** Use of Cord Blood derivatives, such as cord plasma factors (Eur J Immunol. 2015 Aug; 45(8):2324-34 EP 14382348.2 (16/09/2014) or NK cells (PLoS ONE 11/2015; 10(10). DOI:10.1371; Patent EP 15382429.2); **C.** Regulatory Framework: Stem Cells Dev. 23(10): 1074-83, 2014, Br Med Bull 105: 85-105, 2013); **D.** Clinical Trials Our group both expanded mesenchymal stromal cells and coordinated several Clinical Trials (NCT00872326; NCT01079403; NCT00987363; NCT01056471) using autologous mesenchymal stromal cells to avoid leg amputation in diabetic patients (Cell Transpl 20(10): 1329-1639, 2011).

## Publication Highlights

- Soria B., Montanya E., Martín F., Hmadcha A. 2016. A Role for the Host in the Roadmap to Diabetes Stem Cell Therapy. Diabetes. 65(5):1155-7
- Acosta L., Hmadcha A., Escacena N., Pérez-Camacho I., de la Cuesta A., Ruiz-Salmeron R., Gauthier B.R., Soria B. 2013. Adipose mesenchymal stromal cells isolated from type 2 diabetic patients display reduced fibrinolytic activity. Diabetes. 62(12):4266-9
- Soria B., Gauthier B.R. 2013. Dual Trade of Bcl-2 and Bcl-xL in islet physiology: balancing life and death with metabolism secretion coupling. Diabetes; 62(1):18-21
- Calvanese V., Lara E., Suárez-Alvarez B., Abu Dawud R., Vázquez-Chantada M., Martínez-Chantar M.L., Embade N., López-Nieva P., Horrillo A., Hmadcha A., Soria B., Piazzolla D., Herranz D., Serrano M., Mato J.M., Andrews P.W., López-Larrea C., Esteller M., Fraga M.F. 2010. Sirtuin 1 regulation of developmental genes during differentiation of stem cells. Proc Natl Acad Sci U S A. 107(31):13736-41
- Soria B., Roche E., Berná G., León-Quinto T., Reig J.A., Martín F. 2000. Insulin-secreting cells derived from embryonic stem cells normalize glycemia in streptozotocin-induced diabetic mice. Diabetes. 49(2):157-62

## Grants

Ministerio de Economía y Competitividad/Ministerio de Sanidad/Consejería de Economía, Innovación y Ciencia y Consejería de Salud (Junta de Andalucía). European Union (BIOREG-SO3/P1/E750/ COST-A FACTT BM 1305)





## Pancreatic Islets and Stem Cell

**Dr. Franz Martín**

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GROUP LEADER



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#### Research associates

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#### Postdoctoral

Lourdes María Varela Pérez

#### PhD Student

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#### Technicians

Raquel Araujo

Antonio Cárdenas

Amparo Luque

### Academic Background of PI

- 1986: Degree. University of Seville, B.Sc. in Medicine and Surgery
- 1994: PhD. University of Seville, PhD in Medicine and Surgery

### Current Position

- Since 2006: Research Scientist CABIMER/Seville, Spain
- Since 2007: Full Professor at University Pablo de Olavide, Seville, Spain

### Positions Held

- 1991: Postdoctoral Fellowship, Joslin Diabetes Center, Harvard University
- 1992-1993: Visiting Professor, Department of Physiology, University of Alicante, Valencia
- 1993-1997: Lecturer, Department of Physiology, University of Alicante, Valencia
- 1997-2005: Lecturer, Department of Applied Biology, University Miguel Hernández, Alicante, Valencia
- 2005-2007: Lecturer, Department of Molecular Biology and Biochemistry Engineering, University Pablo Olavide, Seville, Spain

### Research Activity

#### OVERVIEW

Our main research lines are: i) to study the role of nutrients and diets in the pathogenesis of diabetes, obesity, diabetes, metabolic syndrome and non-alcoholic fatty liver disease and ii) the development of differentiation protocols, from embryonic, induced pluripotent and adult stem cells, to insulin producing cells, as well as, their employment in diabetes cell therapy.

#### RESEARCH HIGHLIGHTS

Our main research highlights are.

- 1.Excess dietary fat has been implicated in the development and progression of non-alcoholic fatty liver disease (NAFLD) and may be an important modifiable factor involved in the development and progression of NAFLD and NASH. We have found that dietary extra virgin olive oil intake repair high fat diet (HFD)-induced hepatic damage, via an anti-inflammatory effect in adipose tissue and modifications in the liver lipid composition, gene expression and signaling pathways.
- 2.Glucolipotoxicity is one of the key factors in the development of type 2 diabetes. We have identified the mechanisms involved in beta cell toxicity induced by saturated fatty acids HFD. In addition, we have found that monounsaturated fatty acids and phenolic compounds exert



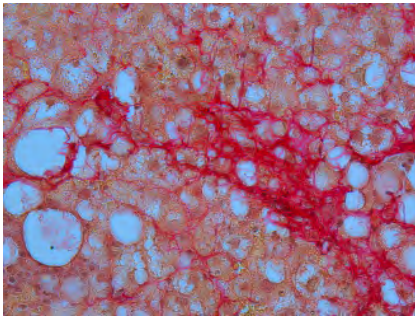


Figure 1. SiriusRed 40x, HFD-L 68 32w

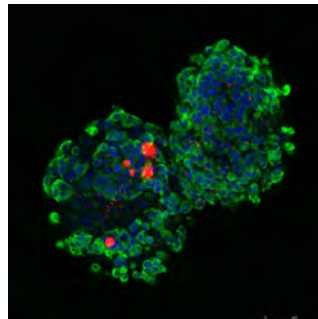


Figure 2. Pancreatic Islets replication

a protective action on regulation of glucose homeostasis. This action is throughout an improve in insulin sensitivity, a reduction in beta cell apoptosis and increase in beta cell number and a better glucose-induced insulin release (GSIS).

3. In an “humanized” hyperlipidemic mice, we have shown that phenolic compounds and monounsaturated fatty acids are able reduce liver damage and improve glucose homeostasis. In the case of glucose homeostasis the mechanisms involved were: i) improvement of insulin resistance; ii) reduction of beta cell inflammation and iii) reduction of beta cell oxidative stress. For the liver the mechanisms were the modification of the expression of the genes involved in fibrosis, inflammation, oxidative stress, lipid metabolism and insulin resistance. In addition, adipose tissue had lower levels of inflammation and oxidative stress.

4. Gain-of-function mutations in glucokinase gene (GCK) have been involved in the pathogenesis of hypoglycemia in neonates and infants. Glucokinase (GK) regulates GSIS and establish beta cell physiological threshold for GSIS. The gain-of-function GCK mutation V91L (GCK-V91L) produced larger islets that are more efficient controlling glucose homeostasis. The introduction of the activating GCK mutation V91L in adult mouse and human pancreatic islets increased their beta cell proliferation and improved GSIS, thereby improving glucose homeostasis.

## Publication Highlights

- Pertusa J.A.G., León-quinto T., Berná G., Tejedo J.R., Hmadcha A., Bedoya F.J., Martín F., Soria B. 2017. Zn<sup>2+</sup> chelation by serum albumin improves hexameric Zn<sup>2+</sup>-insulin dissociation into monomers after exocytosis. *PLOS One*. 12(11):e0187547
- Ortega A., Berná G., Rojas A., Martín F., Soria B. 2017. Gene-diet interactions in type 2 diabetes: the chicken and egg debate. *Int J Mol Sci*. 18(6)
- Jurado-Ruiz E., Varela L.M., Berná G., Cahuana G., Martínez-Force E., Gallego-Durán R., Soria B., De Roos B., Romero-Gomez M., Martín F. 2017. Olive oil rich diet intervention ameliorates non-alcoholic steatohepatitis induced by high-fat “western type” diet in mice. *Mol Nutr Food Res*. 61(3)
- Porciuncula A., Kumarc A., Rodríguez-Atarid M., Araña M., Martín F., Soria B., Prosper F., Verfaillie C., Barajas M. 2016. Pancreatic differentiation of Pdx1-GFP reporter mouse induced pluripotent stem cells. *Differentiation*. 92: 249-256
- Berná G., Oliveras-López M.J., Jurado-Ruiz E., Tejedo J., Bedoya F., Soria B., Martín F. 2014. Nutrigenetics and nutrigenomics insights into diabetes etiopathogenesis. *Nutrients*. 6:5338-5369

## Grants

2017-2019: PC-0111-2016-0111. Consejería de Salud, Junta de Andalucía

2017-2019: AGL-2017-86927-R. Ministerio de Economía, Industria y Competitividad





## Pancreatic Islet Development and Regeneration Unit

**Dr. Benoit R. Gauthier**

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GROUP LEADER



### Group Members updated

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#### Research Associate

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José Manuel Mellado Gil

#### PhD Students

Irene de Gracia Herrera Gomez  
Livia López Noriega

#### Technician

Leopoldo Pérez Rosendo

### Academic Background of PI

- 1987: BSc in Biology. University of Ottawa, Canada
- 1990: MS in Biology. University of Ottawa, Canada
- 1996: PhD in Biochemistry. Queen's University, Canada

### Current Position

- Since 2009: Research Scientist, CABIMER, Seville, Spain

### Positions Held

- 1996-1998: Research Fellow. Ottawa Heart Institute, Canada
- 1999-2000: Postdoctoral Fellow. Geneva University Hospital, Switzerland
- 2001-2007: Research Associate. University of Geneva, Switzerland
- 2007-2009: Assistant Professor. University of Geneva, Switzerland

### Research Activity

#### OVERVIEW

The overall research goal of the Pancreatic Islet Development and Regeneration Unit (PIDRU) focuses on developing innovative therapies for inflammatory/immune related diseases such as Diabetes Mellitus (DM) as well as for promoting healthy aging. To this end, the group has developed a research and development (R&D) pipeline that progresses from basic projects to preclinical and clinical studies. Within the basic program our work focuses on: 1) characterizing anti-diabetic targets that foster survival, regeneration and functionality of pancreatic islet insulin-producing beta cells 2) elucidating the cross talk between immune cells and islets in safeguarding glucose homeostasis and 3) exploring whether modulating levels of cytosolic/nuclear Acetyl-Coenzyme A (Ac-CoA) can prevent/revert metabolic diseases and delay aging. Further up the pipeline, our translational program includes: 1) pre-clinical assessment of anti-inflammatory/-diabetic drugs, 2) development of a novel viral delivery system for inducing immune tolerance against INSULIN in T1DM, and 3) genetic screening of specific targets in patients with Type 2 as well as with gestational diabetes mellitus (T2DM and GDM).

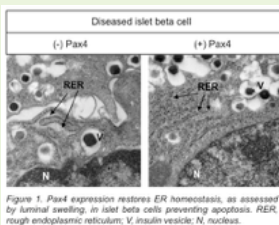
#### RESEARCH HIGHLIGHTS

##### PAX4, a key player safeguarding pancreatic islet beta cells health under stress

Pax4 is a key transcription factor essential for the generation of insulin producing beta cells during pancreatic islet development. The importance of this transcription factor in adult beta cell functionality has been inferred through the association of mutations in Pax4 with the development of diabetes, independently of its aetiology. Our work demonstrate that in adult islets, Pax4 expression is sequestered to a subset of beta cells that are prone to proliferation and more resistant to stress-induced apoptosis. Overexpression of this factor in adult islets stimulates beta cell proliferation and increases their resistance to apoptosis via improved ER homeostasis in several mouse models of diabetes (Figure 1). Our data pinpoint Pax4 as an important target for novel regenerative therapies for diabetes.

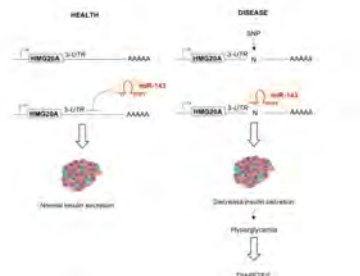
##### The chromatin-remodelling protein HMG20A orchestrates expression of beta cell enriched factors promoting maturity and functionality

HMG20A is a chromatin factor involved in neuronal differentiation and maturation. Recently small nucleotide polymorphisms (SNPs) in the HMG20A gene have been linked to type 2 diabetes mellitus (T2DM) yet neither expression nor function of this T2DM candidate gene in islets is known. We have

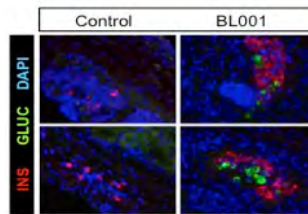


*Figure 1: Pax4 expression restores ER homeostasis, as assessed by luminal swelling, in islet beta cells preventing apoptosis. RER, Rough endoplasmic reticulum; V, insulin vesicle; N, nucleus.*





**Figure 2: HMG20A is essential to maintain beta cell functional maturity.** A diabetes-linked SNP within the 3'UTR of the HMG20A gene results in a 'disallowed' interaction with miRNA leading to beta cell dysfunction and diabetes.



**Figure 3: BL001 improves human islet engraftment in mouse xenotransplantation.** Immunostaining of insulin (INS, red) and glucagon (GLUC, green). Nuclei stained using DAPI.

shown that HMG20A expression is decreased in islets from T2DM donors meanwhile is transiently upregulated by high glucose concentration and during pregnancy. Our data also demonstrate that HMG20A expression in islet is essential for metabolism-insulin secretion coupling and beta cell de-differentiation. Additionally, reduced expression of HMG20A in islets impairs insulin release in respond to glucose, revealing a HMG20A-dependent mechanism of insulin secretion. Furthermore, we found that the T2DM-associated rs7119 SNP, located at 3' UTR of HMG20A transcript reduced protein levels. This rs7119 allele is associated with a new binding site for miR-143. Our study provides first evidence that a diabetes-linked SNP causes the creation of a miRNA binding sites resulting in the potential 'disallowed' interaction between the highly expressed miR-143 and HMG20A the outcome of which results in profound genetic reprogramming and beta cell dysfunction (Figure 2).

### Innovative drug therapies for Type 1 Diabetes Mellitus

Type 1 diabetes mellitus (T1DM) is defined as an autoimmune disease that results in the selective destruction of pancreatic islet beta cells by infiltrating immune cells (insulinitis). As a result, the organism is no longer able to produce insulin and develops hyperglycaemia and, if untreated, death. Despite advances in medical device technology and longer-acting insulin as well as strives in generating in vitro insulin-producing cells from various cell sources, there is still no robust therapy to substitute and protect beta cells that are lost in T1DM. The ultimate therapy should aim on one hand to restore immune self-tolerance and on the other to replenish the beta cell mass in T1DM patients. In this context, we have recently demonstrated that activation of the nuclear receptor, liver receptor homolog 1 (LRH-1/NR5A2) using a novel small chemical activator/agonist (BL001) can induce immune self-tolerance, increase beta cell survival and promote beta cell regeneration through a putative mechanism of alpha-to-beta cell phenotypic switch. More importantly, BL001 improved human islet engraftment in mouse xenotransplantation (Figure 3). Members of the group along with a European business consortium founded in 2016 the Biotechnology Spinoff ARIDDAD Therapeutics S.L. with the mission to develop second-generation LRH-1 agonists for an unprecedented T1DM therapy.

We also assessed the impact of the thyroid hormone, levothyroxine, in blunting development of hyperglycaemia in a mouse model of experimental autoimmune diabetes (EAD). Long-term levothyroxine supplementation enhanced glucose clearance and reduced circulating glucose levels. More importantly, levothyroxine blunted the onset of EAD via the induction of beta cell proliferation and the preservation of insulin expressing cells. Our results suggest a potential T1DM therapeutic intervention based on the use of thyroid hormones or thyromimetics.

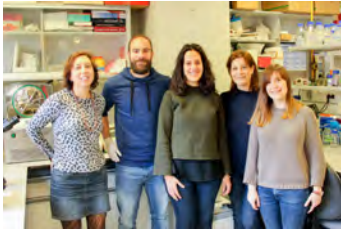
### Publication Highlights

- Lopez-Noriega L., Cobo-Vuilleumier N., Narbona-Perez A.J., Araujo-Garrido J.L.D., Lorenzo, P.I., Gauthier B.R., Martin-Montalvo A\*. 2017. Levothyroxine enhances glucose clearance and blunts the onset of experimental type 1 diabetes mellitus in mice. *Bri. J. Pharmacol.* 174: 3795-3810 (\*Corresponding authors)
- Romero-Zerbo Y.S., Ruz-Maldonado I., Espinosa-Jimenez V., Rafacho A., Gomez-Conde A.I., Sanchez-Salido, L., Cobo-Vuilleumier N., Gauthier B.R., Tinahones, F.J., Persaud, S., Bermudez-Silva, F. 2017. The cannabinoid ligand LH-21 reduces anxiety and improves glucose handling in diet-induced obese pre-diabetic mice. *Sci. Rep.* 21:3946
- Lorenzo P.L., Juarez-Vicente F., Cobo Vuilleumier N., Garcia-Dominguez M., Gauthier B.R. 2017. The diabetes-linked transcription factor Pax4: From gene to functional consequences. *Genes* 9:E101
- Martin-Montalvo A., López Noriega L., Lorenzo P.I. Gauthier B.R. 2017. Targeting pancreatic expressed PAX genes for the treatment of diabetes mellitus and pancreatic neuroendocrine tumors. *Expert Opin. Ther. Targets* 21:77-89
- Mellado-Gil J.M., Jiménez-Moreno C.M., Martin-Montalvo. A., Álvarez-Mercado A.I., Fuente-Martin E., Cobo-Vuilleumier N., Lorenzo, P.I., Bru-Tari E., Herrera Gómez I.G., López Noriega L., Pérez Florido J., Santoyo-López J., Spyranis A., Meda P., Boehm B.O., Quesada I. and Gauthier B.R. 2016. PAX4 preserves endoplasmic reticulum integrity preventing beta cell degeneration in a mouse model of type 1 diabetes mellitus. *Diabetologia*, 59:755-65

### Grants

2017-2021: BFU2017-83588-P. Ministerio de Economía y Competitividad  
2017-2018: Fundación DiabetesCero  
2017-2019: IJCI-2015-26238. Ministerio de Economía y Competitividad  
2017-2020: PI-0006-2016. Consejería de Salud, Junta de Andalucía  
2016-2017: Asociación Lucha y Sonríe por la Vida de Pilas (ALUSVI)  
2016-2017: Amarna Therapeutics S.L.





## Pancreas and Liver Development and Disease

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GROUP LEADER



### Group Members updated

#### Postdoctoral

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#### PhD student

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#### Technician

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### Academic Background of PI

- 1996: Degree. University of Seville, B.Sc. in Biology
- 2001: PhD: University of Seville, Biology

### Current Position

- 2015-present: Investigador Ramón y Cajal, CABIMER, Seville, Spain

### Positions Held

- 2002-2007: Postdoctoral position, University of California San Francisco, UCSF
- 2008-2013: Miguel Servet Investigator, CABIMER, Seville, Spain
- 2014-2015: Miguel Servet II Investigator, CABIMER, Seville, Spain

### Research Activity

#### OVERVIEW

Defects in organogenesis or function of liver and pancreas lead to debilitating diseases, including diabetes and cirrhosis. Understanding the processes by which these organs form during development and how cells are regenerated upon injury in adult tissue is critical to further our insights into how disease affecting these organs and how they might be treated in a more efficient manner than currently possible. Indeed, there is an urgent need for generating liver hepatocytes and pancreatic endocrine islets to treat severe liver failure and diabetes by transplantation. To accomplish this goal, it is imperative to fully understand how these organs are formed within the embryo and how they function at adult stages.

#### RESEARCH HIGHLIGHTS

##### 1. Molecular mechanisms of embryonic pancreas formation and adult pancreatic function

The pancreas is an essential organ that serves two vital functions: it makes digestive enzymes that aid in digestion and produce hormones that control blood glucose levels. Dysfunction of this organ might be caused by failures in the genetic program controlling the organogenesis process. Interestingly, many of these pancreatic embryonic pathways are also active in adult pancreas during normal and pathological conditions (diabetes, pancreatitis or pancreas cancer). Previous studies from our lab have identified new the transcription factors GATA4 and GATA6 as new players in pancreas organogenesis. These two transcription factors are required for the proliferation and differentiation of pancreatic progenitor cells. More recently, our group has uncovered a role of GATA6 in the adult beta cell function. Loss of Gata6 in mice leads to glucose intolerance. Analyses of Gata6-deficient pancreatic islets shows a markedly decrease in the expression of insulin, key components of insulin synthesis and secretion machinery and  $\beta$  cell-enriched transcription factors demonstrating an essential role for GATA6 in  $\beta$  cell function.

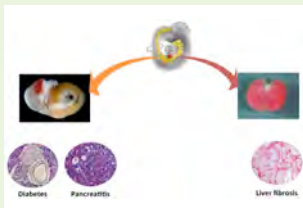


Figure 1: Liver and pancreas;  
from development to disease



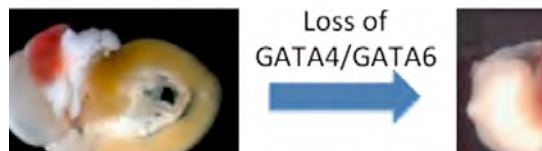


Figure 2: *Gata4* and *Gata6* conditional knockout mice in pancreatic progenitors leads to pancreas agenesis in mice

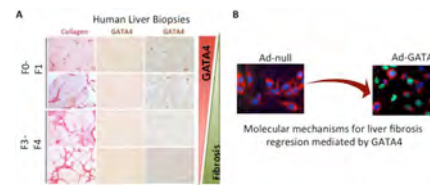


Figure 3: *Gata4* expression in HSCs decreases in advanced stage of liver fibrosis. B: Overexpression of *GATA4* in human LX2 cell line decreases the expression of fibrogenic markers, including laminin.

## 2. Molecular basis for hepatic fibrosis induction and progression

Liver fibrosis is a pathophysiological response to chronic injury produced mainly by alcohol abuse, virus infection or bile duct obstruction. A hallmark of liver fibrosis is the transformation of quiescent hepatic stellate cells (HSCs) into an active and proliferative myofibroblastic phenotype. Activated HSCs are the main source of liver extracellular matrix components (ECM), such as collagen and laminin that form fibrotic scars. Liver fibrosis can be reversed if the damage agent is removed. The regression of liver fibrosis implies breakdown of ECM by metalloproteinases and the clearance of activated HSCs, by apoptosis or reversion to an inactive phenotype, thus allowing the hepatocyte to repopulate the damaged hepatic tissue. However, if the injury is sustained, liver fibrosis can progress and lead to cirrhosis, which is marked by an excessive accumulation of ECM, distorted liver architecture, and impairment of hepatic function. Currently, one of the emerging therapies for liver fibrosis focuses in the inhibition of HSCs activation and proliferation. Although the signals that trigger HSCs activation in liver fibrosis are moderately known, the underlying molecular mechanisms are not well understood and in particular, the transcription factors that mediate this process are unidentified. Our previous studies have uncovered a new role for *GATA4* transcription factor in liver fibrosis. We have shown that loss of *GATA4* in HSCs leads to HSCs activation, which results in severe liver fibrosis in mice. Moreover, the overexpression of *GATA4* in LX2 cells, an activated human hepatic stellate cell line, reduces the accumulation of ECM, suggesting the ability of *GATA4* to revert the phenotype of HSCs from an active to an inactive state. More recent studies from our lab have show the ability of *GATA4* to revert liver fibrosis in vivo in mice treated with CCl<sub>4</sub>, a widely used model for induction of liver fibrosis. Overexpression of *GATA4* by adenovirus infection in CCl<sub>4</sub>-treated mice decreased the expression of fibrotic markers, such collagen (stained with Sirius red) and smooth muscle alpha actin (Sma- $\alpha$ ). Currently, we are the studying the molecular mechanisms underlying the reversion of HSCs phenotype mediated by *GATA4* and examining the potential of *GATA4* as an anti-fibrogenic agent. Our studies will shed light into the regulation of the induction/progression of hepatic fibrosis and could ultimately help to develop novel therapeutic alternatives to treat hepatic fibrosis regardless the etiology.

## Publication Highlights

- Carrasco M., Delgado I., Soria, B., Martín F., Rojas A. 2012. *GATA4* and *GATA6* control mouse pancreas organogenesis. *Journal of Clinical Investigation*. 122(10):3504-3515
- Delgado I., Carrasco M., Cano E., Carmona R., García-Carbonero R., Marín-Gómez L., Soria B., Martín F., Cano D.A., Muñoz-Chápuli R., Rojas A. 2014. *GATA4* loss in the Septum transversum promotes liver fibrosis. *Hepatology*. 59(6):2358-2370
- Cano D.A., Soria B., Martín F., Rojas A. 2013. Transcriptional control of mammalian pancreas organogenesis. *Cellular and Molecular Life Sciences*. 71(13):2383-402
- Cañete A., Carmona R., Ariza L., Sánchez M.J., Rojas A., Muñoz-Chápuli R. 2017. A population of hematopoietic stem cells derives from *GATA4*-expressing progenitors located in the placenta and lateral mesoderm of mice. *Haematologica*. 102(4):647-655
- Carmona R., Cañete A., Cano E., Ariza L., Rojas A., Muñoz-Chápuli R. 2016. Conditional deletion of *WT1* in the septum transversum mesenchyme causes congenital diaphragmatic hernia in mice. *Elife*. 19;5. pii: e16009

## Grants

2015-2017: PI14/00804. Instituto de Salud Carlos III





## Beta Cell Regeneration

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GROUP LEADER



### Group Members

#### Research Associates

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#### Technicians

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Irene Díaz Contreras

### Academic Background of PI

- 1979: Degree. University of Sevilla, B.Sc. in Medicine and Surgery
- 1982: PhD. University of Sevilla. PhD. In Medicine and Surgery

### Current Position

• Since 2007: Professor in Biochemistry and Molecular Biology, Department of Molecular Biology and Biochemical Engineering. University Pablo de Olavide, Seville, Spain

### Positions Held

- 1980-1983: Hospital Virgen Macarena, Seville Medical Internship in Clinical Biochemistry
- 1985-1985: Teaching Associate. University of Seville, Spain
- 1986-2007: Lecturer, University of Seville, Spain

### Research Activity

#### OVERVIEW

Our research interest focuses on two topics: stem cell self-renewal and differentiation and pancreatic beta cell survival. Studies carried out by our group over the past 5 years show that Nitric Oxide (NO) plays a role in the control of self-renewal and differentiation towards insulin producing cells. Thus, we have found that low concentration of this cell messenger elicits an hypoxia-like state and preserves mouse and human ESC pluripotency, thus being revealed as an efficient tool to control spontaneous differentiation events during 'in vitro' culture of these cell types (fig 1). On the other hand, high levels of NO triggers cell death events and differentiation processes in surviving cells. We have reported that this action is instrumental for designing optimized strategies for in vitro differentiation towards insulin secreting cells. Regarding beta cell survival, we have reported that this small molecule mediator is involved in the protective action of insulin and IGF-1 against metabolic stress in pancreatic beta cells. All in all, our research focuses on implementing strategies for a better treatment of Diabetes. In this respect, we are currently interested in studying the impact of NO and metabolic stressors in beta cell survival and stem cell biology

#### RESEARCH HIGHLIGHTS

Our group reported previously that NO delays mouse embryonic stem cell (mESC) differentiation by regulating well described genes controlling pluripotency and differentiation. We next sought to explore the impact of NO at the genome wide level. Culture of mESCs with 2  $\mu$ M of the NO donor diethylenetriamine/NO (DETA/NO) in the absence of leukemia inhibitory factor

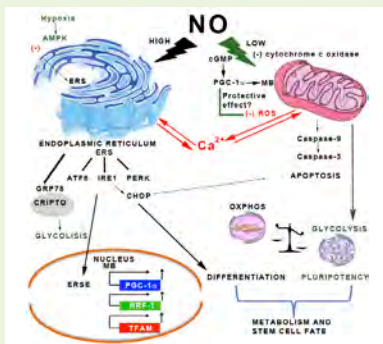


Figure 1



(LIF) induced significant changes in the expression of 16 genes of the pluripotency transcriptional core. Furthermore, treatment with DETA/NO resulted in a high occupancy of activating H3K4me3 at the Oct4 and Nanog promoters and repressive H3K9me3 and H3K27me3 at the Brachyury promoter. Additionally, the activation of signaling pathways involved in pluripotency, such as Gsk3- $\beta$ / $\beta$ -catenin, was observed, in addition to activation of PI3K/Akt, which is consistent with the protection of mESCs from cell death. Finally, a decrease in cell proliferation coincides with cell cycle arrest in G2/M. Our results provide novel insights into NO-mediated gene regulation and cell proliferation and suggest that NO is necessary but not sufficient for the maintenance of pluripotency and the prevention of cell differentiation. These results have been published in: *J Cell Biochem.* 2016 Feb 8. doi: 10.1002/jcb.25513. [Epub ahead of print] PMID:26853909.

Pancreatic and duodenal homeobox (Pdx1) is a transcription factor that regulates the embryonic development of the pancreas and the differentiation towards beta cells. Previously, we have shown that exposure of mouse embryonic stem cells (mESCs) to high concentrations of NO donor diethylenetriamine nitric oxide adduct (DETA-NO) triggers differentiation events and promotes the expression of Pdx1. Here we report evidence that Pdx1 expression is associated with release of Polycomb Repressive Complex 2 (PRC2) and P300 from its promoter region. These events are accompanied by epigenetic changes in bivalent marks of histone H3K27me3 and H3K4me3, site specific changes in DNA methylation, and no change in H3 acetylation. Based on these findings, we developed a protocol to differentiate mESCs towards insulin producing cells consisting of sequential exposure to DETA-NO, valproic acid, and P300 inhibitor (C646) to enhance Pdx1 expression and a final maturation step of culture in suspension to form cell aggregates. This small molecule- based protocol succeeds in obtaining cells that express pancreatic beta cell markers such as PDX1, INS1, GCK and GLUT2 and respond in vitro to high-glucose and KCl. This results have been published in: *Cell Transplant.* 2016 Homeostatic levels of nitric oxide (NO) protect efficiently against apoptotic death in both human and rodent pancreatic beta cells, but the protein profile of this action remains to be determined. We have applied a two dimensional LC-MS-MALDI-TOF/TOF-based analysis to study the impact of protective NO in rat insulin-producing RINm5F cell line and in mouse and human pancreatic islets (HPI) exposed to serum deprivation condition. 24 proteins in RINm5F and 22 in HPI were identified to undergo changes in at least one experimental condition. These include stress response mitochondrial proteins (UQCRC2, VDAC1, ATP5C1, ATP5A1) in RINm5F cells and stress response endoplasmic reticulum proteins (HSPA5, PDIA6, VCP, GANAB) in HPI. In addition, metabolic and structural proteins, oxidoreductases and chaperones related with protein metabolism are also regulated by NO treatment. Network analysis of differentially expressed proteins shows their interaction in glucocorticoid receptor and NRF2-mediated oxidative stress response pathways and eNOS signalling. The results indicate that exposure to exogenous NO counteracts the impact of serum deprivation on pancreatic beta cell proteome. Species differences in the proteins involved are apparent. These results were published in: *Islets.* 2014;6(5-6):e995997.

## Publication Highlights

- Tapia-Limonchi R., Díaz I. Cahuana G.M., Bautista M., Martín F., Soria B., Tejedo J.R., Bedoya F.J. 2014. Impact of exposure to low concentrations of nitric oxide on protein profile in murine and human pancreatic islet cells. *Islets.* 6(5-6):e995997
- Mora-Castilla S., Tejedo J.R., Tapia-Limonchi R., Díaz I., Hitos A.B., Cahuana G.M., Hmadcha A., Martín F., Soria B., Bedoya F.J. 2014. Transient Downregulation of Nanog and Oct4 Induced by DETA/NO Exposure in Mouse Embryonic Stem Cells Leads to Mesodermal/Endodermal Lineage Differentiation. *Stem Cells Int.* 379678.
- Beltran-Povea A., Caballano-Infantes E., Salguero-Aranda C., Martín F., Soria B., Bedoya F.J., Tejedo J.R., Cahuana G.M. 2015. Role of nitric oxide in the maintenance of pluripotency and the regulation of the hypoxia response in stem cells. *World J Stem Cells.* 7(3):605-17
- Tapia-Limonchi R., Cahuana G.M. Caballano-Infantes E., Salguero-Aranda C., Beltrán-Povea A., Hitos A.B., Martín F., Soria B., Bedoya F.J., Tejedo JR. 2016. Nitric Oxide prevents mouse embryonic stem cell differentiation through regulation of gene expression, cell signalling and control of cell proliferation. *J. Cell. Biochem.* 117:2078-2088
- Salguero-Aranda C., Tapia-Limonchi R., Cahuana G.M., Hitos A.B., Díaz I., Hmadcha A. Fraga M., Martín F., Soria B., Tejedo J.R. Bedoya F.J. 2016. Differentiation of Mouse Embryonic Stem Cells toward Functional Pancreatic  $\beta$ -Cell Surrogates through Epigenetic Regulation of Pdx1 by Nitric Oxide. *Cell Transplant.* 25(10):1879-1892

## Grants

2013-2018: CTS-7127/2011. Consejería de Economía, Innovación, Ciencia y Empleo





## Retinal Degeneration: from Genetics to Therapy

**Dr. Shom Shanker Bhattacharya**

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GROUP LEADER



### Group Members

#### Postdoctorals

Berta De La Cerda Haynes  
Elena Reales Rodríguez  
Sofia M. Calado  
Francisco J. Díaz Corrales

#### PhD Student

Ana B. García Delgado

#### Technicians

Andrea Díez Lloret  
M. Lourdes Valdés Sánchez  
Adoración Montero Sánchez

#### Research Assistant

Abhay Krishan

### Academic Background of PI

- 1969: Degree: University of Bombay, B.Sc. Upper Second Division with Honors in Chemistry
- 1971: University of Newcastle upon Tyne, U.K, M.Sc. in Clinical Biochemistry
- 1977: PhD. Newcastle University upon Tyne, UK, PhD in Clinical Biochemistry

### Current Position

- PI of the "Retinal degeneration" research group in CABIMER
- Emeritus Professor in Institute of Ophthalmology, UCL, London, UK

### Positions Held

#### Postdoctoral positions

- Research Associate in the University Department of Clinical Biochemistry, Royal Victoria Infirmary, Newcastle Upon Tyne, UK
- Scientific staff and Senior Research Fellow, MRC Human Genetics Unit, Edinburgh and University of Edinburgh
- Head of Molecular Genetics Unit, Department of Human Genetics, University of Newcastle upon Tyne, UK

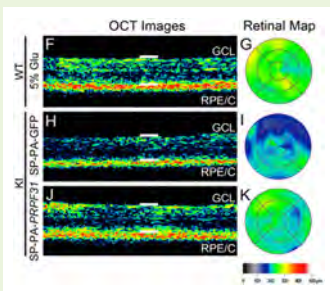
#### Permanent positions

- Sembal Professor of Experimental Ophthalmology, Institute of Ophthalmology, University College London, UK
- Group Leader & Full Professor, Institute de la Vision, Pierre et Marie Curie University, Paris, France
- Director of CABIMER, Seville, Spain
- Head of Department of Cell Therapy and Regenerative Medicine, CABIMER.
- Ministry of Health appointed Associate Director, Genetics Plan for Andalusia

### Research Activity

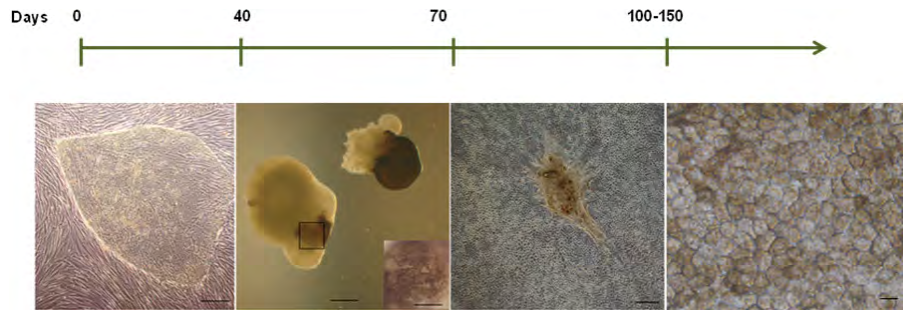
#### OVERVIEW

Our research starts with the genetics of inherited retinal degeneration (IRD), looking for the identification of new genes and regulatory elements involved in IRD. From the genetic information we pursue the study of the specific molecular mechanisms of disease. In a more general approach to photoreceptor (PR) cell-death, we focus on PR-specific response to DNA damage and RNA synthesis-related stress. Our research interest naturally leads us to explore therapeutics for retinal degenerative diseases currently without treatment. We work both on IRD and multifactorial retinal disease, like Age-related macular degeneration (AMD) and diabetic retinopathy. Through reprogramming and differentiation, we obtain cellular models of the affected retinal cell types: PRs and retinal pigmented epithelium (RPE). We use these cellular models and animal models to pre-clinically test different therapeutical approaches that may help in different stages of the disease: cell therapy, gene therapy and testing new neuroprotective compounds.



*Recovery on retinal thickness after  
nonviral gene therapy replacement for  
PRPF31 gene in PRPF31 mouse model.*





Differentiation protocol for human iPS towards RPE.

## RESEARCH HIGHLIGHTS

The identification of new genes involved in IRD is currently approached through our participation in the “European Retinal Degeneration Consortium” in which we partner in the validation of a new diagnostic chip for IRD through our collaboration with the Ophthalmic Unit of University Hospital Virgen Macarena. New genes and candidates have been identified and the genomic tool will soon be licensed for the prospective study of candidate patients for gene therapy. We are also involved in the Eyerisk European consortium effort to study AMD-associated genomic variants with the objective of providing a risk-measurement tool for ophthalmologists.

To study the molecular mechanisms of disease, we use different animal models of retinal degeneration: RD1, RD10, ATR and PRPF31 mutant mice. Molecular mechanisms of PR cell death associated with ciliary defects have been found for the ATR mouse model and work is in progress to deeply analyse the ATR role in the ciliary development in collaboration with the Cell Biology Dpt. In Yale University. For PRPF31, a thorough characterization of the effects of the mutation in the mouse RPE has led to the description of morphologic, functional and molecular consequences, and currently we are developing a PRPF31-patient-derived cellular model to check those findings in a human model. Additionally, PR specific mechanisms to deal with DNA damage and RNA synthesis-related stress have also been studied using IRD models to search for a therapeutic target in degenerating PRs.

In our search of new therapeutic approaches for retinal degeneration we have worked on gene therapy using viral and non-viral vectors, finding a therapeutic effect of the non-viral gene-therapy for the PRPF31 defect with recovery of retinal thickness and spatial vision in the treated mice. As a very useful tool in our lab, we have produced and characterized several PR and RPE cellular models for IRD related to different genes: CEP290 and AIPL1 for Leber congenital amaurosis, EYS and PRPF31 for retinitis pigmentosa, to dissect the mechanisms leading to cell death of PRs caused by different genetic defects. After characterization of the models, we are now working on the gene correction via CRISPR-Cas9 technology. We are also preparing and studying AMD cellular models to dissect the impact of the genetic variants associated with the disease. On preclinical therapeutics, we are mainly focused on the dry form of AMD, testing gene, cell and pharmacological approaches to help patients in different stages of the disease. We have obtained neuroprotection in mouse models of retinal degeneration using a new molecule, which has led to the patent of this neuroprotective drug and further work is ongoing with the goal of a clinical trial. We have also described the re-purposing of a known drug (rasagiline) for retinal neuroprotection in our pre-clinical setting. For early AMD we are checking the effect of gene therapy using neuroprotective genes; but this approach will not suffice for the more advanced cases, which will need to replace damaged tissue; so we are using human iPS-derived RPE patches grown on biocompatible matrices to study the therapeutic possibilities of the RPE transplant. We have so far completed the preclinical assays in mice and the viability and safety study will be done in pigs.

## Publication Highlights

- Dopazo J., Amadoz A., Bleda M., Garcia-Alonso L., Alemán A., García-García F.J., Rodríguez A., Daub J.T., Muntané G., Rueda A., Vela-Boza A., López-Domingo F.J., Florido J.P., Arce P., Ruiz-Ferrer M., Méndez-Vidal C., Arnold T.E., Spleiss O., Alvarez-Tejado M., Navarro, Bhattacharya S.S., Borrego S., Santoyo-López J., Antiñolo G. 2016. 267 Spanish Exomes Reveal Population-Specific Differences in Disease-Related Genetic Variation. *Mol Biol Evol.* 33(5):1205-18
- Calado S.M., Diaz-Corrales F., Silva G.A. 2016. pEPito-driven PEDF Expression Ameliorates Diabetic Retinopathy Hallmarks. *Hum Gene Ther Methods.* 27(2):79-86
- Pensado A., Diaz-Corrales F.J., De la Cerda B., Valdés-Sánchez L., Del Boz A.A., Rodríguez-Martínez D, García-Delgado A.B., Seijo B., Bhattacharya S.S., Sanchez A. 2016. Span poly-L-arginine nanoparticles are efficient non-viral vectors for PRPF31 gene delivery: An approach of gene therapy to treat retinitis pigmentosa. *Nanomedicine.* 12(8):2251-2260
- Rose A.M., Shah A.Z., Venturini G., Krishna A., Chakravarti A., Rivolta C., Bhattacharya S.S. 2016. Transcriptional regulation of PRPF31 gene expression by MSR1 repeat elements causes incomplete penetrance in retinitis pigmentosa. *Sci Rep.* 6:19450
- Colijn JM et al. 2017. Prevalence of Age-Related Macular Degeneration in Europe: The Past and the Future. *Ophthalmology.* 124(12):1753-1763

## Grants

2018-2021. PI17/01026. Instituto de Salud Carlos III  
 2015-2019: 634479. EU Horizon-2020  
 2017-2019: CABIMER-IDIBEL Project. CELLEX Foundation  
 2016-2018: CP15/00071. Instituto de Salud Carlos III  
 2017-2018: DMAE-Cells project. Consejería de Igualdad, Salud y Políticas Sociales, Junta de Andalucía





## Cell-Based Therapies for Neuropathologies

**Dr. Manuel Alvarez-Dolado**

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GROUP LEADER



### Group Members updated

#### Postdoctoral

Magdalena Martínez-Losa.

#### Master Student

M<sup>a</sup> Mercedes Pérez Fernández.

#### Technician

Flora Priego Montes

### Academic Background of PI

- 1992: Degree. Universidad Autónoma de Madrid, B.Sc. in Biological Sciences
- 1997: PhD. Universidad Autónoma de Madrid, Biological Sciences

### Current Position

- Since 2008 Research Scientist. CSIC/ CABIMER, Seville, Spain

### Positions Held

- 1998-2000: Instituto de Investigaciones Biomédicas, CSIC, Madrid, Spain
- 2000-2003: Investigator, University of California San Francisco
- 2003-2006: FISS Researcher at Hospital General Universitario de Valencia, Spain
- 2006-2008: Group Leader at Centro de Investigación Príncipe Felipe, Valencia, Spain
- 2009-2013: Associated Professor in Cell Biology at UPO, Seville, Spain

### Research Activity

#### OVERVIEW

Repairing the brain function is one of the biggest challenges that Regenerative Medicine faces. For this, it is necessary to study and characterize an appropriate stem cells source; understand the effects of the stem cell transplants on animal models of the diseases; and develop the technical conditions to facilitate the translation of the lab discoveries to the clinic. Thus, our laboratory develops pre-clinical cell-based assays in animal models of diverse neuropathologies, such as ataxias, Alzheimer's disease and epilepsy. We work with two different stem cell sources. The first type are bone marrow derived stem cells (BMSC), which we apply for the treatment of ataxias. The second are GABAergic neuronal progenitors derived from the medial ganglionic eminence (MGE), which are being applied in animal models of epilepsy, infantile encephalopathies, and Alzheimer's disease. Our main goal is the development of new cell-based therapies for the treatment of these important diseases. For this, we study their mechanism of action and effects at the electrophysiological, histological, cellular, molecular, and behavioural levels.

#### RESEARCH HIGHLIGHTS

Many studies have reported the contribution of BMSC to the CNS, raising the possibility of using them as a new source to repair damaged brain tissue or restore neuronal function. Ataxias are locomotor disorders that can have an origin both neural and muscular, although both impairments are related. Unfortunately, ataxia has no cure, and the current therapies are aimed at motor re-education or muscular reinforcement. Nevertheless, cell therapy is becoming a promising approach to deal with incurable neural diseases, including neuromuscular ataxias. We have used a model of ataxia, the Purkinje Cell Degeneration (PCD) mutant mouse, to study the effect of healthy (wild-type) bone marrow transplantation on the restoration of defective mobility. Bone marrow transplants (from both mutant and healthy donors) were performed in wild-type and PCD mice. Our results demonstrated that the transplant of wild-type bone marrow restores the mobility of PCD mice, increasing their capabilities of movement (52-100% of recovery), exploration (20-71% of recovery), speed (35% of recovery), and motor coordination (25% of recovery). Surprisingly, our results showed that bone marrow transplant notably improves the skeletal

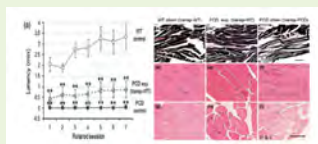
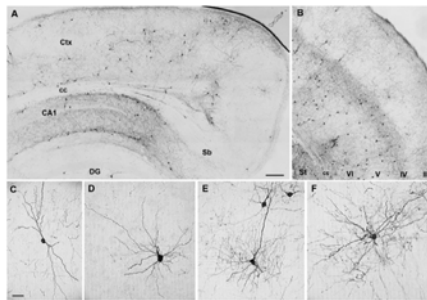


Figure 1. Left graph present a rotarod test showing the improvement of PCD mice in their locomotor coordination after the BMSC transplant. Right panel shows the histological characterization of the muscle. (a–c) Longitudinal view of fibres labelled with Masson's trichrome (TriM); (d–i) Haematoxylin and eosin (H & E) staining of muscular cells in longitudinal (d–f) and transversal (g–i) views. Note the differences in thickness amongst experimental groups. Scale bar: 100  $\mu$ m for a–c; 30  $\mu$ m for d–i





**Figure 2.** Detection of cryopreserved MGE-derived interneuron precursors by immunohistochemistry against GFP in the neocortex (Cbx) and hippocampus four weeks after their transplant. (B) Note the wide distribution of cryopreserved grafted cells in multiple cortical layers and the dark background due to dendrite concentration in layer IV. (C-F) MGE-derived cells in the cortex differentiated into neurons presenting typical morphology of interneuron subtypes e.g., bitufted (C), multipolar (D), chandelier cells with synaptic boutons resembling candlesticks (E), or basket cells (F). In hippocampus, grafted cells accumulated in CA1 and dentate gyrus (DG). Corpus callosum (cc); cortical layers (III-VI); striatum (St); subiculum (Sb). Scale bars for A and B: 200µm; in C for D-F: 25µm.

muscle structure, which is severely damaged in the mutants, rather than ameliorating the central nervous system (Fig.1). Although a multimodal effect of the transplant is not discarded, muscular improvements appear to be the basis of this motor recovery. Furthermore, the results from our study indicate that bone marrow stem cell therapy can be a safe and effective alternative for dealing with movement disorders such as ataxias.

The second main research line of the group is to develop a cell therapy with GABAergic neuronal progenitors for the treatment of interneuron related diseases. We performed transplants in animal models of temporal lobe epilepsy, west syndrome, and Alzheimer's disease (AD) to show the therapeutic potential of these neuronal precursors. In all this models we observed an anticonvulsant activity of the precursors together with restoration of normal brain rhythms and improvement of behavioural and cognitive deficits. In the AD model we only observed this improvement when the transplanted progenitors over-expressed the Nav1.1 channel. The results strongly suggest that naïve or genetically-modified GABAergic neuronal precursors are a promising source of cells for regenerative medicine to treat psychiatric conditions. In this sense, cryopreservation protocols are essential for stem cells storage in order to apply them in the clinic. We have describe a new standardized cryopreservation protocol for these GABAergic neural precursors. We used 10% Me2SO as cryoprotectant and assessed the effects of cell culture amplification and cellular organization, as in toto explants, neurospheres, or individualized cells, on post-thaw cell viability and retrieval. We confirmed that in toto cryopreservation of MGE explants is an optimal preservation system to keep intact the interneuron precursor properties for cell transplantation, together with a high cell viability (>80%) and yield (>70%). In addition, their migration capacity, acquisition of mature neuronal morphology, and potency to differentiate into multiple interneuron subtypes were also confirmed in vivo after transplantation (Fig. 2). The results show that the cryopreserved precursor features remained intact and were similar to those immediately transplanted after their dissection from the MGE. We hope this protocol will facilitate the generation of biobanks to obtain a permanent and reliable source of GABAergic precursors for their clinical application.

Finally, we have also studied, in collaboration with Drs. Santín and Castilla-Ortega from the University of Malaga, the role of the GABAergic system in the cocaine addiction disorder. We analysed whether a persistent cognitive/emotional dysregulation in mice withdrawn from cocaine holds a neurobiological correlate within the hippocampus. The cocaine-withdrawn mice showed no remarkable exploratory or emotional alterations but were consistently impaired in all the analysed cognitive tasks (object and place recognition memory, cocaine-induced conditioned place preference, continuous spontaneous alternation). All the cocaine-withdrawn groups, independent of whether they were submitted to behavioural assessment or not, showed enhanced basal c-Fos expression and an increased number of GABA<sup>+</sup> cells in the dentate gyrus. Moreover, the cocaine-withdrawn mice previously submitted to behavioral training displayed a blunted experience-dependent regulation of PV<sup>+</sup> and NPY<sup>+</sup> neurons in the dentate gyrus, and neurogenesis in the hippocampus. These results highlight the relevance of hippocampal neuroplasticity for the ingrained cognitive deficits present during chronic cocaine withdrawal.

## Publication Highlights

- Paradells S., Zipancic I., Martínez-Losa M.M., García Esparza M.Á., Bosch-Morell F., Alvarez-Dolado M., Soria J.M. 2015. Lipic acid and bone marrow derived cells therapy induce angiogenesis and cell proliferation after focal brain injury. *Brain Inj.* 29(3):380-95
- Rodríguez-Martínez D., Martínez-Losa M., Alvarez-Dolado M. 2017 Cryopreservation of GABAergic Neuronal Precursors for Cell-Based Therapy. *PLoS One* 12(1):e0170776
- Ladrón de Guevara-Miranda D., Millón C., Rosell-Valle C., Pérez-Fernández M., Missiroli M., Serrano A., Pavón F.J., Rodríguez de Fonseca F., Martínez-Losa M., Álvarez-Dolado M., Santín L.J., Castilla-Ortega E. 2017. Long-lasting memory deficits in mice withdrawn from cocaine are concomitant with neuroadaptations in hippocampal basal activity, GABAergic interneurons and adult neurogenesis. *Dis Model Mech.* 10(3):323-336
- Díaz D., Piquer-Gil M., Recio J.S., Martínez-Losa M.M., Alonso J.R., Weruaga E., Álvarez-Dolado M. 2017. Bone marrow transplantation improves motor activity in a mouse model of ataxia. *J Tissue Eng Regen Med.* 12(4):e1950-e1961

## Grants

2014-2018: CTS-2563. Proyectos de Excelencia. Consejería de Economía, Innovación Ciencia y Empleo, Junta de Andalucía





## Cellular and Molecular Neuroimmunology

### Dr. David Pozo Perez

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GROUP LEADER



### Group Members

#### Senior Researcher

Cintia Roodveldt

#### Postdoctorals

Jaime Muñoz-Franco

Zaira I. González Sánchez

#### PhD Students

Maria Magdalena Leal

María Gómez-Lima

#### Master Student

Jesús Pérez-Cabello

### Academic Background of PI

- 1992: Degree. University of Seville, B.Sc. in Biological Sciences
- 1998: PhD. University of Seville, PhD in Biochemistry & Molecular Biology

### Current Position

- Since 2008: Associate Professor (Profesor Titular) of Biochemistry and Molecular Biology (Habilitación Nacional Univ. Salamanca). Dept. Medical Biochemistry, Molecular Biology and Immunology. University of Seville.

### Positions Held

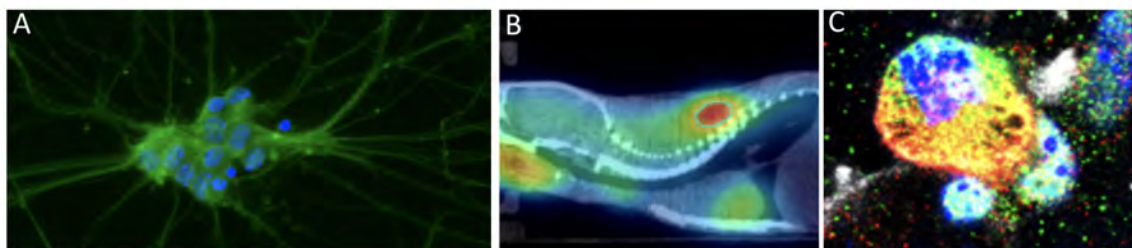
- 2000-2003: University of Cambridge. Division of Immunology. Cambridge, UK. LT-EMBO and Marie Curie postdoctoral fellowships
- 2004: Weizmann Institute of Science. Department of Immunology. Rehovot, Israel. FEBS invited scientist fellowship
- 2006: Short stints as visiting scientist: Weizmann Institute of Science, Israel
- 2011-2013-2015: Visiting Scientist. Harvard University. Center for Neurologic Diseases, Boston, USA
- Scientific Director. BIONAND (Andalusian Center for Nanomedicine and Biotechnology). Malaga. 2010-2014.

### Research Activity

#### OVERVIEW

The Cellular and Molecular Neuroimmunology Laboratory of the University of Seville at CABIMER is focused on understanding molecular and cellular mechanisms that regulate immune homeostasis and contribute to neuronal dysfunction and death, with particular emphasis on the role of key cell populations as microglia, dendritic cells and different T regulatory cell subsets in the development of Parkinson's disease (PD), Multiple Sclerosis (MS), and Amyotrophic Lateral Sclerosis (ALS). The activities at NIR laboratory merge basic disease-oriented research on primary cell cultures and cell line cultures (mouse and human), preclinical studies in mouse models of human diseases (PD, MS, ALS) and patient-driven research in clinical studies in MS and ALS. The active research lines are as follows: a) Immunotherapy and mechanisms of disease-linked protein aggregation in neurodegeneration (PD and MS) b) Modulation of innate and adaptive immunity by endogenous neuropeptides in neurodegeneration c) Nanoparticles for diagnostics and for controlled and targeted drug delivery: improving the drugability of neuropeptides d) Role of immune-mediated mechanisms in Amyotrophic Lateral Sclerosis (ALS).





## RESEARCH HIGHLIGHTS

Related to basic immune mechanisms and interventional approaches in PD, we found that exogenous, 'native'  $\alpha$ -synuclein primes microglia and determines subsequent response elicited by TLR stimulation, which could help explain association between certain infections and the onset of sporadic Parkinson's disease. In addition, we showed that combination of  $\alpha$ -synuclein with Hsp70, or with certain other chaperones, can induce differential immune responses in vitro and in vivo as a result of immunization of mice. We also identified a variety of other potential 'immuno-chaperones' by in vitro screening of a set of chaperones with  $\alpha$ -synuclein as an 'amyloid' protein model.

A common feature among several neurodegenerative diseases including PD, MS or ALS is an impairment of neuroprotective mechanisms associated to immune imbalance. In this sense, the characterization of endogenous molecules with both neuroprotective and immunoregulatory properties is of special interest not only in terms of new therapeutic strategies, but particularly taking into consideration the increasing role of immune mediators in central nervous system (CNS) plasticity and homeostasis. The neuropeptide activity-dependent neuroprotective protein (ADNP) was originally cloned from mouse neuroglial cells as a vasoactive intestinal peptide (VIP) responsive gene. ADNP is known to harbour neuroprotective activities that map to the derived sequence peptide NAPVSIQ (termed NAP), which provides potent neuroprotection both, in vitro and in vivo. In this context, we have disclosed for the first time that the neuroprotective peptide NAP regulates neuroinflammatory mediators. Using a model of acute brain inflammation, we reveal that NAP acts as a potent suppressor of inflammation in vivo by inhibiting leukocyte recruitment, microglia activation, and proinflammatory cytokine-chemokine axis. Remarkably, our results in a mouse model of Adnp haploinsufficiency demonstrate and emerging role of the activity-dependent neuroprotective protein (ADNP) in brain immune homeostasis. In a pathological context, in the transgenic mouse model of ALS (SOD1G93A), we characterized a differential profile of VPAC receptors relevant to therapeutic and prophylactic interventions based on NAP and VIP treatments. In this sense, we have delineated for the first time the physiological role of VIP in the progression and disease severity in the ALS mouse model, using SOD1G93AVIP+/- KO mice. The establishment of the SOD1 colony in the laboratory together with access to samples (CSF and PBMCs) from ALS patients have allowed us to identify the role of innate peripheral immune response and intracellular adaptor molecules as potential biomarkers of early versus established ALS.

Peptide-based interventions (immunochaperones or neuropeptides) can be greatly improved in terms of drugability after surface functionalization of noble metal or organic nanoparticles. We have developed smart delivery platforms that enhance the half-life of our bioactive peptides, retaining full biological performance and gaining particular T1/T2 features with extra values as diagnostic tools in MRI.

## Publication Highlights

- Jimenez-Pacheco A., Franco J.M., Lopez S., Gomez-Zumaquero J.M., Magdalena Leal-Lasarte M., Caballero-Hernandez D.E., Cejudo-Guillén M., Pozo D. 2017. Epigenetic Mechanisms of Gene Regulation in Amyotrophic Lateral Sclerosis. *Adv Exp Med Biol.* 978:255-275
- Leal-Lasarte M.M., Franco J.M., Labrador-Garrido A., Pozo D., Roodveldt C. 2017. Extracellular TDP-43 aggregates target MAPK/MAK/MRK overlapping kinase (MOK) and trigger caspase-3/IL-18 signaling in microglia. *FASEB J.* 31:2797-2816
- Yeste A., Takenaka M.C., Mascanfroni I.D., Nadeau M., Kenison J.E., Patel B., Tukpah A.M., Babon J.A., DeNicola M., Kent S.C., Pozo D., Quintana F.J. 2016. Tolerogenic nanoparticles inhibit T cell-mediated autoimmunity through SOCS2. *Science Signaling.* 9(433):ra61
- Caballero-Hernandez D., Toscano M.G., Cejudo-Guillén M., García-Martin M.L., Lopez S., Franco J.M., Quintana F.J., Roodveldt C., Pozo D. 2016. The 'Omics' of Amyotrophic Lateral Sclerosis. *Trends Molecular Medicine.* 22:53-67
- Alcantara D., Lopez S., García-Martin M.L., Pozo D. 2016. Iron oxide nanoparticles as magnetic relaxation switching (MRSw) sensors: Current applications in nanomedicine. *Nanomedicine.* 12:1253-62

## Grants

2015-2018: Retos-Colaboración Projects. MINECO- Ministry of Economy and Competitiveness of Spain. RTC-2015-3309-1







## Genomics

### Scientific Core Services

The main aim of CABIMER Genomics Core Facility, established in 2007, is to provide internal and external researchers resources and services to support their research needs regarding High-throughput Functional Genomics. In recent years, the Microarray and NGS (next generation sequencing) technologies have become essential in biology to perform studies of transcriptomes and genomes at a global scale. At present, there are several different platforms to carry out these studies.

The Facility is equipped with two platforms for Microarray analyses (Affymetrix and Agilent) able to provide services that include analyses on Molecular Cytogenetics, Expression profiles at the mRNA and Gene/Exon Level, Alternative Splicing, miRNA and Chip-on-Chip. In addition, CABIMER possess two NGS (Next Generation Sequencing) platforms, the Ion- Torrent PGM sequencing and recently, Illumina NextSeq500. The Core Facility developed and standardized protocols for whole-genome sequencing, ChipSeq, DRIP-Seq, MNase-Seq, RNA-Seq and many others applications for different eukaryotic species using both platforms. The Core Facility also offers advice for experimental design and data analysis.

The manipulation of a vast amount of samples processed in a reduced period of time, with accuracy and high reproducibility in the Core Facility, allows the researchers to move to a second phase in their studies on either a wide selection of genes or DNA elements as well as single ones. This is possible due to the diverse high content performance technologies that have been heavily improved in the Core Facility by the use of different robots and high-throughput microscopy.



**Andrés Aguilera López**  
Scientific Coordinator



**Eloísa Andújar**  
**Mónica Pérez**  
Technicians



## Biological Resources

### Scientific Core Services

Researchers in Cabimer -50% of research groups in 2016-2017- are already using mouse models in a variety of ways, from basic to translational research. The Biological Resources Unit enables animal experimentation in Cabimer providing the necessary resources under conditions required by national and EU legislation (Spanish RD 53/2013 and EU Directive 2010/63) for the protection of animals used for scientific purposes. The unit ensures observance of all legal and ethical standards related to the use of animals for research at Cabimer. The mission is to provide for the care, health and well-being of animals as well as to provide specialized techniques and equipment for research.

The unit has capacity for some 6000 mice maintained in Specific Pathogen Free (SPF) condition, a health status monitored through a comprehensive health surveillance programme. Cages, bedding and water are sterilized by autoclaving and food is irradiated. Equipment and material for research is decontaminated by hydrogen peroxide vapour. Laboratory space and equipment is available for in vivo imaging, stereotaxic surgery and general procedures. A transgenic laboratory provides techniques for the generation and maintenance of transgenic mice including microinjection of DNA into zygotes, microinjection of ES cells into blastocysts and rederivation by embryo transfer. During this period, the unit has been successful in applications for personnel and equipment required for our activity. Whenever possible, the unit also collaborates with research groups in Cabimer providing specific technical expertise required in their research projects.

**Luis Sánchez Palazón**

Director of the Unit and

**Benoît Gauthier**

(until June 2016)

Scientific Coordinators

**Itziar Benito Latasa de Aranibar**

Veterinarian

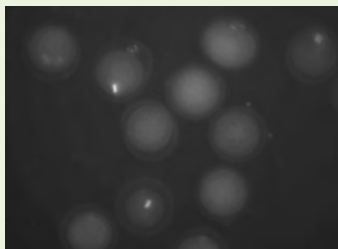
**Flora Guerrero Iglesias**

**Laura Canas Calvo**

**Miriam González Fernández**

**Rosario Segarra Bermúdez**

Technicians



*Enucleation of mouse oocytes*



## Microscopy

Microscopy is an invaluable tool to directly analyze events that take place in the cell or in a live organism. The diverse microscopy techniques facilitate the analysis of the function of proteins, their behavior in some structures, and the relationship among components within a signaling pathway. These studies can be carried out in CABIMER with the support of our Microscopy Facility. This core service provides technical support to scientists from both CABIMER and external entities (public institutions, hospitals and private companies), helping them in all aspects regarding the preparation and development of microscopy experiments: from the experimental design and the use of the instruments to the processing of the data and the analysis of the images. The Microscopy Unit also assists scientists in interpreting and shaping final results. Finally, and in close collaboration with industry partners, the unit is also responsible for the maintenance of the microscopes, to provide the best possible service to our users.

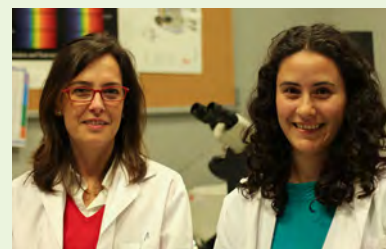
The Microscopy Facility presently counts with state-of-the-art equipment for the development of advanced microscopy techniques, including:

- Three Leica DM6000B vertical fluorescence microscopes for regular microscopy experiments.
- A Nikon Ni-E automated vertical fluorescence microscope, designed for the acquisition of large images or so-called mosaic experiments. The acquisition of a compound big image allows to either show a whole mount/tissue section image at higher detail or to quantify cell phenotypes massively in a single image while maintaining in both cases a high magnification and resolution.
- A Zeiss Axio-Imager 2 inverted fluorescence microscope, equipped with a device (ApoTome) that projects light in a structured way on the sample at different focal planes, creating several images. These subsequent images are processed in real time using an algorithm, which removes the out-of-focus information before reconstructing them into a final optical section. The resulting image is therefore enhanced in terms of quality and sensitivity.
- A Leica DMI6000 inverted fluorescence microscope for real time experiments with live cells.
- Two Leica TCS SP5 confocal microscopes, one of which is also capable of visualizing live cells in real time.

Being the Microscopy facility an essential core service for CABIMER, and despite counting with the previously described equipment, the unit is additionally making a constant effort to increase the number of microscopes, thus allowing the implementation of new microscopy methodologies and providing a better service to all our users. This effort has been rewarded during the 2016-2017 period with the concession of a spinning-disc confocal microscope in the context of a call from the Ministry of Economy and Competitiveness to acquire new scientific and technological infrastructure for research centers. This new equipment will potentiate the capacity of the Microscopy Facility to carry out real time experiments with live cells at a higher resolution, since the spinning-disc confocal microscope allows the acquisition of images at high speed and with high sensitivity, causing less damage and photo-toxicity to the cells.



**Fernando Monje-Casas**  
Scientific Coordinator



**Paloma Dominguez Giménez**  
**Ana Cristina Rivas Rivas**  
Technicians



## GMP

### Scientific Core Services

CABIMER's Good Manufacturing Practices (GMP) core facility is a Unit for ensuring that pharmaceutical products for HUMAN use are CONSISTENTLY manufactured, controlled and documented according to quality standards. GMP is designed to minimize the Risk and ensure the SAFETY of patients enrolled in clinical trials (Regulation (EU) NO 536/2014). The GMP unit of CABIMER is a cell production core facility (UAPC-CABIMER) engaged in the manufacturing of investigational medicinal products "human cells" considered as Advanced Therapy Medicinal Products (ATMPs) in accordance with article 17 of Regulation (EC) n° 1394/2007. The production of ATMPs is carried out in accordance with GMP standards and handled with appropriate controls to ensure their safety, quality, and efficacy as a final medicinal product. UAPC-CABIMER was the first in Andalucía to obtain the Certification from the Spanish Agency of Medicines and Medical Devices (AEMPS, Agencia Española del Medicamento y Productos Sanitarios) to produce cellular medicaments (16 November 2009 and later on February 2012 and April 2015). In order to ensure conformity with the principles and guidelines of good manufacturing practice for investigational medicinal products, a new inspection by the competent authority AEMPS is scheduled for the next April 2018.

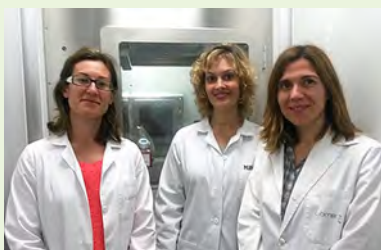
The UAPC-CABIMER facility is a fully equipped 57m<sup>2</sup> installation, with 2 Grade B rooms (ISO 14644-1) for manufacturing ATMPs to use in Clinical Trials and Compassive Use and a fully equipped and independent Quality Control Laboratory. The UAPC-CABIMER follows the strict regulations established by Standard Operating Protocols (SOPs), which cover all aspects of ATMPs manufacturing, from the starting material, recordkeeping, premises, personnel qualifications, sanitation, cleanliness, equipment verification, process validation, and complaint handling to the training and personal hygiene of staff. The full responsibility corresponds to the Scientific Coordinator and the Technical Manager (Technical Director) of the Unit. The UAPC-CABIMER has the adequate number of personnel with the necessary qualifications and practical experience. The responsibilities placed on each individual are not so extensive as to present any risk to quality. The key personnel of UAPC-CABIMER include the head of Production, the head of quality units and the authorized persons. The quality units comprise Quality Assurance (QA) and Quality Control (QC) functions and are combined in one department, the Head of Production and Quality Units are independent of each other. Full-time qualified personnel occupy these key positions ensures running the unit under quality standards at GMP level for the application of investigational medicinal products "human stem cells" as Regenerative Therapies for Cancer, Genetic, Metabolic, Autoimmune, Inflammatory and Degenerative Diseases and, more specifically, to develop autologous cell-based therapies for type 1 and type 2 Diabetes Mellitus and its Complications.

Regulation (EC) No 1394/2007 of the European Parliament and of the Council of 13 November 2007 on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004 (OJ L324, 10.12.2007, p.121).

Regulation (EU) No 536/2014 of the European Parliament and of the Council of 16 April 2014 on clinical trials on medicinal products for human use, and repealing Directive 2001/20/EC (OJ L158, 27.5.2014, p.1).



**Bernat Soria**  
Scientific Coordinator



**Karim Hmadcha**  
Technical Director

**Natalia Escacena**  
**Maria Galvez**  
Quality Units

**Victoria Jiménez**  
Production Unit



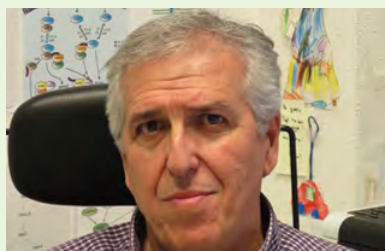
## Citometry and Sorter

Flow cytometry is a powerful tool that measures functional and structural characteristics of heterogeneous mixtures of cells and particles in suspension. Measurements are performed in liquid suspension of cell samples, which flow one cell at a time, through a stream focused to a laser beam at rates up to several thousand particles per second. Both scattered light and fluorescence emitted by the cells are collected, filtered, digitized and sent to a computer for analysis.

The main applications of flow cytometry include immunophenotyping, measurement of cell cycle progression, apoptosis/necrosis, protein expression, among many others. These assays can also utilize the so-called cell sorting technology, which allows the physical isolation of distinct populations of cell of interest for further downstream applications including cell culture, RNA or protein analysis and single cell cloning.

The Cytometry Core Facility of Cabimer provides researchers the opportunity to analyze and sort cells of interest. It is equipped with two BD FACSCalibur Analyzers and one BD FACSAria Cell Sorter. At the end of 2017, the FACSAria Cell Sorter has been upgraded thanks to an equipment grant from the “Ministerio de Economía y Competitividad” (MINECO). The incorporation of a fourth source of excitation, the 561nm laser into the FACSAriaIIIu Cell Sorter, allows to physically separate the excitation and emission of two commonly used fluorochromes, FITC and PE, resulting in the elimination of spillover between the emission spectra of these fluorochromes. In addition, the 561nm laser efficiently excites fruit fluorescent proteins such as mCherry and mTomato. On the other hand, the fluidics upgrade has allowed us to have a more stable, simple and less prone to contamination equipment.

The new version of FACSAriaIIIu Cell Sorter will allow to incorporate the use of new fluorochromes, the design of complex multicolor panels, improve the separation of dim populations and preserve the natural obsolescence of the equipment.



**Abelardo López**  
Scientific Coordinator



**María José Quintero**  
Technician



## Cell Culture

### Scientific Core Services

The Cell Culture Core Facility in CABIMER contains different restricted areas where primary and cell lines cultures are carried out. Five rooms are destined to established cell lines, one room to non-human primary cultures, and a biosafety level II room to infecting cells with viruses. The Facility attends to the requirements from the researchers in order to facilitate the use of equipments in the Facility and provides main reagents used in cell cultures as serum, trypsin, antibiotics, glutamine and PBS. In addition, different fetal bovine serum (FBS) batches are tested yearly in order to select one for common use.

Nowadays, the Facility is equipped with numerous normoxic and hypoxic incubators, safety cabinets, centrifuges, electroporation systems and microscopes. As more specific equipment, the Cell Culture Facility has incorporated a BioSpherix XVivo Incubation System (a workstation for hypoxic conditions) and a cell analyzer xCELLigence® RTCA DP to quantify cell proliferation and morphology changes in a real-time manner. Recently, the infrastructure has been improved with the acquisition of a new ultracentrifuge for isolation of lentiviral vectors.

The Facility makes continuous effort to adapt to the increasing number of users, either by incorporating new areas or by redistributing and optimizing the available space.



**Mario García Domínguez**  
Scientific Coordinator



**M. Mercedes Dana Jiménez**  
Technician



## Model Organisms

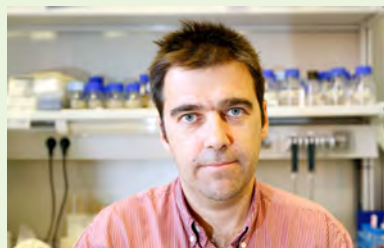
Scientific CABIMER's objectives encompass both the advance in the knowledge of the molecular mechanisms responsible for genetic disorders and cancer and the development of new cellular therapies to address efficiently these diseases. Consistent with these general aims, CABIMER offers a large number of facilities to develop a high quality research based on cell lines and mice. Additionally, CABIMER's research requires the use of different model organisms at two levels:

A. Organisms used as general research tools (required for most research groups). They include the bacteria *Escherichia coli*, which is required for genetic engineering, ectopic expression of recombinant proteins for purification, and in vivo assays of gene expression, the yeast *Saccharomyces cerevisiae*, which is required for in vivo assays for physical interactions between proteins, in vivo assays of gene expression, ectopic expression of recombinant proteins for purification, and vectors for cloning large human and mouse DNA fragments into yeast minichromosomes (YACs), and *Drosophila melanogaster* cell lines infected with baculovirus, which are required for ectopic expression of recombinant proteins for purification.

B. Organisms used as living models by specific research groups to understand the molecular causes of genetic instability and defects in cell cycle progression as two major features of cancer and many genetic disorders. These organisms include the yeast *Saccharomyces cerevisiae*, the worm *Caenorhabditis elegans*, and chicken embryos.

The main objective of this Service is to provide specific facilities for a convenient research with these model organisms. More specifically, this Service is aimed at:

1. Organization, maintenance and handling of specific cell collections
2. Preparation of specific and general solutions and buffers
3. Preparation of media for the growth of different model organisms
4. Growth and collection of high volumes of cell cultures for protein purifications
5. Preparation of competent cells for transformation and electroporation



**Félix Prado**  
Scientific Coordinator



**Cristina Hernández**  
Technician



## Histology

### Scientific Core Services

Histology, as a branch of the morphological sciences, is a very relevant discipline that allows to understand the shape and structure of tissues, and the characterization of abnormalities at the cellular level.

CABIMER has established a very specialized histology service in order to respond the needs of the researches, including tumor tissue characterization, embryo histology, and animal pathology. The samples collected for analysis are treated with the highest quality standards and with the latest technology, providing a full range of histology services to our research community, as well as the neighbor academic and private sectors.

The Histology Core Facility was created in May 2010 as an internal service and since then it has observed an important increment in the demand of the offered services. In last years, we have extended our techniques to different species, including invertebrates, becoming an important support for other academic and research institutions.

The histology laboratory offers advice, protocols and equipment allowing fixation techniques, sectioning of tissues and classical staining for easy viewing of samples. Specific protocols will be provided on demand and upon availability.

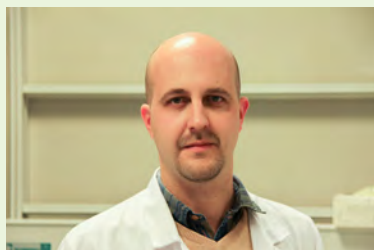
The facility offers methods for the histological analysis of human and animal biological samples. Some of the available methods in this service include the preparation of paraffin embedded samples in the automatic processor of tissue, which simplifies the work of the researches regarding to the manipulation of samples and duration of the protocol. For paraffin blocks and frozen tissues, histological sections can be obtained with an automatic microtome and cryostat, respectively.

For floating samples a vibratome is used. Then, sections can be histological stained or assigned for posterior analysis by immunohistochemistry. The facility is also equipped with a Cytospin for the processing of biological fluids and cell cultures.

The unit is also responsible for new users training and advice in the available equipment. Advanced users have free access to the core facility under internal online booking.



**Anabel Rojas González**  
Scientific Coordinator



**Daniel Rodríguez Martínez**  
Technician



## Washing and Sterilization

The Washing and Sterilization Unit is a basic and fundamental support service for the dynamics of the Research Center. This Unit is responsible for the collection, processing, washing, sterilization and distribution of all the laboratory material as well as the sterilization of the medium and solutions for the whole Research Center (glassware, plastic and consumables). It also handles the processing of the biological waste generated by the research groups as well as by the different support units, meeting all safety regulations for Biohazardous material.

To carry out this work the Unit is in continuous contact with the different research groups and associated support units, in order to offer them a better service and speed up all new demands that arise.

However, due to the incorporation of new research groups to the Centre, the growth of the existing groups, as well as the generation of new services, the Unit has been adapted to provide a more personalized service mainly focusing on the needs of each research group. We had to increase by more than 50% the ordering of glassware plastic material and consumables, since each group works with different types of materials that have to be process in different ways. As a consequence of this adaptation, the equipment of the Unit (autoclaves, thermo-disinfector ...) is continuously in service.

All work processes are executed in accordance with the bio-safety regulations, ensuring at all times the quality of the Sterilization Unit, its management and control.



**Pablo Huertas Sánchez**  
Scientific Coordinator



**Mª Jose Figueroa**  
**Mª Dolores Carrión**  
Technicians



## Biological Safety Unit

### Scientific Core Services

The Unit of Biological Safety provides guidance and advice on all aspects of biological safety at CABIMER, including protection against biological agents, chemicals and radiations. CABIMER is authorized to work with non-encapsulated as well as with encapsulated radioactivity sources and have two different radioisotopes laboratories equipped with all required means of shielding, containment and detection of ionizing radiation. The Unit also has a biological irradiator BioBeam 8000 that allows the study, among other applications, of the repair of genetic damage in different experimental models. The Unit is also in charged, together with the Cell Culture Unit, of a Biosafety level 2 laboratory (P2) equipped to work with biological agents of level 2 such as lentiviral or retroviral vectors. The proper management of biosanitary, toxic and radioactive waste generated in a research center like CABIMER is considered a cornerstone in risks prevention. Improvements in working protocols with chemical or biological agents, information on the risks of each scientific activity, and increase the level to training to researchers, are the main goals of the unit in the last few years. In this context, the continuous incorporation of researcher groups has led to an increase in management and waste generation until reach a production of 24 Tm in 2016-2017.



**José Carlos Reyes Rosa**  
Scientific Coordinator



**Juan Carlos Ostos Vega**  
Technician



## Management and General Services



Management and General Services

CABIMER has an experienced team that provides management support to the scientific community so they can keep their effort on research. The Management and General Services team is in charge of economic control, compliance, purchasing, payments, personnel, labor risk prevention, maintenance and IT issues.

**MANAGER**  
**Pilar Cebolla**

**ASSISTANT**  
**Berta Ferrer**  
Executive Assistant

**HUMAN RESOURCES**  
**Irene González**  
HR Technician

**LABOR RISK PREVENTION**  
**Juan Carlos Ostos**  
Responsible

**ECONOMIC CONTROL**  
**Carmen Ramos**  
Responsible of Economic Control

**Inmaculada Uclés**  
Payment Officer

**PURCHASING**  
**Francisco J. Dorantes**  
Purchasing Manager

**María Isabel Tovaruela**  
Warehouse manager

**IT SERVICE**  
**Arturo Fernández**

**MAINTENANCE**  
**Rafael León**  
Maintenance Manager



## Publications

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## Publications

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- Sastre-Moreno G, Pryor JM, Moreno-Oñate M, Herrero-Ruiz AM, Cortés-Ledesma F, Blanco L, Ramsden DA, Ruiz JF. 2017. Regulation of human pol-lambda by ATM-mediated phosphorylation during non-homologous end joining. *DNA Repair (Amst).* 51:31-45



## Publications

- Schellenberg MJ, Lieberman JA, Herrero-Ruiz A, Butler LR, Williams JG, Muñoz-Cabello AM, Mueller GA, London RE, Cortés-Ledesma F, Williams RS. 2017. ZATT (ZNF451)-mediated resolution of topoisomerase 2 DNA-protein cross-links. *Science*. 357(6358):1412-1416
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- Rodríguez-Jiménez F.J., Alastrue-Agudo A., Erceg S., Stojkovic M., Moreno-Manzano V. 2012. FM19G11 favors spinal cord injury regeneration and stem cell self-renewal by mitochondrial uncoupling and glucose metabolism induction. *Stem Cells*. 30:2221-2233

### Book Chapters 2017

- García-Rubio M, Barroso SI, Aguilera A. 2017. "Detection of DNA-RNA Hybrids In Vivo". *Methods Mol Biol*. 1672:347-361.
- Huertas P, Cruz-García A. 2017. "Single Molecule Analysis of Resection Tracks". *Methods Mol Biol*. 1672:147-154.
- Muñoz-Barrera M, Monje-Casas F. 2017. "Analysis of Cell Cycle Progression in *Saccharomyces cerevisiae* Using the Budding Index and Tubulin Staining". *Methods Mol Biol*. 1505:35-44
- Baro B, Queralto E, Monje-Casas F. 2017. "Regulation of Mitotic Exit in *Saccharomyces cerevisiae*". *Methods Mol Biol*. 1505:3-17

### Editorial 2017

- Roodveldt C, Outeiro TF, Braun JEA. 2017." Molecular Chaperones and Neurodegeneration." *Front Neuroscience*. 11:565. Ed.

### PATENTS

#### 2016

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- Soria B, Hmadcha A, Tejedo J, Bedoya F. Improved cell culture medium for human progenitor cells (HPC). 2016. PCT/EP2016/068767
- Soria B, Salguero C, Hmadcha A, Bedoya F, Tejedo J, Martín F, Tapia R. Methods for obtaining pancreatic beta cell surrogates by increasing pancreatic and duodenal homeobox 1 (PDX-1) expression. 2016 .WO2016/038038
- Morales J, Díaz-Corrales FJ, Valdés L, García-Delgado AB, Montero A. Compuestos acilados para el tratamiento de patologías oculares. 2016. ES1641.1252

#### 2017

- Roodveldt C, Leal-Lasarte M, Franco JM, Pozo D. Agentes moduladores de MOK para el tratamiento de enfermedades con componente inflamatorio, enfermedades neurodegenerativas, enfermedades inflamatorias y cáncer. 2017. P201730383
- Montalvo AM, Gauthier B, Lopez Noriega L, Cobo-Vuilleumier N. Compuestos para el tratamiento de la Diabetes Mellitus. 2017. P201731031
- Soria B, Capilla González V, Hmadcha A, López Beas EJ, Aguilera García Y. Stem Cell-Based Therapy to Improve Neurological functions and prevent radiation-induced brain injury. 201. EP17382607

### SPIN OFF

- Al-Andalus Biopharma S.L. Founder: Bernat Soria
- ARIDDAD Therapeutics S. L. Founders: Benoit R. Gauthier, Nadia Cobo-Vuilleumier and Consortium of European business partners



# Doctoral Theses

## 2016

### • Raquel Araujo Lejido

“Empleo de Mutaciones Activadoras de la Proteína Glucoquinasa en la Terapia Génica Celular de la Diabetes Mellitus” Thesis Supervisors: Dr. Franz Martín and Dr. Antonio L. Cuesta. Universidad Pablo de Olavide y Universidad de Copenhagen.

### • Manuel Carrasco Fernández

“Contribución de los factores GATA a la organogénesis pancreática y a la función del páncreas adulto” Thesis Supervisors: Dra. Anabel Rojas and Dr. Franz Martín. Universidad de Sevilla

### • Natalia Escacena Acosta

“Medicamento Celular como alternativa terapéutica en la Isquemia crónica crítica de miembros inferiores en pacientes diabéticos sin posibilidades de revascularización” Thesis Supervisors: Dr. Bernat Soria and Dr. Abdelkrim Hmadcha. Universidad de Sevilla

### • Carmen Salguero Aranda

“Terapia celular de la Diabetes Mellitus: optimización de los procedimientos de diferenciación e inducción de tolerancia” Thesis Supervisor: Dr. Francisco Bedoya and Dr. Bernat Soria. Universidad Pablo de Olavide

### • Amparo Beltrán Povea

“Estudios sobre la regulación de la funcionalidad de las células pluripotentes por el óxido nítrico” Thesis Supervisors: Dr. Juan Tejedo and Dr. Gladys M. Cahuana. Universidad Pablo de Olavide

### • Marta Cejudo Guillén

“Esclerosis Lateral Amiotrófica: Investigaciones en el modelo SOD1 G93A y correlaciones de interés clínico” Thesis Supervisors: Dra. Diana C Caballero, Dra. Cintia Roodveldt and Dr. David Pozo. Universidad de Sevilla

### • María Jesús Fernández Ávila

“Regulación de la reparación de los cortes de doble cadena en el ADN: papel de la nedilación de proteínas” Thesis Supervisors: Dr. Pablo Huertas and Dra. Sonia Jimeno. Universidad de Sevilla

### • María José Cabello Lobato

“Estudio genético y molecular de mecanismos de resistencia a bilis en salmonella entérica” Thesis Supervisor: Dr. Felix Prado. Universidad de Sevilla

## 2017

### • Andrés Cruz García

“Control de la procesividad de la resección por las proteínas CTIP, BRCA1 y las Topoisomerasas del tipo II” Thesis Supervisor: Dr. Pablo Huertas. Universidad de Sevilla

### • Marta Muñoz Barrera

“Consecuencias de las Alteraciones en la expresión de Aurora Quinasa B sobre la segregación cromosómica y la progresión del ciclo celular” Thesis Supervisor: Dr. Fernando Monje. Universidad de Sevilla

### • Juan Luis Herrera Cabello

“Papel inmunomodulador del péptido neuroprotector NAP derivado de la proteína Activity-dependent Neuroprotective Protein (ADNP)” Thesis Supervisor: Dr. David Pozo Pérez. Universidad de Sevilla/CABIMER

### • Margarita Romero Durán

“A Novel Locus for Cone-Rod Dystrophy and Evaluation of Fox 12 as a Candidate Gene for Retinal Degeneration “ Thesis Supervisor: Dr. Shom S. Bhattacharya and Dr. Kunka Kameranova. Universidad de Sevilla





• **Alejandro Álvarez Quilón**

"Role of ATM in the repair of blocked DNA Double-strand breaks". Thesis Supervisor: Dr. Felipe Cortés. Universidad de Sevilla

• **Macarena Guijo Molero**

"Papel de TDRD9 en oncogénesis " Thesis Supervisor: Dr. José Carlos Reyes. Universidad de Sevilla

• **Marina Arjona Muñoz**

"Papel de Alpha-Tat1 en la dinámica del centrosoma" Thesis Supervisor: Dra. Rosa Ríos. Universidad de Sevilla

• **Ana Cano González**

"Regulación múltiple de la regulación del Sistema TRAIL en apoptosis" Thesis Supervisor: Dr. Abelardo López. Universidad de Sevilla

• **Emilio Javier López Beas**

"Implicación de los MicroRNAs en la obtención de células productoras de insulina a partir de células troncales pluripotentes". Thesis Supervisors: Dr. Bernat Soria and Dr. Abdelkrim Hmadcha. Universidad de Sevilla

• **Adahir Labrador Garrido**

"Alpha-Synuclein and Immune system crosstalk in Parkinson's disease;therapeutic approaches of its modulation by molecular chaperones" Thesis Supervisors: Dra. Cintia Roodveldt and Dr. David Pozo. Universidad de Sevilla

• **Ana López Saavedra**

"Identificación de nuevas proteínas que interaccionan con CTIP: Implicación de PRMT 5 y CCAR2 en la resección del ADN" Thesis Supervisor: Dr. Pablo Huertas. Universidad de Sevilla

• **Francisco de Paula Juárez Vicente**

"Función de la Sumoilación en el desarrollo del sistema nervioso central de vertebrados" Thesis Supervisor: Dr. Mario García Domínguez. Universidad de Sevilla

• **Desiré García Pichardo**

"Papel de la Cromatina en la Formación de R-Loops" Thesis Supervisors: Dr. Andrés Aguilera and Dra. Ana García-Rondón. Universidad de Sevilla

• **Irene Salas Armenteros**

"Nuevos Genes Humanos Asociados a la Biogénesis de mRNPs necesarios para la integridad del Genoma " Thesis Supervisors: Dr. Andrés Aguilera and Dra. Rosa Luna. Universidad de Sevilla

• **Ana Isabel de los Santos Velázquez**

"Búsqueda de nuevos reguladores de salida de mitosis: Hit1 y su papel en el control del ciclo celular" Thesis supervisor: Dr. Fernando Monje Casas. Universidad de Sevilla

• **Cristina Rodríguez Mateo**

"Identificación y Análisis funcional de LNC-SPRY1 como regulador temprano de la EMT. Desarrollo de una nueva metodología para la generación de Gemotecas de esponjas anti-small RNAs" Thesis Supervisor: Dr. José Antonio Pintor. Universidad de Sevilla

• **Isabel Soria Bretones**

"New Insights into CTIP protein: expression inbreast cancer and regulation by sumoylation" Thesis Supervisor: Dr. Pablo Huertas. Universidad de Sevilla

• **Juan Francisco Lafuente Barqueros**

"Papel de factores de la transcripción y la replicación del ADN en el origen de la inestabilidad genómica" Thesis Supervisor: Dr. Andrés Aguilera. Universidad de Sevilla

• **Jenna Lieberman**

"Regulation of TDP2 Function by Sumo Interactions" Thesis Supervisor: Dr. Felipe Cortés. Universidad de Sevilla

• **Estefanía Caballano Infantes**

"Papel del óxido nítrico en la regulación de la respuesta a hipoxia y en la función mitocondrial en células madre" Thesis Supervisors: Dr. F. Bedoya and Dr. Juan Tejedo. Universidad Pablo de Olavide



## Seminar Speakers

### January 2016

"Individual cell contributions to pancreas organogenesis : stochasticity, heterogeneity and self organization" January 15th. **Anne Grapin-Botton**. The Danish Stem Cell Center, Copenhagen, Denmark.

"Limits and thresholds of protein pathways that keep our genomes stable: A conceptual framework to understand cancer" January 29th. **Jiri Lukas**. The Novo Nordisk Foundation Center for Protein Research Copenhagen, Denmark.

### February 2016

"Immune regulatory mechanisms in type 1 diabetes" February 12th. **Lucy Walker**. Institute of Immunity and Transplantation - UCL London, UK.

"Implication of the VRK1 chromatin kinase in the cellular responses to DNA Damage" February 26th. **Pedro Lazo**. Centro de Investigación del Cáncer, Salamanca, Spain.

### March 2016

"Molecular control of self-renewal and differentiation in neural stem cells and brain tumors" March 4th. **Hai-kun Liu**. German Cancer Research Center, Heidelberg, Germany.

"Spatial patterning of recombination by DNA damage response checkpoint kinases" March 11th. **Matt Neale** Genome Damage and Stability Centre, Brighton, UK.

### April 2016

"Regulation of DNA-end resection by CtIP modification" April 8th. **Alessandro Sartori**. Institute of Molecular Cancer Research, Zurich, Switzerland.

"Genotype is not everything: transgenerational epigenetic memory in worms and somatic mutations in tumours" April 29th. **Ben Lehner**. Centre for Genomic Regulation, Barcelona, Spain.

### May 2016

"Coping with DNA damage during chromosome replication" May 13th. **José A. Tercero**. Centro de Biología Molecular Severo Ochoa, Madrid, Spain.

"Topoisomerase-blocked DNA breaks" May 27th. **Felipe Cortés**. CABIMER, Sevilla, Spain.

### June 2016

"The DNA damage response, between genome stability and cellular homeostasis, between bench and bedside" June 10th. **Yossi Shiloh**, The David and Inez Myers Laboratory for Cancer Research, Tel Aviv, Israel.

"Aplicaciones Biomédicas del CNA" June 23rd. **Joaquín Gómez**, Centro Nacional de Aceledadores, Sevilla, Spain.

### September 2016

"Protein oxidation in mitochondria" September 21st, **Johannes M. Herrmann** University of Kaiserslautern, Germany.

### October 2016

"Origin-independent replication priming" October 28th, **Ralf Wellinger**, Cabimer, Sevilla, Spain.

### November 2016

"Dynamic Interplay between chromatin structure, DNA replication and transcription elongation to maintain genome stability" November 4th **María Gómez**. Centro de Biología Molecular "Severo Ochoa", Madrid, Spain.

"Chemistry for drug target validation and identification" November 18th. **María Luz López Rodríguez**. Departamento de Química Orgánica I, Facultad de Ciencias Químicas, Universidad Complutense de Madrid, Madrid, Spain.

### December 2016

"Charting the response to DNA double-strand breaks" December 2nd. **Dan Durocher**. The Lunenfeld Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, Canada.

### January 2017

"Beta-cell reconstitution by islet cell-type interconversion" January 13th. **Pedro Herrera**. Department of Genetic Medicine and Development, University of Geneva Medical Center, Geneva, Switzerland.

"Endocrine disruptors and insulin resistance: is hyperinsulinemia cause or consequence?" January 27th. **Angel Nadal**. CIBER de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Universidad Miguel Hernández de Elche, Spain.



## Seminar Speakers

### February 2017

"Molecular competition and cell size control: a link to cell ageing?" February 10th **Martí Aldea**. Molecular Biology Institute of Barcelona (IBMB), Consejo Superior de Investigaciones Científicas (CSIC), Barcelona, Spain.

"Chromosome and chromatin dynamics during DNA Double Strand Break repair". February 17th. **Gaelle Legube** Université de Toulouse, UPS, LBCMCP, Toulouse, France.

### March 2017

"Endogenous DNA damage: How cells deal with unavoidable". March 10th.. **Jiri Lukas**. Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, Copenhagen, Denmark.

"Reprogramming somatic cell fate and identity: Biomedical application. March 15th. **Angel Raya**. Center of Regenerative Medicine in Barcelona, Barcelona, Spain.

"Genome-wide nucleosome specificity and function of chromatin remodelers in embryonic stem cells". March 24th. **Matthieu Gerard**. Institute for Integrative Biology of the Cell (I2BC), IBITECS, CEA, CNRS, Université Paris-Sud, Université Paris-Saclay, Gif-sur-Yvette, France.

"Tumor genomes shed light into somatic mutational processes and cancer vulnerabilities". March 27th. **Nuria López**. Instituto de Investigación Biomédica, Barcelona, Spain.

### April 2017

"Understanding muscle stem cell regenerative decline with aging". April 7th. **Purificación Muñoz**. Universidad Pompeu Fabra, Barcelona, Spain and Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain.

"ECM remodelling in the stem cell niche: lessons from the Drosophila ovary". April 21st. **Acaimo González Reyes**. Centro Andaluz de Biología del Desarrollo, CSIC, Sevilla, Spain.

### May 2017

"How to fix a broken chromosome". May 12th. **Pablo Huertas**. Centro Andaluz de Biología Molecular y Medicina Regenerativa, Universidad de Sevilla, Sevilla, Spain.

### September 2017

"Establishing nuclear organisation after mitosis". September 21st. **Abderrahmane Kaidi**. Nuclear Dynamics Laboratory, School of Cellular and Molecular Medicine, University of Bristol, Bristol, UK.

### October 2017

"Save the  $\beta$ -cell! A bench-to-bed approach for diabetes". October 2nd. **Kathrin Maedler**. Centre for Biomolecular Interactions Bremen, University of Bremen, Germany.

### November 2017

"Pathway activity models as precision diagnostic and prognostic biomarker linked to cell fusion that uncover disease mechanism". November 10th. **Joaquín Dopazo**. CDCA, Hospital Virgen del Rocío, Seville, Spain.

"Genome Organization Drives Chromosome Fragility. November 17th. **Andre Nussenzweig**. Center for Cancer Research (NIH), Bethesda, USA.

"The Aging Phenomene. Using monogenic DNA repair disorders to understand aging". November 29th. **Morten Scheibye-Knudsen**. Department of Cellular and Molecular Medicine, University of Copenhagen, Denmark.

### December 2017

"Radiobiology in prontontherapy. The synergy between biology and physics". December 19th. **Consuelo Guardiola**. Institut national de physique nucléaire et de physique des particules Université Paris-Saclay, Orsay Ville (France).



## Master Students

### 2016-2017

- Luis Miguel Buch Lorente. Supervisor: Mario García Domínguez. Universidad de Sevilla, (España)
- Ernesto López de Alba. Supervisor: Fernando Monje Casas. Universidad de Sevilla (España)
- Maria Eugenia del Carmen Soler Oliva. Supervisor: Jose Carlos Reyes. Universidad de Sevilla, (España)
- Jody de Jong. Supervisor: Manuel Álvarez Dolado. University of Amsterdam ( The Netherlands)
- M<sup>a</sup> Mercedes Pérez Fernández. Supervisor: Manuel Álvarez Dolado. Universidad de Cadiz (España)
- Mónica González Moreno. Supervisor: Manuel Álvarez Dolado. Universidad de Sevilla (España)
- Laura Galán Pérez. Supervisor: Manuel Álvarez Dolado. Universidad Pablo de Olavide (España)
- Laura Hiraldo Glez. Supervisor: Abelardo López Rivas. Universidad de Sevilla (España)
- Cristina Guillén Mendoza. Supervisor: Andrés Aguilera López. Universidad de Sevilla (España)
- Julia Salmerón Villalobos. Supervisor: Anabel Rojas González. Universidad Pablo de Olavide (España)
- Gonzalo Rafael Vázquez Gómez. Supervisor: Benoit Gauthier. Universidad Pablo de Olavide (España)
- Evie Egelmeers. Supervisor: Benoit Gauthier. Wageningen University (Netherlands)
- Juan Gomez Pinto. Supervisor: Benoit Gauthier. Universidad Pablo de Olavide (España)
- Luz María López Ruíz. Supervisor: Félix Prado Velasco. Universidad de Sevilla, (España)
- Marta Moreno Oñate. Supervisor: Felipe Cortes Ledesma. Universidad de Sevilla (España)
- Raquel Romero Bueno. Supervisor: Felipe Cortes Ledesma. Universidad de Sevilla (España)
- Alejandra Álvarez Llamas. Supervisor: Fernando Monje Casas. Universidad de Sevilla (España)
- Carlos Ruíz Torres. Supervisor: Ralf Wellinger. Universidad de Sevilla (España)
- Rosa Camarillo Daza. Supervisor: Pablo Huertas Sánchez and Sonia Jimeno González. Universidad de Sevilla (España)
- Julia Salmerón Villalobos. Supervisor: Anabel Rojas González. Universidad de Sevilla (España)
- Marta Mozo Mulero. Supervisor: Berta de la Cerda Haynes. Universidad de Sevilla (España)
- Gloria María de Jesús Romero Beltrán. Supervisor: David Pozo. Universidad Nacional Autónoma de Nuevo León, UNANL (México)



## Internal Workshops

**Retiro de Jóvenes Científicos CABIMER**  
**28 Abril 2017**


<b>09.15h</b>	Llegada al Cortijo del Alamillo y Presentación
<b>09.30h</b>	<b>Hayat Heluani</b> "New colorimetric assay for antioxidant capacity and photostability"
<b>10.00h</b>	<b>Carlos Gómez Marín</b> "3D genome architecture in development, evolution and human diseases. An approach to Chromosome Conformation Capture techniques".
<b>10.30h</b>	<b>Irene de Gracia Herrera Gómez</b> "From men to mice: RIP-B7.1 as a novel experimental autoimmune diabetes model"
<b>11.00h</b>	Descanso
<b>11.30h</b>	<b>Javier Manzano López</b> "Herencia Asimétrica y envejecimiento"
<b>12.00h</b>	<b>Cristina González Aguilera</b> "New advances in chromatin purification techniques"
<b>12.30h</b>	<b>Pedro Ortega Moreno</b> "Studying DSB repair in yeast"
<b>13.30h</b>	Comida "paella"
<b>15.30h</b>	Juego colaborativo
<b>17.00h</b>	Fin Jornadas



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RETIREMENT OF YOUNG SCIENTIST 2017

**CABIMER WORKSHOP**  
**December 14<sup>th</sup>-15<sup>th</sup>, 2017**

**cabimer** 

**Thursday Dec. 14<sup>th</sup>**

**9:30 Welcome**

**9:40-10:15 Alejandro Martín-Montalvo**  
*Metabolic interventions for successful aging: CARE4Health*

**10:15-10:50 Tatiana García-Muse**  
*DNA damage dependent phosphorylations during meiosis*

**10:50-11:25 Carmen Palacios**  
*Death under Metabolic Stress in a Breast Tumor Model: Role of the TRAIL system*

**11:25-11:45 Break**

**11:45-12:20 Douglas Maya**  
*Interplay between histone deposition and cohesins modulates chromatin structure and DNA topology*

**12:20-12:55 Elisabet Fernández**  
*MPK1 and its role as a swiss knife to coordinate different stress responses related to manganese*

**15:00-15:35 Vivian Capilla González**  
*Stem cell therapy to prevent radiation-induced brain injury*

**15:35-16:10 Helene Gaillard**  
*New roles for the Nup84 complex in nucleotide excision repair and in the replication of damaged DNA*

**16:10-16:45 Cintia Roodveldt**  
*Molecular chaperones and immunity in protein misfolding diseases*

**Friday Dec. 15<sup>th</sup>**

**10:00-10:35 Francisco Díaz-Corrales**  
*Translating Basic Research to Advanced Retinal Therapies*

**10:35-11:10 Sonia Jimeno**  
*RNA editing affects early steps of homologous recombination*

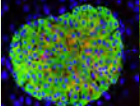
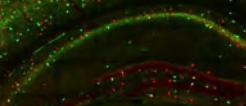
**11:15-11:30 Break**


**11:30-12:05 Magdalena Martínez**  
*Restoring Brain Functions in Alzheimer Model by Transplantation of GABAergic Interneuron Precursors*

**12:05-12:40 Silvia Jimeno-González**  
*Topological crosstalk between transcription initiation and promoter-proximal pausing*

**12:40 Conclusion Remarks**

**Coordinator: Anabel Rojas**

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CABIMER WORKSHOP 2017



## Scientific Advisory Board



**Dr. Francisco Sánchez-Madrid**  
Hospital de La Princesa  
Madrid (Spain)  
Immunology



**Dr. Pier Paolo Di Fiore**  
IFOM- FIRC Institute of Molecular  
Oncology. Milan (Italy)  
Oncology



**Dr. Tony Kouzarides**  
The Gurdon Institute Tennis  
Court Road. Cambridge (UK)  
Cancer Research



**Dr. Roland Kanaar**  
Erasmus University  
Rotterdam (Netherlands)  
Molecular Biology



**Dr. Vivek Malhotra**  
Centre de Regulació Genòmica  
-CRG, Barcelona (Spain). Barcelona  
Coordinator of the Cell & Develop-  
mental Biology Programme



**Dr. Jose M. García Verdugo**  
University of Valencia (Spain)  
Molecular Biology



**Dr. Manuel Serrano**  
Spanish National Cancer Research  
Center-CNIO Madrid (Spain). Director  
of the Molecular Oncology Program



**Dr. Juan Valcárcel**  
ICREA Research Professor at Centre  
de Regulació Genòmica -CRG, Barce-  
lona (Spain). Coordinator of the Gene  
Regulation, Stem Cells and Cancer  
Programme



**Dr. Ramón Gomis**  
Director of IDIBAPS Institut  
d'investigacions Biomèdiques Au-  
gust Pi i Sunyer, Barcelona (Spain)







## Where we are

Europe > Spain > Andalusia > Seville



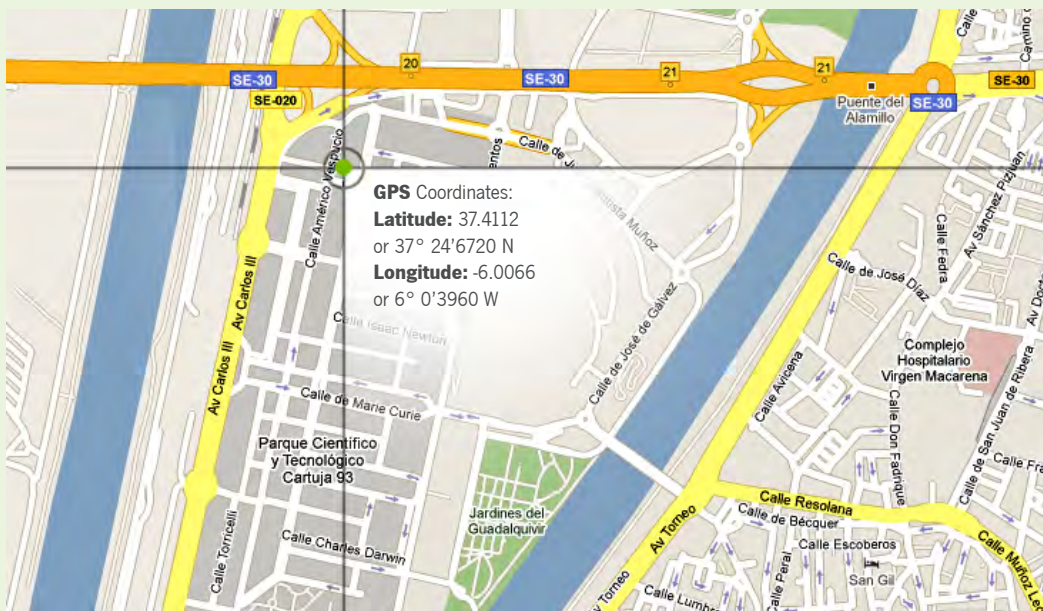
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### Seville Map







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