

RESEARCH
DEVELOPMENT
INNOVATION
2017&2018

CNBREPORT



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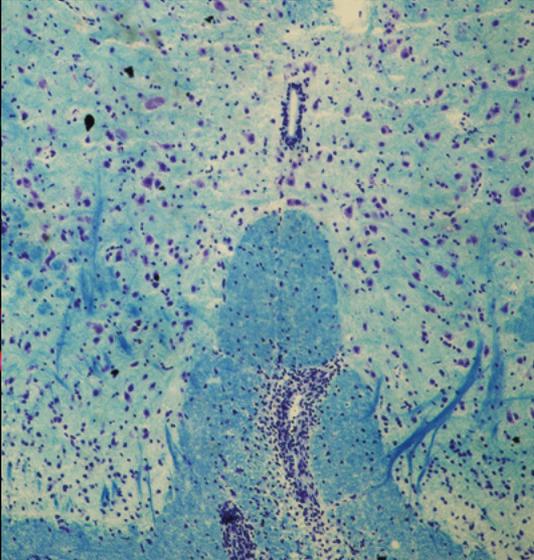
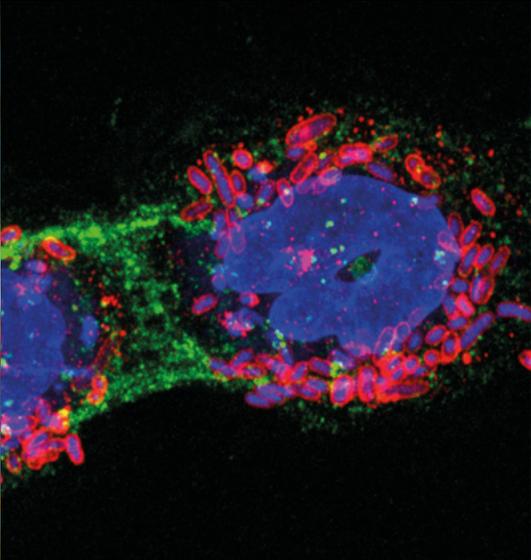
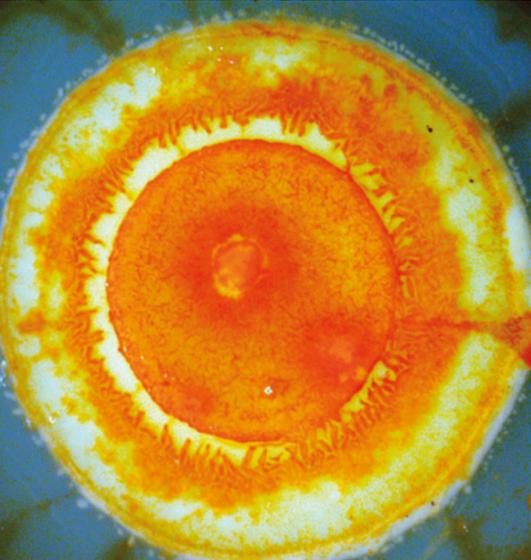
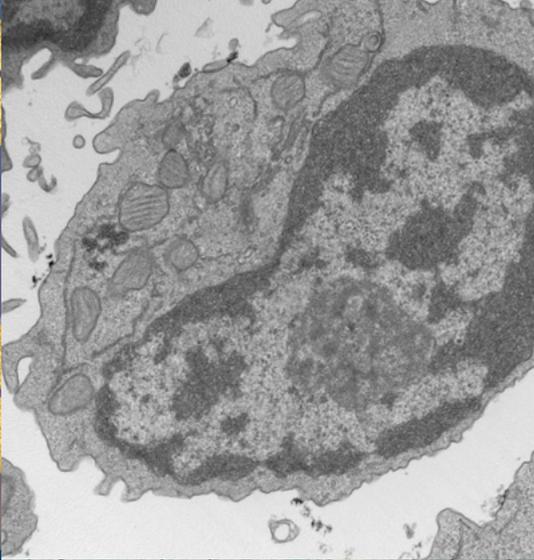
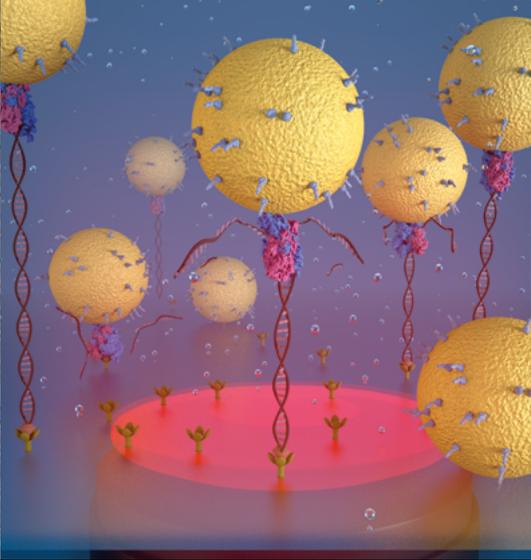
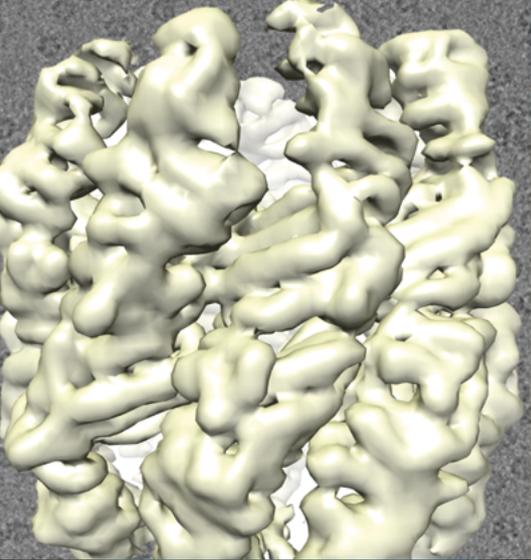
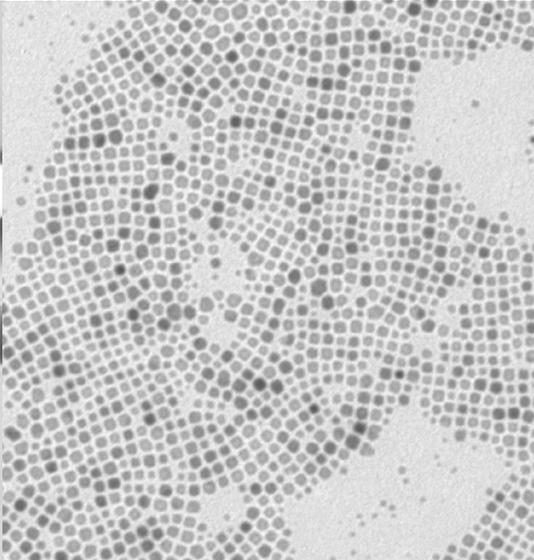
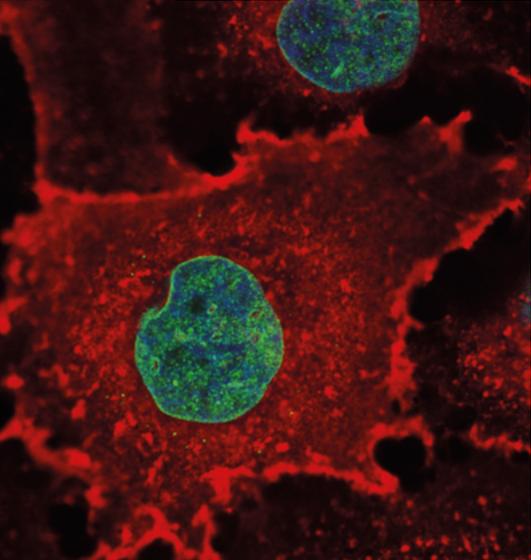
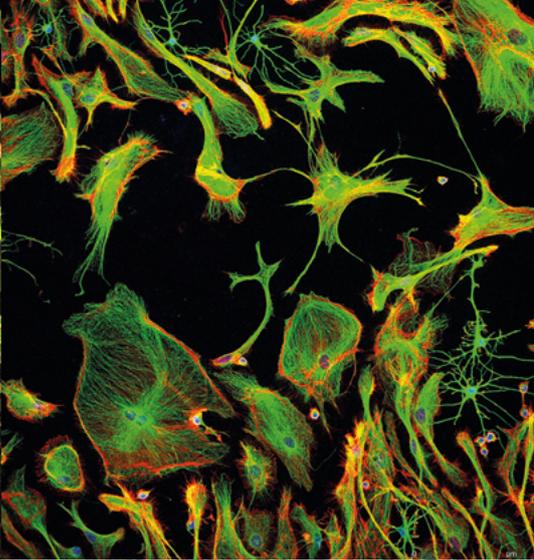
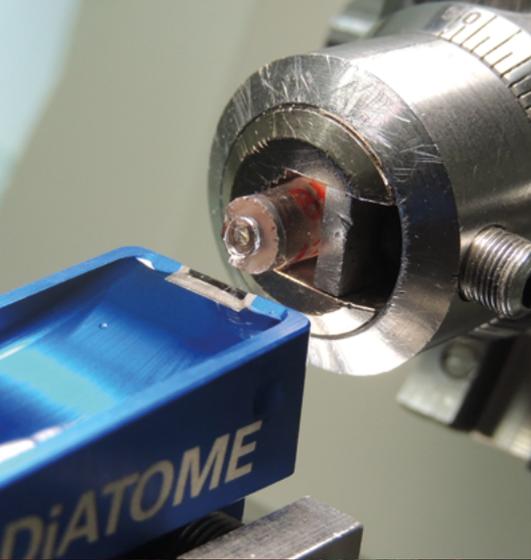
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Welcome to the CNB

Fernando Rojo

DIRECTOR

In 2017, we celebrated the 25th anniversary of our centre. Celebrations were inaugurated by the Secretary of State for Research, Development and Innovation in the presence of the CSIC Vice-president and the Chancellor of the Autónoma University; they included two symposia, a social event and the installation of a time line in the entrance hall with milestones in the history of the CNB. The jubilee year also brought a facelift to the CNB, such as complete refurbishing of its north facade, renovation of seats in the auditorium and painting of the entrance hall and adjacent corridors.

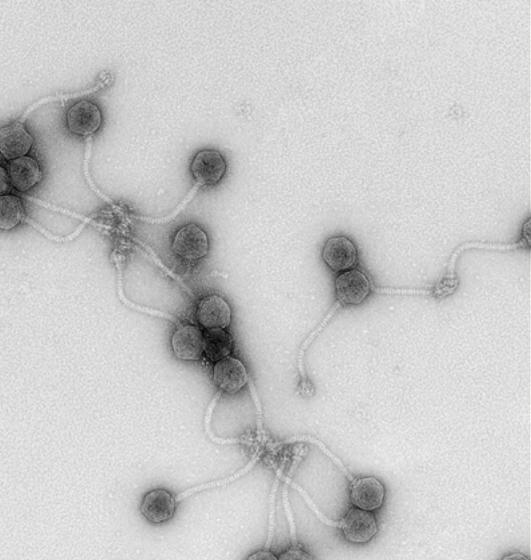
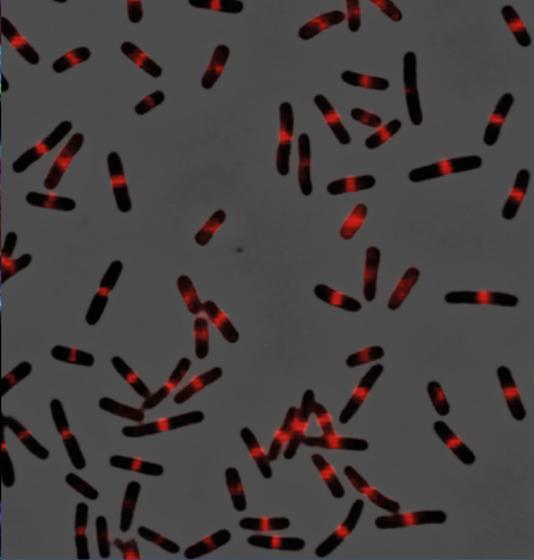
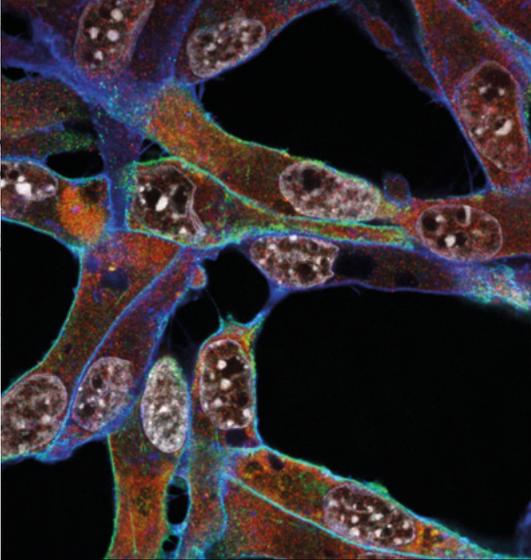
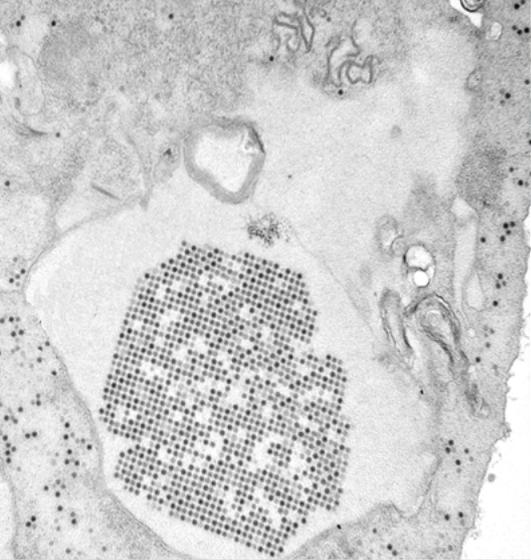
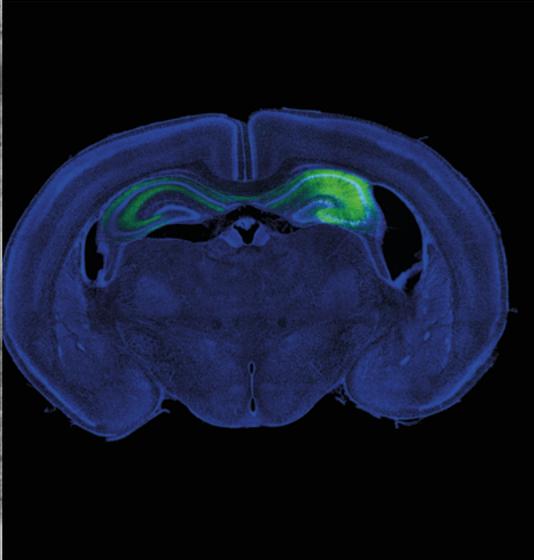
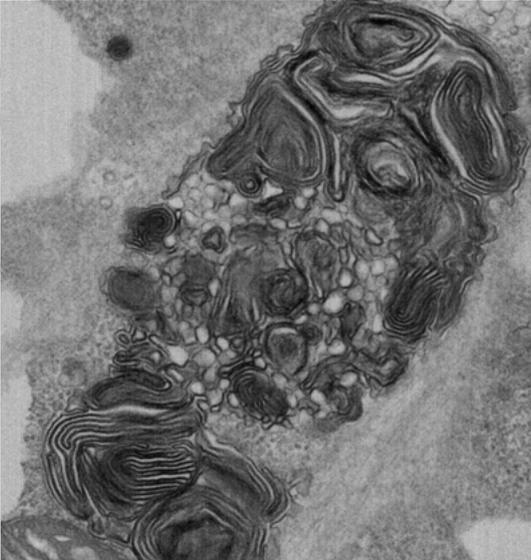
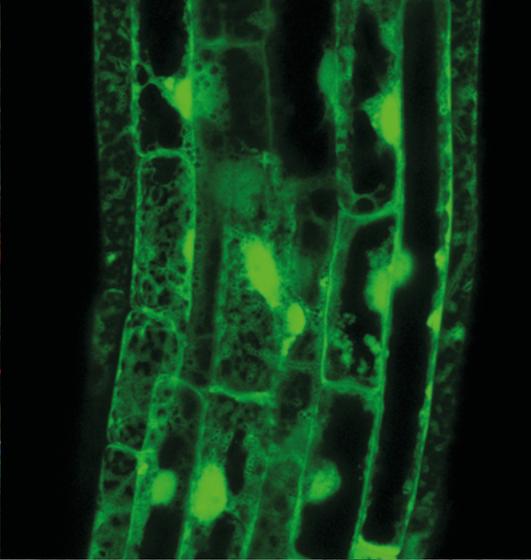
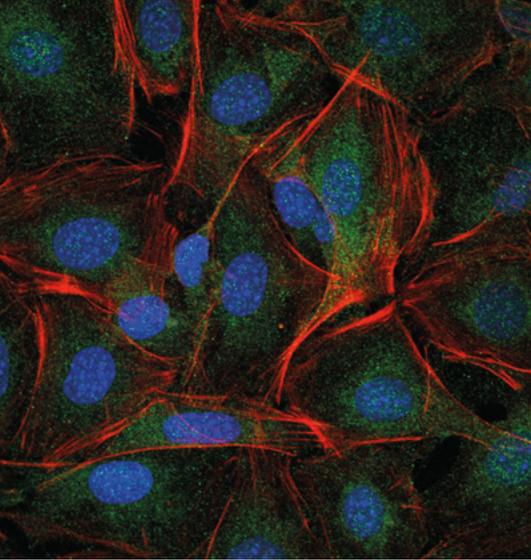
In 2017, we also faced the challenge to renew the accreditation of the CNB as Severo Ochoa Centre of Excellence, which we obtained for the first time in 2014. We successfully passed the 2016 mid-term review by an international panel of experts who concluded that *"the CNB did well and has made good use of the Severo Ochoa funding, which has been essential to help overcome some of the recent shortages."* By the end of 2017, we presented a new 4-year strategic plan focussed on three main areas, namely building a state-of-the-art bio-imaging platform, strengthening capabilities for imaging data analysis and mathematical modelling of biological systems, and becoming a leader in synthetic biology and engineering of biotechnologically interesting bacteria. Our efforts were successful and, in late 2018, we were notified the renewal of our Severo Ochoa distinction. Reviewers indicated that *"CNB has received the SO award in the past and has proven to make good use of this support... The number of researchers has stayed relatively steady during years 2013-2017. The management has been efficient. Overall the CNB has performed very well and maintained its excellent level in a difficult context for Spanish science"*.

Our distinction as Severo Ochoa Centre of Excellence would not have been possible without a distinguishing high activity over the past years. In 2017 and 2018, scientific productivity and fund raising continued at levels similar to previous years. Our scientists published more than 500 articles in JCR-indexed journals. Ranking these journals by their impact factor, we find that 79% and 36% of the centre's scientific output have been published, respectively, in the top 25% and top 10% of journals in their corresponding field of knowledge; the mean impact factor was close to 6.

The filing of 11 new patents, 7 PCT/NPE applications and 10 license agreements reflect our efforts to push the development of research results into marketable products. Apart from numerous activities that established contacts between companies and researchers, the Knowledge Transfer Office organised seminars, round-tables and workshops to raise awareness about diverse aspects of innovation and increase the visibility of the centre's technology offer.

The incorporation of 7 young researchers and 2 senior scientists, who obtained a CSIC position or moved in from other institutes during the past 2 years, will certainly help to keep up with or even improve these benchmarks. We are also confident that ongoing efforts to consolidate a considerable number of temporal positions will provide the stability we need to resist the ups and downs of research funding.

We have continued our efforts to communicate our work to the society. Our Communication and Outreach Office kept up to date our web site, launched a blog, and actively managed our Facebook and Twitter accounts, respectively, that count already on 2,500 and 12,800 followers. The Office organised numerous guided visits to our centre and coordinated our participation in outreach events, such as the European Researcher's Night, the National Science and Technology Week and the 100XCiencia 2 and 3 meetings of SOMMA, the alliance of Spanish Centres of Excellence.



The Communication and Outreach Office was also instrumental in coordinating the centre's training programmes, such as the CNB Course on Introduction to Research for university students or our PhD training programme, which includes a yearly welcome event, a scientific symposium and soft skill training courses. Furthermore, the Office helped with the coordination of more than 300 seminars and courses, which included talks by 43 distinguished speakers in the CNB and CNB Junior seminar series. We are confident that our constant efforts to offer excellent training opportunities and a rich academic life will keep attracting students to the CNB, who follow the example of the 47 brilliant students who, in 2017 and 2018, obtained their PhD degree under the supervision of CNB researchers.

By end of 2018, we finished the renewal of our Scientific Advisory Board with our eyes already on the forthcoming 5-year review of the centre's strategy and performance during the 2016-2020 period.

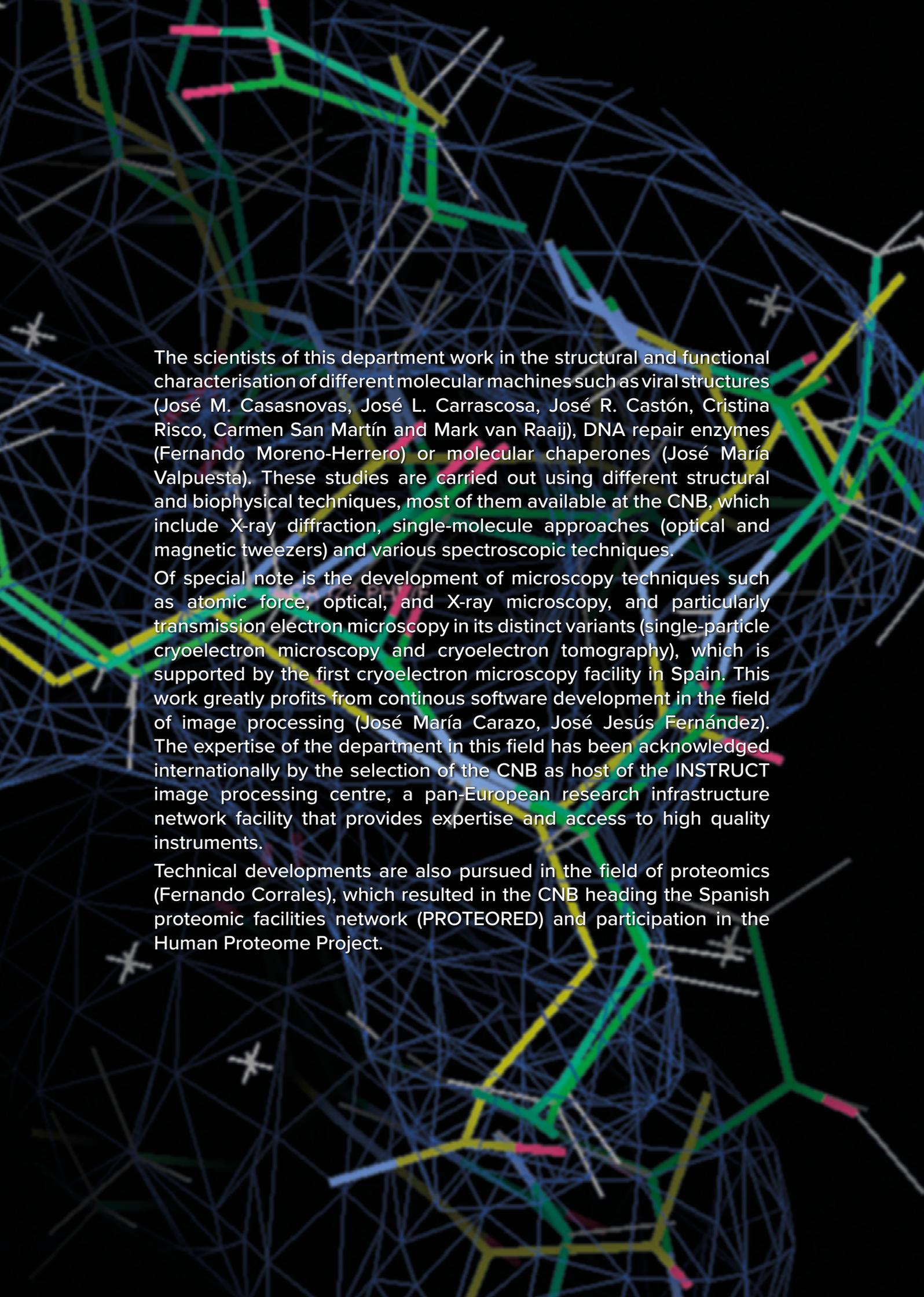
This look back on the past two years cannot finish without expressing my deepest gratitude to Mario Mellado and Peter Klatt, Vice-directors of the CNB, for their outstanding contribution to solve many and frequently complicated scientific, organisation and administrative issues.

Fernando Rojo

Director

If you want to know more about the CNB, please check the following links:

- CNB website: <http://www.cnb.csic.es>
- Blog CNB divulga: <http://divulga.cnb.csic.es>
- CNB publications: <http://www.cnb.csic.es/index.php/en/science-society/publications>
- CNB YouTube: <http://www.youtube.com/user/CNBcsic>
- CNB Facebook: <http://www.facebook.com/CNB.csic>
- CNB Twitter: http://twitter.com/CNB_CSIC



The scientists of this department work in the structural and functional characterisation of different molecular machines such as viral structures (José M. Casasnovas, José L. Carrascosa, José R. Castón, Cristina Risco, Carmen San Martín and Mark van Raaij), DNA repair enzymes (Fernando Moreno-Herrero) or molecular chaperones (José María Valpuesta). These studies are carried out using different structural and biophysical techniques, most of them available at the CNB, which include X-ray diffraction, single-molecule approaches (optical and magnetic tweezers) and various spectroscopic techniques.

Of special note is the development of microscopy techniques such as atomic force, optical, and X-ray microscopy, and particularly transmission electron microscopy in its distinct variants (single-particle cryoelectron microscopy and cryoelectron tomography), which is supported by the first cryoelectron microscopy facility in Spain. This work greatly profits from continuous software development in the field of image processing (José María Carazo, José Jesús Fernández). The expertise of the department in this field has been acknowledged internationally by the selection of the CNB as host of the INSTRUMENT image processing centre, a pan-European research infrastructure network facility that provides expertise and access to high quality instruments.

Technical developments are also pursued in the field of proteomics (Fernando Corrales), which resulted in the CNB heading the Spanish proteomic facilities network (PROTEORED) and participation in the Human Proteome Project.

Macromolecular Structures

HEAD OF DEPARTMENT

José María Valpuesta

RESEARCH GROUPS

- 1. Biocomputing unit**
José María Carazo & Carlos Óscar Sorzano Sánchez
- 2. Structure of macromolecular assemblies**
José L. Carrascosa
- 3. Cell-cell and virus-cell interactions**
José M. Casasnovas
- 4. Viral molecular machines**
José R. Castón
- 5. Functional proteomics**
Fernando Corrales
- 6. Electron tomography and image processing of cell structures**
José Jesús Fernández
- 7. Ultrastructure of viruses and molecular aggregates**
Jaime Martín-Benito
- 8. Molecular biophysics of DNA repair nanomachines**
Fernando Moreno-Herrero
- 9. Cell structure laboratory**
Cristina Risco
- 10. Structural and physical determinants of complex virus assembly**
Carmen San Martín
- 11. Structure and function of molecular chaperones**
José María Valpuesta
- 12. Structural biology of virus fibres**
Mark J. van Raaij



Biocomputing unit

12 MACROMOLECULAR STRUCTURES

GROUP LEADERS

José María Carazo
Carlos Oscar Sorzano Sánchez

SENIOR SCIENTISTS

Joan Segura Mora
Marta Martínez
Roberto Melero del Río

POSTDOCTORAL SCIENTISTS

Amaya Jiménez
David Maluenda
Yunior Fonseca Reyna
Peter Horvath
Erney Ramírez

TECHNICIANS

Pablo Conesa Mingo
Laura del Caño Novales
Carmen Yaiza Rancel Gil

ADMINISTRATION

Blanca E Benítez

PHD STUDENTS

Javier Mota
José Luis Vilas
Rubén Sánchez García
David Strelak

MASTER'S STUDENT

Estrella Fernández Giménez

UNDERGRADUATE STUDENTS

Ana Sanmartín Domenech
Federico de Isidro Gómez
David Herreros Calero

VISITING SCIENTISTS

Roberto Marabini Ruiz
Lisandro Otero

SELECTED PUBLICATIONS

Ljubetič A, Lapenta F, *et al.* Design of coiled-coil protein-origami cages that self-assemble *in vitro* and *in vivo*. *Nat Biotechnol* 2017; 35: 1094-1101.

Segura J, Sanchez-García R, *et al.* 3DBIONOTES v2.0: a web server for the automatic annotation of macromolecular structures. *Bioinformatics* 2017; 33: 3655-3657.

Sacristán C, Ahmad MUD, *et al.* Dynamic kinetochore size regulation promotes microtubule capture and chromosome biorientation in mitosis. *Nat Cell Biol* 2018; 20: 800-810.

Sorzano COS, Vargas J, *et al.* A new algorithm for high-resolution reconstruction of single particles by electron microscopy. *J Struct Biol* 2018; 204: 329-337.

Vilas JL, Gómez-Blanco J, *et al.* MonoRes: Automatic and Accurate Estimation of Local Resolution for Electron Microscopy Maps. *Structure* 2018; 26: 337-344.

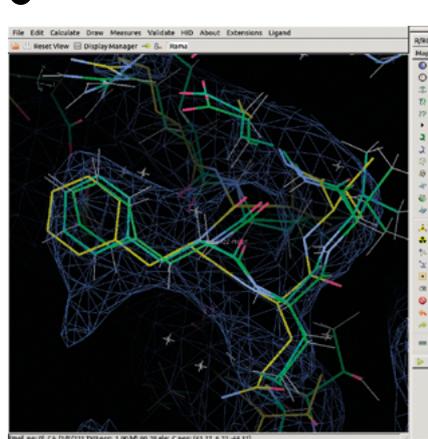
Electron microscopy under cryogenic conditions (cryo EM) is nowadays one of the key technologies to unravel biological complexity, offering the possibility to analyse large and flexible macromolecules in close to their native state to quasi atomic resolution. Indeed, cryo EM was awarded the Nobel Prize in Chemistry in 2017.

Our group is focused on the development of new image processing algorithms capable of dealing with the low signal-to-noise ratios characteristic of these images and, at the same time, trying to extract all the information present in them. We are especially active in the domain of single particle analysis where we cover the whole image processing pipeline from the processing of the movies acquired by the microscope to the accurate 3D reconstruction of the macromolecular structural model under study. We have also proposed new ways of validating the results. All our algorithms are publicly available within the Xmipp software package. We actively collaborate with structural biologists participating in the image processing and structural elucidation of several biological macromolecules of high scientific interest. Additionally, we give structural analysis support to European scientists through the Instruct and iNext platforms, and we are the reference centre for image processing in the European Infrastructure for Structural Biology Instruct-ERIC.

Beside the development of new algorithms, we also develop software infrastructure for the community. In particular, an image processing workflow engine that allows the traceability and reproducibility of the data analysis steps taken from the raw data to the final structure. This workflow engine, called Scipion, is installed in several electron microscopy facilities worldwide, and it has served several thousands of projects around the world.

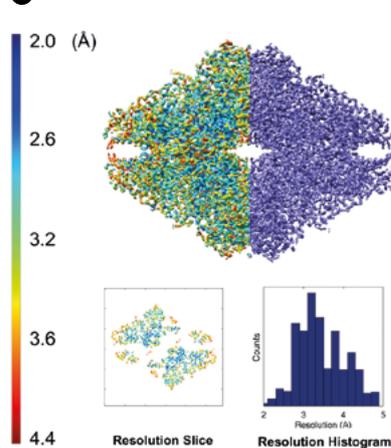
We also play a relevant role in Structural Bioinformatics by connecting the structural information obtained from the electron microscope with atomic models of these macromolecules and a whole range of genomic, proteomic and interactomic information publicly available in bioinformatics databases. This connection helps to better understand the biological properties of the reconstructed structures and has been recognised as one of the few Recommended Interoperability Resources of the European Infrastructure for Life Science Information, ELIXIR.

1



1 Detail of a macromolecular map and the variability of several atomic models fitted to it.

2



2 Example of local resolution result for a macromolecular map.



Structure of macromolecular assemblies

MACROMOLECULAR STRUCTURES 13

GROUP LEADER

José L. Carrascosa

SENIOR SCIENTISTS

Ana Cuervo Gaspar
Rebeca Bocanegra Rojo
Francisco Javier Chichón
M^a Josefa Rodríguez Gómez

TECHNICIAN

Encarna Cebrián

PHD STUDENT

María del Mar Pérez Ruiz

VISITING SCIENTIST

Aleikar José Vásquez Suarez
(Universidad de Concepción, Chile)

SELECTED PUBLICATIONS

Otón J, Pereiro E, Conesa JJ, Chichón FJ, Luque D, Rodríguez JM, Pérez-Berná AJ, Sorzano COS, Klukowska J, Herman GT, Vargas J, Marabini R, Carrascosa JL, Carazo JM. XTEND: Extending the depth of field in cryo soft X-ray tomography. *Sci Rep* 2017; 7: 45808.

Mingorance L, Castro V, Ávila-Pérez G, Calvo G, Rodríguez MJ, Carrascosa JL, Pérez-Del-Pulgar S, Forns X, Gastaminza P1. Host phosphatidic acid phosphatase lipin1 is rate limiting for functional hepatitis C virus replicase complex formation. *PLoS Pathog* 2018; 14: e1007284.

Mata CP, Mertens J, Fontana J, Luque D, Allende-Ballester C, Reguera D, Trus BL, Steven AC, Carrascosa JL, Castón JR. The RNA-Binding Protein of a Double-Stranded RNA Virus Acts like a Scaffold Protein. *J Virol* 2018; 92: e00968-18.

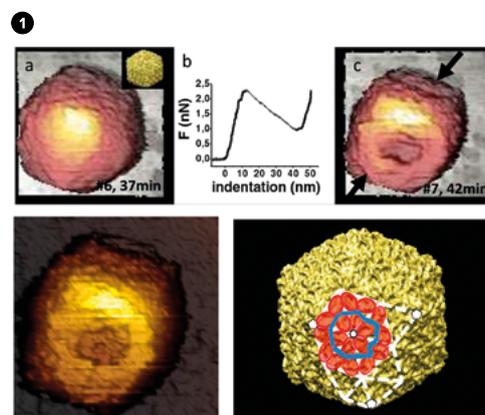
Mertens J, Bondia P, Allende-Ballester C, Carrascosa JL, Flors C, Castón JR. Mechanics of Virus-like Particles Labeled with Green Fluorescent Protein. *Biophys J* 2018; 115: 1561-1568.

López-Andarías A, López-Andarías J, Atienza C, Chichón FJ, Carrascosa JL, Martín N. Tuning optoelectronic and chiroptic properties of peptide-based materials by controlling the pathway complexity. *Chemistry* 2018; 24: 7755-7760.

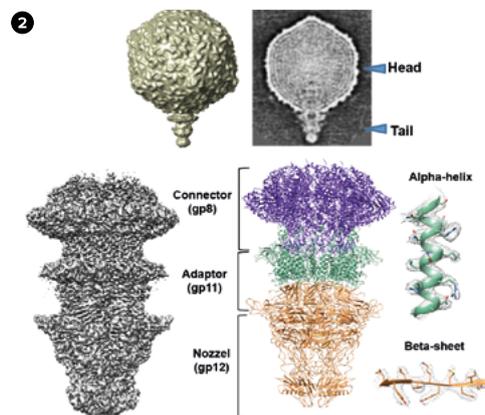
We have continued our work on the analysis of the molecular bases of assembly and nanoscopic properties of different macromolecular complexes, using a combination of cryo-electron microscopy and other biophysical methods such as atomic force microscopy. Our studies have covered different resolution levels. We have carried out whole cell correlation of chemical characterisation and structural determination (introducing soft X-ray microscopy and spectroscopy) for nanoparticle interaction with eukaryotic cells with potential medical applications, as well as for the detailed analysis of the morphogenesis of complex eukaryotic viruses. At a more detailed level (molecular up to atomic resolution), we have studied different aspects of the molecular behaviour of viral-derived nanocontainers as potential vectors for site-directed delivery.

One of the main efforts of our work has been devoted to the study on how viral particles incorporate DNA inside the virus, how the DNA is stabilised, and which are the virus components involved in its ordered delivery upon infection. Extensive use of cryo-electron microscopy on different virus components of phage T7 have revealed the atomic structure of the machinery involved in DNA translocation (the connector and several tail components) which, in turn, has provided the bases for understanding how the DNA is securely packaged inside the viral shell, and how the different tail components assemble sequentially.

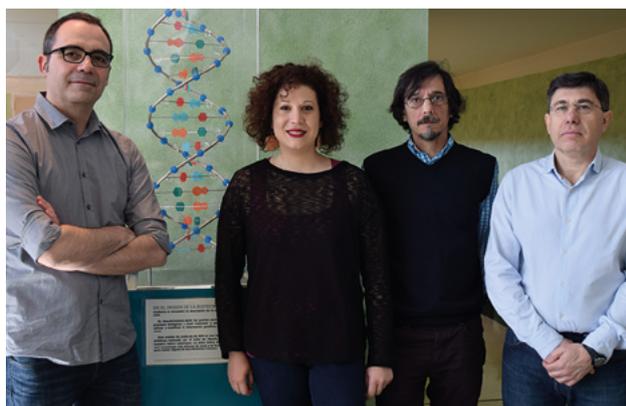
Another main component of the phage head is the core, which is composed of several proteins that dissociate upon viral interaction with the bacterial receptor, and they reassemble again to build a conduit for DNA delivery to the cell cytoplasm. Different complexes derived from core proteins are currently being studied providing first insights on how this complex process is accomplished.



1 Single indentation assay. Phage T7 particles (a) indented using AFM (b) produced the shell breakage following a discrete pattern (c). In the images below, the breakage area (darker) is interpreted on the bases of the T7 shell structure obtained by cryo-TEM. White: lattice lines. Red: capsid subunits. Blue: border of the breakage area following the subunits of one hexamer. Adapted from: De Pablo, Hernández-Pérez, Carrasco and Carrascosa. *J Biol Phys* 2018; 44: 225-235.



2 Cryo-EM structure of the tail of phage T7 at atomic resolution. Upper panel, left: CryoEM reconstruction of phage T7 particles. Upper panel, right: section along a longitudinal axis revealing the features of the head and the tail. Lower panel, left: Cryo-EM reconstruction of isolated tail particles. Secondary structure elements are clearly visible at 3.5 Å resolution. Lower panel, right: Atomic structure of the three components of the tail. As an example, the quality of the resolved data is shown in the case of one α -helix and one β -sheet.



Cell-cell and virus-cell interactions

14 MACROMOLECULAR STRUCTURES

GROUP LEADER

José M. Casasnovas

SENIOR SCIENTIST

César Santiago

POSTDOCTORAL SCIENTISTS

José Luis Torán

Manuela Zonca

TECHNICIAN

David Almendral

UNDERGRADUATE STUDENT

Nuria Sanchez

Universidad de Alcalá de Henares,
Spain

VISITING PhD STUDENT

Ana Belén Peñaherrera

Universidad Tecnológica Nacional,
Nacional, Argentina

Sara Insausti

Universidad Politécnica Valencia, Spain

SUMMER STUDENTS

Ignacio Ramírez Gallego

Silvia Calvo Serrano

SELECTED PUBLICATIONS

Santiago C, Mudgal G, Reguera J, Recacha R, Albrecht S, Enjuanes L, Casasnovas JM. Allosteric inhibition of aminopeptidase N functions related to tumor growth and virus infection. *Sci Rep* 2017; 7: 46045.

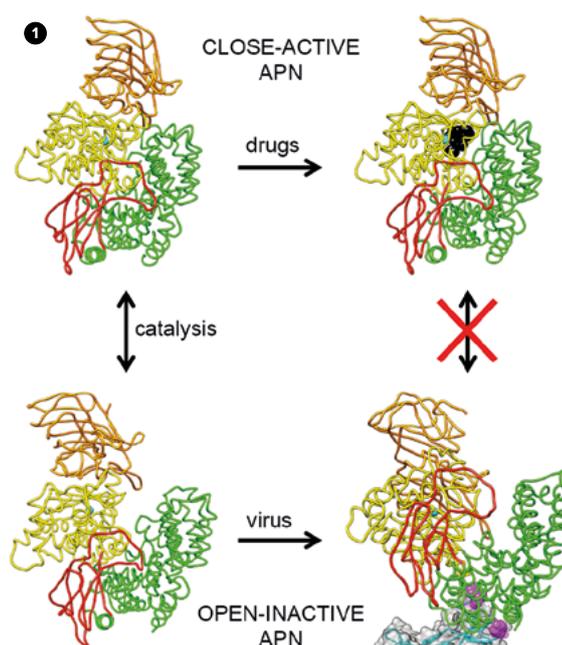
Muñoz-Alía MA, Casasnovas JM, Celma ML, Carabaña J, Liton PB, Fernández-Muñoz R. Measles Virus Hemagglutinin epitopes immunogenic in natural infection and vaccination are targeted by broad or genotype-specific neutralizing monoclonal antibodies. *Virus Res* 2017; 236: 30-43.

Baggen J, Hurdiss DL, Zocher G, Mistry N, Roberts RW, Slager JJ, H Guo H, van Vliet ALW, Wahedi M, Benschop K, Duizer E, de Haan CAM, de Vries E, Casasnovas JM, de Groot RJ, Arnberg N, Stehle T, Ranson NA, Thibaut H, van Kuppeveld FJM. A role of enhanced receptor engagement in the evolution of a pandemic acute haemorrhagic conjunctivitis virus. *Proc Natl Acad Sci USA* 2018; 115: 397-402.

Our group studies the cell surface molecules that regulate the immune system and virus entry into host cells. We analyse receptor-ligand interactions related to immune processes, as well as virus binding to cells. In addition, we characterise virus neutralisation by humoral immune responses and its correlation with virus entry into cells. Our research has provided key observations regarding immune receptor function, and has identified viral epitopes essential for virus infection, some of which are targeted by neutralising antibodies. Our multidisciplinary research applies structural (X-ray crystallography), biochemical and cell biology approaches.

Cell-surface ectoenzymes. Recently, we reported the dynamic conformation of aminopeptidase N (APN), a membrane-bound ectoenzyme associated to various physiological processes and diseases such as blood pressure control, angiogenesis, cell adhesion and motility, tumour cell growth and virus infections. Inhibitors of APN catalysis prevent tumour growth and invasion. We determined several crystal structures that defined the dynamic motion of the APN ectodomain and distinct functional forms (Figure 1), which are probably responsible for its “moonlighting” activity. Viral proteins bound to an inactive APN open form, prevented transition to a closed form and inhibited catalysis. In addition, drugs that target the active site and prevent tumour growth mediated allosteric inhibition of coronavirus cell infection. Blocking APN dynamics can thus be a valuable approach for the development of drugs that target this ectoenzyme.

Antibody neutralisation of viruses. We are analysing how antibodies prevent and neutralise virus infection. Presently we are characterising a human antibody that neutralise Human Immunodeficiency Virus (HIV) and designing bispecific molecules against HIV-1. In addition, we built human antibody libraries that comprise the immunoglobulin repertoire of convalescent patients of Ebola. We aim to identify human antibodies that neutralise Ebola virus.



1 Allosteric inhibition of APN by preventing ectodomain motions. Structures of APN define the dynamic conformation of its ectodomain and different functional forms (closed to open). Peptide catalysis requires the transition between the closed and open APN forms. Coronavirus bind to the open form and prevent transition to the closed form and peptide hydrolysis. Drugs block formation of the open form and inhibit coronavirus infections.



Viral molecular machines

MACROMOLECULAR STRUCTURES 15

GROUP LEADER

José R. Castón

SENIOR SCIENTIST

Javier María Rodríguez Martínez

POSTDOCTORAL SCIENTISTS

Alvaro Ortega Esteban

Carlos Pérez Mata

TECHNICIAN

Esther Marín Forero

PhD STUDENTS

Carolina Allende Ballestero

David Gil Cantero

Celia Gutiérrez García

MASTER'S STUDENT

M^a Jesús Rodríguez Espinosa

UNDERGRADUATE STUDENT

Diego Pérez Dones

VISITING SCIENTIST

Daniel Luque

(Instituto de Salud Carlos III, Spain)

SELECTED PUBLICATIONS

Mata CP, Luque D, Gómez-Blanco J, Rodríguez JM, González JM, Suzuki N, Ghabrial SA, Carrascosa JL, Trus BL, Castón JR. Acquisition of functions on the outer capsid surface during evolution of double-stranded RNA fungal viruses. *PLoS Pathog* 2017; 13: e1006755.

Putri RM, Allende-Ballestero C, Luque D, Klem R, Rousou KA, Liu A, Traulsen CH, Rurup WF, Koay MST, Castón JR, Cornelissen JJLM. Structural characterization of native and modified encapsulins as nanoplatforms for *in vitro* catalysis and cellular uptake. *ACS Nano* 2017; 11: 12796-12804.

Sato Y, Castón JR, Suzuki N. The biological attributes, genome architecture and packaging of diverse multicomponent fungal viruses. *Curr Opin Virol* 2018; 33: 55-65.

Mata CP, Mertens J, Fontana J, Luque D, Allende-Ballestero C, Reguera D, Trus BL, Steven AC, Carrascosa JL, Castón JR. The RNA-binding protein of a double-stranded RNA virus acts like a scaffold protein. *J Virol* 2018; 92: e00968-18.

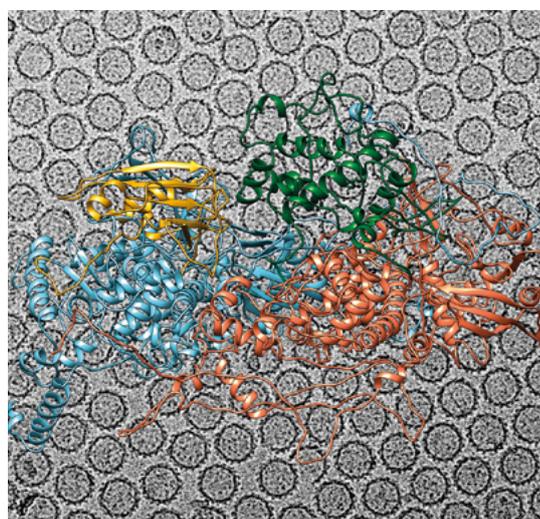
Mertens J, Bondia P, Allende-Ballestero C, Carrascosa JL, Flors C, Castón JR. Mechanics of Virus-like Particles Labeled with Green Fluorescent Protein. *Biophys J* 2018; 115: 1561-1568.

Our studies aim to elucidate structure-function-evolution relationships of viral macromolecular complexes, also known as viral nanomachines, which control many fundamental processes in virus life cycle. Our model systems are the viral capsid and other viral macromolecular complexes, such as helical tubular structures and replication and ribonucleoprotein complexes.

Capsids should be considered dynamic structures, defining different functional states, that participate in multiple processes: virus morphogenesis, selection of the viral genome, recognition of the host receptor, and release of the genome to be transcribed and replicated; some capsids even participate in genome replication. Structural analysis of viruses is therefore essential to understand their properties. To reveal the three-dimensional structure of such complex assemblies, we use a multidisciplinary approach that has led to structural analysis by three-dimensional cryo-electron microscopy combined with atomic structures (hybrid approach). We have incorporated state-of-the-art approaches to obtain near-atomic resolution structure directly from two-dimensional micrographs. Structural analysis of viruses is complemented by study of mechanical properties by atomic force microscopy (AFM), to examine the relationship between physical properties such as rigidity and mechanical resilience, and virus biological function. Finally, our research establishes the basis for incorporation of heterologous proteins and/or chemicals into viral capsids (considered as nanocontainers), of potential use for future biotechnological applications.

Our group studies several viral systems with varying levels of complexity, focused on a number of double-stranded RNA viruses such as birnaviruses (infectious bursal disease virus, IBDV), human picobirnaviruses and several fungal viruses, as well as single-stranded RNA viruses such as rabbit hemorrhagic disease virus (RHDV). Some of these viruses cause serious diseases, and structural characterisation of their macromolecular assemblies will offer new alternatives for altering their function, as well as possible vaccination strategies. We extend our studies to other viruses and eukaryotic complexes in collaboration with several national and international groups.

1



1 Rosellinia necatrix quadrivirus 1 (RnQV1) is a fungal double-stranded RNA virus associated with latent infections of a pathogenic ascomycete. RnQV1 capsid is based on a single-shelled T=1 lattice built of heterodimers of P2 (blue) and P4 (orange). Domain insertions in P2 and P4 provide additional functions at the capsid outer surface. The P2 insertion has a fold similar to that of proteins related to cytoskeleton metabolism (yellow), and the P4 insertion suggests protease activity involved in P2 cleavage (green). The background shows a cryo-electron microscopy image of RnQV1.



Functional proteomics

16 MACROMOLECULAR STRUCTURES

GROUP LEADER

Fernando J. Corrales

SENIOR SCIENTIST

Alberto Paracela

POSTDOCTORAL SCIENTISTS

Miguel Marcilla

Adán Alpízar

Lorena Carmona

PhD STUDENTS

Rosana Navajas

Antonio Ramos

UNDERGRADUATE STUDENT

José Ignacio Granero

SELECTED PUBLICATIONS

Álvarez-Sola G, Uriarte I, Latasa MU, Fernandez-Barrena MG, Urtasun R, Elizalde M, Barcena-Varela M, Jiménez M, Chang HC, Barbero R, Catalán V, Rodríguez A, Frühbeck G, Gallego-Escuredo JM, Gavaldà-Navarro A, Villarroja F, Rodríguez-Ortigosa CM, Corrales FJ, Prieto J, Berraondo P, Berasain C, Ávila MA. Fibroblast growth factor 15/19 (FGF15/19) protects from diet-induced hepatic steatosis: development of an FGF19-based chimeric molecule to promote fatty liver regeneration. *Gut* 2017; 66: 1818-1828.

Mora MI, Molina M, Odriozola L, Elortza F, Mato JM, Sitek B, Zhang P, He F, Latasa MU, Ávila MA, Corrales FJ. Prioritizing Popular Proteins in Liver Cancer: Remodelling One-Carbon Metabolism. *J Proteome Res* 2017; 16: 4506-4514.

Guruceaga E, Garin-Muga A, Prieto G, Bejarano B, Marcilla M, Marín-Vicente C, Perez-Riverol Y, Casal JI, Vizcaíno JA, Corrales FJ, Segura V. Enhanced Missing Proteins Detection in NCI60 Cell Lines Using an Integrative Search Engine Approach. *J Proteome Res* 2017; 16: 4374-4390.

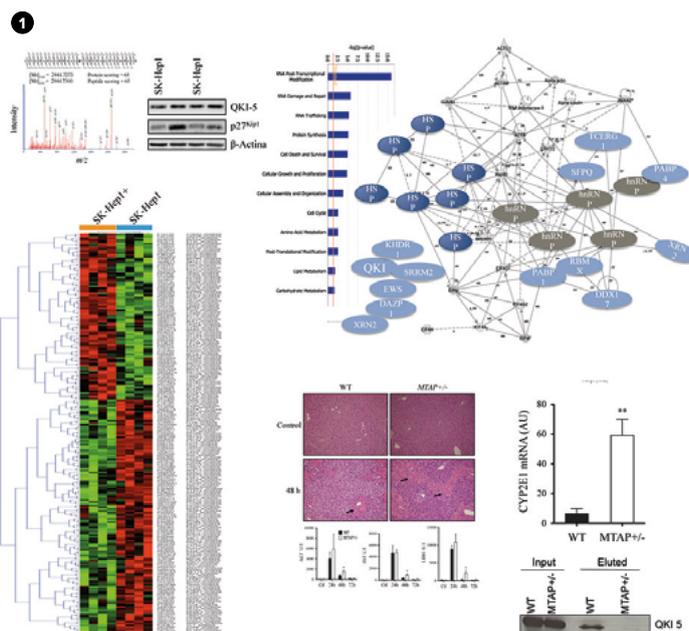
Clemente LF, Hernández ML, Ramos-Fernández A, Ligeró G, Gil C, Corrales FJ, Marcilla I. Identification of the Missing Protein Hyaluronan Synthase 1 in Human Mesenchymal Stem Cells Derived from Adipose Tissue or Umbilical Cord. *J Proteome Res* 2018; 17: 4325-4328.

Mato JM, Elortza F, Lu SC, Brun V, Paracela A, Corrales FJ. Liver cancer-associated changes to the proteome: what deserves clinical focus? *Expert Rev Proteomics* 2018; 9: 749-756.

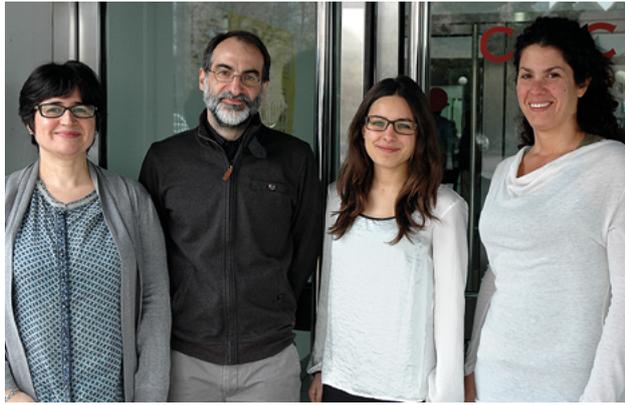
Our main interest is the investigation of the molecular mechanisms underlying the progression of chronic liver disorders. We have identified one carbon metabolism (1CM) as an essential process to preserve the differentiated phenotype of quiescent hepatocytes. 1CM is a master connection between the intermediate metabolism and epigenetic regulation that must be finely tuned since its unbalance triggers a progressive liver ailment leading to non-alcoholic steatohepatitis (NASH) and hepatocellular carcinoma (HCC).

Our work has contributed to elucidate protein networks that explain the molecular pathogenesis of chronic liver diseases and HCC using a functional proteomics approach. Deficiencies on key 1CM enzymes, such as methionine adenosyltransferase (MAT1A) and methylthioadenosine phosphorylase (MTAP) lead to a deregulated methylation capacity of liver cells and a deep reshape of their protein methylation profile (including RNA binding proteins). Assuming the key role of 1CM remodelling in the progression of liver disorders, we have developed a multiplexed mass spectrometry-based method to quantify the enzymes catalysing 1CM reactions. This quantification kit is a straightforward method to follow up chronic liver disease patients.

All these studies are part of the Liver Initiative of the Biology and Disease-Driven Human Proteome Project (B/D-HPP), currently coordinated by our lab. We are also actively participating in the functional annotation of unknown proteins in the framework of the Chromosome Centric Human Proteome Project (C-HPP). Our research has been funded with grants for more than 20 years and our results have been published in more than 140 peer reviewed scientific articles.



1 Functional proteomics analysis reveals changes of protein abundance and methylation that explain mechanisms of liver disease progression.



Electron tomography and image processing of cell structures

GROUP LEADER

José Jesús Fernández

SENIOR SCIENTIST

María del Rosario Fernández

PHD STUDENT

Eva Martín Solana

SELECTED PUBLICATIONS

Fernández-Fernández MR, Ruiz-García D, Martín-Solana E, Chichón FJ, Carrascosa JL, Fernández JJ. 3D Electron Tomography of brain tissue unveils distinct Golgi structures that sequester cytoplasmic contents in neurons. *J Cell Sci* 2017; 130: 83-89.

Moreno JJ, Martínez-Sánchez A, Martínez JA, Garzón EM, Fernández JJ. TomoEED: fast edge-enhancing denoising of tomographic volumes. *Bioinformatics* 2018; 34: 3776-3778.

Fernández JJ, Li S, Bharat TAM, Agard DA. Cryo-tomography tilt-series alignment with consideration of the beam-induced sample motion. *J Struct Biol* 2018; 202: 200-209.

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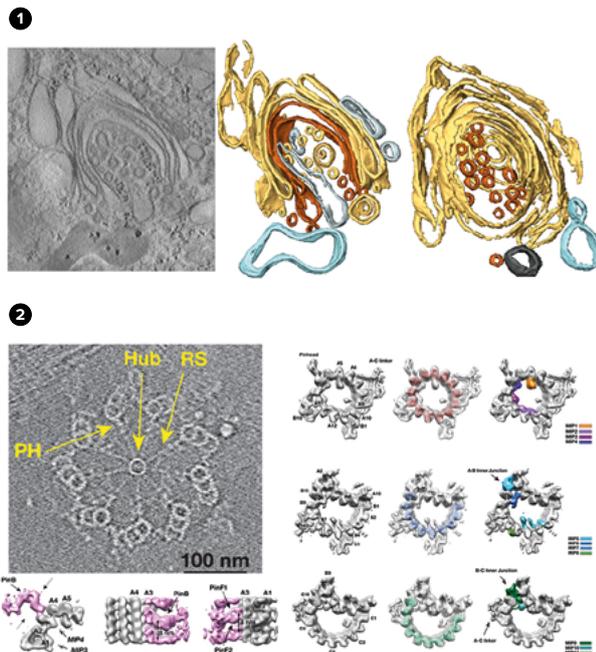
Li S, Fernández JJ, Marshall W, Agard DA. Electron Cryo-Tomography Provides Insight into Procentriole Architecture and Assembly Mechanism. *BioRxiv* 2018; 442590.

Our group is interested in the unique ability of electron tomography (ET) to visualise in three-dimensions the subcellular architecture and macromolecular organisation of cells and tissues *in situ* at a resolution of a few nanometres. Combined with image processing, ET has emerged as a powerful technique to address fundamental questions in molecular and cellular biology.

One of our research interests is focused on the 3D analysis of the neuronal subcellular architecture. Here, ET and image processing are the central techniques along with protocols that ensure preservation of brain tissue samples in close-to-native conditions. With this approach, we are exploring the structural alterations that underlie neurodegenerative diseases, particularly Huntington's disease.

We are also working in close collaboration with Dr. Sam Li (UCSF) in structural elucidation of the microtubule-organising centre (MTOC). This is an important and complex cellular organelle whose dysfunction is linked to many diseases. In addition, we actively collaborate with other teams at the CNB and other international groups in experimental structural studies.

Another important focus of our research is the development of new image processing techniques and tools for the advancement of ET. We are working on new methods for the different computational stages involved in structural studies by ET: image alignment, correction for the transfer function of the microscope, tomographic reconstruction, noise reduction, automated segmentation and sub-tomogram analysis.



1 Three-dimensional visualisation of neuronal subcellular architecture with electron tomography and image processing. Distinct Golgi structures that sequester cytoplasmic contents for their potential degradation were unveiled by electron tomography of brain tissue. These structures are composed of several concentric double-membraned layers that appear to be formed simultaneously by the direct bending and sealing of discrete Golgi stacks.

2 Molecular architecture of procentrioles revealed by electron cryo-tomography and image processing.



Ultrastructure of viruses and molecular aggregates

18 MACROMOLECULAR STRUCTURES

GROUP LEADER

Jaime Martín-Benito

SENIOR SCIENTIST

Rocío Coloma Ciudad

PhD STUDENT

Diego Carlero Carnero

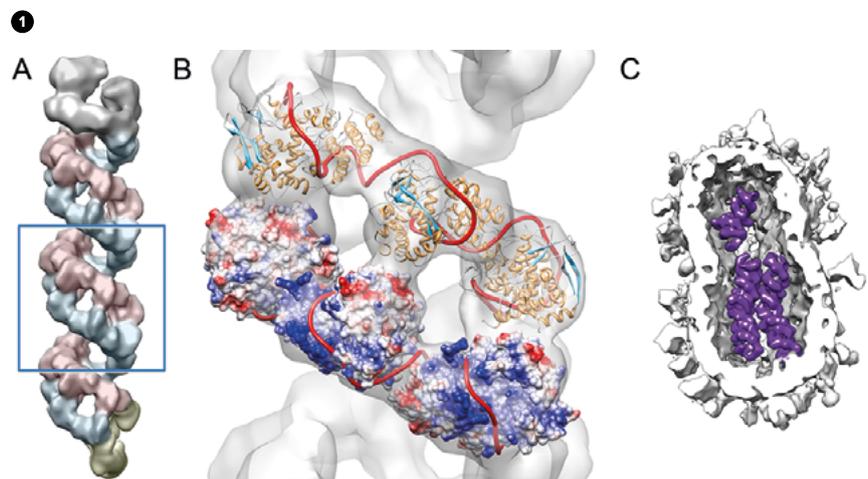
SELECTED PUBLICATIONS

Arranz R, Martín-Benito, Valpuesta JM. Structure and Function of the Cochaperone Prefoldin. *Adv Exp Med Biol* 2018; 1106: 119-131.

Scheffer MP, Gonzalez-Gonzalez L, Seybert A, Ratera M, Kunz M, Valpuesta JM, Fita I, Querol E, Piñol J, Martín-Benito J, Frangakis AS. Structural characterization of the NAP; the major adhesion complex of the human pathogen *Mycoplasma genitalium*. *Mol Microbiol* 2017; 105: 869-879.

The main research line of our group is the study of the influenza A ribonucleoproteins (RNPs) that conform the virus nucleocapsid. RNPs are macromolecular complexes, composed of the genomic RNA bound to multiple monomers of a nucleoprotein, and a single copy of the polymerase. In recent years, our laboratory has determined the structure of isolated RNPs at medium resolution, and by cryoelectron tomography we have verified that this structure is present in native virions.

We are currently pursuing two major lines of research in this field that will extend into coming years. The first is improving the resolution of the RNP structure. For this purpose, we will use the state-of-the-art cryoelectron microscope, equipped with a direct electron detector, that has been installed recently at the CNB. We have already discovered enormous conformational variability in RNP structure, made possible by the design of a new protocol able to classify and reconstruct helical structures. We hypothesised that this extreme conformational variability is closely related to the biological roles of the RNP. With this idea in mind, we opened the second major line of our research, elucidation of the transcription mechanism. We plan to complement structural data with biochemical assays that will allow us to establish the mechanism of action that underlies the biological function of RNPs.



1 A) Structure of the influenza virus ribonucleoprotein (RNP), the RNA polymerase is shown in grey. B) Detail of the helical part showing the nucleoprotein and the modelled RNA position (red thread). C) Tomogram of an influenza A virion showing the arrangement of the RNPs inside the virus.



Molecular biophysics of DNA repair nanomachines

MACROMOLECULAR STRUCTURES 19

GROUP LEADER

Fernando Moreno-Herrero

SENIOR SCIENTISTS

Adriana Gil Gil
Carolina Carrasco Pulido
Silvia Hormeño Torres

COMPUTER SCIENTIST

María Teresa Arranz

POSTDOCTORAL SCIENTISTS

Clara Aicart Ramos
César López Pastrana

PhD STUDENTS

Julene Madariaga Marcos
Alejandro Martín González
Alberto Marín González

SELECTED PUBLICATIONS

Fisher GL, Pastrana CL, Higman VA, Koh A, Taylor JA, Butterer A, Craggs T, Sobott F, Murray H, Crump MP, Moreno-Herrero F, Dillingham MS. The structural basis for dynamic DNA binding and bridging interactions which condense the bacterial centromere. *Elife* 2017; 6: e28086.

Marin-Gonzalez A, Vilhena JG, Perez R, Moreno-Herrero F. Understanding the mechanical response of double-stranded DNA and RNA under constant stretching forces using all-atom molecular dynamics. *Proc Natl Acad Sci USA* 2017; 114: 7049-7054.

Fuentes-Pérez ME, Núñez-Ramírez R, Martín-González A, Juan-Rodríguez D, Llorca O, Moreno-Herrero F, Oliva MA. TubZ filament assembly dynamics requires the flexible C-terminal tail. *Sci Rep* 2017; 7: 43342.

Martín-García B, Martín-González A, Carrasco C, Hernández-Arriaga AM, Ruiz-Quero R, Díaz-Orejas R, Aicart-Ramos C, Moreno-Herrero F, Oliva MA. The TubR-centromere complex adopts a double-ring segrosome structure in Type III partition systems. *Nucleic Acids Res* 2018; 46: 5704-5716.

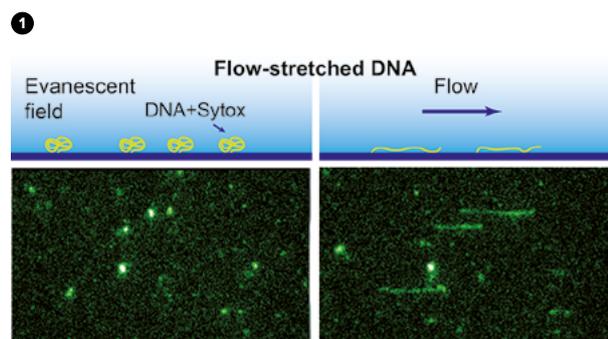
Madariaga-Marcos J, Hormeño S, Pastrana CL, Fisher GLM, Dillingham MS, Moreno-Herrero F. Force determination in Lateral Magnetic Tweezers combined with TIRF microscopy. *Nanoscale* 2018; 10: 4579-4590.

Our group develops and employs single-molecule techniques to study the inner workings of protein machines involved in the repair, replication and maintenance of chromosome structures. We use novel single-molecule approaches based on atomic force microscopy and magnetic tweezers, as well as molecular dynamics simulations.

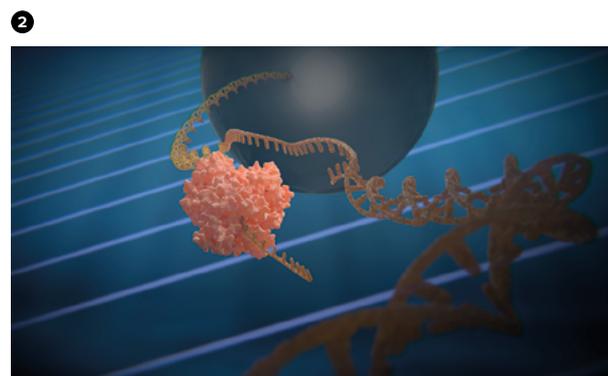
In the last two years, we have developed and implemented a module to laterally stretch DNA molecules at a constant force, in our regular and combined magnetic tweezers (MT)–TIRF setups. The compatibility of the module with TIRF microscopy and the parallelisation of measurements was shown by characterising DNA binding by the ParB protein from *Bacillus subtilis*. Simultaneous MT pulling and fluorescence imaging demonstrated the non-specific binding of *BsParB* to DNA and its dynamic interaction under conditions restrictive to condensation. We also found that the central DNA binding domain of ParB is essential for anchoring at *parS*, but this interaction is not required for DNA condensation. Further work on the mechanism of generation of ParB networks unveiled a dual role for the C-terminal domain of ParB as both DNA binding and bridging interface.

We also investigated the architecture of the segrosome complex in Type III partition systems. We presented the particular features of the centromere site, *tubC*, of the model system encoded in *Clostridium botulinum* prophage c-st. Within the same project, we unravelled the molecular basis for TubZ filaments assembly and dynamics combining electron and atomic force microscopy and biochemical analyses.

Other line of work was the study of the mechanical properties of double-stranded DNA and double-stranded RNA subjected to force and torque by using constant-force, all-atom, microsecond-long molecular dynamics. The methodology and results have opened the field to explore larger forces to test experimental measurements, and to challenge the predictions given by our simulations.



1 Visualisation of fluorescence-labelled DNA molecules by stretching them with a flow in a combined Magnetic Tweezers TIRF setup.



2 Representation of an experiment using a Magnetic Tweezers setup where the action of individual proteins on DNA can be monitored in real time.



Cell structure laboratory

20 MACROMOLECULAR STRUCTURES

GROUP LEADER

Cristina Risco

SENIOR SCIENTISTS

Isabel Fdez. de Castro Martín
Raquel Tenorio Vela
Beatriz Pacheco González

TECHNICIAN

Sara Yolanda Fdez. Sánchez

PHD STUDENTS

Moisés García Serradilla
Paula Ortega González
Alberto Fernández Oliva

SELECTED PUBLICATIONS

Fernández de Castro I, Barajas D, Fernández JJ, Nagy PD, Risco C. Three-dimensional imaging of the intracellular assembly of a functional viral RNA replicase complex. *J Cell Sci* 2017; 130: 260-268.

de Castro Martín IF, Fournier G, Sachse M, Pizarro-Cerda J, Risco C, Naffakh N. Influenza virus genome reaches the plasma membrane via a modified endoplasmic reticulum and Rab11-dependent vesicles. *Nat Commun* 2017; 8: 1396.

Tenorio R, Fernández de Castro I, Knowlton JJ, Zamora PF, Lee CH, Mainou BA, Dermody TS, Risco C. Reovirus σ NS and μ NS proteins remodel the endoplasmic reticulum to build replication neo-organelles. *MBio* 2018; 9: e01253-18.

Sachse M, Fernández de Castro I, Fournier G, Naffakh N, Risco C. Electron microscopy imaging of influenza A virus ribonucleoproteins transport and packaging. *Methods Mol Biol* 2018; 1836: 281-301.

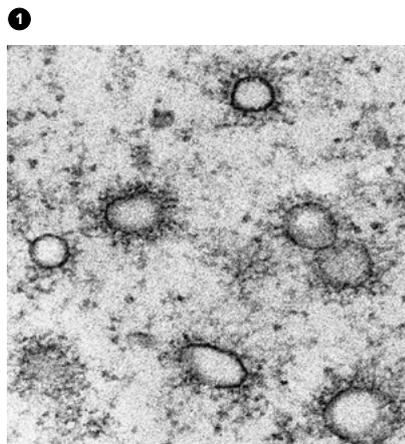
Knowlton J, Fernández de Castro I, Gestaut D, Zamora P, Ashbrook A, Frydman J, Risco C, Dermody T. The TRIC chaperonin controls reovirus replication through outer-capsid folding. *Nat Microbiol* 2018; 3: 481-493.

Antiviral drugs to treat many of the most troubling viruses have not been approved yet. One of the current strategies for antiviral drug development is the search for host cellular pathways used by many different viruses. Viruses alter lipid synthesis and flows to build replication neo-organelles or factories. Despite the importance of factories in viral infections, there are key gaps in knowledge about how they form and mediate their functions.

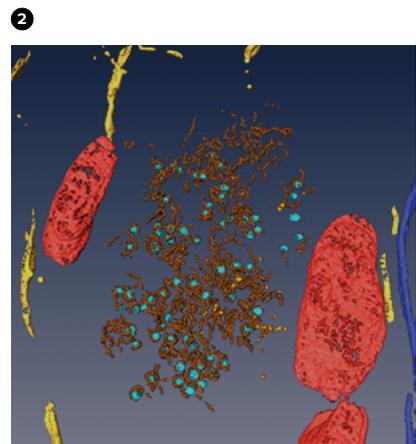
Our lab is pioneer in combining light and electron microscopy techniques to study viral infections. We have discovered that influenza virus builds a new organelle to transport its genome inside infected cells. This work received the European Microscopy Society 2017 Outstanding Paper Award in Life Sciences. With correlative light and electron microscopy (CLEM) we demonstrated that the replication neo-organelles of the human reovirus, also known as viral inclusions (VI), form by major remodelling of the endoplasmic reticulum and that ER tubulation and vesiculation are mediated by the reovirus σ NS and μ NS proteins, respectively.

High-throughput screening of clinically tested compounds provides a rapid means to identify undiscovered, antiviral functions for well-characterised therapeutics. We have selected 9 different drugs and studied their capacity to block bunyavirus infection. Four of these compounds are repurposed drugs validated originally to treat other pathologies. Our studies included CLEM, cell sorting and proteomics. One of these compounds has been selected for additional studies due to its low toxicity and high efficiency against bunyavirus infection.

We are currently studying how RNA viruses hijack lipid transfer proteins (LTPs) and mitochondrial proteins to build replication factories. Furthermore, we are looking for inhibitors of these LTPs and mitochondrial proteins using state-of-the-art computational tools and databases of clinically approved drugs. Our goal is to identify new targets for antiviral drug development and to validate broad-spectrum antivirals to treat many pathogenic viruses.



1 Transmission electron microscopy of ICVs (irregularly coated vesicles), a new organelle built by influenza virus to transport its genome.



2 Double-tilt electron tomography of a reovirus inclusion. The VI is a collection of tubules and vesicles with viral particles (blue) attached to membranes (brown). Mitochondria are coloured in red.



Structural and physical determinants of complex virus assembly

MACROMOLECULAR STRUCTURES 21

GROUP LEADER

Carmen San Martín

SENIOR SCIENTIST

Roberto Marabini Ruiz
(Visiting scientist, Universidad Autónoma de Madrid, Spain)

POSTDOCTORAL SCIENTISTS

Gabriela N. Condezo Castro
Mercedes Hernando Pérez

TECHNICIAN

Mara I. Laguna Castro

PhD STUDENTS

Marta Pérez Illana
Natalia Martín González
José Gallardo Hernanz

MASTER'S STUDENT

Eva Arriero País

UNDERGRADUATE STUDENTS

Marina Moreno Mayorga
Manuel Palacios Pérez
Adrián Plaza Pegueroles
Clara Rivero Rodríguez
Marina Botello Marabotto

SELECTED PUBLICATIONS

Condezo GN and San Martín C. Localization of adenovirus morphogenesis players, together with visualization of assembly intermediates and failed products, favor a model where assembly and packaging occur concurrently at the periphery of the replication center. *PLoS Pathog* 2017; 13: e1006320.

Marion S, San Martín C, Siber A. Role of Condensing Particles in Polymer Confinement: A Model for Virus-Packed "Minichromosomes". *Biophys J* 2017; 113: 1643-1653.

Menéndez-Conejero R, Nguyen TH, Singh AK, Condezo GN, Marschang RE, van Raaij MJ, San Martín, C. Structure of a Reptilian Adenovirus Reveals a Phage Tailspike Fold Stabilizing a Vertebrate Virus Capsid. *Structure* 2017; 25: 1562-1573.

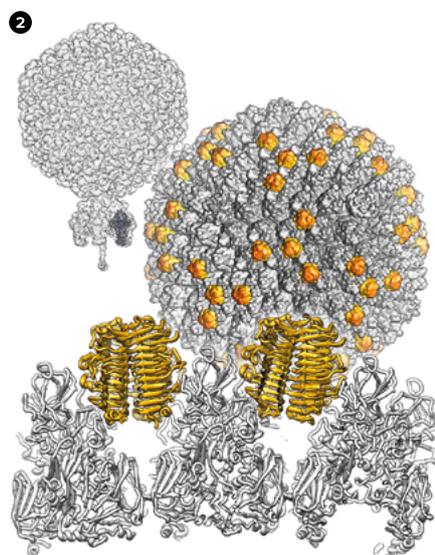
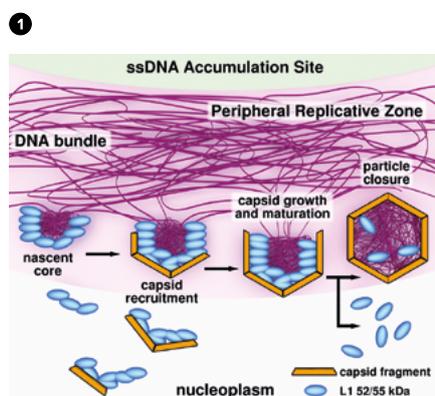
San Martín C and van Raaij MJ. The so far farthest reaches of the double jelly roll capsid protein fold. *Virology* 2018; 15: 181.

Ortega-Esteban A, Martín-Gonzalez N, Moreno-Madrid F, Llauro A, Hernando-Pérez M, San Martín C, de Pablo PJ. Structural and Mechanical Characterization of Viruses with AFM. *Methods Mol Biol* 2019; 1886: 259-278.

We are interested in the principles governing complex virus assembly. Our main model system is adenovirus, a specimen of interest in both basic virology and nanobiomedicine. Adenoviruses are human pathogens but can be engineered as therapeutic tools. With a 95 nm capsid composed of more than 10 different proteins, adenovirus is among the most complex non-enveloped icosahedral viruses. Of the approximately 200 adenovirus types found so far in nature, only a few have been characterised, and there are still considerable open questions regarding the infectious particle architecture and assembly.

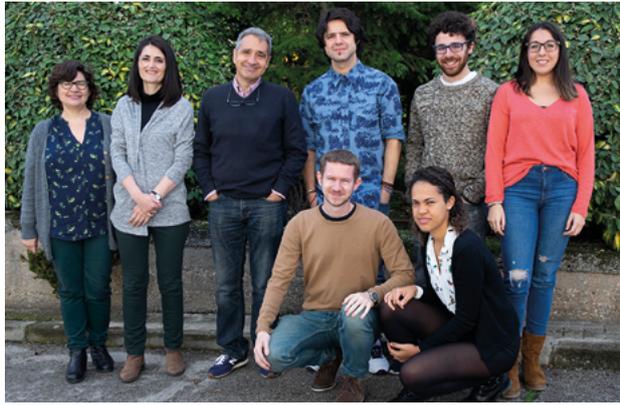
Our research lines focus on answering some of these questions, such as: how the adenovirus capsid is assembled and the genome packaged within the capsid; what are the key elements modulating virion stability; and what are the physicochemical properties of uncharacterised adenoviruses, with potential uses as alternative vectors. We use a multidisciplinary approach to this problem that combines biophysics, structural and molecular biology techniques. Within the past two years, we have used immunofluorescence and immunoelectron microscopy to define the location of the human adenovirus assembly factory in the infected cell. We have also provided evidence

indicating that adenovirus capsid assembly and genome packaging occur in a concerted manner, rather than sequential like in other dsDNA viruses. As a consequence of these studies, we have proposed a model for the mechanism of adenovirus assembly (Figure 1). Using a combination of cryo-electron microscopy and protein crystallography (in collaboration with Mark J. van Raaij at the CNB), we have discovered that the capsids of adenoviruses infecting reptiles are stabilised by a cementing protein with a trimeric beta-helix fold that, in viruses, had only been previously found in receptor binding proteins of bacteriophages (Figure 2).



1 A model for the adenovirus assembly mechanism (modified from Condezo & San Martín, *PLoS Pathog* 2017; 13: e1006320).

2 Structure of snake adenovirus type 1. The cementing protein LH3 (highlighted in gold), has a fold typical of bacteriophage tailspikes, and makes multiple contacts with the major coat protein (hexon, in grey) to stabilise the capsid. Bacteriophage P22 capsid (EMDB-8005) with the tailspike protein in blue (PDB-1TSP) is shown in the top left corner for comparison.



Structure and function of molecular chaperones

22 MACROMOLECULAR STRUCTURES

GROUP LEADER

José María Valpuesta

SENIOR SCIENTISTS

Jorge Cuéllar
Rosario Fernández

POSTDOCTORAL SCIENTIST

Lucía Quintana

PHD STUDENTS

Teresa Bueno
Marcos Gragera
Moisés Maestro
Lissette Ochoa

MASTER'S STUDENTS

Miguel Cantero
José Gallardo
Juan García-Bernalt

SELECTED PUBLICATIONS

Fernández-Fernández MR, Gragera M, Ochoa-Ibarrola L, Quintana-Gallardo L, Valpuesta JM. Hsp70 - a master regulator in protein degradation. *FEBS Lett* 2017; 591: 2648-2660.

Sot B, Rubio-Muñoz A, Leal-Quintero A, Martínez-Sabando J, Marcilla M, Roodveldt C, Valpuesta JM. The chaperonin CCT inhibits assembly of α -synuclein amyloid fibrils by a specific, conformation-dependent interaction. *Sci Rep* 2017; 7: 40859.

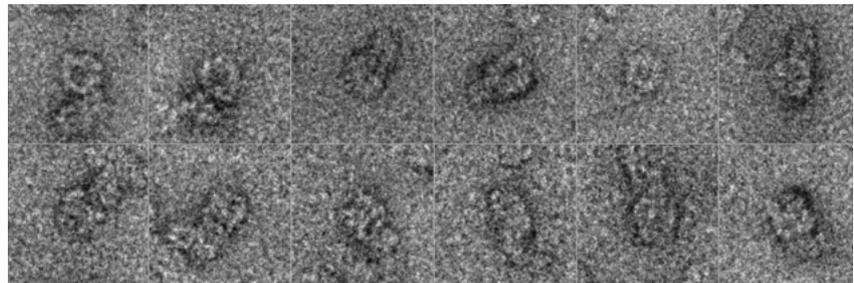
Cuellar J, Valpuesta JM, Wittinghofer A, Sot B. Domain Topology of Human Rasal. *Biol Chem* 2017; 399: 63-72.

Fernández-Fernández MR, Valpuesta JM. Hsp70 chaperone: a master player in protein homeostasis. *F1000Res* 2018; 7: 1497.

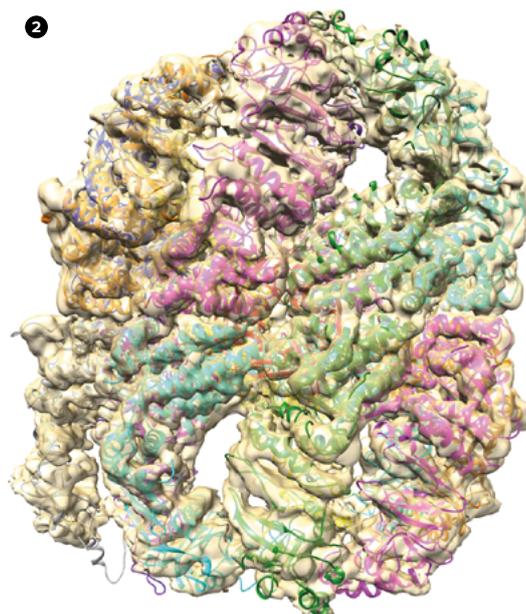
Pouchucq L, Lobos-Ruiz P, Araya G, Valpuesta JM, Monasterio O. The chaperonin CCT promotes the formation of fibrillar aggregates of γ -tubulin. *Biochim Biophys Acta Proteins Proteom* 2018; 1866: 519-526.

Most of the cellular processes are executed by sets of proteins that work like molecular machines in a coordinated manner, thus acting like an assembly line and making the process a more efficient one. One of such assembly lines is the one formed by molecular chaperones, a group of proteins involved in cell homeostasis through two opposite functions, protein folding and degradation. Over the last years it has been found that chaperones are not only devoted to assist the folding of other proteins but also, given the right conditions and the presence of specific cochaperones, they can be active players in protein degradation. The two processes are carried out through the transient formation of complexes between different chaperones and cochaperones. Our goal is the structural characterisation, at the highest possible resolution, of some of these complexes, using as a main tool state-of-the-art cryoelectron microscopy and image processing techniques.

1

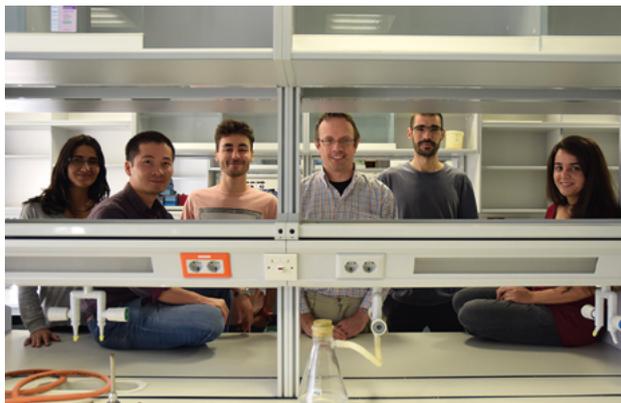


2



1 Gallery of images, obtained by transmission electron microscopy, of complexes between the eukaryotic chaperonin CCT and oligomers of the α -synuclein A53T mutant. The chaperonin has been shown to inhibit the formation of α -synuclein fibres.

2 Image of a high-resolution map (3.9 Å) of the eukaryotic chaperonin CCT obtained by cryoelectron microscopy.



Structural biology of virus fibres

MACROMOLECULAR STRUCTURES 23

GROUP LEADER

Mark J. van Raaij

POSTDOCTORAL SCIENTISTS

Pablo Soriano Maldonado
Thanh Hong Nguyen

TECHNICIAN

Mara Inmaculada Laguna Castro

PhD STUDENTS

Marta Sanz Gaitero
Mateo Seoane Blanco
Antonio Pichel Beleiro

MASTER'S STUDENTS

Daniel Salas Lloret
Laida Pagalday Arrizabalaga

UNDERGRADUATE STUDENTS

Daniel Gutiérrez Tejedor
David Nuñez Nepomuceno

SELECTED PUBLICATIONS

Granel M, Namura M, Alvira S, Kanamaru S, van Raaij MJ. Crystal structure of the carboxy-terminal region of the bacteriophage T4 proximal long tail fiber protein gp34. *Viruses* 2017; 9: E168.

Menéndez-Conejero R, Nguyen TH, Singh AK, Condezo GN, Marschang RE, van Raaij MJ, San Martín C. Structure of a reptilian adenovirus reveals a phage tailspike fold stabilizing a vertebrate virus capsid. *Structure* 2017; 25: 1562-1573.

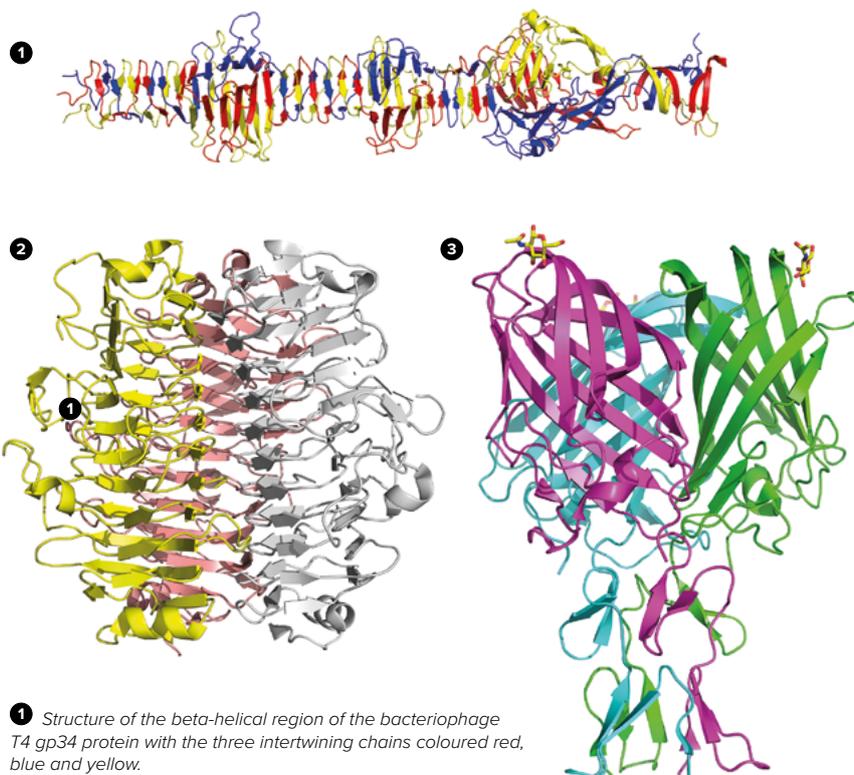
Taylor NMI, van Raaij MJ, Leiman PG. Contractile injection systems of bacteriophages and related systems. *Mol Microbiol* 2018; 108: 6-15.

Kokotidou C, Jonnalagadda SVR, Orr AA, Seoane-Blanco M, Apostolidou CP, van Raaij MJ, Kotzabasaki M, Chatzoudis A, Jakubowski JM, Mossou E, Forsyth VT, Mitchell EP, Bowler MW, Llamas-Saiz AL, Tamamis P, Mittraki A. A novel amyloid designable scaffold and potential inhibitor inspired by GALLG of amyloid beta and the HIV-1 V3 loop. *FEBS Lett* 2018; 592: 1777-1788.

Singh AK, Nguyen TH, Vidovszky MZ, Harrach B, Benkő M, Kirwan A, Joshi L, Kilcoyne M, Berbis MÁ, Cañada FJ, Jiménez-Barbero J, Menéndez M, Wilson SS, Bromme BA, Smith JG, van Raaij MJ. Structure and N-acetylglucosamine binding of the distal domain of mouse adenovirus 2 fibre. *J Gen Virol* 2018; 99: 1494-1508.

Recognition of the correct host cell to infect is of crucial importance to a virus. Many viruses bind to their host cell receptors via specialised spike proteins or via specialised fibre proteins, like adenoviruses and bacteriophages. These fibres all have the same basic architecture: they are trimeric and contain an N-terminal virus or bacteriophage attachment domain, a long, thin, but stable shaft domain and a more globular C-terminal cell attachment domain. These trimeric, fibrous proteins are very stable to denaturation by temperature or detergents.

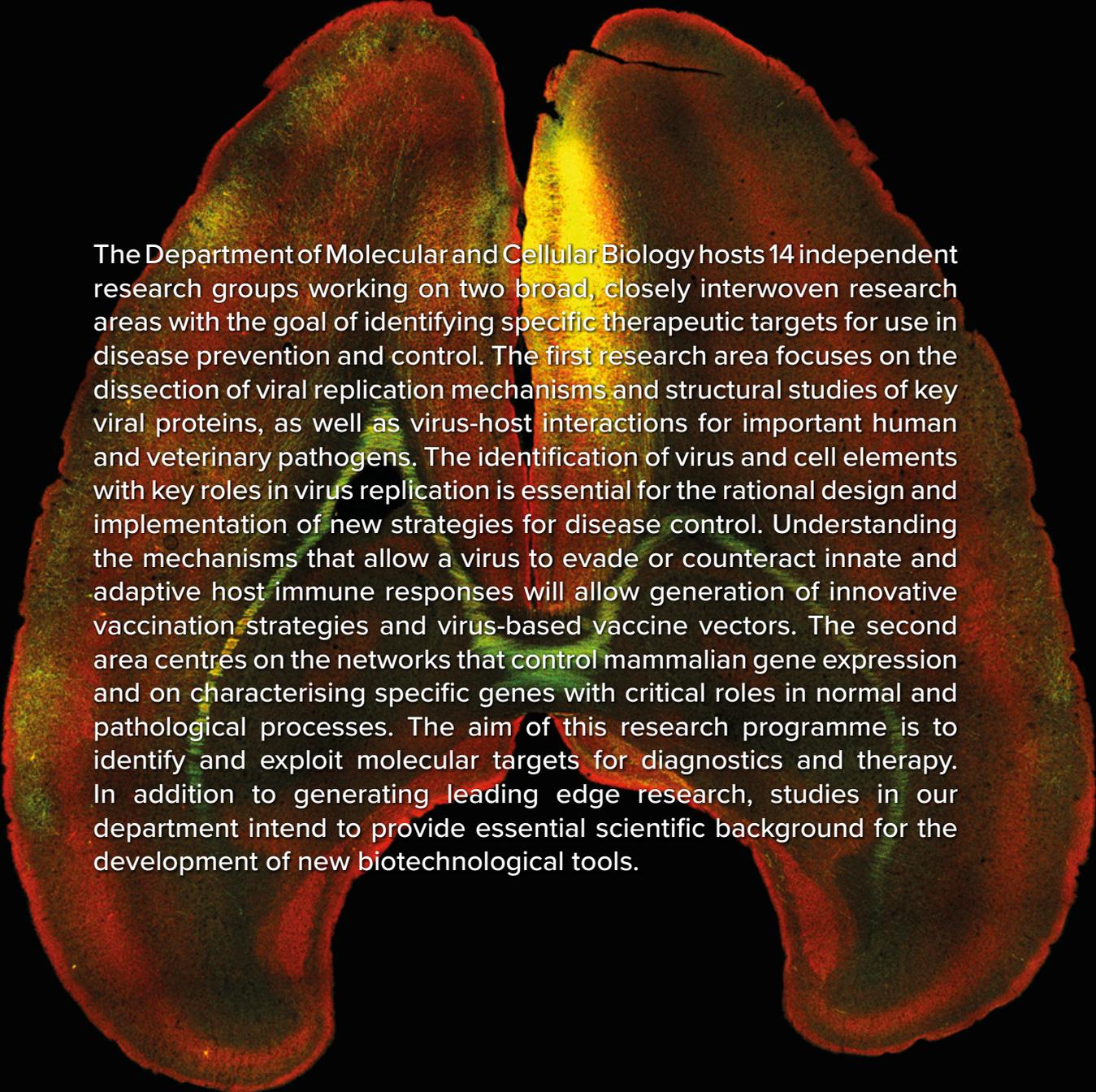
In the years 2017 and 2018, we have determined the structures of three receptor-binding proteins of Staphylococcal bacteriophage K and of two endolysin proteins. In addition, we collaborated with other research groups in crystallisation and structure solution of the proteins and peptides they produce. Knowledge of the structures of bacteriophage receptor-binding and endolysin proteins may lead to different biotechnological applications. Modification of the bacteriophage fibre receptor binding specificities may lead to improved detection of specific bacteria, while a better understanding of endolysin structure, stability and specificity may lead to better elimination of pathogenic or otherwise unwanted bacteria.



1 Structure of the beta-helical region of the bacteriophage T4 gp34 protein with the three intertwining chains coloured red, blue and yellow.

2 Structure of the snake adenovirus 1 LH3 protein.

3 Structure of the mouse adenovirus 2 fibre receptor-binding domain bound to N-acetyl-glucosamine. The three protein chains are coloured green, cyan and magenta.



The Department of Molecular and Cellular Biology hosts 14 independent research groups working on two broad, closely interwoven research areas with the goal of identifying specific therapeutic targets for use in disease prevention and control. The first research area focuses on the dissection of viral replication mechanisms and structural studies of key viral proteins, as well as virus-host interactions for important human and veterinary pathogens. The identification of virus and cell elements with key roles in virus replication is essential for the rational design and implementation of new strategies for disease control. Understanding the mechanisms that allow a virus to evade or counteract innate and adaptive host immune responses will allow generation of innovative vaccination strategies and virus-based vaccine vectors. The second area centres on the networks that control mammalian gene expression and on characterising specific genes with critical roles in normal and pathological processes. The aim of this research programme is to identify and exploit molecular targets for diagnostics and therapy. In addition to generating leading edge research, studies in our department intend to provide essential scientific background for the development of new biotechnological tools.

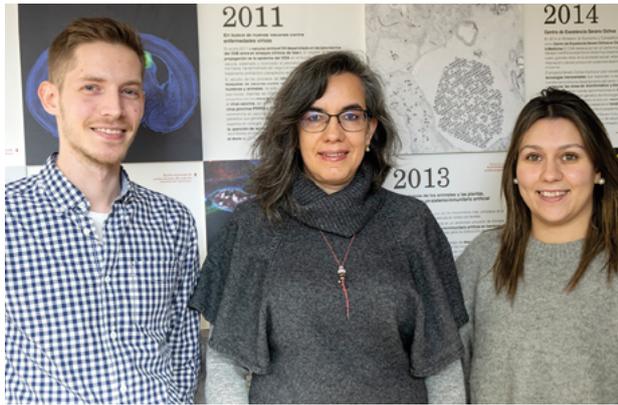
Molecular and Cellular Biology

HEAD OF DEPARTMENT

Francisco J. Iborra

RESEARCH GROUPS

- 1. Molecular bases of actin cytoskeleton reorganisation in cell motility, tumour generation and invasiveness**
Inés M. Antón
- 2. Coronavirus: replication, virus-host interactions, and protection**
Luis Enjuanes & Isabel Sola
- 3. Poxvirus and vaccines**
Mariano Esteban
- 4. Virus-host interactions in hepatitis B virus infection**
Urtzi Garaigorta
- 5. Hepatitis C virus infection**
Pablo Gastaminza
- 6. Biological noise and its physiopathological implications**
Francisco J. Iborra & Fernando Almazán
- 7. Animal models by genetic manipulation**
Lluís Montoliu
- 8. Functional analysis of the transcriptional repressor DREAM**
José Ramón Naranjo
- 9. Mechanisms of interaction between the influenza virus and the infected cell**
Amelia Nieto
- 10. Cerebral cortical development**
Marta Nieto
- 11. Molecular characterisation and epidemiology of torovirus**
Dolores Rodríguez
- 12. Molecular biology of birnaviruses**
José F. Rodríguez
- 13. Embryonic development and differentiation in vertebrates**
Juan José Sanz-Ezquerro
- 14. Cellular immunobiology and microbiology**
Esteban Veiga Chacón



Molecular bases of actin cytoskeleton reorganisation in cell motility, tumour generation and invasiveness

GROUP LEADER

Inés M. Antón

TECHNICIAN

Carla Gómez-Oro

PHD STUDENT

Sergio Rivas

SELECTED PUBLICATIONS

Pfäffer L, Seidel MG, Houmadi R, Rey-Barroso J, Hirschmugl T, Salzer E, Antón IM, Urban C, Schwinger W, Boztug K, Dupré L. WIP deficiency severely affects human lymphocyte architecture during migration and synapse assembly. *Blood* 2017; 130: 1949-1953.

Escoll M, Gargini R, Cuadrado A, Anton IM, Wandosell F. Mutant p53 oncogenic functions in cancer stem cells are regulated by WIP through YAP/TAZ. *Oncogene* 2017; 36: 3515-3527.

Rivas S, Antón IM, Wandosell F. WIP-YAP/TAZ as a new pro-oncogenic pathway in glioma. *Cancers (Basel)* 2018; 10: E191.

Rivas S, Gómez-Oro C, Antón IM, Wandosell F. Role of Akt isoforms controlling cancer stem cell survival, phenotype and self-renewal. *Biomedicines* 2018; 6: E29.

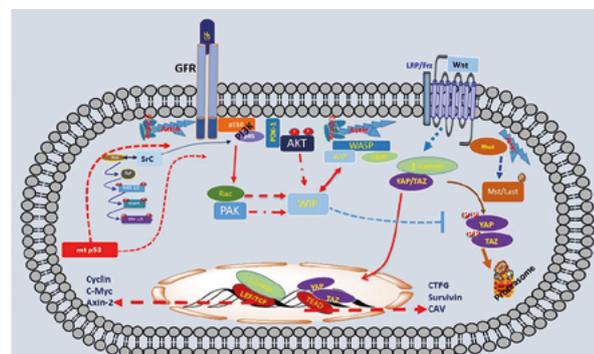
Antón IM, Gómez-Oro C, Rivas S, Wandosell F. Crosstalk between WIP and Rho family GTPases. *Small GTPases* 2018; 29: 1-7.

Alterations in the PI3K-Akt-GSK3/mTORC pathways are the basis of ageing-related pathologies like neurodegenerative disorders (Alzheimer's disease) or cancer. Our research interest focuses on the role of actin-binding elements in the regulation of Akt-mediated signalling routes that control motility, invasiveness and survival and how they contribute to cell differentiation/degeneration or tumourigenesis. Our model actin-binding proteins are (N)WASP (neural Wiskott-Aldrich Syndrome Protein), WIP (WASP Interacting Protein) and WIRE (WIP Related).

Using animal models, recombinant lentivirus and advanced imaging techniques (two and three dimensional cultures) in combination with biochemical and proteomic approaches, we have described a relevant role for WIP in neuronal differentiation through the regulation of survival pathways (PI3K-Akt and mTORC1). Most tumours are initiated and maintained from a subpopulation of migratory and invasive mesenchymal cancer stem cells (CSC) which are responsible for the acquisition of aggressive tumour phenotypes being more resistant to many therapeutic approaches and responsible for tumour recurrence, hence they are attractive targets for novel treatments.

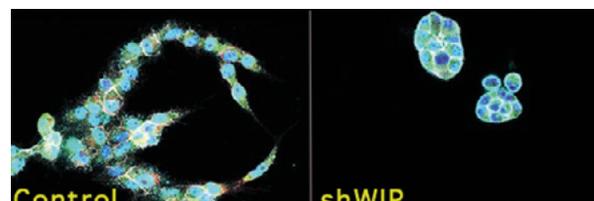
Our work showed that WIP is preferentially expressed in invasive tumour samples (glioblastoma and breast cancer) and affect tumour phenotype at two levels: 1) **initiation**, as it participates in the establishment and maintenance of CSC preventing their apoptotic caspase-dependent cell death; 2) **progression**, as it promotes CSC capacity to degrade the extracellular matrix contributing to invasiveness and metastasis. WIP expression leads to the sequestration of the destruction complex in multivesicular bodies and thus promotes the stability of the transcriptional co-activators YAP/TAZ. WIP is also an essential part of a p53-mediated oncogenic cascade that maintain tumour growth capacity and the stem phenotype. Our findings demonstrate an oncogenic role for WIP through an Akt-related developmental/oncogenic axis and hopefully they will contribute to find new biomarkers and therapeutic targets to fight neurodegenerative disorders and metastases.

1



1 Schematic of several routes that control glioma progression, including the novel WIP-YAP/TAZ pathway that connects mutant p53 and growth-factor-receptor signals via Akt with oncogenesis and cell invasiveness.

2



2 WIP expression increases cell invasiveness. Control (left) or shWIP interfered (right) MDA-MB-231 cells were seeded on Matrigel and stained for YAP/TAZ in green, actin in red, integrin in cyan and nuclei in blue.



Coronavirus: replication, virus- host interactions, and protection

MOLECULAR AND CELLULAR BIOLOGY 27

GROUP LEADERS

Luis Enjuanes
Isabel Sola

SENIOR SCIENTISTS

Sonia Zuñiga
Marta López de Diego

POSTDOCTORAL SCIENTISTS

Lucía Morales
Carlos Castaño

TECHNICIANS

Carlos M. Sánchez
Raúl Fernández
Margarita González

PHD STUDENTS

Alejandro Pascual
Javier Gutiérrez
Javier Cantón
José Manuel Honrubia
Li Wang
Gonzalo Vigara

VISITING SCIENTIST

C. Muñoz-Fontela
*Heinrich Pette Institute, Leibniz Institute
for Experimental Virology, Hamburg,
Germany*

SELECTED PUBLICATIONS

Morales L, Oliveros JC, Fernandez-Delgado R, tenOever BR, Enjuanes L, Sola I. SARS-CoV-encoded small RNAs contribute to infection-associated lung pathology. *Cell Host Microbe* 2017; 21: 44-355.

Canton J, ..., Enjuanes L, Sola I. MERS-CoV 4b protein interferes with the NF-kappaB-dependent innate immune response during infection. *PLoS Pathog* 2018; 14: e1006838.

Castaño-Rodríguez C, Honrubia JM, Gutierrez-Álvarez J, ..., Sola I, Enjuanes L. Role of severe acute respiratory syndrome coronavirus viroporins E, 3a and 8a in replication and pathogenesis. *mBio* 2018; 9: e02325-17

Stalin Raj V, Okba NMA, Gutierrez-Álvarez J, Fernandez-Delgado R, Sola I, ..., Enjuanes L, Haagmans BL. Chimeric camel/human heavy-chain antibodies protect against MERS-CoV infection. *Sci Adv* 2018; 4: eaas9667.

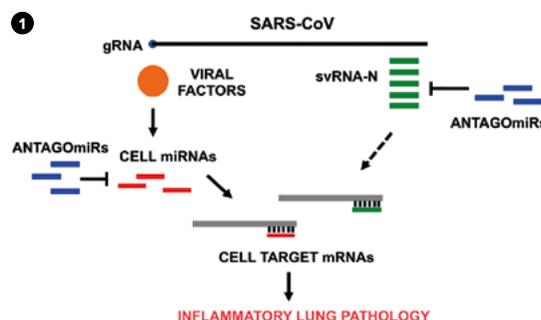
Letko M, Miazgowiec K, McMinn R, Seifert SN, Sola I, Enjuanes L, Carmody A, van Doremalen N, Munster V. Adaptive evolution of MERS-CoV to species variation in DPP4. *Cell Rep* 2018; 24: 1730-1737.

Human infections causing pneumonia and acute respiratory distress syndrome (ARDS) are a growing health problem. In 2015, respiratory diseases were the third most common cause of death in the EU. The problem is even greater in the elderly population, which responds with significant lower efficacy to vaccination.

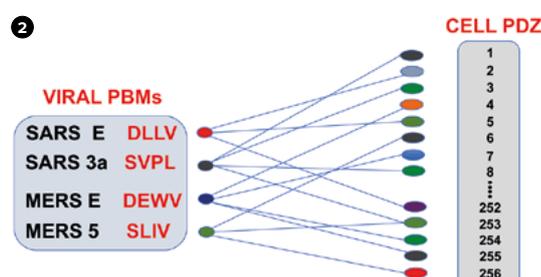
Viruses are responsible for most respiratory infections. Among them, human coronaviruses (CoV) are the cause of up to 15% of all respiratory problems. Six human CoVs have been described, HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, SARS-CoV and MERS-CoV, the last two leading to deadly infections. Our laboratory focuses on the design of vaccines and selection of antivirals to protect against human respiratory CoV infections by modulating the innate immune response in young and elderly populations.

The main aims of our research are to:

- **Identify CoV genes responsible for virus virulence**, to delete or modify these genes using reverse genetics in order to develop new generation vaccines such as replication-competent propagation-deficient RNA replicons, which are safe and promising vaccine candidates, and to determine their effectiveness in animal model systems. The expression of micro-RNAs with immunomodulating capacities will provide enhanced efficiency in older adults.
- **Identify cell-signalling pathways involved in CoV replication and pathology**, and to select antiviral drugs that inhibit these pathways interfering with virus replication or pathology. In particular, we study PBM-PDZ protein-protein interactions involved in the innate immune and inflammatory responses, since overstimulation of these pathways seems responsible for an increase in fatalities during SARS-CoV and MERS-CoV epidemics.
- **Determine the contribution of host miRNAs and virus-derived small RNAs to the inflammatory lung pathology** induced by CoV infection. These small non-coding RNAs represent targets for antivirals.
- **Study the effect of specific IFN-stimulated genes on the replication and innate immune responses of respiratory viruses**, such as influenza and CoVs, which induce diseases associated with excessive immune signalling.



1 Interactions of viral protein-PBMs with cellular PDZ-containing proteins. Highly virulent human coronaviruses, such as SARS-CoV and MERS-CoV, include at least two proteins each containing PDZ binding motifs (PBMs) (left) that may interact with 256 unique PDZ motifs present in more than 400 cellular protein isoforms (right).



2 Contribution of host and viral small non-coding RNAs to SARS-CoV lung pathology. Infection by a virulent SARS-CoV induces the differential expression of host microRNAs (miRNAs), which are mainly associated with the regulation of cell target mRNAs related to cytokine-mediated inflammatory pathways, suggesting that the viral-induced inflammatory pathology is in part coordinated by miRNAs.



Poxvirus and vaccines

28 MOLECULAR AND CELLULAR BIOLOGY

GROUP LEADER Mariano Esteban

SENIOR SCIENTISTS

Juan García Arriaza
Carmen E. Gómez
Beatriz Perdiguero

TECNICIANS

Victoria Jiménez
Cristina Sánchez

PhD STUDENTS

Mauro Di Pilato
Ernesto Mejías
Suresh Chithathur
María Quirós
Patricia Pérez
Adrián Lázaro

MASTER'S STUDENTS

Pilar Mediavilla
Eleftheria Sideris
Lidia Saiz
Beatriz Isla
Sara Moreno

SELECTED PUBLICATIONS

Vijayan A, Mejías-Pérez E, *et al.* A prime/boost PfCSI4KM/MVA-sPfCSM vaccination protocol generates robust CD8⁺ T cell and antibody responses to Plasmodium falciparum circumsporozoite protein and protects mice against malaria. *Clin Vaccine Immunol* 2017; 24: e00494-16.

Di Pilato M, Mejías-Pérez E, Sorzano COS, Esteban M. Distinct roles of vaccinia virus NF- κ B inhibitor proteins A52, B15, and K7 in the immune response. *J Virol* 2017; 91: e00575-17.

Enamorado M, Iborra S, *et al.* Enhanced anti-tumour immunity requires the interplay between resident and circulating memory CD8⁺ T cells. *Nat Commun* 2017; 8: 16073.

Lázaro-Frías A, Gómez-Medina S, *et al.* Distinct immunogenicity and efficacy of poxvirus-based vaccine candidates against ebola virus expressing GP and VP40 proteins. *J Virol* 2018; 92: e00363-18.

Pérez P, Q Marín M, *et al.* A vaccine based on a modified vaccinia virus ankara vector expressing zika virus structural proteins controls zika virus replication in mice. *Sci Rep* 2018; 8: 17385.

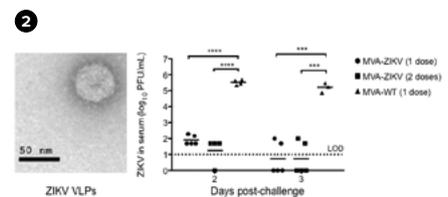
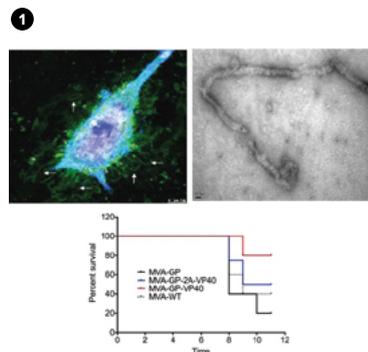
The research interest of the Poxvirus and Vaccines laboratory focuses on the control of human pathogens by developing safe and effective vaccines. This is accomplished by studying the biology of poxviruses and their application as vaccine candidates, using cultured cells as well as various animal models (mice and monkeys) together with human clinical trials. By studying the behaviour of replication competent and incompetent poxvirus vectors MVA and NYVAC, our group has made important contributions to the immune biology of vaccinia virus, the mechanisms of T cell and B cell humoral immune responses, and engineering vaccine candidates against diseases like HIV/Aids, hepatitis C, chikungunya, ebola, zika, malaria and leishmaniasis, some of which have advanced to phase I/II clinical trials (HIV).

In the 2017-2018 period, in collaboration with other groups, we have made the following achievements:

- We identified immune mechanisms and established T and B cell immune responses relevant to protection against HIV-1 in preclinical studies (mice and monkeys).
- We defined, in a phase I clinical trial, the immunogenicity of a prophylactic clade C vaccine (MVA-C) against HIV-1 developed by our group, as well as the long-term immune responses to the other clade B vaccine candidate (MVA-B).
- We generated novel vaccine candidates and established protocols of immunisation that are safe and efficiently protect animals against pathogens: chikungunya, ebola, zika, malaria and leishmaniasis.

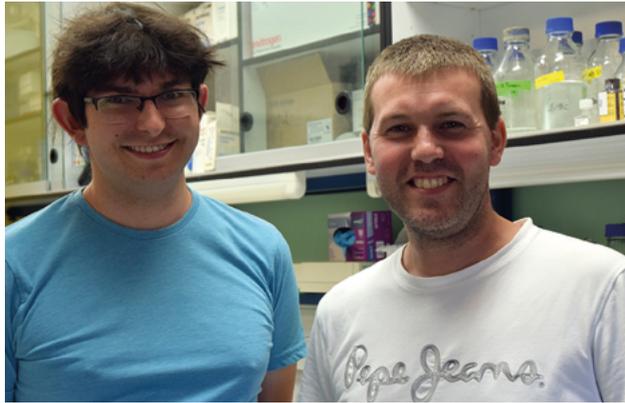
Our current research aims to optimise the immunogenicity of the poxvirus vectors MVA and NYVAC as recombinant vaccine candidates, alone and in combination with other immunogens (DNA, mRNA, alphavirus replicon, protein), to identify immune mechanisms in preclinical and clinical trials, and to establish correlates of protection. Our goal is to develop the best-in-class immunogens and vaccination protocols to be applied against prevalent human diseases.

Our research is supported by national and international grants, resulted in the publication of over 350 papers in international journals, 11 patents and the direction of 32 PhD theses. We maintain a fruitful collaboration with HIV vaccine experts in the USA and Europe through CAVD projects financed by the Bill and Melinda Gates Foundation and EU H2020 program, as well as with other EU colleagues developing HIV, ebola, chikungunya and zika vaccines.



1 Ebola vaccine MVA-EBOV, showing by confocal microscopy the cell destruction, by electron microscopy the VLPs and in mice the efficacy of a single dose against ebola virus (line in red).

2 Zika vaccine MVA-ZIKA, showing the VLP formation and efficacy in mice.



Virus-host interactions in hepatitis B virus infection

MOLECULAR AND CELLULAR BIOLOGY 29

GROUP LEADER

Urtzi Garaigorta de Dios

PhD STUDENT

Andoni Gómez Moreno

MASTER'S STUDENT

Pilar Gomollón Zueco

SELECTED PUBLICATIONS

Gómez-Moreno A, Garaigorta U. Hepatitis B virus and DNA damage response: Interactions and consequences for the infection. *Viruses* 2017; 9: E304.

Kruse RL, Shum T, Tashiro H, Barzi M, Yi Z, Whitten-Bauer C, Legras X, Bissig-Choisat B, Garaigorta U, Gottschalk S, Bissig KD. HBsAg-redirected T cells exhibit antiviral activity in HBV-infected human liver chimeric mice. *Cytherapy* 2018; 20: 697-705.

Hepatitis B virus (HBV) represents an important human pathogen causing acute and chronic hepatitis. Over 250 million people are chronically infected, and more than 780,000 people die every year due to complications of HBV, including liver cirrhosis and hepatocellular carcinoma. Currently, approved therapies suppress very effectively virus replication and viremia, but they are not curative because they do not eliminate the nuclear viral episomal DNA (cccDNA), a hallmark of HBV persistence. Therefore, there is an imminent need to develop novel cccDNA-targeting therapies that eradicate HBV from chronically infected patients.

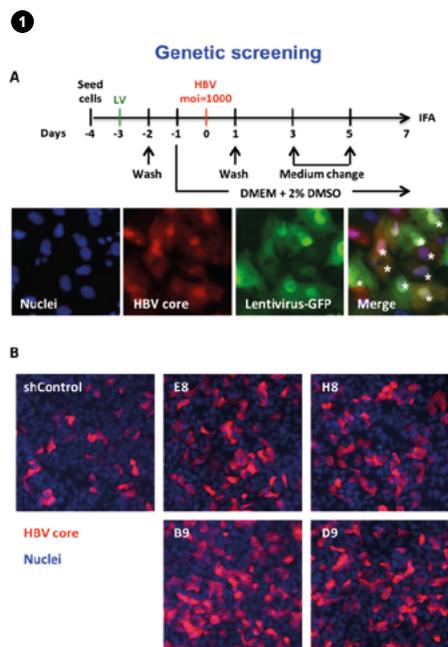
We believe that, by understanding basic aspects of cccDNA biology, we will unravel new aspects of viral pathogenesis and identify vulnerabilities in the virus life cycle that could be exploited for the development of novel and safe curative antiviral strategies.

Thus, our group is interested in deciphering the cellular factors, pathways and mechanisms that regulate cccDNA levels in infected cells. To this end, we make use of HBV infection cell culture models and apply genetic, molecular, biochemical and virological approaches to understand cccDNA formation and homeostasis.

The specific aims of our research are to:

Identify and characterise cellular proteins and pathways that regulate cccDNA formation. We have performed loss-of-function genetic and pharmacological screenings that identified three DNA damage response proteins, namely Aquarius, Senataxin and DNA-PK, as factors restricting the accumulation of HBV cccDNA during infection. Experiments to understand the mechanism by which these cellular factors regulate the HBV life cycle are underway.

- Develop an in-house cell-based ELISA for antiviral small molecules screening.
- Visualise cccDNA formation in infected cells. We have recently started a project aimed to chemically label cccDNA while it is being formed in the infected cells. This technique will allow us to perform cccDNA localisation studies that are difficult with current technologies.



1 A) Genetic screening set up performed to identify cellular proteins that regulate HBV infection. B) Increased HBV infection in Aquarius (AQR:E8 & H8) and Senataxin (SETX: B9 and D9) deficient cells.



Hepatitis C virus infection

30 MOLECULAR AND CELLULAR BIOLOGY

GROUP LEADER

Pablo Gastaminza Landart

POSTDOCTORAL SCIENTIST

Ginés Ávila Pérez

TECHNICIAN

Gema Calvo Gutiérrez

PHD STUDENT

Victoria Castro Illana

UNDERGRADUATE STUDENTS

Alba Escalera

Nicolás Aguillón

SELECTED PUBLICATIONS

Coto-Llerena M, Koutsoudakis G, Boix L, López-Oliva JM, Caro-Pérez N, Fernández-Carrillo C, González P, Gastaminza P, Bruix J, Forns X, Pérez-Del-Pulgar S. Permissiveness of human hepatocellular carcinoma cell lines for hepatitis C virus entry and replication. *Virus Res* 2017; 240: 35-46.

Moreno E, Gallego I, Gregori J, Lucía-Sanz A, Soria ME, Castro V, Beach NM, Manrubia S, Quer J, Esteban JI, Rice CM, Gómez J, Gastaminza P, Domingo E, Perales C. Internal disequilibria and phenotypic diversification during replication of Hepatitis C Virus in a noncoevolving cellular environment. *J Virol* 2017; 91: e02505-16.

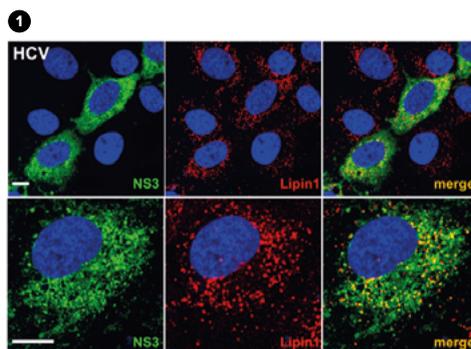
Sepúlveda-Crespo D, Jiménez JL, Gómez R, De La Mata FJ, Majano PL, Muñoz-Fernández MA, Gastaminza P. Polyanionic carboxylate dendrimers prevent hepatitis C virus infection in cell culture. *Nanomedicine* 2017; 13: 49-58.

Perez-Berna AJ, Valcarcel R, Rodríguez MJ, Chichón FJ, Sorrentino A, Carrascosa JL, Gastaminza P, Pereiro E. The dual-axes for soft X-ray cryotomography reveals ultrastructural alterations of the host cell during hepatitis C infection by increasing the isotropic axial resolution. *Microsc Microanal* 2017; 23(S1), 976-77.

Mingorance L, Castro V, Ávila-Pérez G, Calvo G, Rodríguez MJ, Carrascosa JL, Pérez-Del-Pulgar S, Forns X, Gastaminza P. Host phosphatidic acid phosphatase lipin1 is rate limiting for functional hepatitis C virus replicase complex formation. *PLoS Pathog* 2018; 14: e1007284.

Our laboratory studies pathogenic human viral infections and focuses on understanding the molecular basis of viral pathogenesis and identifying new molecular targets for antiviral therapy. Our final aim is to propose new therapeutic approaches for antiviral treatment and for reversion of virus-induced pathogenesis. We consider that determining the cellular and molecular mechanisms by which the virus replicates provides new opportunities in the fight against clinically relevant human pathogens. To achieve these general aims, we use cell culture models of infection, in which pharmacological and genetic manipulation enables the study of fundamental aspects of virus-host interactions.

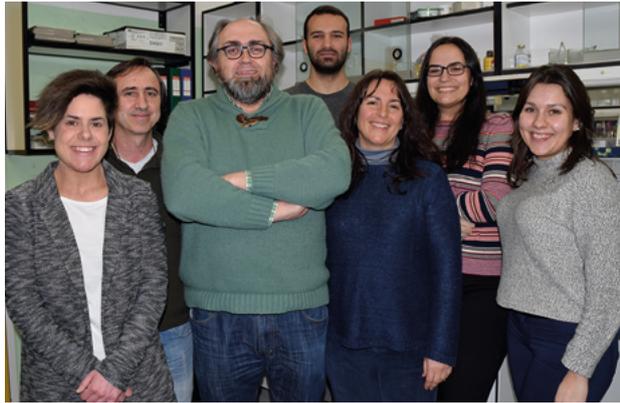
During the 2017-2018 period, we have been focused on the role of lipins, a family of lipid-modifying enzymes, in hepatitis C virus infection. Lipins control glycerophospholipid homeostasis, including triglyceride and phospholipid biosynthesis. They also control the levels of very important lipid second messengers such as phosphatidic acid and diacylglycerol (DAG). We have recently demonstrated that the best characterised member of the lipin family, lipin1, is rate-limiting for the production of HCV replicase complexes, probably because it provides an optimal lipid environment for recruitment of host and viral factors that assemble the viral replicase. We have verified that lipin1 dependency is exquisitely specific for hepatitis C virus infection; infection by flaviviruses dengue and Zika or by respiratory viruses such as coronavirus hCoV-229E or influenza virus is not limited in lipin1-deficient cells. Thus, we have defined a new HCV-specific molecular target for HCV-specific antiviral intervention. Future studies will involve the study of other members of the family, including lipin2. Lipin2-deficient cells display a completely different phenotype, as they are refractory to infection by all the aforementioned viruses. Overall, our results provide not only new antiviral targets for therapeutic intervention but also molecular insights into the different functions of host genes involved in lipid homeostasis.



1 Immunofluorescence microscopy images showing lipin1 (red) colocalisation with viral protein NS3 (green) in HCV-infected human hepatoma cells (Huh-7).

2 Transmission electron microscopy of human hepatoma (Huh-7) cells expressing HCV replicase components and forming characteristic double and multiple membrane vesicles (DMV and MMV). The number of HCV-induced membranous structures are significantly reduced in lipin1-deficient cells (*PLoS Pathog* 2018; 14: e1007284).





Biological noise and its physiopathological implications

MOLECULAR AND CELLULAR BIOLOGY 31

GROUP LEADERS

Francisco J. Iborra
Fernando Almazán

POSTDOCTORAL SCIENTISTS

Silvia Márquez Jurado
Verónica Martín García

TECHNICIAN

Carla Gómez Oro

PhD STUDENT

Juan Díaz Colunga

UNDERGRADUATE STUDENTS

Patricia Suárez Ara
Manuel Laguía Fernández-Chinchilla

VISITING SCIENTIST

Petros Papadopoulos
(Hospital Clínico San Carlos, Madrid, Spain)

SELECTED PUBLICATIONS

Márquez-Jurado S, Díaz-Colunga J, das Neves RP, Martínez-Lorente A, Almazán F, Guantes R, Iborra FJ. Mitochondrial levels determine variability in cell death by modulating apoptotic gene expression. *Nat Commun* 2018; 9: 389.

Lima AF, May G, Díaz-Colunga J, Pedreiro S, Paiva A, Ferreira L, Enver T, Iborra FJ, das Neves RP. Osmotic modulation of chromatin impacts on efficiency and kinetics of cell fate modulation. *Sci Rep* 2018; 8: 7210.

Diot A, Agnew T, Sanderson J, Liao C, Carver J, das Neves, RP, Gupta R, Guo Y, Waters C, Seto S, Daniels MJ, Dombi E, Lodge T, Morten K, Williams SA, Enver T, Iborra FJ, Votruba M, Poulton J. Validating the RedMIT/GFP-LC3 mouse model by studying mitophagy in autosomal dominant optic atrophy due to the OPA1Q285STOP mutation. *Front Cell Dev Biol* 2018; 6: 103.

Márquez-Jurado S, Nogales A, Ávila-Pérez G, Iborra F, Martínez-Sobrido L, Almazán, F. An alanine-to-valine substitution in the residue 175 of Zika virus NS2A protein affects viral RNA synthesis and attenuates the virus *in vivo*. *Viruses* 2018; 10: E547.

Ávila-Pérez G, Nogales A, Martín V, Almazán F, Martínez-Sobrido L. Reverse genetic approaches for the generation of recombinant Zika virus. *Viruses* 2018; 10: E597.

The non-genic heterogeneity of cell populations (phenotypic variability), which is due mainly to differential gene expression, plays an important role in many biological processes such as cell differentiation, development, apoptosis, cancer and viral infections. Our laboratory is interested in understanding the origins of this phenotypic variability and its impact on different physiological processes. The knowledge of this variability will improve our understanding of phenomena like tumour resistance to drugs, virus infection, or cell fate choice.

During the years 2017-2018, we have made important achievements in two main areas:

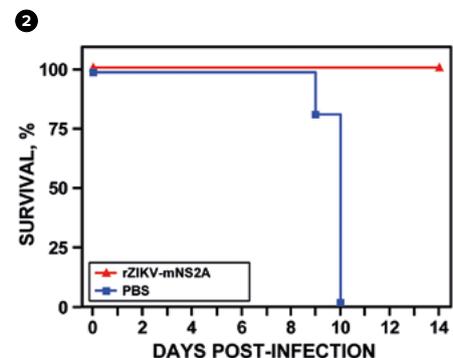
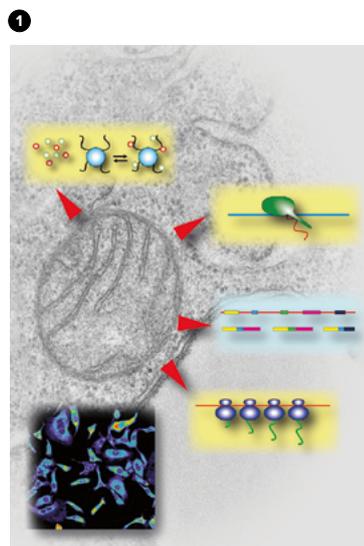
1. Origin of phenotypic variability.

We have found that mitochondrial content is a main factor of phenotypic variability in a clonal cell population. The mitochondrial content of each cell contributes to heterogeneity in gene products and has a large impact on alternative splicing, thus modulating both the abundance and type of mRNAs, which ultimately leads to phenotypic diversity.

2. Physiopathological implications of the variability of mitochondrial content in biological processes.

2.1. Effect of mitochondria variability on apoptosis in response to chemotherapy. We have found that the cellular mitochondrial content determines the apoptotic fate and modulates the time to death in response to TRAIL treatment. This finding could have a great impact on the understanding of tumour relapse and partial response to chemotherapy.

2.2. Effect of mitochondrial variability on viral infections. Using vaccinia virus and transmissible gastroenteritis coronavirus as experimental models, we have determined that there is an inverse correlation between the mitochondrial content and viral replication. Due to the importance of Zika virus (ZIKV) in human health, we have extended these studies to ZIKV and we have initiated a new investigation line focused on the study of the molecular bases of ZIKV pathogenesis. In that sense, we have developed a ZIKV reverse genetic system that has allowed us to generate an attenuated recombinant virus providing fully protection against ZIKV in a mouse model. These studies will improve our understanding of ZIKV biology and facilitate the development of vaccine and antiviral strategies.



1 Mitochondria and gene expression. Electron micrograph of one mitochondrion with radiating arrows to the steps of gene expression where mitochondria play an important role.

2 Protection efficacy of mutant virus rZIKV-mNS2A. Female 4-to-6-week old A129 mice (*IFN α /-*) were mock-vaccinated (PBS) or vaccinated with rZIKV-mNS2A. At 21 days after vaccination, mice were challenged with a lethal dose of ZIKV and survival were monitored daily for 14 days.



Animal models by genetic manipulation

32 MOLECULAR AND CELLULAR BIOLOGY

GROUP LEADER Lluís Montoliu

POSTDOCTORAL SCIENTISTS

Almudena Fernández
Marcos Rubio
Diego Muñoz

TECHNICIAN

Marta Cantero

PHD STUDENTS

Santiago Josa
Juliana Fernández
Andrea Montero
Ana María Guardia

MASTER'S STUDENTS

Yaiza López
Celia de Lara

UNDERGRADUATE STUDENTS

Marta Sánchez Díez
Miguel Ángel Pollino
Marta Martín López

VISITING SCIENTISTS

Iván Caballero
José Joaquín Serrano
Gonzalo Muñoz
Ana Belén Sanz
Juan Manuel Rosa

SELECTED PUBLICATIONS

Montoliu L, Marks MS. A new type of syndromic albinism associated with mutations in AP3D1. *Pigment Cell Melanoma Res* 2017; 30: 5-7.

Mato-Berciano A,... Montoliu L, Fillat C. A NOTCH-sensitive uPAR-regulated oncolytic adenovirus effectively suppresses pancreatic tumor growth and triggers synergistic anticancer effects with gemcitabine and nab-paclitaxel. *Oncotarget* 2017; 8: 22700-22715.

Raspa M, Guan M, Paoletti R, Montoliu L, *et al.* Dry ice is a reliable substrate for the distribution of frozen mouse spermatozoa: A multi-centric study. *Theriogenology* 2017; 96: 49-57.

Fernández A, Josa S, Montoliu L. A history of genome editing in mammals. *Mamm Genome* 2017; 28: 237-246.

Rubio-Fernández M ..., Montoliu L, de la Villa P, Martín-Nieto J, Cruces J. Impairment of photoreceptor ribbon synapses in a novel *Pom1* conditional knockout mouse model of dystroglycanopathy. *Sci Rep* 2018; 8:8543.

Our laboratory is interested in understanding the underlying pathological mechanisms of a group of human rare diseases globally known as albinism, a heterogeneous genetic condition associated with mutations in at least 20 genes, characterised by visual impairment and, often, pigmentation alterations. These research projects on human rare diseases are performed within our participation in the CIBERER-ISCIII.

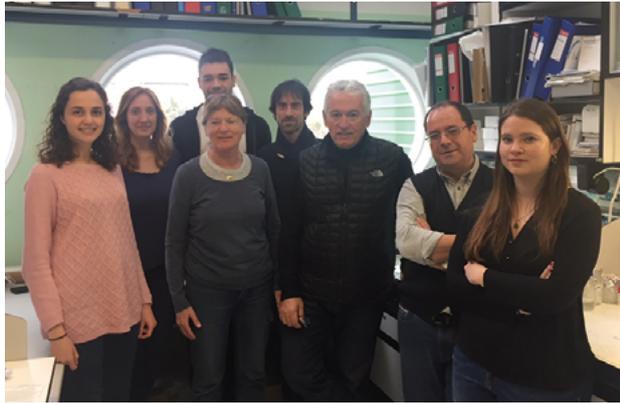
Our laboratory has generated and analysed new animal models to study visual abnormalities and different anomalies affecting retina development that are associated with albinism and other retinopathies such as achromatopsia. Furthermore, using mouse models, we have explored the use of small molecules as potential therapeutic candidates for albinism. In collaboration with Angel Carracedo (USC) and Carmen Ayuso (FJD), we have devised, within the CIBERER-ISCIII, a proposal for the universal genetic diagnose of all known mutations in albinism. We are already applying this knowledge in cooperation with ALBA, the Spanish association in support of people with albinism. Within CIBERER, we are actively contributing to the genetic diagnosis of many people with albinism in Spain.

We are also interested in understanding the function of regulatory elements that are required to define gene expression domains in mammalian genomes and contribute to specify its expression pattern in space and time. The mouse tyrosinase locus, used as experimental model, has allowed us to identify several genome boundaries or insulators, which protect the locus from surrounding genes. We use transgenic animals, zebrafish and mice to introduce different type of gene constructs in order to investigate the relevance of specific sequences. The functional analysis of regulatory elements found within the intergenic sequences can now be addressed more efficiently thanks to the new genome editing system, CRISPR-Cas9. In Spain, we pioneered the application of this technology in mice, successfully implemented it in our laboratory and disseminated its use among colleagues by hosting short stays and organising *ad-hoc* workshops, seminars and courses.

1



1 Genome-edited mouse generated with CRISPR tools displaying the classical mosaicism characteristic of these animal models.



Functional analysis of the transcriptional repressor DREAM

MOLECULAR AND CELLULAR BIOLOGY 33

GROUP LEADER

José R. Naranjo

SENIOR SCIENTISTS

Britt Mellström
Alberto Rábano

TECHNICIANS

Paz González
Xosé M. Dopazo

PHD STUDENTS

Alejandro López-Hurtado
Valentina González
María D. Arrabal

MASTER'S STUDENTS

Rocío Naranjo
Daniel Fernández

UNDERGRADUATE STUDENTS

Lidia Amigo
Isabel Díaz-Pinés

SELECTED PUBLICATIONS

Benedet T, Gonzalez P, Oliveros JC, Dopazo JM, Ghimire K, Palczewska M, Mellstrom B, Naranjo JR. Transcriptional repressor DREAM regulates trigeminal noxious perception. *J Neurochem* 2017; 141: 544-552.

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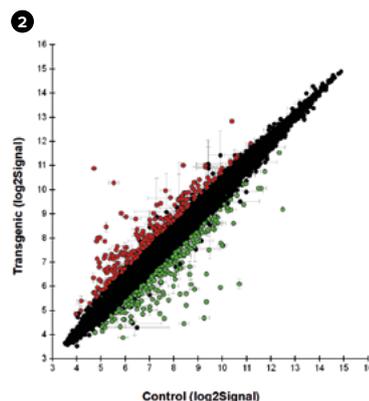
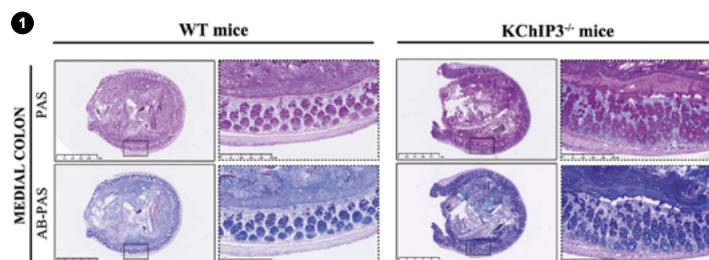
Momtazi G, Lambrecht BN, Naranjo JR, Schock BC. Regulators of A20 (TNFAIP3): new drug-able targets in inflammation. *Am J Physiol Lung Cell Mol Physiol* 2019; 316(3): L456-L469.

Our major research focus is on the multifunctional protein DREAM (downstream regulatory element antagonist modulator) and its role in the control of calcium homeostasis in health and disease.

DREAM, also known as calsenilin or KChIP3, is a Ca²⁺ binding protein of the neuronal calcium sensors (NCS) superfamily that interacts with specific sites in the DNA to repress transcription of target genes in a Ca²⁺-dependent manner. In addition, DREAM interacts with specific proteins to exert various specialised functions in different subcellular compartments. Thus, through the control of activity-dependent gene expression and specific protein-protein interactions, DREAM participates in many physiological processes in and outside the central nervous system. Work reported by us and other groups has shown important regulatory roles for DREAM in learning and memory in the hippocampus, in pain control in the spinal cord as well as in the immune response, inflammation, thyroid gland and placenta. Moreover, recent studies have shown the involvement of DREAM in several neurodegenerative disorders including Huntington's disease (HD) and Alzheimer's disease (AD).

DREAM was originally associated with AD because of its interaction with presenilins. However, altered neuronal calcium homeostasis and early compensatory changes in transcriptional programs are common features of many neurodegenerative disorders, which open the opportunity to explore a role for DREAM in these pathologies.

In physiological conditions, binding of calcium or arachidonic acid regulate the interaction with DNA or with potassium channels, respectively. Newly identified molecules, including gliinides, modify DREAM conformation and activity upon binding. In this respect, our interest is to contribute to the definition of more specific DREAM binding molecules, to reveal the molecular mechanisms underlying their effect upon binding to DREAM, and to assess their potential therapeutic actions on appropriate cellular and/or mouse models of target pathologies.



1 *DREAM/Kcnip3*^{-/-} mice show increased mucus layer at the medial colon (*Elife* 2018; 7: e39729).

2 Scatter Plot. Transcriptomic analysis of trigeminal ganglia in *daDREAM* transgenic mice



Mechanisms of interaction between the influenza virus and the infected cell

34 MOLECULAR AND CELLULAR BIOLOGY

GROUP LEADER

Amelia Nieto

SENIOR SCIENTISTS

Ana Falcón
Laura Marcos

POSTDOCTORAL SCIENTIST

Alejandra Pazo

TECHNICIAN

Noelia Zamarreño

MASTER'S STUDENTS

Celia Gutiérrez
Verónica Gomollón

SELECTED PUBLICATIONS

Martínez I, Oliveros JC, Cuesta I, de la Barrera J, Ausina V, Casals C, de Lorenzo A, García E, García-Fojeda B, Garmendia J, González-Nicolau M, Lacoma A, Menéndez M, Moranta D, Nieto A, Ortín J, Pérez-González A, Prat C, Ramos-Sevillano E, Regueiro V, Rodríguez-Frandsen A, Solís D, Yuste J, Bengoechea JA, Melero JA. Apoptosis, Toll-like, RIG-I-like and NOD-like receptors are pathways jointly induced by diverse respiratory bacterial and viral pathogens. *Front Microbiol* 2017; 8: 276.

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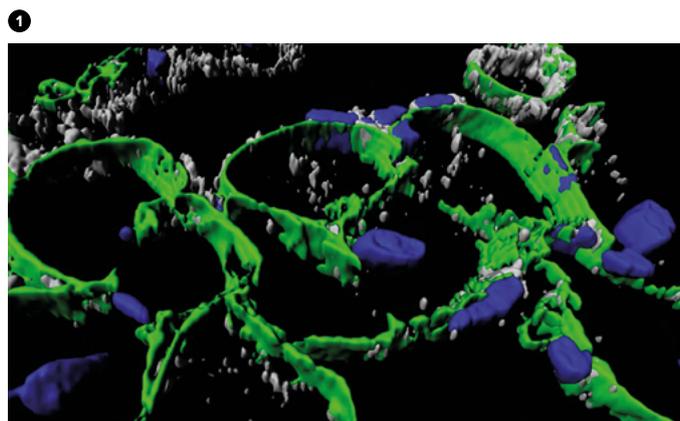
Vasiljevic J, Zamarreño N, Oliveros JC, Rodríguez-Frandsen A, Gómez G, Rodríguez G, Pérez-Ruiz M, Rey S, Barba I, Pozo F, Casas I, Nieto A, Falcón A. Reduced accumulation of defective viral genomes contributes to severe outcome in influenza virus infected patients. *PLoS Pathog* 2017; 13: e1006650.

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Influenza A virus (IAV) polymerase plays a crucial role controlling the expression of the viral and the host genome as well as viral pathogenicity. IAV uses a variety of mechanisms to control the antiviral response, such as the induction of epigenetic changes in specific histone residues that control the interferon signalling pathway, possibly through alterations in cellular sensors of viral RNAs, including methylation of lysine 79 of histone 3 together with a general decrease of histone acetylation.

Defective viral genome RNAs (DVGs) have been detected in virus particles. They have the 3' and 5' ends of the parental RNA segments, and most have a single large central deletion that generates viral RNAs of 180-1,000 nucleotides. DVGs potentiate the host response possibly through recognition of double-stranded RNA by receptors that activate antiviral signalling cascades. Specific point mutations at the viral polymerase control DVGs production, and decreased levels of DVGs correlate with increased pathogenesis in mice; conversely increased levels diminish *in vivo* pathogenesis.

Performing genomic analysis of viruses isolated from a cohort of previously healthy IAV infected patients with highly severe/fatal outcome, we showed that these viruses accumulated fewer DVGs than viruses isolated from a cohort of mildly infected patients, suggesting that low DVGs abundance constitutes a new virulence pathogenic marker in humans and that reduced accumulation of DVGs constitutes a virulent factor itself. Further characterisation of a recombinant virus with a point mutation in the PA subunit that produces low amount of DVGs, showed that the virus replicates efficiently in the heart, causes cardiac disorders and induces sudden death in infected animals. Influenza virus uses a plethora of mechanisms to increase its pathogenicity; viral proteins and viral RNAs work coordinately to accomplish an efficient infection mediated by this virus that possesses low genomic information.



1 Presence of influenza virus protein in cardiomyocytes of infected mice. White: viral nucleoprotein; blue: nuclei; green: lamina.



Cerebral cortical development

MOLECULAR AND CELLULAR BIOLOGY 35

GROUP LEADER

Marta Nieto López

SENIOR SCIENTIST

Linnea A. Weiss

POSTDOCTORAL SCIENTIST

Carlos García Briz

PHD STUDENTS

Noelia Sofía de León Reyes
Fernando Martín González
Lorena Bragg Gonzalo

MASTER'S STUDENT

Andrea de la Fuente Revenga

UNDERGRADUATE STUDENT

Diego Mañanes Cayero

SELECTED PUBLICATIONS

Nieto Guil AF, Oksdath M, Weiss LA, Grassi DJ, Sosa LJ, Nieto M, Quiroga S. IGF-1 receptor regulates dynamic changes in neuronal polarity during cerebral cortical migration. *Sci Rep* 2017; 7: 7703.

Briz CG, Navarrete M, Esteban JA, Nieto M. In utero electroporation approaches to study the excitability of neuronal subpopulations and single-cell connectivity. *J Vis Exp* 2017; 120: e55139.

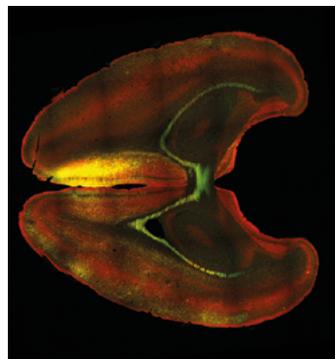
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Weiss LA and Nieto M. The crux of Cux genes in neuronal function and plasticity. *Brain Res* 2019; 1705: 32-42.

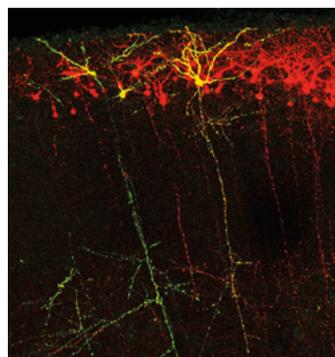
Our brain contains an extraordinary number and diversity of neurons, which together form one of the most complex functional networks found in biological systems. These circuits wire during development in manners that guarantees the brain's optimal responses to the external world and its capacity for intellectual processing and common social behaviours.

Using the mouse as a model, we aim to understand how neurons encode for the molecular information necessary to reproduce and build precisely the stereotyped circuits of the cortex; and how neurons translate this information into selective connectivity while dialoging with their environment. We focused on the extreme plasticity of this process for its potential therapeutic implications. While cortical neurons seem predetermined to follow strict rules of wiring, they also show a remarkable rewiring capacity that ensures optimal functional connectivity in abnormal scenarios, such as the loss of sensory organs, developmental defects or ischemic injury. Understanding and manipulating these intrinsic properties of developing neuronal networks (highly reproducible selective connectivity and plasticity) is key to understand normal brain functions and to manage and treat neurodevelopmental disorders, such as autism spectrum disorders (ASD), intellectual disabilities, bipolar, schizophrenia or epilepsy; it has also implications for the treatment of neurodegeneration and brain injury.

1



2



Our investigation is based on *in vivo* manipulation of wild type circuits by modifying gene expression, sensory input and circuit activity. For this purpose, we use transgenic approaches, including CRISPR/Cas-mediated knock-in, *in utero* electroporation, electrophysiology, stereotaxic retrotracing injections, pharmacological interventions and RNA-sequencing.

The main projects are:

- i. Investigating the action and downstream programs of Cux1 and Cux2 transcription factors in the specification of the cortical upper layer neurons.
- ii. Understanding the development of interhemispheric connections of the *corpus callosum*, its plasticity and its involvement in neurodevelopmental disorders.
- iii. The role of sensory circuits in the coordinated wiring of cortical networks; pharmacological interventions.
- iv. The action of interneurons.

1 Tracing of corpus callosum connections in the mouse using *in utero* electroporation. Confocal image of a section of a mouse brain electroporated at embryonic day 15. Yellow and green label the electroporated neurons and their axonal ramifications through the corpus callosum.

2 A Cre recombinase-diluted strategy enables sparse labeling of cortical neurons. Images obtained after vectors were delivered to layer II-III neurons by *in utero* electroporation at embryonic day 15.5 and coronal sections were made at postnatal day 16. Low amounts of CAG-CRE were electroporated. The CAG-DsRed vector was co-transfected as control (red). GFP (green) is expressed only in those neurons that also incorporated Cre, allowing the recombination of the LoxP sites in the CALNL-GFP vector. The sparse labelling allows distinguishing individual neurons and to trace their branching.



Molecular characterisation and epidemiology of torovirus

36 MOLECULAR AND CELLULAR BIOLOGY

GROUP LEADER

Dolores Rodríguez Aguirre

PhD STUDENTS

Liliana Lilibeth Cubas Gaona

Elisabet Díaz Beneitez

Óscar Candelas Rivera

MASTER'S STUDENTS

Ana Benito Zafra

Aileen Santini Santiago

VISITING SCIENTIST

Maroš Kompas

(Dpt. Genetics and Microbiology,
Faculty of Sciences, Charles University,
Prague, Czech Republic)

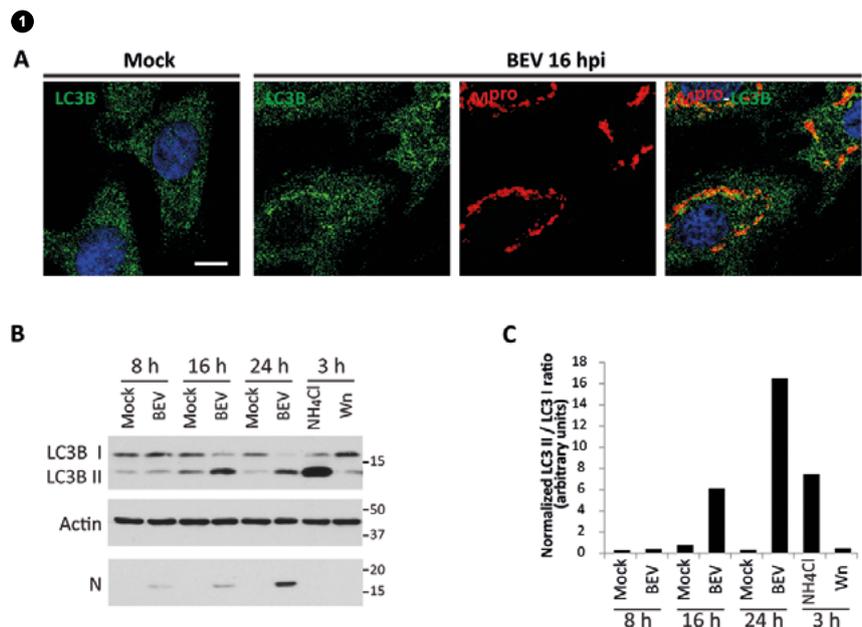
SELECTED PUBLICATIONS

Méndez F, Romero N, Cubas LL, Delgui L, Rodríguez D, Rodríguez JF. Non-lytic egression of infectious bursal disease virus (IBDV) particles from infected cells. *PLoS One* 2017; 12: e0170080.

Cubas-Gaona LL, Diaz-Beneitez E, Ciscar M, Rodríguez JF, Rodríguez D. Exacerbated apoptosis of cells infected with infectious bursal disease virus (IBDV) upon exposure to Interferon alpha (IFN- α). *J Virol* 2018; 92: e00364-18.

Toroviruses are positive-sense single-stranded RNA viruses belonging to the *Nidovirales* order that cause gastrointestinal disease in different domestic animal species and in humans. Toroviruses are distributed worldwide, and high prevalence has been reported in porcine and bovine livestock in different countries, including ours. Our research during this period has been focussed on the virus-host interaction to uncover the signalling pathways induced by torovirus infection that may contribute to either benefit or control virus infection. In this regard, we have shown that BEV, the prototype member of the *Torovirus* genus, activates the autophagy machinery as a cellular defence mechanism.

On the other hand, we have recently started a collaboration with the group of José F. Rodríguez at the CNB aimed at deciphering the molecular bases of infectious bursal disease virus (IBDV) pathogenesis. IBDV infection is responsible for the immunosuppression and/or death of infected birds, causing heavy losses to the poultry industry worldwide. We are focussed on the study of the effect of IFN- α/β and IFN- γ secreted by infected macrophages on IBDV-mediated cell death, a phenomenon that appears to be critical in the depletion of B-cell populations and the destruction of the *bursa of Fabricius* (BF), the major lymphoid organ in chickens, and also the main IBDV target organ. An intriguing hypothesis that arises from our results, to explain the destruction of the BF in IBDV infected chicken, is that IFN secreted by infected macrophages and lymphocytes may contribute to exacerbate cell death by apoptosis, leading to a chicken state of immunosuppression. Therefore, our objective is to characterise in depth the process leading to apoptosis of infected cells upon IFN treatment, both in cultured cells but also in bursal cells from IBDV-infected animals.



1 BEV infection activates the autophagy pathway.

(A) Immunofluorescence analysis of LC3 (green) and BEV Mpro (red) proteins in BEV-infected *E. Derm* cells. The nuclei were stained with DAPI. Scale bar, 10 μ m.

(B) Western blot analysis of the conversion of LC3B I to LC3B II in extracts of *E. Derm* cells, mock-infected or infected with BEV, collected at 8, 16 and 24 hpi. Extracts of *E. Derm* cells treated with ammonium chloride (20 mM, NH₄Cl) or wortmannin (5 μ m, Wn) during 3 h were included as control. Anti-BEV-N and anti-actin antibodies were used as infection and load control respectively.

(C) Quantification of the ratio LC3B II / LC3B I by densitometry.



Molecular biology of birnaviruses

MOLECULAR AND CELLULAR BIOLOGY 37

GROUP LEADER

José F. Rodríguez

POSTDOCTORAL SCIENTIST

Fernando Méndez

TECHNICIAN

Antonio Varas

PHD STUDENTS

Daniel Fuentes
Laura Broto

MASTER'S STUDENT

Alejandro Cenalmor

UNDERGRADUATE STUDENTS

Luis Morán
Sergio Nieves

VISITING SCIENTIST

Ikbel Hassine
(Higher Institute of Biotechnology,
University of Monastir, Tunisia)

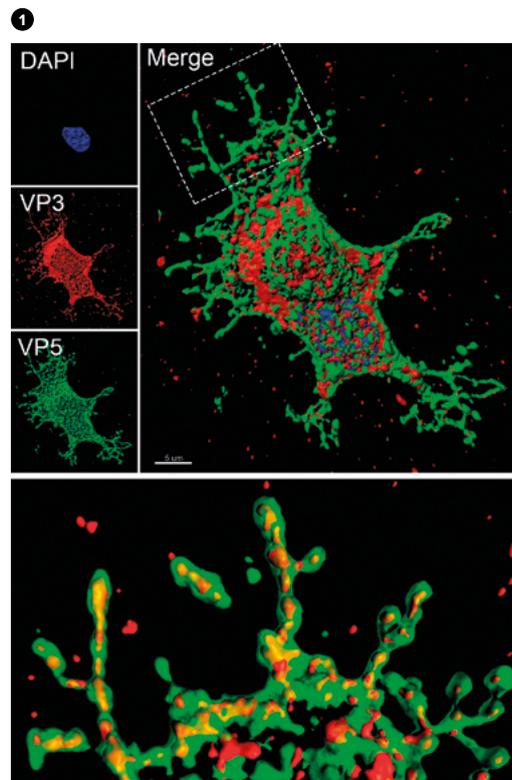
SELECTED PUBLICATIONS

Méndez F, Romero N, Cubas LL, Delgui L, Rodríguez D, Rodríguez JF. Non-lytic egression of infectious bursal disease virus (IBDV) particles from infected cells. *PLoS One* 2017; 12: e0170080.

Cubas-Gaona LL, Diaz-Beneitez E, Ciscar M, Rodríguez JF, Rodríguez D. Exacerbated apoptosis of cells infected with infectious bursal disease virus (IBDV) upon exposure to Interferon alpha (IFN- α). *J Virol* 2018; 92: e00364-18.

Birnaviruses are unconventional double-stranded (dsRNA) RNA viruses displaying unique structural features and molecular strategies to ensure genome expression/replication whilst controlling the onset of cellular innate antiviral responses. Our main birnavirus model is the infectious bursal disease virus (IBDV), the etiological agent of a devastating immunosuppressive avian disease, causing major losses to the poultry industry worldwide. Our work is currently focussed on two major topics, namely i) the mechanism underlying the non-lytic release of infectious virions, and ii) the molecular basis of IBDV-induced pathogenesis and virulence. We are currently closely collaborating with the group led by Dr. Dolores Rodríguez at the CNB to unravel the molecular mechanisms used by IBDV to counteract the antiviral cellular immune responses.

In addition to our IBDV work, we maintain collaborative links with academic and industrial research groups for vaccine development.



1 Non-lytic IBDV release. Upper left panels correspond to immunofluorescence images from an IBDV-infected QM7 cell stained with antibodies against the VP5 (green) and VP3 (red) IBDV polypeptides. Cell nuclei were stained with DAPI (blue). The right panel shows a 3D volume rendering of the image shown in left panels. The lower panel shows an isosurface 3D volume rendering detail (2.5x) from the upper right image. Images were acquired with a confocal multispectral Leica TCS SP8 system and processed using the Imaris software at the CNB Advanced Light Microscopy Core facility.



Embryonic development and differentiation in vertebrates

38 MOLECULAR AND CELLULAR BIOLOGY

GROUP LEADER

Juan José Sanz Ezquerro

TECHNICIAN

Elena Fernández-Cortés Navia

SELECTED PUBLICATIONS

Cuenda A. and Sanz-Ezquerro JJ. p38 γ and p38 δ : from spectators to key physiological players. *Trends Biochem Sci* 2017; 42: 431-442.

Sanz-Ezquerro JJ, Musterberg AE, Stricker S. Signalling pathways in embryonic development. *Front Cell Dev Biol* 2017; 5: 76.

Alsina-Beauchamp D, Escós A, Fajardo P, González-Romero D, Díaz-Mora E, Risco A, Martín-Serrano MA, Del Fresno C, Domínguez-Andrés J, Aparicio N, Zur R, Shpiro N, Brown GD, Ardavin C, Netea MG, Alemany S, Sanz-Ezquerro JJ, Cuenda A. Myeloid cell deficiency of p38 γ /p38 δ protects against candidiasis and regulates antifungal immunity. *EMBO Mol Med* 2018; 10: 8485.

Our group is interested in understanding the molecular and cellular basis of organ formation during embryonic development. This knowledge is important for identifying the origin of congenital malformations and to understand the basis of morphological evolution. We use animal models (mouse and chicken embryos) to address several biological questions related to development, such as morphogenesis and cell differentiation. We also study the role of developmental pathways in adult tissue homeostasis and regeneration.

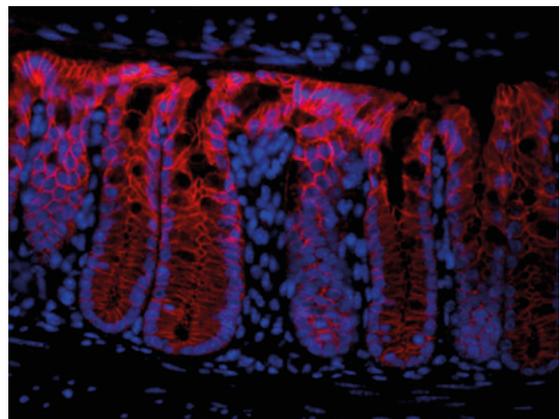
We have previously shown that Fgf signalling controls the formation of phalanges in digits. Moreover, we provided evidence that the last phalange has special features, including the presence of a specific molecular program that could be related to nail formation and the regenerative potential of the digit tips. We have continued the analysis of the Fgf signalling pathway during digit development, investigating how the maintenance of Fgf signals can induce the production of extra phalanges (hyperphalangy). We have also studied the expression and regulation in digits of MKP3 (a phosphatase that negatively regulates Fgf signalling) and the possible role of a MKP3 negative feedback loop in producing truncation of avian digits.

We are also interested in the relationship between inflammation and regeneration. The role of inflammation in regenerative processes is controversial. In some cases, it has been shown to improve tissue healing, but in other instances it has been shown to be detrimental to regeneration. In collaboration with the group of Ana Cuenda (Department of Immunology and Oncology, CNB) we are using a model of cancer associated to inflammation (colon cancer associated to colitis, CAC) to address this problem. By chemically inducing damage to the colon, which triggers an inflammatory response, we are investigating the role of p38MAPK signalling in the regeneration of the epithelium, the control of inflammation and in the induction of tumour formation.

1

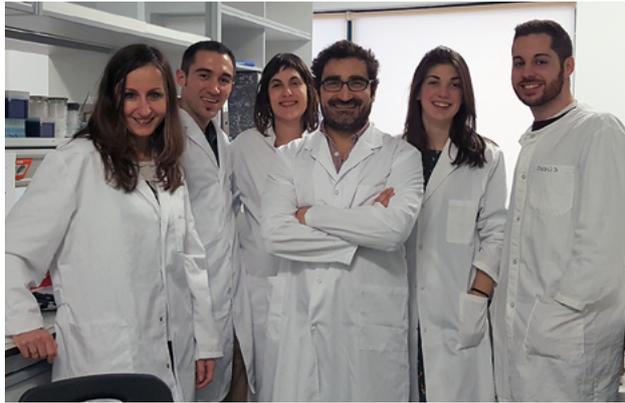


2



1 Expression of MKP3 in a chicken embryo wing detected by RNA in situ hybridisation.

2 Immunohistochemistry staining of mouse colon crypts showing expression of E-Cadherin (red). Cell nuclei are visualised in blue by DAPI staining.



Cellular immunobiology and microbiology

MOLECULAR AND CELLULAR BIOLOGY 39

GROUP LEADER

Esteban Veiga

SENIOR SCIENTIST

Aránzazu Cruz Adalia

TECHNICIAN

Mónica Torres Torresanoz

PhD STUDENTS

Guillermo Ramírez Santiago
Jesús Osuna Pérez
Raquel García Ferreras

SELECTED PUBLICATIONS

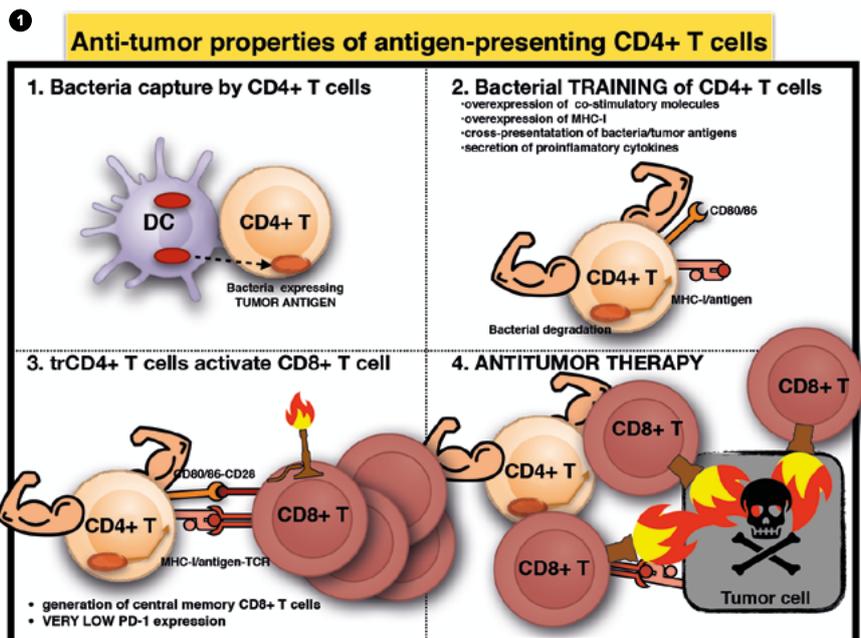
Cruz-Adalia A, Ramirez-Santiago G, Osuna-Pérez J, Torres-Torresano M, Zorita V, Martínez-Riaño A, Boccasavia V, Borroto A, Martínez Del Hoyo G, González-Granado JM, Alarcón B, Sánchez-Madrid F, Veiga E. Conventional CD4+ T cells present bacterial antigens to induce cytotoxic and memory CD8+ T cell responses. *Nat Commun* 2017; 8: 1591.

Antitumour therapies potentiating the immune response (mainly antibodies anti check point inhibitors) have emerged in recent years, allowing to treat previously intractable tumours. The response rates, however, remain low and these treatments are associated in many cases to undesirable side effects. It is therefore necessary to find novel therapies with increased response rates, minimising side effects and ideally leading to complete and lasting removal of the tumour.

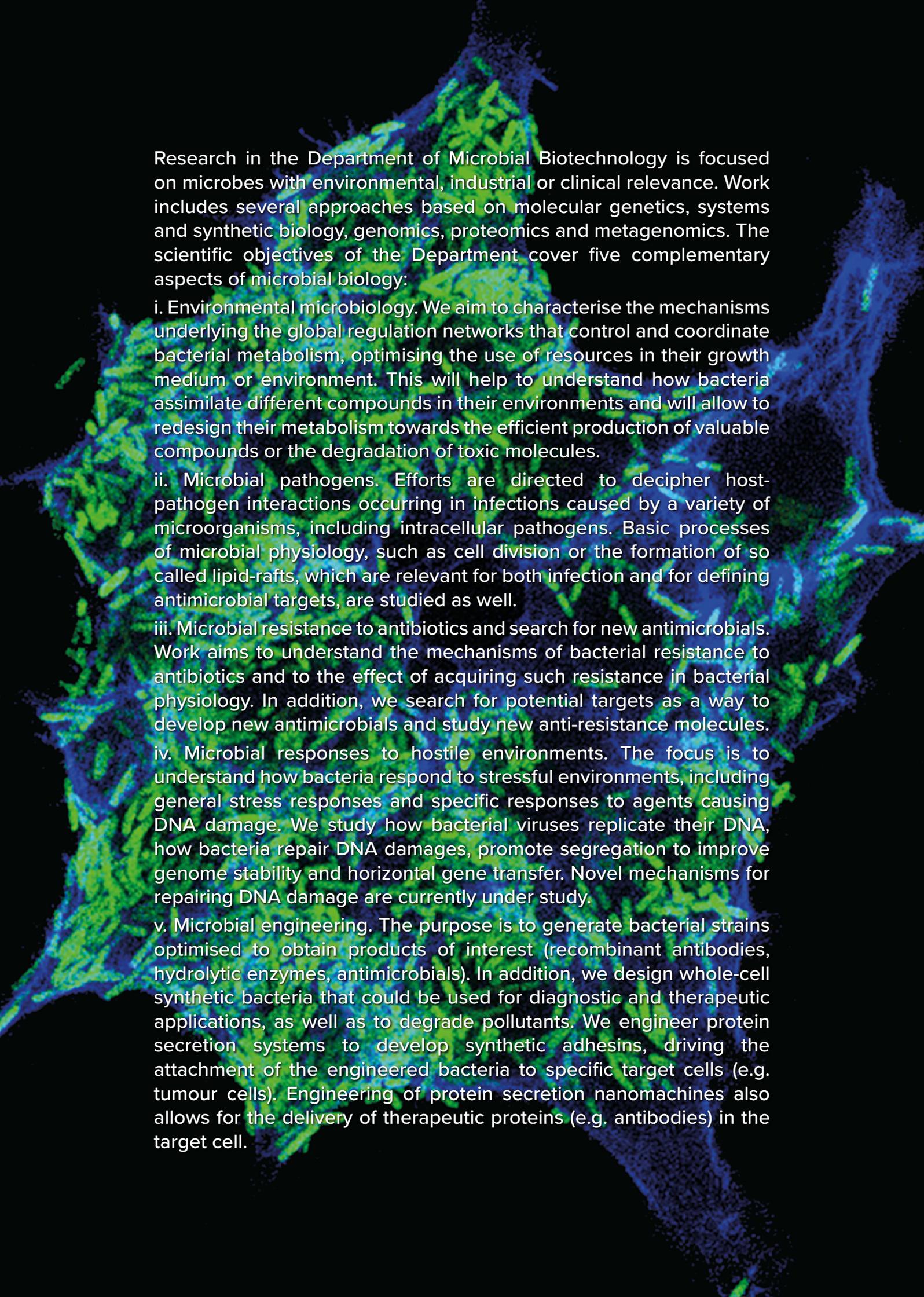
We have discovered that conventional CD4+ T cells can be “trained” after bacteria capture. Trained (tr), CD4+ T cells became potent antigen presenting cells able to (1) cross-present antigens from captured bacteria, activating naïve CD8+ T cells that became effective cytotoxic cells and (2) generating central memory; activities involved in the removal of tumours. Note that actually there exist huge efforts to generate central memory CD8+ T cells from tumour infiltrating lymphocytes. These effects, together with (3) the localised secretion of inflammatory cytokines by trCD4+ T cells, which could block the immunosuppressive environment generated by solid tumours, prompted us to hypothesised that trCD4+ T cells could be useful in antitumour therapies.

This hypothesis was tested in a proof-of-concept model of aggressive mouse melanoma. Mice treated with trCD4+ T cells that have captured/killed bacteria expressing tumour antigens (we generated bacteria expressing neoantigens from known tumour models) were protected against tumour development.

Now we are studying the antitumour potential of trCD4+ T cells (using different models of solid and liquid tumours) and the molecular mechanisms of trCD4+ T cells-driven antitumour responses.



1 CD4+ T cell capture and destroy bacteria by transphagocytosis (1). Exposure to bacteria “trains” CD4+ T cells that overexpress MHC-I and coestimulatory molecules and secrete locally inflammatory cytokines (2). Moreover, bacteria-trained (tr) CD4+ T cells differentiate into antigen cross-presenting cells, potentially activating naïve CD8+ T cells, and generate central memory CD8+ T cells expressing very low levels of PD-1(3). These activities are all involved in the removal of tumours. We are testing the antitumour activity of trCD4+ (4).

A fluorescence microscopy image of a cell, likely a bacterium, showing internal structures. The cell is stained with green and blue dyes, highlighting various components. The green staining appears to be distributed throughout the cell, while the blue staining is more concentrated in certain areas, possibly representing the cell wall or specific organelles. The background is dark, making the stained cell stand out.

Research in the Department of Microbial Biotechnology is focused on microbes with environmental, industrial or clinical relevance. Work includes several approaches based on molecular genetics, systems and synthetic biology, genomics, proteomics and metagenomics. The scientific objectives of the Department cover five complementary aspects of microbial biology:

i. Environmental microbiology. We aim to characterise the mechanisms underlying the global regulation networks that control and coordinate bacterial metabolism, optimising the use of resources in their growth medium or environment. This will help to understand how bacteria assimilate different compounds in their environments and will allow to redesign their metabolism towards the efficient production of valuable compounds or the degradation of toxic molecules.

ii. Microbial pathogens. Efforts are directed to decipher host-pathogen interactions occurring in infections caused by a variety of microorganisms, including intracellular pathogens. Basic processes of microbial physiology, such as cell division or the formation of so called lipid-rafts, which are relevant for both infection and for defining antimicrobial targets, are studied as well.

iii. Microbial resistance to antibiotics and search for new antimicrobials. Work aims to understand the mechanisms of bacterial resistance to antibiotics and to the effect of acquiring such resistance in bacterial physiology. In addition, we search for potential targets as a way to develop new antimicrobials and study new anti-resistance molecules.

iv. Microbial responses to hostile environments. The focus is to understand how bacteria respond to stressful environments, including general stress responses and specific responses to agents causing DNA damage. We study how bacterial viruses replicate their DNA, how bacteria repair DNA damages, promote segregation to improve genome stability and horizontal gene transfer. Novel mechanisms for repairing DNA damage are currently under study.

v. Microbial engineering. The purpose is to generate bacterial strains optimised to obtain products of interest (recombinant antibodies, hydrolytic enzymes, antimicrobials). In addition, we design whole-cell synthetic bacteria that could be used for diagnostic and therapeutic applications, as well as to degrade pollutants. We engineer protein secretion systems to develop synthetic adhesins, driving the attachment of the engineered bacteria to specific target cells (e.g. tumour cells). Engineering of protein secretion nanomachines also allows for the delivery of therapeutic proteins (e.g. antibodies) in the target cell.

Microbial Biotechnology

HEAD OF DEPARTMENT

José Luis Martínez

RESEARCH GROUPS

1. Genetic stability

Juan C. Alonso

2. Recombination-dependent DNA replication

Silvia Ayora

3. Stress and bacterial evolution

Jesús Blázquez

4. Bacterial engineering for biomedical applications

Luis A. Fernández-Herrero

5. Laboratory of intracellular bacterial pathogens

Francisco García-del Portillo

6. Molecular infection biology

Daniel López

7. Ecology and evolution of antibiotic resistance

José Luis Martínez

8. Heterologous gene expression and secretion in gram-positive bacteria with industrial applications

Rafael P. Mellado

9. Regulation of gene expression and metabolism in bacteria

Fernando Rojo

10. Genetic control of cell cycle

Miguel Vicente



Genetic stability

GROUP LEADER

Juan C. Alonso

SENIOR SCIENTISTS

Begoña Carrasco
María Moreno del Alamo

POSTDOCTORAL SCIENTIST

Ester Serrano

TECHNICIAN

Chiara Marchisone

PhD STUDENTS

Héctor Romero
Ruben Torres

UNDERGRADUATE STUDENTS

Feras Barak
Gabriela Bello
Ophélie Danneels
Pauline Feurgard
Eleni Pretridi
Lucía Sánchez
Soraya Tadrissi
Kristina Tramm
Sophia Vorri

SELECTED PUBLICATIONS

Le S, Serrano E, Kawamura R, Carrasco B, Yan J, Alonso JC. *Bacillus subtilis* RecA with DprA-SsbA antagonizes RecX function during natural transformation. *Nucleic Acids Res* 2017; 45: 8873-8885.

Moreno-Del Álamo M, Tabone M, Liroy VS, Alonso JC. Toxin ζ triggers a survival response to cope with stress and persistence. *Front Microbiol* 2017; 8: 1130.

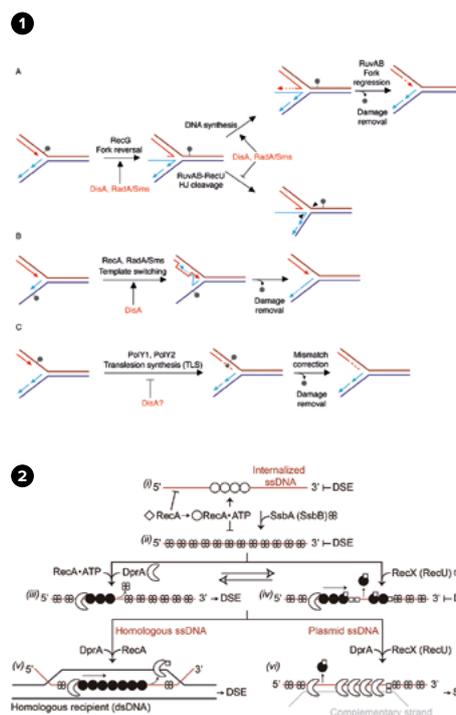
Raguse M, Torres R, Seco EM, Gándara C, Ayora S, Moeller R, Alonso JC. *Bacillus subtilis* DisA helps to circumvent replicative stress during spore revival. *DNA Repair (Amst)* 2017; 59: 57-68.

Serrano E, Carrasco B, Gilmore JL, Takeyasu K, Alonso JC. RecA regulation by RecU and DprA during *Bacillus subtilis* natural plasmid transformation. *Front Microbiol* 2018; 9: 1514.

Carrasco B, Seco EM, López-Sanz M, Alonso JC, Ayora S. *Bacillus subtilis* RarA modulates replication restart. *Nucleic Acids Res* 2018; 46: 7206-7220.

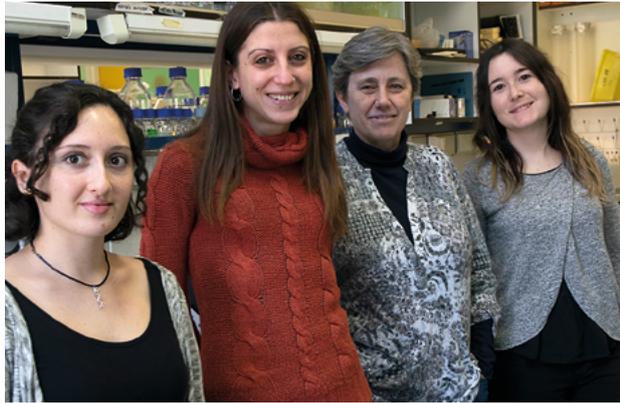
Our research focuses on the study of the molecular mechanisms that bacteria of the *Firmicutes phylum* use to secure genomic stability, to promote horizontal gene transfer, and to control cell proliferation and accurate plasmid segregation. Using *Bacillus subtilis* as a model, we have shown that the DNA damage response recruits different complex molecular machineries depending on the type of DNA damage and the growth conditions. We have shown that, in the presence of stalled or reversed forks (Holliday junction, HJ), DisA may trigger error-free and error-prone DNA damage tolerance (DDT) responses. DisA, in concert with the RecA recombinase, branch migration translocases (RecA/Sms, RecG and RuvAB) and the HJ resolvase (RecU) RadA/Sms, recognises the displaced loops and HJ intermediates and suppress c-di-AMP synthesis that in turn halts cell proliferation during exponential growth or during revival of haploid spores (Figure 1).

Starved *B. subtilis* cells develop natural competence, with DprA, SsbB, SsbA, RecO(R), RecX, and CoiA contributing to help RecA to increase genetic diversity. Studying the functions that control RecA activities, we are addressing how mediators and modulators contribute to the maintenance of the species. SsbA or SsbB inhibits RecA loading onto ssDNA. DprA enhances the polymerisation of RecA onto SsbA-coated ssDNA and RecX or RecU facilitates RecA depolymerisation from ssDNA. Activated RecA or DprA counters RecX or RecU negative regulation on RecA nucleoprotein filament assembly (Figure 2). Plasmid transformation, which is a RecA-independent event, requires RecX or RecU to promote RecA depolymerisation from the linear plasmid ssDNA, and DprA to catalyse DNA strand annealing of the complementary strands coated by SsbA or SsbB, and circularisation of the redundant tailed ends to render an active replicon.



1 Proposed DDT mechanisms. An unrepaired DNA lesion on the leading strand template (black dot) (A and C) or an unrepaired DNA lesion on the lagging strand (B) causes replication fork blockage. (A) DisA regulates branch migration of the stalled fork. Synthesis of the DNA complementary to the damaged site (dotted line) overcomes blockage upon fork regression. B, DisA regulates RecA-mediated strand invasion on the undamaged sister strand (template switching). C, the replicative DNA polymerase is replaced by PolY1 and/or PolY2 that catalyse nucleotide mis-incorporation (lesion bypass, denoted with X). After damage removal and mismatch correction replication continues.

2 Model for RecA-mediated natural transformation. Apo RecA cannot nucleate on the ssDNA. RecA bound to ATP undergoes its first structural transition (empty circle). RecA-ATP can nucleate onto ssDNA, but it cannot catalyse DNA strand exchange (DSE) (step i). RecA-ATP cannot nucleate on the SsbA-ssDNA complexes (step ii). DprA interacts with and loads RecA onto SsbA-coated ssDNA. RecA undergoes its second transition (filled circle) E (step iii). RecU (or RecX) promotes RecA disassembly from the ssDNA (step iv). DprA assists RecA-ATP assembly and favour homologous chromosomal transformation (step v). In the absence of homology, DprA binds to the complementary plasmid strand (gray line) and catalyses single strand annealing (SSA) (step vi).



Recombination-dependent DNA replication

MICROBIAL BIOTECHNOLOGY 43

GROUP LEADER

Silvia Ayora

TECHNICIAN

María López Sanz

PHD STUDENTS

Cristina Ramos
Laura Torralba

UNDERGRADUATE STUDENTS

Alvaro Quevedo
Iva Gramatikov
Bernadett Dávid
Christopher Sánchez

SELECTED PUBLICATIONS

Valero-Rello A, López-Sanz M, Quevedo-Olmos A, Sorokine A, Ayora S. Molecular mechanisms that contribute to horizontal transfer of plasmids by the bacteriophage SPP1. *Front Microbiol* 2017; 8: 1816.

Seco EM and Ayora S. *Bacillus subtilis* DNA polymerases, PolC and DnaE, are required for both leading and lagging strand synthesis in SPP1 origin-dependent DNA replication. *Nucleic Acids Res* 2017; 45: 830-8313.

Neamah MM, Mir-Sanchis I, López-Sanz M, Acosta S, Baquedano I, Haag AF, Marina A, Ayora S, Penades JR. Sak and Sak4 recombinases are required for bacteriophage replication in *Staphylococcus aureus*. *Nucleic Acids Res* 2017; 45: 6507-6519.

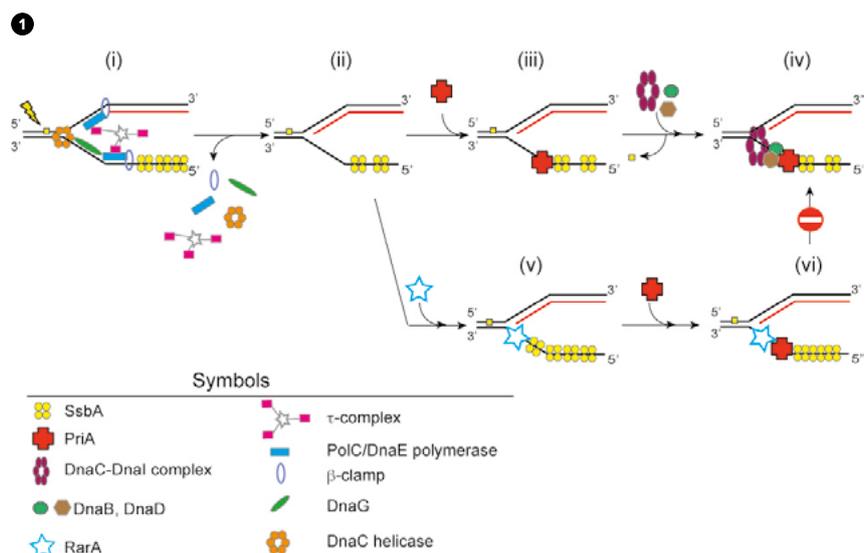
Khavnekar S, Dantu SC, Sedelnikova S, Ayora S, Rafferty J, Kale A. Structural insights into dynamics of RecU-HJ complex formation elucidates key role of NTR and stalk region toward formation of reactive state. *Nucleic Acids Res* 2017; 45: 975-986.

Carrasco B, Seco EM, López-Sanz M, Alonso JC, Ayora S. *Bacillus subtilis* RarA modulates replication restart. *Nucleic Acids Res* 2018; 46: 7206-72220.

Genomic instability is minimised by four major mechanisms: high-fidelity DNA replication, precise chromosome segregation, error free repair of DNA damage and coordinated cell cycle progression. Our research focuses on the study of the mechanisms that cells use to continue DNA replication when this process encounters impediments, which may eventually stall or collapse the fork, producing DNA gaps or broken DNA ends. Replication restart is then mediated by proteins that were initially identified by their roles in homologous recombination and repair of DNA double-strand breaks. We use a simple model system, *Bacillus subtilis* and its bacteriophage SPP1, and several biophysics, molecular biology and genetic techniques to study the recombination mechanisms that contribute to genome stability.

One of our lines of research focuses on the effect of recombination proteins in DNA replication. In the last years, we have reconstituted *in vitro* the replisome of *B. subtilis* and its phage SPP1. Mimicking stalled and collapsed replication forks with artificial substrates, we have analysed how the conserved RarA protein modulates replication restart. Currently, the effects of other recombination proteins in DNA replication are under study.

Recombination also leads to evolution, and recombination proteins are frequently encoded in the genome of many bacteriophages. Our study has demonstrated that viral DNA-single strand annealing proteins of the Sak, Sak4 and RecT families are absolutely required for phage DNA replication and for the horizontal transfer of plasmids, independently of their mechanism of replication. The contribution of other viral recombination proteins to DNA replication and horizontal gene transfer was also analysed.



2 Model of RarA action on blocked forks. (i) When a lesion blocks DNA replication, DNA synthesis is stopped and the replisome disassembles. (ii to iv) SsbAA-PriA load the DnaC helicase. Then the damage is repaired, and the helicase DnaC recruits DnaG primase and the replisome and DNA replication can restart. (v and vi) If the DNA damage is not repaired, SsbA loads RarA at the stalled fork. The SsbA-RarA-PriA-DNA complex impedes the recruitment of the replisome and initiation of DNA synthesis is inhibited.



Stress and bacterial evolution

44 MICROBIAL BIOTECHNOLOGY

GROUP LEADER

Jesús Blázquez

SENIOR SCIENTISTS

Susanne Gola
Alfredo Castañeda
Meritxel García

TECHNICIAN

Pablo García

PhD STUDENTS

Esmeralda Cebrían
Vanessa Baldir

UNDERGRADUATE STUDENTS

Félix Martínez
Isabel Martín
Violeta de Anca

SELECTED PUBLICATIONS

Castañeda-García A, Prieto AI, Rodríguez-Beltrán J, Alonso N, Cantillon D, Costas C, Pérez-Lago L, Zegeye ED, Herranz M, Płociński P, Tonjum T, García de Viedma D, Paget M, Waddell SJ, Rojas AM, Doherty AJ, Blázquez J. A non-canonical mismatch repair pathway in Prokaryotes. *Nat Commun* 2017; 8: 14246.

Martín-Gutiérrez G, Docobo-Pérez F, Rodríguez-Beltrán J, Rodríguez-Martínez JM, Aznar J, Pascual A, Blázquez J. Urinary tract conditions affect fosfomicyn activity against *Escherichia coli* strains harboring chromosomal mutations involved in fosfomicyn uptake. *Antimicrob Agents Chemother* 2017; 62: e01899-17.

Recacha E, Machuca J, Díaz de Alba P, Ramos-Güelfo M, Docobo-Pérez F, Rodríguez-Beltrán J, Blázquez J, Pascual A, Rodríguez-Martínez JM. Quinolone resistance reversion by targeting the SOS response. *MBio* 2017; 8: e00971-17.

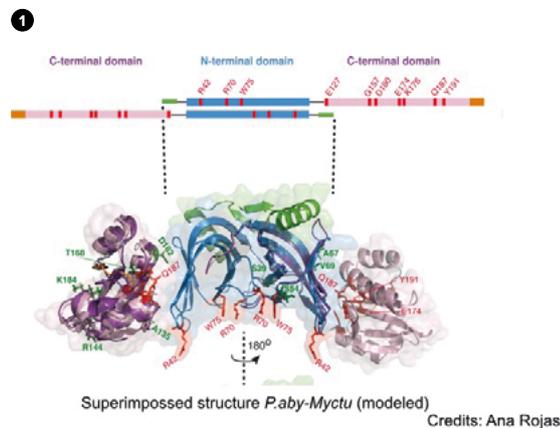
Blázquez J, Rodríguez-Beltrán J, Ivan Matic I. Antibiotic-induced genetic variation: How it arises and how it can be prevented. *Annu Rev Microbiol* 2018; 72: 209-230.

Ibacache-Quiroga C, Oliveros JC, Couce A, Blázquez J. Parallel evolution of high-level aminoglycoside resistance in *Escherichia coli* under low and high mutation supply rates. *Front Microbiol* 2018; 9: 427.

We try to understand the genetic mechanisms involved in genome stability in bacteria and their roles in evolution and adaptation. Specifically, we study the genetic basis of both stable and induced hyper-mutation/hyper-recombination as bacterial “strategies” to speed adaptation to environmental stresses such as antibiotics.

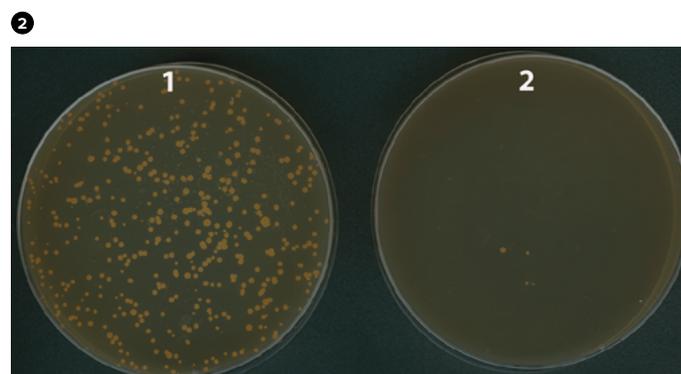
We have discovered a novel non-canonical mismatch repair system in prokaryotes (present in Archaea and Actinobacteria), responsible for maintaining genome stability in *Mycobacterium*. Mutants in the key protein of this system, NucS, display increased mutation and recombination rates. We are trying to disentangle its genetics and biochemical bases in *Mycobacterium* and *Streptomyces*.

We are applying this knowledge to i) understand and prevent the development of antibiotic resistance in bacterial pathogens (including *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Mycobacterium abscessus* and *Mycobacterium tuberculosis*) and ii) improve prokaryotic species of biotechnological interest (including *Streptomyces*, *Mycobacterium*, *Bifidobacterium*, *Rhodococcus*, *Corynebacterium*, *Pyrococcus* and *Halobacterium*).



1 Domain characterisation of *M. tuberculosis* NucS, the key protein of the novel non-canonical mismatch repair system. Credits: Ana Rojas.

2 NucS is a guardian of the genome stability in *Mycobacterium*. Increased production of mutant clones resistant to rifampicin in a *Mycobacterium smegmatis* strain lacking nucS gene (1) in comparison with the nucS-proficient strain (2).





Bacterial engineering for biomedical applications

MICROBIAL BIOTECHNOLOGY 45

GROUP LEADER

Luis Ángel Fernández Herrero

SENIOR SCIENTISTS

Beatriz Álvarez González
Elena M. Seco Martín

POSTDOCTORAL SCIENTIST

Massiel Cepeda Molero

TECHNICIAN

Yago Margolles Azpiazu

PhD STUDENTS

Carmen Mañas Torres
Lidia Cerdán García
Alejandro Asensio Calavia
Eva Pico Sánchez
Álvaro Ceballos Munuera

VISITING SCIENTIST

Ronald Jara Gacitua

SELECTED PUBLICATIONS

Álvarez B and Fernández LA. Sustainable therapies by engineered bacteria. *Microb Biotechnol* 2017; 10: 1057-1061.

Salema V and Fernández LA. *Escherichia coli* surface display for the selection of nanobodies. *Microb Biotechnol* 2017; 10: 1468-1484.

Cepeda-Molero M, Berger CN, Walsham A, Ellis S, Wemys-Holden S, Schüller S, Frankel G, Fernández LA. Attaching and effacing (A/E) lesion formation by enteropathogenic *E. coli* on human intestinal mucosa is dependent on non-LEE effectors. *PLoS Pathog* 2017; 13: e1006706.

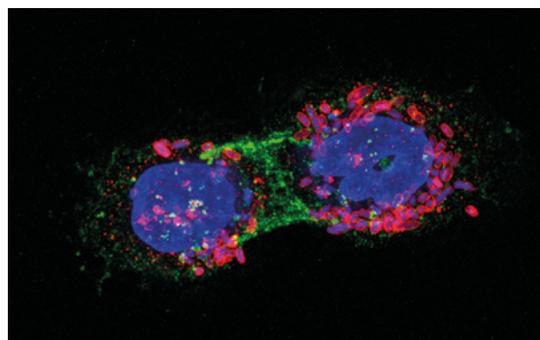
Kalogiraki I, Abellán Flos M, Fernández LA, Menéndez M, Vincent S, Solís D. Direct evaluation of live uropathogenic *E. coli* adhesion and efficiency of anti-adhesive compounds using a simple microarray approach. *Anal Chem* 2018; 90: 12314-12321.

Our research is aimed to engineer *E. coli* bacteria for biomedical applications, including the selection of small recombinant antibodies and the design of bacteria for diagnostic and therapeutic use *in vivo*. We study protein secretion systems found in pathogenic *E. coli* strains, such as enteropathogenic *E. coli* (EPEC), and engineer them to develop protein nanomachines that can be applied for selection of recombinant antibodies and the delivery of therapeutic proteins by non-pathogenic *E. coli* strains. Among the recombinant antibodies, we employ single-domain antibodies (sdAbs) or nanobodies, the smallest antibody fragments known-to-date with full antigen-binding capacity. We use synthetic biology approaches and genome engineering to combine the expression of these modular parts in the designed bacteria.

The specific projects in which we have worked are:

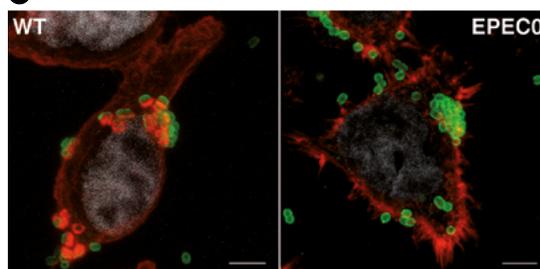
- E. coli* display technology for selection of nanobodies from libraries.** Bacterial outer membrane proteins of the Intimin-Invasin and autotransporter families have been used to display nanobody libraries on the surface of *E. coli* and for the selection of high-affinity binders against antigens relevant in infection diseases and cancer.
- Re-programming *E. coli* adhesion to tumours with synthetic adhesins.** The display of nanobodies on the surface of *E. coli* has allowed us to generate “synthetic adhesins” that can drive the attachment of bacteria to target tumour cells expressing cell surface antigens.
- Injection of therapeutic proteins from *E. coli* into human cells.** We are engineering the type III protein secretion system (T3SS) from EPEC to directly deliver therapeutic proteins and nanobodies from *E. coli* into the cytosol of tumour cells. We are engineering the controlled expression of EPEC T3SS in the non-pathogenic *E. coli* K-12 strain to specifically deliver cytotoxins in tumour cells. In addition, we have generated an effector-less EPEC strain (EPECO) that can inject specific combination of proteins of interest to human cells.

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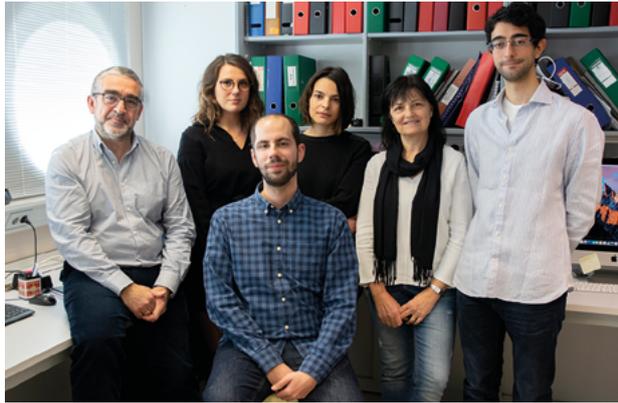


1 Fluorescence confocal microscopy image showing *E. coli* bacteria (red fluorescence) with synthetic adhesins targeting an antigen (green fluorescence) expressed on the surface of human tumour cells (nuclei and bacterial DNA stained in blue).

2



2 Fluorescence confocal microscopy image showing enteropathogenic *E. coli* (EPEC) (green fluorescence) wild type (WT) and effector-less strain (EPECO) infecting HeLa cells. EPECO bacteria do not inject any effector present in the pathogen, like the translocated intimin receptor Tir that polymerises F-actin under the attached bacteria. (F-actin in red; nuclei in grey).



Laboratory of intracellular bacterial pathogens

46 MICROBIAL BIOTECHNOLOGY

GROUP LEADER

Francisco García del Portillo

SENIOR SCIENTIST

María Graciela Pucciarelli

POSTDOCTORAL SCIENTISTS

Sonia Castanheira
Joaquín Giner

PhD STUDENTS

Juan José Cestero
Charlotte Dessaux
David López
Alberto Díaz

TECHNICIAN

Pablo García

MASTER'S STUDENT

Sandra Laborda

UNDERGRADUATE STUDENTS

Adrian Plaza
Carmen Morales
Jonathan Ruiz

VISITING SCIENTIST

Rodolfo Ortíz
(Univ. Nacional Cuyo, Mendoza, Argentina)

VISITING STUDENTS

Nicolás Figueroa (Argentina)
Julieta Bisio (Uruguay)
María Laura Echarren (Argentina)
Isela Serrano (Mexico)

SELECTED PUBLICATIONS

Castanheira S, *et al.* A specialized peptidoglycan synthase promotes *Salmonella* cell division inside host cells. *MBio* 2017; 8: e01685-17.

Impens F, *et al.* N-terminomics identifies Prli42 as a membrane miniprotein conserved in Firmicutes and critical for stressosome activation in *Listeria monocytogenes*. *Nat Microbiol* 2017; 2: 17005.

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Castanheira S, Cestero JJ, García-del Portillo F, Pucciarelli MG. Two distinct penicillin binding proteins promote cell division in different *Salmonella* lifestyles. *Microb Cell* 2018; 5: 165-168.

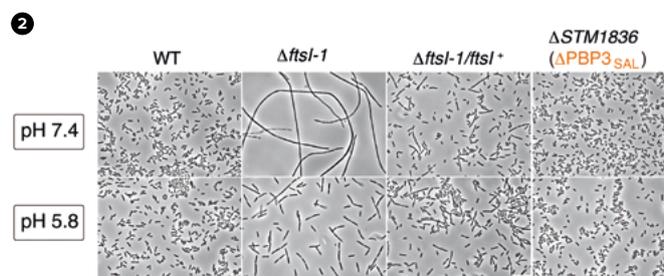
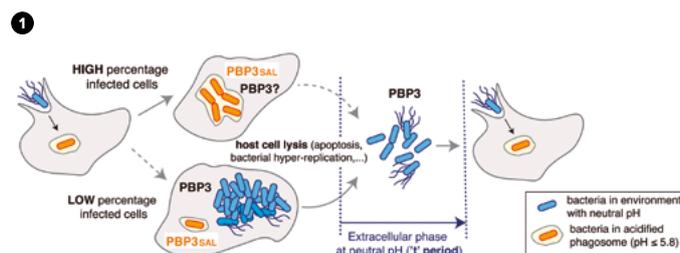
Casino P, Miguel-Romero L, Huesa J, García P, García-del Portillo F, Marín A. Conformational dynamism for DNA interaction in the *Salmonella* RcsB response regulator. *Nucleic Acids Res* 2018; 46: 456-472.

Our lab aims to understand the physiological changes that accompany the adaptation to an intracellular lifestyle of the model pathogens *Listeria monocytogenes* and *Salmonella enterica*, both causing food-borne diseases of high incidence in humans and livestock.

During these two years, we have focused on deciphering mechanisms responsible for: i) the adaptation of *Listeria monocytogenes* to stress conditions such as high osmolarity and cold (4°C), as well as the assembly dynamics of the 'stressosome' complex; and, ii) the remodelling of the main cell wall component, peptidoglycan, in *Salmonella enterica* when this pathogen inhabits the intracellular niche of eukaryotic cells. These studies have allowed us to unravel novel phenomena with no precedents in the literature.

These novel findings include the induction of a subset of cell surface proteins and regulatory small RNAs (sRNAs) when *L. monocytogenes* grows at 4°C and the chemical modification of the peptidoglycan when *S. enterica* persists inside vacuolar compartments of eukaryotic cells. We have also obtained preliminary evidence linking these peptidoglycan modifications to alteration of defence responses in the infected cell.

Most of our current interest is now directed to: i) unravel the global regulatory network of *L. monocytogenes* at 4°C (temperature at which this food-borne pathogen proliferates) using RNASeq approaches and classical genetics; ii) assess how the stressosome complex of *L. monocytogenes* responds to the intracellular infection; iii) identify the enzymatic activities of *S. enterica* that alter peptidoglycan structure in response to host signals; and, iv) define how intra-vacuolar *S. enterica* interferes host innate immunity signalling to establish a persistent infection.



1 Model proposed for the difference use of penicillin-binding proteins PBP3 and PBP3SAL by *Salmonella enterica* during the course of the infection. Note that PBP3SAL promotes cell division of the pathogen inside acidic phagosomes of eukaryotic cells.

2 Phenotype of a *Salmonella enterica* mutant lacking the *ftsI* gene that encodes penicillin binding 3 (PBP3), a peptidoglycan enzyme essential for cell division in *Escherichia coli*. Note that this $\Delta ftsI$ mutant, which can only use the *Salmonella*-specific PBP3SAL for building the division septum, divides exclusively in acidified media.



Molecular infection biology

MICROBIAL BIOTECHNOLOGY 47

GROUP LEADER

Daniel López

SENIOR SCIENTIST

Ana Isabel Rico

POSTDOCTORAL SCIENTISTS

Esther García Fernández
María del Mar Cordero Alba
Julia García Fernández
Ilaría Peschería
Marta Ukleja
Marcin Krupka

PROJECT MANAGER

Irene Sánchez

TECHNICIAN

Ana Marina Cabrerizo

PHD STUDENTS

Rabea Wagner
Lara Kricks
Iván Camilo Acosta
Diana Damián
Elena Pedrero
Samuel García
César Omar Domínguez

SELECTED PUBLICATIONS

García-Fernandez E, Koch G, Wagner RM, ... López D. Membrane microdomain disassembly inhibits MRSA antibiotic resistance. *Cell* 2017; 171: 1354-1367.

Mielich-Süß B, ... López D. Flotillin scaffold activity contributes to type VII secretion system assembly in *Staphylococcus aureus*. *PLoS Pathog* 2017; 13: e1006728.

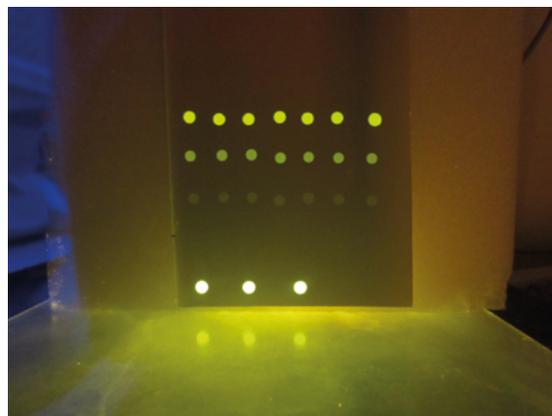
García-Betancur JC, ... López D. Cell Differentiation defines acute and chronic infection cell types in *Staphylococcus aureus*. *eLife* 2017; 6: e28023

Koch G, Wermser Ch, Acosta IC, Kricks L, ... López D. Attenuating *Staphylococcus aureus* virulence by targeting flotillin protein scaffold activity. *Cell Chem Biol* 2017; 24: 845-857.

Dragos A, Martin M, Falcón-García C, Kricks L, ... López D, Lieleg O & Kovács AT. Collapse of genetic division of labour and evolution of autonomy in pellicle biofilms. *Nat Microbiol* 2018; 3: 1451-1460.

A number of bacterial cell processes are confined in platforms termed functional membrane microdomains, some of whose organisational and functional features resemble those of lipid rafts of eukaryotic cells. How bacteria organise these intricate platforms and their biological significance remains an important question. Our laboratory is a key laboratory in the field of functional membrane microdomain bacterial compartmentalisation and its role during infections, using MRSA (Methicillin-resistance *Staphylococcus aureus*) as model organism. Our research is supported by competitive funding, such as ERC-StG-2013 or H2020 RIA Biotech-03-2016. We aim to identify the structure and molecular mechanisms that lead to bacterial membrane compartmentalisation and their role during staphylococcal infections that are resistant to antibiotic treatments. To this end, we work at the interface of molecular and cellular biology with other scientific disciplines, such as structural, infection, synthetic and systems biology. This interactive and multidisciplinary environment provides to my laboratory a means to open novel areas of research, unravel the mechanisms of bacterial infections and discover innovative antimicrobial strategies to fight antibiotic resistance and multi-drug resistance pathogens, with special emphasis on those associated with hospital infections.

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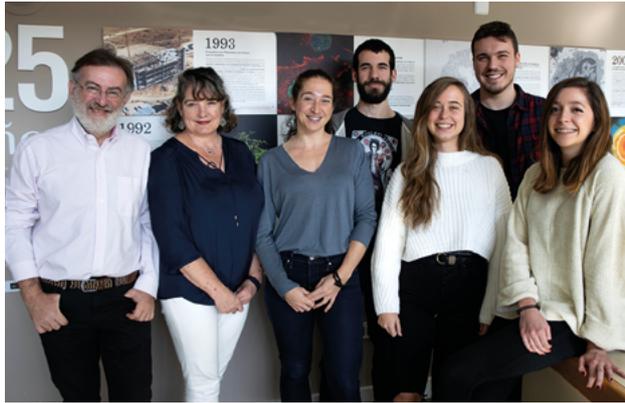


1 Several different fluorescently labeled lipids spotted on a TLC plate and visualised under blue light.

2 Competitive behavior of two distinct strains of *Staphylococcus aureus* growing in a single macrocolony.

2





Ecology and evolution of antibiotic resistance

48 MICROBIAL BIOTECHNOLOGY

GROUP LEADER

José Luis Martínez

SENIOR SCIENTISTS

María Blanca Sánchez
Sara Hernando-Amado

POSTDOCTORAL SCIENTISTS

Manuel Alcalde-Rico
Fernando Corona
Felipe Lira
Aida Pitarch

PhD STUDENTS

Paula Blanco
Teresa Gil
Pablo Laborda
Fernando Sanz-García

TECHNICIAN

Trinidad Cuesta

MASTER'S STUDENTS

Cristina Rosco
Alejandro Tejada

UNDERGRADUATE STUDENTS

Irene Clares
Isabel Alonso
Kepa Arbe
Rafael Amieva
Beatriz Barrientos
Patricia Blanco

SELECTED PUBLICATIONS

Baquero F, Martínez JL. Interventions on metabolism: making antibiotic-susceptible bacteria. *mBio* 2017; 8: e01950-17-e01950-17.

Bernardini, A, Martínez, JL. Genome-wide analysis shows that RNase G plays a global role in the stability of mRNAs in *Stenotrophomonas maltophilia*. *Sci Rep* 2017; 7: 16016.

Olivares J, Álvarez-Ortega C, Alcalde Rico M, Martínez JL. Metabolic compensation of fitness costs is a general outcome for antibiotic resistant *Pseudomonas aeruginosa* mutants overexpressing efflux pumps. *MBio* 2017; 8: e00500-17.

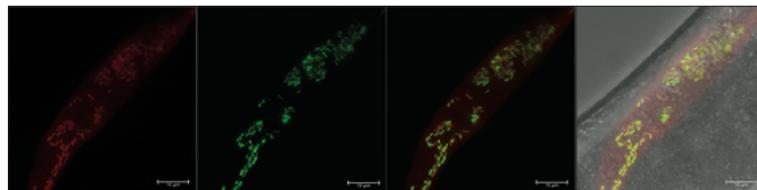
Blanco P, Corona F, Martínez JL. Biolog phenotype microarray is a tool for the identification of multidrug resistance Efflux pump inducers. *Antimicrob Agents Chemother* 2018; 62: e01263-18.

Sanz-García F, Hernando-Amado S, Martínez JL. Mutation-driven evolution of *Pseudomonas aeruginosa* in the presence of either ceftazidime or ceftazidime-avibactam. *Antimicrob Agents Chemother* 2018; 62: e01379-18.

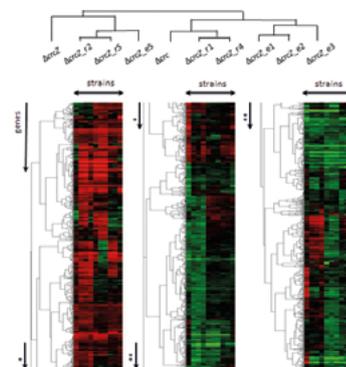
We study the biology of opportunistic pathogens, focusing on the networks and the evolutionary processes that connect resistance and virulence. In particular, in the last years we have proposed several rules for predicting the emergence of antibiotic resistance and are currently standardising these tools, which are based on experimental evolution, whole-genome sequencing and functional assays. One important element in our studies is determining the stochastic and deterministic elements that modulate the evolutionary trajectories towards antibiotic resistance of bacterial pathogens. Using this approach, we characterised mechanisms of resistance to latest-generation antibiotics and identified the target and the mechanisms of resistance to antibiotics still under development.

Since evolution towards resistance presents some degree of predictability, it is important to address the elements shifting stochastic evolution towards determinism. Among them, we are particularly interested in the epistatic interactions between antibiotic resistance elements and also between elements involved in antibiotic resistance and virulence of bacterial pathogens. Finally, we are currently studying the epigenetic events leading to transient resistance, in particular the signals that trigger such resistance, as well as identification of the differential elements modulating antibiotic resistance at the populational and the single cell levels.

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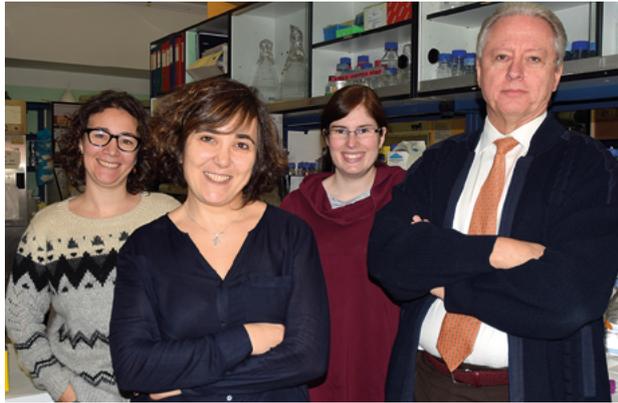


2



1 Heterogeneous gene expression of *Pseudomonas aeruginosa* cells colonising the gut of *Caenorhabditis elegans*.

2 Transcriptional analysis of a *crcZ*-deficient *Pseudomonas* mutant in comparison with pseudo-revertant mutants selected in complex growth medium.



Heterologous gene expression and secretion in Gram-positive bacteria with industrial applications

MICROBIAL BIOTECHNOLOGY 49

GROUP LEADER
Rafael P. Mellado

SENIOR SCIENTIST
Sonia Gullón

POSTDOCTORAL SCIENTIST
Rebeca López Vicente

TECHNICIAN
Silvia Marín

VISITING SCIENTIST
Sara Fuentes Saborío
(Instituto Tecnológico de Costa Rica,
San José, Costa Rica)

SELECTED PUBLICATIONS

Gullón S and Mellado RP. The cellular mechanisms that ensure an efficient secretion in *Streptomyces*. *Antibiotics* (Basel) 2018, 7: E33.

Valverde JR, Gullón S, Mellado RP. Modelling the metabolism of protein secretion through the Tat route in *Streptomyces lividans*. *BMC Microbiol* 2018; 18: 59.

Our group has a long-standing interest in the physiological and molecular characterisation of the protein secretory routes of the soil Gram-positive bacteria *Streptomyces lividans*, a well-known efficient producer of extracellular hydrolytic enzymes and other compounds of industrial application.

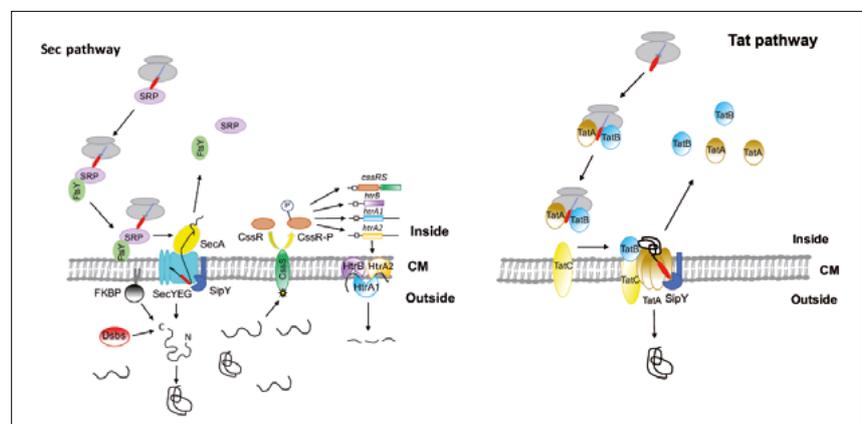
We are currently advancing in the characterisation of the components affecting the quality and quantity of the protein secreted in *S. lividans*. Hence, we are studying the functioning of the three specific proteases acting in a cooperative manner (Vicente, Gullón, Marín and Mellado, *PLoS One* 2016; 11: e0168112), which degrade the secreted misfolded or unfolded polypeptides extracellularly accumulating outside the cell when overproduced, presumably, forming part of the quality control factors present in the bacteria. Moreover, we are characterising the bacterial proteins involved in the correct folding of the secreted proteins (peptidyl-prolyl cis-trans isomerases and thiol-disulphide oxidoreductases) to warrant a suitable balance between all of them so as to ensure an efficient secretion of the oversynthesised secretory proteins.

We also look at the structural characteristics that may favour the secretion of proteins via the major secretory route (Sec), which releases incorrectly folded proteins versus the minor route (Tat) which releases properly folded polypeptides to the culture medium, thereby facilitating the engineering of the most potentially effective transport routes to the culture medium.

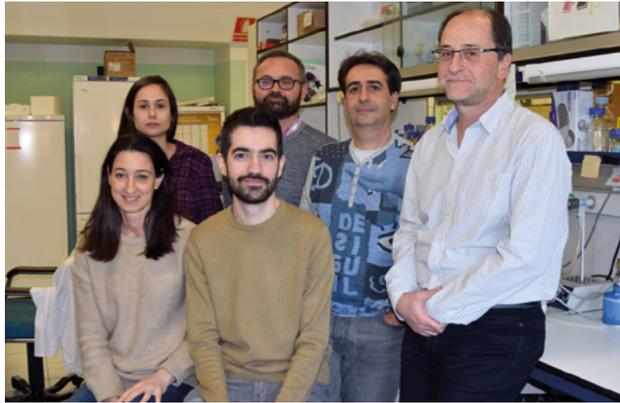
Additionally, we are using metabolic flux analyses to study the metabolic changes induced by secretory protein overproduction. This will allow us to estimate the metabolic cost of that overproduction which, in turn, would enable us to design secretory protein production processes, consisting of a suitable balance between the amount of product obtained and its relative cost.

The obtained results would be applied first-hand at an industrial level for scaling up secretory protein production, allowing its optimisation, as well as favouring the design and construction of new and efficient secretory strains in *S. lividans*.

1



1 *Streptomyces lividans* major secretory route (Sec pathway, left) releases secretory proteins out of the cell in an unfolded configuration; the action of thiol-disulphide oxidoreductases (DsbA) and peptidyl-prolyl cis-trans isomerases (FKBP) procure the correctly folded structure that will protect secretory proteins from degradation by three specific HtrA-like proteases. The minor secretory route (Tat pathway, right) releases secretory proteins properly folded.



Regulation of gene expression and metabolism in bacteria

50 MICROBIAL BIOTECHNOLOGY

GROUP LEADER

Fernando Rojo

SENIOR SCIENTISTS

Renata Moreno
Lázaro Molina

PhD STUDENTS

Dione Sánchez
Guillermo Gómez

TECHNICIAN

Luis Yuste

MASTER'S STUDENT

Javier Alaiza

SELECTED PUBLICATIONS

Kuiroz-Rocha E, Moreno R, Hernández-Ortiz A, Fragosó-Jiménez JC, Guzmán J, Rojo F, Espín G, Núñez C. Glucose uptake in *Azotobacter vinelandii* occurs through a GluP transporter that is under the control of the CbrA/CbrB-Crc/Hfq system. *Sci Rep* 2017; 7: 858.

Sevilla E, Yuste L, Moreno R, Rojo F. Differential expression of the three *Alcanivorax borkumensis* SK2 genes coding for the three P450 cytochromes involved in the assimilation of hydrocarbons. *Environ Microbiol Rep* 2017; 9: 797-808.

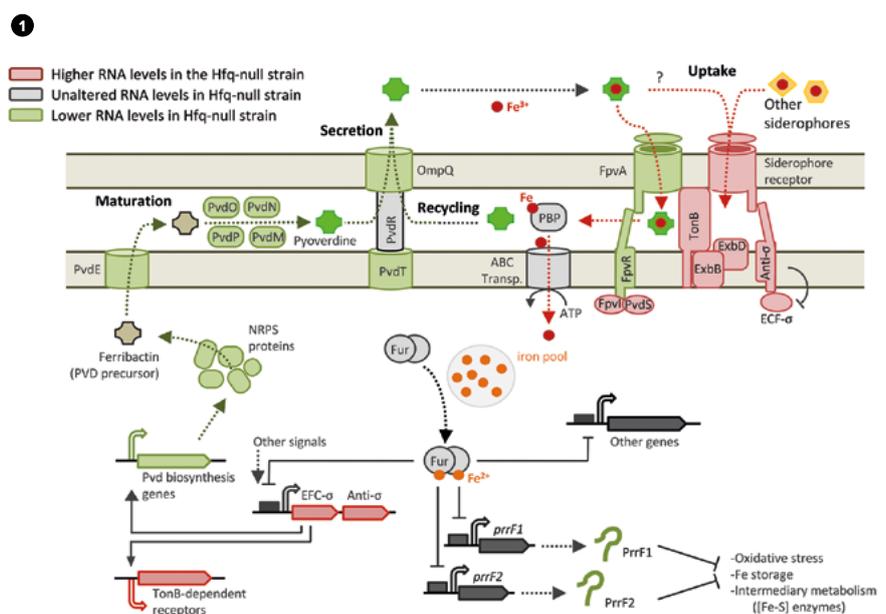
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Sánchez-Hevia D, Yuste L, Moreno R, Rojo F. Influence of the Hfq and Crc global regulators on the control of iron homeostasis in *Pseudomonas putida*. *Environ Microbiol* 2018; 20: 3484-3503.

Val-Calvo J, Luque-Ortega JR, Crespo I, Miguel-Arribas A, Abia D, Sánchez-Hevia D, Serrano E, Gago-Córdoba C, Ares S, Alfonso C, Rojo F, Wu LJ, Boer R, Meijer WJJ. Novel regulatory mechanism of establishment genes of conjugative plasmids. *Nucleic Acids Res* 2018; 46: 11910-11926.

To be competitive in the environments they colonise, bacteria must optimise their metabolism to attain maximum gain from available nutrients at minimum energetic cost. Not all potential carbon sources are equally effective in this respect. Probably for this reason, when confronted with a mixture of potentially assimilable compounds at sufficient concentrations, many bacteria preferentially use one of them, leaving others aside until the preferred one is consumed. This selection implies a complex regulatory process termed catabolite repression. Unravelling the molecular mechanisms that underlie these regulatory events helps to understand how bacteria coordinate their metabolism and gene expression programs and optimise growth. It also aids in the design and optimisation of biotechnological processes and to understand how bacteria degrade compounds in nature. This is particularly true for compounds that are difficult to degrade and accumulate in the environment, posing pollution problems. Hydrocarbons are a clear example of this kind of non-preferred compounds.

The regulatory proteins and molecular mechanisms responsible for catabolite repression differ among microorganisms. Our work is focused on *Pseudomonas putida*, a bacterium that has a very versatile and robust metabolism, colonises very diverse habitats, and is widely used in biotechnology. In the last few years we have been analysing a regulatory network that relies on the Crc and Hfq proteins, which ultimately inhibit translation of mRNAs containing a specific A-rich sequence motif within their translation initiation region. Two small RNAs named CrcZ and CrcY, the levels of which vary greatly depending on growth conditions, antagonise the inhibitory effect of Hfq and Crc. Our aim is to characterise the influence of Crc, Hfq, CrcZ and CrcY in the physiology of *P. putida*, the signals to which they respond, and the molecular mechanisms by which they regulate gene expression. We pretend to determine how they modulate metabolism in response to fluctuating environmental conditions.



1 Effect of the Hfq global regulator on the expression of genes involved in iron metabolism in *Pseudomonas putida*. Scheme of the synthesis, export and uptake of pyoverdine, and involvement of the Fur protein and the PrrF1 and PrrF2 sRNAs in the regulation of iron homeostasis.



Genetic control of the cell cycle

MICROBIAL BIOTECHNOLOGY 51

GROUP LEADER Miguel Vicente

SENIOR SCIENTISTS

Ana Isabel Rico
Susanne Gola

PHD STUDENT

Laura Cueto

TECHNICIANS

Pilar Palacios
Mercedes Casanova
Marina Cabrerizo

MASTER'S STUDENTS

David López
Eduardo Ruiz

UNDERGRADUATE STUDENT

Martín Sastre

VISITING STUDENT

Ida Thalsø-Madsen
(University of Copenhagen, Denmark)

SELECTED PUBLICATIONS

Mura A, Fadda D, Perez AJ, Danforth ML, Musu D, Rico AI, Krupka M, Denapaite D, Tsui HT, Winkler ME, Branny P, Vicente M, Margolin W, Massidda O. Roles of the essential protein FtsA in cell growth and division in *Streptococcus pneumoniae*. *J Bacteriol* 2017; 199: e00608-16.

Ortiz C, Casanova M, Palacios P, Vicente M. The hypermorph FtsA⁺ protein has an *in vivo* role in relieving the *Escherichia coli* proto-ring block caused by excess ZapC+. *PLoS One* 2017; 12: e0184184.

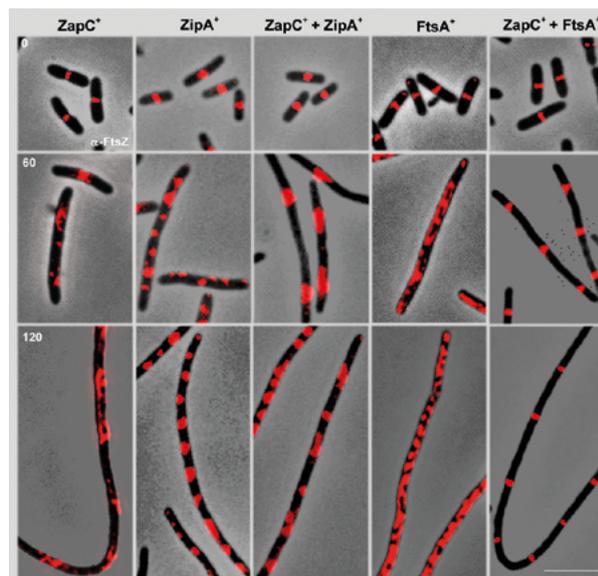
Matsumoto S, Kawai Y, Miyagawa S, Iwamoto Y, Okuda S, Sánchez-Gorostiaga A, Vicente M, Tsuneda S. Unique transcriptional profile of native persisters in *Escherichia coli*. *J Biosci Bioeng* 2018; 125: 15-22.

Pazos M, Peters K, Casanova M, Palacios P, VanNieuwenhze M, Breukink E, Vicente M, Vollmer W. Z-ring membrane anchors associate with cell wall synthases to initiate bacterial cell division. *Nat Commun* 2018; 9: 5090.

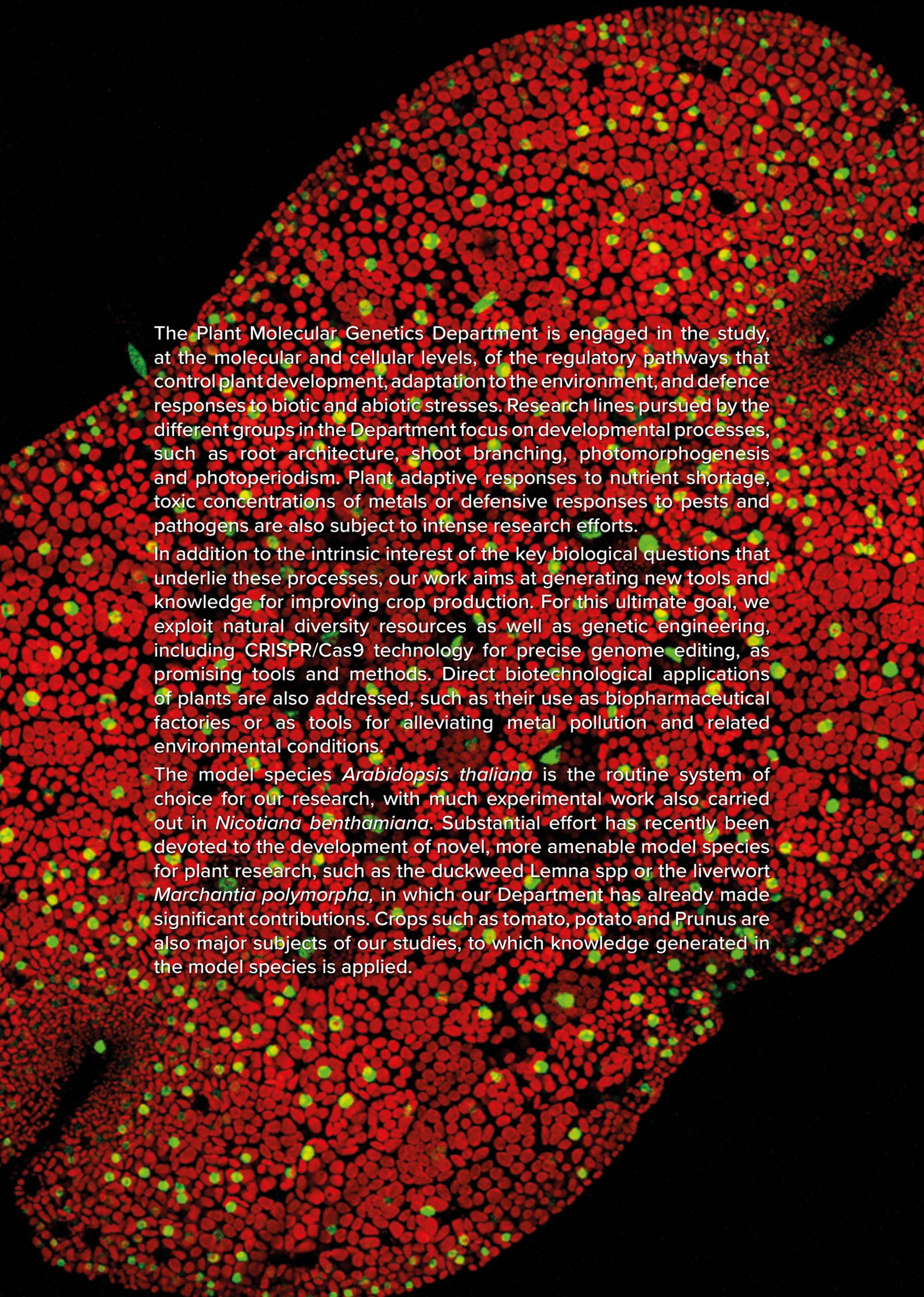
The success of bacteria results from their ability to survive and multiply even under adverse conditions. To proliferate, *Escherichia coli*, an important inhabitant of the human gut, assembles a very efficient complex, called divisome. The divisome is exactly placed at the centre of the cell, avoiding the region occupied by the chromosome until it is fully replicated and segregated. A main component of the divisome is FtsZ, an analogue of the human tubulin, that polymerises forming a contractile ring to initiate cell division. FtsZ needs two proteins, ZipA and FtsA, to be anchored to the membrane. In addition, FtsZ is prevented by other proteins called Min from polymerising at the poles, whereas another protein SlmA, excludes the polymers from the region around the chromosome. On the other hand, a group of Zap proteins, as ZapC, serve to stabilise the FtsZ polymers. All these proteins that interact with FtsZ have collectively received the name of "The Keepers of the Ring".

Differently from their role in the division of *E. coli*, in *Streptococcus pneumoniae*, a bacterium having a coccal rather than bacillar shape, FtsA and FtsZ coordinate both peripheral and septal PG synthesis and are codependent for their localisation at midcell. Although *E. coli* cells can grow in the absence of FtsZ, they cannot divide and form filaments. In addition, the very low levels of FtsZ present in synthetically FtsZ-deprived cells have unexpected and severe pleiotropic effects on the global physiology of *E. coli*, culminating in a reduced resilience that compromises bacterial survival. Studying the properties of FtsZ and its keepers, offers then the bright possibility to discover compounds to neutralise the ring. These would be the much needed new antibiotics essential to fight against the Dark Powers of the Ring, the antibiotic resistant pathogens.

1



1 The assembly of FtsZ to produce defined division rings is modified by an excess of ZapC, FtsA or ZipA. Only the combination of ZapC with FtsA (right column) allows the production of otherwise inactive rings. The frames show images of *E. coli* cells in which the FtsZ protein (red) is visualised. Each row corresponds to different times (indicated in minutes) after the induction of the corresponding genes cloned in suitable plasmids. The FtsZ protein was revealed using anti-FtsZ and Alexa 594-conjugated anti-rabbit antibody. Bar: 5 μ m. (From Ortiz et al., 2017).

A fluorescence microscopy image of a plant tissue section, likely a root or stem, showing a dense array of cells. The cells are primarily stained red, with numerous bright green spots scattered throughout, indicating specific cellular components or gene expression patterns. The overall structure is curved and shows some internal vascular or cellular organization.

The Plant Molecular Genetics Department is engaged in the study, at the molecular and cellular levels, of the regulatory pathways that control plant development, adaptation to the environment, and defence responses to biotic and abiotic stresses. Research lines pursued by the different groups in the Department focus on developmental processes, such as root architecture, shoot branching, photomorphogenesis and photoperiodism. Plant adaptive responses to nutrient shortage, toxic concentrations of metals or defensive responses to pests and pathogens are also subject to intense research efforts.

In addition to the intrinsic interest of the key biological questions that underlie these processes, our work aims at generating new tools and knowledge for improving crop production. For this ultimate goal, we exploit natural diversity resources as well as genetic engineering, including CRISPR/Cas9 technology for precise genome editing, as promising tools and methods. Direct biotechnological applications of plants are also addressed, such as their use as biopharmaceutical factories or as tools for alleviating metal pollution and related environmental conditions.

The model species *Arabidopsis thaliana* is the routine system of choice for our research, with much experimental work also carried out in *Nicotiana benthamiana*. Substantial effort has recently been devoted to the development of novel, more amenable model species for plant research, such as the duckweed *Lemna* spp or the liverwort *Marchantia polymorpha*, in which our Department has already made significant contributions. Crops such as tomato, potato and *Prunus* are also major subjects of our studies, to which knowledge generated in the model species is applied.

Plant Molecular Genetics

HEAD OF DEPARTMENT

José Juan Sánchez Serrano

RESEARCH GROUPS

- 1. Natural variation in plant development**
Carlos Alonso-Blanco
- 2. Plant immunity strategies against microbial pathogen infection**
Carmen Castresana
- 3. Genetic control of bud dormancy**
Pilar Cubas
- 4. Plant-pathogen interaction in viral infections**
Juan A. García & Carmen Simón
- 5. Mechanisms underlying nutrient uptake and phytoremediation**
Antonio Leyva
- 6. Regulation of gene activity in plants: the phosphate starvation rescue system**
Javier Paz-Ares
- 7. Light signalling and day length control of potato tuber formation**
Salomé Prat
- 8. Role of ubiquitin in the control of plant growth and stress tolerance**
Vicente Rubio
- 9. Signalling networks in plant development and defence responses**
José J. Sánchez-Serrano & Enrique Rojo
- 10. Jasmonate signalling and plant defence**
Roberto Solano



Natural variation in plant development

GROUP LEADER

Carlos Alonso Blanco

SENIOR SCIENTIST

Belén Méndez de Vigo

POSTDOCTORAL SCIENTIST

Almudena Mollá

TECHNICIAN

Mercedes Ramiro Rivero

PhD STUDENTS

Noelia Arteaga Ramos

Alberto Fuster Pons

MASTER'S STUDENT

Teresa Zúñiga

UNDERGRADUATE STUDENTS

Isabel López

Sara Escudero

SELECTED PUBLICATIONS

Durvasula A, Fulgione A, Gutaker RM, Alacakaptan SI, Flood PJ, Neto C, Tsuchimatsu T, Burbano HA, Picó FX, Alonso-Blanco C, Hancock AM. African genomes illuminate the early history and transition to selfing in *Arabidopsis thaliana*. Proc Natl Acad Sci USA 2017; 114: 5213-5218.

Lee CR, Svardal H, Farlow A, Exposito-Alonso M, Ding W, Novikova P, Alonso-Blanco C, Weigel D, Nordborg M. On the post-glacial spread of human commensal *Arabidopsis thaliana*. Nat Commun 2017; 8:14458.

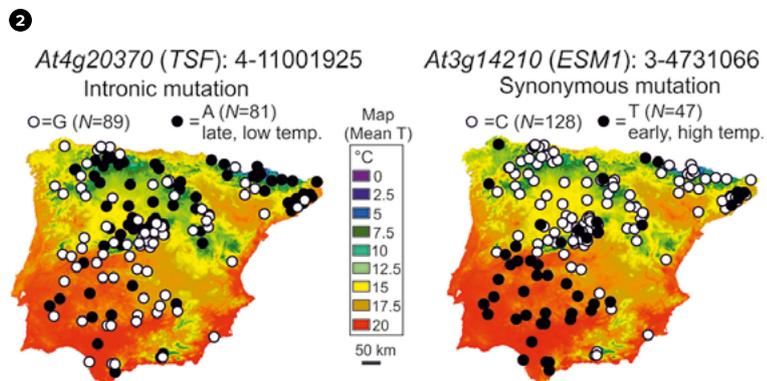
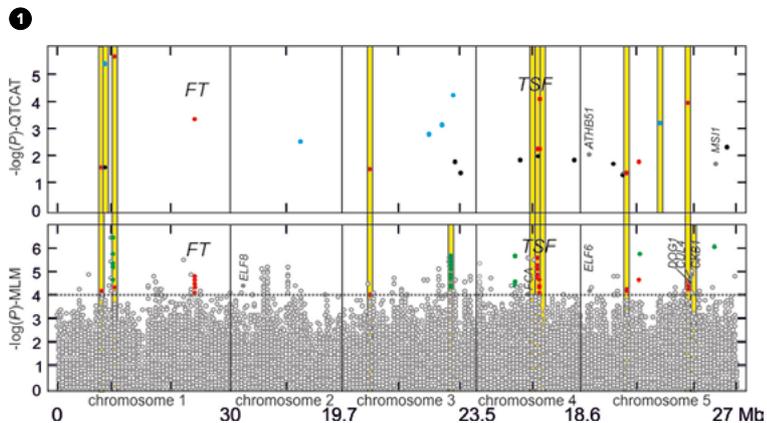
Tabas-Madrid D, Méndez-Vigo B, Arteaga N, Marcer A, Pascual-Montano A, Weigel D, Xavier Picó F, Alonso-Blanco C. Genome-wide signatures of flowering adaptation to climate temperature: Regional analyses in a highly diverse native range of *Arabidopsis thaliana*. Plant Cell Environ 2018; 41: 1806-1820.

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The main goal of our laboratory is to understand the genetic, molecular and evolutionary mechanisms involved in plant adaptation. In particular, we are interested in understanding how developmental traits, such as flowering time, seed dormancy or vegetative growth, allow plant adaptation. To address this question, we are exploiting the genetic variation that exists in nature within the wild, annual, and model plant *Arabidopsis thaliana*.

Given the relevance of climate change, our research is currently focused on identifying new genes and natural alleles that are involved in the adaptation to different climates. To this end, we are exploiting an *A. thaliana* regional collection of more than 400 wild accessions collected in the Iberian Peninsula (Tabas-Madrid *et al.*, 2018; Marcer *et al.*, 2018). We are carrying out multiple phenotypic and environmental genome-wide association analyses (GWAS), using the genome sequence of 174 Iberian accessions (Figure 1). We have identified known genes, such as *TWIN SISTER OF FT (TSF)*, and new genes, like *VOLTAGE DEPENDENT ANION CHANNEL 5 (VDAC5)* as candidates for adaptation to climate temperature by altered flowering time (Figure 2). Furthermore, we are analysing the adaptive and demographic history of *A. thaliana* not only in this region (Exposito-Alonso *et al.*, 2018), but also in Eurasia (Lee *et al.*, 2017) and Africa (Durvasula *et al.*, 2017).



1 GWAS of flowering time in the Iberian Peninsula. Genomic regions associated with flowering time and climate temperature are shown in yellow colour.

2 Geographic and climatic distribution of genes involved in flowering adaptation. Each panel shows the Iberian distribution of polymorphisms at TSF (left panel) and VDAC5 in relation to mean or maximum temperature, respectively.



Plant immunity strategies against microbial pathogen infection

GROUP LEADER

Carmen Castresana

SENIOR SCIENTISTS

Luis Muñiz
Ana López Sánchez

POSTDOCTORAL SCIENTIST

Yovanny Izquierdo

TECHNICIAN

Tomás Cascón

PhD STUDENTS

Ada Martínez
Ruben Fernández
Bran López

MASTER'S STUDENTS

Alfonso González de Padrena
Pablo Benito

UNDERGRADUATE STUDENT

Blanca Ureña

SELECTED PUBLICATIONS

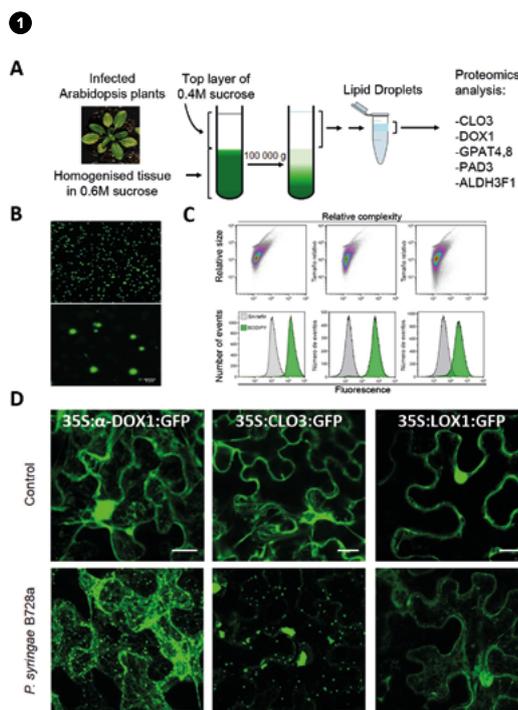
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Plant pathogens cause diseases in many economically important crop plants, leading to severe losses in food production that are also of fundamental importance for forestry, other plant-derived products and for the sustainability of natural environments. This circumstance, together with an increasing augmentation of the world population, pose a severe threat to agriculture and plant sustainability. An important requirement for the development of successful plant disease control strategies is the understanding of host-pathogen interactions and, in particular, of the molecular mechanisms evolved in plants to avoid pathogen infection. This knowledge will be critical to devise effective approaches to minimise plant losses due to infection by microbes.

To this end, we focus our research on exploring the activities of oxylipins, a family of lipid derivatives activating immune responses in plants. Over the last years, our research has revealed that oxylipins, produced by the biosynthetic pathways initiated by fatty acid alpha-dioxygenases (alpha-DOXs) and 9-lipoxygenases (9-LOXs), contribute to the activation of local and systemic defence. Moreover, we found that specific derivatives from these pathways act as inducers of defence responses and modulators of hormone homeostasis. In our recent studies, we showed that cellular organelles such as lipid droplets (Figure 1) and mitochondria are important players during the response to pathogen infection and that global translational reprogramming contribute to activation of plant immunity. Presently, we focus our research in examining the participation of oxylipins in these defence mechanisms and in defining the relevance of these processes as part of the plant defence responses to control pathogen infection. Our experiments are performed in Arabidopsis and tomato plants helping to examine the translation of results from a model plant to crops as well as to compare the responses and defence mechanisms of both types of plants. The characterisation of the processes mentioned will contribute to define new defence mechanisms, as well as the signals, pathways, and genes involved in controlling plant immunity.



1 Formation and characterisation of lipid droplets (LDs) in Arabidopsis thaliana leaves responding to *Pseudomonas syringae* pv. tomato DC3000 *avrRpm1* inducing a hypersensitive defence reaction (HR). (A) The protocol allows reproducible isolation of highly LD-enriched fractions from limited amounts of tissue. Mass spectrometry analyses showed the LD proteome of *Pseudomonas-syngae*-infected and senescent leaves. (B) Confocal microscopy and flow cytometry of unstained (grey) and BODIPY stained (green) LD samples. (C) LD-localisation of selected proteins was examined by transient expression in leaves of *N. benthamiana*. According to our proteomic analyses, alpha-DOX and CLO3 localise in LDs whereas LOX1 was not detected in these cellular structures.



Genetic control of bud dormancy

56 PLANT MOLECULAR GENETICS

GROUP LEADER

Pilar Cubas

POSTDOCTORAL SCIENTIST

Michael Nicolas

TECHNICIANS

Isabel Domínguez Cañete
José Rodrigo García del Campo

PHD STUDENTS

Elena Sánchez
Carlos Tarancón
Aitor Muñoz

UNDERGRADUATE STUDENT

Coral Fernández

SELECTED PUBLICATIONS

Tarancón C, González-Grandío E, Oliveros JC, Nicolas M, Cubas P. A conserved carbon starvation response underlies bud dormancy in woody and herbaceous species. *Front Plant Sci* 2017; 8: 788.

González-Grandío E, Pajoro A, Franco-Zorrilla JM, Tarancón C, Immink RG, Cubas P. Abscisic acid signaling is controlled by a BRANCHED1/HD-ZIP I cascade in Arabidopsis axillary buds. *Proc Natl Acad Sci USA* 2017; 114: E245-E254

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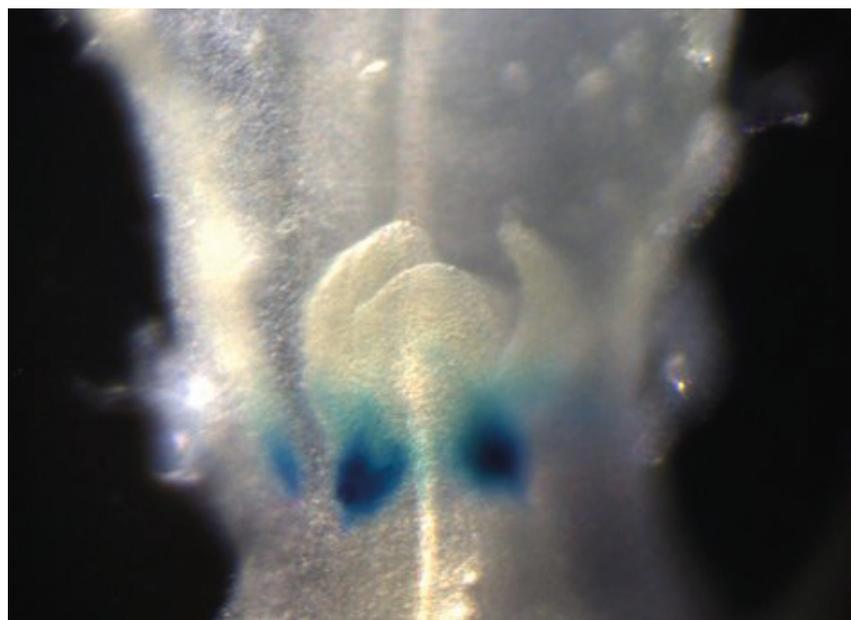
Sanchez E, Artuso E, Lombardi C, Visentin I, Lace B, Saeed W, Lolli ML, Kobauri P, Ali Z, Spyrakis F, Cubas P, Cardinale F, Prandi C. Structure-activity relationships of strigolactones via a novel, quantitative in planta bioassay. *J Exp Bot* 2018; 69: 2333-2343.

Martín-Fontecha ES, Tarancón C, Cubas P. To grow or not to grow, a power-saving program induced in dormant buds. *Curr Opin Plant Biol* 2018; 41: 102-109.

Shoot branching patterns depend on a key developmental decision: whether axillary buds grow out to give a branch or remain dormant in the leaf axils. This decision is controlled by hormone-mediated endogenous and environmental stimuli. The Arabidopsis gene BRANCHED1, which encodes a TCP transcription factor, negatively controls shoot branching. We are trying to understand the growth-to-dormancy transition in axillary buds and the role of BRC1 in this process by using transcriptomic and ChIP-seq studies to build transcriptional networks induced in dormant Arabidopsis axillary buds. We have identified several direct BRC1 targets that mediate BRC1 function and are essential for ABA synthesis and signalling in bud entering dormancy. We are also studying the evolution and divergence of the BRC1 function in *Solanaceae*, in particular in potato and tomato.

Strigolactones (SL) are phytohormones that regulate shoot branching. SL perception and signalling involve the F-box protein MAX2 and the hydrolase D14, proposed to act as a SL receptor. We are using strong loss-of-function alleles of the D14 gene to characterise its function. Our data showed that D14 distribution overlaps that of MAX2 at tissue and subcellular levels, allowing physical interactions between these proteins. Grafting studies indicated that neither D14 mRNA nor the protein move upwards over a long range in the host. Like MAX2, D14 is needed locally in the aerial part of the plant to suppress shoot branching. We also identified a mechanism of SL-induced MAX2-dependent proteasome-mediated D14 degradation. This negative feedback loop would cause a substantial drop in SL perception, which would effectively limit SL duration and signalling intensity.

1



1 Beta-glucuronidase expression driven by the HB53 promoter in Arabidopsis thaliana axillary buds. HB53 is a direct target of the BRANCHED1 gene and promotes bud growth arrest in short day photoperiods and under canopy shade.



Plant-pathogen interactions in viral infections

PLANT MOLECULAR GENETICS 57

GROUP LEADERS

Juan Antonio García Álvarez
Carmen Simón Mateo

SENIOR SCIENTISTS

Adrián Valli
Bernardo Rodamilans Ramos
Sandra Martínez Turiño

POSTDOCTORAL SCIENTIST

Hongying Shan

TECHNICIAN

Beatriz García García

PhD STUDENTS

Araiz Gallo
Frida Mesel
Marta Hervás García
Alfonso González de Prádena

MASTER STUDENT

María Ribaya Muñoz

UNDERGRADUATE STUDENTS

M^a del Carmen Nevado Blasco
Magdalena de Arriba Sánchez
de la Campa

VISITING SCIENTISTS

Zhenyu Liu
Andrés Mauricio Pinzón Núñez

SELECTED PUBLICATIONS

Pasin F, Bedoya L, Bernabé-Orts JM, Gallo A, Simón-Mateo C, Orzaez , García JA. Multiple T-DNA delivery to plants using novel mini binary vectors with compatible replication origins. *ACS Synth Biol* 2017; 6: 1962-1968.

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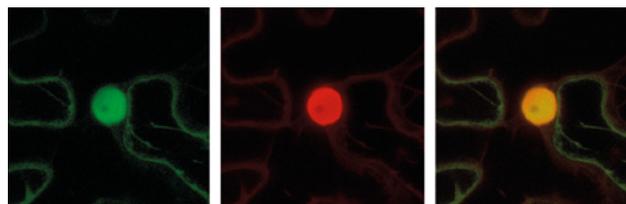
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Rodamilans B, Valli A, Mingot A, San León D, López-Moya JJ, García JA. An atypical RNA silencing suppression strategy provides a snapshot of the evolution of sweet potato-infecting potyviruses. *Sci Rep* 2018; 8: 15937.

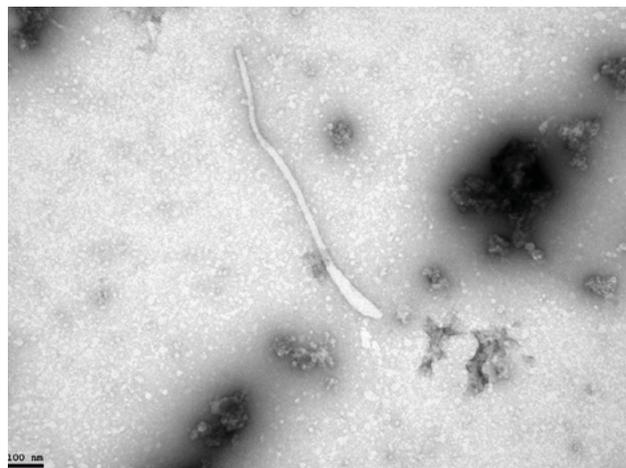
A complex plant-virus interactive network modulates infectivity and symptom severity. Most plant viruses cannot infect all their potential hosts, and when they do, serious illness is not the norm, as revealed by recent metagenomic studies. Our laboratory studies this interaction network, which facilitates virus replication and propagation, but also induces plant defense responses and disease symptoms. *Plum pox virus*, our main subject of study, belongs to the family Potyviridae, the largest group of plant RNA viruses and causes sharka, a serious disease of stone fruit trees. We are especially interested in defense responses related to RNA silencing and its viral suppressors. The typical silencing suppressor of potyvirids is HCPro, but the existence of additional silencing suppressors in different potyvirids, prompted us to suggest that escaping RNA silencing-mediated antiviral defenses is a powerful driving force of virus evolution. Potyvirus genomic RNAs are expressed through the synthesis of large polyproteins, processed by viral-encoded endopeptidases. We are studying how host-specific modulation of this processing can contribute to potyvirus pathogenicity and host range definition. Encapsidation of potyvirus genome is an active process. We have demonstrated a functional link between potyvirus RNA replication and virion assembly, and we are studying how posttranslational modifications of the capsid protein can contribute to sort the potyvirus RNA into translation, replication or encapsidation. An important goal of our laboratory is applying our basic research results to control viral diseases through novel strategies. For instance, we are attenuating plant viruses by recoding their genomes in order to use them as cross-protection agents. We are also interested in developing other valuable biotools, such as a novel T-DNA delivery system, which can be used to efficiently inoculate plants with infectious viral cDNA clones, among other multiple applications.

1



1 *Confocal microscope image of the nuclear interaction between Cucurbit yellowing virus, P1b and Importin 7 of Nicotiana benthamiana, observed by Bimolecular fluorescence complementation.*

2



2 *Distorted virion of a Plum pox virus mutant emulating phosphorylation at the capsid protein N-terminal region*



Mechanisms underlying nutrient uptake and phytoremediation

58 PLANT MOLECULAR GENETICS

GROUP LEADER

Antonio Leyva

SENIOR SCIENTIST

Cristina Navarro

TECHNICIAN

Yolanda Leo del Puerto

PHD STUDENTS

Cristian Mateo Elizalde
Micaela A. Navarro Correa

MASTER'S STUDENT

Alvaro Segovia San Benito

UNDERGRADUATE STUDENTS

María Silvia Rodríguez Gómez
María García González
Carolina Jiménez de la Sen
Odette Den Rozalén
Irene Beltrán de Heredia
Amaya Escribano Ortega
Carlos Fernández Celdrán

VISITING SCIENTIST

Sarra Arbaoui
(Fellow of the Woman in Science Foundation, Higher Institute of Agronomic Science, Chott-Mariem, Tunisia).

SELECTED PUBLICATIONS

Puga MI, Rojas-Triana M, de Lorenzo L, Leyva A, Rubio V, Paz-Ares J. Novel signals in the regulation of Pi starvation responses in plants: facts and promises. *Curr Opin Plant Biol* 2017; 39: 40-49.

Navarro C, Arbaoui S, Mateo C, Bettaieb T, Leyva A. Arsenic, the Silent Threat: New Phytoremediation strategies for contaminated soils and waters. In *Phytoremediation Methods, Management and Assessment, Environmental Research Advances 2017*; ed. Vladimir Matichenkov, pp 27-59. Nova Science Publishers, New York.

In our group, we are interested in the characterisation of the molecular mechanisms involved in arsenic perception in plants. Our aim is to improve the efficiency of plants to extract arsenic from contaminated waters and soils. Plants have an extraordinary capacity to capture large quantities of nutrients and toxic compounds, including heavy metals and arsenic. Indeed, arsenic contamination is responsible for the worst mass poisoning ever suffered by man and is considered a silent threat to public health. Arsenic can enter into the food chain through water consumption or crops irrigated with arsenic contaminated water, particularly rice. This chemical threat was critical for the evolution of sessile organisms such as plants, which were forced to develop rapid tolerance responses when arsenic was present.

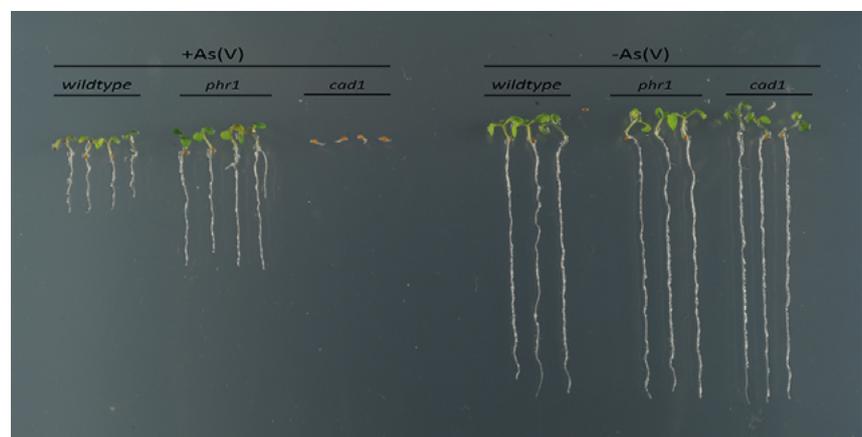
For the last two years we kept working on the characterisation of the molecular mechanisms involved in arsenic perception and detoxification using genetic and molecular approaches. We have identified several candidates of the signal transduction pathway that are currently being functionally characterised.

We have published two reviews in collaboration with other groups which outline the latest discoveries of the phosphate starvation response and the new strategies of arsenic phytoremediation.

In collaboration with Dr. Carlos Alonso Blanco at the CNB, we also study the application of natural isolates of duckweed aquatic plants for water phytoremediation. In particular, our laboratory is currently involved in the project "Duckweed technology for improving nutrient management and resource efficiency in pig" (www.life-lemna.eu), funded by the LIFE Programme of the European Commission. The collection is currently being used to identify highly efficient natural isolates for arsenic phytoextraction from contaminated waters.

The research lines in progress within our laboratory will allow us to understand the mechanisms that underlie arsenic perception, which will open up new possibilities for phytoremediation of arsenic-contaminated soils and waters.

1



1 Arsenic tolerance phenotype of *Arabidopsis* wildtype and mutant plants altered in arsenate uptake (*phr1*) or in arsenic detoxification (*cad1*) in the presence [+As(V)] or without [-As(V)] arsenate.



Regulation of gene activity in plants: the phosphate starvation rescue system

PLANT MOLECULAR GENETICS 59

GROUP LEADER

Javier Paz-Ares

SENIOR SCIENTISTS

María Isabel Puga
César Poza

TECHNICIAN

Erica Gil

PhD STUDENTS

Miguel Miñambres
Sergio Díaz

SELECTED PUBLICATIONS

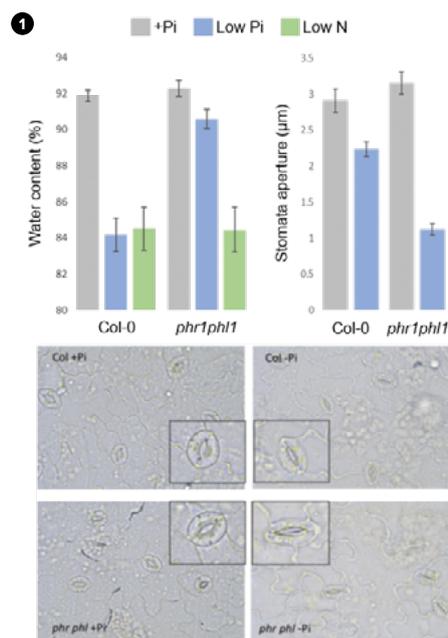
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Puga MI, Rojas-Triana M, de Lorenzo L, Leyva A, Rubio V, Paz-Ares J3 Novel signals in the regulation of Pi starvation responses in plants: facts and promises. *Curr Opin Plant Biol* 2017; 39: 40-49.

Our research is focused on the phosphate (Pi) starvation rescue system (PSR) of plants, a model system for studies of gene activity. In addition, the study of this rescue system is important to generate tools and strategies towards the effective implementation of low-input, sustainable agricultural practices. Significant progress has been made in the last 20 years in the dissection of the signalling pathway governing the activation of this system, in which our laboratory has made key contributions, including among others the identification of PHR1 and related transcription factors as master regulators of PSR; the implication of SPX1 and related proteins in the Pi sensing system, and the identification of the targets and mechanisms of regulation of miRNAs by non-coding RNAs.

In the past two years, we have found that PHR1 targets induced by Pi starvation are also induced by drought. In line with this observation, we found that Pi starvation reduces water content (Figure 1). This effect on water content is mediated by PHR1, which strongly supports the notion that plant water content management contributes to the PSR. In fact, an obvious consequence of water content reduction is a concomitant increase in Pi concentration. Our studies on PHR1 targets have also served to identify a key role of PHR1 in the attenuation of plant defence responses to pathogens, indicating that plants prioritise Pi homeostasis over plant defence during Pi starvation.

Additionally, we embarked on a project to study the effect of Pi starvation on the formation of extra-chromosomal circular DNA (ecc DNA). So far, we have implemented the method for eccDNA isolation and characterisation (based on paired end sequencing) and found that there are more than 1,000 eccDNAs produced during Pi starvation. The Pi starvation specificity of these eccDNAs and their biological significance is currently under investigation.





Light signalling and day length control of potato tuber formation

60 PLANT MOLECULAR GENETICS

GROUP LEADER

Salomé Prat

SENIOR SCIENTISTS

Cristina Martínez
Cristina Nieto

POSTDOCTORAL SCIENTISTS

Scott Hayes
Evyatar Steiner

TECHNICIAN

Ana Beloso

PHD STUDENT

Alejandro Navarro Galiano

UNDERGRADUATE STUDENT

Silvia de Dios

SELECTED PUBLICATIONS

Martínez C, Espinosa-Ruiz A, de Lucas M, Bernardo-García S, Franco-Zorrilla JM, Prat S. PIF4-induced BR synthesis is critical to diurnal and thermomorphogenic growth. *EMBO J* 2018; 37: e99552.

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Espinosa-Ruiz A, Martínez C, de Lucas M, Fàbregas N, Bosch N, Caño-Delgado AI, Prat S. TOPLESS mediates brassinosteroid control of shoot boundaries and root meristem development in *Arabidopsis thaliana*. *Development* 2017; 144: 1619-1628.

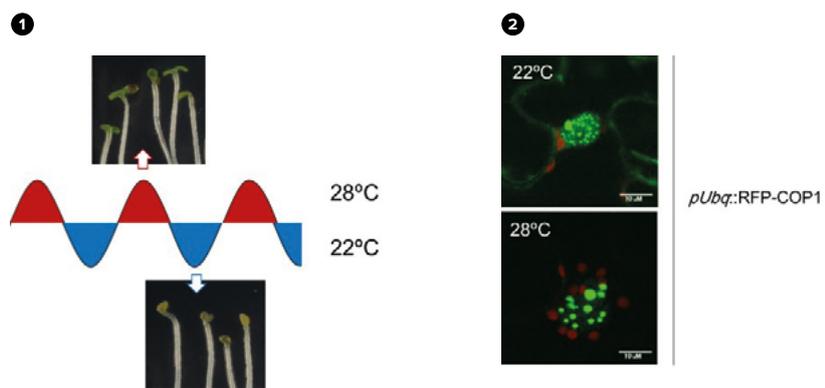
Day-length and temperature vary both daily and seasonally. Plants are not simply passive onlookers to these environmental fluctuations; in fact they constantly monitor their environment to pre-empt and prepare for the challenges that lie ahead. For example, a gradual decrease in temperatures and low light indicate the coming of night, and decreasing daylength and temperatures suggest that winter is just around the corner.

Plants sense the quality and quantity of daylight through a suite of photoreceptors: UVR8, cryptochromes, phototropins, zeitlupes and phytochromes. Information from these photoreceptors is integrated with the circadian clock and this enables plants to adjust their growth and development to match diurnal light-cycles and seasonal variation. Developmental processes tightly regulated by light and temperature cues and diel conditions include seed germination and early seedling development, the transition to flowering, and in potato, the differentiation of storage organs (tuberisation).

Research by our group aims to decipher the mechanisms by which these external cues interact with endogenous growth and hormonal programs. Our work spans across multiple developmental stages, from de-etiolation and seedling establishment, through to stress tolerance and storage-organ formation. We achieve this through work in the model species *Arabidopsis* and potato. Understanding how environmental factors are integrated into plant development is of utmost importance if we are to develop crops that are resilient to the negative impact of global warming.

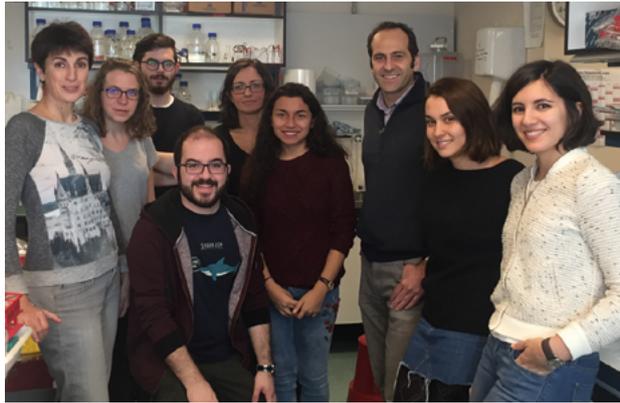
In this context, specific biological questions addressed by our research are:

- How do warm temperatures and light affect plant morphology and how are these signals integrated with hormonal pathways?
- How are temperature oscillations sensed by the endogenous clock and what are the consequences of this for early plant development?
- How do elevated temperatures suppress the function of the clock “evening complex” whilst promoting the activity of the COP1 E3 ligase?
- What are the mechanisms underlying suppression of thermomorphogenic hypocotyl elongation by drought and salinity?
- Can we de-couple the effects of plant hormones on morphology and their effects on stress tolerance?
- What are the mechanisms responsible for temperature suppression of the FT mobile tuberisation signal?
- Are potato tubers derived from the vascular cambium?



1 Oscillations in temperature entrain seedlings to de-etiolation on exposure to light.

2 Effect of temperature on COP1 distribution.



Role of ubiquitin in the control of plant growth and stress tolerance

PLANT MOLECULAR GENETICS 61

GROUP LEADER

Vicente Rubio

SENIOR SCIENTIST

Sandra Fonseca

POSTDOCTORAL SCIENTIST

Elisa Iniesto

TECHNICIAN

Yolanda Fernández

PHD STUDENTS

Amr Nassrallah

Marta García León

Esther Cañibano

MASTER'S STUDENTS

Camila García

Daniela Gómez

Marcos Peñalver

UNDERGRADUATE STUDENTS

Nicolás Manosalva

Marina Martín

Daniel Lillo

Francisco Avila

VISITING SCIENTISTS

Evyatar Steiner

(Institute of Plant Sciences and Genetics, Rehovot, Israel)

Carolina Rossi

(Universidade do Sao Paulo, Brazil)

SELECTED PUBLICATIONS

Perea-Resca C,... Iniesto E, Rubio V, Salinas J. Prefoldins Negatively Regulate Cold Acclimation in *Arabidopsis thaliana* by promoting nuclear proteasome-mediated H2B degradation. *Mol Plant* 2017; 10: 791-804.

Puga M,... Rubio V, Paz-Ares J. Novel signals in the regulation of Pi starvation responses in plants: facts and promises. *Curr Opin Plant Biol* 2017; 39: 40-49.

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Park J, ... Iniesto E, Rubio V, ... Yun DJ. Epigenetic switch from repressive to permissive chromatin in response to cold stress. *Proc Natl Acad Sci USA* 2018; 115: E5400-E5409.

Nassrallah A, ... Fonseca S, Iniesto E, ... Bowler C, Rubio V, Barneche F. 2018 DET1-mediated degradation of a SAGA-like deubiquitination module controls H2Bub homeostasis. *Elife* 2018; 7: e37892.

The relevance of protein ubiquitination as an integral mechanism of many signalling pathways in plants has been demonstrated extensively. Ubiquitin (Ub) conjugation to proteins (i.e. ubiquitination) may trigger degradation of protein targets at the 26S proteasome or changes in their properties (e.g., protein activity, localisation, assembly and interaction ability), depending on the extent or specific Ub chain configurations. Protein ubiquitination is mediated by an enzymatic cascade in which different types of E3 Ub ligases provide the substrate specificity. Among them, Cullin4 RING E3 ubiquitin ligases (CRL4) have been involved in biological processes spanning the plants' whole life, including embryogenesis, seedling photomorphogenesis, circadian clock function, flowering and tolerance to different stresses (i.e., drought, high salinity, cold, osmotic stress) by promoting degradation of specific targets controlling those processes.

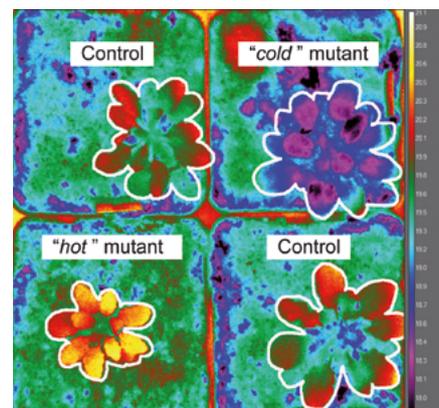
As an example, we have recently shown that DDA1, a substrate adaptor of CRL4-CDDD complexes, recognises abscisic acid (ABA) receptors, triggering their ubiquitination and proteasomal degradation (Irigoyen *et al.*, *The Plant Cell* 2014). Therefore, CRL4-CDDD complexes act as repressors of ABA-mediated water stress responses under optimal growth conditions. Interestingly, CRL4-CDDD function is performed in close proximity to chromatin, which should enable rapid translation of environmental and stress signals into changes in gene expression. Indeed, recent results from our laboratory showed that CRL4-CDDD complexes are part of a molecular pathway controlling epigenetic homeostasis (including Histone2B ubiquitination) in response to external stimuli (i.e., light conditions; Nassrallah *et al.*, *eLife* 2018). Our current objective is to identify and characterise additional mechanisms by which CRL4-CDDD controls the accumulation of specific epigenetic marks across the plant genome in response to environmental changes, thereby regulating the expression of specific sets of genes that confer plants the ability to adapt to changing climate conditions.

1



1 We investigate the molecular mechanisms by which light conditions influence growth and development through plants' life.

2



2 Infra-red imaging techniques allow quantitation of foliar temperature, which is indicative of water loss by transpiration, in mutant and control plants.



Signalling networks in plant development and defence responses

62 PLANT MOLECULAR GENETICS

GROUP LEADERS

José Sánchez-Serrano
Enrique Rojo

SENIOR SCIENTIST

Maite Sanmartín

POSTDOCTORAL SCIENTIST

Mary Paz González

TECHNICIAN

Yolanda Fernández

PhD STUDENT

Aleksandra Lazarova

MASTER'S STUDENTS

Iris Merino

Beatriz Larruy

UNDERGRADUATE STUDENT

Alba González

SELECTED PUBLICATIONS

Muñoz A, Mangano S, González-García MP, Contreras R, Sauer M, De Rybel B, Weijers D, Sánchez-Serrano JJ, Sanmartín M, Rojo E. RIMA-dependent nuclear accumulation of IYO triggers Auxin-irreversible cell differentiation in Arabidopsis. *Plant Cell* 2017; 29: 575-588.

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Lynch, CJ, Bernad R, Calvo I, Nobrega-Pereira S, Ruiz S, Ibarz N, Martínez-Val A, Grana-Castro O, Gomez-López G, Andres-Leon E, Espinosa Angarica V, Del Sol A, Ortega S, Fernandez-Capetillo O, Rojo E, Munoz J, and Serrano M. The RNA Polymerase II factor RPAP1 is critical for mediator-driven transcription and cell identity. *Cell Rep* 2018; 22, 396-410.

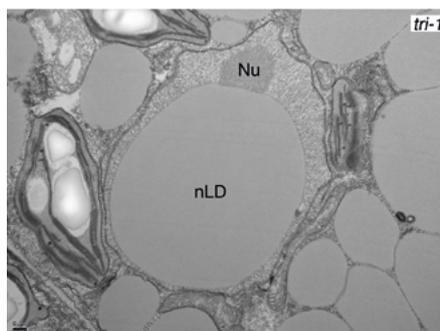
Our group studies how plants adjust their growth and development to changes in the environment and, in particular, to challenges from pests and pathogens.

One of our research lines is focused on understanding the molecular mechanisms that initiate cell differentiation and organogenesis in plants. Our previous work identified MINIYO (IYO) and RIMA as essential genes for cell differentiation in Arabidopsis. Analysis of these two genes has led to a working model suggesting that, upon displacement of stem cells from the meristem core, IYO and RIMA migrate from the cytosol to the nucleus where they modulate RNA polymerase II activity and reprogram the transcriptome to activate cell differentiation. We are now trying to elucidate how the IYO/RIMA nuclear switch is controlled by developmental and environmental cues and how it activates downstream targets to drive cell differentiation. IYO and RIMA homologues are found throughout eukaryotes, and the IYO transcriptional reprogramming module for stem cell differentiation appears to be conserved in mammals, suggesting that findings made on this molecular switch in Arabidopsis will be broadly applicable to crop species and potentially to animal systems.

A second line of research in our laboratory focuses on the signalling pathways that activate defence responses in plants. Our work has unravelled a crucial role of protein phosphatases 2A (PP2As) at the crossroads of hormonal and innate immunity signalling in plants. Previously, we have demonstrated the importance of PP2As in auxin and abscisic acid signalling, as well as in plant immunity against bacteria. We have now gained

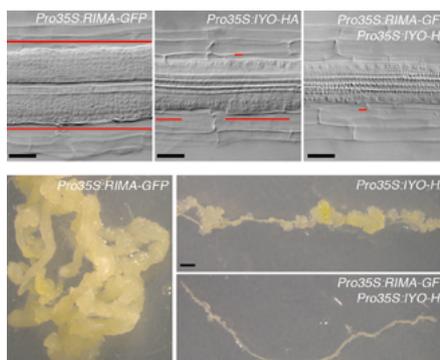
evidence that PP2As also play a key role in jasmonate and brassinosteroid signal transduction. By learning how these phosphatases integrate hormonal and defence signalling, we hope to develop technology to improve crop protection without negatively affecting plant growth and yield.

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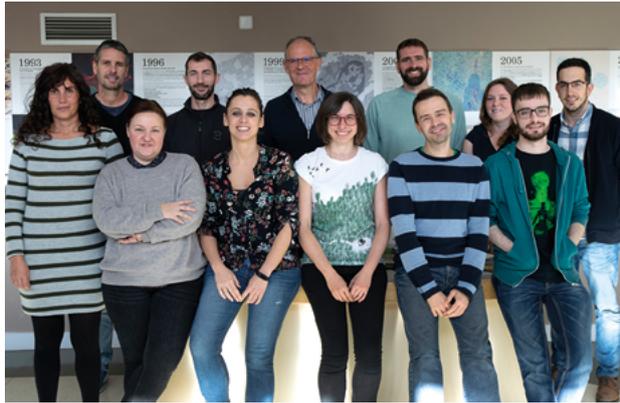


1 Nuclear lipid droplets accumulate in seipin triple mutants. Nu: nucleolus; nLD: nuclear lipid droplet.

2



2 Concomitant overexpression of IYO and RIMA blocks auxin-induced callus growth. Root explants from plants overexpressing RIMA-GFP, IYO-HA or RIMA-GFP and IYO-HA under a constitutive 35S promoter were incubated for 5 days (upper panels) or 51 days (lower panels) in media containing 300ng/ml 2,4D.



Jasmonate signalling and plant defence

PLANT MOLECULAR GENETICS 63

GROUP LEADER

Roberto Solano

SENIOR SCIENTIST

Andrea Chini

POSTDOCTORAL SCIENTISTS

José Manuel Chico
Selena Giménez Ibáñez
Isabel Monte
Guillermo Giménez-Alemán
Sophie Kneeshaw
Gonzalo Soriano

TECHNICIAN

Gemma Fernández Barbero

PHD STUDENTS

María Peñuelas
Andrés Ortigosa
Alberto Iniesta

SELECTED PUBLICATIONS

Bowman JL, ... Monte I, Solano R, ... *et al.* Insights into Land Plant Evolution Garnered from the *Marchantia polymorpha* Genome. *Cell* 2017; 171: 287-304.

Gimenez-Ibanez S, Boter M, Ortigosa A, García-Casado G, Chini A, Lewsey MG, Ecker JR, Ntoukakis V, Solano R. JAZ2 controls stomata dynamics during bacterial invasion. *New Phytol* 213: 1378-1392.

Chini A, Monte I, Zamarreño AM, Hamberg M, Lassueur S, Reymond P, Weiss S, Stintzi A, Schaller A, Porzel A, García-Mina JM, Solano R. An ORP3-independent pathway uses 4,5-didehydro-jasmonate for jasmonate synthesis. *Nat Chem Biol* 2018; 14: 171-178.

Monte I, Ishida S, Zamarreño AM, Hamberg M, Franco-Zorrilla JM, García-Casado G, Gouhier-Darimont C, Reymond P, Takahashi K, García-Mina JM, Nishihama R, Kohchi T, Solano R. Ligand-receptor co-evolution shaped the jasmonate pathway in land plants. *Nat Chem Biol* 2018; 14: 480-488.

Ortigosa A, Gimenez-Ibanez S, Leonhardt N, Solano R. Design of a bacterial speck resistant tomato by CRISPR/Cas9-mediated editing of SIJAZ2. *Plant Biotechnol J* 2019; 17: 665-673.

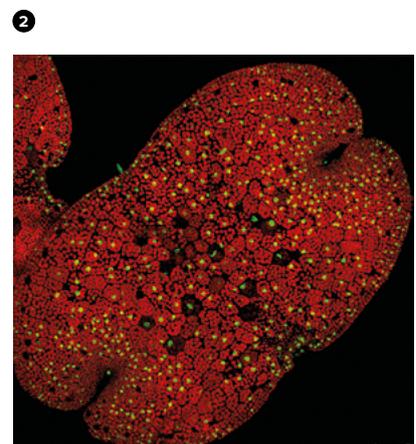
Jasmonates (JAs) are fatty acid-derived signalling molecules essential for the survival of plants in nature since they are important activators of stress responses and developmental programs. The main focus of our lab is to understand mechanistically the JA signalling pathway in plants; knowledge that is basic to design biotech and agronomical applications that improve plant resistance to stresses and plant yield. We have traditionally worked in the model plant *Arabidopsis thaliana*, but have recently focused in the Liverwort *Marchantia polymorpha* due to its remarkable genetic advantages, such as very low gene redundancy.

Our major achievements in the last two years:

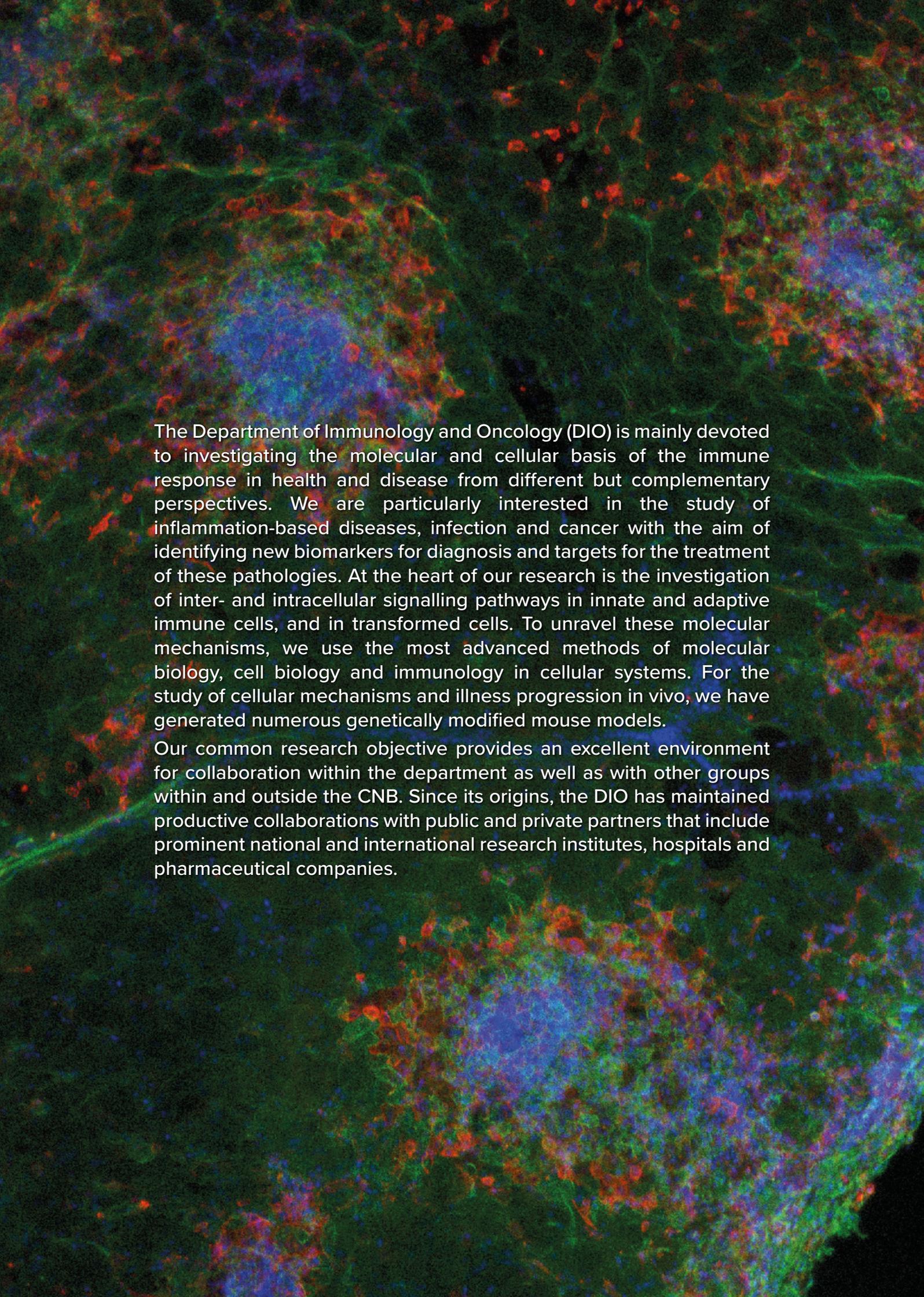
- Discovery of a new pathway for JA biosynthesis (Chini *et al.*, *Nature Chem Biol* 2018)
- Discovery of the bioactive jasmonate in Bryophytes (Monte *et al.*, *Nature Chem Biol* 2018)
- Participation in the sequencing and analysis of the genome of *Marchantia polymorpha* (Bowman *et al.*, *Cell* 2017)
- Identification of the tomato orthologue of AtJAZ2 and design of tomato plant resistant to *Pseudomonas syringae* by CRISPR/Cas9-based mutation of SIJAZ2 (Gimenez-Ibanez *et al.*, *New Phytol* 2017; Ortigosa *et al.*, *Plant Biotechnol J* 2018).
- Filing of a patent for the biotech application of JAZ2 to improve resistance to plant pathogens: PCT/EP17/078493 owned by the CSIC and licensed to PBL (Plant Biotech Limited, UK).
- Characterisation of the TIFY family in wheat (Ebel *et al.*, *PLoS One* 2018).
- Identification and characterisation of a new fungal jasmonate (Chini *et al.*, *J Exp Bot* 2018).
- Collaboration in the characterisation of a JAZ subtype-selective agonist of jasmonate perception (Takaoka *et al.*, *Nat Commun* 2018).
- Identification of the DNA target sequence of many plant transcription factors using previously developed tools and in collaboration with several groups (Molina-Hidalgo *et al.*, *J Exp Bot* 2017; Gallemi *et al.*, *New Phytol* 2017; Hichri *et al.*, *Front Plant Sci* 2017; Yan *et al.*, *PNAS* 2017; Matthijs *et al.*, *EMBO J* 2017).



1 Air pore in the epidermis of WT *Marchantia polymorpha* plants



2 Nuclear localisation of JAZ protein in young *Marchantia polymorpha* plants.

A fluorescence microscopy image of a cell. The cell is stained with three different dyes: green, red, and blue. The green signal highlights the cytoskeleton, showing a network of filaments. The red signal appears as small, punctate spots, likely representing organelles or specific proteins. The blue signal is distributed throughout the cell, representing the nucleus and other DNA-containing structures. The overall appearance is that of a complex, interconnected cellular structure.

The Department of Immunology and Oncology (DIO) is mainly devoted to investigating the molecular and cellular basis of the immune response in health and disease from different but complementary perspectives. We are particularly interested in the study of inflammation-based diseases, infection and cancer with the aim of identifying new biomarkers for diagnosis and targets for the treatment of these pathologies. At the heart of our research is the investigation of inter- and intracellular signalling pathways in innate and adaptive immune cells, and in transformed cells. To unravel these molecular mechanisms, we use the most advanced methods of molecular biology, cell biology and immunology in cellular systems. For the study of cellular mechanisms and illness progression in vivo, we have generated numerous genetically modified mouse models.

Our common research objective provides an excellent environment for collaboration within the department as well as with other groups within and outside the CNB. Since its origins, the DIO has maintained productive collaborations with public and private partners that include prominent national and international research institutes, hospitals and pharmaceutical companies.

Immunology and Oncology

HEAD OF DEPARTMENT

Ana Cuenda

RESEARCH GROUPS

- 1. Dendritic cell and macrophage immunobiology**
Carlos Ardavín
- 2. Immune hyperactivity in autoimmunity and hyporesponsiveness in cancer depend on the level of mitochondrial activity**
Dimitrios Balomenos
- 3. Nanomedicine, cancer immunotherapy and autoimmune diseases**
Domingo F. Barber
- 4. Adult heart turnover**
Antonio Bernad
- 5. B cell dynamics**
Yolanda R. Carrasco
- 6. Molecular targets in health and disease: focus on PI3-kinase**
Ana Clara Carrera
- 7. Stress-activated protein kinase p38MAPK in inflammation and cancer**
Ana Cuenda
- 8. Physiopathology of chemokine receptor interactions**
Leonor Kremer
- 9. Signalling networks in inflammation and cancer**
Santos Mañes
- 10. Stem cells and immunity**
Carlos Martínez-A
- 11. Chemokine receptors: new targets for therapeutic intervention**
Mario Mellado
- 12. Diacylglycerol kinases in the control of immune response and cancer progression**
Isabel Mérida
- 13. Transcriptional control of B lymphocyte differentiation**
Ignacio Moreno de Alborán
- 14. Receptor-ligand interactions in immune responses to cancer and viruses**
Hugh T. Reyburn
- 15. T cell signalling in autoimmune diseases and cancer**
Jesús M. Salvador
- 16. Tumour immune activation and evasion**
Mar Valés-Gómez



Dendritic cell and macrophage immunobiology

66 IMMUNOLOGY & ONCOLOGY

GROUP LEADER Carlos Ardavín

SENIOR SCIENTISTS

María López-Bravo
Cristina Godio

PHD STUDENTS

Jorge Domínguez
Lidia Feo
Laura Hernández
Adrián Vega

VISITING PHD STUDENT

Julieta Alcaín

SELECTED PUBLICATIONS

Domínguez-Andrés J, Feo-Lucas L, Minguito de la Escalera M, González L, López-Bravo M, Ardavín C. Inflammatory Ly6C^{high} monocytes protect against candidiasis through IL-15-driven NK cell/neutrophil activation. *Immunity* 2017; 46: 1059-1072.

Domínguez-Andrés J, ..., Ardavín C, Netea MG. Rewiring monocyte glucose metabolism via C-type lectin signaling protects against disseminated candidiasis. *PLoS Pathog* 2017; 13: e1006632.

Nieto C, ..., Ardavín C, Castrillo A, Vega MA, Puig-Kröger A, Corbí AL. The Activin A-PPAR γ axis contributes to the transcriptome of GM-CSF-conditioned human macrophages. *Front Immunol* 2018; 9: 31.

Beceiro S, ..., Ardavín C, Partida-Sanchez S, Rodríguez-Fernández JL, Nagy L, Tontonoz P, Castrillo A. LXR nuclear receptors are transcriptional regulators of dendritic cell chemotaxis. *Mol Cell Biol* 2018; 38: 00534-17.

Alsina-Beauchamp D, ..., Ardavín C, Netea MG, Alemany S, JJ Sanz-Ezquerro, Cuenda A. Myeloid cell deficiency of p38 γ /p38 δ protects against candidiasis and regulates antifungal immunity. *EMBO Mol Med* 2018; 10: e8485.

Our research program aims at exploring the role of inflammatory monocytes and macrophages during infection, allergy and intraperitoneal tumour metastasis, and encompasses the following research lines:

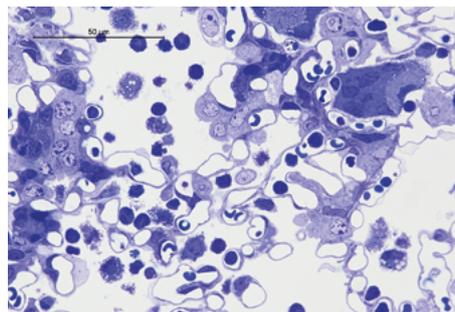
- Role of monocytes and type-I interferon in NK cell and neutrophil activation during the innate immune response against systemic *Candida albicans* infection.
- Alveolar macrophage dynamics and immunophysiology during airway allergic reactions caused by house dust mite-derived allergens.
- Role of the innate immune system of the peritoneal cavity in defence against intraperitoneal bacterial infections and colorectal tumour metastasis.

The experimental approach designed to address the role of monocytes during *Candida* infection involves the analysis of the early and cooperative spleen and kidney innate immune responses against intravenous infection with the fungus *Candida albicans* in a mouse model of systemic candidiasis, using wild type mice of the C57BL/6 strain as well as mice deficient in the type-I interferon receptor (IFNAR), the chemokine receptor CCR2 and the cytokine IL-15.

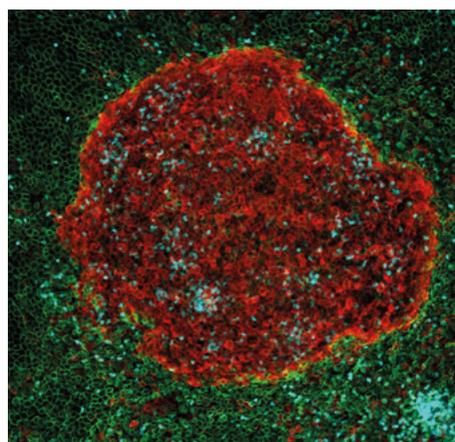
Our project on the dynamics of alveolar macrophages involves the study of the alveolar damage caused by strong airway allergic reactions against house dust mite-derived allergens, the process by which the alveolar macrophage subset is regenerated once the allergic process is resolved, and the mechanisms ensuring alveolar tissue repair and surfactant homeostasis. Wild type C57BL/6 and CCR2-deficient mice, parabiosis and progenitor transfer experiments, and immunofluorescent and electron microscopy are currently used in our laboratory to address these issues.

Experimental sepsis, peritoneal bacterial infection models using mouse intestinal strains of *E. coli*, and mouse models of intraperitoneal metastasis of colorectal tumours in wild type C57BL/6 and CCR2-deficient mice are employed to explore the role of the innate immune system of the peritoneal cavity in defence against intraperitoneal infection and tumour metastasis.

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1 Alveolar damage and pneumocyte hyperplasia caused by house-dust mite airway allergy. Light microscopy of a 1 μ m semithin section of the lung 4 days after intratracheal allergen challenge.

2 Macrophage cluster attached to the peritoneal wall mesothelium 4h after intraperitoneal bacterial infection. Confocal microscopy after whole mount immune-fluorescence using anti-F4/80 (macrophage marker; red), anti-Ly6G (neutrophil marker; blue) and anti-podoplanin (mesothelium marker; green) antibodies.



Immune hyperactivity in autoimmunity and hyporesponsiveness in cancer depend on the level of mitochondrial activity

GROUP LEADER
Dimitrios Balomenos

PhD STUDENTS
Rahman Shokri
Parinaz Tavakoli
Sara Colomo del Pino

MASTER'S STUDENTS
Pauline Rault
Karla Svaguhša

UNDERGRADUATE STUDENTS
Blanca Castell Miñón
Laura Guerrero González
Esther Díaz Maroto

SELECTED PUBLICATIONS

Balomenos D, Shokri R, Daszkiewicz L, Vázquez-Mateo C, Martínez-A C. On how Fas apoptosis-independent pathways drive T cell hyperproliferation and lymphadenopathy in lpr mice. *Front Immunol* 2017; 8: 237.

Rodríguez-Frade JM, Guedán A, Lucas P, Martínez-Muñoz L, Villares R, Criado G, Balomenos D, Reyburn HT, Mellado M. Use of lentiviral particles as a cell membrane-based mFasL delivery system for *in vivo* treatment of inflammatory arthritis. *Front Immunol* 2017; 8: 460.

Rackov G, Shokri R, Álvarez De Mon M, Martínez-A. C, Balomenos D. The role of IFN-beta during the course of sepsis progression and its therapeutic potential. *Front Immunol* 2017; 8: 493.

In autoimmune diseases, hyperactive immunity provokes self-reactivity. In order to neutralise this damaging effect, the immune response needs to be deactivated. Alternatively, in cancer, immunosuppressed immunity requires reactivation.

Our studies suggest that p21 is a regulator of mitochondrial activity, controlling the balance between hyperactivation and immunosuppression. Therefore, high expression of p21 tempers T cell overactivity, while lack of p21 enhances T cell and macrophage responses (Figure 1).

Increased mitochondrial activity and lack of p21 increase memory T cell responses

Compared to normal memory T cells, autoreactive T cells become overactivated due to their repeated encounters with autoantigens. We have shown therapeutic potential for p21, as its overexpression deactivates hyperactivated autoreactive T cells (Daszkiewicz L *et al*, *Sci Rep* 2015; 5: 7691).

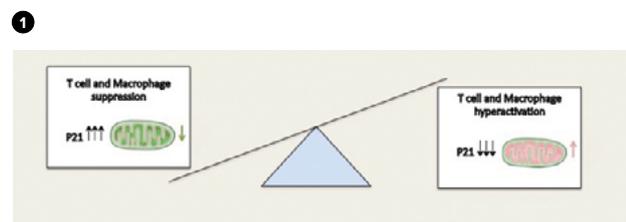
Our current work indicates that p21 does not act as a cell cycle inhibitor but modulates the activation of autoreactive T cells. Lack of p21 concurs with increased mitochondria activation, which drives T cell responses. We are currently examining a potential association between p21 and mitochondrial function.

The effect of p21 and mitochondrial activation in macrophage responses and its possible effects in cancer immunotherapy

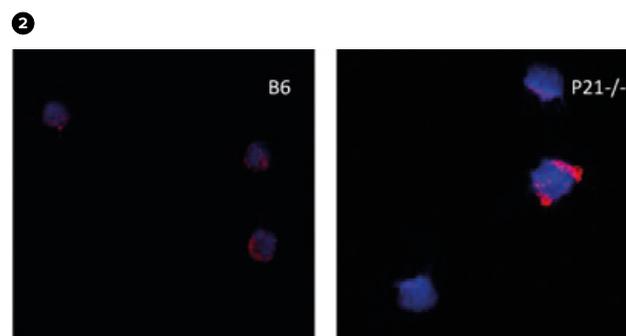
We have shown a dual regulatory role for p21; first, in macrophage activation to M1 state and, second, in macrophage reprogramming from M1 to the M2 unresponsive state. Lack of p21 prevents macrophage reprogramming to M2 status (Rackov G *et al*, *J Clin Invest* 2016; 126: 3089-3103).

Our data show that macrophage activation to M1 status is also associated to mitochondrial activation, which is linked to lack of p21 expression (Figure 2).

The role of p21 and mitochondria in macrophage activation may have an effect in immunotherapy of cancer, as tumour persistence neutralises M1 macrophages and attracts deactivated M2 cells.



1 Increased p21 expression is associated with controlled mitochondrial activity which contributes to T cell and macrophage hyporesponsiveness, while lack or decreased levels of p21 lead to increased mitochondrial activity that drive T cell and macrophage hyperactivation.



2 Lack of p21 increases mitochondrial activity in activated macrophages.



Nanomedicine, cancer immunotherapy and autoimmune diseases

68 IMMUNOLOGY & ONCOLOGY

GROUP LEADER

Domingo F. Barber Castaño

TECHNICIAN

Sonia Pérez-Yagüe

PhD STUDENTS

Laura Sanz Ortega
Patricia Hernández Flores
Sara de Bernardo
Hernández-Coronado
Yadileiny Portilla Tundidor

MASTER'S STUDENT

Andrés París Muñoz

UNDERGRADUATE STUDENT

Sara Mellid López

SELECTED PUBLICATIONS

Rojas JM, Gavilán H, Del Dedo V, Lorente-Sorolla E, Sanz-Ortega L, da Silva GB, Costo R, Pérez-Yagüe S, Talelli M, Marciello M, Morales MP, Barber DF, Gutiérrez L. Time-course assessment of the aggregation and metabolism of magnetic nanoparticles. *Acta Biomater* 2017; 58: 181-195.

Gonzalo-Gil E, García-Herrero C, Toldos O, Usategui A, Criado G, Pérez-Yagüe S, Barber DF, Pablos JL, Galindo M. Microthrombotic renal vascular lesions are associated to increased renal inflammatory infiltration in murine lupus nephritis. *Front Immunol* 2018; 9: 1948.

Fortes Brollo ME, Hernández Flores P, Gutiérrez L, Johansson C, Barber DF, Morales MDP. Magnetic properties of nanoparticles as a function of their spatial distribution on liposomes and cells. *Phys Chem Chem Phys* 2018; 20: 17829-17838.

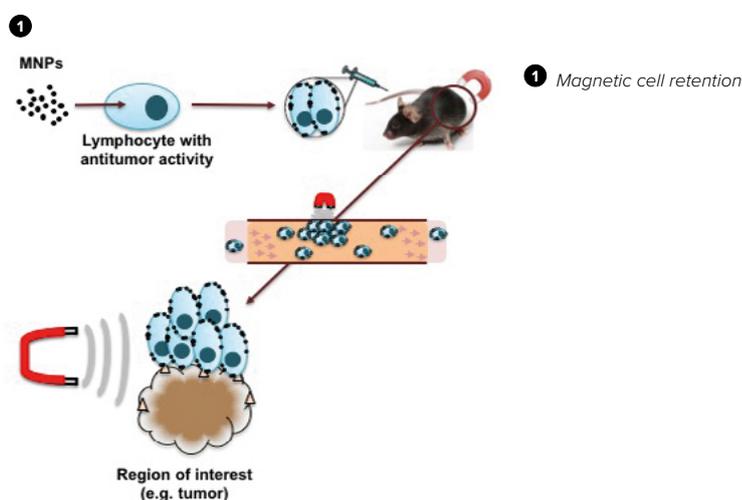
Risco A, Martín-Serrano MA, Barber DF, Cuenda A. p38γ and p38δ are involved in T lymphocyte development. *Front Immunol* 2018; 9: 65.

Mejías R, Hernández Flores P, Talelli M, Tajada-Herraiz JL, Brollo MEF, Portilla Y, Morales MP, Barber DF. Cell-promoted nanoparticle aggregation decreases nanoparticle-induced hyperthermia under an alternating magnetic field independently of nanoparticle coating, core size and subcellular localization. *ACS Appl Mater Interfaces* 2019; 11: 340-355.

Due to their small size and physicochemical properties, superparamagnetic iron oxide nanoparticles (SPION) have great potential as a nanomedicine in the fight against cancer, as they have proven effective for targeted drug release and in diagnosis by magnetic resonance imaging. SPIONs also show considerable promise for two additional cancer therapies, intracellular hyperthermia induction and targeting in cell transfer. Adoptive cell transfer is a type of immunotherapy that exploits the antitumor capacity of cytotoxic lymphocytes. The application of alternating magnetic fields (AMF) can magnetically induce intracellular hyperthermia in SPION-loaded cells, which can be used in cancer treatment. Results to date, using SPIONs *in vitro* studies and in animal models, suggest potential for rapid translation of these technologies to clinical practice. This development has nonetheless been delayed in part by a lack of basic knowledge of SPION-induced molecular and cellular mechanisms and the routes that regulate SPION degradation in the organism, both of which affect the therapeutic effectiveness of SPIONs and their accumulation and long-term toxicity.

The overall objective of our group is to understand SPION-mediated molecular and cellular mechanisms in distinct biomedical applications oriented to cancer and autoimmunity treatment, and to use this knowledge to improve SPION functional design for specific biomedical purposes in antitumour therapy. We pursue six specific objectives.

1. Investigate the ability of intracellularly loaded SPIONs to induce biological effects following the application of an AMF, to identify effects that depend on temperature increase or that are mediated by other mechanisms.
2. Comparative analysis of the effectiveness of various nanoparticle-targeting approaches in antitumour therapies.
3. Study SPION-induced immunogenic and epigenetic changes in cells and the possible contribution of AMF application in intracellular hyperthermia treatment on these changes.
4. Study of SPION degradation and transformation within lysosomes.
5. Potentiation of antitumour therapy by adoptive transfer of NK cells and CD8+ T cells using SPIONs.
6. Potentiation of autoimmunity treatment by adoptive transfer of immunosuppressive cells using SPIONs.





Adult heart turnover

IMMUNOLOGY & ONCOLOGY 69

GROUP LEADER
Antonio Bernad

SENIOR SCIENTIST
Susana Cañón

POSTDOCTORAL SCIENTIST
Diego Herrero

RESEARCH ASSISTANT
Susana Aguilar

TECHNICIAN
Rosa M. Carmona

PHD STUDENT
Guillermo Albericio

UNDERGRADUATE STUDENTS
Paloma Ruiz
Adrian Holguín

SELECTED PUBLICATIONS

Sanz-Ruiz R *et al.* Rationale and design of the first-in-man clinical trial to evaluate the safety and efficacy of intracoronary infusion of allogeneic human CARDiac stEm cells in patients with acute Myocardial Infarction and left ventricular dysfunction: the randomized multicenter double-blind controlled CAREMI trial. *Circulation Res* 2017; 121: 71-80.

Escudero B *et al.* Polu deficiency induces moderate shortening of P53^{-/-} mouse lifespan and modifies tumor spectrum. *DNA Repair (Amst)* 2017; 54: 40-45.

Herrero D *et al.* A. Redox-dependent BMI1 activity drives *in vivo* adult cardiac progenitor cell differentiation. *Cell Death Differ* 2018; 25: 807-820.

Herrero D *et al.* Bmi1-Progenitor Cell Ablation Impairs the Angiogenic Response to Myocardial Infarction. *Arterioscler Thromb Vasc Biol* 2018; 38: 2160-2173.

García-López S *et al.* Deregulation of the imprinted DLK1-DIO3 locus ncRNAs is associated with replicative senescence of human adipose-derived stem cells. *PLoS One* 2018; 13: e0206534.

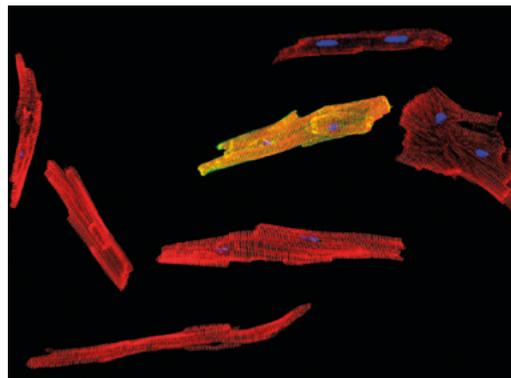
Adult mammalian heart can refresh damaged or aged cells during their lifetime but with low rate. However, mechanisms involved in the turnover remain highly controversial. We have defined a population of non-cardiomyocytic cells that expresses high levels of the polycomb Bmi1 transcription factor (Bmi1⁺), which contributes to the turnover of the three main cardiac lineages. In response to a variety of acute cardiac insults, the Bmi1⁺ cells progeny increases their contribution to the mature lineages.

In adult tissues, progenitors and stem cells are lodged in specialised structures (niches) that provide a protective microenvironment, essential for their correct regulation, and usually associated to low oxidative stress. In agreement with our working hypothesis, we found that Bmi1⁺ cells show low levels of reactive oxygen species (ROS). Interestingly, in homeostasis conditions, the analysis of cell distribution showed that Bmi1⁺ cardiac progenitor cells were located close to the vasculature, with an enrichment in quiescent Bmi1⁺ cells close to endothelial structures. These results strongly suggest the instructive role of cardiac vasculature that was confirmed by *in vitro* co-culture experiments with endothelial cells. In agreement with this role, *in vivo* genetic ROS amelioration altered the perivascular location of Bmi1⁺ cells, which resumed adolescent-like gene expression profiles. Altogether, we concluded that cardiac vasculature provides a protective and low-stress niche-like microenvironment that contributes to the maintenance of Bmi1⁺ cardiac progenitor cells in adult heart.

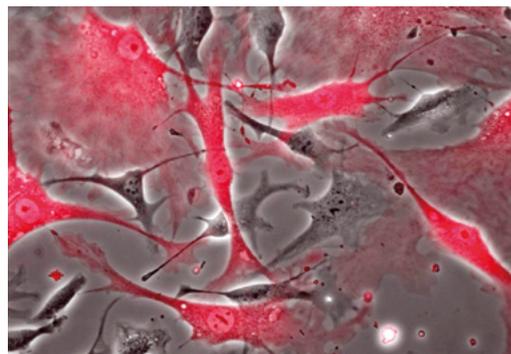
Finally, *in vivo* genetic depletion of the Bmi1⁺ population demonstrated that, although the population is not essential in homeostasis, its deletion in the context of acute

infarct recovery is highly deleterious; depleted animals demonstrated a significantly increased mortality, associated with major structural and functional heart alterations. Bmi1⁺-deficient infarcted hearts showed a severe decrease in neo-angiogenesis and ejection fraction function, that seemed to account for a severe ischemic-dilated cardiac phenotype.

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1 Plated murine cardiomyocytes isolated from adult heart. Cardiomyocytes are labelled with sarcomeric beta-actinin (red) and nuclei are labelled with DAPI (blue). *In vivo* lineage tracing study uncovers mature cardiomyocytes derived from Bmi1⁺ cardiac progenitor cells assessed by yellow fluorescent protein expression (yellow).

2 Cardiac progenitor cells (tomato+) in co-culture with 1g11 endothelial cells (not coloured).



B cell dynamics

70 IMMUNOLOGY & ONCOLOGY

GROUP LEADER

Yolanda R. Carrasco

PhD STUDENTS

Sara Román García
Sara Violeta Merino Cortés
Sofía R. Gardeta Castillo

MASTER'S STUDENT

Esther Ferreira Alonso

UNDERGRADUATE STUDENTS

Cristina Rodilla Hernández
Raúl Izquierdo Serrano
Eduardo Martín Quintana

SELECTED PUBLICATIONS

Roman-García S, Merino-Cortés SV, Gardeta SR, de Bruijn MJW, Hendriks RW, Carrasco YR. Distinct roles for Bruton's tyrosine kinase in B cell immune synapse formation. *Front Immunol* 2018; 9: 2027.

Carrasco YR. *In vivo* tracking of particulate antigen localization and recognition by B lymphocytes at lymph nodes. *Methods Mol Biol* 2018; 1707: 163-169.

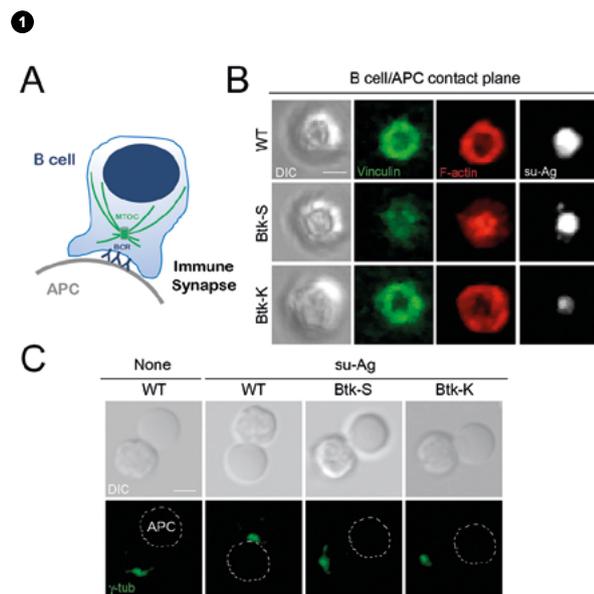
Perez-Olivares M, Trento A, Rodríguez-Acebes S, González-Acosta D, Fernández-Antoran D, Roman-García S, Martínez D, López-Briones T, Torroja C, Carrasco YR, Méndez J, Moreno de Alborán I. Functional interplay between c-Myc and Max in B lymphocyte differentiation. *EMBO Rep* 2018; 19: e45770.

Martínez-Muñoz L, Rodríguez-Frade JM, Barroso R, Sorzano COS, Torreño-Pina JA, Santiago CA, Manzo C, Lucas P, García-Cuesta EM, Gutiérrez E, Barrio L, Vargas J, Cascio G, Carrasco YR, Sánchez-Madrid F, García-Parajo MF, Mellado M. Separating actin-dependent chemokine receptor nanoclustering from dimerization indicates a role for clustering in CXCR4 signaling and function. *Mol Cell* 2018; 70: 106-119.

Bruton's tyrosine kinase (Btk) has a key role in the signalling pathways of receptors essential for the B cell response. Given its implication in B cell related immunodeficiency, leukaemias/lymphomas and autoimmunity, Btk is studied intensely and used as a target for therapy. Numerous clinical trials are ongoing, using Btk kinase activity inhibitors to treat patients suffering of B cell malignancies or B cell-related autoimmune disorders; while the clinical results are excellent, the underlying molecular mechanisms are hardly known. BCR recognition of antigen triggers the formation of the immune synapse (IS); this B cell/APC interaction platform provides a framework for signalling and polarised membrane trafficking to achieve B cell activation and antigen extraction. The actin cytoskeleton remodelling and adhesion-site dynamics have a crucial role in IS formation and stability. We reported that Btk controls the B cell ability to trigger IS formation and its appropriate intramolecular organisation mainly through shuttling/scaffold activities. Btk kinase function determines antigen accumulation at the IS by controlling the PLC γ 2/Ca $^{2+}$ axis. Impaired Btk shuttling/scaffold activity leads to defects in B cell activation and proliferation equivalent to those due to Btk kinase inhibition.

We also investigated Diacylglycerol kinases (DGK), a therapeutic target for fighting against immunosuppression in tumours. DGK limits antigen receptor signalling by DAG consumption, but the relevance of their product, phosphatidic acid (PA), in lymphocyte responses is quite unknown. Our findings suggest that PA generated by the DGK ζ isoform shapes B cell responses by controlling actin/adhesion-mediated force generation and cell polarity-related events at the synapse. An appropriate DAG/PA balance is key for B cell function.

The results derived from our studies reveal important aspects of Btk and DGK ζ /PA functions that enrich our knowledge and aid in the therapeutic targeting of these proteins.



1 Distinct roles for Btk scaffold and kinase activities in B cell synapse formation.

A) Explicative scheme of the B cell IS established with an APC.

B) DIC and fluorescence images of vinculin, F-actin and antigen (su-Ag) at the contact plane of the mature IS for representative B cells with normal Btk (WT), lacking kinase activity (Btk-K) or defective for the scaffold function (Btk-S); scale Bar, 2.5 μ m. C) DIC and fluorescence images of γ -tubulin (MTOC) for representative conjugates of B cells with APC unloaded (none) or antigen-loaded (su-Ag). Scale Bar, 5 μ m.



Molecular targets in health and disease: focus on PI3-kinase

IMMUNOLOGY & ONCOLOGY 71

GROUP LEADER

Ana Clara Carrera

SENIOR SCIENTIST

Ana María González García

POSTDOCTORAL SCIENTISTS

Sudhansu Yadav
Africa Millán Uclés
Jesus Vallejo Díaz

TECHNICIANS

Carmen Hernández Agüero
Lorena Sanz González

PhD STUDENTS

Miriam Sánchez Ortega
Manuel Olazabal Morán

MASTER'S STUDENT

Pablo Ballesteros

UNDERGRADUATE STUDENTS

Elena Ramís Bravo
María Martínez Vega

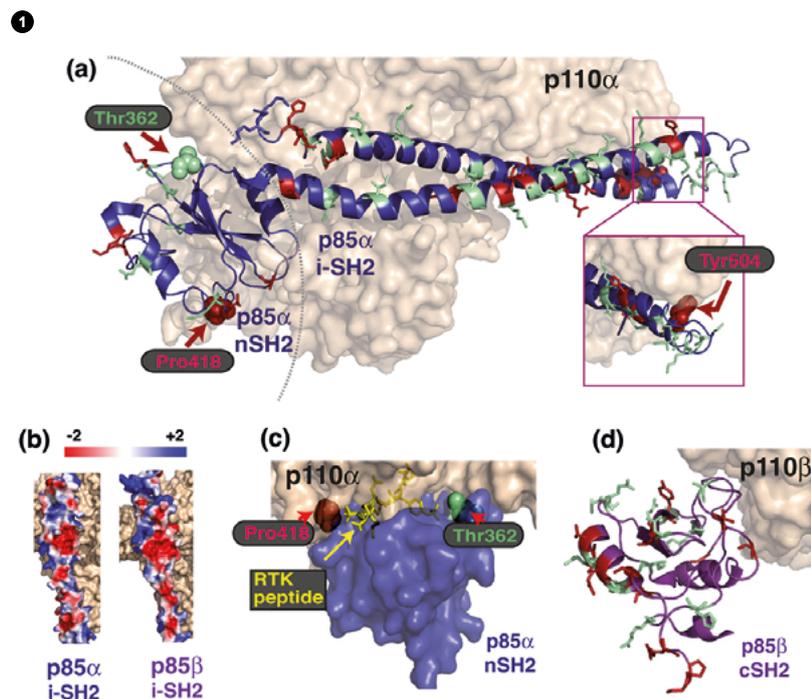
SELECTED PUBLICATIONS

Varona R, Ramos T, Escribese MM, Jimeno L, Galán A, Würtzen PA, Vega F, Marín A, Martín S, Carrera AC, Blanco C, Barber D. Persistent regulatory T-cell response 2 years after 3 years of grass tablet SLIT: Links to reduced eosinophil counts, sIgE levels, and clinical benefit. *Allergy* 2019; 74: 349-360.

Olazabal-Morán M, González-García A, Carrera AC. Functions of Nuclear polyphosphoinositides. *Handbook of Experimental Pharmacology* 2018; Chapter 8 (Springer, Heidelberg).

Two biological problems have occupied the activity of our team of 12 to 15 members: cancer and inflammation. Our work is based on the assumption that the same biological activities that control physiological responses also control pathology when deregulated. The team is currently working on class I phosphoinositide 3-kinase (PI3K), with emphasis on examining the specific function of each of different catalytic and regulatory isoforms in physiology and disease.

- Function of PI3K in cancer using animal models and biochemistry approach.
- Alternative cancer treatments based on interfering molecules.
- New therapeutic targets on cancer based on the tumour hypoxic and oxidative environment.
- Mechanism for PI3-kinase beta action on DNA/chromatin remodelling.



1 Structural differences between p85α and p85β. Residue differences of p85α and p85β in complex with PI3K/p110. Distinct residues could explain why the tumour suppressor p85α restrains p110 activity, while p85β is less inhibitory and drives tumour progression.

(a) p110α (ochre)/p85α (blue) interacting surface. Non-conserved (red) and semi-conserved (turquoise) residues. Only three residues: Thr362, Pro418 and Tyr 504 are in contact with PI3K (b) Different electrostatic surface of p85α and p85β (red-blue), p110α surface behind. (c) p110α (ochre) and nSH2 region of p85α (blue) showing that Thr362 and Pro418 are close to receptor Tyr Kinases (yellow). (d) p110β (ochre) and p85β iSH2 domain (purple) showing that distinct residues are not in contact with p110β and most likely mediate association with different partners.



Stress-activated protein kinase p38MAPK in inflammation and cancer

GROUP LEADER

Ana Cuenda

SENIOR SCIENTIST

Ana Risco

ADMINISTRATION

Ruth Gómez-Caro
Elena Fernández Cortes Navia

PhD STUDENTS

Alejandra Escós
Miguel Ángel Martín Serrano
Diego González Romero
Pilar Fajardo Flores
Ester Díaz Mora

MASTER'S STUDENT

José Martín Gómez

SELECTED PUBLICATIONS

Cuenda A, Sanz-Ezquerro JJ. p38 γ and p38 δ : from spectators to key physiological players. Trends Biochem Sci 2017; 42: 431-442.

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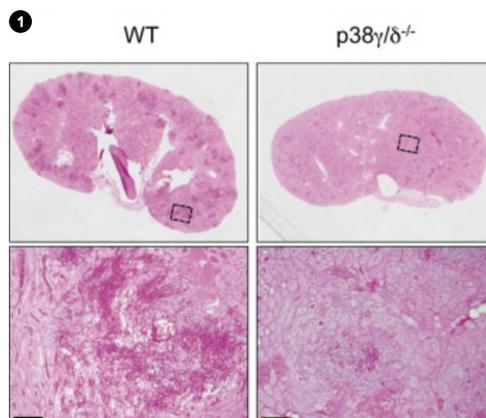
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Cuenda A. MAP Kinases. Editorial: Encyclopedia of Cancer, Third Edition (editors in chief: Paolo Boffetta and Pierre Hainaut), Elsevier.

Our group is studying the physiological and pathological functions of the p38MAPK family in the context of inflammation and cancer. Inflammation, in the right place and at the right time, controls a healthy host response; however, uncontrolled inflammation causes many diseases, including some types of cancer. In these two years, we have expanded our knowledge on the molecular mechanisms involved in the inflammatory response in the setting of infection.

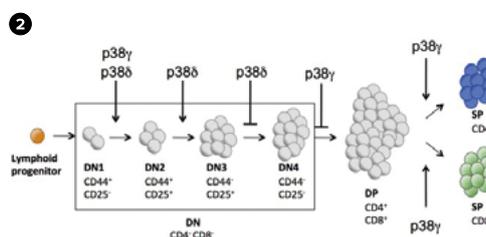
Previously, we have demonstrated that alternative p38MAPK (p38 γ and p38 δ) are key elements in the control of the inflammatory response in several models of inflammatory disease. p38 γ and p38 δ regulate many immune cell functions such as cytokine production, migration, or T cell activation. More recently, we have shown that p38 γ /p38 δ deletion protects against *Candida albicans* infection and increases mice survival. *C. albicans* is normally a benign member of the microbiota that colonise the gastrointestinal tract. p38 γ /p38 δ -/- mice exhibit increased fungicidal activity and decreased systemic inflammation. We also have defined a novel Dectin-1 signalling pathway by which p38 γ and p38 δ are essential for ERK pathway activation and contribute to production of inflammatory cytokines in macrophages infected by *C. albicans*. We demonstrated that genetic and chemical inhibition of p38 γ /p38 δ reduce fungal burden in mice, establishing p38 γ /p38 δ as potential therapeutic targets in humans.

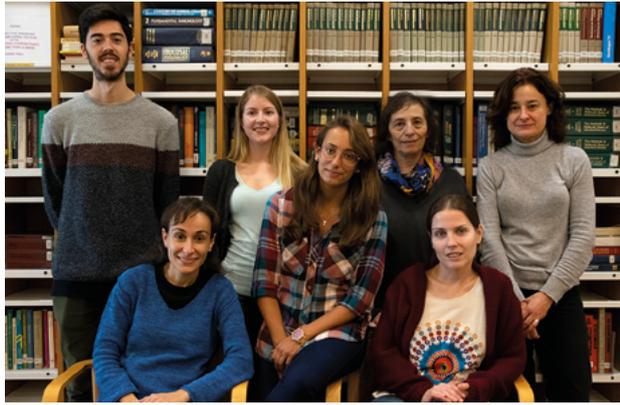
We have also investigated the role of alternative p38MAPK and in the development of immune cells such as T lymphocytes. We found that the T cell differentiation program in thymus was affected at different stages in p38 γ -, p38 δ -, and p38 γ / δ -deficient mice; peripheral T cell homeostasis was also compromised. Particularly, p38 δ deletion affects different stages of early CD4-CD8- double-negative thymocyte development, whereas lack of p38 γ favours thymocyte positive selection from CD4+CD8- double-positive to CD4+ or CD8+ single-positive cells. Our results have identified unreported functions for p38 γ and p38 δ in T cells.



1 Haematoxylin and eosin staining of kidney sections infected with *Candida albicans*, where fungus hyphae are seen as filaments.

2 Schematic representation indicating the different stages of T cell development partially controlled by p38 γ and/or p38 δ .





Physiopathology of chemokine receptor interactions

IMMUNOLOGY & ONCOLOGY 73

GROUP LEADER Leonor Kremer

TECHNICIANS

Mercedes Llorente Gómez
Ana María García Cabrero
María Lozano Alonso

PhD STUDENTS

Isabel Corraliza Gorjón
Beatriz Somovilla Crespo

MASTER'S STUDENTS

Lisa Kochsiek
Sergio Ruiz Saavedra

UNDERGRADUATE STUDENT

Celia López Sanz

SELECTED PUBLICATIONS

Corraliza-Gorjón I, Somovilla-Crespo B, Santamaría S, García-Sanz JA, Kremer L. New strategies using antibody combinations to increase cancer treatment effectiveness. *Front Immunol* 2017; 8: 1804.

Pose-Utrilla J, García-Guerra L, Del Puerto A, Martín A, Jurado-Arjona J, De León-Reyes NS, Gamir-Morralla A, Sebastián-Serrano A, García-Gallo M, Kremer L, Fielit J, Ireson C, Pérez-Álvarez MJ, Ferrer I, Hernández F, Ávila J, Lasa M, Campanero MR, Iglesias T. Excitotoxic inactivation of constitutive oxidative stress detoxification pathway in neurons can be rescued by PKD1. *Nat Commun* 2017; 8: 2275.

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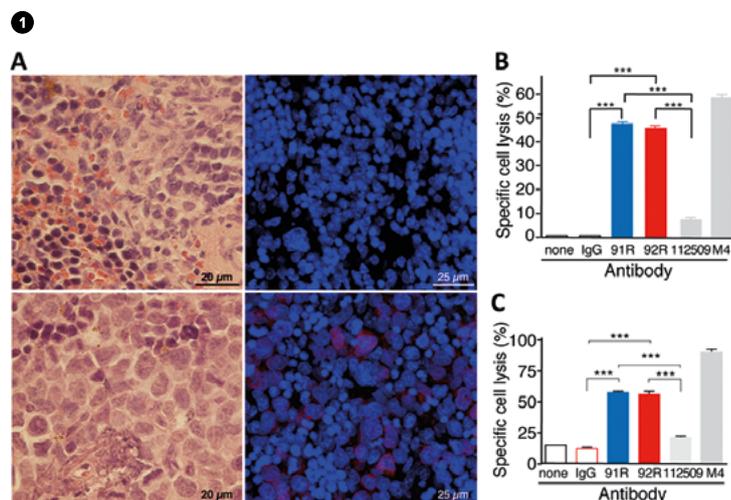
There is an increasing interest in the development of new immunotherapies for cancer treatment. This interest correlates with the therapeutic success obtained using these strategies, in particular with Chimeric Antigen Receptor (CAR) T-cell therapies and antibody-based medicines.

Chemokines and their receptors are key players in cancer biology, where they have relevant roles in tumour progression and metastasis. These proteins modulate tumour-associated angiogenesis and host anti-tumour immunological responses, and stimulate tumour cell survival and proliferation.

Our group studies the physiopathology of chemokine receptors involved in inflammatory diseases and cancer, and is currently focused on the human CCR9 receptor, a seven-transmembrane domain receptor that is highly expressed in a number of different haematological malignancies.

We have generated a panel of CCR9-specific monoclonal antibodies. Two of them were selected based on their effectiveness in reducing the growth of human CCR9+ tumours in immunodeficient mouse models. The results of *in vitro* experiments suggest that these antibodies might eliminate tumour cells through complement- and antibody-dependent cellular cytotoxicity. These antibodies have been licensed to a biopharmaceutical company.

Recent results of our work, in collaboration with Dr. J. A. García-Sanz (CIB-CSIC) and SunRock Biopharma, demonstrated that both the chimeric and humanised variants of these antibodies have the same specificity, affinity and *in vivo* anti-tumour activity as the original antibodies. We also observed that these antibodies strongly inhibit the growth of human CCR9+ leukaemia cell tumours in an immunodeficient NSG mouse model; these mice lack T cells, B cells and have compromised NK and complement activities. These findings support the notion that other mechanisms, including antibody-dependent cellular phagocytosis or direct apoptosis, might also play a role in tumour elimination mediated by these anti-CCR9 antibodies.



1 (A) Haematoxylin and eosin staining (left) and fluorescent immunohistochemical analysis (right) of spleens of NSG mice. Images of structural changes in the spleen an untreated mouse (top) and of a mouse inoculated with CCR9+ human tumour cells stained with red fluorescence (bottom). (B) Specific complement-dependent cytotoxicity and (C) specific NK cell-mediated cytotoxicity of CCR9+ human tumour cells opsonised with anti-CCR9 mAb (91R, 92R or 112509), isotype-matched mAb or positive control M4 serum, evaluated by flow cytometry.



Signalling networks in inflammation and cancer

GROUP LEADER

Santos Mañes

SENIOR SCIENTISTS

Emilia Mira
Rosa Ana Lacalle
Raquel Blanco
Laura del Barrio

POSTDOCTORAL SCIENTISTS

Lorena Carmona-Rodríguez
Jesús Ogando

TECHNICIAN

Rosa M. Peregil

PhD STUDENTS

Ana Martín Leal
Javier Santos

MASTER'S STUDENTS

Carolina Castro Hernández
Diego Martínez Rey

UNDERGRADUATE STUDENTS

Weronika Spaleniak
Raquel Ruiz Palomares
Rita Caracuel
Irati Rincón
Patricia Villalba

SELECTED PUBLICATIONS

Lacalle RA, Blanco R, Carmona-Rodríguez L, Martín-Leal A, Mira E, Mañes S. Chemokine receptor signaling and the hallmarks of cancer. *Int Rev Cell Mol Biol* 2017; 331:181-244.

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Inflammation is a defence response of the organism against internal and external harmful stimuli. Nonetheless, a deregulated inflammatory response can promote cancer and other diseases such as Alzheimer's.

In the 2017-2018 period we worked in the following four areas.

1. Extracellular superoxide dismutase (SOD3) in normalisation of tumour-associated vasculature.

The endothelium is a semipermeable barrier that regulates the transfer of oxygen, endo- and xenobiotics. Progressing tumours are characterised by an exacerbated angiogenesis but, unexpectedly, they are highly hypoxic. We have shown that elevation of extracellular superoxide dismutase (SOD3) in the tumour microenvironment or in perivascular regions normalise the tumour vasculature through a nitric oxide-dependent mechanism. We are now studying how SOD3 regulates tumour infiltration by effector immune cells.

2. Identification signalling pathways associated to PD-1-induced immunosuppression.

The immune system is able to identify and delete neoplastic cells, and blockade of immune checkpoints (such as PD-1) has transformed the clinical practice. Nevertheless, little is known about how PD-1 blocks the effector function in T cells. We have used RNA-seq and bioinformatics to identify the metabolism and mitochondrial structure as new targets of the inhibitory program elicited by PD-1 in CD8+ T cells.

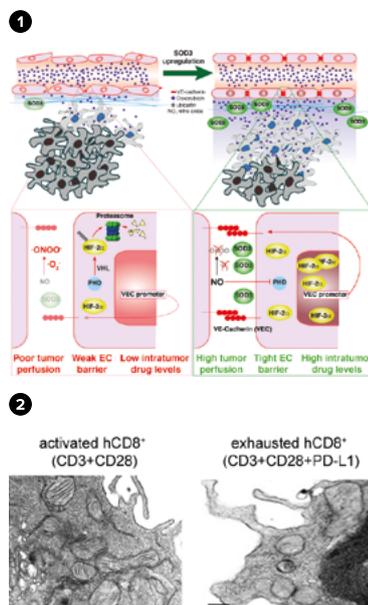
3. CCR5 effects on T-cell receptor (TCR) organisation and the response of memory CD4+ T cells.

The chemokine receptor CCR5 not only work as a chemoattractant receptor for immune cells, but also provides costimulatory signals required for optimal CD4+ T cell activation. We have found that in addition to its role in primary activation of these cells, it regulates the function of CD4+ memory T cells *in vivo*. This activity is associated to changes in the nanoscale organisation of the TCR due to alterations on sphingolipid metabolism.

4. Innate immune cell differentiation in neurological diseases.

Innate immune cells, particularly macrophages, are major conductors of the inflammatory reaction. Depending on their polarisation, macrophages may activate a "healing program", which in the case of cancer, or a "tissue destruction program" as this occurs in inflammatory diseases.

As part of a multidisciplinary European consortium, we are investigating the metabolic changes associated to apolipoprotein E (APOE) epsilon-4 genotype, a variant associated to late-onset Alzheimer's disease, and its influence on monocyte/macrophage/microglia functionality.



1 SOD3 increases the delivery of chemotherapeutics into tumours. tumours express low levels of SOD3, and prolyl hydroxylases (PHD) trigger HIF-2α degradation. SOD3 re-expression prevents nitric oxide (NO) oxidation, which stabilizes HIF-2α by NO-mediated PHD inhibition. HIF-2α then enhances VE-cadherin (VEC) transcription.

2 PD-1 induces structural defects in the mitochondria of human CD8+ T lymphocytes. Magnified mitochondria from human CD8+ T cells activated ex-vivo with anti-CD3 and anti-CD28 antibodies, or with the same anti-CD3 and anti-CD28 antibodies plus the ligand for PD-1 (Fc-PD-L1).



Stem cells and immunity

IMMUNOLOGY & ONCOLOGY 75

GROUP LEADER Carlos Martínez-A

SENIOR SCIENTISTS

Karel HM van Wely
Thierry Fischer
Ricardo Villares
Julio Gutiérrez Hernández
Cristina Pacios Bras
María Ángeles García
Tirso Pons
Jesús de Celis

SENIOR TECHNICIAN

Agnes Fütterer

TECHNICIAN

Ainhoa Sánchez de Diego

PHD STUDENTS

Amaia Talavera Gutiérrez
Fernando Gutiérrez del Burgo
Carmen Mora Gallardo

MASTER'S STUDENT

Marta González Eraso

UNDERGRADUATE STUDENT

José Jiménez Villegas

SELECTED PUBLICATIONS

Fütterer A, de Celis J, Navajas R, Almonacid L, Gutiérrez J, Talavera-Gutiérrez A, Pacios-Bras C, Bernascone I, Martín-Belmonte F, Martínez-A C. DIDO as a switchboard that regulates self-renewal and differentiation in embryonic stem cells. *Stem Cell Rep* 2017; 8: 1062-1075.

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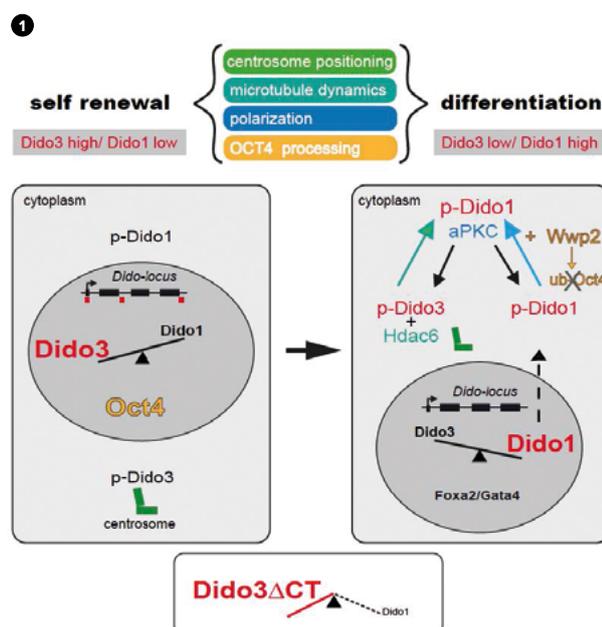
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We work to identify the molecular mechanisms of stem cell renewal and differentiation, and their role in regulating transcription and the cell cycle. Genome-wide screens have identified potential regulators including the *Dido* (death-inducer obliterator) gene, a locus that encodes three proteins generated by alternative splicing. From smallest to largest, *Dido1*, *Dido2*, and *Dido3* have a common N-terminal region with a PHD domain, and isoform-specific C-terminal parts.

Mice carrying the *Dido3* C-terminal truncation (*Dido3 Δ CT*) die at day 8 *post coitum*, and embryonic stem cells (ESC) derived from these mutants retain self-renewal capacity but fail to undergo differentiation, a process rescued by ectopic expression of wild type *DIDO3*. The mechanism (see Figure 1) shows that *Dido3* binds the *Dido* locus through the PHD domain via H3K4me3 and RNA PolII, and induces *DIDO1* expression, necessary for lineage commitment and differentiation into the primitive endoderm [PE]. In addition, *Dido3* must be phosphorylated and translocate to centrosomes, which ensures their correct positioning for PE cell polarisation and maintenance of daughter cell self-renewal capacity. The interesting ability of the *Dido* gene itself to regulate production of the distinct *Dido* isoforms is the subject of further study.

The PHD binding of *Dido* to H3K4me3, an epigenetic marker involved in histone recognition, indicates a possible role of *Dido* in transcription regulation. Using cells from the (*Dido3 Δ NT*) mice lacking the PHD domain, we showed that *Dido* is expelled from the histones during chromatin condensation. To preserve long-term histone trimethylation, adjacent residues are rapidly phosphorylated by mitotic kinases. This process ejects the transcription machinery through steric hindrance, promoting access for cohesins and condensins, and subsequent chromatin compaction. At the end of cell division, dephosphorylation unmasks the prior epigenetic state, allowing for resumption of an unchanged transcription program.



1 *DIDO* act as a switchboard that regulates the genetic programs for Embryonic Stem Cells (ESC) transition from symmetric to asymmetric cell division. *DIDO3*, binds the *Dido* locus via H3K4me3 and RNA POL II and induces *DIDO1* expression. *DIDO1*, which is exported to cytoplasm, associates with, and is N-terminally phosphorylated by PKC α . It binds the E3 ubiquitin ligase WWP2, which contributes to cell fate by OCT4 degradation, to allow expression of primitive endoderm (PE) markers. PE formation also depends on phosphorylated *DIDO3* localisation to centrosomes, which ensures their correct positioning for PE cell polarisation.



Chemokine receptors: new targets for therapeutic intervention

76 IMMUNOLOGY & ONCOLOGY

GROUP LEADER

Mario Mellado

SENIOR SCIENTISTS

José Miguel Rodríguez Frade
Ricardo Villares

POSTDOCTORAL SCIENTISTS

Eva M^o García Cuesta
Blanca Soler Palacios

TECHNICIANS

Pilar Lucas
Nuria Andrés

PhD STUDENTS

Pablo Martínez
Sofía Gardeta

SELECTED PUBLICATIONS

Collins PJ, McCully ML, Martínez-Muñoz L, Santiago C, Wheelodon J, *et al.* Epithelial chemokine CXCL12 synergizes with CXCL12 via allosteric modulation of CXCR4. *FASEB J* 2017; 31: 3084-3097.

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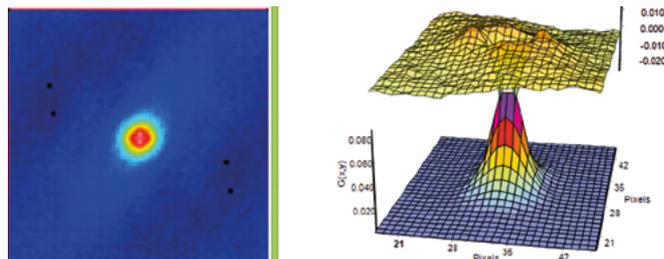
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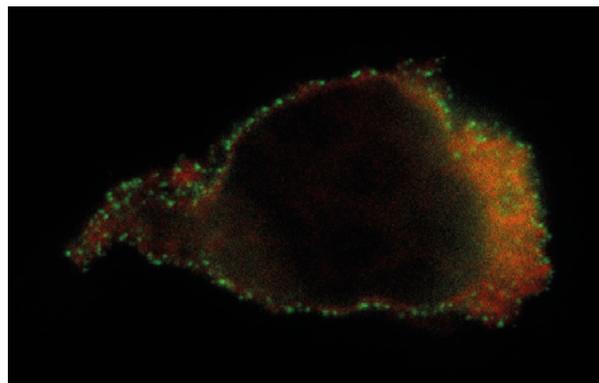
A broad array of biological responses including cell polarisation, movement, immune and inflammatory responses, cancer metastasis and prevention of HIV-1 infection are triggered by the chemokines, a family of secreted chemoattractant proteins that bind to class A-specific G protein-linked seven-transmembrane receptors.

In the last quarter century, the field has accumulated much information regarding the implications of these molecules in different immune processes, as well as mechanistic insight into the signalling events activated through their binding to their receptors. Today, we know that chemokine receptors must not only be considered isolated entities that are activated following ligand binding; rather, they are found as dimers and/or higher order oligomers at the cell surface, even in the absence of ligands. These complexes form organised arrays that can be modified by receptor expression and ligand levels, indicating that they are dynamic structures. The way in which these receptor complexes are stabilised modulates ligand binding as well as their pharmacological properties and the signalling events activated. These conformations thus represent a mechanism that increases the broad variety of chemokine functions. However, in the last five years, the use of new biophysical approaches, i.e. super-resolution microscopy and total internal reflection microscopy that allow precise analysis of protein–protein interactions in living cells, are revealing an unanticipated level of complexity among chemokine receptors at the cell surface. The dynamic interactions between these receptors, as well as their interplay with other proteins co-expressed by the cells, lipids that form the cell membrane, the cytoskeleton, and downstream signalling machinery will be crucial for defining the context-specific functions triggered. This new information is transforming our working model of chemokine-associated functions and allows us to identify new targets and to devise innovative pharmacological therapies to modulate certain cell activities without affecting others.

1



2



1 Analysis of lipid diffusion in Jurkat cells using the RICS (Raster Image Correlation Spectroscopy) approach.

2 Analysis by STED (Stimulated Emission Depletion) microscopy of a polarized primary CD4⁺ T cell. (Green: human CXCR4; Red: Phalloidin)



Diacylglycerol kinases in the control of immune response and cancer progression

GROUP LEADER

Isabel Mérida

SENIOR SCIENTIST

Juana Antonia Ávila-Flores

TECHNICIANS

Rosa Mª García Liébana
Alejandra Cordero Cantos

ADMINISTRATION

Raquel Arcos Pérez

PhD STUDENTS

María Tello-Lafoz
Elena Andrada Romero
Javier Arranz-Nicolás
Cristina Rodríguez Rodríguez
Natalia González Mancha
Miguel Martín Salgado

MASTER'S STUDENTS

Daniel Meraviglia
Marta Marina
Laura Vicente
Isabel Wang

UNDERGRADUATE STUDENTS

Luis González Valle
Tania Vaiolopolou

SELECTED PUBLICATIONS

Tello-Lafoz M, Rodríguez-Rodríguez C, *et al.* SNX27 links DGKζ to the control of transcriptional and metabolic programs in T lymphocytes. *Sci Rep* 2017; 7: 16361.

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Arranz-Nicolás J, Ogando J, *et al.* Diacylglycerol kinase α inactivation is an integral component of the costimulatory pathway that amplifies TCR signals. *Cancer Immunol Immunother* 2018; 67: 965-980.

The Diacylglycerol Kinase (DGK) family of lipid kinases regulate the conversion of Diacylglycerol (DAG) into phosphatidic acid (PA). Altered DAG/PA homeostasis resulting from DGK malfunction causes several human diseases (Figure 1). In T lymphocytes, DGKs limit DAG-dependent activation of effector functions. We work to get a better understanding of the mechanisms underlying DGK regulation in T cells so that steps of the process can be manipulated for therapeutic benefit.

1. DGK and cancer

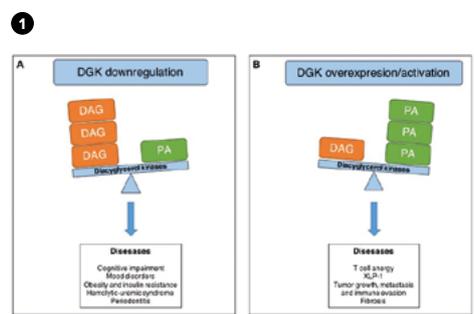
Overcoming the hypofunctional state imposed by solid tumours to T cells has become a critical strategy in the fight against cancer. Clinical progress is challenging due to the complex strategies that tumours employ to evade the immune system. Our group works to demonstrate that targeting specific DGK isoforms represents a novel and understudied strategy to manipulate antitumoural immune responses (Figure 2).

2. DGK and aplastic anaemia

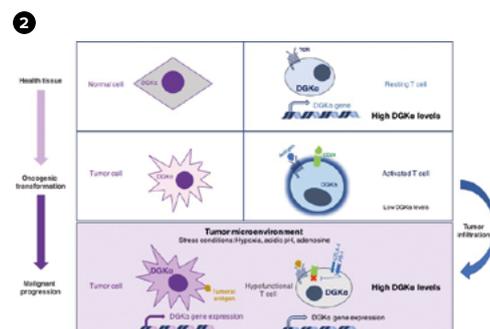
Aplastic anaemia (AA) is a disease in which the bone marrow gradually stops producing cells. In most cases, AA results from spontaneous T cell attack to bone marrow cells. We recently discovered that deficiency of specific DGK isoforms facilitates T cell activation in the bone marrow. We are working to better understand whether DGK malfunction may contribute to AA triggering.

3. DGK and Alzheimer's disease in Down Syndrome

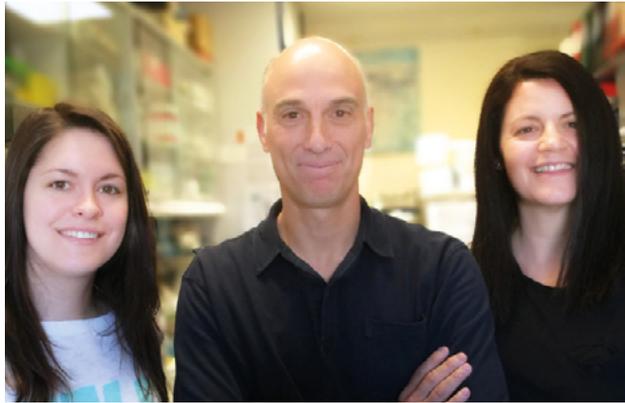
Down syndrome (DS), the most prevalent chromosomal disorder, results in mild cognitive impairment, high frequency of infections and elevated risk of leukaemia and autoimmune diseases. Virtually all DS people develop Alzheimer's disease (AD) by their 40s and at least 70% develop dementia. Notably, people with DS are protected from solid tumours. We recently identified SNX27, a protein that is diminished in DS, as a DGKζ partner and have demonstrated that SNX27/DGKζ interaction contributes to the control of T cell responses. We work to investigate whether reduced SNX27 expression, due to trisomy 21, favours immune disorders that may contribute to DS associated pathologies.



1 General model of DGK contribution to human disease. (A) Deficiency of selected DGK isoforms results in an excess of DAG that impairs synaptic transmission, favours insulin resistance and triggers abnormal platelet or neutrophil activation. (B) Upregulation of DGK isoforms limits T and NK cytotoxic function and contributes to oncogenic transformation.



2 High DGKζ expression in tumours and tumour infiltrating T cells. Expression of DGKζ decrease in activated T lymphocytes and increases during oncogenic transformation. Stress conditions lead to enhanced expression of DGKζ in tumour and tumour infiltrating T cells. These cells become non functional since TCR and costimulatory signals are blocked by the high expression of immune checkpoints such as CTLA-4 and PD-1. This limits the T cell response to cancer cells and leads to tumour persistence.



Transcriptional control of B lymphocyte differentiation

78 IMMUNOLOGY & ONCOLOGY

GROUP LEADER

Ignacio Moreno de Alborán

POSTDOCTORAL SCIENTIST

María Alfonsina Trento

PhD STUDENT

Mercedes Pérez-Olivares

MASTER'S STUDENT

Patricia Rico

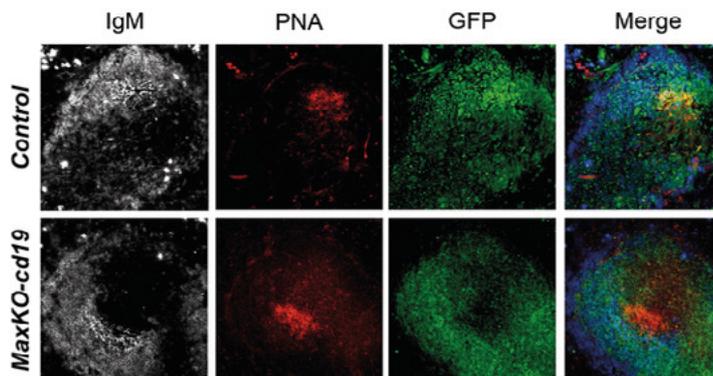
SELECTED PUBLICATIONS

Pérez-Olivares M, Trento A, Rodríguez-Acebes S, González-Acosta D, Fernández-Antorán D, Román-García S, Martínez D, López-Briones T, Torroja C, Carrasco YR, Méndez J, Moreno de Alborán I. Functional interplay between c-Myc and Max in B lymphocyte differentiation. *EMBO Rep* 2018; 19: e45770.

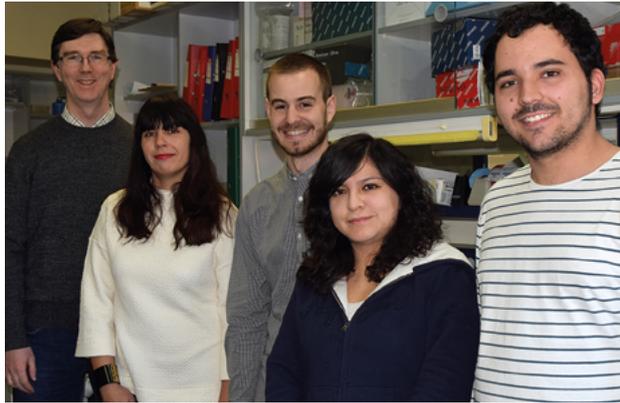
B lymphocytes are essential cellular components of the immune response. They undergo a differentiation process in the bone marrow and in secondary lymphoid organs in which a number of transcription factors play a prominent role. Our general biological question is to understand the transcriptional program that governs this process. Among the spectrum of transcription factors involved, we focused our attention on the function of the proto-oncogene *c-myc* for two reasons. First, the c-Myc protein is a member of the Myc family (N-, L- and c-Myc) of transcription factors involved in numerous biological functions, including the regulation of cell proliferation, differentiation and apoptosis in various cell types. This pleiotropic function confers this protein an essential and distinct role at different differentiation stages in numerous cell types. Second, in animal models and humans, deregulated c-Myc expression leads to the development of tumours, including B and T lymphomas. This oncogenic potential provides an interesting dimension in terms of possible therapeutic applications of our research.

The Myc proteins contain a basic region/helix-loop-helix/leucine zipper domain that mediates DNA binding and heterodimerisation with its mandatory partner Max. It is generally assumed by the majority of scientific reports that, in order to activate or repress target genes, Myc proteins must heterodimerise with Max and bind to specific regulatory regions. However, no definitive data have addressed the role of this Myc/Max interplay *in vivo*. Previous data from our group showed that Myc/Max functional collaboration in B lymphocytes is more complex than initially anticipated. In our lab, we are currently interested in the study of the functional relationship between Myc and Max in physiological and pathological scenarios *in vivo*. For this purpose, we have generated new and complex genetically modified mouse models that specifically allow to address these questions. Due to the central role of Max, we expect that our results will have a relevant impact on the current knowledge of Myc biology.

1



1 Analysis of Germinal Centre (GC) formation in the spleens of *MaxKO-cd19* and heterozygous control mice immunised with TNP-KLH. Representative images of frozen spleen sections stained with IgM (grey/blue), PNA (GC marker; red), and GFP (*Max*-deficient B cells; green).



Receptor ligand interactions in immune responses to cancer and viruses

GROUP LEADER

Hugh T. Reyburn

POSTDOCTORAL SCIENTIST

Daniela Dukovska

TECHNICIAN

Ruth Gómez Caro Gil

PHD STUDENTS

Alfonso Blázquez Moreno
Adriana Pérez Portilla

MASTER'S STUDENTS

Daniel Fernández Soto
Ane Calvo Apalategui

UNDERGRADUATE STUDENT

Jesús Cantoral

VISITING SCIENTIST

Sedaminou Judith Gbenodoun
(Fellow of the Woman in Science Foundation, Institute for Applied Biomedical Sciences, Cotonou, Republic of Benin)

SELECTED PUBLICATIONS

Esteso G, Guerra S, Valés-Gómez M, Reyburn HT. Innate immune recognition of double-stranded RNA triggers increased expression of NKG2D ligands after virus infection. *J Biol Chem* 2017; 292: 20472-20480.

Blázquez-Moreno A, Pérez-Portilla A, Agúndez-Llaca M, Dukovska D, Valés-Gómez M, Aydogmus C, Ikinociogullari A, Regueiro JR, Reyburn HT. Analysis of the recovery of CD247 expression in a PID patient: insights into the spontaneous repair of defective genes. *Blood* 2017; 130: 1205-1208.

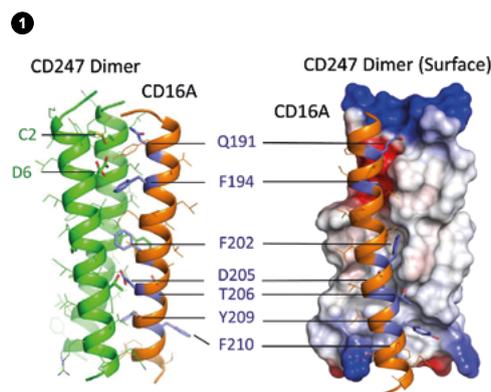
Blázquez-Moreno A, Park S, Im W, Call MJ, Call ME, Reyburn HT. Transmembrane features governing Fc receptor CD16A assembly with CD16A signaling adaptor molecules. *Proc Natl Acad Sci USA* 2017; 114: E5645-E5654.

Bravo García-Morato M *et al.* New human combined immunodeficiency caused by interferon regulatory factor 4 (IRF4) deficiency inherited by uniparental isodisomy. *J Allergy Clin Immunol* 2018; 141: 1924-1927.

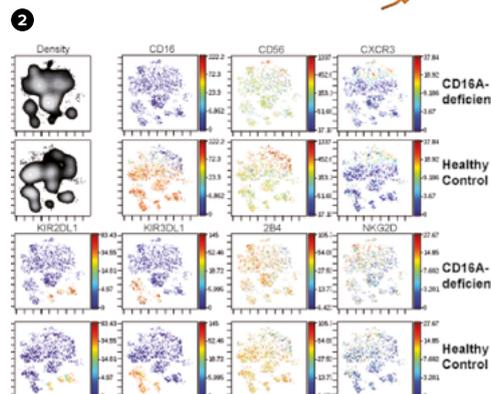
Dukovska D, Fernández-Soto D, Valés-Gómez M, Reyburn HT. NKG2H-expressing T cells negatively regulate immune responses. *Front Immunol* 2018; 9:390.

Natural killer (NK) cells kill infected cells and secrete cytokines, to play an important role in defence against viral infection. Although NK cells are often perceived as rather primitive lymphocytes; always ready to kill unless checked by inhibitory receptors binding to MHC Class I molecules. It is now clear that the behaviour of an NK cell when confronted by a potential target cell depends on the integration of multiple signals coming from a range of activating and inhibitory receptors. Inhibitory receptor expression is largely under genetic control, whereas activation receptor expression is heavily environmentally influenced, and NK cells adapt their expression of activating receptors in response to pathogens and tumours so giving rise to the multiple discrete NK cell subpopulations that can be found in human peripheral blood. Thus, to understand NK cells in disease requires detailed knowledge of the biochemistry of individual activating and inhibitory receptors and the subpopulations of NK cells expressing different receptor repertoires.

We have contributed extensively to the knowledge of the cell biology of various NK cell receptors and their ligands. Recently, to address the wider roles of NK cells in immunity, we have initiated collaborations with clinical colleagues to study patients suffering from primary immunodeficiencies that affect NK cell function. Inherited human immunodeficiencies are experiments of nature in which gene defects compromise immune function, and our hypothesis is that the study of congenital defects affecting NK cells will help to increase our understanding of NK cell biology and function *in vivo*. We use innovative flow cytometry and molecular genetic technologies to characterise these primary immunodeficiency diseases at high resolution. These studies are complemented and enhanced by *in vitro* experiments involving the study of NK cells and the use of genome-editing technologies to investigate in detail the molecular bases of the changes observed *in vivo*.



1 Model of CD16A assembled with CD247 dimer. The key residues for this interaction, identified in experiments of site-directed mutagenesis, are highlighted.



2 ViSNE plots of NK cells from a healthy control and a CD16A-deficient patient. Cryopreserved PBMC were thawed, stained with a panel of 45 mAbs and analysed by mass cytometry. ViSNE plots for the indicated NK receptors are shown. Density plots are shown in grayscale, and visualise cell quantities in each area of the plot. Expression plots are represented in a colour scale, from blue (low) to red (high).



T cell signalling in autoimmune diseases and cancer

80 IMMUNOLOGY & ONCOLOGY

GROUP LEADER

Jesús María Salvador

POSTDOCTORAL SCIENTIST

María Salvador-Bernáldez

PHD STUDENTS

Umberto Rosato

MASTER'S STUDENTS

Adrián Méndez Carreira
Nicolás de Miguel Rubio

UNDERGRADUATE STUDENT

Daniel Díaz Rodríguez

VISITING SCIENTIST

Christian Duarte Varela

SELECTED PUBLICATIONS

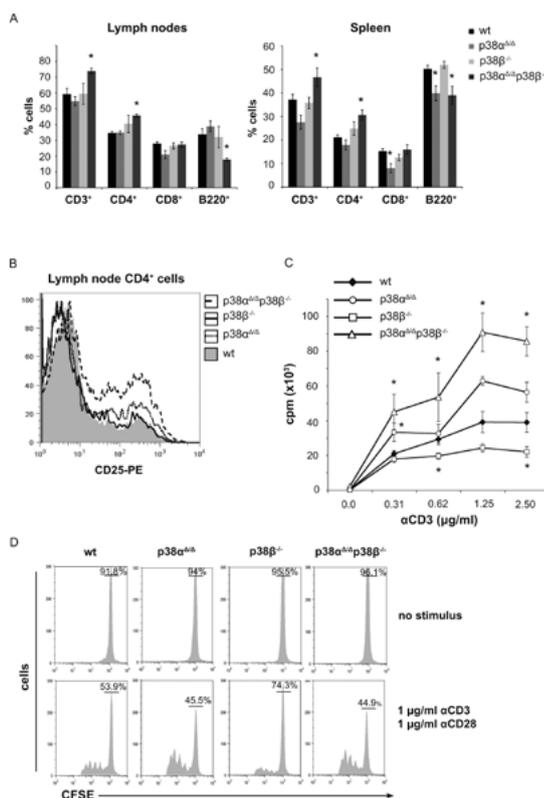
Salvador-Bernáldez M, Mateus SB, Del Barco Barrantes I, Arthur SC, Martínez-A C, Nebreda AR, Salvador JM. p38 α regulates cytokine-induced IFN γ secretion via the Mnk1/eIF4E pathway in Th1 cells. *Immunol Cell Biol* 2017, 95: 814-823.

Herrero D, Cañon S, Pelacho B, Salvador-Bernáldez M, Aguilar S, Pogontike C, Carmona RM, Salvador JM, Pérez-Pomares JM, Klein OD, Prósper F, Jiménez-Borreguero LJ and Bernad A. Bmi1+ progenitor cell ablation impairs the angiogenic response to myocardial infarction. *Arterioscler Thromb Vasc Biol* 2018, 38: 2160-2173.

Our group is focused on the identification and characterisation of the molecular mechanisms that regulate T cell functions involved in the development of autoimmune diseases and cancer. p38 MAPKs pathways have a critical role in the regulation of the immune response and inflammatory processes. The precise function of p38 α and p38 β in T cell proliferation and cytokine production nonetheless remains controversial, because it has been addressed mostly using chemical inhibitors. To dissect p38 α and p38 β functions in T cells, we have characterised mice deficient for each isoform. Since p38 α -deficient mice are not viable, we used a conditional knockout mouse model to analyse p38 α function in CD4 $^+$ T cells; in addition, we characterised mice lacking p38 β , and generated double-knockout mice. Notably, our results indicate that p38 α and p38 β have distinct regulatory roles in CD4 $^+$ T cell proliferation: p38 α is a negative regulator, whereas p38 β plays the opposite function.

We have analysed the role of p38 α and p38 β in Th1 and Th2 effector function as well as cytokine production. Our results demonstrate that p38 α and p38 β are essential for normal Th1, but not for Th2 effector function. p38 α and p38 β control T cell receptor-induced IFN γ and TNF α production, but only p38 α modulates cytokine-induced IFN γ production. Our findings demonstrate that p38 α , but not p38 β , controls IFN γ production through the activation of the Mnk1/eIF4E pathway of translation initiation in T cells.

These findings could be useful in generating new anti-inflammatory treatments. Our data indicate that selective inhibition of p38 α activity is not sufficient to block production of proinflammatory cytokines, and that combined inhibition of p38 α and p38 β should be considered for targeting the inflammatory response in autoimmune diseases.



1 Opposite roles of p38 α and p38 β in CD4 $^+$ T cell proliferation. (A) Percentage of lymph node and spleen cells showing increased CD4 $^+$ T cell population in p38 α -/- p38 β -/- mice. (B) Representative histogram overlay of CD25 expression in CD4 $^+$ T cells from wt, p38 α -/-, p38 β -/- and p38 α -/-p38 β -/- mice. (C) Compared to wt cells, p38 α -/- and p38 α -/-p38 β -/- CD4 $^+$ T cells showed more rapid proliferation in response to TCR activation. (D) CD4 $^+$ T cells from wt, p38 α -/-, p38 β -/- and p38 α -/-p38 β -/- mice were activated with anti-CD3 and anti-CD28 antibody and the proliferative response was monitored by CFSE staining.



Tumour immune activation and evasion

IMMUNOLOGY & ONCOLOGY 81

GROUP LEADER

Mar Valés Gómez

POSTDOCTORAL SCIENTIST

Gloria Esteso Tornero

PHD STUDENTS

Sheila López Cobo
Carmen Campos Silva
Maren K. Kramer

UNDERGRADUATE STUDENTS

Eva Escudero
Basilio Campillo
Lucía Téllez

VISITING SCIENTIST

Adrián Fernández Martín

SELECTED PUBLICATIONS

López-Cobo S, Pieper N, Campos-Silva C, García-Cuesta EM, Reyburn HT, Paschen A and Valés-Gómez M. Impaired NK cell recognition of vemurafenib-treated melanoma cells is overcome by simultaneous application of histone deacetylase inhibitors. *Oncoimmunology* 2017; 7: e1392426.

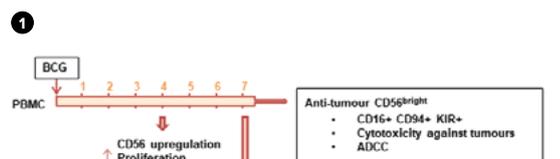
García-Cuesta EM, Esteso G, Ashiru O, López-Cobo S, Álvarez-Maestro M, Linares A, Ho M, Martínez-Piñeiro L, Reyburn HT and Valés-Gómez M. Characterisation of a human anti-tumoral NK cell population expanded after BCG treatment of leukocytes. *Oncoimmunology* 2017; 6: e1293212.

S López-Cobo, C Campos-Silva, A Moyano, M Oliveira-Rodríguez, A Paschen, M Yáñez-Mó, MC Blanco-López, M. Valés-Gómez. Immunoassays for scarce tumour-antigens in exosomes: detection of the human NKG2D-Ligand, MICA, in tetraspanin-containing nanovesicles from melanoma. *J Nanobiotechnology* 2018; 16: 47.

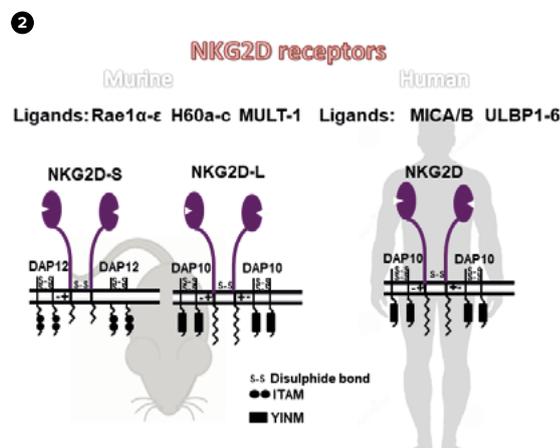
Campos-Silva C., Kramer MK, Vales-Gomez M. NKG2D-ligands: Putting everything under the same umbrella can be misleading. *HLA* 2018; 91: 489-500.

The group is interested in cancer immunity mediated by Natural Killer (NK) cells. These studies pose many difficulties, because of the complexity of the response, arising from the large number of cell subtypes and soluble factors that can be recruited to the tumour environment. NK cells can be affected by tumour recognition or evasion events and, thus, directly contribute to the outcome of the immune interaction. We use the treatment of bladder cancer patients with intra-vesical instillations of BCG as a model for the study of the stimulation of immune cells to eliminate tumours. In this context, *in vitro* experiments involving the culture of PBMCs with non-pathogenic Mycobacteria have shown that, after stimulation with BCG, a subpopulation of CD56^{bright} NK cells expands and acquires the ability to recognise tumour cells, including bladder cancer. CD56^{bright} cells were defined in other systems as a subpopulation of immature NK cells, usually with a high ability to secrete cytokines. However, the subpopulation that we have discovered derives from CD56^{dim} cells and mediates cytotoxic activity due to the presence of other NK receptors.

We have also described that, in melanoma, NKG2D-mediated immune modulation can occur in the context of therapies directed to proliferation pathways, such as the activation of BRAF. This could represent a mechanism of immune evasion for therapies directed against the MAPK route. Since NKG2D-ligands can be released as soluble molecules or in extracellular vesicles, the consequence of their modulation in the context of BRAF inhibitors could be followed analysing patient plasma. To investigate this idea, we have developed techniques for the study of NKG2D-ligands in extracellular vesicles.



1 Immune activation during one week of co-incubation of PBMCs with BCG. PBMCs from healthy donors incubated with BCG provoke the expansion of an anti-tumour NK cell population characterised by the upregulation of CD56 and CD16. This CD56^{bright} NK population has an increased capacity to eliminate different bladder cancer cell lines by cytotoxic degranulation. The activation depends on cytokine release, presumably by other immune cell subtypes.



2 Murine and human NKG2D receptors and their ligands: different things under the same umbrella. Mouse NKG2D (left) exists as two different splicing forms and can trigger different signalling routes. Human NKG2D only associates with DAP10. Many different proteins bind to human NKG2D: the genes for MICA and MICB are highly polymorphic and ULBPs also have different variants (modified from Campos-Silva, Kramer and Valés-Gómez, *HLA* 2018).

One of the transformative novelties in Life Sciences research of the last couple of decades is the onset and growth of the so-called Systems Biology, which brings about a radical change in the way we address biological problems. Although molecular biology has been traditionally considered to have been founded by physicists, this circumstance did not result in a quantitative culture and an accurate, standardised descriptive language characteristic of the hard sciences. On the contrary, with very few exceptions, the biosciences that developed since that time seldom took the opportunity to formalise the mechanisms and functions of living systems with accurate languages and codes. Systems Biology occupies this niche by analysing biological entities as comprehensible physicochemical objects with a functioning and relational logic that can be modelled, understood and reshaped.

By the same token, Synthetic Biology is not just a contemporary update of the recombinant DNA technologies of the past 30 years, along with a descriptive language imported from electrical and industrial engineering. It is also a new interpretive key for living systems as well as a declaration of intent on the use and reprogramming of biological objects for human benefit. In the same way that scientific chemistry as initiated by Lavoisier evolved into the chemical engineering that is the basis of our industrial society, biology has acquired a transforming potential that could lead to a type of industry and economy very different of the current paradigm.

The CNB SysBio Program maps in the contemporary landscape of the field by developing active research lines in environmental genomics, network biology, systemic computation and metabolic engineering. This frame (which many consider to be a veritable paradigm change) seeks to address the complexity of living systems as such, not to divide them into smaller parts—unlike the traditional reductionism of Molecular Biology. The scientific and technological potential of Systems and Synthetic Biology is immense, both in the field of Biomedicine and Industrial, Agricultural and Environmental Biotechnology.

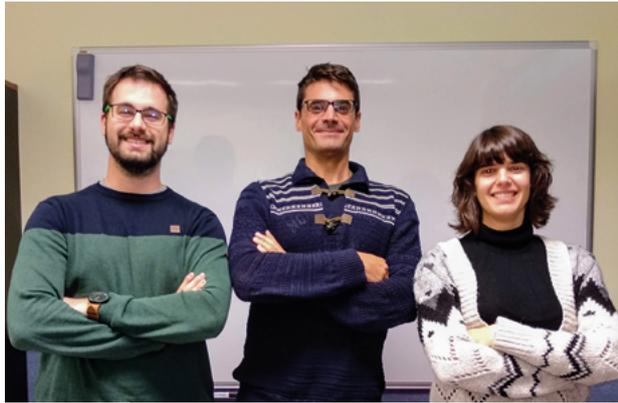
Systems Biology

HEAD OF DEPARTMENT

Víctor de Lorenzo

RESEARCH GROUPS

- 1. Clocks and rulers in life**
Saúl Ares
- 2. Molecular environmental microbiology**
Víctor de Lorenzo
- 3. Evolutionary systems**
Susanna Manrubia
- 4. Systems biotechnology**
Juan Nogales
- 5. Computational systems biology**
Florencio Pazos Cabaleiro
- 6. Logic of genomic systems**
Juan F. Poyatos
- 7. Microbiome analysis**
Javier Tamames & Carlos Pedrós



Clocks and rulers in life

GROUP LEADER

Saúl Ares

PhD STUDENT

Pau Casanova

MASTER'S STUDENTS

Christian Camilo Cortés
Gabriel Rodríguez Maroto

SELECTED PUBLICATIONS

Val-Calvo J, Luque-Ortega JR, Crespo I, Miguel-Arribas A, Abia D, Sánchez-Hevia DL, Serrano E, Gago-Córdoba C, Ares S, Alfonso C, Rojo F, Wu LJ, Boer DR and Meijer WJJ. Novel regulatory mechanism of establishment genes of conjugative plasmids, *Nucleic Acids Res* 2018; 46: 11910-11926.

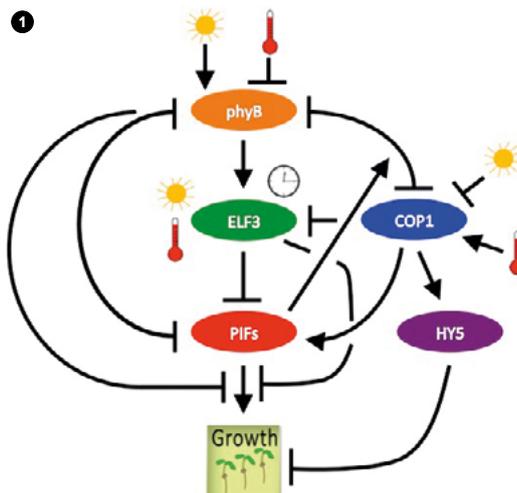
We are interested in spatiotemporal phenomena in living systems: oscillations, pattern formation and dynamics of gene expression. We work in collaboration with experimentalists to build theories of these spatiotemporal phenomena, using ideas from physics and mathematics to build models and computer simulations that help us understand the nature of the interactions underlying the dynamics of life.

We pursue this goal on a variety of research lines. Cyanobacteria are important organisms for the environment for their photosynthetic activity and their capacity to fix nitrogen into chemical forms usable for other life forms. They are also of biotechnological interest as the source of fertilisers or biofuels. We study how filamentous cyanobacteria differentiate into nitrogen-fixing cells called heterocysts, and how the patterns of heterocysts on the filament are formed.

The ability of plants to sense light and temperature allows them to tune their growth to environmental conditions. In a context of global climate change and endangered crops, it is important to understand how this occurs. In collaboration with the group of Salomé Prat in the Department of Plant Molecular Genetics, we formulate mathematical models that help to understand what are the key molecular factors to the response of plants in light and temperature.

Bacterial resistance to antibiotics is becoming a major health hazard. One of the ways in which this resistance can spread is through bacterial conjugation, a process through which bacterial cells can share pieces of DNA. We work together with the lab of Wilfried Meijer at CBMSO to understand the dynamics and regulation of conjugation in Gram-positive bacteria.

Finally, embryos are constantly growing and reshaping, differentiating new cell types and organs. Developmental biology is always an interest of the lab, and the problem of how the body of vertebrate embryos is segmented is especially close to our hearts.



1 Regulatory logic of the control of hypocotyl growth in the model plant *Arabidopsis thaliana*. Suns and thermometers represent effects of light and temperature, respectively.



Molecular environmental microbiology

SYSTEMS BIOLOGY PROGRAMME 85

GROUP LEADER

Víctor de Lorenzo

SENIOR SCIENTISTS

Belén Calles
Esteban Martínez

POSTDOCTORAL SCIENTISTS

Yamal Al-Ramahi
David Rodríguez Espeso
Tomás Aparicio
Pavel Dvorak

TECHNICIANS

Sofía Fraile
Elena Algar

LAB EXECUTIVE ASSISTANT

Inés Merino

PHD STUDENTS

Mª de los Ángeles Hueso Gil
Huseyin Tas
Elena Velázquez

VISITING SCIENTISTS

Joseph Mougous
(Washington University, USA)
Hiren Joshi
(WSCD, India)

VISITING STUDENTS

Estela Ricaurte
Jillian Silbert
Ananda Sanches
Hannes Löwe
Coral García Gutiérrez

SELECTED PUBLICATIONS

Dvořák P, *et al.* Bioremediation 3.0: Engineering pollutant-removing bacteria in the times of systemic biology. *Biotechnol Adv* 2017; 35: 845-866.

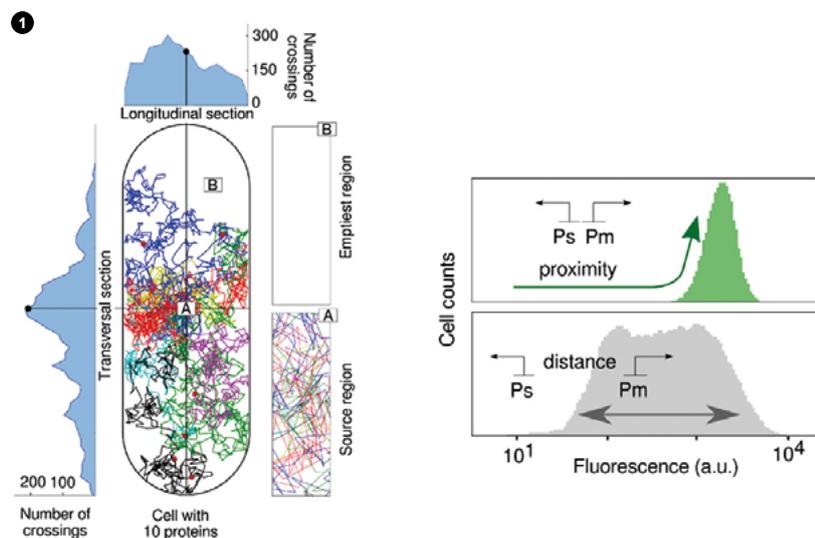
Goñi-Moreno A, *et al.* Deconvolution of gene expression noise into spatial dynamics of transcription factor-promoter interplay. *ACS Synth Biol* 2017; 6: 1359-1369.

Akkaya O, *et al.* The metabolic redox regime of *Pseudomonas putida* tunes its evolvability towards novel xenobiotic substrates. *mBio* 2018; 9: e01512-18

Dvorak P, de Lorenzo V. Refactoring the upper sugar metabolism of *Pseudomonas putida* for co-utilization of cellobiose, xylose, and glucose. *Metab Eng* 2018; 48: 94-108

de Lorenzo V. *et al.* The power of synthetic biology for bioproduction, remediation and pollution control. *EMBO Rep* 2018; 19: e45658.

The longstanding mission our team is the production of biological agents for biosensing, remediation and (wherever possible) valorisation of chemical waste that is otherwise dumped into the Environment by urban and industrial activities. The workhorse to this end is the soil bacterium *Pseudomonas putida*, which combines the ease of genetic programming that is typical of *Escherichia coli* with the safety, robustness and metabolic capabilities required in whole-cell catalysts for applications in harsh biotechnological settings. Specific activities include: [i] Development of *P. putida* as a reliable chassis for implantation of genetic and metabolic circuits. This involves a profound editing of the extant genome of this microorganism for enhancing desirable properties and eliminating drawbacks. Also, the exploitation of surface-display systems for designing complex catalytic properties altogether separated from the cell metabolism and even the design of artificial communities by means of ectopic adhesins. [ii] Genetic tools for deep refactoring of metabolic properties of *P. putida*. The list of new assets that we are developing includes a large collection of standardised plasmid and transposon vectors as well as dedicated reporter systems for parameterisation of the gene expression flow and for switching entire metabolic regimes. [iii] The TOL system borne by plasmid pWWO as a reference for metabolic circuit implantation. The two operons for toluene and m-xylene biodegradation encoded in pWWO offer a natural case of expansion of the metabolic repertoire of environmental bacteria through acquisition of new genes. [iv] Deep metabolic engineering of *P. putida*. Currents efforts attempt to develop strains that can be entirely programmed to deliver catalytic phenotypes of choice upon exposure and computation of both external and internal cues. This endeavour combines direct rational engineering with fine-tuning of gene expression by means of site-specific diversification of genomic sequences of choice through adaptation to *P. putida* of multiple automated genome engineering (MAGE) technology.



1 Gene expression noise detected as changes in the fluorescent output of individual cells bearing transcriptional fusions to the promoters of interest reflects the distance and the intracellular microgranularity that cognate transcription factors have to go through the cytoplasm to find its target sequence (see Goñi-Moreno *et al.* 2017 for details).



Evolutionary systems

GROUP LEADER

Susanna Manrubia

SENIOR SCIENTIST

Jacobo Aguirre

POSTDOCTORAL SCIENTIST

Juan Antonio García-Martín

PhD STUDENTS

Adriana Lucía-Sanz
Pilar Somovilla

MASTER'S STUDENTS

Oscar Gómez Balaguer
Sergio Javier Avero Rodríguez
Luis Marchante

VISITING STUDENTS

Víctor Buendía
María Caro

SELECTED PUBLICATIONS

Lucía-Sanz A, Manrubia S. Multipartite viruses: adaptive trick or evolutionary treat? NPJ Syst Biol Appl 2017; 3: 34.

Yubero P, Manrubia S, Aguirre J. The space of genotypes is a network of networks: implications for evolutionary and extinction dynamics. Sci Rep 2017; 7: 13813.

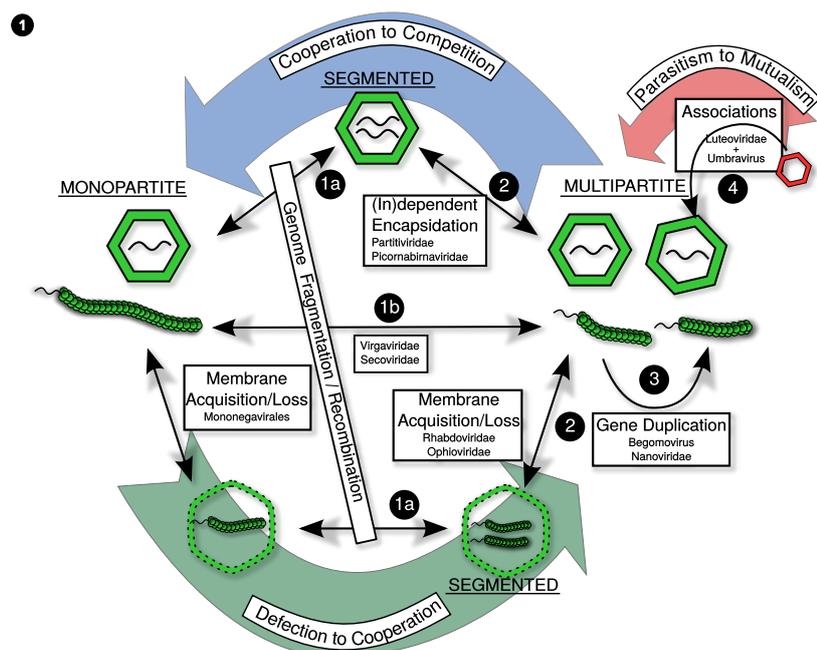
Iranzo J, Cuesta JA, Manrubia S, Katsnelson M, Koonin EV. Disentangling the effects of selection and loss bias on gene dynamics. Proc Natl Acad Sci USA 2017; 114: E5616-E5624.

Lucía-Sanz A, Aguirre J, Manrubia S. Theoretical approaches to disclosing the emergence and adaptive advantages of multipartite viruses. Curr Opin Virol 2018; 33: 89-95.

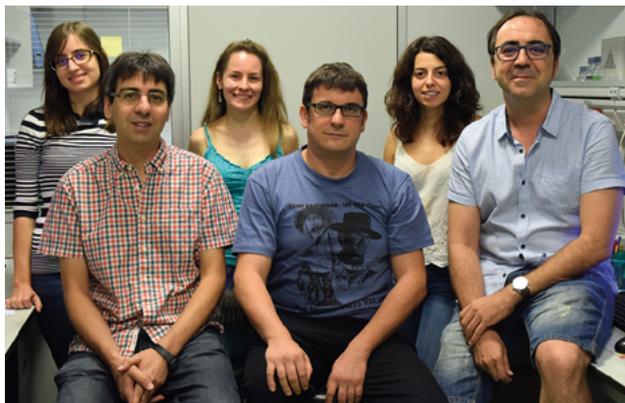
Aguirre J, Catalán P, Cuesta JA, Manrubia S. On the networked architecture of genotype spaces and its critical effects on molecular evolution. Open Biol 2018; 8: 180069.

The main interest of the group is the theoretical investigation of evolutionary systems of different kinds. We develop models inspired by the phenomenology observed in natural systems, chiefly molecular populations, viruses, and interacting agents from the cellular level and up. Our approach addresses either the study of fundamental properties of adapting systems—with a strong emphasis on their evolutionary origin—or, at a more specific level, tries to reproduce and eventually predict the response of such populations to endogenous and exogenous change. In this context, we investigate the properties of the genotype-phenotype map through models such as the folded state of RNA sequences, focusing on the topological structure of neutral networks of genotypes and its relevance in adaptation and molecular innovation. Another main subject is the understanding of the survival strategies of viruses, among others the relevance of multipartite genomes or the ecological effect of viral satellites.

At a higher organisational level, we are also interested in the modelisation of the interaction between agents organised in networks that vie e.g. for resources, food, or mates, as competitive interactions represent one of the driving forces behind evolution and natural selection in biological systems. Finally, we explore the application of complex systems to biotechnology through the development of analysis techniques with environmental and health purposes. We have applied graph theory to antibody microarrays in order to improve the characterisation of experimental samples, with direct application to allergy control, toxin detection in fresh water ecosystems and planetary sciences. Our studies of viral response to antiviral treatments have determined optimal modes of drug administration to minimise viral load and mutant escape



1 Multipartite viruses have genomes formed with fragments encapsidated in separated viral particles. The evolutionary origin of multipartitism is uncertain, though it could have emerged on several occasions. Current evidence suggests that *de novo* associations between independent genes, as well as fragmentation or duplication of monopartite genomes are plausible evolutionary pathways leading to multipartite genomes.



System biotechnology

GROUP LEADER

Juan Nogales Enrique

POSTDOCTORAL SCIENTISTS

Beatriz García Jiménez
Jesús Torres Bacete
Blas Blázquez Castiñeira
Igor Martínez Sánchez
María Santos Merino

PhD STUDENTS

Lucía Agudo Algibe
Irene Sánchez Pascual

MASTER'S STUDENTS

Alejandro Ronco Campaña
Francisco J. Canalejo Molero
Antonio Mesa Galán

UNDERGRADUATE STUDENT

Jorge Carrasco Muriel

VISITING SCIENTISTS

Cansu Uluseker
University of Trento, Italy
Silvana Díaz Herrera
University of Buenos Aires, Argentina
Fabian Moreno
Universidad Nacional Autónoma de Mexico, Mexico

SELECTED PUBLICATIONS

Gudmundsson S, Agudo L, Nogales J. Applications of genome-scale metabolic models of microalgae and cyanobacteria in biotechnology. Chapter 4 pp.93-111 in González-Fernández C and Muñoz R (eds). *Microalgae-Based Biofuels and Bioproduct*, Woodhead Publishing Series in Energy.

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Nogales J et al. BO. Expanding the computable reactome in *Pseudomonas putida* reveals metabolic cycles providing robustness. bioRxiv 2017: 139121.

García-Jiménez B, García JL, Nogales J. FLYCOP: metabolic modeling-based analysis and engineering microbial communities. *Bioinformatics* 2018; 34: 1954-1963.

Our foundational aim is the system-level understanding of microbial metabolism as a framework for developing a broad range of novel and non-intuitive biotechnological processes. Taking advantage of metabolic modelling, systems and synthetic biology we are addressing, at different levels, the understanding and full taming of bacterial systems emergence.

Increasing the completeness and scope of metabolic reconstructions

We are involved in the high-quality metabolic modelling of a large set of metabolically diverse bacteria including *P. putida*, *Synechocystis*, *S. elongatus*, *A. platensis*, *Azoarcus* CIB, *S. sTFA*, *P. pseudoalcaligenes* and *B. bacteriovorus*. This effort is enabling the system-level analysis of new metabolic processes while providing new computational test-beds for biotechnological applications. We are also interested in the inclusion of new metabolic modules. Current efforts are targeted on i) the modelling of endogenous reactive oxygen species (ROS), ii) the implementation of dynamic condition-specific models and iii) the inclusion of underground metabolism.

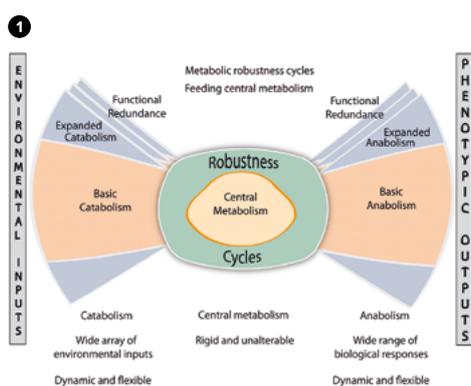
System-level analysis of metabolic robustness in bacteria

The robustness of a system is the property that allows it to maintain its functions despite external and internal perturbations. Through the metabolic modelling analysis of *P. putida*, we have identified metabolic cycles providing metabolic robustness. By using synthetic biology, ongoing efforts are focused on the rational engineering of such cycles under diverse biotechnological scenarios.

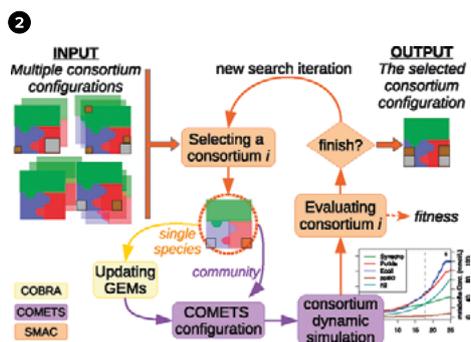
System-level analysis and designing of microbial communities

The division of labour in microbial consortia allows an expanded complexity and functionality in bacteria. We are interested in: i) understanding how these expanded capabilities emerge within a community and ii) how we can engineer this community-

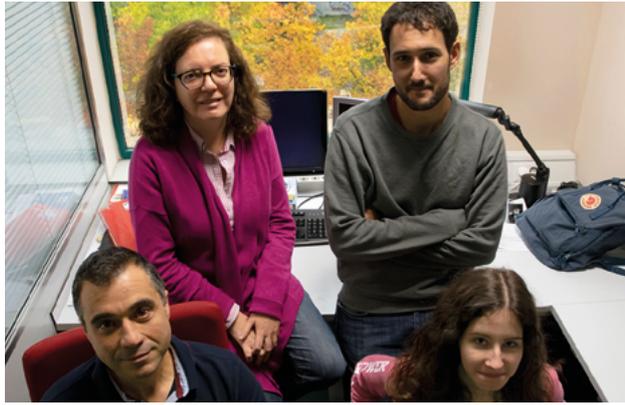
level functionality towards biotechnological endeavours. To address these two fundamental questions, we have developed a computational platform called FlyCop for modelling and engineering synthetic microbial consortia. Further implementation of these model-based designs is allowing us to develop new synthetic biology tools for engineering microbial communities.



1 The bow-tie structure of the *P. putida* metabolism. *P. putida* metabolic network has increased its metabolic robustness by: i) expanding catabolism and anabolism; ii) increasing functional redundancy for many metabolic processes; and iii) incorporating buffering cycles around central metabolism (metabolic robustness cycles), acting as metabolic capacitors to promote the stable connection of catabolism and anabolism with the central metabolism under perturbations.



2 FLYCOP pipeline: Graphical representation of FLYCOP workflow. Multiple consortium configurations are systematically evaluated providing the best one maximising a given fitness.



Computational systems biology

GROUP LEADER

Florencio Pazos Cabaleiro

SENIOR SCIENTIST

Mónica Chagoyen Quiles

PHD STUDENT

Javier López-Ibañez Infante

UNDERGRADUATE STUDENTS

Claudia Corona

Julia Pérez

Laura T. Martín

SELECTED PUBLICATIONS

González-Pérez S, Pazos F, Chagoyen M. Factors affecting interactome-based prediction of human genes associated with clinical signs. *BMC Bioinformatics* 2017; 18: 340.

Alpizar A, Marino F, Ramos-Fernández A, Lombardía M, Jeko A, Pazos F, Paradelo A, Santiago C, Heck AJR, Marcilla M. A molecular basis for the presentation of phosphorylated peptides by HLA-B antigens. *Mol Cell Proteomics* 2017; 16: 181-193.

Pazos F, Garcia-Moreno A, Oliveros JC. Automatic detection of genomic regions with informative epigenetic patterns. *BMC Genomics* 2018; 19: 847.

Garrido-Martín D, Pazos F. Effect of the sequence data deluge on the performance of methods for detecting protein functional residues. *BMC Bioinformatics* 2018; 19: 67.

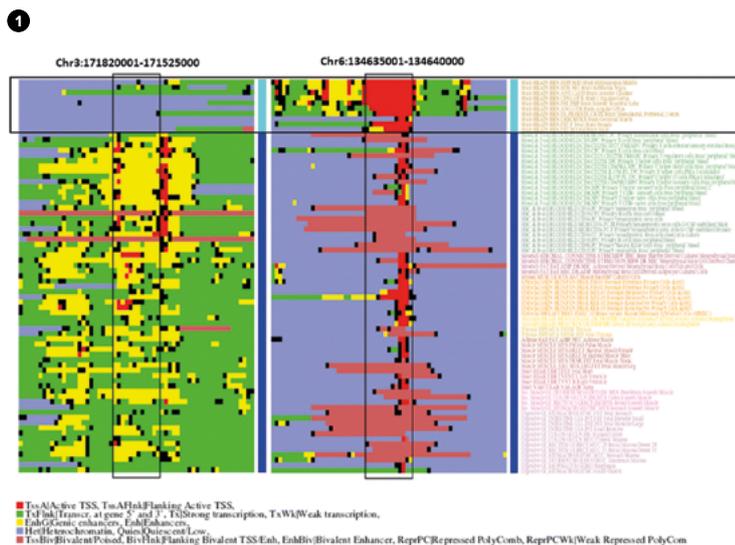
Pazos F, Chagoyen M. Characteristics and evolution of the ecosystem of software tools supporting research in molecular biology. *Brief Bioinform* 2018; doi: 10.1093/bib/bby001.

Our group is interested in different aspects of bioinformatics, computational biology and systems biology. Our goal is to obtain new biological knowledge with an *in-silico* approach which complements the *in-vivo* and *in-vitro* methodologies of biology. This mainly involves mining the massive amounts of information stored in biological databases. Besides our lines of scientific research, we also collaborate with experimental groups providing them with bioinformatics support for their specific needs and participate in different teaching projects.

We have developed evolutionary-based method for predicting sites with some functional importance in protein sequences and structures. These are based on the fact that functional sites are subject to certain evolutionary constraints whose landmarks can be detected on multiple sequence alignments.

We have also developed evolutionary-based methods for predicting interaction partners which have been accepted and followed by the community. These methods are mainly based on the hypothesis that interacting or functionally related proteins adapt to each other during the evolutionary process (co-evolution). We try to detect the landmarks that this co-evolutionary process left in the sequences and structures of the proteins.

We are studying metabolic networks (central metabolism and biodegradation) and protein interaction networks from a top-down systemic approach. Of special interest for us is the study of the complex phenomenon of protein function from a systemic perspective, trying to understand how complex functions arise by combining the molecular functions of proteins when these interact in intricate networks. We are also interested in applying this systemic approach to the study of human diseases.



1 Example of two genomic regions with an epigenetic pattern related to brain. The sample classification is indicated with light blue (brain) and dark blue (others). The brain samples are further highlighted with a box. The sample names are on the right, coloured using the tissue-based colour schema of the Roadmap Epigenomics Consortium.



Logic of genomics systems

SYSTEMS BIOLOGY PROGRAMME 89

GROUP LEADER

Juan F. Poyatos

PhD STUDENTS

Clara Moreno
Pablo Yubero

MASTER'S STUDENT

Alvar Alonso

SELECTED PUBLICATIONS

Rodrigo G, Bajic D, Elola I, Poyatos JF. Deconstructing a multiple antibiotic resistance regulation through the quantification of its input function. *NPJ Syst Biol Appl* 2017; 3: 30.

Moreno-Fenoll C, Cavaliere M, Martinez-Garcia E, Poyatos JF. Eco-evolutionary feedbacks can rescue cooperation in microbial populations. *Sci Rep* 2017; 7: 42561.

Yubero P, Poyatos, JF. Large-scale analysis of the global transcriptional regulation of bacterial gene expression. *BioRxiv* 2018; 405894.

Chagoyen M, Poyatos JF. Complex genetic and epigenetic regulation deviates gene expression from a unifying global transcriptional program. *BioRxiv* 2018; 390419.

Sánchez-Gorostiaga A, Bajic D, Osborne ML, Poyatos JF, Sánchez A. High-order interactions dominate the functional landscape of microbial consortia. *BioRxiv* 2018; 333534.

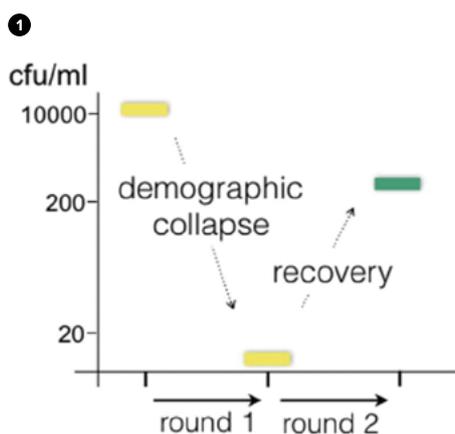
Research at the Logic of Genomic Systems Laboratory searches for design principles in biological systems. During the last years, we examined the dynamics of microbial communities, the integration of numerous regulatory signals in the triggering of an antibiotic response, and the impact of physiology on genome-wide expression.

In our study of microbial communities, we demonstrated the consequences of the coupling between ecology and evolution on community resilience (Figure 1), and the difficulty of anticipating community function. For the study of eco-evolutionary feedbacks, we used synthetic communities constituted by engineered *Escherichia coli* variants; for the study of community function, we assembled an artificial consortium constituted by natural species. Both approaches emerge as tractable experimental models to recognise ecosystem properties.

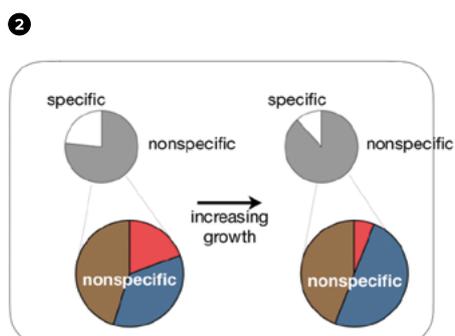
To study the intricate modulation of an *E. coli*'s response to antibiotics, we deconstructed its regulation with the use of input functions. These functions quantify the rate of transcription of the genes constituting the response with respect to the signals acting on its cognate regulators. By examining how the shape of the function changes in different situations, e.g., when a given regulator is mutated, we identified the functional implications of the associated control architecture and emphasised the role of a core dual auto-regulatory motif.

We also completed two studies in which we inspected how cellular physiology influences gene expression, what is termed the global program of regulation. We first studied the impact of the global program on gene order in bacteria. The study incorporated a large-scale characterisation of the global program, with experiments validating the analysis. In a second study, we integrated two models of resource allocation (Figure 2, cellular

“economics” is an intrinsic feature coupled to physiology) to evaluate how genetic and epigenetic regulation combine with the global regulation for the genome-wide control of gene expression. Both topics considerably advance our previous understanding of Genome Biology.



1 While the initial dynamics of a community constituted by producers (Ps) and non-producers of a public good (20% Ps, represented by a yellow rounded rectangle) leads to a demographic collapse of the full population (measured in colony forming units per ml), it also generates specific conditions, in a second round of the dynamics, to allow the recovery of Ps (green rectangle). This confirms the eco-evolutionary feedback in the community.



2 Partition of genome expression into five sectors that combine two previous resource allocation models. A partition based on three sectors in which the expression of their constituent genes increases (positive genes, blue), decreases (negative genes, red), or remains constant (invariant genes, brown) with increasing growth rate appears as a fine structure of a broad partition between specific and nonspecific genes.



Microbiome analysis

GROUP LEADERS

Javier Tamames de la Huerta
Carlos Pedrós-Alió

POSTDOCTORAL SCIENTISTS

Fernando Puente Sánchez
Beatriz Cámara Gallego
Arturo Marín Alguacil

PHD STUDENTS

Marta Cobo Simón
Natalia García García
Diego Jiménez Lalana

MASTER'S STUDENTS

Luis Alberto Macías Pérez
Elena Montenegro de la Borbolla

SELECTED PUBLICATIONS

Royo-Llonch M, Ferrera I, Cornejo-Castillo FM, Sánchez P, Salazar G, Stepanauskas R, González JM, Sieracki, ME, Speich, S, Stemmann, L, Pedrós-Alió C, Acinas SG. Exploring microdiversity in novel *Kordia* spp. (Bacteroidetes) with proteorhodopsin from the tropical Indian Ocean via single amplified genomes. *Front Microbiol* 2017; 8: 1317.

Cobo M, Tamames J. Relating genomic characteristics to environmental preferences and ubiquity in different microbial taxa. *BMC Genomics* 2017; 18: 499.

Pedrós-Alió C, Acinas SG, Logares R, Massana R. The HTS contribution to marine microbial diversity. Chapter 2, pp.47-97, 2018; In JM Gasol and D Kirchman (eds.) *Microbial Ecology of the Oceans*, 3rd edition.

Alcamán-Arias ME, Pedrós-Alió C, Tamames J, Fernández C, Pérez-Pantoja D, Vázquez M, Díez B. Diurnal changes in active carbon and nitrogen pathways along the temperature gradient in Porcelana hot spring microbial mat. *Front Microbiol* 2018; 9: 2353.

Narciso-da-Rocha C, Rocha J, Vaz-Moreira I, Lira F, Tamames J, Henriques I, Martínez JL, Manaia CM. Bacterial lineages putatively associated with the dissemination of antibiotic resistance genes in a full-scale urban wastewater treatment plant. *Environmental Int* 2018; 118: 179-18.

Microbial communities (microbiomes) are key players in many scenarios, from how the biosphere works to industrial and biotechnological processes, as well as human health and wellness. We study microbiomes of diverse environments trying to learn the rules that govern the assemblage of these microbial communities. This knowledge will help to understand how they function, and to predict the effects of disturbances. Eventually, this will lead to rational design and manipulation of microbiomes.

We focus mostly on marine microbial communities, but we are actively working in many other microbiomes from different environments. We study extreme environments because their microbiotas show fascinating adaptations to the harsh conditions. We work with human-associated microbiomes, such as the gut and the vagina, because of their potential to improve our health. We are also interested in other habitats, such as wastewaters and soils.

We use mostly bioinformatics tools to study the composition and functionality of microbiomes. Metagenomics is the basis of our work, since it provides the basic material: DNA sequences from environmental samples. The analysis of these sequences informs about the presence of diverse organisms and the content of their genomes, and the latter

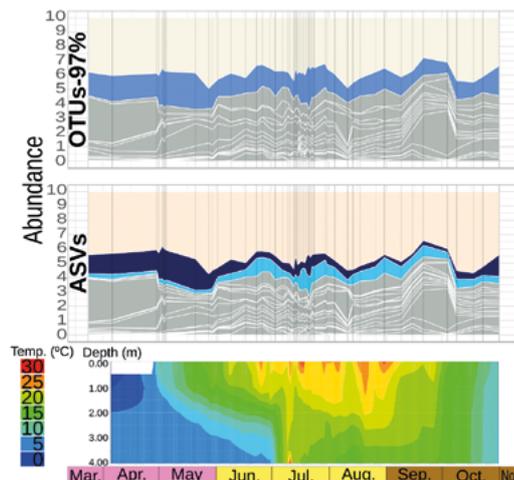
can be linked to functionality. We also carry out experimental work addressing interactions between members of microbiomes.

1

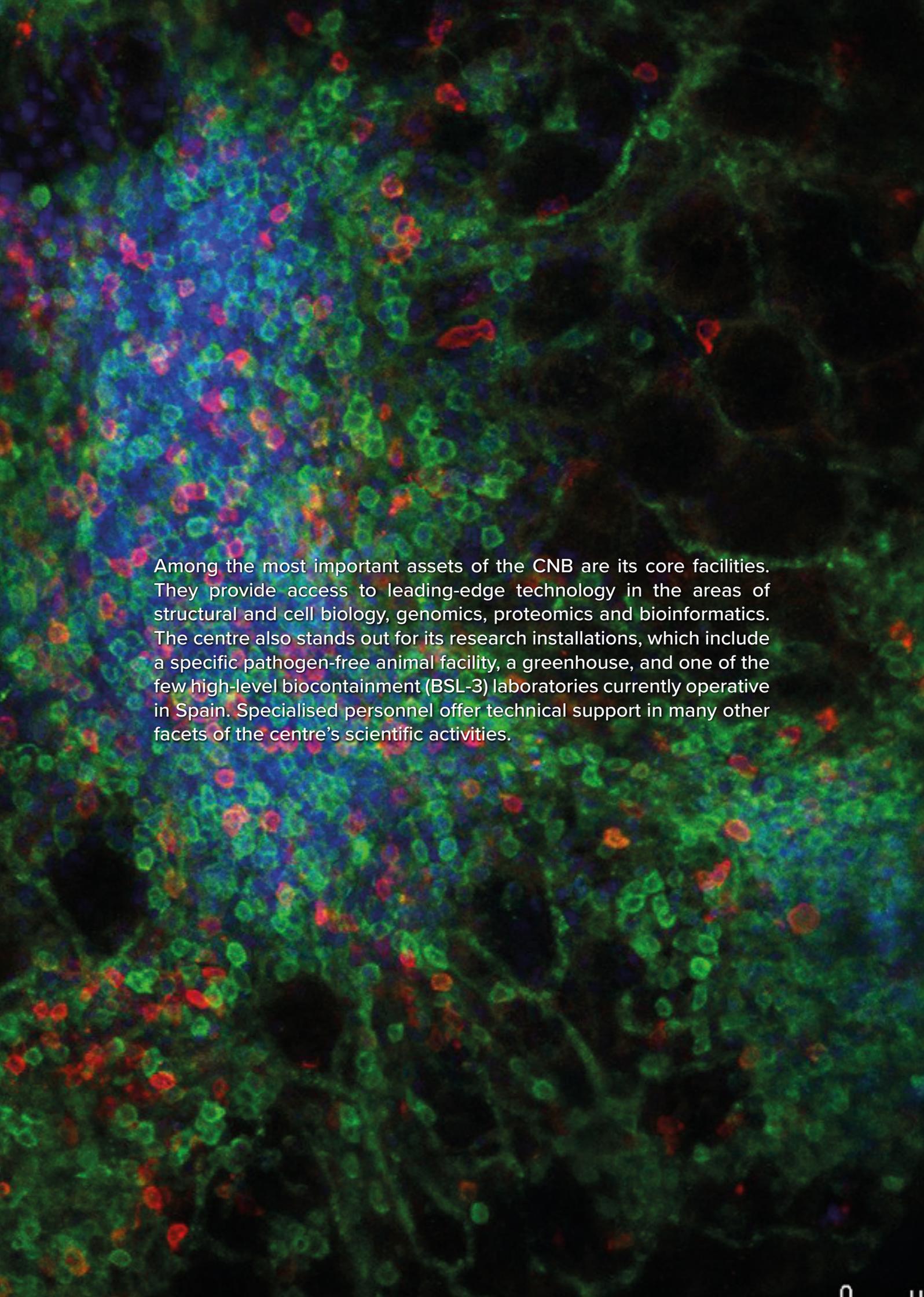


1 *Microbial mats in Porcelana Geyser (Southern Chile), where we are studying metagenomics and metatranscriptomics of the thermophilic bacteria.*

2



2 *Bacterial community composition in a Wisconsin Lake. Blue colours show the presence of Polynucleobacter OTU (97%, top panel) or as its component strains (Amplicon Sequence Variants, middle panel). While OTUs show a fairly constant presence along the year, ASVs (corresponding to different ecotypes) alternate. Lake temperature is shown in bottom panel.*

A fluorescence microscopy image showing a dense population of cells. The cells are stained with three different dyes: green, red, and blue. The green staining highlights the cytoplasm and some organelles, the red staining highlights other organelles or structures, and the blue staining highlights the nuclei. The overall appearance is a complex, multi-colored cellular network.

Among the most important assets of the CNB are its core facilities. They provide access to leading-edge technology in the areas of structural and cell biology, genomics, proteomics and bioinformatics. The centre also stands out for its research installations, which include a specific pathogen-free animal facility, a greenhouse, and one of the few high-level biocontainment (BSL-3) laboratories currently operative in Spain. Specialised personnel offer technical support in many other facets of the centre's scientific activities.

Scientific Services

STRUCTURAL AND CELL BIOLOGY

Advanced light microscopy

Sylvia Gutiérrez

Cryoelectron microscopy

Rocío Arranz

Electron microscopy

Cristina Patiño

Macromolecular X-ray crystallography

César Santiago

Flow cytometry

M^a Carmen Moreno-Ortiz

Histology

Lluís Montoliu

Mouse embryo cryopreservation

Lluís Montoliu

Protein tools

Leonor Kremer

Transgenesis

Belén Pintado

GENOMICS, PROTEOMICS AND BIOINFORMATICS

Bioinformatics for genomics and proteomics

Juan Carlos Oliveros

Genomics

José Manuel Franco

Proteomics

Fernando Corrales

Sequence analysis and structure prediction

Mónica Chagoyen

Scientific computing

José Ramón Valverde

RESEARCH INSTALLATIONS

Animal facility

Ángel Naranjo

Greenhouse

Tomás Heras

Radioactive facility and biosafety level 3 laboratory

Fernando Usera

TECHNICAL SUPPORT

Cell culture, washing and sterilisation

Rosa María Bravo

Instrumentation

Ismael Gómez

Photography

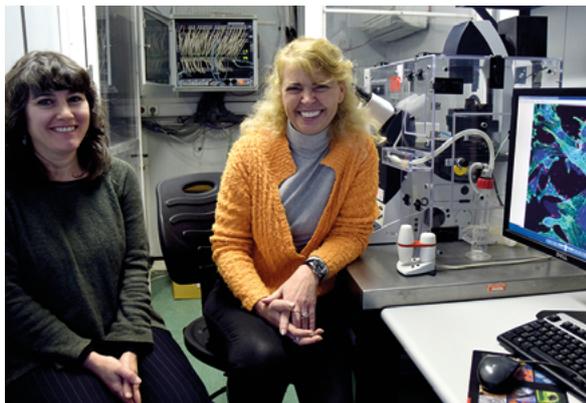
Inés Poveda

Radiation protection and biological safety

Fernando Usera

Workshop

Daniel Pastora



94 SCIENTIFIC SERVICES

Advanced light microscopy

HEAD

Sylvia Gutiérrez Erlandsson

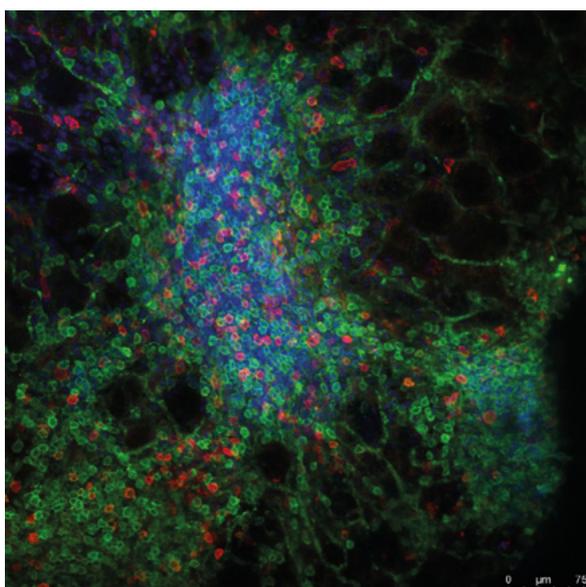
PERSONNEL

Ana Oña Blanco

The presence of fluorescent markers in the sample allows location of cell components in single sections and various experimental approaches, involving single or multiple fluorescent labelling in fixed cells and tissues.

The facility provides infrastructure for epifluorescence, confocal laser scanning microscopy, TIRF, STED, SMLM nanoscopy and image processing tools, covering most experimental light microscopy imaging approaches.

The equipment and services are available to all CNB personnel as well as to researchers from the public and private sectors. Technical staff offers assistance and training in the use of equipment, experimental methods and image processing and analysis procedures. The facility also provides cell culture support and aliquots of probes and secondary antibodies with broad use in fluorescence microscopy applications.



Cryoelectron microscopy

HEAD

Rocío Arranz

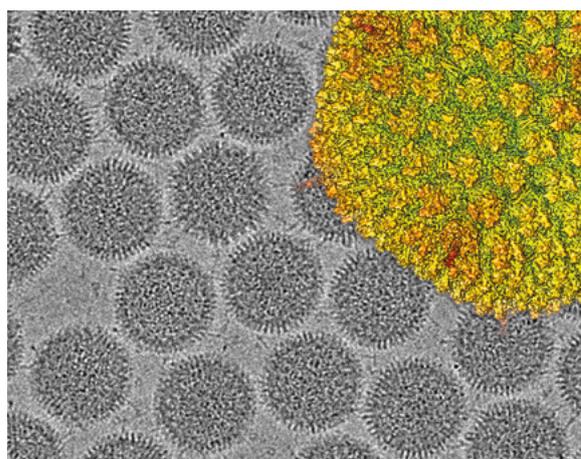
PERSONNEL

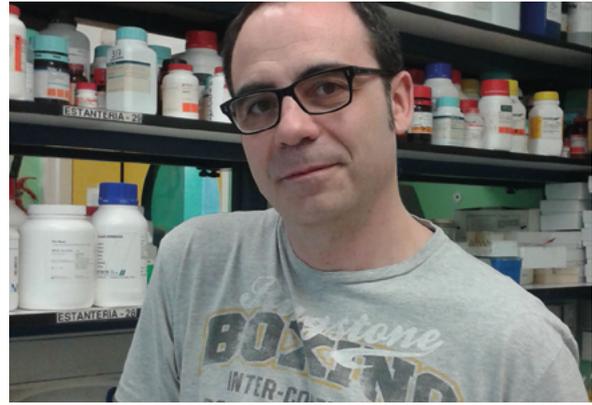
Francisco Javier Chichón
Rafael Núñez (CIB-CSIC)

The cryoelectron microscopy core facility is located at the CNB and jointly operated with the Centro de Investigaciones Biológicas (CIB-CSIC). The services offered by the facility include sample preparation and image collection for cryoelectron microscopy.

The facility provides access to two microscopes for cryoelectron microscopy of unstained biological material. One of them, a 200 kV FEI TALOS Arctica, equipped with an autoloader and with a Falcon III direct electron detector, is ideally suited for the collection of large amounts of high-resolution data. The other microscope, a 200 kV FEI Tecnai G2, equipped with a CCD camera, is available for general routine cryoEM.

The service also has two different apparatus for specimen vitrification, a FEI Vitrobot and a Leica EM CPC.





Electron microscopy

HEAD

Cristina Patiño Martín

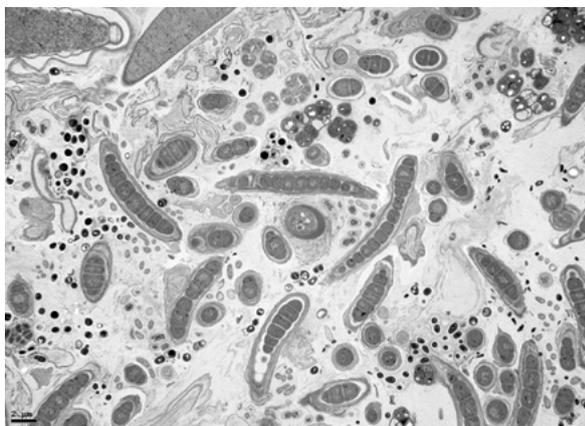
PERSONNEL

Beatriz Martín Jouve

The transmission electron microscope (TEM) operates on many of the same optical principles as the light microscope but uses electrons as the illumination source. Their much shorter wavelength makes it possible to obtain much higher resolution, which allows to study ultrastructure of organelles, viruses and macromolecules.

The aim of this facility is to deliver scientific and technical support to researchers at the CNB and to users from public or private institutions by providing access to the necessary equipment, advising on the most appropriate techniques, offering specialised sample preparation, performing microscopy, data collection and support for the interpretation of the obtained data.

The facility is equipped with a 100 kV transmission electron microscope with a digital camera. For sample processing, the facility disposes of a Reichert ultramicrotome, Leica UC6-FC6 cryo-ultramicrotome, Leica AFS2 automatic freeze substitution system, Leica AC 600 carbon coating equip and Leica EM PACT2 high-pressure vitrification unit.



Macromolecular structures and X-ray crystallography

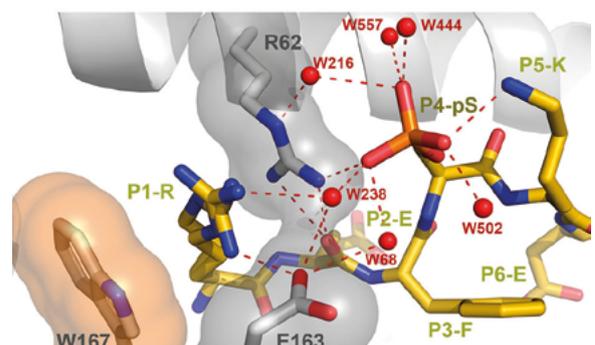
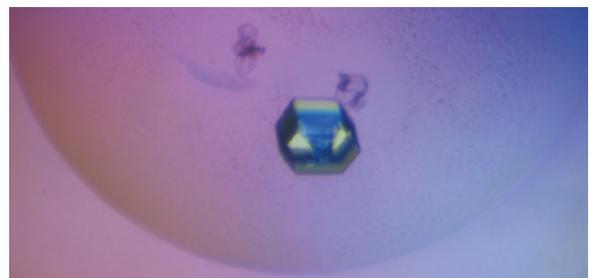
HEAD

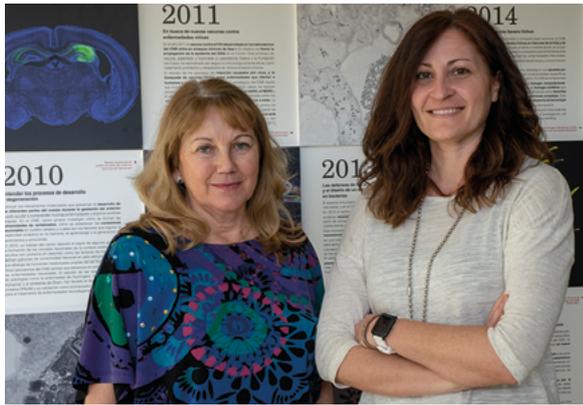
César Santiago

Protein X-ray crystallography is a high-resolution technique that allows to study protein structure at the atomic level. This method provides a detailed view of protein function, ligand and protein interactions, supramolecular organisation and mutants related to human diseases. Substantial improvements, both in crystallisation techniques and in software for structure resolution and refinement, have been achieved in the last decade, which increases the success of solving a macromolecule structure.

The facility provides both advice and supervision of protein production from cloning to expression in bacterial, yeast and eukaryotic systems, as well as support and training in protein purification to obtain crystal-grade protein for automated crystallisation using an automated nanodispensing robot.

The facility also offers crystal optimisation and freezing for diffraction data at the synchrotron. Structure resolution and interpretation are also provided for both data coming from X-ray crystallography or data produced with any other structural biology technique.





Flow cytometry

HEAD

M^a del Carmen Moreno-Ortiz Navarro

PERSONNEL

Sara Isabel Escudero García

The facility provides scientific and technological support to CNB groups and other researchers from the public and private sectors.

The facility offers training and advice on the principles and the applications of analytical flow cytometry, assistance to plan, design, and optimise flow cytometry experiments, including the implementation of new technologies and reagents as well as sample preparation, instrument operation and data analysis. The facility provides quantification of secreted cytokines by multiplexed assays, cellular isolation by cell sorting, antibodies and commonly used reagents. Analysis of results can be performed using specialised software packages.

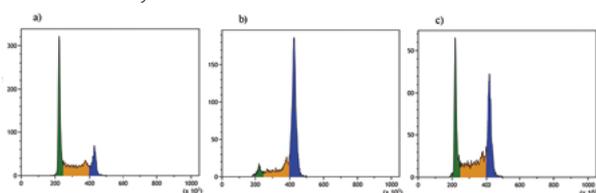
Equipment

1BD FACSCalibur: 4 colours. 2 Beckman Coulter CYTOMICS FC 500: 5 colors.1 BD LSRII: 8 colours. 1 Beckman Coulter GALLIOS: 10 colours. 1 Beckman Cytoflex: 13 colours. 1 Luminex 100 IS Multiparametric Analyzer. 1 Cell Sorter Beckman Coulter Moflow XDP: 10 colours.

Common applications

Cell viability, either fresh or fixed cells; apoptosis; cell cycle and ploidy levels in eukaryotic cells; mitotic population studies, proliferation assessment using BrDU, EdU, CFSE or CELLTRACE, gene expression of fluorescent proteins; immunophenotyping up to 13 colours; intracellular signalling; cell migration; calcium mobilisation; intracellular cytokines, quantitation of soluble molecules by multiplexed assays and cell sorting.

Fluorescence intensity (propidium iodide) to measure T Cell arrest at G2M phase after treatment with nocodazole: a) untreated cells, b) after nocodazole treatment, c) cell division recovery after nocodazole withdrawal.



Histology

HEAD

Lluís Montoliu

TECHNICAL MANAGER

Soledad Montalbán

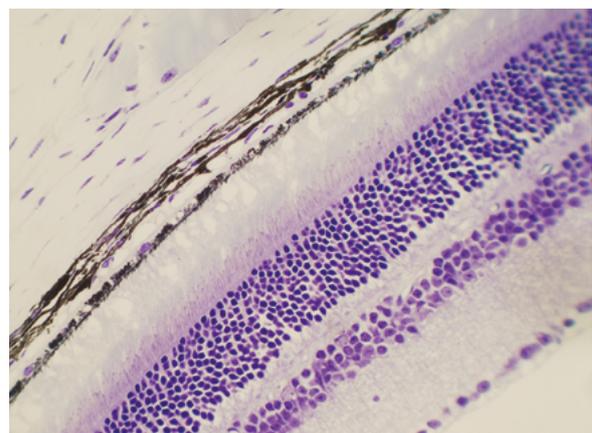
PERSONNEL

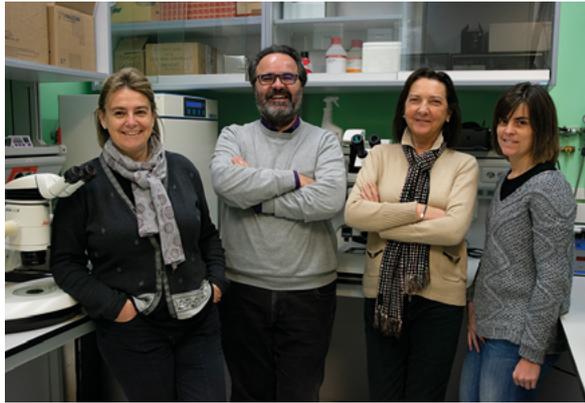
Óscar Sánchez

The CNB histology facility offers the preparation of animal and plant biological samples for their histological analysis. All requests are received and processed electronically, through the facility's web site, available in Spanish and in English.

Offered methods and procedures include the preparation of wax (paraffin) and plastic (resin) blocks with biological specimens embedded, and the corresponding generation of histological sections with one of the two available automated microtomes. The facility also offers the preparation and sectioning of frozen blocks with the cryostat. The orientation, width and arrangement of the sections can be specified by the user. All sections can be counterstained with any of the available staining procedures (haematoxylin/eosin, cresyl violet, PAS, Mason's trichrome, elastin fibers/Van Gieson/Sirius Red, etc.) or can be processed subsequently for immunohistochemistry. The facility implements new staining procedures or histological methods upon request. The CNB histology facility has an ample experience in processing a large variety of animal and plant tissues and organs.

The CNB histology facility coordinates a joint platform with the IIB-UAM/CSIC histology facility, offering to CNB and IIB researchers a larger processing capacity for histological samples.





Mouse embryo cryopreservation

HEAD

Lluís Montoliu

TECHNICAL MANAGER

Julia Fernández Punzano

PERSONNEL

María Jesús del Hierro Sánchez

Marta Castrillo Labrado,

Isabel Martín-Dorado Caballero

The CNB mouse embryo cryopreservation facility offers to researchers the possibility to freeze, maintain and rescue transgenic and knockout mouse lines in the form of embryos and/or sperm, hence contributing to current animal welfare recommendations and complying with the associated legislation on animal experimentation. Current methods available include freezing sperm, oocytes and/or embryos, the thawing of sperm, oocytes and/or embryos previously frozen and the subsequent revitalisation of the cryopreserved mouse lines through *in vitro* fertilisation, assessment and/or logistical support for importing/exporting frozen or refrigerated embryos or sperm, from and to the CNB, and quality controls and genotyping procedures.

The CNB hosts the Spanish node of the European scientific-technological infrastructure INFRAFRONTIER-EMMA, whose objective is the cryopreservation, organised archiving and coordinated distribution of mouse lines of interest for the scientific community in biomedicine.

The CNB mouse embryo cryopreservation facility has scientific cooperation agreements with the Spanish National Cancer Centre (CNIO) and the University of Kumamoto for the archiving and distribution of mutant mouse lines of interest in biomedical research.

Lluís Montoliu and Julia Fernández participate regularly as instructors in mouse cryopreservation workshops and courses, organized in collaboration with CARD-University of Kumamoto, INFRAFRONTIER-EMMA, ISTT and CIEMAT-SECAL, among other institutions.



Protein tools

HEAD

Leonor Kremer

PERSONNEL

María Teresa Martín

Mónica García-Gallo

Ana María García

Mercedes Llorente

María Lozano

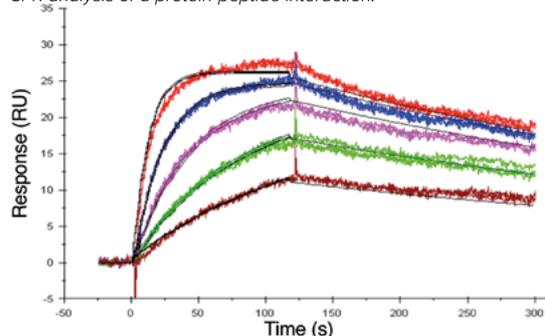
The CNB protein tools unit offers scientific services related to the design, generation and characterisation of custom monoclonal antibodies (mAb); immune response studies; customised immunoassays; antibody purification and labelling, and analysis of biomolecular interactions.

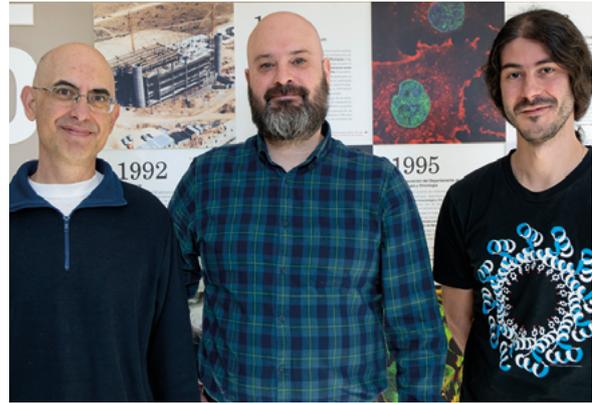
The facility has expertise in immunobiology and immunochemistry, and provides technical assistance, data analysis, training in specific techniques, introduction of new methodologies and technical advice. It also organises theoretical and practical courses and assists with the preparation of manuscripts and oral presentations.

The facility also has a surface plasmon resonance (SPR) based biosensor, Biacore 3000, that allows the characterisation of biomolecular interactions in real time and the determination of kinetic and affinity constants. SPR analysis can be applied to a wide range of molecules or particles such as proteins, nucleic acids, carbohydrates, lipids, low molecular weight compounds, liposomes and viruses.

Protein tools unit is a founder member of the EuroMAbNet, the first European non-profit organisation of multidisciplinary academic laboratories specialised in mAb production, which offers to researchers working in the field a framework for exchange of knowledge, methods and materials, recommendations and training in antibody validation. The facility provides research tools and services to scientists from the CNB, other CSIC institutes, universities, public research organisations and private companies.

SPR analysis of a protein-peptide interaction.





Transgenesis

HEAD

M^a Belén Pintado Sanjuanbenito

PERSONNEL

Verónica Domínguez Plaza (CBMSO)
Marta García Flores
Alicia Llorente

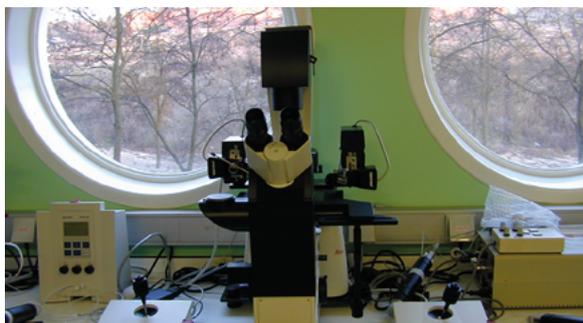
The CNB-CBMSO transgenesis unit is a joint scientific service shared between CNB and CBMSO. The facility is integrated in the scientific-technological platform INNOTEK (UAM+CSIC) and member of the Merck CRISPR Core Partnership Program to test new CRISPR/Cas9-related products.

It supports research groups in all the required steps to obtain the desired mouse model: from the creation of a genetically modified model to the establishment and management of lines in order to achieve the desired genotype. The facility provides the animal resources and technology to produce models based on additive transgenesis, targeted mutagenesis (KO, KI) or genome edition based on CRISPR/cas9 technology. The facility designs and tests procedures to produce KI and conditional mouse models in a single step, supporting and complementing the expertise of its customers.

The facility has two microinjection settings, dissecting microscopes, a standard molecular biology laboratory and a fully equipped laboratory for ES cells handling, as well as full access to the animal facilities of CBMSO and CNB.

Services

- Pronuclear microinjection of plasmidic, BAC and YAC DNA
- Genome editing based on CRISPR/cas9 technology, either by microinjection or embryo electroporation, including guide design and in vitro validation
- Injection of ES cell lines generated indoors or from international consortia
- Embryo rederivation through IVF or embryo transfer from external animal facilities



Bioinformatics for genomics and proteomics (BioinfoGP)

HEAD

Juan Carlos Oliveros

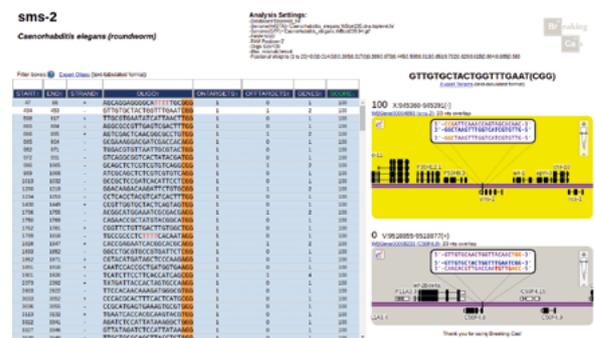
PERSONNEL

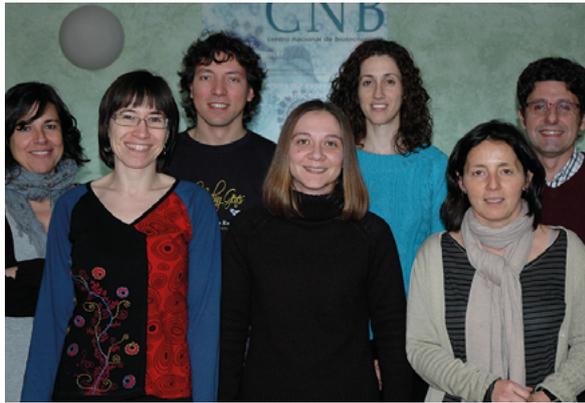
Rafael Torres-Pérez
Juan Antonio García-Martín

The BioinfoGP facility provides CNB's research groups with bioinformatics support for the analysis, visualisation and interpretation of both genomics and proteomics-related projects, including:

- Assistance in the experimental design of deep sequencing and DNA microarray experiments
- Biostatistical support for extracting quantitative results from genomics or proteomics projects
- Functional annotation of relevant lists of genes or proteins.
- Periodic courses and tutorials on bioinformatics

In short, the BioinfoGP facility aims to fill the gap between the complex outcome of the many powerful biostatistical methods available and the researchers' needs.





Genomics

HEAD

José Manuel Franco Zorrilla

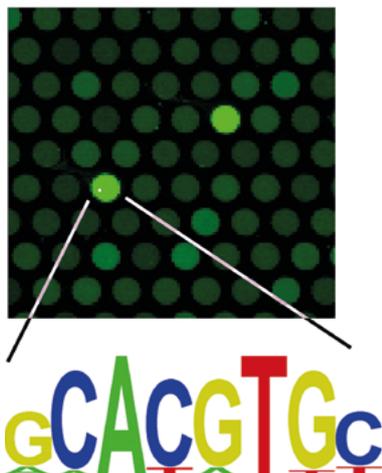
PERSONNEL

Irene López-Vidriero
 Marta Godoy
 Luis Almonacid
 Gloria García Casado
 Beatriz Martín

The genomics facility is focused on the analysis of gene expression from biological samples using microarrays, interrogating the activity of complete genomes in a single experiment, and contributing to the elucidation of the genetic basis of the biological processes. The facility routinely hybridises and analyses one- and two-channels microarrays, including Agilent, Affymetrix, and custom microarrays.

The services offered by the facility include microarray printing and design, analysis of RNA integrity and microarray hybridisations. Raw data are statistically analysed using “state-of-the-art” algorithms, and filtered results are supplied to customers in a web-based easy-to-use tool developed by the facility. The facility offers support in the use of several bioinformatics tools for functional analysis, helping customers in the biological interpretation of their results. The facility also offers the possibility of validating gene expression data by real time qPCR.

Research projects are being constantly developed by the facility with the goal to implement new services and technologies for customers, including novel high-throughput technologies for studying DNA-protein interactions to decipher the molecular basis of transcriptional regulation.



Proteomics

HEAD

Fernando Corrales

PERSONNEL

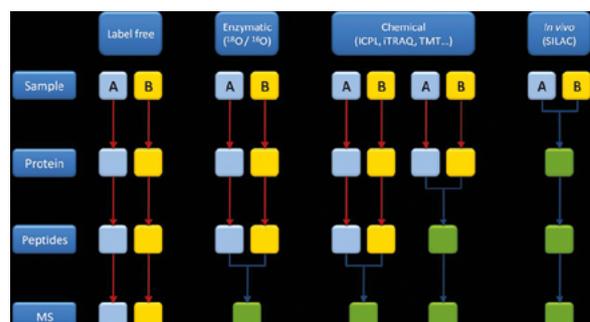
Adan Alpizar
 Gema Bravo
 Lorena Carmona
 Sergio Ciordia
 Manuel Lombardía
 Miguel Marcilla
 Rosana Navajas
 Alberto Paradelo
 Antonio Ramos

Created in 1999, the CNB Proteomics Facility maintains a technological platform suitable for large-scale protein identification and characterisation, with more than 3,000 proteomic analyses performed in 2017. Massive protein identification, characterisation (including posttranslational modifications) and quantification are performed by multidimensional nano-HPLC chromatography coupled to a nano-electrospray mass spectrometers (MS). Finally, prolamin detection and characterisation by ELISA, quantitative PCR and mass spectrometry are also included in our analysis portfolio.

For educational purposes, we organise practical courses on quantitative proteomics and bioinformatics. The head of the CNB Proteomics Facility (Dr. Fernando Corrales) also coordinates Proteored-ISCIII (Plataforma en Red de Proteómica-Carlos III).

Services

- Protein identification and characterisation
- Protein quantitation by label-free, metabolic and chemical stable isotopic labelling (SILAC, ICPL, ITRAQ, TMT)
- Selected/multiple reaction monitoring (S/MRM-MS)
- Identification and characterisation of post-translational modifications
- Peptide synthesis and membrane-bound peptide array design
- Gluten analysis by ELISA, PCR and mass spectrometry





Sequence analysis and structure prediction

HEAD

Mónica Chagoyen

Sequence analysis and protein structure prediction methods can explain, simplify and further guide experimental work. The facility specialises in *ad hoc* analysis of protein sequences to solve specific problems or questions.

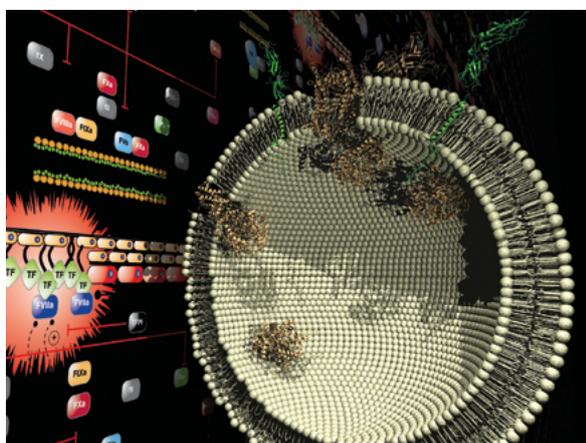
This analysis commonly aims to:

- Predict protein structure
- Search for homologous proteins
- Generate multiple sequence alignments
- Produce structural organisation drafts
- Study relevant residues for protein structure/function
- Extract sequence features from full proteomes

Additional services include:

- DNA/RNA motif discovery
- Consultancy on the use of sequence-based methods
- Generation of high-quality protein sequence/structure images for publication

These services are available to CNB researchers as well as to users from other academic institutions and private organisations.



Scientific computing service

HEAD

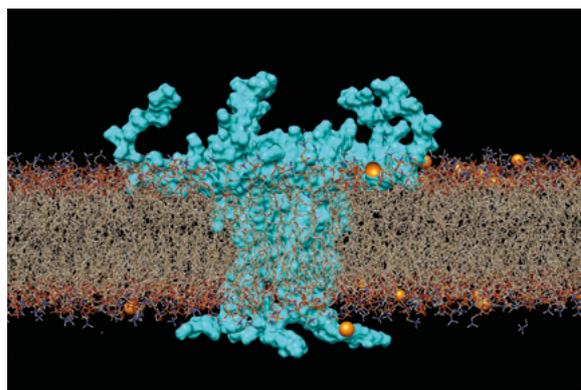
José R. Valverde

The Scientific Computing Service provides advanced support for scientific data analysis in Bioinformatics and Biocomputing through delivery of international courses, close collaboration with research groups and international institutions.

Our areas of expertise span Bioinformatics, Genomics, Metagenomics, Metabolomics, Biostatistics, Artificial Intelligence/Machine Learning, and Computational Biology, including molecular docking, drug screening, in silico mutagenesis, Molecular Dynamics, QM and QM/MM models, Quantum Dynamics and reaction modelling, as well as computer programming in numerous languages.

Sample applications of these techniques involve cancer diagnosis, the effect of pesticides on soil, identifying ecological indicators, sequencing new bacterial strains, dynamic metabolic networks for improving biotechnological processes in *S. lividans*, activity of the ϵ - ζ toxin-antitoxin system, analysis of viral structures and virus-host interactions in Phage T7, human SARS-CoV, and HIV for the development of new antiviral therapies.

The service has participated in several Networks of Excellence in Bioinformatics, Grid computing, Genomics and CBRN, and advised students' theses at Graduate, Master and PhD levels from U. Autónoma of Madrid, U. of Alcalá de Henares, U. Politécnica of Madrid, U. of Colombo in Sri Lanka. Additionally, the service collaborates in academic activities organised by other institutions in numerous countries in Europe, Latin America, Asia and Africa.





Animal facility

HEAD

Ángel Naranjo

RESEARCH TECHNICIAN

Javier Martín

SHIPMENT COORDINATOR AND ADMINISTRATION

Alberto García

AREA AND COLONY MANAGERS

Antonio Morales
Raquel Gutiérrez
Eladio Martínez

ANIMAL TECHNICIANS

Sergio Magallón
Ruví Jaramillo
Lola García
Ivan Jareño
Raquel Castañera

The CNB laboratory animal facility is an area dedicated to the production and maintenance of experimental animals, aiding in research, essential techniques, and legal support for this duty. Most of the experimentation is carried out with genetically modified mice. The laboratory animal service provides a controlled environment for the animals, with periodic control of diet, water, temperature, air, housing, and husbandry conditions. The unit is separated into several areas: quarantine, conventional, and specific pathogen-free (SPF), depending on the microbiological status of the animals. The facility provides special housing conditions for conventional, genetically modified, and immunodeficient animals, depending on the experimental objectives. At the same time, a totally isolated biosafety area is dedicated to *in vivo* experiments using biological agents.

The animal facility staff delivers services to laboratories for obtaining commercial lines and strains of animals, shipping animals for collaboration with other institutes, as well as maintenance, breeding, and generation of transgenic, knock-out and knock-in animals. These services allow control of the microbiological and genetic quality of the animals used in experimentation. The animal facility staff provides services for various techniques used in mouse research models, research assistance in surgical techniques, selection of animal models, animal health surveillance, laboratory animal care, and animal well-being. The facility also organises courses for continued education and to obtain accreditation for working with animals and manage colonies of genetically modified animals.

The facility's goal is to achieve research excellence following the 3R principles: reduction, refinement, and replacement of animal experiments.

Greenhouse

HEAD

Tomás Heras Gamo

PERSONNEL

Alejandro Barrasa Fustes
Joaquín Rivera Cuesta

The CNB greenhouse takes care of the following facilities specific for plant cultivation:

- A standard greenhouse with 8 cabinets (total growth surface: 180 m²)
- A P2 safety level greenhouse with 4 cabinets (total growth surface: 83 m²)
- 16 climate chambers

The facility carries out the following tasks:

- Growth and propagation of plants under controlled environmental conditions
- Growth and propagation of mutant and transgenic lines under controlled environmental conditions
- Identification, selection and phenotypic analysis of mutant and transgenic plants





Radioactive facility and biosafety level 3 laboratory

HEAD

Fernando Usera Mena

PERSONNEL

María Teresa Bartolomé Jiménez
 Aranzazu de la Encina Valencia
 Jessica Gaspar Navarro

This unit directly operates the centre's gamma irradiator, radioactive facility and biosafety level 3 laboratory.

Radioactive facility

The CNB radioactive facility is a category 2, non-encapsulated sources type installation, equipped with all the required means of shielding, containment and detection of ionising radiation.

The following equipment is available for users:

- Two cabinets for radioisotopes beta and gamma
- Biosafety class IIA cabinet
- CO2 incubator
- Ultracentrifuge
- Centrifuges and microfuges
- Vacuum concentrator (SpeedVac)
- Hybridisation oven

Biosafety level 3 laboratory

The laboratory has three sub laboratories with all the necessary equipment for safely handling biological agents included in risk group 3.

The installation is equipped with:

- Three biosafety class IIA cabinets
- Three cell culture incubators
- Microbiological culture incubator
- Refrigerated ultracentrifuge
- Three bench-top refrigerated centrifuges
- Three refrigerated microfuges
- Three inverted optical microscopes
- Fluorescence microscope
- Liquid nitrogen tank with capacity for 6,000 samples
- Double door steam steriliser
- Pass through box for biological inactivation of small items
- Air lock for biological inactivation of large items
- Five ultra-freezers (-80°C)
- Data transmission network (computers and telephone)
- Several alarm systems to alert of incidents, accidents or malfunctions

Cell culture, washing and sterilisation

HEAD

Rosa M^a Bravo Igual

PERSONNEL

Carmen Berdeal
 Esther Dorado
 Carlos Enríquez Casas
 Margarita Felipe Hombrados
 Isabel Martín-Dorado
 Ana Montero
 Ana Isabel Nieto Jiménez
 Josefa Pérez Alfaro
 Rosa Ramos Hernández
 Aránzazu Rodríguez Martínez
 Sonia Rodríguez Murcia
 Anunciación Romero
 M^a Angeles Sánchez
 Ángel Valera

Services

- Preparation of cell culture media
- Routine cell culture procedures
- Washing, sterilisation and replacement of laboratory material



Instrumentation

HEAD

Ismael Gómez López

PERSONNEL

Juan Ignacio Golpe de la Fuente
Carlos González Redondo
Rodrigo López Manzano

Services

- Calibration and validation of scientific instrumentation
- Maintenance and repair of scientific instrumentation
- Technical advice during the acquisition of scientific-technical equipment
- Supervision of the installation of scientific-technical equipment
- User training for scientific-technical equipment



Photography

HEAD

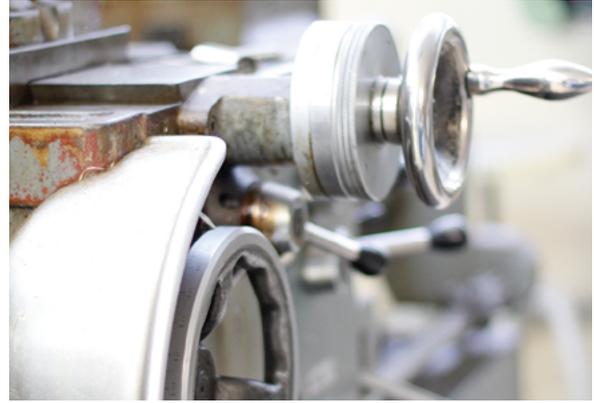
Inés Poveda

The CNB photography service supports scientists with the photographic material necessary for their research and the dissemination of their results.

Photos are taken on a reprographic table with continuous lighting or with studio flashes against an adjustable background, and illumination with white or ultraviolet light, as needed.

The photography service also manages image processing and, when required, photo retouching; digital images are made accessible to clients on dedicated servers.

The service offers digital color printing of large format posters and, on request, also provides advice for graphic and image design.



Radiation protection and biological safety

HEAD

Fernando Usera Mena

PERSONNEL

María Teresa Bartolomé Jiménez
 Aranzazu de la Encina Valencia
 Jessica Gaspar Navarro

Services

- Evaluation of biological, chemical and radiological risks
- Management of official authorisations for research facilities
- Management of the acquisition of radioisotopes and equipment for protection
- Design of laboratories and other facilities potentially exposed to risks
- Issuing of the CNB Basic Guide, the CNB Safety & Health Manual and other safety guidelines and standard operating procedures
- Training of personnel related to specific risks in research laboratories
- Control of the accomplishment of safety and health rules related with research activities
- Management of medical and dosimetry of the exposed personnel in the laboratories
- Management of accidents and emergency situations following established procedures
- Managing of biologic, toxic and radioactive waste

Workshop

HEAD

Daniel Pastora

Services

- Machining metal and plastic parts
- Custom manufacture of metal structures
- Welding and repair of steel carts

Equipment

- Parallel lathe
- Milling machine
- Power welding set
- Spot welding equipment
- Mitre saw
- Reciprocating saw
- Automatic slitter
- Bending machine
- Grinding machine
- Column drilling machine



The unique know-how and cross-disciplinary expertise of CNB's scientists and technologists provides excellent opportunities to transfer leading-edge knowledge and technologies to society and industry. The purpose of the CNB Knowledge Transfer Office (KTO) is to facilitate the process of innovation by:

raising **awareness** among CNB's researchers about the potential socioeconomic impact of their research and facilitating their implication in technology development and innovation,

enhancing the **visibility** of the CNB as a source of transferrable knowledge and partner for industry in the development of innovative technologies, and

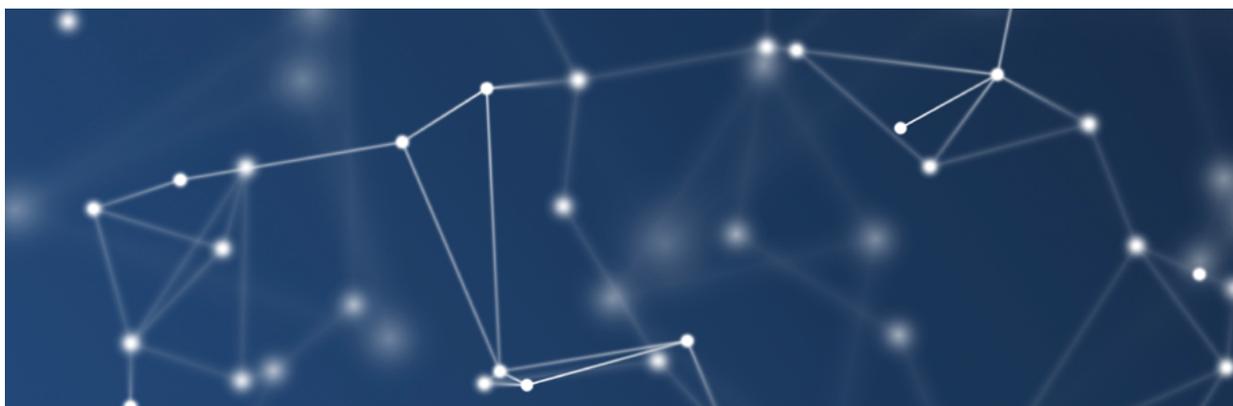
potentiate the centre's innovation **capabilities** across all aspects of knowledge protection, commercialisation and entrepreneurship.

Innovation

KNOWLEDGE TRANSFER MANAGER

Cristina Merino Fernández (since June 2017)

Ana Sanz Herrero (until March 2017)



Awareness

The CNB Knowledge Transfer Office (KTO) organises innovation events to foster the entrepreneurial spirit of CNB scientists and familiarise them with the basic principles and benefits of knowledge transfer. Activities in the 2017-2018 period included round tables and workshops on News on European RTD Funds (Eurostars, Eureka, SME Instrument), Wizard of OTT (Technology Transfer Office) and the centre's Course on Introduction to Research. The KTO participated in the 1st RAFTS4BIOTECH Industrial Event, the Round Table: IPR in different scientific fields within the 100xCiencia.2 conference: Co-creating Value in Scientific Research.

Furthermore, with the aim of showcasing success stories of spin-off companies promoted by scientists from academia, the KTO and the CNB Science Communication and Outreach Office teamed up to organise guided tours to the biotech company NIMGenetics.

Visibility

To enhance the visibility of CNB's technology offer, the KTO has established contacts with stakeholders in the public and private sectors, including INNOMADRID, AINIA, ASAJA, San Carlos Hospital, Infanta Leonor Hospital, TTO-CIRCLE, business angles (WOMENANGELS), investors, REDTRANSFER. KTO has also participated in meetings, seminars, and congresses related with innovation (Innovatia 8.3: Women and Entrepreneur Universities, wegate, ESOF, EIT-HEALTH, FarmaForum, Biospain).

Furthermore, in close collaboration with CNB's Science Communication and Outreach Office, the KTO disseminates the capacities and expertise of CNB scientists in the media and social networks.

Capabilities

The CNB KTO works in close collaboration with the CSIC Deputy Vice-presidency for Knowledge Transfer, covering all aspects of innovation management from the protection of intellectual property to the development and commercialisation of new technologies.

CONTRACTUAL RESEARCH

The KTO supports and manages contractual relations between the CNB and partners in industry. In the 2017-2018 period, these contracts have generated revenues of 2.4 M€.

Major areas of collaboration and industrial partners include:

- Detection of gluten in food (Damm, R-Biopharm, Ingenasa, Operon)
- Production and study of recombinant bacteria, antibodies and proteins for diagnostic and therapeutic purposes (Mediagnost, Biopolis, Synlogic, Genmed, G2 Therapies, eBioscience, Millipore, Alergovet, Ingenasa, Protein Alternatives, Bacmine, Sanofi-Aventis, Bioncotech, Thrombotargets, Landsteiner)
- Vaccine development (Sanofi-Aventis, Labopat, Syva, Ceva Santé Animale)
- Development of applications for clinical analytics (Immunostep)
- Improvement of crop production and resistance to pathogens (Plant Response Biotech, Plant Bioscience Limited, Globachem, Agro Innovation International, Electro Transformación Industrial)
- Electron microscopy and image analysis (FEI Electron Optics, Thermo Fischer Scientific)
- Biotech consulting (Lab Safety Consulting, Elzaburu)

Furthermore, the KTO is in charge of managing Material Transfer Agreements (MTA) with other research institutions and companies all around the world, in which the CNB acts as the material provider in almost half of them. These agreements reflect on the international reputation of the CNB as a provider of leading-edge materials for research in the life sciences.

PUBLIC-PRIVATE RTD ALLIANCES

The KTO is in charge of collecting information about skills, results and activities of research at the CNB with the aim of identifying potential partners for technology transfer and opportunities for joint research projects.

Furthermore, the office also provides CNB researchers relevant and timely information on funding opportunities for



public-private research projects and assists them in grant preparation, contractual and follow-up issues. During the past two years, CNB scientists presented 5 innovative projects in the framework of private-public partnership funding schemes, such as the Retos Colaboración, INTERCONECTA or AECC programmes, and GlaxoSmithKline's Discovery Fast Track Program.

INTELLECTUAL PROPERTY PROTECTION

The KTO provides support in all aspects of intellectual property protection of research results, identifies appropriate business partners for outsourcing the development of new technologies, supervises the activities of license holders, and oversees the payment of royalties.

ENTREPRENEURSHIP

The KTO helps to identify business opportunities and provides advice on the creation of spin-off companies. In 2017 and 2018, the office supported 3 initiatives for the creation of technology-based companies.

TECHNOLOGY OFFER

Patents available for licensing are summarised below. Companies interested in a patent license or investors for the creation of a technology-based company are being sought.

OUTCOME OF THE SUPPORT PROVIDED BY THE KTO IN 2017-2018

Technological support contracts



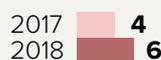
Confidential disclosure agreements



R&D contracts



License agreements



Material transfer agreements (material provider)



Material transfer agreements (material receptor)



Public-private research grants



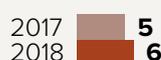
Co-ownership agreements



Inventions

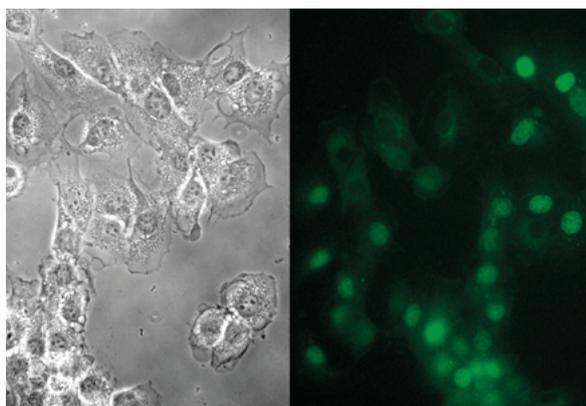


Priority patent applications

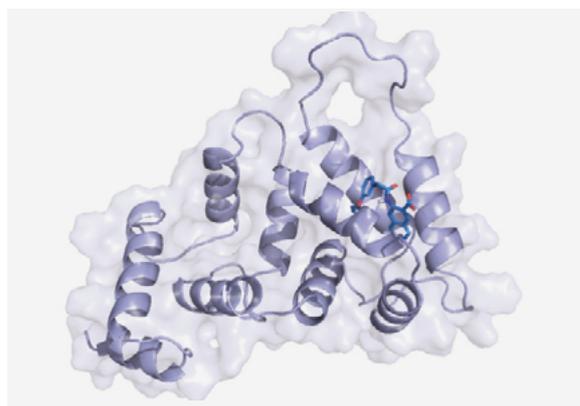


International patent applications





110 INNOVATION



New anti-inflammatory molecules based on natural products of bacterial origin

CSIC and the Autónoma University of Madrid have discovered anti-inflammatory properties in a natural peptide that has its origin in the bacterial cell wall. The structure of this peptide, which differs from the canonical peptidoglycan structure, is only observed during intracellular infections.

CNB INVENTORS

Francisco García del Portillo
Estel Ramos Marquès
Gadea Rico Pérez

APPLICATION NUMBER AND PRIORITY DATE

P201831219, 14/12/2018

MAIN INNOVATIONS AND ADVANTAGES

- The peptide may have less side effects than other steroid-based anti-inflammatory agents.
- It can be potentially used to reduce inflammation in autoimmune disorders and infections caused by other intracellular pathogens.

KChIP2 modulating compounds and their use for the treatment of cardiovascular pathologies

Dr José Ramón Naranjo's research group has developed new compounds able to activate the KChIP2/Kv4 interaction without directly affecting Kv4 channels or other potassium currents.

CNB INVENTOR

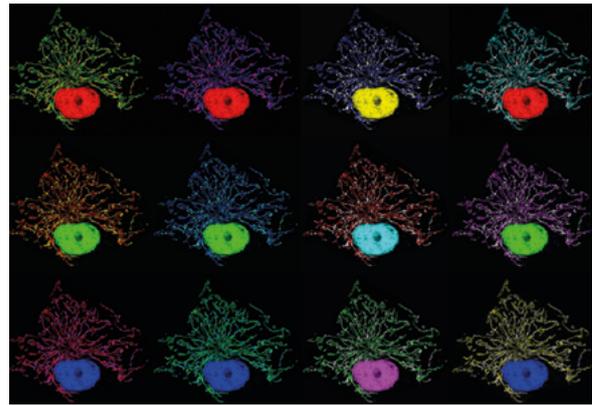
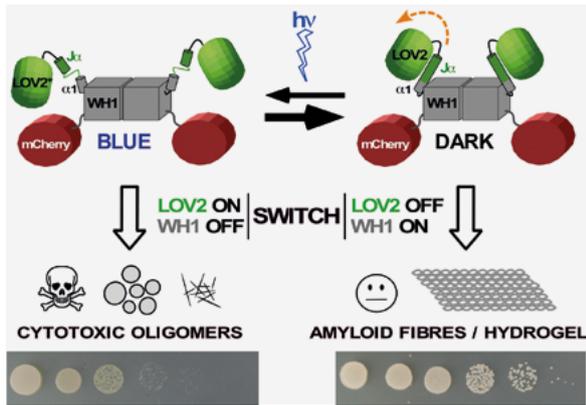
José Ramón Naranjo Orovio

APPLICATION NUMBER AND PRIORITY DATE

EP18382890, 04/12/2018

MAIN INNOVATIONS AND ADVANTAGES

- KChIP2 is the main accessory subunit of Kv4 channels. It is expressed in the heart and regulates voltage-dependent potassium currents (I_{To}) through the interaction with Kv4 potassium channels. The I_{To} current is essential to control cardiac excitability and is reduced in various cardiac pathologies.
- The new KChIP2 modulating compounds activate the KChIP2/Kv4 interaction without interfering with Kv4 channels or other potassium currents.
- These compounds could potentially be useful to treat cardiac pathologies, such as cardiac hypertrophy, arrhythmias, infarct or ischemic failure.



OPTOBIOTICS: novel antibacterial proteins activated by blue light

The group of Dr Rafael Giraldo has developed a chimeric protein that, when expressed in bacteria, changes its structure upon absorption of blue light, which results in the generation of protein aggregates (amyloids) that inhibit bacterial growth.

CNB INVENTOR

Rafael Giraldo Suárez

APPLICATION NUMBER AND PRIORITY DATE

EP18382882.1, 03/12/2018

MAIN INNOVATIONS AND ADVANTAGES

- A synthetic protein (LOV2-WH1) is activated as an anti-microbial by a physical, harmless stimulus: blue light.
- Blue light absorption by LOV2-WH1 stimulates the assembly of anti-bacterial protein particles.
- Mobilisation of LOV2-WH1 through vectors/bacteriophages would enable its usage against a broad spectrum of bacterial pathogens.
- LOV2-WH1 is amenable to photo-therapy approaches, aiming to combat bacterial skin infections or to decontaminate pathogens from surfaces.
- In mixed bacterial populations assembled in an industrial bioprocess, optogenetic activation of LOV2-WH1 can be used to selectively eliminate a specific subpopulation once it has accomplished its task.

New method for detection of fluorescent proteins in cells

Dr Francisco Iborra's research group, in collaboration with scientists from the Autónoma University of Madrid and IMDEA Nanoscience, developed a new method for detection of fluorescent proteins in cells.

CNB INVENTOR

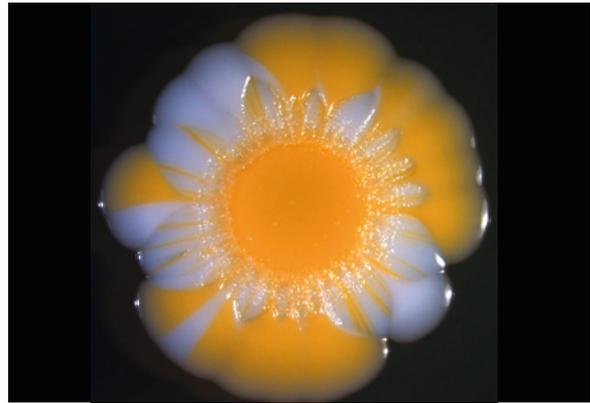
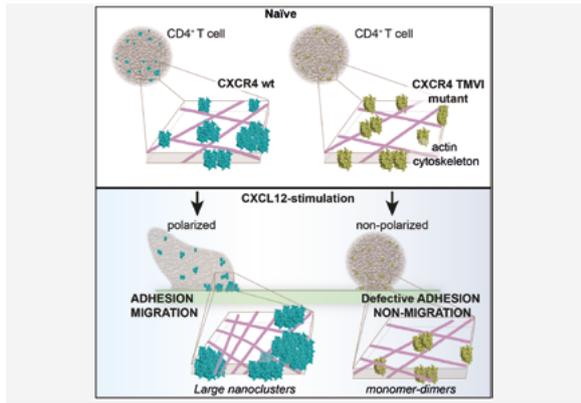
Francisco José Iborra Rodríguez

APPLICATION NUMBER AND PRIORITY DATE

201830775, 27/07/2018

MAIN INNOVATIONS AND ADVANTAGES

- The new method for the detection of fluorescent markers in cells provides high resolution images with less phototoxicity than other approaches available so far.
- The new method is based on the use photoactive nanoparticles and optical tweezers.



112 INNOVATION

Therapeutic targets in chemokine receptors to select useful compounds for the treatment of pathologies that intervene in chemokine responses

CNB researchers have identified a molecular target in the transmembrane VI (TMVI) region of the chemokine receptor CXCR4. This target is now being used to design and detect selective compounds that modulate/antagonise chemokine-mediated function by altering receptor oligomerisation. Antagonists of receptor oligomerisation block cell movement towards chemoattractant gradients, which renders this target potentially useful for altering immune cell infiltration in tissues during autoimmune and inflammatory diseases or metastasis. As CXCR4 is also the main coreceptor for T-tropic HIV-1 viruses, these antagonists may be useful to block HIV-1 infection and to reduce the viral load of AIDS patients.

CNB INVENTORS

Mario Mellado García
 José Miguel Rodríguez Frade
 Laura Martínez Muñoz
 César Augusto Santiago Hernández

APPLICATION NUMBER AND PRIORITY DATE

PCT/ES2018/070484, 05/07/2018

MAIN INNOVATIONS AND ADVANTAGES

- The invention provides a novel approach for the screening of compounds that are potentially useful for the treatment of pathological conditions triggered by chemokine signalling, such as inflammatory and autoimmune diseases, cancer or HIV-1 infection.
- The identification of molecular targets in chemokine receptors/chemokine complexes provides a completely new and effective approach to antagonise specific chemokine responses, opposed to classical strategies based on ligand binding blockade that have failed so far to obtain drugs for clinical purposes.
- The invention has been tested in *in vivo* experiments using a murine model for neutrophil homing to the bone marrow.

Mol. Cell 2018 70:106-119

Overcoming resistance to Beta-lactam antibiotics in Bacterial species

New use of statins for overcoming resistance to beta-lactam antibiotics in bacterial species synthesising isoprenoids using the mevalonate synthetic pathway.

CNB INVENTOR

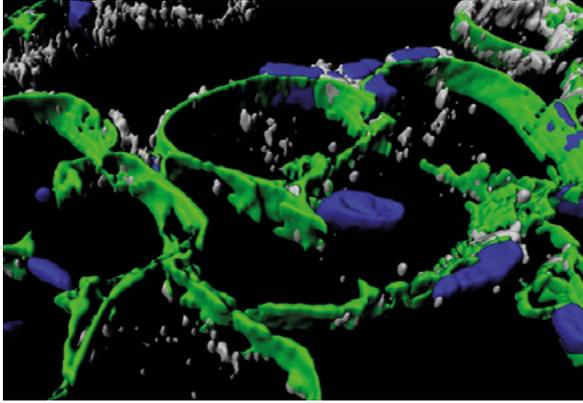
Daniel López Serrano

APPLICATION NUMBER AND PRIORITY DATE

EP17382734, 02/11/2017

MAIN INNOVATIONS AND ADVANTAGES

- This method can be used to eliminate Methicillin-resistant *Staphylococcus aureus* (MRSA) infections that are resistant to multiple antibiotics, which cause hard-to-treat infections in hospitals that sometimes become lethal to the patient. As there are limited strategies to eliminate these infections, this method represents an outstanding possibility to treat patients with complicated infections.
- This method can be used to recycle conventional antibiotics that were discarded because of the spread of antibiotic resistance. Due to the lack of new antibiotics and the necessity to increase the arsenal to fight antibiotic resistance of bacterial infections, recycling conventional antibiotics to fight new multi-drug resistant infections is a promising strategy to reduce the number of infection in clinical settings.



Construction of an attenuated influenza virus and its use as vaccine

Drs Ana Falcón and Amelia Nieto have developed an influenza A virus that contains an amino acid change in the PB2 subunit of the viral polymerase that attenuates the virus *in vivo*, but replicates efficiently in cultured cells. The virus accumulates high amounts of defective viral genomes (DVGs) within its viral particles, and the content of DVGs is related with the antiviral response. Accordingly, the mutant virus elicits an efficient stimulation of the host-response, both in cell cultures and in infected mice. Mice infected with this mutant virus do not lose body weight, indicating that the virus is not pathogenic, although it replicates efficiently in the lungs.

CNB INVENTORS

Ana María Falcón Escalona
Amelia Nieto

APPLICATION NUMBER AND PRIORITY DATE

PCT/ES18/070566, 21/08/2017

MAIN INNOVATIONS AND ADVANTAGES

- The invention applies reverse genetics to generate an attenuated Influenza virus that contains a point mutation in a subunit of the viral polymerase that is stable in the circulating viruses.
- The mutant virus is not pathogenic in mice but replicates efficiently in the lung where it elicits an effective antiviral response.
- These properties make the PB2 mutant virus an ideal candidate for vaccine development.



INNOVATION 113

Bacterium for conversion of plant biomass waste into valuable chemicals

Dr Victor de Lorenzo's group is developing KT2440 strains of robust stress-resistant and safe (GRAS-certified) *Pseudomonas putida* that are able to utilise plant biomass-derived sugars – glucose, cellobiose, and xylose – and convert them into valuable bioplastics, polyhydroxyalkanoates (PHA) and the platform chemical xylonate. Xylonate can be used as a complexing agent, chelator, or a precursor for co-polyamides, polyesters, hydrogels, 1,2,4-butanetriol, ethylene glycol or glycolate, and may also provide a cheap, non-food derived alternative for D-gluconic acid, which is widely used (about 80 kton/year) in industry.

CNB INVENTORS

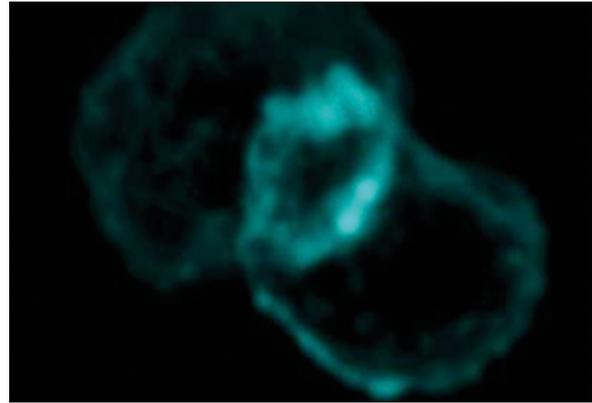
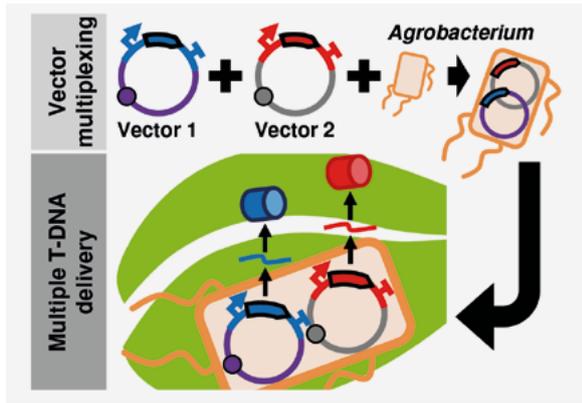
Pavel Dvorak
Víctor de Lorenzo Prieto

APPLICATION NUMBER AND PRIORITY DATE

PCT/EP2018/068347, 06/07/2017

MAIN INNOVATIONS AND ADVANTAGES

- Engineered strains with reduced genome show better physiological vigour, higher ATP and NADPH availability, higher resistance to oxidative stress, and more stable heterologous gene expression when compared with wild-type *Pseudomonas putida*.
- The new *Pseudomonas putida* strain EM42 β grows rapidly on cellobiose, converts this sugar into PHA and, in addition, oxidizes xylose to xylonate. Thus, two desirable bioproducts are obtained in parallel.
- Xylonate is also produced from xylose with high yield (up to 0.97 g g⁻¹) by non-growing *Pseudomonas putida* whole cells which can be recycled to reduce process cost.
- An engineered *Pseudomonas putida* strain that can co-utilise xylose with glucose and cellobiose is already available, and new strains that can co-utilise multiple plant biomass-derived sugars and convert them into valuable chemicals are under development.



114 INNOVATION

Next-generation binary T-DNA vectors for (plant) synthetic biology

Researchers at the CNB and the Polytechnical University of Valencia have jointly developed an expression vector system that provides a flexible framework for plant biotechnology and synthetic biology applications. The system includes novel mini binary vectors with compatible origins. Vectors have been used successfully in plant transient and stable expressions, CRISPR/Cas9-targeted genome mutagenesis, and for genetic circuit component and viral expression vector delivery.

CNB INVENTORS

Fabio Pasin
Juan Antonio García Álvarez
Carmen Simón Mateo

APPLICATION NUMBER AND PRIORITY DATE

PCT/ES18/070421, 12/06/2017

MAIN INNOVATIONS AND ADVANTAGES

- Compactness: the reduced size (< 3.8 kb) of the vectors facilitates cloning and reverse genetic studies.
- Flexibility: vectors autonomously replicate in *Escherichia coli* and *Agrobacterium*.
- Stability: vectors include bacterial terminators that avoid transcriptional read-through to T-DNA cassettes and help to stabilise large/complex inserts.
- Scalability: vectors are suitable for standard as well as high-throughput cloning methods (e.g. Golden Gate, GoldenBraid, and Gibson assembly)
- Standardisation: vectors meet current plant synthetic biology standards and allow the use of publicly available DNA parts libraries.
- Compatibility: vectors can be multiplexed with binary vectors commonly used by plant scientists for multi T-DNA delivery in a two-vector/one-strain expression approach.

Transphagocytic T cells as anti-cancer immunotherapy

Dr Esteban Veiga's research group, in collaboration with scientists from the Centre of Molecular Biology "Severo Ochoa" (CBMSO-CSIC), the Autónoma University of Madrid and the Health Research Institute of the "La Princesa" University Hospital, developed a new method for anti-cancer immunotherapy based on transphagocytic lymphocytes (tiCD4+ T cells).

CNB INVENTOR

Esteban Veiga Chacón

APPLICATION NUMBER AND PRIORITY DATE

Patent filed in USA, Canada, Australia and EU, 7/8/2015

MAIN INNOVATIONS AND ADVANTAGES

- The invention can be used to prevent/treat tumours and/or stimulation of an immune response against tumour antigens.
- tiCD4+ T cells (trained by bacteria) are newly defined antigen-presenting cells that can be useful as a cancer immunotherapy tool.
- tiCD4+ T cells mediated antigen presentation potently cross-prime naive CD8+ T cells.
- tiCD4+ T cells mediated antigen presentation generates central memory C8+ T cells with very low levels of PD-1.
- The anti-tumour activity of tiCD4+ T cells has been tested in mouse melanoma models. The invention can be applied for melanoma and other highly immunogenic tumours.

The Communication and Outreach Office works to increase the awareness of the research carried out by CNB scientists and to strengthen our bonds with other academic institutions, private partners of the industrial biotechnology sector, as well as journalists and the media.

During 2017 and 2018, several press releases have been issued regarding the scientific achievements by CNB researchers in our continuous effort to establish relations with the media.

A new CNB Blog has been implemented by the office in 2017 as a channel to keep society informed of the centre's latest news. The office also maintains a dialogue with the public through the social networks, which have increased their communities to 2,800 and 12,500 followers on Facebook and Twitter, respectively.

The presence of CNB in national and international outreach events has consolidated in the last two years. With the dedicated and indispensable involvement of the centre's scientists, the office has coordinated activities in the framework of the European Researcher's Night, the National Science and Technology Week and the celebration of the International Day of Women and Girls in Science (11 February). The office also coordinates monthly guided visits for secondary school students.

The office acts as a liaison in the organisation of the annual CNB Seminar Series, CNB Scientific Workshop, PhD Students' Workshop, Advances in Molecular Biology by Young Researchers Abroad Workshop, and the CNB Course on Introduction to Research. In addition, the office collaborates in arranging the training activities for PhD students organised by the CNB Training Advisory Committee, and the Innovation Events conducted by the Knowledge Transfer Office.

Finally, the CNB participated in 100XCiencia 2 and 3 meetings, the international science communication forum organised by the Severo Ochoa and María de Maeztu Centres and Units of Excellence (SOMMA). We are particularly indebted to Julia García, who managed the office for most of the last two years period, for her invaluable contributions to increase the centre's visibility and improve the quality of its outreach and training activities. We would also like to acknowledge Dr Miguel Vicente's support, advice and involvement in the office's activities.

Communication and Outreach

COMMUNICATION AND OUTREACH MANAGER

Susana de Lucas (since October 2018)

Julia García (until July 2018)

Media appearances



118 COMMUNICATION AND OUTREACH



Diario Médico, 17-05-2017



SINC, 01-06-2017



Diario Médico, 06-03-2017



Vozpopuli, 09-05-2017



Cadena Ser, 10-12-2017



El País, 17-07-2017

Social media followers

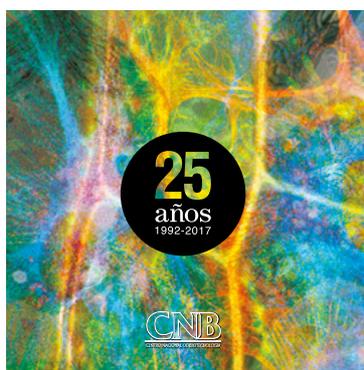
			
12,500	2,800	1,740	140

120 COMMUNICATION AND OUTREACH

Publications



Blog CNB Divulga. Since 2017 we have published more than 20 blog posts sharing news, interviews and life at CNB with the aim to promote scientific culture and interest in society.



CNB 25th Anniversary celebrations included the edition of the brochure "25 years (1992-2017)" illustrating CNB research since its opening.



CNB Newsletter. From 2016, we have issued a regular newsletter with relevant information from CNB. We have more than 500 subscribers.

 <p>Structure of phage proteins: Mark J. van Raaij 467 visualizaciones • Hace 1 año Subtítulos</p>	 <p>Microscopía confocal: Sylvia Gutiérrez Erlandsson 539 visualizaciones • Hace 1 año Subtítulos</p>	 <p>Sistemas evolutivos: Susanna Manrubia 289 visualizaciones • Hace 1 año Subtítulos</p>	 <p>Laboratorio de nivel 3 de contención biológica: 773 visualizaciones • Hace 1 año Subtítulos</p>	 <p>Bioinformática para genómica y proteómica: Juan 720 visualizaciones • Hace 1 año Subtítulos</p>
 <p>Criomicroscopía electrónica: Rocío Arranz 541 visualizaciones • Hace 1 año Subtítulos</p>	 <p>Biotecnología para optimizar procesos industriales: Daniel 387 visualizaciones • Hace 1 año</p>	 <p>La proteína p38 en enfermedades inflamatorias 716 visualizaciones • Hace 1 año</p>	 <p>Plantas para descontaminar el medioambiente: Antonio 713 visualizaciones • Hace 1 año</p>	 <p>Inmunoterapia contra el cáncer: Esteban Veiga 1 mil visualizaciones • Hace 1 año</p>

10 minivideos explaining our researchers' work were produced with the support of FECYT and have received more than 6,000 views in a year.

Outreach activities

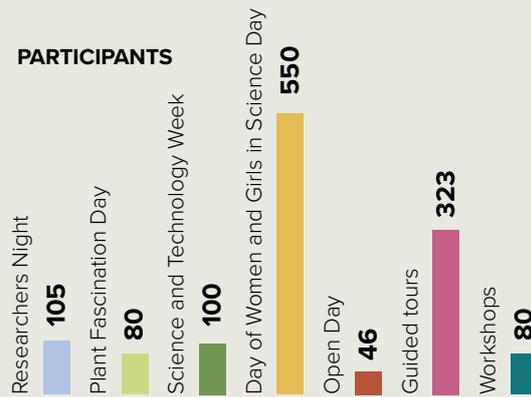


Guided visits for secondary school students. More than 300 students visited our installations in 2017 and 2018, including students from schools in the new CSIC program "Ciencia en el Barrio".



As part of the 2017 **National Science and Technology Week**, we held an **Open day** visited by more than 50 people.

PARTICIPANTS



COMMUNICATION AND OUTREACH 121



European Researchers' Night 2018. "Tricks not to get lost in a sea of information" More than 20 researchers from CNB collaborated in this event with talks and practical workshops to exercise critical spirit and to recognise (mis)information.



Workshop to bridge science and society.



2018 International Day of Women and Girls in Science. In collaboration with RNE and Principe de Asturias School, we recorded a radio program with interviews to researchers women from CNB. In addition, CNB researchers collaborated in the activity "Adventurous women researchers" held in "La Casa Encendida" reaching more than 300 primary school students.



Brain Awareness Week

In the 2017-2018 period, our centre hosted 98 undergraduate, 71 master's and 168 PhD students. 35 PhD students received competitive fellowships (e.g., INPhINIT, FPU, FPI) to perform their PhD theses at our institute, and 47 students obtained their PhD degree under the supervision of a CNB scientist.

We are making continuous efforts to attract young people who wish to pursue a scientific career. For instance, in summer 2017 and 2018, we organised the 5th and 6th edition of the "CNB course on introduction to research", providing undergraduate students first-hand experience in biotechnology research. In collaboration with the CSIC and funding from the Severo Ochoa Centres of Excellence Program, we offered fellowships to attract brilliant master's students, and our researchers showcased the centre's outstanding training opportunities through their participation in numerous university and master's degree programs.

Over the past two years, we have consolidated our PhD training program, launched in 2014 as part of the Severo Ochoa Centres of Excellence Program. The PhD Student's Committee, together with the Training Advisory Committee and with the invaluable help of our Science Communication and Outreach Office (Julia García) organised predoctoral scientific workshops, new PhD student's welcome events, as well as courses fostering public presentation skills or teaching how to write a scientific paper; and Lluís Montoliu spearheaded the implementation of an interactive workshop on ethics and integrity in research.

A rich program of seminars, conferences, workshops and courses, more than 300 in the 2017-2018 period, provide optimal opportunities for our researchers to keep up with the latest advances in biotechnology. Highlights from the past two years include workshops on plant systems biology (Pilar Cubas and Antonio Leyva) and genomic editing in bacteria (Laura Cueto and Miguel Vicente), the launch of the CNB Severo Ochoa "Challenges in Life Sciences" workshop series with the topic "Redefining disease" (Susanna Manrubia) and the organisation of a crash course on how to manage a biotech startup (Ana Sanz).

Training

SCIENTIFIC ACTIVITIES COMMITTEE

Juan Carlos Alonso
Antonio Leyva
Florencio Pazos
Hugh T. Reyburn
Juan José Sanz
José María Valpuesta

TRAINING ADVISORY COMMITTEE

Yolanda R. Carrasco
Vicente Rubio
Juan José Sanz
Javier Tamames
Mark Van Raaij
Miguel Vicente

PhD STUDENTS COMMITTEE

Alejandro Asensio
Sara de Bernardo
María Teresa Bueno
Lorena Bragg
Marcos Gragera
Noelia de León
Jesús Ogando
Andrés Ortigosa
Elena Sánchez
Adriana Sanz
Jesús Vallejo



PhD fellowships

2 “LA CAIXA” PhD FELLOWSHIPS

“LA CAIXA” FOUNDATION

Moisés Maestro López

Andrés Paris Muñoz

4 INTERNATIONAL INPHINIT FELLOWSHIPS

“LA CAIXA” FOUNDATION

Lorena Bragg Gonzalo

César Omar Domínguez Márquez

Natalia González Mancha

David Strelak

5 FPU FELLOWSHIPS

MINISTRY OF EDUCATION,
CULTURE AND SPORT

Álvaro Ceballos Munuera

Ester Díaz Mora

Andoni Gómez Moreno

Pablo Laborda Martínez

Micaela Andrea Navarro
Correa

24 FPI FELLOWSHIPS

MINISTRY OF ECONOMY
AND COMPETITIVENESS &
MINISTRY OF ECONOMY,
INDUSTRY AND
COMPETITIVENESS

Guillermo Albericio Bonilla

Esther Cañibano Morejón

María del Pilar Fajardo Flores

Alberto Fernández Oliva

Alberto Fuster Pons

José Gallardo Hernanz

Raquel García Ferreras

Natalia García García

Guillermo Gómez García

Alfonso González de Prádena

Diego González Romero

Alberto Iniesta Saiz

Diego Esteban Jiménez Lalana

Aleksandra Lazarova

David López Escarpa

Bran López Luengo

Andrea Montero Atalaya

Javier Mota García

Lisette Ochoa Ibarrola

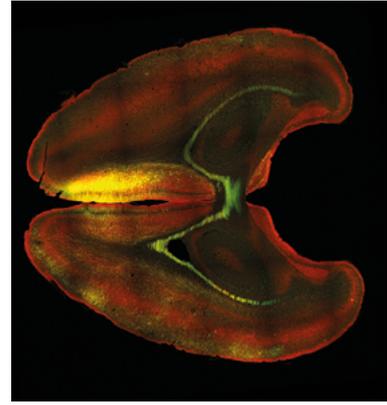
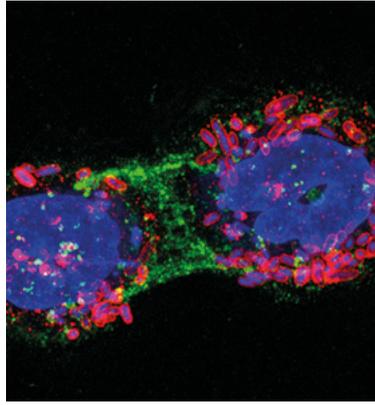
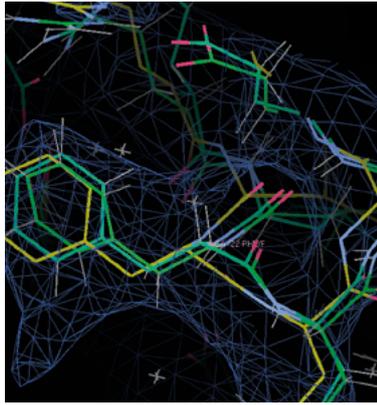
Cristina Ramos Andrades

Miriam Sánchez Ortega

Adrián Vega Pérez

Gonzalo Vígara Astillero

Pablo Yubero Bernabé



Doctoral theses

In 2017 and 2018, 47 students obtained the PhD degree under the supervision of CNB researchers.

2017

AMR ABDELMOTAGALY NASSRALLAH

Target destabilization and chromatin remodelling are coordinated by CRL4-CDDD E3 ubiquitin ligases to repress photomorphogenesis in *Arabidopsis thaliana*.

(Vicente Rubio)

MANUEL ALCALDE RICO

Efecto de la sobre-exposición de los sistemas de bombeo múltiple de drogas, MexAB-OprM y MexCD-OprJ, sobre el sistema de señalización por quorum sensing de *Pseudomonas aeruginosa*.

(José Luis Martínez and Jorge A. Olivares)

ELENA ANDRADA ROMERO

Papel diferencial de las diacilglicerol quinasas alfa y zeta en el control de la respuesta T citotóxica: implicaciones para la inmunoterapia antitumoral.

(Isabel Mérida)

LORENA CARMONA RODRÍGUEZ

Función de la superóxido dismutasa extracelular (SOD3) en el endotelio tumoral. Implicaciones sobre la eficacia de la quimioterapia y la migración de linfocitos T.

(Santos Mañes and Emilia Mira)

FERNANDO CORONA PAJARES

Impacto del regulador global Crc en la fisiología de *Pseudomonas aeruginosa*.

(José Luis Martínez)

EDUARD CRUZ ORÓ

Control of potato tuberization by Gl and proteins of the FT family.

(Salomé Prat)

LILIANA LILIBETH CUBAS GAONA

Contribución del interferón a la citopatogenicidad causada por el virus de la bursitis infecciosa (IBDV).

(Dolores Rodríguez and Francisco Rodríguez)

JOSÉ MIGUEL DE LA ROSA TREVÍN

Scipion: a software framework toward integration, reproducibility and validation in 3D Electron Microscopy.

(Roberto Marabini and José María Carazo)

JORGE DOMÍNGUEZ ANDRÉS

Papel de los monocitos frente a infección sistémica por *Candida albicans*.

(Carlos Ardavín)

ARAÍZ GALLO GALLARDO

Conexiones entre la replicación del RNA, su empaquetamiento y la proteína HC en la infección de un potyvirus.

(Juan Antonio García and Adrián Valli)

CARLOS GARCÍA BRIZ

Control transcripcional de las redes neuronales en la corteza somatosensorial.

(Marta Nieto)

YOVANNY IZQUIERDO NÚÑEZ

La proteína NOXY7 de *Arabidopsis thaliana* regula la activación de la inmunidad y la adaptación a estrés a través de una ruta no canónica de control de síntesis de proteínas.

(Carmen Castresana)

SHEILA LÓPEZ COBO

Ligandos de NKG2D: nuevos aspectos sobre su bioquímica, su papel en interacciones celulares y su modulación por fármacos antitumorales.

(Mar Valés-Gómez)

CÉSAR LÓPEZ PASTRANA

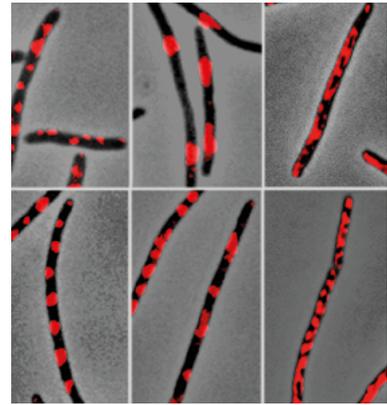
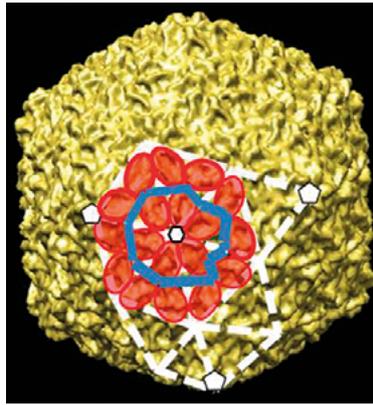
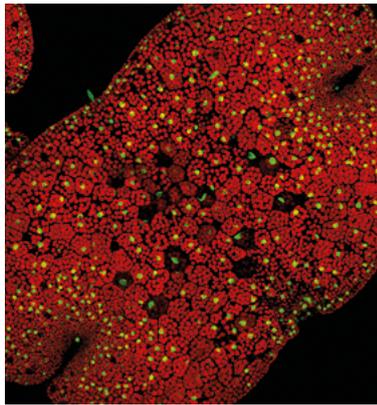
Magnetic Tweezers: Applications to the study of DNA-protein interactions in DNA-replication, condensation and segregation.

(Fernando Moreno-Herrero)

ERNESTO MEJÍAS

Vectores virales basados en poxvirus como agentes oncolíticos.

(Mariano Esteban)



ISABEL MONTE GRONDONA

Evolutionary divergence in the bioactive jasmonate in land plants.

(Roberto Solano)

MARÍA LUCÍA MORALES RODRÍGUEZ

Contribución de RNAs no codificantes pequeños del hospedador y del virus a la patología pulmonar inflamatoria causada por el coronavirus del síndrome respiratorio agudo y grave.

(Luis Enjuanes and Isabel Sola)

CLARA MORENO FENOLL

How genetic, social, and evolutionary interactions shape the many levels of biological complexity.

(Juan Poyatos)

PILAR MUÑOZ RUIZ

Estudio de la penetración intratubular, áreas de vacío y la filtración bacteriana de un material biocerámico.

(José Luis Martínez and Rafael Cisneros)

LUCIA QUINTANA GALLARDO

Structural insights into the chaperone-dependent ubiquitin/proteasome protein degradation pathway.

(José María Valpuesta and Jaime Martín-Benito)

GUILLERMO RAMÍREZ

Descripción de la transfagocitosis, una vía para la captura de bacterias y la presentación antigénica por parte de los linfocitos T CD4+: posibles aplicaciones en biomedicina.

(Esteban Veiga and Aranzazu Cruz Adalia)

UMBERTO ROSATO

Dissecting the role of Gadd45b in hepatocellular carcinoma development.

(Jesús Salvador)

ALBERTO SÁNCHEZ-PASCUALA JEREZ

Deep refactoring of central carbon metabolism in the soil bacterium *Pseudomonas putida*.

(Victor de Lorenzo and Pablo Iván Nike)

RAHMAN SHOKRI

p21 regulates IFN- γ production and autoimmunity by controlling recurrent T cell activation.

(Dimitrios Balomenos)

MARÍA TELLO-LAFOZ

Role of SNX27 in protein transport and lipid signaling in cell models of polarized trafficking.

(Isabel Mérida)

CLAUDIA VASALLO VEGA

Estudio sistemático sobre las funciones celulares del Receptor Sigma-1: implicaciones para la infección por el virus de la hepatitis C.

(Pablo Gastaminza)

2018

ALFONSO BLÁZQUEZ MORENO

Insights into the genetics and biochemistry of signaling adaptor modules and NK cell receptors from study of primary immunodeficiency.

(Hugh Reyburn)

CARLOS CASTAÑO RODRÍGUEZ

Papel de las viroporinas E, 3a y 8a del coronavirus del SARS en replicación y virulencia.

(Luis Enjuanes)

SURESH CHITHATHUR RAMAN

Enhancing B and T cell immune responses against the HIV-1 envelope using protein and poxvirus-based vaccines.

(Mariano Esteban)

RAMÓN CONTRERAS DE LUNA

La partición núcleo/citoplásmica de IYO como interruptor binario de la diferenciación.

(Enrique Rojo and Maite Sanmartín)

LAURA CUETO BURDIEL

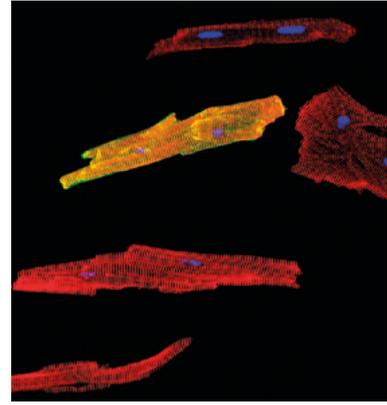
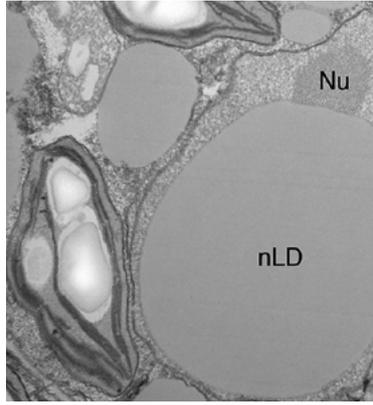
The effects of the synthesis and localization of ZipA, an essential component of the *Escherichia coli* divisome, on division and membrane dynamics.

(Miguel Vicente)

LIDIA FEO LUCAS

Estudio de los macrófagos alveolares durante las reacciones alérgicas.

(Carlos Ardavín and María López-Bravo)

**RUBÉN FERNÁNDEZ SANTOS**

Proteómica de cuerpos lipídicos en la senescencia y la infección por *Pseudomonas syringae* en *Arabidopsis thaliana*.

(Carmen Castresana)

PATRICIA HERNÁNDEZ FLORES

Estudio de los efectos de la aplicación de un campo magnético alterno sobre células cargadas con nanopartículas magnéticas de óxido de hierro utilizando diferentes aproximaciones experimentales.

(Domingo F. Barber)

DIEGO HERRERO ALONSO

Relevancia de los progenitores cardíacos Bmi1+ asociados a un nicho vascular con bajo estrés oxidativo.

(Antonio Bernad)

GUILLERMO MARTÍN GUTIERREZ

Effect of urinary tract physiological conditions on quinolones and fosfomicin activity against *Escherichia coli*.

(Jesús Blázquez and Jerónimo Rodríguez)

ADA MARTÍNEZ AYALA

Participación de las mitocondrias en la respuesta de defensa de *Arabidopsis thaliana* frente a la infección de microorganismos patógenos.

(Carmen Castresana and Yovanny Izquierdo)

FERNANDO MÉNDEZ HERNÁNDEZ

Estudio de la función y localización de la proteína no estructural VP5 del virus de la bursitis infecciosa.

(Francisco Rodríguez Aguirre)

FRIDA MESEL

microRNAs artificiales como fuente de resistencia antiviral y diversidad.

(Carmen Simón Mateo and Juan Antonio García)

JESÚS OGANDO CASTRO

La regulación de la función efectora de macrófagos por miR-223 y de linfocitos T CD8+ por PD-1.

(Santos Mañes and Rosa Ana Lacalle)

ANA ISABEL RODRÍGUEZ

Bases moleculares de la hiperrecombinación en cepas patógenas de *Escherichia coli*.

(Jesús Blázquez and Jerónimo Rodríguez)

HECTOR ROMERO GONZÁLEZ

Single-molecule dynamics in protein interactions: characterization of RarA and RecD2 of *Bacillus subtilis*.

(Juan Carlos Alonso and Peter Graumann)

GUILLERMO RUANO BLANCO

Caracterización funcional de las proteínas MTV9 y MTV11 y su implicación en el tráfico vacuolar.

(Enrique Rojo and Jan Zouhar)

DIONE SÁNCHEZ HEVIA

Estudio de los regulones de las proteínas Hfq y Crc de *Pseudomonas putida*, y de la expresión del ARN pequeño CrcZ que controla la actividad de estas proteínas.

(Fernando Rojo and Renata Moreno)

ESTER SERRANO ÁLVAREZ

Bacillus subtilis RecA accessory proteins at the stage of homology search during natural transformation.

(Juan Carlos Alonso and Begoña Carrasco)

HONGYING SHAN

Characterization of P1 leader proteases of the *Potyvirus* family and identification of the host factors involved in their proteolytic activity during viral infection.

(Juan Antonio García and Bernardo Rodamilans)

JESÚS VALLEJO DÍAZ

Papel de la proteína p85 β en el cáncer de células escamosas de pulmón.

(Ana Clara Carrera)

JUNIOR SEMINARS

XI CNB JUNIOR SEMINAR SERIES 2017/2018

- NOVEMBER 17 **Patricia Osseweijer**
Delft University of Technology, The Netherlands
- DECEMBER 1 **Nathalie Leonhardt**
Institut de Biosciences et Biotechnologies d'Aix-Marseille (IBBM), France
- JANUARY 19 **Rudi W. Hendriks**
Erasmus MC, Rotterdam, The Netherlands
- FEBRUARY 2 **Gijs J. L. Wuite**
Vrije Universiteit Amsterdam, The Netherlands
- APRIL 20 **Veronica Grieneisen**
John Innes Centre, Norwich, UK
- MAY 4 **Paula da Fonseca**
MRC Laboratory of Molecular Biology, Cambridge, UK
- MAY 25 **Caroline Gutjahr**
Ludwig-Maximilians-Universität München, Germany
- JUNE 15 **Karla Kirkegaard**
Stanford University School of Medicine, USA

12.00h
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SEMINARS

XXII CNB SEMINAR SERIES 2017/2018

- SEPTEMBER 29 **Fernando Martín**
IMDEA Nanociencia, GMA, Madrid, Spain
- NOVEMBER 3 **Áitor Hierro**
OCINGENE, Burgos, Spain
- FEBRUARY 9 **Eduard Batlle**
IBB Barcelona, Barcelona, Spain
- FEBRUARY 16 **Carles Úbeda Morant**
FISABIO, Valencia, Spain
- MARCH 16 **Friedrich Foester**
Bijvoet Center for Biomolecular Research, Utrecht University, The Netherlands
- MARCH 23 **Luca Guidotti**
Ospedale San Raffaele, Milan, Italy
- APRIL 27 **Florian Greten**
Institute for Tumor Biology and Experimental Medicine, Frankfurt, Germany
- MAY 18 **Ian Rosenshine**
The Hebrew University of Jerusalem, Israel
- JUNE 1 **Bernard Malissen**
Centre d'Immunologie de Marseille-Luminy, Marseille, France
- JUNE 2 **Isabel Allona Alberich**
CSG UPM-INIA, Madrid, Spain
- JUNE 22 **Manuel Rodríguez Concepción**
CRAG CSIC-IRTA-UIB-UB, Barcelona, Spain

12.00h
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JUNIOR SEMINARS

XII CNB JUNIOR SEMINAR SERIES 2018/2019

- SEPTEMBER 23 **Francesco Pedaci**
Principal Investigator, Centre de Biochimie Structurale de Montpellier, France
- OCTOBER 26 **Moussa Benhamed**
Institute of Plant Science Paris-Saclay, France
- NOVEMBER 15 **Ulrich Keyser**
University of Cambridge, UK
- NOVEMBER 23 **Karla Kirkegaard**
Stanford University, USA
- JANUARY 11 **Tom Bennett**
University of Leeds, UK
- FEBRUARY 8 **Arjen Jakobi**
Kavli Institute, Delft, The Netherlands
- FEBRUARY 22 **Mariana Gomes de Pinho**
Principal Investigator, Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Portugal
- MARCH 8 **Magdalena Zernicka-Goetz**
University of Cambridge, UK
- MARCH 15 **Benedikt Berninger**
King's College London, UK
- MAY 19 **Benjamin D. Towbin**
Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland
- JUNE 14 **Jan Lammerding**
Weill Institute for Cell and Molecular Biology, Cornell University, USA

12.00h
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Centro Nacional
de Biotecnología



SEMINARS

XXIII CNB SEMINAR SERIES 2018/2019

- SEPTEMBER 25 **Richard Henderson**
Laboratory of Molecular Biology, Cambridge, UK, Nobel Prize in Chemistry 2017
- NOVEMBER 30 **Maria Antonia Llamas**
Estación Experimental de Zaidín, Granada, Spain
- FEBRUARY 1 **Nick Blackburn**
Borås Aps, Kivstgård, Denmark
- MARCH 1 **Manuel Talón**
Director Centro de Genómica, IVA, Moncada, Valencia, Spain
- MARCH 22 **Stefan Rausner**
Director, Max Planck Institute of Molecular Physiology Dortmund, Germany
- MARCH 29 **Mike Skinner**
Imperial College London, UK
- APRIL 5 **Diarmuid Hughes**
Uppsala University, Sweden
- APRIL 12 **Malcolm Bennett**
School of Biosciences, Division of Plant Sciences, University of Nottingham, UK
- APRIL 26 **Lydia Sorokin**
Institute of Physiological Chemistry and Pathobiochemistry, University of Münster, Germany
- MAY 17 **Jochen Schwenk**
School of Biotechnology, KTH Royal Institute of Technology Stockholm, Sweden
- MAY 24 **Roberto Chiarle**
Boston Children Hospital, Harvard Medical School, USA

12.00h
CONFERENCE ROOM
Centro Nacional
de Biotecnología
www.cnb.csic.es
91265400

CNB
Centro Nacional
de Biotecnología





Scientific meetings and courses

In 2017 and 2018, the CNB hosted 246 seminars, including talks by 41 speakers from internationally renowned institutions who presented their latest findings in the CNB Seminar Series and the CNB Junior Seminar Series.

Furthermore, CNB scientists participated in the organisation of more than 100 conferences, workshops and courses.

2017

14 JANUARY 2017

AUSTIN, USA

DABE pre-conference symposium at the 43 annual conference of the IETS

Lluís Montoliu

16 JANUARY 2017 (CNB)

V Colloquium of the systems and synthetic biology program on multi-scale biological design

Víctor de Lorenzo

24-27 JANUARY 2017

SANTIAGO DE CHILE, CHILE

Workshop “Análisis estructural de proteínas y complejos macromoleculares mediante técnicas de microscopía electrónica y procesamiento de imagen”

José María Valpuesta

27 FEBRUARY - 2 MARCH 2017

BADALONA, SPAIN

GEIVEX/REDIEX workshop on isolation and characterization of extracellular vesicles obtained from different biological fluids

Mar Valés-Gómez

27-28 MARCH 2017 (CNB)

Madrid meeting on dendritic cells and macrophages 2017

Carlos Ardavín

3-7 APRIL 2017

PRAGUE, CZECH REPUBLIC

2nd Programmable nucleases (CRISPR/Cas9) transgenesis course

Lluís Montoliu

7 APRIL 2017 (CNB)

Workshop mycobacterium: molecular microbiology

Susanne Gola and Miguel Vicente

5 MAY 2017 (CNB)

25th Anniversary scientific meeting

Fernando Rojo, Mario Mellado and Julia García

2 JUNE 2017 (CNB)

Technical workshop

Noelia Sofia de Leon

9 JUNE 2017 (CNB)

II Workshop by CNB PhD students

CNB PhD Students Junior Committee and Julia García

12-16 JUNE 2018 (CNB)

VII Curso de proteómica cuantitativa

Alberto Paradela

3-28 JULY 2018 (CNB)

V Curso de introducción a la investigación

Julia García

10 JULY 2017 (CNB)

NanoBIOSOME: design, development and production of nanocontainers and nanovehicles

José María Valpuesta

11 JULY 2017 (CNB)

CIBERER practical course on CRISPR genome-editing techniques in human rare diseases

Lluís Montoliu

31 AUGUST - 1 SEPTEMBER 2017 (CNB)

EUSynBioS symposium 2017: engineering biology for a better future

Huseyin Tas

3-6 SEPTEMBER 2017

VALENCIA, SPAIN

XIV Solanaceae and III cucurbitaceae genomics joint conference

Pilar Cubas



4-8 SEPTEMBER 2017

LYON, FRANCE

Workshop on living architectures. 14th European conference on artificial life 2017

Juan Nogales

6-9 SEPTEMBER 2017

CARDIFF, UNITED KINGDOM

2nd European chemokine and cell migration conference

Mario Mellado

10-14 SEPTEMBER 2017

JERUSALEM, ISRAEL

Symposium "Molecular machines in action" at the 42nd FEBS congress

José María Valpuesta

17 SEPTEMBER 2017

DUBLIN, IRELAND

Human Proteome Project - principal investigator's meeting

Fernando Corrales

22 SEPTEMBER 2017 (CNB)

CNB Severo Ochoa workshop series "Challenges in life sciences"

Susanna Manrubia

22-24 SEPTEMBER 2017

TERUEL, ESPAÑA

XI Jornadas ALBA sobre albinismo

Lluís Montoliu

24 SEPTEMBER - 1 OCTOBER 2017

HEIDELBERG, GERMANY

EMBO practical course synthetic biology in action: programming bacteria to do amazing things

Víctor de Lorenzo

1-4 OCTOBER 2017

SALT LAKE CITY, USA

14th Transgenic technology meeting

Lluís Montoliu

5-6 OCTOBER 2017

BARCELONA, SPAIN

2nd Meeting of the Spanish adenovirus network

Carmen San Martín

5-6 OCTOBER 2017 (CNB)

2nd Meeting of the B cell network (Net-B)

Yolanda Carrasco and Ignacio Moreno de Alborán

9-10 OCTOBER 2017

SEGOVIA, SPAIN

Workshop on systems biology 2017

Jacobo Aguirre

22-26 OCTOBER 2017

AWAJI CITY, JAPAN

20th International congress on "Calcium-binding proteins and calcium function in health and disease"

José R. Naranjo

23 OCTOBER 2017

BARCELONA, SPAIN

2nd Emerging scientist workshop at the XL SEBBM congress

Fernando Moreno

23-26 OCTOBER 2017

LA LAGUNA, SPAIN

Workshop on annotation of transporters

Javier Tamames

24-27 OCTOBER 2017

GIJÓN, SPAIN

Joint SEBD/SEG/SEBC congress 2017

Pilar Cubas

27 OCTOBER 2017 (CNB)

25th Anniversary scientific meeting

Fernando Rojo, Mario Mellado and Julia García

30 OCTOBER - 1 NOVEMBER 2017

MOSCOW, RUSSIA

Clinical proteomics - postgenome medicine

Fernando Corrales

13 NOVEMBER 2017

PARIS, FRANCE

Fostering responsible research with CRISPR-Cas9

Lluís Montoliu

13-15 NOVEMBER 2017

DERIO, SPAIN

GEIVEX/REDIEX workshop on bioinformatics tools to study exosomes' effects

Mar Valés-Gómez

28-29 NOVEMBER 2017

BILBAO, SPAIN

VIII Reunión científica de proteómica clínica

Fernando Corrales



18 DECEMBER 2017 (CNB)

XXV CNB Scientific workshop
Julia García

20 DECEMBER 2017 (CNB)

XXV Workshop Advances in Molecular Biology by Young Researchers Abroad
Domingo F. Barber, Mar Valés-Gómez and Julia García

2018

17-19 JANUARY 2018 (CNB)

Instruct course on image processing for electron microscopy in the cloud
José María Carazo

19 JANUARY 2018

MADRID, SPAIN
VIII Jornada científica de la Sociedad de Inmunología de la Comunidad de Madrid (SICAM)
Yolanda R. Carrasco

25-26 JANUARY 2018

MADRID, SPAIN
International Symposium: applications of gene editing on research and therapy of human rare diseases
Lluís Montoliu

14-20 FEBRUARY 2018 (CNB)

Theoretical-practical workshop on plant systems biology
Pilar Cubas and Antonio Leyva

7-10 MARCH 2018

OSLO, NORWAY
4EDA: 4th European days of albinism
Lluís Montoliu

22-23 MARCH 2018

PARIS, FRANCE
ARRIGE kick-off meeting
Lluís Montoliu

5 APRIL 2018 (CNB)

FELASA workshop on the severity classification and reporting under EU directive 2010/63/EU
Belén Pintado

6-7 APRIL 2018

SAN ILDEFONSO, SPAIN
3rd Retreat of the European Academy of Microbiology
Laura Cueto and Miguel Vicente

13 APRIL 2018 (CNB)

CNB workshop: genomic editing in bacteria
Laura Cueto and Miguel Vicente

16-20 APRIL 2018

PRAGUE, CZECH REPUBLIC
3rd Programmable nucleases (CRISPR/Cas9) transgenesis course
Lluís Montoliu

9 MAY 2018 (CNB)

Ciencia, periodismo y sociedad
Julia García

21 MAY 2018 (CNB)

Fascinados por las plantas: botánicos y artistas en las expediciones españolas del siglo XVIII
Julia García

4-6 JUNE 2018

MADRID, SPAIN
15th Experimental chaos and complexity conference
Jacobo Aguirre

11 JUNE 2018 (CNB)

III Workshop by CNB PhD students
CNB
CNB PhD Students Junior Committee and Julia García

11-15 JUNE 2018 (CNB)

VIII Curso de proteómica cuantitativa
Alberto Paradela

16-17 JUNE 2018

SANTIAGO DE COMPOSTELA, SPAIN
19th C-HPP symposium
Fernando Corrales

16-20 JUNE 2018

SANTIAGO DE COMPOSTELA, SPAIN
XII EuPA congress
Fernando Corrales

26-27 JUNE 2018 (CNB)

2nd I2PC cryoEM facilities meeting
José María Carazo

18 JUNE 2018

SANT FELIU DE GUIXOLS, SPAIN
3rd RedDevNeural annual meeting
Marta Nieto



27-29 JUNE 2018 (CNB)

Instruct – I2PC- FEI: facility-based image processing for electron microscopy

José María Carazo

2-27 JULY 2018 (CNB)

VI Curso de introducción a la investigación

Julia García

4-6 JULY 2018

SALAMANCA, SPAIN

XIV Reunión de Biología Molecular de Plantas

Pilar Cubas

9 JULY 2018 (CNB)

Workshop on ethics and integrity in research

Lluís Montoliu

10-12 JULY 2018

GIF-SUR-YVETTE, FRANCE

CNRS-CSIC workshop “Microbial adaptation to environmental stresses”

Francisco García del Portillo

12-13 JULY 2018 (CNB)

NanoBIOSOME: design, development and production of nanocontainers and nanovehicles

José María Valpuesta

10 SEPTEMBER 2018

SANTANDER, SPAIN

3rd Emerging scientist workshop at the XLI SEBBM congress

Fernando Moreno-Herrero

26-29 SEPTEMBER 2018

CUERNAVACA, MEXICO

13th International adenovirus meeting

Carmen San Martín

28-29 SEPTEMBER 2018

SEGOVIA, SPAIN

XII Jornadas ALBA sobre albinismo “rompiendo mitos”

Lluís Montoliu

30 SEPTEMBER 2018

ORLANDO, USA

Human Proteome Project - principal investigator’s meeting

Fernando Corrales

9-12 OCTOBER 2018

ALBI, FRANCE

SFEAP 2018 congress

Fernando Corrales

15-17 OCTOBER 2018

BAEZA, SPAIN

UNIA Workshop “Current trends in biomedicine - the cell biology behind the oncogenic PI3K lipids”

Ana Clara Carrera

17-18 OCTOBER 2018

MADRID, SPAIN

IX Reunión científica de proteómica clínica

Fernando Corrales

18 OCTOBER 2018 (CNB)

CIBERER workshop on working group on genome editing and gene therapy

Lluís Montoliu

26 OCTOBER 2018

MADRID, SPAIN

Evaluating a combination of immune-based therapies to achieve a functional cure of HIV infection (HIVACAR)

Mariano Esteban

26-28 OCTOBER 2018

SHANGHAI, CHINA

VI ICGEB Workshop on human RNA viruses

Luis Enjuanes

12 NOVEMBER 2018

HANGZHOU, CHINA

International symposium on root development and nutrients/water absorption

Javier Paz-Ares

3-4 DECEMBER 2018

MUNICH, GERMANY

INFRAFRONTIER / IMPC stakeholder meeting 2018 - advancing rare disease research and gene therapy applications with animal models

Lluís Montoliu

13 DECEMBER 2018 (CNB)

II Reunión científica Immunothercan

Santos Mañes

17-19 DECEMBER 2018 (CNB)

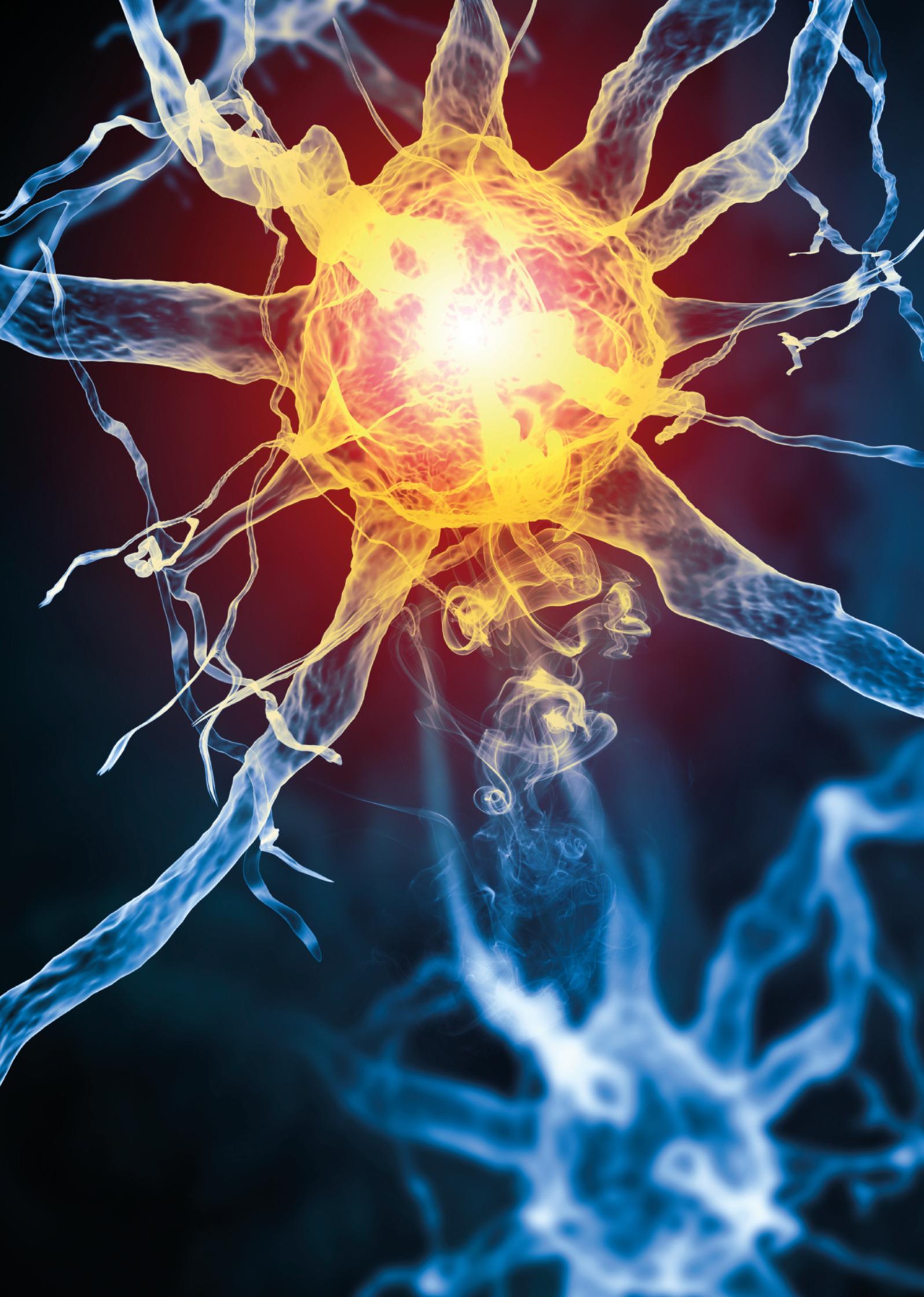
XXVI CNB Scientific workshop

Susana de Lucas

21 DECEMBER 2018 (CNB)

XXVI Workshop Advances in Molecular Biology by Young Researchers Abroad

Domingo F. Barber, Mar Valés-Gómez and Susana de Lucas



Direction and management

- 1. Direction team**
- 2. Management team**
- 3. CNB Staff**
- 4. Publications**
- 5. Research Funding**
- 6. Scientific Advisory Board**



DIRECTOR
Fernando Rojo



VICEDIRECTOR
Mario Mellado



TECHNICAL VICEDIRECTOR
Peter Klatt



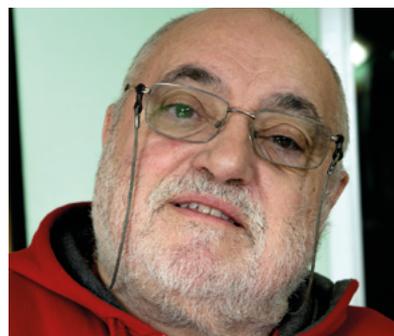
SCIENTIFIC COMMUNICATION AND
OUTREACH MANAGER
Susana de Lucas
Julia García (until July 2018)



TECHNOLOGY TRANSFER
MANAGER
Cristina Merino
Ana Sanz (until March 2017)



ASSISTANT TO THE DIRECTOR
Yolanda García



OUTREACH ADVISER
Miguel Vicente



GENERAL MANAGER

Isabel Sevillano
Miguel Anchuelo (until April 2017)



PROJECT MANAGEMENT

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Soraya Olmedilla María

PERSONNEL

Pilar Ara Laúna
Daniel Martín Hernando
Diana Gloria Pastor Calero

DIRECTION AND MANAGEMENT 137



PURCHASING AND SUPPLIES

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Julio Díez Álvarez
Ramón Serrano Coronado
(until June 2017)

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M^a José Caballero Martín
(until July 2018)
M^a Ángeles Lumbreras Carrasco
M^a Carmen Pascual Martínez
Antonio Pastor Encabo
Iris Roldán Zuasti
(until September 2018)



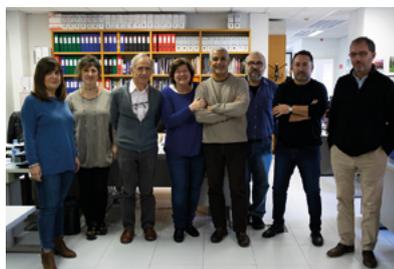
HUMAN RESOURCES

HEAD

Marina Hernando Bellido

PERSONNEL

Aurora Cabrerizo Alonso
Pilar Corral Cid
Mario Pérez Arranz
Gloria del Sastre Martín
(until February 2018)
Javier Tortosa Nieto



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Francisco Luis Aparicio Reyes
Santos Esteban Barranco Sierra
M^a Carmen Berreiros Cano
Fco Javier Hernández Izquierdo
Rufino Fernández Senso
M^a José Gregorio Usano
Rafael López Laso
M^a Carmen Vaz Pereña



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Iñigo Oficialdegui
(until September 2017)

Alberto Sánchez Castaño
(until February 2018)



LIBRARIAN

Mª Dolores Aparicio

SCIENTIFIC EDITING

Catherine Mark (until April 2018)



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Alfonso García

Jesús González

Enrique Mejías

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INFRASTRUCTURE PLANNING

Javier Zarco



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Sergio Jiménez Antón

Pilar Cutillas

Lourdes Sánchez Díaz

Adela Gracia Díaz

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Beatriz García (until March 2018)

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Beyca López Milla

Juan Pablo Illescas Muñoz

Juana González

Ana María Puerto Collantes

José Miguel de la Hoz Calderón

Paloma González (until October 2018)

Santa López Almena

Carolina Nogales Mauro

Daniel Pérez Sánchez

Aileen Notario Bonsol

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Sócrates Gutiérrez

PERSONNEL

Fernando Albarrán

Abderrahim Asgais

Tomás Castro González

Marcos Fuentes

Jesús Payán

María Esther Rodríguez Baltasar



667 **631**
2017 2018

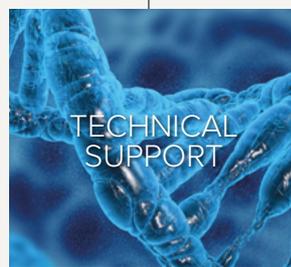
DIRECTION AND MANAGEMENT **139**



507 **467**
2017 2018



95 **93**
2017 2018



56 **54**
2017 2018



34 **31**
2017 2018

GROUP LEADERS
74 **75**
2017 2018

POSTDOCTORAL & STAFF SCIENTISTS
122 **99**
2017 2018

RESEARCH TECHNICIANS
74 **73**
2017 2018

PHD STUDENTS
148 **146**
2017 2018

SHORT-TERM TRAINEES AND VISITING SCIENTISTS
89 **74**
2017 2018

FEMALE
58% **294** **253** 54%
2017 2018

MALE
42% **213** **214** 46%
2017 2018

SPANISH
88% **447** **401** 86%
2017 2018

INTERNATIONAL
12% **60** **66** 14%
2017 2018

PUBLICATIONS

EVOLUTION OF RESEARCH FUNDING

PUBLICATIONS IN JCR-INDEXED JOURNALS

241 **263**
2017 2018

AVERAGE IMPACT FACTOR

6.3 **5.5**
2017 2018

PUBLICATIONS IN FIRST QUARTILE (Q1) JOURNALS

83% **201** **197** 75%
2017 2018

PUBLICATIONS IN FIRST DECILE (D1) JOURNALS

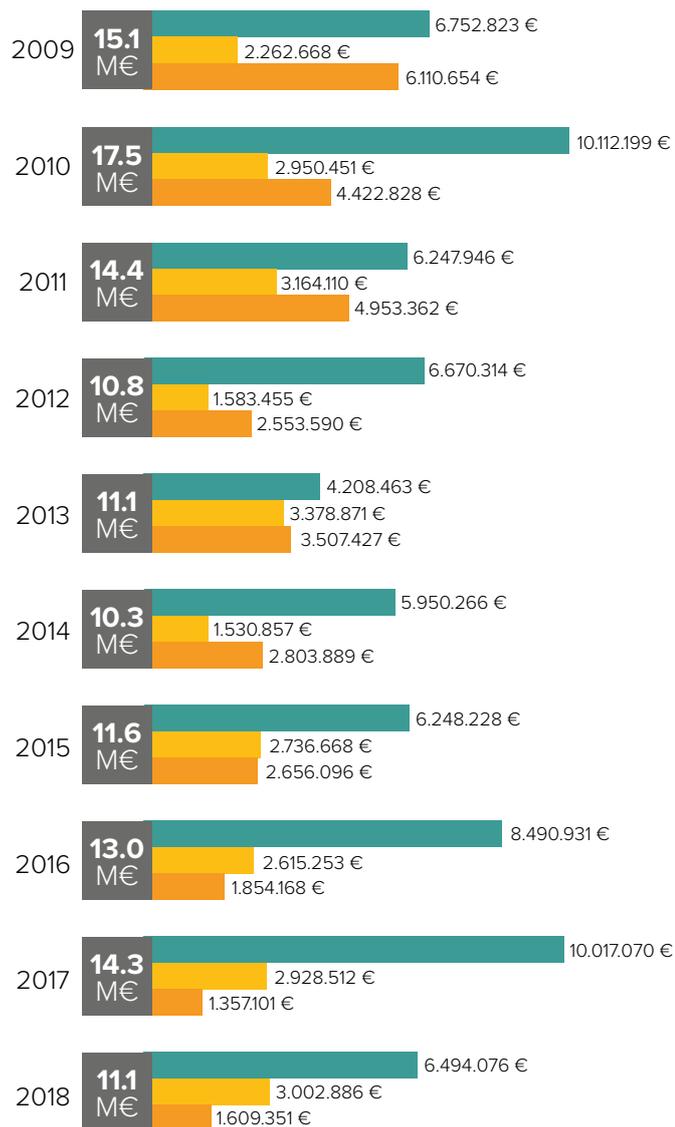
36% **86** **99** 38%
2017 2018

PUBLICATIONS WITH CNB SCIENTIST AS SENIOR AUTHOR

48% **116** **128** 49%
2017 2018

PUBLICATIONS IN INTERNATIONAL COLLABORATION

49% **117** **171** 65%
2017 2018



■ Research Grants (w/o European Commission Grants)
■ European Commission Grants
■ Research Contracts

Scientific advisory board

Our Scientific Advisory Board (SAB) has been recently renewed having in mind the forthcoming 5-year review of the centre's strategy and performance during the 2016-2020 period. Members of the new SAB are 6 eminent scientists in the centre's major research areas.

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Wolfgang Baumeister

Director of the Department of Structural Biology, Max Planck Institute for Biochemistry, Martinsried, Germany.



Yaakov Benenson

Professor for Synthetic Biology, Department of Biosystems Science and Engineering, ETH Zurich, Switzerland.



Martin Crespi

Director of the Institute of Plant Sciences Paris-Saclay (IPS2), Gif-sur-Yvette, France.



José Luis García-López

CSIC Research Professor for Environmental Biotechnology, Centro de Investigaciones Biológicas (CIB), Madrid, Spain.



George Kollias

President and Director of the Biomedical Sciences Research Center (BSRC) "Alexander Fleming", Vari, Greece.



Geoffrey L. Smith

Head of the Department of Pathology, Division of Virology, University of Cambridge, UK.

CNBREPORT

2017_2018 / RESEARCH_DEVELOPMENT_INNOVATION

REPORT COORDINATORS

Susana de Lucas
Peter Klatt
Fernando Rojo

SCIENTIFIC PHOTOGRAPHY

Inés Poveda

GRAPHIC DESIGN

Lucía Bajos

D.L.: M-14667-2019



Centro Nacional de Biotecnología

Campus de Cantoblanco
Darwin 3, Madrid 28049, Spain
Tel.: [+ 34] 91 585 4500 / Fax: [+ 34] 91 585 4506
www.cnb.csic.es



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Y UNIVERSIDADES

