R+D=

2011-2012 REPORT

CNB
Centro Nacional de Biotecnología

CSIC
Consejo Superior de Investigaciones Científicas

R+D=

2011-2012 REPORT

INDEX

Welcome to the CNB

CNB DIRECTOR:

Carmen Castresana

In November 2012, we celebrated the 20th anniversary of the inauguration of the CNB, an important age for an institute and an important time to acknowledge the contribution of all CNB personnel who have devoted their efforts to the creation of an excellent research institute, of which we have many reasons to feel proud.

The pathway has not been and it is not easy. We have faced many difficulties, in particular those derived from the constraints imposed by a system that lacks the flexibility needed to solve our daily problems and maintain the centre at the forefront of scientific development. The unconditional dedication of our personnel and their devotion to science have nonetheless allowed us to raise the funds needed to cope with these problems and to achieve scientific results of exceptional quality.

In the last two years, CNB researchers have contributed to the publication of 424 papers in ISI-listed journals, 35 of which were published in journals with an impact factor above 10. As proof of its dynamism, the CNB obtained 125 grants (11 from the European Union VII Framework Programme), submitted 54 PhD theses, taught more than 400 hours in Master's degree programmes and workshops in Spain and abroad, hosted 322 seminars and organised 18 international workshops and meetings. The numbers speak for themselves of the international nature of the CNB; near 40% of the papers published by our scientists are the result of collaborations with international scientific groups. As a result of this, the CNB is attractive to young scientists from abroad, who currently make up 38% of the pre- and post-doctoral personnel

of the centre. At this point, I want to acknowledge the initiative by the Fundación La Caixa in offering fellowships for international PhD students to carry out their thesis projects at the CNB. As a result of the 2011 and 2012 calls, the CNB selected 20 excellent students from all over the world to initiate their PhD studies at the centre.

All these scientific indicators were appreciated by the CNB Scientific Advisory Board, who participated in a special act in November 2012 to commemorate the CNB's 20th anniversary. For this occasion, Her Majesty Sofia, Queen of Spain visited the CNB in February 2013, accompanied by relevant authorities from the government, the CSIC and the Universidad Autónoma de Madrid. All had the opportunity to listen to a few words from previous CNB Directors and to visit some of our most attractive installations.

In recent times, we are encountering a new and worrying situation derived from the economic crisis in Spain, which translates into a 36% reduction in our funding in the last two years. These cuts have already caused the loss of nearly 20% of the people working at the CNB, and if it is not stopped we will soon suffer other negative effects that might severely affect the progress of our research.

At this point, I would like to acknowledge the Universidad Autónoma de Madrid (UAM), on whose campus the CNB is located, for establishing the UAM+CSIC International Campus of Excellence. As part of this initiative, the UAM has contributed to the creation of a number of technological platforms in which the CSIC institutes collaborate in close interaction with the university. This initiative promotes the acquisition of new equipment and infrastructure that will partially alleviate the shortage of funds we presently face.

Despite the difficult situation, we are making the efforts necessary to maintain our position and develop our scientific potential. One of our principal challenges is to strengthen the biotechnological value of the CNB. As an initial effort in this direction, in the present report we include a specific section devoted to innovation, which highlights results patented at the CNB and licensed to companies for translation into benefits for society. This section also includes a technological offer that describes patented CNB discoveries for which we are searching for an industrial partner. Our compromise with the innovation facet of our research will be strongly pursued through the organisation of meetings, seminars and projects in which the CNB will open its doors, facilities, knowledge and discoveries to biotechnology companies. Equally important, and as part of our compromise with society, we want to transmit and help the public understand our need as a country to maintain the scientific position we have achieved in recent years. In these difficult times, we seek and hope to find the help of foundations and organisations that are conscious of the situation and could play a critical role in helping us to preserve the present and future value of our scientists and technological developments.

Finally, I want to acknowledge the agencies and institutions that have funded CNB research in the last two years and to express again my admiration of all CNB personnel who, by performing excellent work, help to keep the centre running and moving towards the accomplishment of our objectives.

01 Macromolecular Structures

The activity of the department is focussed on the area of Structural Biology. The groups are involved in different aspects of the determination of the structure of macromolecules, their interactions and the molecular basis of their function.

One of the main strengths in this department is the presence of several groups with ample experience in advanced microscopy methods, ranging from cryo-electron microscopy and three-dimensional single particle reconstruction, tomography and correlative methods. This unique critical mass of expert microscopists host the Instruct Image Processing Centre, and maintain strong collaborations with ALBA and other European synchrotrons to develop X-ray imaging methods.

X-ray crystallography has grown in the department in the last few years to become a major activity, developing interfaces with the microscopy groups as well as with many other groups in the CNB and abroad. The analysis and manipulation of isolated macromolecules and complexes is also a main topic in the activity of the department, including a formal collaboration with the IMDEA Nanoscience.

The department also hosts the coordinating node for the Spanish Proteomic Network, running several projects based on advanced methods in mass spectrometry, with emphasis on high-throughput analyses of post-translational modifications. The incorporation of a Functional Bioinformatics Unit has reinforced the activity of the department in different “Omics” projects.

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SELECTED PUBLICATIONS

R. Navajas, A. Paradela, J. P. Albar, Immobilized metal affinity chromatography/reversed-phase enrichment of phosphopeptides and analysis by CID/ETD tandem mass spectrometry. Methods Mol Biol 681, 337-48 (2011).

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S. Gharbi *et al.*, Diacylglycerol kinase zeta controls diacylglycerol metabolism at the immunological synapse. Mol Biol Cell 22, 4406 (2011).

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Functional proteomics

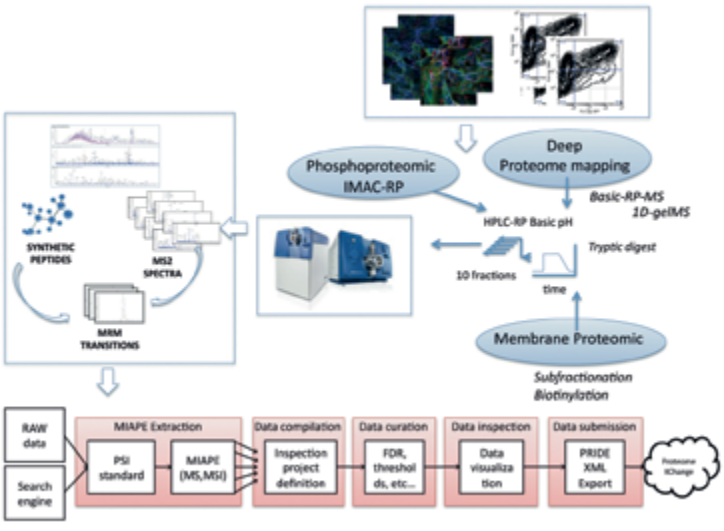
Functional proteomics aspires to draw a complete map of protein dynamics, interactions and posttranslational modifications that take place in the cell. Our goals within the CNB Functional Proteomics Group are to develop and apply state-of-the-art tools to monitor proteins involved in molecular interactions and pathways relevant to pathologies in a variety of tissues, cell types and organisms after various experimental treatments/conditions. We incorporate the latest methodologies to specific functional proteomic projects:

1. Human Proteome Project: This project was launched by the HUPO to systematically map the whole human proteome. The Chromosome-Centric HPP focusses on constructing a protein catalogue on a chromosome-to-chromosome basis. Our main goal is to design experimental approaches to detect and quantify both the "conspicuous" and the "hidden" proteome. This is being driven by the most advanced unbiased shotgun approaches and targeted profiling by selected reaction monitoring (S/MRM).

2. Signal transduction networks untangled by phosphoproteomic analyses: We are focussing on TCR signalling and the role of diacylglycerol production on the control of this response. A combination of phosphopeptide enrichment and SILAC labelling has been implemented for accurate phosphoprotein and phosphopeptide analysis and quantitation.

3. Interactomics: The CAM project "Interactomics of the Centrosome" aims to characterise interactions between centrosomal proteins and to identify macromolecular complex components by proteomics approaches based on affinity tags, stable isotopic labelling, mass spectrometry and peptide arrays.

4. Computational proteomics covers data analysis obtained from large-scale experiments and meta-annotation of proteins and protein complexes. This includes: a) probability-based methods for large-scale peptide and protein identification and quantitation from mass spectrometry data, b) strategies for data mining visualisation, and c) data analysis tools for integration, validation, inspection, deposition and reporting. See ProteoRed MIAPE WTK available at http://www.proteored.org/MIAPE). All within the EU ProteomeXchange project.



5. Quality control and experimental standardisation: Reproducibility and robustness of proteomics workflows are key issues that are being addressed through participation in multi-laboratory studies within the "ProteoRed-ISCIII" project led by our group.

6. Prolamin characterisation in foods using classical and mass spectrometry approaches is being carried out in the context of coeliac diseases.

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SELECTED PUBLICATIONS

Patwardhan A, Carazo JM, Carragher B, Henderson R, Heymann JB, Hill E, Jensen GJ, Lagerstedt I, Lawson CL, Ludtke SJ, Mastronarde D, Moore WJ, Roseman A, Rosenthal P, Sorzano CO, Sanz-García E, Scheres SH, Subramaniam S, Westbrook J, Winn M, Swedlow JR, Kleywegt GJ. Data management challenges in three-dimensional EM. Nat Struct Mol Biol. 2012 Dec, 19(12):1203-7.

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Three-dimensional electron and X-ray microscopies: image processing challenges

During this period, we initiated a strong refocusing of our activities, centering on our role as image processing infrastructure providers for Instruct, the Structural Biology project of the European Strategic Forum for Research Infrastructures. We have thus been particularly active in the area of algorithmic inventions and technological development, aiming to provide not only image processing capabilities, but to link them to the exponentially growing fields of genomics and proteomics. Indeed, Xmipp, the software suite we developed, is being used increasingly in the field of three-dimensional electron microscopy, with hundreds of individualised downloads per year from all over the world; it is the software of choice for a large percentage of all 3D maps being deposited in structural databases.

In 2012, the soft X-ray microscope of the Spanish synchrotron ALBA began operation, the third instrument of its kind in the world. We have studied the image processing issues associated to this instrument in depth, developing the first formulation of its image formation model under incoherent illumination, and have begun to develop tailored 3D reconstruction approaches suited to this new imaging modality.

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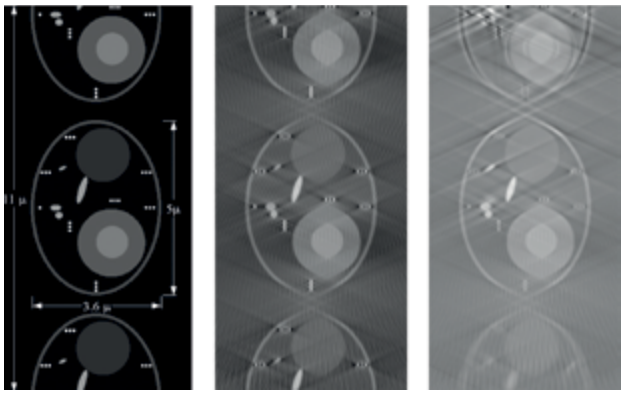

1 *Simulations of different 3D reconstruction from soft X-ray microscopy images under several conditions (left, oiginal phantom, center and right, 3D reconstructions with standard EM algorithms)*

2 *Different DNA-binding modes of p53 tetramers solved by 3D electron microscopy.*

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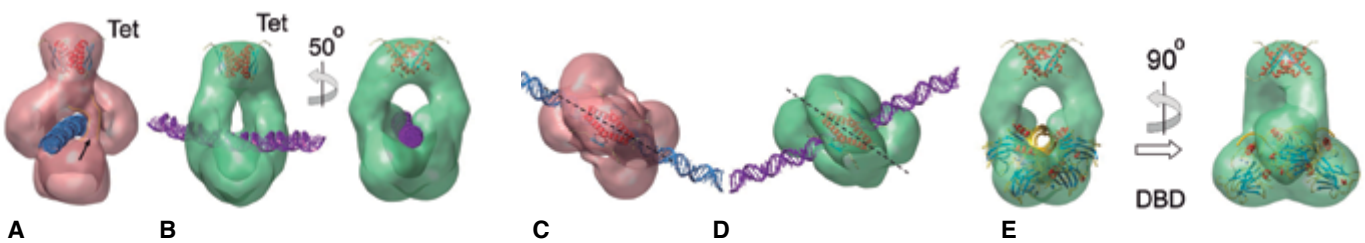

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SELECTED PUBLICATIONS

Reguera J, Santiago C, Mudgal G, Ordoño D, Enjuanes L, Casasnovas JM. Structural bases of coronavirus binding to host aminopeptidase N and its inhibition by neutralizing antibodies. PLoS Pathog. 2012;8(8):e1002859

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Cell-cell and virus-cell interactions

A large variety of glycosylated molecules engaged in cell-cell and virus-cell interactions populate cell and viral membranes. Cell surface glycoproteins connect the cell with its environment; they participate in cell-cell contacts and in virus entry processes.

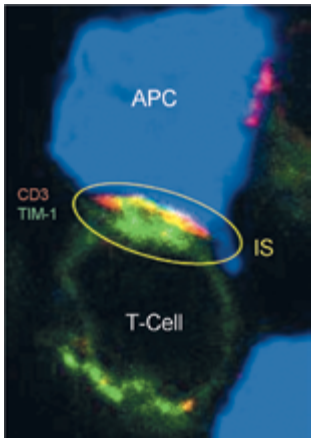
We study cell surface molecules engaged in immune system regulation and virus entry into host cells. We analyse receptor-ligand interactions related to immune processes such as cell adhesion and phagocytosis, as well as to virus binding to cells. We are also characterising virus neutralisation by humoral immune responses and its correlation with virus cell entry. Our research has provided key insights into immune receptor function and has identified viral epitopes essential for virus infection, some of which are targeted by neutralising antibodies. The group carries out multidisciplinary research using structural (X-ray crystallography), biochemical and cell biology approaches. Below we highlight some recent results related to immune processes and viral infections.

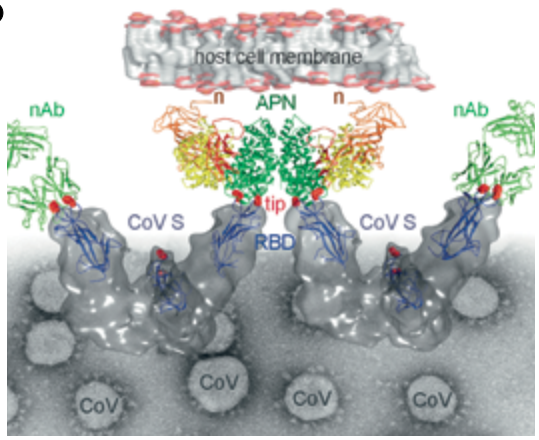
TIM proteins: a family of PtdSer receptors that regulate immunity

The transmembrane, immunoglobulin and mucin domain (TIM) gene family has a critical role in regulating immune responses, including transplant tolerance, autoimmunity, allergy and asthma, and the response to viral infections. We demonstrated that the TIM proteins are pattern recognition receptors, specialised in detecting the phosphatidylserine (PtdSer) cell death signal. The TIM protein bears a motif, the MILIBS, which determines its specificity for phospholipids such as PtdSer. We recently determined that TIM-1 traffics to the immune synapse during antigen presentation (Figure 1), where it may function as a costimulatory molecule.

Virus-receptor interactions and virus neutralisation by antibodies

Our group has been analysing virus-receptor interactions in measles virus and coronavirus, and has determined crystal structures of virus-receptor complexes. These structures define the way in which measles virus and certain coronaviruses bind to cell surface proteins, and identify the major receptor recognition determinants in those viruses. Moreover, our analysis of how antibodies prevent and neutralise virus infections showed that potent measles- and coronavirus-neutralising antibodies target virus residues engaged in binding to cell surface receptors. This indicates that prevention of virus entry into host cells is a major mechanism used by the immune system for virus neutralisation. Figure 2 illustrates how an antibody (nAb) prevents coronavirus (CoV) binding to the aminopeptidase N (APN) receptor.





1 *TIM-1 traffic toward the immunological synapse (IS), formed by a T cell and an antigen presenting cell (APC).*

2 *Structural view of coronavirus (CoV) binding to its host cell aminopeptidase N (APN) receptor and its inhibition by neutralising antibodies.*

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SELECTED PUBLICATIONS

Ionel A, Velázquez-Muriel JA, Luque D, Cuervo A, Castón JR, Valpuesta JM, Martín-Benito J, Carrascosa JL. Molecular Rearrangements Involved in the Capsid Shell Maturation of Bacteriophage T7. J Biol Chem. 2011 Jan 7;286(1):234-42

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Fumagalli L, Esteban-Ferrer D, Cuervo A, Carrascosa JL, Gomila G. Label-free identification of single dielectric nanoparticles and viruses with ultraweak polarization forces. Nat Mater. 2012 Sep;11(9):808-16

Structure of macromolecular assemblies

The activity of the group has focussed on the study of the molecular bases of virus assembly and maturation. We used 3D-cryo-EM and single particle reconstruction approaches to describe, at subnanometer resolution, the reorganisation involved in maturation of the shell of the icosahedral dsDNA caudovirales group. Using the phage T7 as a model, we are presently using a combination of biochemical and microscopy approaches to study structural changes in viral components involved in DNA packaging, virus-cell interaction and DNA ejection.

Viral maturation of complex viruses has been studied at the subcellular level using mainly the vaccinia virus as a model system. Analysis by electron tomography of infected cells has shown the existence of extensive membrane reorganisation during vaccinia maturation. To overcome the intrinsic limitation of electron microscopy in imaging samples thicker than 0.5 microns, we developed novel methods for soft X-ray microscopy of frozen whole cell samples. Successful X-ray imaging of 5- to 10-micron samples in the frozen state allowed us to produce cryo-X-ray tomographic 3D reconstructions of cells. The resolution obtained so far for the tomograms has been sufficient to detect different virus types within the cytoplasm of unfixed, uncontrasted cells. We are currently developing methods for correlative combination of light, electron and X-ray tomography to improve quantitative 3D microscopy.

The structural data obtained by 3D-cryo-microscopy from the experimental systems under study in our group are combined with analysis of the nano-mechanical properties of individual viral particles, using atomic force microscopy and spectroscopy. We are also exploring the application of optical tweezers to test small forces involved in specific viral functions. We are using isolated viral components to study their properties as nanomachines and to build with them synthetic tools. We are studying the deformation behaviour and material properties in individual viral particles for systematic studies to correlate molecular structure, nanoscopic behaviour, and macroscopic properties of viral containers.

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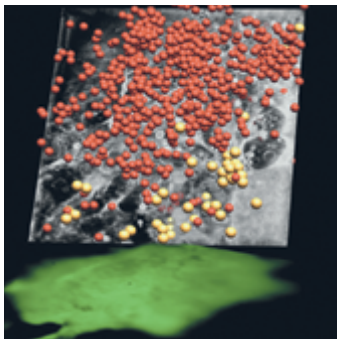

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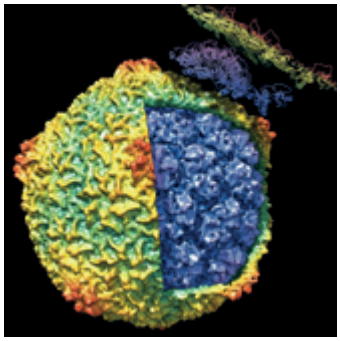

1 *Composite image showing correlative merging of a light microscopy image of a cell (fluorescent green), a plane of an X-ray cryo-tomogram of the same cell, and the segmented virus types (yellow: immature vaccinia virus; red: mature virus) from an X-ray cryo-tomogram of the cell.*

2 *View of the mature capsid of bacteriophage T7 obtained by three-dimensional reconstruction from electron cryo-microscopy at 1 nm resolution.*

3 *Reconstruction of the capsid of bacteriophage T7 by electron cryo-microscopy at 1 nm resolution. The outer shell corresponds to the mature capsid. The inner blue shell is from the immature prohead. The ribbon models are two related structures of the monomers from each shell type, showing the domain reorganisation involved in virus maturation.*

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Ionel A, Velázquez-Muriel JA, Luque D, Cuervo A, Castón JR, Valpuesta JM, Martín-Benito J, Carrascosa JL. Molecular rearrangements involved in the capsid shell maturation of bacteriophage T7. J Biol Chem. 2011 Jan 7;286(1):234-42

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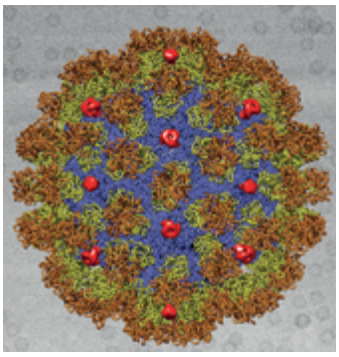
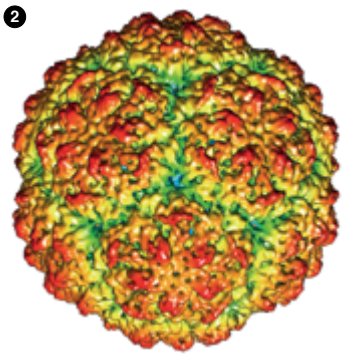
Gómez-Blanco J, Luque D, González JM, Carrascosa JL, Alfonso C, Trus B, Havens WM, Ghabrial SA, Castón JR. *Cryphonectria nitschkei* virus 1 structure shows that the capsid protein of chrysoviruses is a duplicated helix-rich fold conserved in fungal double-stranded RNA viruses. J Virol. 2012 Aug;86(15):8314-8

Viral molecular machines

Our studies address the structure-function-assembly relationships of viral macromolecular complexes, also known as viral nanomachines, that control many fundamental processes in the virus life cycle. Our model systems are the viral capsid and other viral macromolecular complexes, such as helical tubular structures and ribonucleoprotein complexes. We study several viral systems with different levels of complexity: double-stranded (ds)RNA viruses such as infectious bursal disease virus (IBDV), *Penicillium chrysogenum* virus (PcV) and human picobirnavirus (HPBV), and single-stranded RNA viruses such as human rhinovirus 2 (HRV2) and rabbit haemorrhagic disease virus (RHDV). The structure of regular viral capsids, in which capsid proteins make extensive use of symmetry, is a paradigm of the economy of genomic resources. Capsids should not be considered inert closed structures, but as dynamic structures that define different functional states and participate in numerous processes, including 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 at the highest achievable resolution is therefore essential to understand their properties. We are carrying out nanoscopic studies of these biomachines by single-molecule manipulation techniques such as atomic force microscopy (AFM) to correlate structural features of capsomer interactions with their mechanical properties.

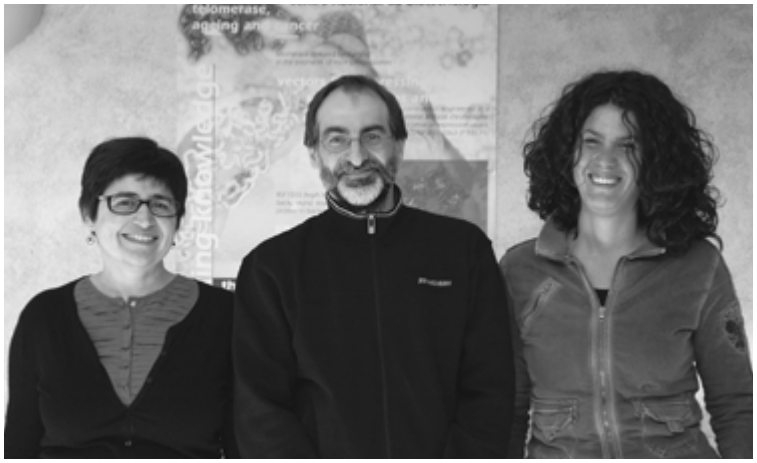
To determine the three-dimensional structure of such complex assemblies, we use hybrid methods that combine cryo-electron microscopy and image processing techniques with high-resolution X-ray structures. Our studies also intend to establish the basis of the conformational flexibility necessary to switch among almost identical conformational states (transient complexes), and their functional implications, which can provide clues for new vaccine design and/or immunisation strategies. We also focus on the structural basis of dsRNA virus replication. All dsRNA viruses, from the mammalian reoviruses to the bacteriophage phi6 and including fungal viruses, share a specialised capsid involved in transcription and replication of the dsRNA genome. Quasiatomic model of the RHDV virion. The RHDV capsid is based on a T=3 lattice containing 90 VP1 dimers. The cryo-EM map allowed modelling of the VP1 backbone structure from X-ray structures of other caliciviruses. Each VP1 monomer has three domains: an internal N-terminal arm, a shell composed of an eight-stranded b-sandwich (purple), and a flexible protruding domain subdivided into two subdomains, P1 (yellow) and P2 (orange).





❶ *Quasiatomic model of RHDV virion. The RHDV capsid is based on a T=3 lattice containing 90 VP1 dimers. The cryo-EM map allowed modeling of the VP1 backbone structure from X-ray structures of other caliciviruses. Each VP1 monomer has three domains, an internal N-terminal arm, a shell composed of an eight-stranded b-sandwich (purple), and a flexible protruding domain subdivided into two subdomains, P1 (yellow) and P2 (orange).*

❷ *Structure of Penicillium chrysogenum virus (PcV), a fungal double-stranded RNA virus. Radially color-coded outer surfaces of three-dimensional cryo-EM reconstruction of the PcV at 8 Å r esolution, which has a T=1 capsid formed by 60 copies of a single polypeptide. Structural subunits have two similar helical domains indicative of gene duplication.*



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SELECTED PUBLICATIONS

Agulleiro JI, Fernandez JJ. Fast tomographic reconstruction on multicore computers. Bioinformatics. 2011 Feb 15;27(4):582-3

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Fernandez-Fernandez MR, Ferrer I, Lucas JJ. Impaired ATF6α processing, decreased Rheb and neuronal cell cycle re-entry in Huntington's disease. Neurobiol Dis. 2011 Jan;41(1):23-32

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Li S, Fernandez JJ, Marshall WF, Agard DA. Three-dimensional structure of basal body triplet revealed by electron cryo-tomography. EMBO J. 2011 Dec 13;31(3):552-62

Computational methods for 3D electron microscopy

Knowledge of the structure of biological specimens is essential to understanding their functions at all scales. Electron microscopy (EM) combined with image processing allows study of the three-dimensional (3D) structure of biological specimens over a wide range of sizes, from cell structures to single macromolecules, providing information at different levels of resolution. Depending on the specimen under study and the structural information sought, different 3D EM approaches are used. Single particle EM makes it possible to visualise macromolecular assemblies at subnanometer or even near-atomic resolution. Electron tomography is a unique tool for deciphering the molecular architecture of the cell. In all cases, the computational methods of image processing play a major role. Computational advances have contributed significantly to the current relevance of 3D EM in structural biology.

Our research interests focus mainly on structural analysis of specimens of biological relevance, using 3D EM in general and especially electron tomography. We are exploring the structural alterations in the subcellular architecture in normal and pathological conditions in neurodegenerative diseases, particularly Huntington's disease. This project is led by Dr. MR Fernández Fernández, who has substantial expertise in different aspects of the molecular and cell biology of Huntington's disease neurodegeneration. We are also interested in structural elucidation of the microtubule-organising centre (MTOC), an important and complex cell organelle in eukaryotes. We are conducting projects to understand the spindle pole body and centriole/basal body in collaboration with Dr. Sam Li (UCSF, CA, USA). We also collaborate with other national and international groups in experimental structural studies. Another important focus of our research is the development of new image processing methods and tools for the advancement of electron tomography. In the last few years, we have worked on implementation of sophisticated tomographic reconstruction methods that are robust in our experimental conditions. We have also developed new methods to address an important challenge in electron tomography, that is, the automated segmentation of tomograms for 3D visualisation of subcellular landscapes.

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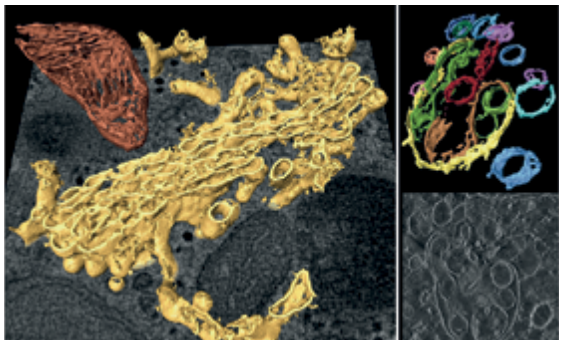
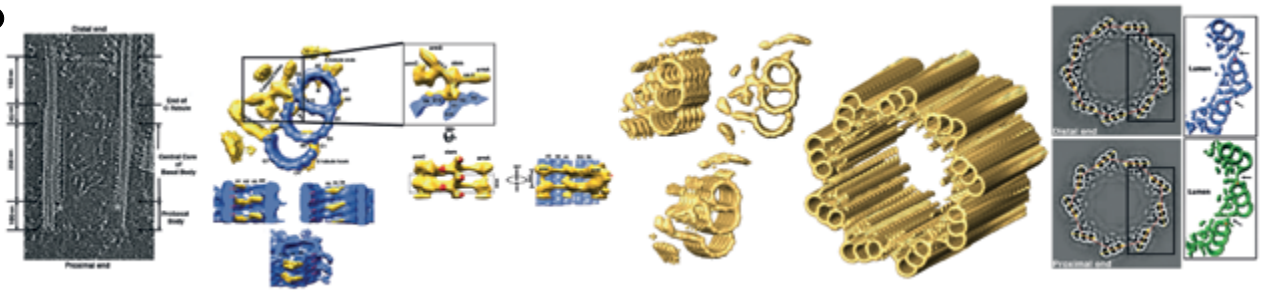


Three-dimensional visualisation of subcellular architecture with electron tomography and image processing techniques. Golgi complex and mitochondrion from a human cytotoxic T lymphocyte (left). Multivesicular body from a wild type mouse striatal sample (right).

2



Elucidation of the structure at close-to-molecular resolution of the basal body triplet and 3D model for the whole basal body.

GROUP LEADER:
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César López Pastrana

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María Teresa Arranz Sarmiento

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Fuentes-Perez ME, Gwynn EJ, Dillingham MS, Moreno-Herrero F. Using DNA as a fiducial marker to study SMC complex interactions with the Atomic Force Microscope. Biophys J. 2012 Feb 22;102(4):839-48

Yeeles JT, van Aelst K, Dillingham MS, Moreno-Herrero F. Recombination hotspots and single-stranded DNA binding proteins couple DNA translocation to DNA unwinding by the AddAB helicase-nuclease. Mol Cell. 2011 Jun 24;42(6):806-16

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Hormeño S, Ibarra B, Carrascosa JL, Valpuesta JM, Moreno-Herrero F, Arias-Gonzalez JR. Mechanical Properties of High G•C-content DNA with A-type base-stacking. Biophys J. 2011 Apr 20;100(8):1996-2005

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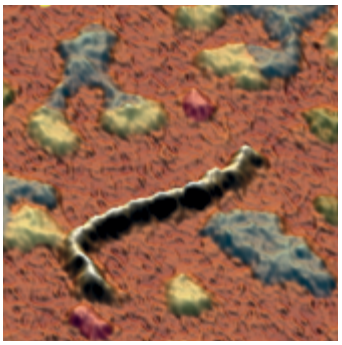
Molecular biophysics of DNA repair nanomachines

The molecular biophysics group aims to develop single-molecule techniques to study the mechanisms of protein machines involved in DNA repair processes. We also study the mechanical properties of nucleic acids and their interaction with proteins using these single-molecule approaches.

Over the last two years, we completed the construction of a magnetic tweezers (MT) machine that can manipulate single DNA molecules and measure force and torque applied by molecular motors. The group also has two custom-adapted atomic force microscopes (AFM) and a home-built optical tweezers setup (OT). We have used AFM and MT to image and monitor the dynamics of binding and processing of DNA breaks by the AddAB helicase-nuclease and to study the role of SSB in these reactions. We found that recombination hotspot sequences activate DNA unwinding by the translocating AddAB helicase-nuclease. This is the first example of stimulation of a DNA helicase by interaction with a specific sequence during translocation. This phenomenon will ensure the formation of ssDNA downstream of recombination hotspots as is required for homologous recombination. We have also investigated the structure and oligomerisation state of SMC (structural maintenance of chromosome) complex from *Bacillus subtilis*. Using a novel AFM method developed by the group, we have determined how the binding of ScpA and ScpB affect the overall structure of the SMC complex. Finally, we have further developed our knowledge in the mechanical properties of DNA and how these are affected by sequence and condensation.

1



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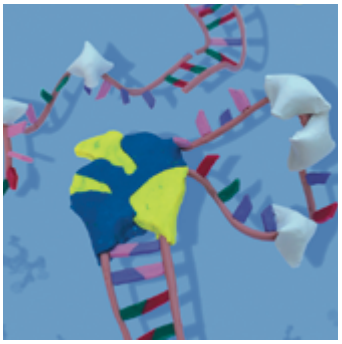

1 *SMC complex interactions studied with the AFM using DNA as a fiducial marker to quantify volumes of proteins with high precision. AFM results were used to color code the different protein components of the SMC complex: monomers of ScpA (red); monomers and dimers of ScpB (green); ScpA-ScpB complexes (yellow); and SMC proteins (blue). The fiducial DNA molecule used in the study appears in white at the bottom part of the picture. Size of the image is 500 nm x 500 nm.*

2 *Three-dimensional model illustrating that DNA translocation and unwinding are coupled through interactions of the AddAB helicase-nuclease with recombination hotspots (Chi). In the drawing, the AddAB complex pictured (yellow and blue) has recognised a Chi sequence, provoking the formation of a single-stranded DNA loop and thereby promoting stable DNA unwinding. Single-stranded DNA binding proteins, which also assist DNA unwinding, are shown in white.*

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SELECTED PUBLICATIONS

Zuklys S, Mayer CE, Zhanybekova S, Stefanski HE, Nusspaumer G, Gill J, Barthlott T, Chappaz S, Nitta T, Dooley J, Nogales-Cadenas R, Takahama Y, Finke D, Liston A, Blazar BR, Pascual-Montano A, Holländer GA. MicroRNAs control the maintenance of thymic epithelia and their competence for T lineage commitment and thymocyte selection. J Immunol. 2012 Oct 15;189(8):3894-904

Tabas-Madrid D, Nogales-Cadenas R, Pascual-Montano A. GeneCodis3: a non-redundant and modular enrichment analysis tool for functional genomics. Nucleic Acids Res. 2012 Jul;40(Web Server issue):W478-83

Muniategui A, Nogales-Cadenas R, Vázquez M, Aranguren XL, Agirre X, Luttun A, Prosper F, Pascual-Montano A, Rubio A. Quantification of miRNA-mRNA interactions. PLoS One. 2012;7(2):e30766

Fontanillo C, Nogales-Cadenas R, Pascual-Montano A, De las Rivas J. Functional analysis beyond enrichment: non-redundant reciprocal linkage of genes and biological terms. PLoS One. 2011;6(9):e24289

Pascual-Montano A. Gene classification, gene expression data processing and exploratory data analysis. Wiley Interdisciplinary Reviews Data Mining and Knowledge Discovery. Apr 04 2011. DOI: 10.1002/widm.29

Functional bioinformatics

To understand the biology underlying experimental settings, our group studies the development of new methodologies and analysis techniques to solve very oriented and specific biological questions. We concentrate our efforts in the functional bioinformatics area, which focusses its activities in the functional characterisation of genes and proteins in different experimental conditions. Our group focussed on the development of new methodologies for the analysis and interpretation of biological data. In particular, we have been working in two major areas: transcriptomics and functional analysis.

In the case of gene expression analysis, we have developed several techniques to determine the potential interactions between micro RNAs and their target transcripts. These interactions are predicted by sequence complementarity and quantified using expression information from both mRNAs and miRNAs in the same samples. We have also developed several very novel techniques to obtain the functional characterisation of list of genes or proteins. The novelty of our proposal lies in the fact that we combine several sources of information and determine which combination of functional annotations is significantly enriched in the list of genes or proteins. This has opened a new field of research know as modular functional enrichment. Methodologies to eliminate the redundancy of annotations as well as the existing bias in the annotations databases have been also developed.

In these years, we have continued our policy of developing high-quality bioinformatics software and making it available to the scientific community. The picture summarises the developments of our group in Functional Bioinformatics. More details can be found at http://bioinfo.cnb.csic.es

1 *General overview of the bioinformatics applications developed by the Functional Bioinformatics Group*

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Moisés García Serradilla

MASTER'S DEGREE STUDENT:
Monserrat Lara Rojas

SELECTED PUBLICATIONS

López-Montero N, Risco C. Self-protection and survival of arbovirus-infected mosquito cells. Cell Microbiol. 2011 Feb;13(2):300-15

Risco C, Sanmartín-Conesa E, Tzeng WP, Frey TK, Seybold V, de Groot RJ. Specific, sensitive, high-resolution detection of protein molecules in eukaryotic cells using metal-tagging transmission electron microscopy. Structure. 2012 May 9;20(5):759-66

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PATENTS

P201031880 / PCT ES11/070869: Clonable marker for microscopy

PCT 012/070864: Method for protein detection in cells with a clonable tag and sprectoscopic imaging.

Cell structure lab

Viruses manipulate cell organisation by recruiting materials to build factories, where they replicate their genomes, assemble new infectious particles, and conceal themselves from the antiviral defence sentinels of the cell. Our laboratory studies the biogenesis of virus factories to understand how viruses manipulate cell structure and create new organelles. The group works with important human pathogens such as Bunyaviruses, Togaviruses and Reoviruses; we are also interested in mechanisms of cellular immunity. With longstanding experience in structural biology, the lab is involved in developing new probes for correlative light and electron microscopy (CLEM) and electron tomography.

In the last two years, our group studied the structural transformations of mammalian cells during the last phase of the bunyavirus life cycle, and characterised two unreported structures involved in virus egress and propagation (Sanz & Risco, unpublished). Like many Arboviruses, Bunyaviruses are serious pathogens for mammals but cause little damage to their arthropod vectors. We studied the life cycle of a bunyavirus in mosquito cells (López-Montero & Risco, 2011) and are currently moving from cell culture systems to the arthropod hosts. These studies are necessary to understand key factors for virus spread in the arthropod vectors.

In collaboration with Dr. Raoul J. de Groot (University of Utrecht, The Netherlands), we described an approach, termed metal-tagging transmission electron microscopy (METTEM), that allows detection of intracellular proteins in mammalian cells with high specificity, exceptional sensitivity, and at molecular scale resolution. Based on the metal-binding protein metallothionein as a clonable tag, METTEM was combined with elemental gold imaging for simultaneous visualisation of ultrastructural details and protein molecule location. The applicability and strength of METTEM was demonstrated by a study of Rubella virus replicase and capsid proteins, which identified virus-induced cell structures not seen before (Risco *et al.*, 2012). With the help of METTEM, we recently characterised the biogenesis of the replication organelles of a Tombusvirus, in a study developed in collaboration with Dr. Peter D. Nagy (University of Kentucky, KY USA)(Barajas, Fernández de Castro, Risco & Nagy, unpublished).

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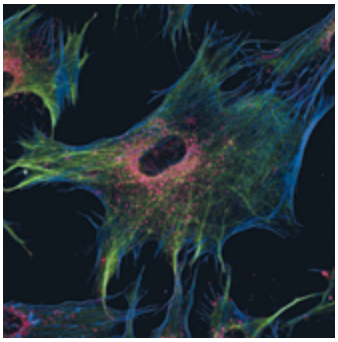

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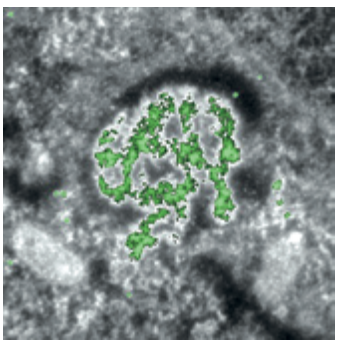

1 *Changes in the cytoskeleton of bunyavirus-infected cells*

2 *Localisation of proteins in cells with metal-tagging TEM and electron spectroscopic imaging*

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SELECTED PUBLICATIONS

Graziano V, Luo G, Blainey PC, Pérez-Berná AJ, McGrath WJ, Flint SJ, San Martín C, Xie XS, Mangel WF. Regulation of a Viral Proteinase by a Peptide and DNA in One-dimensional Space: II. Adenovirus proteinase is activated in an unusual one-dimensional biochemical reaction. J Biol Chem. 2012 Oct 7;288(3):2068-80

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Ortega-Esteban A, Horcas I, Hernando-Pérez M, Ares P, Pérez-Berná AJ, San Martín C, Carrascosa JL, de Pablo PJ, Gómez-Herrero J. Minimizing tip-sample forces in jumping mode atomic force microscopy in liquid. Ultramicroscopy. 2012 Mar;114:56-61

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Structural and physical determinants of adenovirus assembly

We are interested in the structural and physical principles that govern assembly and stabilisation of complex viruses. As a model system we use adenovirus, a challenging specimen of interest both in basic virology and nanobiomedicine. We approach the problem from an interdisciplinary point of view, combining biophysics, computational, structural and molecular biology techniques.

Adenoviruses are pathogens of particular clinical relevance in the immunocompromised population. They are also widely used as vectors for gene therapy, vaccination and oncolysis. The adenovirus genome, a dsDNA molecule, is bound to large amounts of positively charged proteins that help condense it to form the core, which is confined inside an icosahedral capsid composed of multiple copies of seven different viral proteins. The final stage of adenovirus morphogenesis consists of proteolytic processing of several capsid and core proteins. The immature virus, containing all precursor proteins, is not infectious due to an uncoating defect. To determine why the presence of precursor proteins impairs uncoating, we analysed *in vitro* disruption of mature and immature adenovirus capsids subjected to different types of stress: thermal, chemical, or mechanical (in collaboration with Dr. Pedro J. de Pablo, UAM, Madrid, Spain). The results indicated that precursor viral proteins act as scaffolds during assembly, explained how maturation primes the virus for stepwise uncoating in the cell, and revealed the structural changes the virion undergoes in conditions similar to those encountered during entry. In collaboration with Prof. Walter F. Mangel (Brookhaven Natl. Laboratory, NY, USA), we also helped to define the role of the viral genome as a cofactor of the adenovirus protease during maturation, in a newly described one-dimensional chemistry process. Our current research lines focus on the less understood aspects of adenovirus assembly, such as how the viral genome is packaged into the capsid, key elements that modulate virion stability and mechanical properties, how adenovirus evolution relates to that of its hosts, and finally, the organisation of the non-icosahedral virion components. Accurate knowledge of adenovirus structure and biology is fundamental both to the discovery of anti-adenovirus drugs and to the design of new, efficient adenoviral therapeutic tools.

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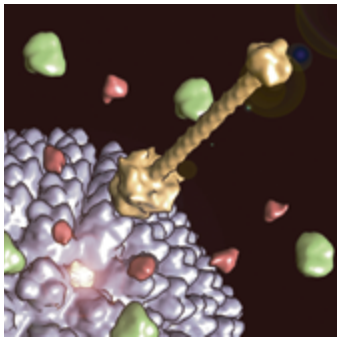

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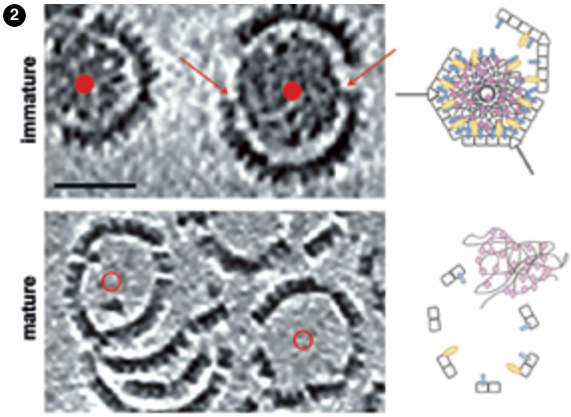

❶ *The first stages of mature adenovirus uncoating. In mildly acidic conditions that mimic those of the early endosome, adenovirus virions (purple) release a few pentons (orange) and internal proteins located at the core periphery (green and red)*

❷ *The final stages of adenovirus uncoating. Cryo-electron tomography data (left panel). In the immature virion, even under high stress conditions, the genome (filled red circles) remains a compact spherical particle attached to capsid fragments (red arrows). In the mature virion, the capsid cracks open and the genome is completely released, leaving an empty shell (empty red circles)*

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SELECTED PUBLICATIONS

Knijnenburg AD, Tuin AW, Spalburg E, de Neeling AJ, Mars-Groenendijk RH, Noort D, Otero JM, Llamas-Saiz AL, van Raaij MJ, van der Marel GA, Overkleeft HS, Overhand M. Exploring the conformational and biological versatility of β-turn-modified gramicidin S by using sugar amino acid homologues that vary in ring size. Chemistry. 2011 Mar 28;17(14):3995-4004

Tizón L, Otero JM, Prazeres VF, Llamas-Saiz AL, Fox GC, van Raaij MJ, Lamb H, Hawkins AR, Ainsa JA, Castedo L, González-Bello C. A prodrug approach for improving antituberculosis activity of potent *Mycobacterium tuberculosis* type II dehydroquinase inhibitors. J Med Chem. 2011 Sep 8;54(17):6063-84

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Garcia-Doval C, van Raaij MJ. Structure of the receptor-binding carboxy-terminal domain of bacteriophage T7 tail fibers. Proc Natl Acad Sci USA. 2012 Jun 12;109(24):9390-5

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Structural biology of viral fibres

Some viruses and bacteriophages attach to their host cell via proteins integral to their capsids, for example poliovirus, coxsackievirus and rhinovirus ('common cold virus'). Other viruses bind to their host cell receptors via specialised spike proteins (for example HIV, the AIDS virus), or via specialised fibre proteins, like adenovirus, reovirus and bacteriophages such us T4, T5, T7 and lambda (Ur). It is these fibre proteins that form the main research interest of our research group. The 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. They are very stable to denaturation by temperature or detergents.

In 2011, we determined the structure of gp17, the fibre of the *Escherichia coli* bacteriophage T7. The structure revealed a pyramid domain of unknown fold and a tip domain with a novel structural topology. Amino acid residues important for determining the host specificity of bacteriophage T7 and related phages are located on the top of this tip domain, facing the bacterium in early stages of bacteriophage infection. Knowledge of the structures of bacteriophage fibre proteins may lead to different biotechnological applications. Modification of the bacteriophage fibre receptor binding specificities may lead to improved detection and elimination of specific bacteria.

As adenovirus is used in experimental gene therapy, modification of its fibre should allow targeting to specific cellular receptors. In 2012 we determined the structures of adenovirus fibre receptor domains from two families of adenovirus for which no structures were known. We also collaborate with other research groups in crystallisation and structure solution of the proteins and peptides they produce. In 2011-2012, we have determined structures of several cyclic antibiotic peptides and bacterial dehydroquinases complexed with inhibitors. We also determined the structure of bacterial (*Thermus thermophilus*) enoyl-acyl carrier protein reductase in the apo-form, in complex with NAD+, and in complex with NAD+ and the antibacterial agent triclosan.

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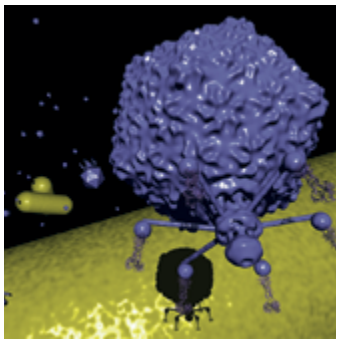

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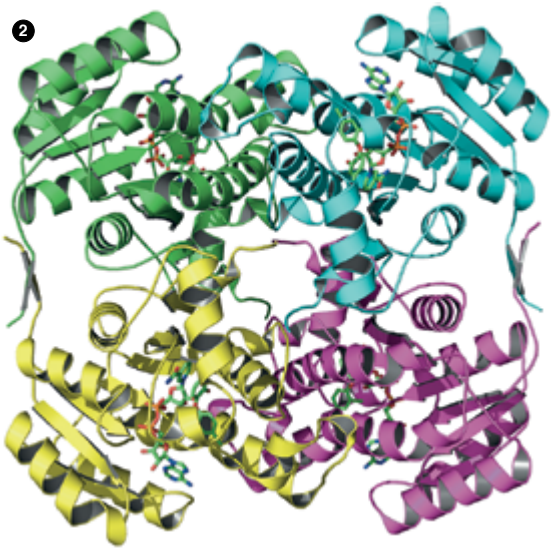

❶ *T7 bacteriophages (purple) at the point of recognising and infecting Escherichia coli bacteria (yellow). At the end of the six fibres, the crystal structure of the lipo-polysaccharide domains is represented.*

❷ *Structure of the tetrameric Thermus thermophilus enoyl-acyl carrier protein reductase in complex with NAD+ and the antibacterial agent triclosan*

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Marina Serna
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Luis Pouchoucq
Fernando Lledías

SELECTED PUBLICATIONS

Muñoz IG, Yébenes H, Zhou M, Mesa P, Serna M, Park AY, Bragado-Nilsson E, Beloso A, de Cárcer G, Malumbres M, Robinson CV, Valpuesta JM, Montoya G. Crystal structure of the mammalian cytosolic chaperonin CCT in complex with tubulin. Nat Struct Mol Biol. 2011 Jan;18(1):14-9

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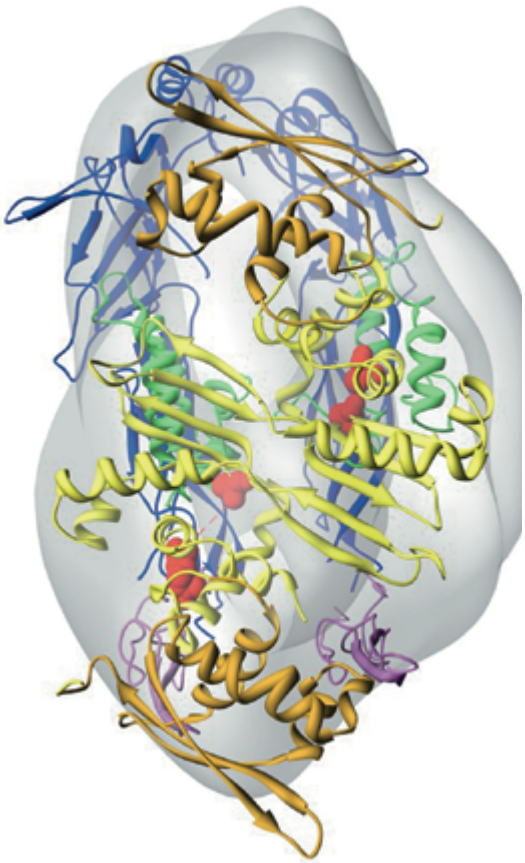
Arranz R, Coloma R, Chichón FJ, Conesa JJ, Carrascosa JL, Valpuesta JM, Ortín J, Martín-Benito J. The structure of native influenza virion ribonucleoproteins. Science. 2012 Dec 21;338(6114):1634-7

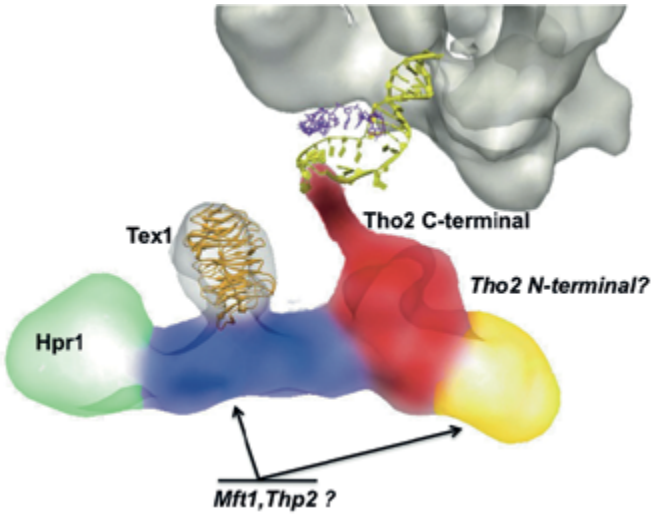
Structure and function of molecular chaperones

Molecular chaperones are proteins that assist the folding of other proteins, although they were also recently found to be involved in protein degradation. Our main line of work deals with the structural and functional characterisation of molecular chaperones and their interaction in the protein folding and degradation assembly pathways. Using various techniques, principally electron microscopy and image processing, we have been working with chaperones such as CCT, Hsp110, Hsp90, Hsp70, Hsp40 and nucleoplasmin, as well as with some of their co-chaperones like Hop, Hip and CHIP. We have characterised several complexes formed by these chaperones and their co-chaperones that constitute part of the various assembly lines involved in protein homeostasis. These techniques were used to study other proteins and macromolecular complexes including various amyloids, RNA processing proteins and centrosomal proteins.

We also studied centrosomes and ciliary components using microscopy techniques such as electron and X-ray tomography.

Finally, we are developing single-molecule techniques such as optical tweezers, collaborating with other groups in the characterisation of the mechanical properties of long polymers like DNA and RNA, and in the mechanochemistry of the phage Φ29 DNA polymerase.





02 Immunology and Oncology

Research in the Department of Immunology and Oncology focusses on the molecular and cellular bases of immune system function and tumour development, to develop improved approaches for immune response modulation during infection and inflammatory reactions, to innovate in vaccination strategies, and to identify targets for the prevention, diagnosis and treatment of cancer.

The various research groups in the department address many aspects of innate and adaptive immunity, with special emphasis on characterising the molecular mechanisms that underlie inflammation, the processes that drive tissue-specific tumour development, as well as tumour immunology and the relationships among stem cells, inflammation and cancer.

From a methodological perspective, the department's activities are multidisciplinary, combining advanced microscopy and flow cytometry, next-generation genomics technologies, the use of animal models, and the generation of complex genetically modified mouse lines.

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Patricia Leiriao
Didier Soulat

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Elena Liarte
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Andrés Domínguez

TECHNICIANS:
Leticia González
Ruth Gómez Caro

SELECTED PUBLICATIONS

Domínguez PM, López-Bravo M, Kalinke U, Ardavín C. Statins inhibit iNOS-mediated microbicidal potential of activated monocyte-derived dendritic cells by an IFN-β-dependent mechanism. Eur J Immunol. 2011 Nov;41(11):3330-9

Leirião P, del Fresno C, Ardavín C. Monocytes as effector cells: activated Ly-6C(high) mouse monocytes migrate to the lymph nodes through the lymph and cross-present antigens to CD8+ T cells. Eur J Immunol. 2012 Aug;42(8):2042-51

Risco A, del Fresno C, Mambol A, Alsina-Beauchamp D, MacKenzie KF, Yang HT, Barber DF, Morcelle C, Arthur JS, Ley SC, Ardavin C, Cuenda A. p38γ and p38δ kinases regulate the Toll-like receptor 4 (TLR4)-induced cytokine production by controlling ERK1/2 protein kinase pathway activation. Proc Natl Acad Sci USA. 2012 Jul 10;109(28):11200-5

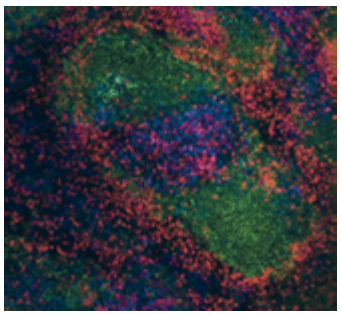
Martin Caballero J, Garzón A, González-Cintado L, Kowalczyk W, Jimenez Torres I, Calderita G, Rodriguez M, Gondar V, Bernal JJ, Ardavín C, Andreu D, Zürcher T, von Kobbe C. Chimeric infectious bursal disease virus-like particles as potent vaccines for eradication of established HPV-16 E7-dependent tumors. PLoS One. 2012;7(12):e52976

Differentiation and functional specialisation of dendritic cells during inflammatory, infectious and allergic processes

Our research explores the functional specialisation of dendritic cells derived from monocytes during inflammatory responses caused by infection by bacteria (*Listeria monocytogenes*), yeasts (*Candida albicans*) and parasites (*Leishmania major*) or by allergic reactions induced by plant- , fungi- or acaridae-derived allergens. Our current research interests are focussed on the following topics:

- Analysis of the effector functions of mouse monocytes in innate and adaptive immunity
- Analysis of the differential migratory properties of mouse monocyte-derived dendritic cells and macrophages.
- Regulation of mouse monocyte differentiation into dendritic cells and macrophages during *in vivo* immune response to *Leishmania major*.
- Functional specialisation of mouse dendritic cells for the induction of Th2 responses against pathogens and allergens.
- Gene expression profile of mouse monocytes and monocyte-derived dendritic cells exposed to allergens and Th2-polarising mediators.
- Effect of statin treatment on proinflammatory cytokine production and nitric oxide metabolism by LPS-activated or *Listeria monocytogenes*-infected mouse monocyte-derived dendritic cells.
- Analysis of the splenic innate immune response to *in vivo Listeria monocytogenes* infection in statin-treated mice.
- Role of type-I interferon in the induction of Th17 immune responses against *Candida albicans* in mice.

1



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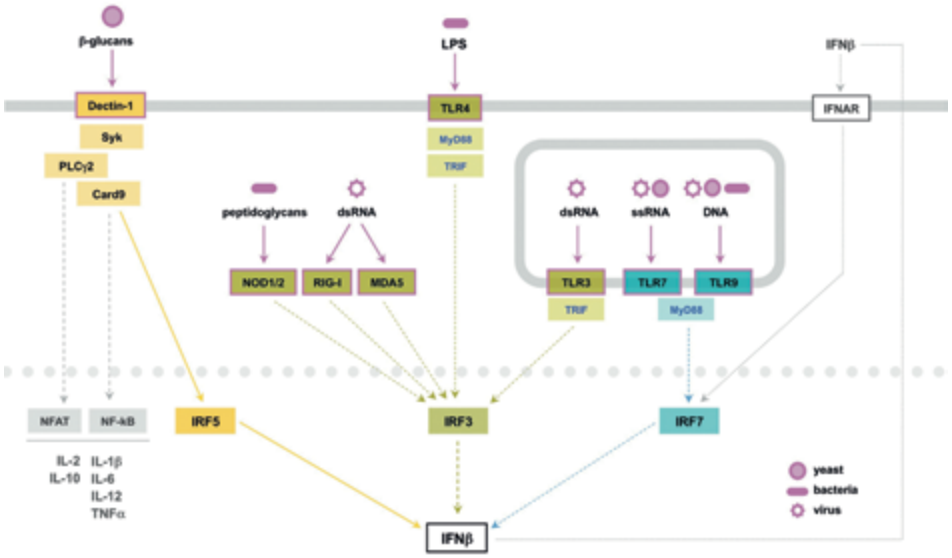

1 *Neutrophilic infiltration of the T cell area of the splenic white pulp during Listeria monocytogenes infection.*

2 *New Dectin-Syk-IRF5-dependent pathway of type-I inteferon production in response to Candida albicans infection.*

GROUP LEADER:
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Adrián Madrigal Avilés

SELECTED PUBLICATIONS

Balomenos D. Cell Cycle regulation and systemic lupus erythematosus. Systemic Lupus Erythematosus (5th Edition). (2011) Chapter: 11 Págs:191-197. Editor:LAHITA/ ELSEVIER.

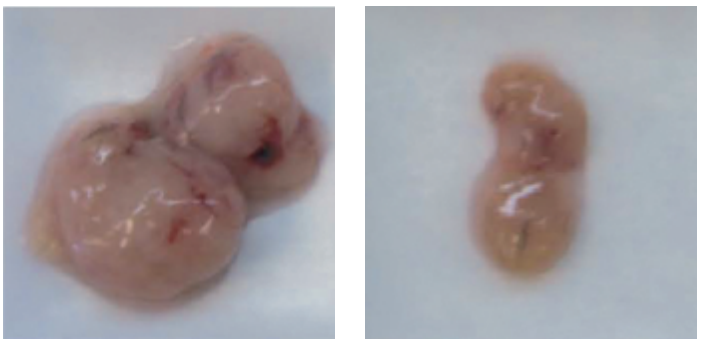
Mavers M, Alexander V. Misharin, Carla M Cuda, Angelica K Gierut, Hemant Agrawal, Evan Weber, G. Kenneth Haines III, Balomenos D, Perlman H. The Cyclin Dependent Kinase Inhibitor p21(WAF1/CIP1/ SDI1) Suppresses and Mediates Resolution of Inflammatory Arthritis via its C-Terminal Domain. Arthritis and Rheumatism (2012).

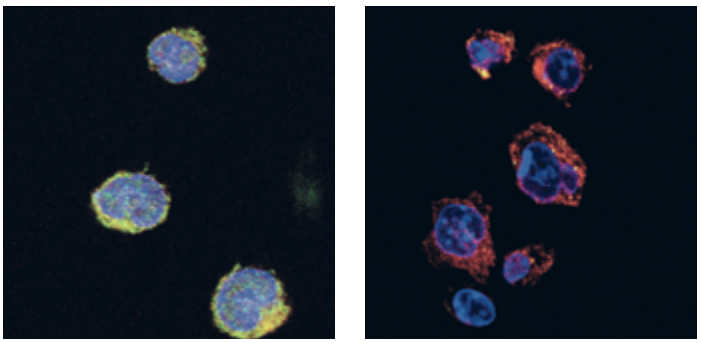
Identification of regulators of activation, apoptosis and proliferation specific for controlling autoimmune T cell memory and inflammation

Novel p21 and Fas functions in normal and autoimmune T memory responses

Apoptosis is considered a basic mechanism for limiting the T cell memory expansion known as homeostasis. Activation and proliferation of memory T cells are also critical for homeostasis control. Autoimmune memory T cells present unique features compared to normal memory T cells, due to their repetitive encounter with autoantigens. Our work focusses on identifying the differences in the control of expansion between naïve, memory and autoimmune memory T cells.

The cell cycle inhibitor p21 suppresses autoimmunity and controls autoimmune but not normal T cell memory responses. We have shown that p21 is an autoimmunity suppressor, since p21 deficiency leads to autoimmunity. Indeed, p21 overexpression in T cells of autoimmune lupus-prone Fas-deficient (B6-*lpr*) mice reduces autoimmunity and lymphadenopathy development (Fig. 1). p21 limits the expansion of autoreactive B6-*lpr* memory T cells but not of normal memory T cells. Our work identifies a p21 function in the regulation of TCR-dependent activation of memory T cells.





❶ *Decreased lymph node size in the presence of the p21 transgene in B6/lpr-p21tg compared to B6/lpr mice at 8 months of age.*

❷ *T cells from B6 (A) and Fas-deficient B6/lpr mice (B) were stained after secondary TCR stimulation, to detect Fas (green) and TCR (red), and mounted with DAPI (blue) for confocal microscopy. Results show strong TCR interaction with Fas shown by color interaction (A), while B6/lpr T cells show only TCR expression and lack of Fas staining, as predicted (B).*

An alternative function for the Fas/FasL apoptosis system. Our analysis of memory T cell activation, apoptosis and cell cycle regulation events in immunity and autoimmune disease revealed a previously unknown function of Fas. We established that Fas also has a crucial attenuating role in the response of previously activated T cells, but not of primary T cells. We focussed on the mechanistic aspects of this role of the Fas/FasL system on preactivated T cells. As shown in Figure 2, Fas interaction with the TCR receptor following secondary stimulation T cell stimulation is evident.

p21 regulates the macrophage activation and inflammation

Independently of its cell cycle inhibitory capacity, p21 regulates macrophage activation by controlling the NF-κB pathway. p21 regulation of NF-κB activation is critical for progression of *in vivo* inflammation, since it decreases sensitivity to LPS-induced septic shock. We are studying the mechanism that defines the role of p21 in NF-κB activation.

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SELECTED PUBLICATIONS

Mejias, R., Barber, D.F. (2012) Tumor targeting of drug-loaded magnetic nanoparticles by an external magnetic field for *in vivo* cytokine delivery in cancer immunotherapy. Hot Topics in Cell Biology, edited by José Becerra and Leonor Santos-Ruiz. Chartridge Books Oxford, Oxford, UK. ISBN 978 1 909287 00 6

Sánchez-Ruiz, J., Mejías, R., García-Belando, M., Barber, D.F., and González-García, A. (2011) Ral GTPases regulate cell-mediated cytotoxicity in NK cells. J Immunol 187: 2433-41.

Gutierrez, L., Mejias, R., Barber, D.F., Veintemillas-Verdaguer, S., Serna, C.J., Lazaro, F.J., and Morales, M.P. (2011) Ac magnetic susceptibility study of *in vivo* nanoparticle biodistribution. J Physics D-Appl Physics, 44, Article Number: 255002.

Mejías, R., Pérez-Yagüe, S., Gutiérrez, L., Cabrera, L.I., Spada, R., Acedo, P., Serna, C.J., Lázaro, F.J., Villanueva, A., Morales, M.P., and Barber, D.F. (2011) Dimercaptosuccinic acid-coated magnetite nanoparticles for magnetically guided *in vivo* delivery of interferon-gamma for cancer therapy. Biomaterials, 32:2938-2952.

PATENT

P201030138: Magnetic nanoparticles to be used in a pharmaceutical composition

Lymphocytes in physiological and pathological processes: autoimmune inflammatory diseases, cancer immunotherapy, and nanobiomedicine

The group's research is organised around the study of molecular and cellular mechanisms that control the immune response and immune tolerance in the regulation of autoimmune disease onset and progression and in the anti-tumour immune response; we also study nanobiomedicine in autoimmunity and cancer.

Autoimmunity and immune-mediated diseases. We analysed atherosclerosis development in the absence ofp110γ. Atherosclerotic plaques in fat-fed LR$^{-/-}$p110γ$^{-/-}$ mice were smaller than in controls, with less immune cell infiltration. This coincided with decreased macrophage proliferation in atherosclerotic lesions, and with higher intracellular cAMP levels.

Altered natural killer (NK) cell activity correlates with the pathogenesis of various autoimmune diseases, although their role in the immunopathogenesis of systemic lupus erythematosus (SLE) is poorly understood. Using MRL/MpJ and MRL/MpJlpr SLE-like murine models, we found greater infiltration of activated NK cells in kidneys of diseased mice, with increased glomerulonephritis. The NKG23D ligands Rae-1 and Mult-1 were expressed in all MRL mouse glomeruli, at higher levels in diseased mice, which correlated with increased NK and CD8^{+} cell infiltration in glomeruli.

Nanobiomedicine and cancer immunotherapy. We developed dimercaptosuccinic acid (DMSA)-coated monodisperse magnetic nanoparticles (MNP) as an IFNγ delivery system. In mouse cancer models, we targeted IFNγ -DMSA-MNP to the tumour site using an external magnetic field, and found a notable reduction in tumour size. We studied long-term biodistribution, toxicity, and *in vivo* biotransformation of DMSA-MNP, which could be a safe, efficient drug delivery system for tumour immunotherapy. We are developing MNP for controlled, localised release of siRNA, microRNA and antagomirs for specific gene silencing as a potential therapy in cancer and autoimmune disorders.

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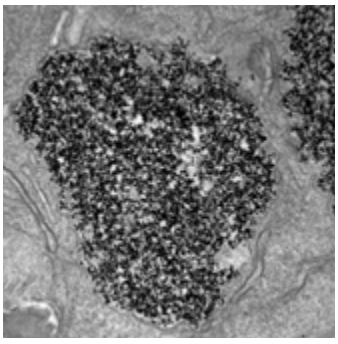

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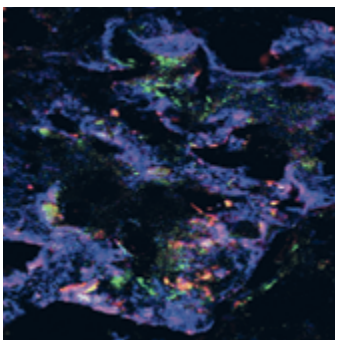

❶ *Magnetic nanoparticles inside vesicles of cells from a pancreatic adenocarcinoma (Photo: R Mejías/C Patiño).*

❷ *Glomerular (purple) infiltration by NK cells (green) in an autoimmune disease process (Photo: R Spada)*

GROUP LEADER:
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Laura Barrio Cano

TECHNICIAN:
Víctor Delgado

SELECTED PUBLICATIONS

Sáez de Guinoa J, Barrio L, Mellado M, Carrasco YR. CXCL13/CXCR5 signaling enhances BCR-triggered B-cell activation by shaping cell dynamics. Blood. 2011 Aug 11;118(6):1560-9

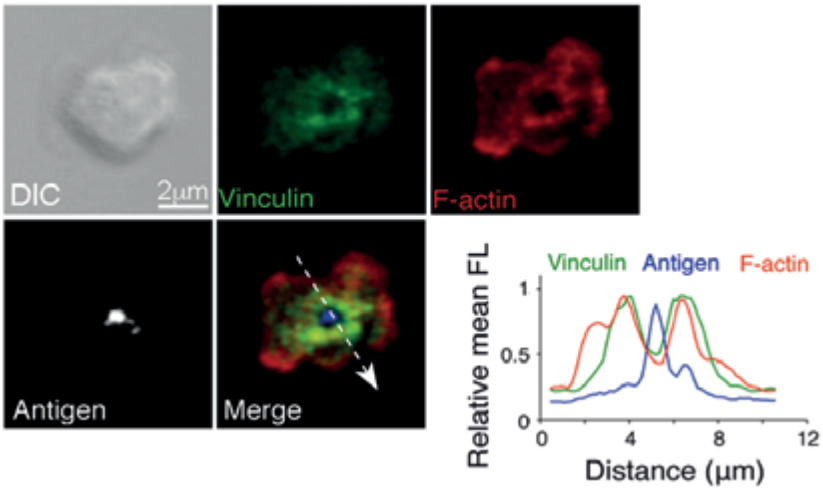
Baixauli F, Martín-Cófreces NB, Morlino G, Carrasco YR, Calabia-Linares C, Veiga E, Serrador JM, Sánchez-Madrid F. The mitochondrial fission factor dynamin-related protein 1 modulates T-cell receptor signalling at the immune synapse. EMBO J. 2011 Apr 6;30(7):1238-50

Rincón E, Sáez de Guinoa J, Gharbi SI, Sorzano CO, Carrasco YR, Mérida I. Translocation dynamics of sorting nexin 27 in activated T cells. J Cell Sci. 2011 Mar 1;124(Pt 5):776-88

B cell dynamics

The regulated interplay between cell adhesion and cell motility is critical for B lymphocyte function. B cells explore entire follicles in secondary lymphoid organs, where antigens are collected and presented by antigen-presenting cells (APC); B cells migrate continuously in response to the chemokine CXCL13, assisted by integrin ligands. Specific B cell receptor (BCR) recognition of antigen leads B cells to establish the immune synapse (IS). This supramolecular structure arrests chemokine-mediated B cell motility and leads to long-lasting B cell adhesion to the APC; a ring-shaped LFA-1 integrin-rich domain surrounds the antigen/BCR cluster formed at the IS. The IS has an important role in B cell activation.

We study the molecular mechanisms used by the CXCL13 receptor CXCR5 and the BCR to coordinate integrin function and thus, B cell adhesion and motility. Our results identified a co-stimulatory function for CXCL13/CXCR5 signalling in shaping the cell dynamics that enhance BCR-triggered B cell activation. At limiting antigen conditions, B cells integrate BCR signals while migrating in response to CXCL13 (kinapse stage). When antigen abundance is sufficient, BCR signalling triggers IS formation (synapse stage) and CXCL13/CXCR5 assist antigen gathering by promoting membrane ruffling. B cells exploit both kinapse and synapse dynamic stages to integrate BCR signals; the use of one or the other will be determined mainly by antigen quality and abundance (Sáez de Guinoa *et al.*, Blood 2011). We recently identified the actin-binding protein vinculin as a major controller of integrin-mediated adhesion dynamics in B cells. BCR recognition of membrane-tethered antigen recruits vinculin to the ring-shaped LFA-1-rich domain of the IS; this is dependent on Syk and actomyosin activity. Lack of vinculin localisation at the IS impairs firm adhesion to the APC and thus, motile B cell arrest. We also focussed on the interplay between CXCR5 and inflammatory/innate receptors of the Toll-like receptor (TLR) family to modulate B cell dynamics and ultimately, B cell fate. We found that TLR4 signalling primes the actin cytoskeleton of B cells for subsequent CXCL13 responses; this might improve B cell abilities to encounter cognate antigen.



1 *Vinculin recruitment and localisation at the B cell immune synapse. Ring-shaped distribution pattern of vinculin and F-actin surrounding the central antigen cluster at the B cell synapse. Profile of relative mean fluorescence distribution of the indicated markers along the white arrow (right).*

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Vicente Pérez García

TECHNICIANS:
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Lorena Sanz González

Functional study of PI3K in survival, cell division and cancer

Our team is currently examining the function of kinases that affect the cell membrane lipid composition on cell behaviour, with special emphasis on phosphoinositide 3-kinases (PI3K).

These enzymes (class IA) phosphorylate the membrane phosphoinositides, giving rise to a lipid product (PIP3) that is normally found at low levels in quiescent cells. PIP3 levels increase transiently when normal cells are activated to execute a cell response, as PIP3 induces cell survival and contributes to triggering cell migration and division. In cancer cells, PIP3 levels are constitutively high (in ~50% of all human cancers).

Although the PI3K enzymatic system is clearly a promising therapeutic target, very few therapeutic approaches are currently directed successfully to this target.

We study the molecular mechanisms by which PI3K isoforms control cell behaviour and its potential implications in disease.

In chronic inflammation, our recent findings show PI3K involvement in systemic lupus erythematosus, due in part to its capacity to mediate memory T cell survival. These studies help to show that haematopoietic PI3K isoforms are a potential target for chronic inflammatory disease treatment.

In cancer, we focus on a nuclear form of PI3K that controls DNA duplication, DNA repair and chromosome division, among other processes. Our goal is to define how this enzyme controls DNA homeostasis and the consequences of its amplification in cancer.

The second aspect of our recent studies is to understand why PI3K regulatory subunit usage changes during tumour progression, how this affects tumour progression and invasion, and the potential therapeutic applications of these findings.

SELECTED PUBLICATIONS

Kumar A, Fernandez-Capetillo O, Carrera AC. Nuclear phosphoinositide 3-kinase beta controls double-strand break DNA repair. Proc Natl Acad Sci USA. 2010 Apr 20;107(16):7491-6

Kumar A, Redondo-Muñoz J, Perez-García V, Cortes I, Chagoyen M, Carrera AC. Nuclear but not cytosolic phosphoinositide 3-kinase beta has an essential function in cell survival. Mol Cell Biol. 2011 May;31(10):2122-33

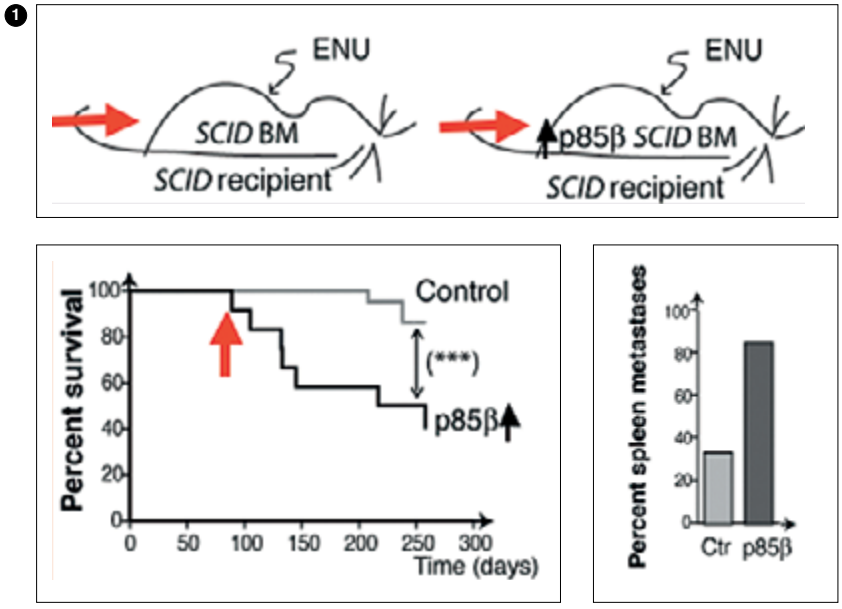
Suárez-Fueyo A, Barber DF, Martínez-Ara J, Zea-Mendoza AC, Carrera AC. Enhanced phosphoinositide 3-kinase δ activity is a frequent event in systemic lupus erythematosus that confers resistance to activation-induced T cell death. J Immunol. 2011 Sep 1;187(5):2376-85

Cortés I, Sánchez-Ruíz J, Zuluaga S, Calvanese V, Marqués M, Hernández C, Rivera T, Kremer L, González-García A, Carrera AC. p85β phosphoinositide 3-kinase subunit regulates tumor progression. Proc Natl Acad Sci USA. 2012 Jul 10;109(28):11318-23

Silió V, Redondo-Muñoz J, Carrera AC. Phosphoinositide 3-kinase β regulates chromosome segregation in mitosis. Mol Biol Cell. 2012 Dec;23(23):4526-42

PATENT

P201031137: Biomarker for cancer diagnosis, prognosis and follow-up.



1 *p85β-driven tumour lymphomagenesis model induced in SCID mice after transplant with control or p85β-infected bone marrow, followed by ENU treatment. Kaplan-Meier survival curves (right); (***) Mantel-Cox test P <0.001 (n = 15 p85β, n = 22 controls) and percentage of mice with spleen metastases of the thymic lymphoma (left).*

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VISITING SCIENTISTS:
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Marilena Palmisano

SELECTED PUBLICATIONS

Cerezo-Guisado MI, del Reino P, Remy G, Kuma Y, Arthur JS, Gallego-Ortega D, Cuenda A. Evidence of p38γ and p38δ involvement in cell transformation processes. Carcinogenesis. 2011 Jul;32(7):1093-9

Risco A, Cuenda A. New insights into the p38γ and p38δ MAPK pathways. J Signal Transduct. 2012;2012:520289

Risco A, del Fresno C, Mambol A, Alsina-Beauchamp D, MacKenzie KF, Yang HT, Barber DF, Morcelle C, Arthur JS, Ley SC, Ardavin C, Cuenda A. p38γ and p38δ kinases regulate the Toll-like receptor 4 (TLR4)-induced cytokine production by controlling ERK1/2 protein kinase pathway activation. Proc Natl Acad Sci USA. 2012 Jul 10;109(28):11200-5

Chen CM, Bentham J, Cosgrove C, Braganca J, Cuenda A, Bamforth SD, Schneider JE, Watkins H, Keavney B, Davies B, Bhattacharya S. Functional significance of SRJ domain mutations in CITED2. PLoS One. 2012;7(10):e46256

Role of stress-activated protein kinase p38MAPK in human diseases

The aim of our group is to discover how members of the p38MAPK family regulate cell function in physiological conditions and in response to environmental stresses, infection and proinflammatory cytokines, and to understand how they are deregulated in several human disease situations such as oncogenic transformation and inflammation.

Our research is focussed on:

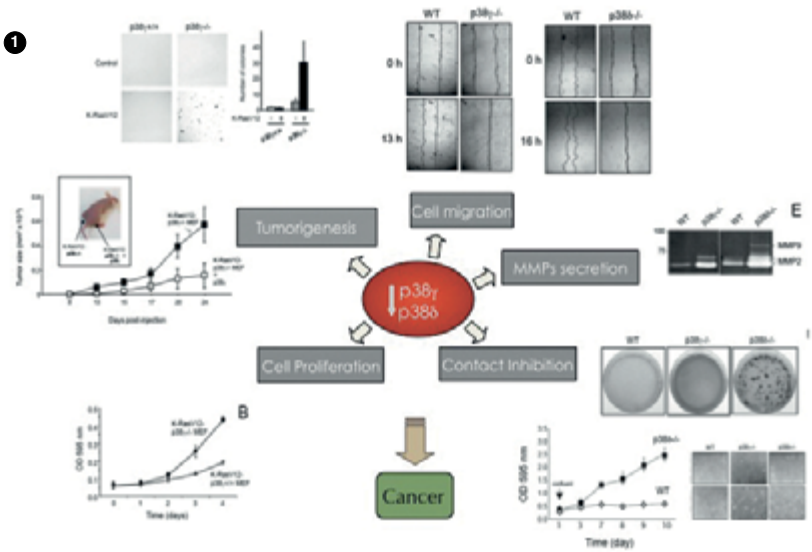
1. The discovery of new substrates, interacting proteins and inhibitors for these kinases, and the study of their physiological roles using mice transgenic for p38 isoforms, and

2. The study of p38MAPK as a link between chronic inflammation and cancer, and as mediators of chronic inflammatory diseases.

These studies use biochemical, cell biology and whole animal model approaches.

There are four p38MAPK family members (p38α, p38β, p38γ and p38δ), which are similar in amino acid sequence but differ in expression patterns, substrate specificities and sensitivity to inhibitors. In recent years, our group has centred on elucidating the regulation and roles of p38γ and p38δ. Using p38γ- or p38δ-deficient cells, we found that these kinases regulate processes involved in malignant cell transformation such as contact inhibition, migration, apoptosis, proliferation and tumourigenesis. Moreover, lack of p38γ in K-Ras-transformed fibroblasts causes an increase in cell proliferation *in vitro* and tumour formation *in vivo*, whereas lack of p38δ in fibroblasts blocks cell growth by inhibition contact and induces focus formation. Additionally, we recently showed that p38γ and p38δ are key components in innate immune responses, since their deletion impaired the innate immune response to septic shock by reducing the production of inflammatory cytokines such as TNFα and IL1β.

We are currently studying how p38γ and p38δ regulate the integrity of nuclear and intercellular-junctional complexes, cell adhesion, migration and polarity in response to many kinds of external stimuli. Little is known about the role of p38γ and p38δ isoforms in chronic inflammatory disease. We are currently undertaking further studies to investigate this as well as their role in the development of cancer associated to inflammation, using the genetically modified mice.



1 *Evidence of p38γ and p38δ as a tumour suppressor in cell transformation processes*

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Cristina Bravo

SELECTED PUBLICATIONS

Urdinguio RG, Fernandez AF, Moncada-Pazos A, Huidobro C, Rodriguez RM, Ferrero C, Martinez-Camblor P, Obaya AJ, Bernal T, Parra-Blanco A, Rodrigo L, Santacana M, Matias-Guiu X, Soldevilla B, Dominguez G, Bonilla F, Cal S, Lopez-Otin C, Fraga MF. Immune dependent and independent anti-tumor activity of GM-CSF aberrantly expressed by mouse and human colorectal tumors. Cancer Res. 2013 Jan 1;73(1):395-405

Fernandez AF, Huidobro C, Fraga MF. *De novo* DNA methyltransferases: oncogenes, tumor suppressors, or both? Trends Genet. 2012 Oct;28(10):474-9

Huidobro C, Urdinguio RG, Rodríguez RM, Mangas C, Calvanese V, Martínez-Camblor P, Ferrero C, Parra-Blanco A, Rodrigo L, Obaya AJ, Suárez-Fernández L, Astudillo A, Hernando H, Ballestar E, Fernández AF, Fraga MF. A DNA methylation signature associated with aberrant promoter DNA hypermethylation of DNMT3B in human colorectal cancer. Eur J Cancer. 2012 Sep;48(14):2270-81

Calvanese V, Fernández AF, Urdinguio RG, Suárez-Alvarez B, Mangas C, Pérez-García V, Bueno C, Montes R, Ramos-Mejía V, Martínez-Camblor P, Ferrero C, Assenov Y, Bock C, Menendez P, Carrera AC, Lopez-Larrea C, Fraga MF. A promoter DNA demethylation landscape of human hematopoietic differentiation. Nucleic Acids Res. 2012 Jan;40(1):116-31

Feil R, Fraga MF. Epigenetics and the environment: emerging patterns and implications. Nat Rev Genet. 2012 Jan 4;13(2):97-109

The role of epigenetics in cancer

Epigenetics has become a fundamental discipline in the study of genome biology. Cell growth and differentiation are largely regulated by epigenetic processes. The alteration of these epigenetic mechanisms is associated with numerous pathologies, notably cancer. Our research currently focusses on the study of locus-specific and genome-wide epigenetic mechanisms such as DNA methylation and histone post-translational modifications during cell differentiation, and their alterations in cancer and aging. We are particularly interested in the role of locus-specific DNA demethylation in health and disease, and are involved in ultra-deep sequencing projects to characterises the genome-wide patterns of specific epigenetic marks in human tumourigenesis. Recent findings in our laboratory include the identification of a DNA methylation signature in human haematopoiesis, the discovery of specific genome-wide DNA methylation patterns associated with aberrant regulation of DNMT3B in colon cancer, characterisation of the molecular mechanisms involved in the aberrant regulation of the bromodomain BRD4 in colon cancer, and identification of an epigenetic alteration responsible for aberrant secretion of the cytokine GM-CSF by colorectal cancer cells.

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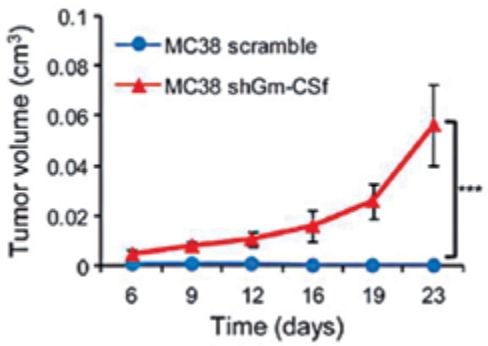

❷

❶ *Inhibition of GM-CSF promotes colon cancer growth.*

❷ *Epigenetic changes during hematopoietic differentiation*

GROUP LEADER:
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COLLABORATORS:
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Mónica García-Gallo
Mercedes Llorente
María Teresa Martín

SELECTED PUBLICATIONS

González-Magaldi M, Postigo R, de la Torre BG, Vieira YA, Rodríguez-Pulido M, López-Viñas E, Gómez-Puertas P, Andreu D, Kremer L, Rosas MF, Sobrino F. Mutations that hamper dimerization of foot-and-mouth disease virus 3A protein are detrimental for infectivity. J Virol. 2012, 86:11013-23.

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PCT ES2010/070718: Monoclonal antibody against the protein GAPDH from *Candida famata.*

PCT ES09/070206: Antibody anti-Dectin-1, hybrodoma producer of this antibody and its application

Chemokine-receptor interactions in physiopathological processes

Our group is interested in understanding how chemokines and their receptors regulate the interaction between cancer cells and their microenvironment, specifically, how these proteins control tumour growth and progression. We also evaluate the potential of chemokine receptors as targets for anti-tumour therapy.

Chemokine receptors are a family of seven transmembrane domain proteins that are coupled to G proteins and regulate cell migration during homeostasis, inflammation and infection. These proteins are important in defining organ-specific metastatic localisation in cancer. The levels of certain chemokine receptors correlate with cell dissemination in lymph nodes, lung, bones, liver or other sites. We focus mainly on CCR9, a receptor expressed almost exclusively on lymphoid cells in the thymus, infiltrating cells in small bowel, a small subset of circulating memory T lymphocytes (CCR9+ $\alpha 4\beta 7$hi), IgA-secreting plasma cells and plasmacytoid dendritic cells. The chemokine CCL25, the CCR9 ligand, is expressed in thymus and small bowel.

Recent reports showed that CCR9 overexpression increases the migratory and invasive capacity of prostate and ovarian cancer cells, directs melanoma cell metastases to the small intestine, and increases proliferation and resistance to apoptosis of acute lymphoblastic leukaemia-derived cell lines. CCR9-mediated intracellular signalling activates anti-apoptotic pathways and downregulates caspase activation, leading to cell survival and increased proliferation.

Our laboratory focusses on determining the role of CCR9 in tumour physiopathology. We have generated mouse anti-human CCR9 monoclonal antibodies that recognise this receptor by flow cytometry, Western blot and immunocytochemistry. Using xenogeneic mouse models and human tumour lines, we study whether tumour cell cycling, survival, migration, invasiveness or growth are inhibited by these antibodies. CCR9-expressing human carcinoma cell lines and RNA interference approaches are also being used to study the molecular mechanisms that underlie CCR9-mediated effects. In addition, we are analysing whether these antibodies can be used for diagnostic imaging and for screening of low molecular weight antagonists.

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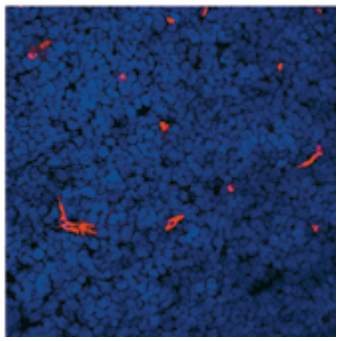

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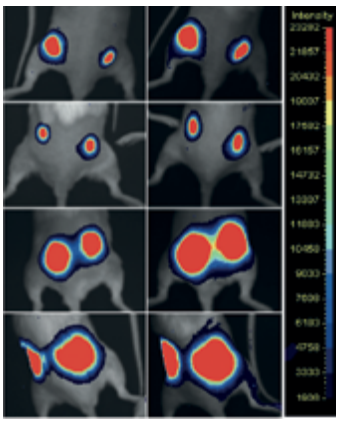

❶ *Immunofluorescence staining of a human xenograft tumour. Paraffin sections were stained with anti-CD31 antibody (red), a specific endothelial marker suitable for the identification of blood vessels and angiogenesis. Cell nuclei were counterstained with DAPI (blue).*

❷ *Bioluminescence images showing differential growth of human leukaemia cell tumours in an immunodeficient $Rag2^{-/-}$ mouse model, in response to treatment with two different monoclonal antibodies. Luciferase activity was measured and transformed to a pseudocoloured image.*

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González-Martín A, Gómez L., Lustgarten J, Mira E, Mañes S. Maximal T cell-mediated antitumor responses rely upon CCR5 expression in both CD4+ and CD8$^+$ T cells. Cancer Res. 2011 Aug 15;71(16):5455-66

González-Martín A, Mira E, Mañes S. CCR5 in cancer immunotherapy: more than an "attractive" receptor for T cells. Oncoimmunology. 2012 Jan 1;1(1):106-108

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PATENTS

P201031015. Use of SOD-3 as adjuvant for immunotherapy

PCT/EP2011/051237: Methods and compositions for the treatment of AIDS. Use of Des-1 inhibitors to prevent HIV infection

Signalling networks in inflammation and cancer

Inflammation is a complex stereotypical response essential for effective defence of the organism against harmful stimuli such as pathogens, irritants or tissue damage. The detailed processes of inflammation indicate a close relationship between the inflammatory reaction and the immune response. A hallmark of inflammation is the directed migration (chemotaxis) of inflammatory cells (mostly leukocytes) through the walls of blood vessels to the site of injury. Once wound healing is complete, inflammation resolves and tissue homeostasis returns. A deregulated response to tissue damage can nonetheless lead to autoimmunity and chronic inflammatory diseases, and can also promote cancer.

Recent clinical and experimental evidence indicates that solid tumours increase inflammation to promote their progression. This leads to a tumour microenvironment largely controlled by inflammatory cells, which alters the metabolic needs of the tissue, promoting neo-angiogenesis, proliferation, survival, mutagenesis, migration and metastasis of malignant cells. Intriguingly, tumour-induced inflammation usually leads to immunosuppression, impeding the surveillance function of the immune system and clearance of the tumour. Breaking immunosuppression has been demonstrated as a useful, efficient means of eradicating cancers. Immune cells might thus provide both anti- and pro-tumourigenic signals, which might be harnessed or attacked for therapeutic purposes.

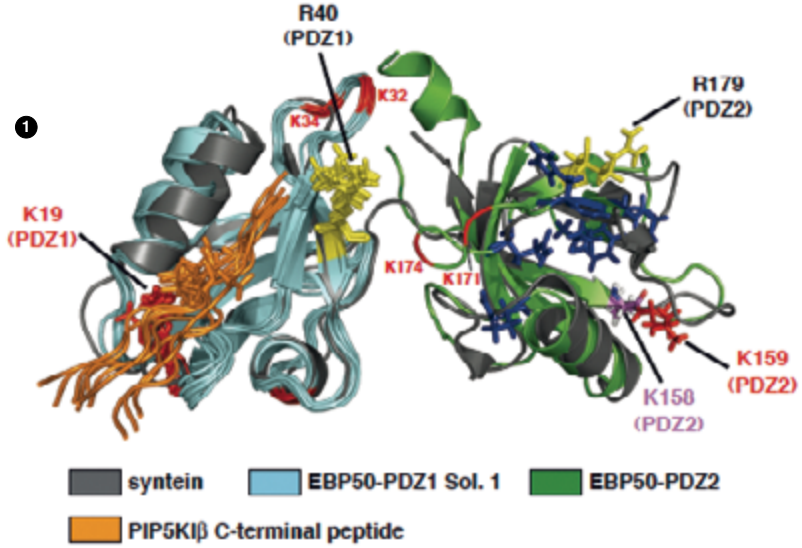
We aim to understand the critical cellular, molecular, and chemical mediators by which tumours promote inflammation and subvert the immune system to favour their own progression. We hope that comprehension of the mechanisms that balance pro- and anti-tumour immunity will lead to the design of more effective anti-cancer therapeutics.

The main achievements of the group in these two years have been

1. The study of signalling pathways elicited by the phosphatidylinositol-4-phosphate-5-kinase during acquisition of a motile phenotype in leukocytes.

2. The characterisation of the specific role of CCR5 and its ligands in antigen cross-presentation and the potentiation of anti-tumour responses.

3. The identification of SOD3 as a molecule involved in normalisation of the tumour vasculature and enhancement of chemo and immunotherapy of cancer.



1 *Model for interaction between the PDZ-binding motif in the type1 PIP5K-beta isoform and the tandem EBP50-PDZ domains (modelled based on syntein structure). The critical EBP50 residues involved in PIP5K binding are indicated.*

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SELECTED PUBLICATIONS

Martínez-A C, van Wely KH. Centromere fission, not telomere erosion, triggers chromosomal instability in human carcinomas. Carcinogenesis. 2011 Jun;32(6):796-803

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Fütterer A, Raya A, Llorente M, Izpisúa-Belmonte JC, de la Pompa JL, Klatt P, Martínez-A C. Ablation of Dido3 compromises lineage commitment of stem cells *in vitro* and during early embryonic development. Cell Death Differ. 2012 Jan;19(1):132-43

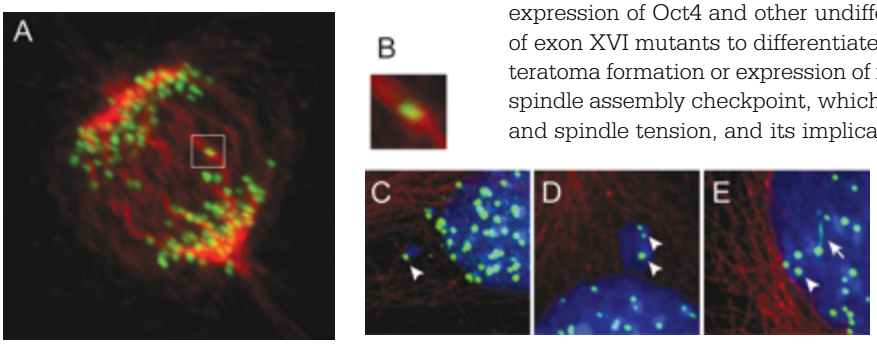
Muñoz LM, Holgado BL, Martínez-A C, Rodríguez-Frade JM, Mellado M. Chemokine receptor oligomerization: a further step toward chemokine function. Immunol Lett. 2012 Jul 30;145(1-2):23-9

Biology of stem cells: genomic instability, cancer and immunoregulation

We work with stem cells to try to understand the connections between chromosome instability and the stem cell theory of cancer. Most tumours have a combination of genetic defects termed chromosome instability (CIN), found in approximately 85% of non-hereditary carcinomas. We study two aspects of CIN, aneuploidy and the combination of translocations, duplications and deletions known collectively as segmental chromosome defects. The data from patient material and human cancer cell lines show that the majority of chromosome defects in human carcinomas comprise pericentromeric breaks that are captured by healthy telomeres; only a small proportion of chromosome fusions can be attributed to telomere erosion or random breakage. After comparing different DNA breakage pathways and by studying the role of mitotic spindle defects in the generation of aneuploidy and chromosome breakage, we postulate that a single mechanism, centromere fission, might be responsible for genetic instability on the cellular, individual and evolutionary scale. We hypothesise that centromere fission, rather than telomere erosion, is the most probable trigger of CIN and early carcinogenesis.

One of our model systems focusses on mutations in Dido, a locus identified by genome-wide screens as a potential regulator of stem cell function. The Dido locus is a gene complex described only in higher vertebrates; it encodes three splice variants, Dido1 (the smallest of these), Dido2 and Dido3. Dido3 also interacts with centrosomes and the synaptonemal complex. N-terminal truncation of Dido3 provokes aneuploidy, centrosome amplification and increased incidence of haematological myeloid neoplasms in the adult mouse. Misexpression of this splice variant is linked to myelodysplastic syndrome/myeloproliferative disease in humans.

In all attempts to date, deletion of the entire Dido locus or of Dido3-specific exons (exon XVI) in cell lines or mice has failed, indicating that its loss is incompatible with life. Stem cells generated from a targeted deletion of exon XVI show aneuploidy, centrosome amplification and inability to differentiate *in vitro*, as indicated by sustained expression of Oct4 and other undifferentiated stem cell markers. The ability of exon XVI mutants to differentiate can be restored by retinoic acid, *in vivo* teratoma formation or expression of full-length Dido3. The role of Dido3 in the spindle assembly checkpoint, which senses and signals centrosome position and spindle tension, and its implication in an early cell-cell contact-induced ES cell differentiation step, render Dido3 an appropriate candidate for future study of proteins that couple centrosome orientation to stem cell decisions.



1 *Merotelic attachments in Dido mutant cells. Dido mutant MEF were seeded on coverslips and double-labelled with antibodies to centromeres (green) and α-tubulin (red). DNA was stained with DAPI (blue), and cells were studied by confocal fluorescence microscopy. (A) Detection of merotelic centromere attachments in Dido mutant MEF. An individual anaphase centromere attached to both spindle poles is shown. The image of a whole cell is from maximum projection (A), and the three-fold amplification (B) is from a single confocal layer. (C-E) Appearance of centromeres in Dido mutant MEF. Centromeres in micronuclei (C) and in a nuclear protrusion (D) are indicated (arrowheads). (E) In some cases, an individual centromere is distorted (arrow), whereas neighbouring centromeres appear normal (arrowhead). From PNAS;107:4159-4164*

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SELECTED PUBLICATIONS

Barroso R, Martínez Muñoz L, Barrondo S, Vega B, Holgado BL, Lucas P, Baíllo A, Sallés J, Rodríguez-Frade JM, Mellado M. EBI2 regulates CXCL13-mediated responses by heterodimerization with CXCR5. FASEB J. 2012 Dec;26(12):4841-54

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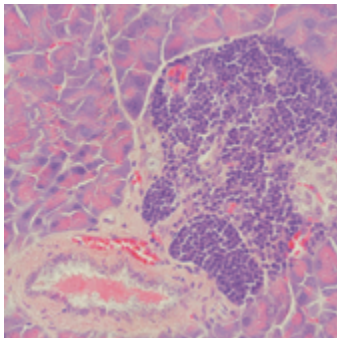
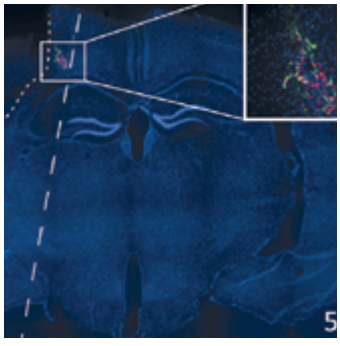
Chemokine receptors: new targets for therapeutic intervention

The chemokines, a family of structurally related chemoattractant proteins that bind to specific seven transmembrane receptors linked to G proteins, trigger a broad array of biological responses that range from cell polarisation, movement, immune and inflammatory responses to prevention of HIV-1 infection. Chemokine-mediated cell activation was thought to be due to the binding of a monomeric chemokine to its monomeric receptor. Chemokine biology is nonetheless more complex than was initially predicted, as several studies suggest that chemokines can dimerise and that their receptors are found as dimers and/or higher order oligomers at the cell surface. These complexes form organised arrays that can be modified by receptor expression and ligand levels, indicating that they are dynamic structures. This oligomerisation could be important to different aspects of their biology, from ontogeny to the regulation of their pharmacological and signalling properties.

Using fluorescence resonance energy transfer (FRET) techniques, we confirmed the functional relevance and regulation of homo- and heterodimeric receptor complexes. CXCR5 and EBI2 form homo- and heterodimers. EBI2 expression induces conformational changes in CXCR5 homodimers, triggering a notable reduction in binding affinity of the ligand (CXCL13) for CXCR5; as a consequence, CXCR5-mediated responses in cell lines and primary B cells is altered. In addition to the receptors, the chemokines themselves oligomerise, which influences cell activation. HMGB1 (high mobility group box 1), a nuclear protein released by necrotic and severely stressed cells, binds CXCL12; the heterocomplexes promote conformational rearrangements of CXCR4 different from those of CXCL12 alone. As a result, HMGB1 influences inflammatory cell recruitment to damaged tissues. We reported that chemokines activate the JANUS kinases (JAK), which associate to the chemokine receptor and promote its rapid tyrosine phosphorylation. Through the JAK/STAT pathway, the chemokines trigger suppressor of cytokine signalling (SOCS) expression. The SOCS intracellular proteins are thus key physiological regulators of cytokine and chemokine responses; they target ubiquitinated signalling intermediates for degradation by the proteasome pathway through ECS (elongin-Cullin-SOCS) E3 ligase formation. As a result of this effect, SOCS1 acts as a tumour suppressor, blocking cell cycling in human melanoma by affecting G1/S and mitosis.





1 *In type I diabetes, immune cells invade the pancreas*

2 *In cerebral infarction, neural stem cells migrate toward the ischaemic area*

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SELECTED PUBLICATIONS

Martínez-Moreno M, García-Liévana J, Soutar D, Torres-Ayuso P, Andrada E, Zhong XP, Koretzky GA, Mérida I, Ávila-Flores A. FoxO-dependent regulation of diacylglycerol kinase ζ gene expression. Mol Cell Biol. 2012 Oct;32(20):4168-80

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Role of diacylglycerol kinases in the control of immune response and cancer progression

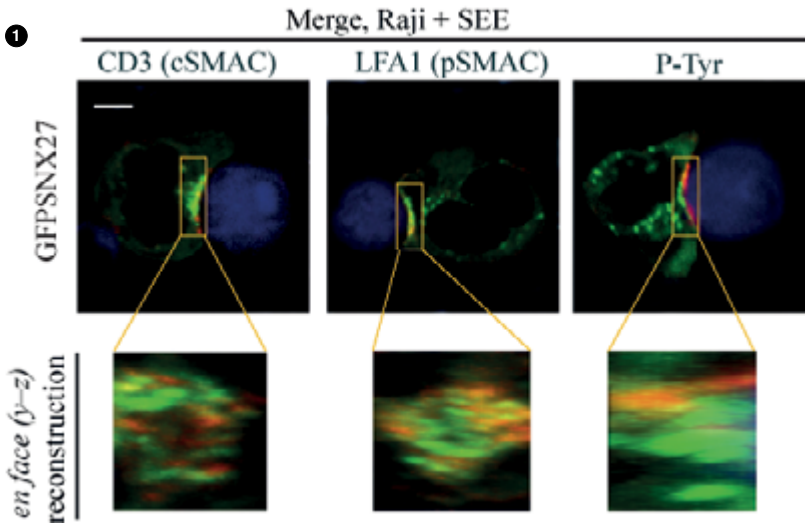
We study the contribution of diacylglycerol kinases (DGK) to T cell functions and oncogenic transformation. We have focussed on two specific isoforms that are important modulators of DAG-dependent functions in T lymphocytes. We expect that our findings will help to assess the therapeutic potential of the DGK enzyme family as tools for better, more effective management of the immune response and treatment of cancer. The work in the laboratory is organised in two different, complementary areas:

1. DGK and as negative regulators of the adaptive immune response

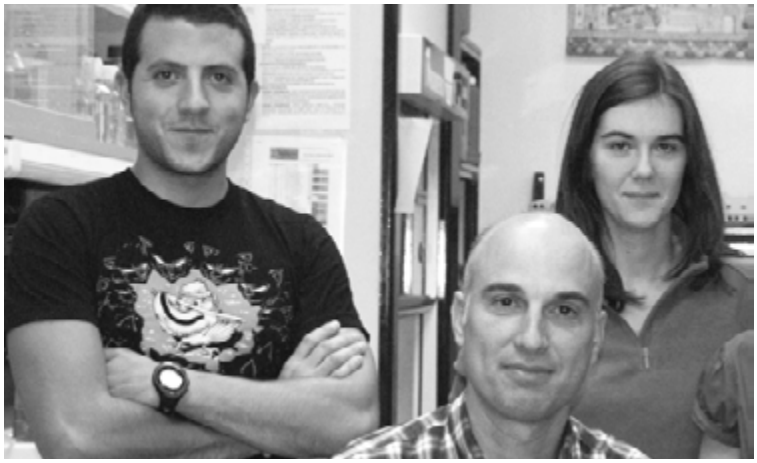
We and others have characterised DGKα and ζ as negative regulators of the DAG that is generated during T cell activation, limiting Ras guanyl-releasing protein (RasGRP1)-dependent activation of Ras. A better understanding of the mechanisms that regulate the expression and activation of these two enzymes will allow T cell functions to be modulated, perhaps even harnessed, to provide new strategies in combating diseases such as autoimmunity and cancer. In the past years, we have contributed to better understand the mechanism that determines DGKα localisation/activation during immune synapse formation, as well as its contribution to DAG metabolism at this site. We also identified DGKα as a gene regulated by the AKT/FoxO axis, and characterised it as a negative regulator of TCR and IL2-regulated functions.

2. DGKα and ζ: the lipid restraint on tumour metabolism

Our studies intend to define the precise contribution of these DGK isoforms to the onset of malignant transformation. We use genetic and biochemical approaches to investigate the role of DGKα in the control of the transition from quiescence to proliferative states in untransformed cells, and to assess its function in the context of malignant transformation. Validation of the distinct regulation of expression as well as careful assessment of DGKα and ζ functions in normal and transformed cells is an important challenge to the full evaluation of the potential of these proteins as therapeutic targets.



1 *SNX27 distribution at the Jurkat T cell mature inmune synapse. GFPSNX27-transfected Jurkat T cells were stimulated with SEE-loaded Raji B cells (blue). Cell-cell conjugates were fixed, processed for IF with anti-CD3 (first column), anti-LFA1 (second column) or anti-PTyr (third column), followed by Cy3-anti-mouse IgG Ab and imaged by confocal microscopy.*



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SELECTED PUBLICATIONS

Vallespinós M, Fernández D, Rodríguez L, Alvaro-Blanco J, Baena E, Ortiz M, Dukovska D, Martínez D, Rojas A, Campanero MR, Moreno de Alborán I. B lymphocyte commitment program is driven by the proto-oncogene *c-myc*. J Immunol. 2011 Jun 15;186(12):6726-36

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Fernandez D, Sanchez-Arevalo VJ, de Alboran IM. The role of the proto-oncogene *c-myc* in B lymphocyte differentiation. Crit Rev Immunol. 2012;32(4):321-34

Function of the c-Myc proto-oncogene *in vivo*

During an immune response, activated B lymphocytes undergo a final stage of differentiation known as terminal B lymphocyte differentiation. This process is characterised by the generation of antibody-secreting cells (ASC) or plasma cells, and memory B cells. Antigen re-challenge can improve the function of the antibodies produced by these specialised cells through two mechanistically linked processes, immunoglobulin class switch recombination (CSR) and somatic hypermutation (SH). The transcriptional program driving terminal B cell differentiation is regulated by the transcriptional repressor Blimp-1. Among its transcriptional targets, Blimp-1 downregulates the gene expression of *c-myc*. c-Myc protein is a member of the MYC family of transcription factors involved in multiple biological processes such as cell proliferation, apoptosis and differentiation.

Despite extensive study of Myc, little is known of its function in terminal B cell differentiation. In recent years, our group has focussed on defining the role of c-Myc in this process. It is assumed that repression of *c-myc* by Blimp-1 during terminal differentiation is intended to cease cell proliferation exclusively. However, we has observed that c-Myc function goes beyond the regulation of cell proliferation during terminal B lymphocyte differentiation. We found that c-Myc is also essential for ASC function and differentiation *in vivo* using several genetically-modified mouse models. Our current efforts in the lab are focussed on the characterisation of the c-Myc-dependent mechanisms governing this process and how these functions might be altered in human pathologies.

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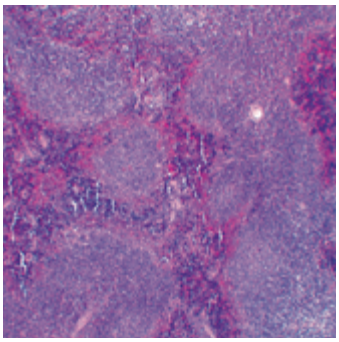

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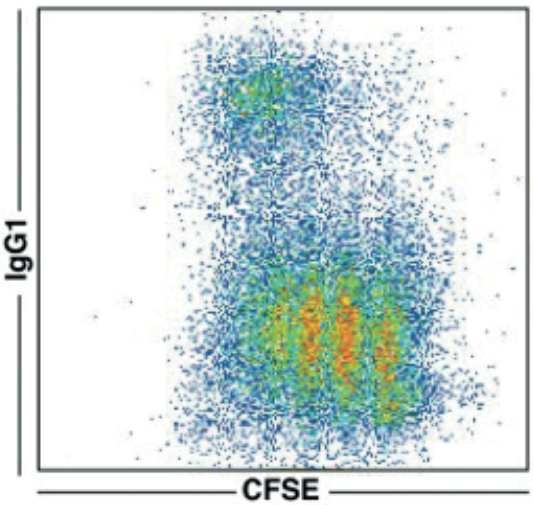

❶ *Haematoxylin / Eosin-stained section of mouse spleen*

❷ *Immunoglobulin class switch recombination (CSR) and cell division of activated B lymphocytes in vitro. Flow cytometry analysis of B lymphocytes were stained with CFSE and anti-IgG1 to monitor cell division and CSR, and analysed by Flow cytometry.*

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SELECTED PUBLICATIONS

Zonca M, Mancheño-Corvo P, DelaRosa O, Mañes S, Büscher D, Lombardo E, Planelles L. APRIL and BAFF proteins increase proliferation of human adipose-derived stem cells through activation of Erk1/2 MAP kinase. Tissue Eng Part A. 2012 Apr;18(7-8):852-9

PATENT

EP10382244,PCT/EP11/065540. Stem cell culture media and methods

Function and regulation of APRIL, a TNF protein: implications in pathology

Our research studies the biological functions of the tumour necrosis factor proteins APRIL (a proliferation-inducing ligand) and BAFF (B cell-activator factor) in- and outside the immune system, and their involvement in disease processes such as cancer. We have three main projects in which we characterise the modulatory role of APRIL on B lymphocytes, examine the dialogue between hASC and APRIL in an inflammatory context, and assess the relevance of APRIL in solid tumours such as breast cancer. We generated MMTV-ErbB2-APRIL-Tg and MMTV-ErbB2-APRIL-KO mouse models and used a panel of human breast carcinoma cell lines to study the implication of APRIL in breast cancer. We found that APRIL is expressed in breast carcinoma cell lines; it supports their basal growth and activates MAP kinase signalling pathways. We are currently analysing the mouse models as well as the clinical potential of APRIL.

We recently characterised the APRIL/BAFF system in hASC. hASC are mesenchymal stem cells with reduced immunogenicity and the ability to modulate immune responses; hASC therapy is currently under clinical investigation. As APRIL and BAFF proteins are overexpressed in many inflammatory and autoimmune diseases, we addressed their potential association with hASC. We found that hASC express APRIL and BAFF as well as their receptors TACI, BCMA and the BAFF-specific receptor (BAFF R). APRIL and BAFF secretion was differentially enhanced by CXCL12 and IFNγ, implicated in hASC-mediated migration and immunosuppression, respectively. In addition, APRIL and BAFF induced rapid phosphorylation of ERK1/2 and Akt kinases and promoted an increase in hASC proliferation, without affecting the immunosuppressive capacity of these cells. The use of specific chemical inhibitors indicated that the PI3K transduction pathway is involved in hASC basal growth, and that APRIL- and BAFF-mediated effects are ERK-dependent.

These results provide new information about the molecular mechanisms that underlie APRIL and BAFF secretion and signalling in hASC, and are of special relevance for the use of allogeneic hASC as therapeutic tools.

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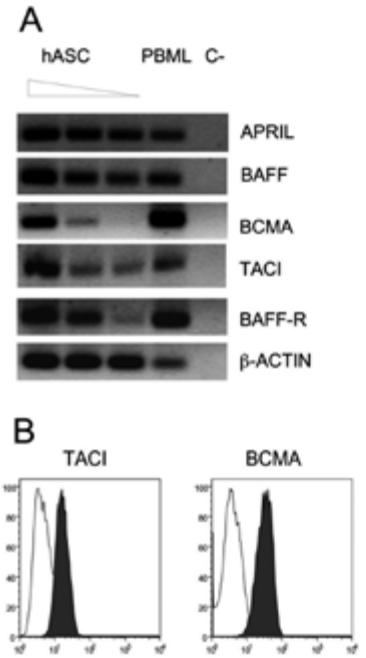

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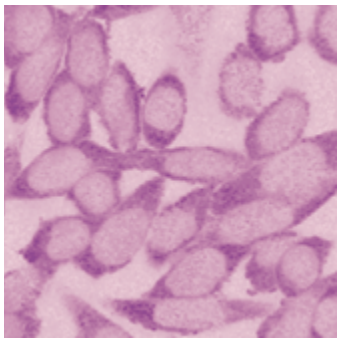
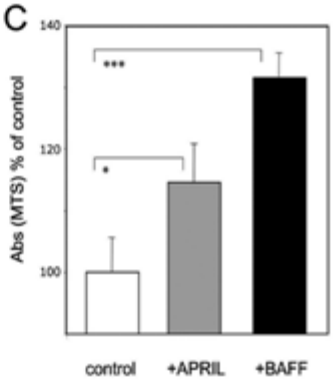
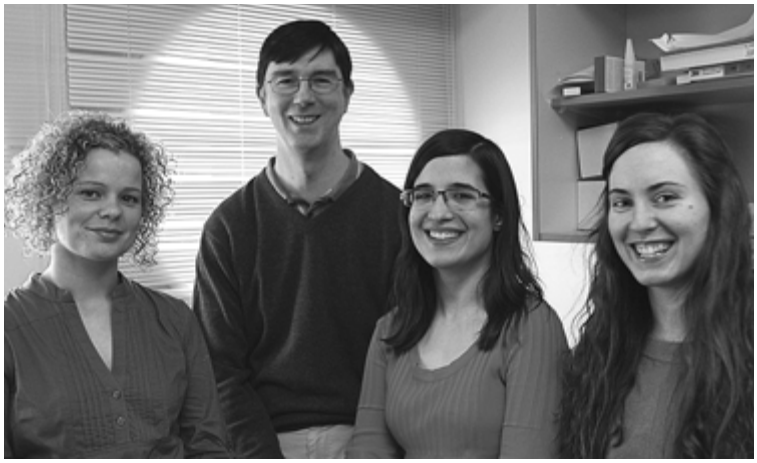




❶ *APRIL expression in MDA-MB231 breast carcinoma cells by IHQ*

❷ **A)** *APRIL, BAFF, BCMA, TACI and BAFF-R transcripts are found in hASC.* **B)** *TACI and BCMA protein expression in hASC as determined by flow cytometry.* **C)** *APRIL and BAFF sustain hASC proliferation (MTS assay).*



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SELECTED PUBLICATIONS

Fernández-Messina L, Reyburn HT, Valés-Gómez M. Human NKG2D-ligands: cell biology strategies to ensure immune recognition. Front Immunol. 2012;3:299

Agüera-González S, Gross CC, Fernández-Messina L, Ashiru O, Esteso G, Hang HC, Reyburn HT, Long EO, Valés-Gómez M. Palmitoylation of MICA, a ligand for NKG2D, mediates its recruitment to membrane microdomains and promotes its shedding. Eur J Immunol. 2011 Dec;41(12):3667-76

Fernández-Messina L, Ashiru O, Agüera-González S, Reyburn HT, Valés-Gómez M. The human NKG2D ligand ULBP2 can be expressed at the cell surface with or without a GPI anchor and both forms can activate NK cells. J Cell Sci. 2011 Feb 1;124(Pt 3):321-7

Receptor ligand interactions in immune responses to cancer and viruses

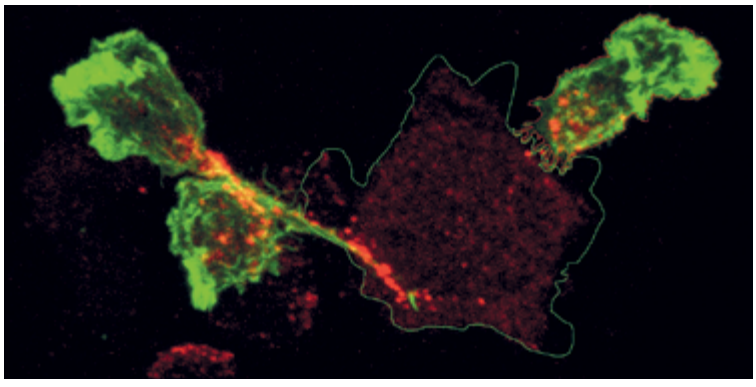
Current research in the laboratory addresses various issues related to the biology of NK cells and in particular, the receptor NKG2D and its ligands:

1. We have shown that shedding of molecules such as MICA and ICAM-1 is increased after human cytomegalovirus (HCMV) infection both *in vitro* and in transplant recipients who develop HCMV infection. This phenomenon depends on an increase in activity of the metalloproteases ADAM17 and MMP14 due to decreased expression of the endogenous inhibitor of metalloproteases TIMP3. Assay of the sheddase activity of ADAM17 could be of use as a biomarker in patients at risk of developing CMV disease.

2. We found that innate immune recognition of double-stranded RNA produced during viral infection plays a key role in the induction of expression of NKG2D ligands. These data suggest interesting parallels between the production of type I interferons and the induction of expression of NKG2D ligands. We identified proteins in both vaccinia and influenza virus whose expression markedly reduces the induction of both IFN-β and NKG2D ligands after infection.

3. The observation that stimulation via PRRs has a key role in the induction of NKG2D ligand expression prompted us to study the cell biology of these molecules in more detail. We have chosen to concentrated on inflammasome protein IFI16, that senses cytosolic DNA and stimulates production of both interferons and IL-1β.

4. Loss of immune function is commonly observed in NK cells isolated from patient tumours. We have established an *in vitro* system to induce hyporesponsiveness in primary human NK cells and have identified changes in specific cell surface receptors as being particularly important mechanistically. We are currently collaborating with the group of Dr. Eric Long (NIAID) to use live single-particle tracking to examine the diffusion properties of these receptors on normal and hyporesponsive NK cells.



1 *Deposition of NKG2D-containing lytic granules from an NK cell onto a tumour cell induced to express NKG2D ligands*

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SELECTED PUBLICATIONS

Johnen H, González-Silva L, Carramolino L, Flores JM, Torres M and Salvador JM. Gadd45g is essential for primary sex determination, male fertility and testis development. PLoS ONE 8(3): e58751. doi:10.1371/journal.pone.0058751, 2013

López-Santalla, M., Salvador-Bernáldez, M., González-Álvaro, I., Castañeda, S., Ortiz, A.M., García, M.I., Eiró, N., Kremer, L., Roncal, F., Mulero, J., Martínez-A, C. and Salvador JM. Tyr323-dependent p38 activation is associated with rheumatoid arthritis and correlates with disease activity. Arthritis Rheum 63:1833-42, 2011

T cell signalling in autoimmune diseases and cancer

The main goal of our group is the identification of novel therapeutic targets in autoimmune diseases and cancer. We focus on the role of key proteins involved in the regulation of T cell activation and differentiation. We are studying the biological functions of *Gadd45* and p38 MAPK in autoimmunity and cancer development. To address this goal, we generated mouse models deficient for each member of the *Gadd45* family and have analysed T cells from patients with different autoimmune diseases including rheumatoid arthritis (RA) and ankylosing spondylitis.

The pathogenesis and progression of RA is complex and involves T-cell-mediated antigen-specific responses. The initial stages of RA-associated autoimmunity, which precede articular inflammation, are the results of breakdown in B and T cell tolerance. p38 MAPK is a key regulator of T cell signalling and controls immune system effector functions. Our group demonstrated that p38 is hyperphosphorylated on Tyr323 in T cells from patients with active RA and that this phosphorylation reflects the disease activity state of RA. We generated and patented a novel method to determine the disease activity state of RA. We designed an intracellular staining method to assess RA disease activity by flow cytometry analysis (Lopez-Santalla *et al.,* 2011). In addition, we showed that the tyrosine kinase lck has a major role in p38 activation in pathological conditions in RA. Our data suggest that specific inhibition of lck can abolish p38 effector functions in T cells.

We recently showed that the *Gadd45* family also has roles in growth and development of specific tissues in the embryo. In mice, *Gadd45* genes are differentially expressed during embryonic development; *Gadd45b* is expressed in the chorion whereas *Gadd45g* is expressed in mouse brain. We found a critical role for *Gadd45g* in gonad development, male fertility and sex determination, as *Gadd45g*-deficient mice show an unexpected male-to-female sex reversal phenotype. In male gonads, SRY expression triggers differentiation of a somatic supporting cell lineage into Sertoli cells, which direct the male developmental pathway. In the absence of SRY, SOX9 is downregulated, leading to differentiation of the somatic supporting lineage into granulosa cells, which aid oocyte development. *Gadd45g*, but not *Gadd45a* or *Gadd45b*, is necessary for activation of the male sex-determining pathway in mice; its absence leads to development of female gonads. Lack of *Gadd45g* decreases SRY expression and blocks SOX9 expression resulting in ovary and Müllerian duct development, whereas lack of *Gadd45a* and/or *Gadd45b* has no effect on testis development.

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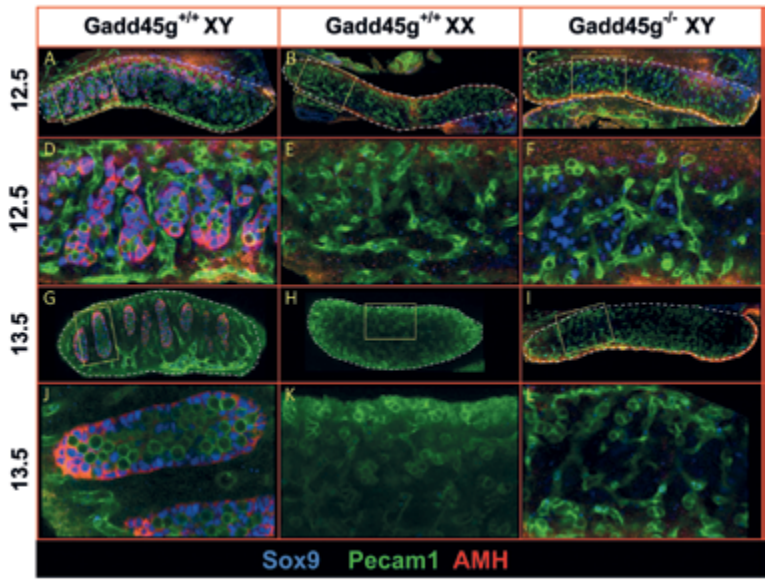

1 *Lack of Sertoli cell differentiation and testis cord formation in XY Gadd45g-/- gonads. (A-L) Confocal optical slices of whole mount immunostained B6 gonads (dashed outline), showing expression of Sertoli cell markers SOX9 (nuclear, blue) and AMH (cytoplasmic, red) and the germ/endothelial cell marker Pecam1 (membrane, green).*

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SELECTED PUBLICATIONS

Fernández-Messina L, Reyburn HT, Valés-Gómez M. Human NKG2D-ligands: cell biology strategies to ensure immune recognition. Front Immunol. 2012;3:299

Agüera-González S, Gross CC, Fernández-Messina L, Ashiru O, Esteso G, Hang HC, Reyburn HT, Long EO, Valés-Gómez M. Palmitoylation of MICA, a ligand for NKG2D, mediates its recruitment to membrane microdomains and promotes its shedding. Eur J Immunol. 2011 Dec;41(12):3667-76

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Biochemical characterisation of ligands for the immune receptor NKG2D: implications of heterogeneity for pathology and therapy

Our research aims to characterise the cellular routes that facilitate tumour elimination by the immune system and those that permit tumour escape. We have focussed recently on the study of the ligands for the NKG2D receptor, molecules that send stress signals to the immune system and that regulate effector cells such as T lymphocytes and natural killer (NK) cells. NKG2D is an activating immune receptor, constitutively expressed in humans in most cytotoxic lymphocytes, including NK and CD8+ T cells, whereas in mice it is expressed on NK cells, but on T cells only after activation. After binding to its ligands, NKG2D activates the mechanisms that lead to lysis and cytokine secretion by immune effector cells. In humans, NKG2D ligands (NKG2D-L) belong to two families of "stress-inducible" proteins: the polymorphic family of MHC-I-related chain A/B (MICA/B) and the multi-gene family of UL16-binding proteins (ULBP, now called RAET1A-E). Besides their activating role after ligand binding, NKG2D-L can be released to the extracellular media and interfere with immune recognition. Recent work from our laboratory focussed on the cellular and molecular differences that explain NKG2D-L heterogeneity. Our data demonstrate that, although both NKG2D-L families, MICA/B and ULBP, are related to MHC molecules and their expression is increased after stress, there are many differences in terms of biochemical properties and cell trafficking. We propose that categorisation of NKG2D-L according to their biological features, rather than their genetic family, might help to achieve a better understanding of the association of these molecules with disease.

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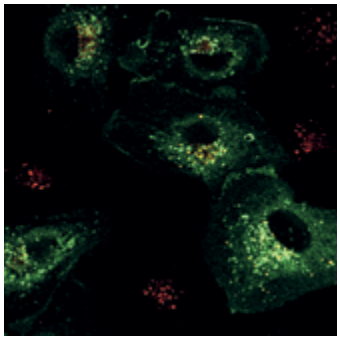

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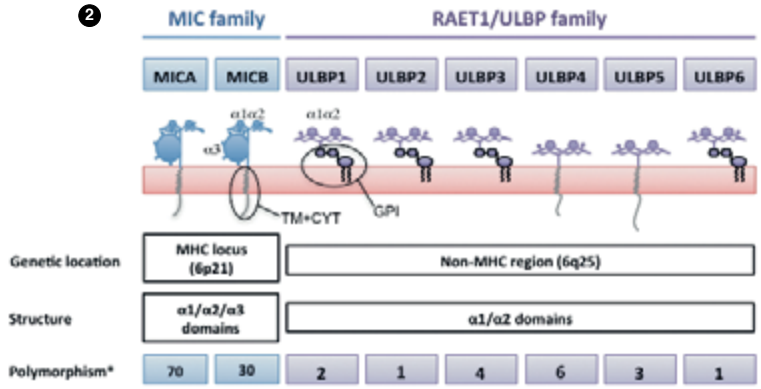

1 *Some NKG2D ligands, in addition to being expressed at the cell surface, are recycled and accumulate in intracellular organelles.*

2 *NKG2D ligands belong to two polymorphic families of MHC-related proteins that respond to cell stress.*

03

Cellular and Molecular Biology

The Department of Cellular and Molecular Biology consists of 12 independent research groups working in two areas: the structural and functional characterisation of virus and cellular elements involved in the progression of infection, and understanding the molecular basis of mammalian gene expression and control of cell processes in normal and pathological conditions. The first area analyses the role of productive virus-host interactions of human and animal pathogens that are highly relevant for health. The identification of cell factors that control viral replication is crucial for the recognition of new therapeutic targets. In addition, this area provides key knowledge for the design of vaccination strategies and virus-based vaccine vectors.

The pivotal point of the second area is the identification and exploitation of relevant diagnostic and therapeutic molecular targets. Studies in the department also intend to provide essential scientific background for the development of new biotechnological tools of biomedical importance.

These topics are highly interconnected. While most established pathogens normally cope with stressful conditions by developing efficient adaptive responses, the opportunistic pathogens are metabolically very versatile, making them efficient biodegraders of pollutants. Fighting against pathogenic microorganisms requires deep understanding of their behaviour during infection and of how resistance develops as pathogens are challenged by antibiotics.

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SELECTED PUBLICATIONS

Bañón-Rodríguez I, Monypenny J, Ragazzini C, Franco A, Calle Y, Jones GE, Antón IM. The cortactin-binding domain of WIP is essential for podosome formation and extracellular matrix degradation by murine dendritic cells. Eur J Cell Biol. 2011 Feb-Mar;90(2-3):213-23

Franco A, Knafo S, Banon-Rodriguez I, Merino-Serrais P, Fernaud-Espinosa I, Nieto M, Garrido JJ, Esteban JA, Wandosell F, Anton IM. WIP is a negative regulator of neuronal maturation and synaptic activity. Cereb Cortex. 2012 May;22(5):1191-202

García E, Jones GE, Machesky LM, Antón IM. WIP: WASP-interacting proteins at invadopodia and podosomes. Eur J Cell Biol. 2012 Nov-Dec;91(11-12):869-77

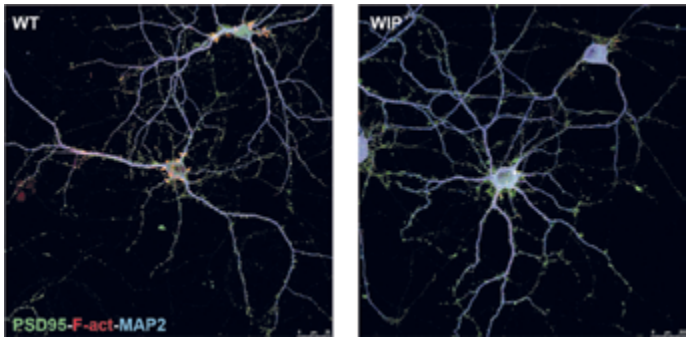
Yu X, Zech T, McDonald L, Gonzalez EG, Li A, Macpherson I, Schwarz JP, Spence H, Futó K, Timpson P, Nixon C, Ma Y, Anton IM, Visegrády B, Insall RH, Oien K, Blyth K, Norman JC, Machesky LM. N-WASP coordinates the delivery and F-actin-mediated capture of MT1-MMP at invasive pseudopods. J Cell Biol. 2012 Oct 29;199(3):527-44

Garber JJ, Takeshima F, Antón IM, Oyoshi MK, Lyubimova A, Kapoor A, Shibata T, Chen F, Alt FW, Geha RS, Leong JM, Snapper SB. Enteropathogenic *Escherichia coli* and vaccinia virus do not require the family of WASP-interacting proteins for pathogen-induced actin assembly. Infect Immun. 2012 Dec;80(12):4071-7

Molecular bases of cytoskeletal reorganisation: role of actin polymerisation in neuritogenesis, inflammation and metastasis

Our aim is to unravel the mechanism of (N)WASP (neural Wiskott-Aldrich syndrome protein)- and WIP (WASP interacting protein)-mediated actin-polymerisation and determine how WIP deficiency affects essential actin-mediated functions such as pathogen motility, migration, invasion (podosome/invadopodia formation), and neuronal differentiation. We hope to better understand the molecular mechanisms that underlie inflammation-mediated affections, tumour invasion and neurological diseases.

Using animal models and novel reagents, including WIP-specific poly- and monoclonal antibodies and recombinant lentivirus to express WIP or mutant forms that lack the binding sites for actin, WASP/N-WASP, nck or cortactin, we identified an essential role for WIP in persistence during amoeboid (B lymphocyte) and mesenchymal (fibroblast) migration. Efficient fibroblast chemotaxis towards PDGF-AA requires WIP binding to Nck, whereas matrix degradation by dendritic cells depends on WIP binding to cortactin. Using digital video microscopy and confocal fluorescence microscopy, we identified the role of WIP in the formation of actin-rich invasive structures (podosomes and invadopodia). At the biochemical level, we defined how WIP phosphorylation affects disengagement of the WIP-WASP complex (and not WIP-Nck) and how the complex contributes to actin flux in podosomes. We also detected elevated WIP levels in highly invasive epithelial cancer cells, and found that WIP knock-down reduces invadopodium formation and invasive capacity. Finally, we described WIP expression in adult brain and in embryonic neurons, where it negatively regulates soma size, neurite sprouting and dendritic branching without affecting axon generation. Biochemical and pharmacological analyses define N-WASP, mTOR and Abl as signalling molecules involved in this process. In mature neurons, WIP modulates synaptic activity and dendritic spine (morphology and actin content) by regulating sphingomyelinase levels and membrane lipid composition to modulate the ROCK-profilin II pathway. We intend to increase our understanding of the contribution of the N-WASP/WIP complex to neuron and astrocyte migration and the pathological effects of WIP deficiency on the murine nervous system. Our work should yield fundamental information on the activity of cytoskeletal components and the molecular mechanisms that underlie actin dynamics and related functions, providing new diagnostic, prognostic and/or therapeutic tools for neurological disorders, inflammation-mediated affections, tumour initiation and metastasis.



1 *Enhanced dendritic maturation in WIP$^{-/-}$ neurons. Hippocampal primary neurons from control (WT) or WIP$^{-/-}$ embryos were grown for 22 days (DIV) on an astrocyte monolayer and stained for PSD95 (green), F-actin (red) and MAP2 (blue).*

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SELECTED PUBLICATIONS

Sola I, Mateos-Gomez PA, Almazan F, Zuñiga S, Enjuanes L. RNA-RNA and RNA-protein interactions in coronavirus replication and transcription. RNA Biol. 2011 8(2):237-48

Cruz JL, Sola I, Becares M, Alberca B, Plana J, Enjuanes L, Zuñiga S. Coronavirus gene 7 counteracts host defenses and modulates virus virulence. PLoS Pathog. 2011 Jun;7(6):e1002090

DeDiego ML, Nieto-Torres JL, Jiménez-Guardeño JM, Regla-Nava JA, Alvarez E, Oliveros JC, Zhao J, Fett C, Perlman S, Enjuanes L. Severe acute respiratory syndrome coronavirus envelope protein regulates cell stress response and apoptosis. PLoS Pathog. 2011 Oct;7(10):e1002315

Nogales A, Márquez-Jurado S, Galán C, Enjuanes L, Almazán F. Transmissible gastroenteritis coronavirus RNA-dependent RNA polymerase and nonstructural proteins 2, 3, and 8 are incorporated into viral particles. J Virol. 2012 Jan;86(2):1261-6

Mateos-Gomez PA, Morales L, Zuñiga S, Enjuanes L, Sola I. Long-distance RNA-RNA interactions in the coronavirus genome form high-order structures promoting discontinuous RNA synthesis during transcription. J Virol. 2013;87(1):177-86

PATENTS

EP11788149: Nucleic acids encoding PRRSV GP5-ecto and M protein

EP10013125: Nucleic acids encoding TGEV and PRRSV sequences for improved expression of PRRSV sequences

EP06762172: Attenuated SARS and use as a vaccine

EP04007406: Artificial chromosome constructs containing nucleic acid sequences capable of directing the formation of a recombinant RNA-virus

Replication, virus-host interactions, and protection in coronavirus

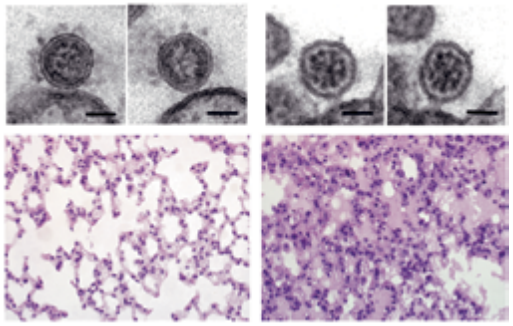
Our group is interested in the molecular basis of replication, transcription, assembly, and virus-host interactions of coronaviruses (CoV) using TGEV and SARS-CoV as models. To control disease, we focus on the impact of the host on infection by these viruses, and on the identification of signalling pathways modified by the virus.

Virus replication and transcription as well as virus-host interactions are mediated by the binding among virus RNA motifs and viral and host cell proteins. High-order virus genome RNA structures have been identified in our laboratory. The relevance of host cell factors involved in these processes has been evaluated by inhibiting their expression using siRNA. Viral polymerases and other viral replicase proteins, together with cell proteins involved in CoV replication within the viral particle, probably represent the identification of a starting replication engine inside the virion.

We described three mechanisms that regulate transcription in coronaviruses at different levels. Base pairing between the nascent minus RNA chain and the leader transcription-regulating sequence controls the amount of all subgenomic mRNAs produced. In addition, a transcription enhancer and long distance RNA-RNA interactions that form high order structures regulate the expression of specific mRNA.

The study of virus-host interaction and cell signalling pathways that affect virus replication, such as those associated with inflammation of respiratory tissues, has provided the basis for selection of antiviral drugs that protect against CoV infection. We showed that specific virus proteins, such as the TGEV protein 7 and SARS-CoV envelope (E) protein, influence virus virulence and modulate cell signalling pathways. Deletion of E protein led to the generation of propagation-deficient TGEV virions, to attenuated phenotypes in the case of SARS-CoV, and to upregulation of the cell stress response, which affects the immune response. Deletion of non-essential virus components such as TGEV protein 7 notably affected viral and cell translation and apoptosis, as a consequence of increased host antiviral response.

Using reverse genetic approaches based on infectious cDNA clones of SARS-CoV, promising vaccine candidates that protect against SARS have been engineered.



1 *Generation of a recombinant vaccine to prevent infection by the severe and acute syndrome virus (SARS-CoV). Electron micrographs of SARS-CoV virions just after completing internal budding (top left). Top right figures show two virions derived from SARS-CoV in which the envelope gene (E) was deleted (SARS-CoV-ΔE) at the end of the internal budding process that is delayed in relation to the virus with E protein. Mice infected with a mouse-adapted SARS-CoV in which the E gene was deleted (SARS-CoV-MA15-ΔE) showed no pathology in their lungs (bottom left). In contrast, the same virus including the E gene caused strong lung inflammation (bottom right) in BALB/c mice. The engineered SARS-CoV-MA15-ΔE provided full protection against challenge with the virulent virus, indicating that this attenuated virus is a very promising vaccine candidate. Bars, 50 nm. Bottom panels, haematoxylin/eosin-stained lung sections.*

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María Victoria Jiménez

SELECTED PUBLICATIONS

García F, Bernaldo de Quirós García F, Bernaldo de Quirós JC, Gómez CE, *et al.* Safety and immunogenicity of a modified pox vector-based HIV/AIDS vaccine candidate expressing Env, Gag, Pol and Nef proteins of HIV-1 subtype B (MVA-B) in healthy HIV-1-uninfected volunteers: A phase I clinical trial (RISVAC02). Vaccine. 2011 Oct 26;29(46):8309-16

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García-Arriaza J, Nájera JL, Gómez CE, Tewabe N, Sorzano CO, Calandra T, Roger T, Esteban M. A candidate HIV/AIDS vaccine (MVA-B) lacking vaccinia virus gene C6L enhances memory HIV-1-specific T-cell responses. PLoS One. 2011;6(8):e24244

Gómez CE, Perdiguero B, Nájera JL, Sorzano CO, Jiménez V, González-Sanz R, Esteban M. Removal of vaccinia virus genes that block interferon type I and II pathways improves adaptive and memory responses of the HIV/AIDS vaccine candidate NYVAC-C in mice. J Virol. 2012 May;86(9):5026-38

Vijayan A, Gómez CE, Espinosa DA, Goodman AG, Sanchez-Sampedro L, Sorzano CO, Zavala F, Esteban M. Adjuvant-like effect of vaccinia virus 14K protein: a case study with malaria vaccine based on the circumsporozoite protein. J Immunol. 2012 Jun 15;188(12):6407-1715;188(12):6407-17

PATENTS

PCT/US2010/032966 Modified immunization vectors

PCT/ES2012/070521 Recombinant vectors based on Ankara modified virus (MVA), with a deletion in 6CL gene, as vaccines against HIV and other diseases

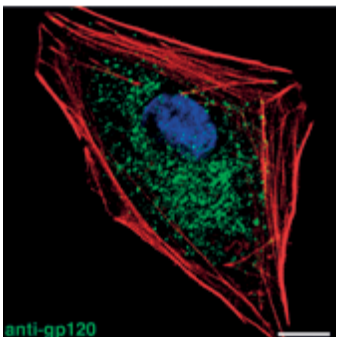
PCT/ES2012/070794 Adjuvant effect of A27 protein from vaccinia virus (14K) and its applications for vaccines

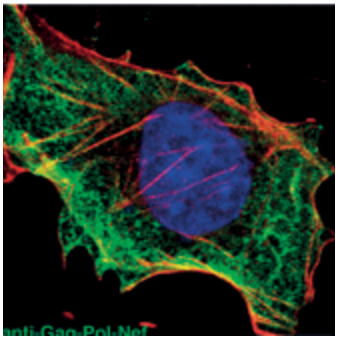
Poxvirus and vaccines

The main objectives of our laboratory are geared to understand the molecular basis in the pathogenesis of infectious agents and their interaction with the host, as well as to use this knowledge in the development of vaccines that might be effective against diseases like AIDS, malaria, leishmaniasis, hepatitis C and prostate cancer. As a model system of infectious agent and as a delivery vector for expression of genes of interest, we used vaccinia virus (VACV) a member of the poxvirus family.

In the current period of 2011-2012, we continued with the development of a vaccine against HIV/AIDS remains a major challenge in the control of this pandemic that has produced more than 25 million deaths since it was first diagnosed in 1981 and continues unabated worldwide. Our group has developed candidate vaccines to HIV/AIDS based on poxvirus vectors (MVA and NYVAC) that have shown excellent immunological profiles in animal models (mouse and macaques) and effectiveness against simian immunodeficiency virus in macaques. Some of these vectors have entered phase I clinical trials, giving positive responses in about 90% of healthy volunteers. The immunisation protocols were further improved in monkeys, providing greater transmission of HIV-specific cellular immune responses from mothers to lactating infants and selective targeting of HIV antigens to dendritic cells. In addition, vectors have been improved by the selective deletion of viral immunomodulatory genes. The aim is to enter phase II clinical trials with some of the candidate vaccines developed in coming years. HIV research in our group is supported by national and international grants (Bill and Melinda Gates Foundation) and we collaborate with international teams in Europe and the USA. We have also developed candidate vaccines against influenza, leishmaniasis and malaria.

1





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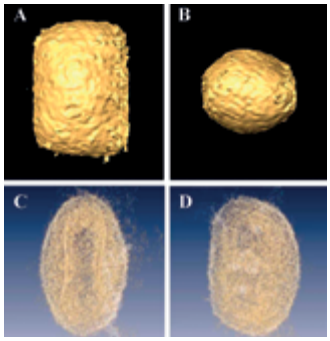

1 *Expression of the HIV antigens Env and Gag-Pol-Nef by the HIV/AIDS vaccine candidate MVA-B". Confocal microscopy of cells infected with MVA-B at 6 hours postinfection. In green, HIV antigens; in red, cytoskeletal filaments; in blue, nuclei.*

2 *Cryo-electron tomography of the infectious mature virus particle (MV) of vaccinia virus at 4-6 nm resolution*

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SELECTED PUBLICATIONS

Polyak SJ, Morishima C, Scott JD, Gastaminza P, Cox A, de Araújo ES, Higgs MR, Loo YM, Golden-Mason L, Lindenbach BD, Baumert TF, Randall G, Gale M Jr. A summary of the 18th International Symposium on Hepatitis C Virus and Related Viruses. Gastroenterology. 2012 Jan;142(1):e1-5

Montero A, Gastaminza P, Law M, Cheng G, Chisari FV, Ghadiri MR. Self-assembling peptide nanotubes with antiviral activity against hepatitis C virus. Chem Biol. 2011 Nov 23;18(11):1453-62

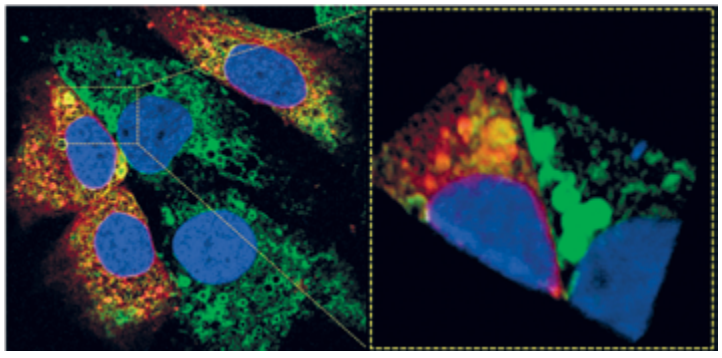
Gastaminza P, Pitram SM, Dreux M, Krasnova LB, Whitten-Bauer C, Dong J, Chung J, Fokin VV, Sharpless KB, Chisari FV. Antiviral stilbene 1,2-diamines prevent initiation of hepatitis C virus RNA replication at the outset of infection. J Virol. 2011 Jun;85(11):5513-23

Cellular factors involved in hepatitis C virus infection and pathogenesis

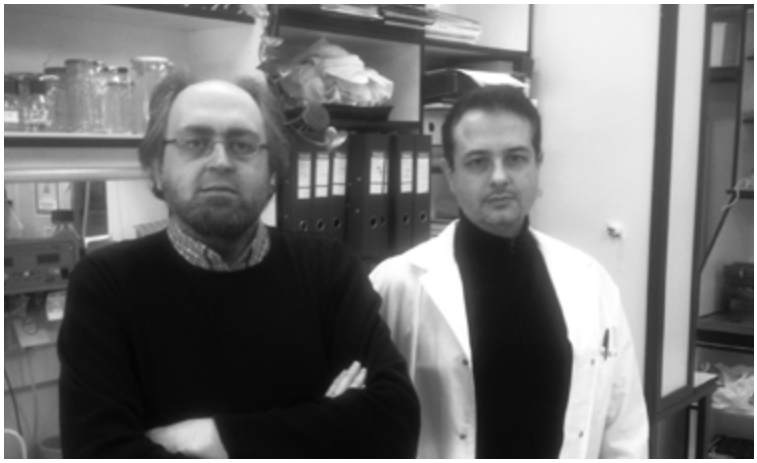
Hepatitis C virus (HCV) is an important pathogen that infects 3% of the human population worldwide. Despite intense efforts to control this pandemic, 3 to 4 million people are infected, and about 350,000 individuals die of HCV-related diseases every year. New strategies to control and eradicate this virus must thus be implemented. Our laboratory is interested in the cellular and molecular processes that underlie different aspects of HCV biology and pathogenesis, to discover new targets for antiviral therapy.

Using a cell culture model of HCV infection, we recently identified a new host factor, the sigma1 receptor (S1R), which plays a specific role at the onset of the HCV life cycle. This cellular factor is an important component of mitochondria-associated endoplasmic reticulum (ER) membranes (MAM) and regulates bidirectional interorganellar transport of lipids and Ca^{2+} ions between mitochondria and the ER. Silencing of this factor resulted in a proportional decrease in susceptibility to HCV infection. Subsequent mechanistic studies indicated that early steps of viral RNA replication, downstream of translation of the incoming viral genomes, is rate-limited by cellular S1R levels. These findings open up the possibility that HCV uses MAM as the gateway to access the cell machinery needed for efficient viral replication. Moreover, S1R functions can be modulated by exogenous synthetic drug-like ligands, thus constituting an interesting cellular target for the control of HCV infection.

In addition to these basic studies, we sought new molecules with antiviral potential against HCV. Using an in-house screening system, we interrogated two different chemical libraries. The first was composed of compounds susceptible to chemical derivatisation using click chemistry, a highly modular and predictive synthetic methodology that is optimal for structure-activity relationship studies. The screening led to identification of a novel family of anti-HCV compounds and its derivatisation permitted optimising the antiviral molecules to obtain compounds with antiviral activity at nanomolar concentrations. A second library was composed of synthetic cyclic peptides able to form nanotubes. Using the same screening system, we found a family of non-toxic, nanotube-forming cyclic peptides that efficiently inhibited viral entry at micromolar concentrations downstream of viral adsorption to the target cell, probably at the level the membrane fusion process.



1 *Micrograph of hepatitis C virus-infected cells expressing S1R-EGFP (green), which forms ring-like structures derived from the endoplasmic reticulum. Viral antigen E2 (red) colocalises with S1R. 3D reconstruction of the selected area shows a network of enlarged S1R-containing ER cisternae*



GROUP LEADER:
Francisco J. Iborra

SELECTED PUBLICATIONS

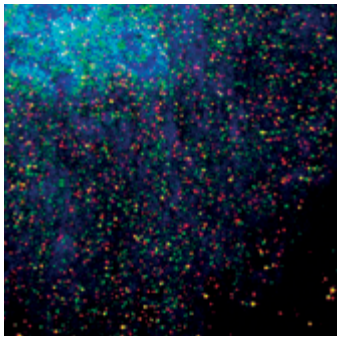
Johnston IG, Gaal B, Neves RP, Enver T, Iborra FJ, Jones NS. Mitochondrial variability as a source of extrinsic cellular noise. PLoS Comput Biol. 2012;8(3):e1002416

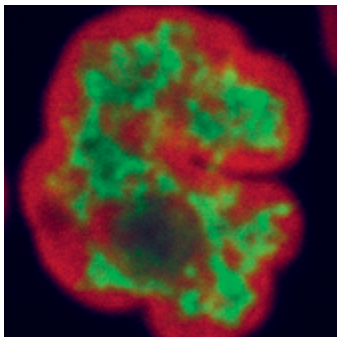
Biological noise

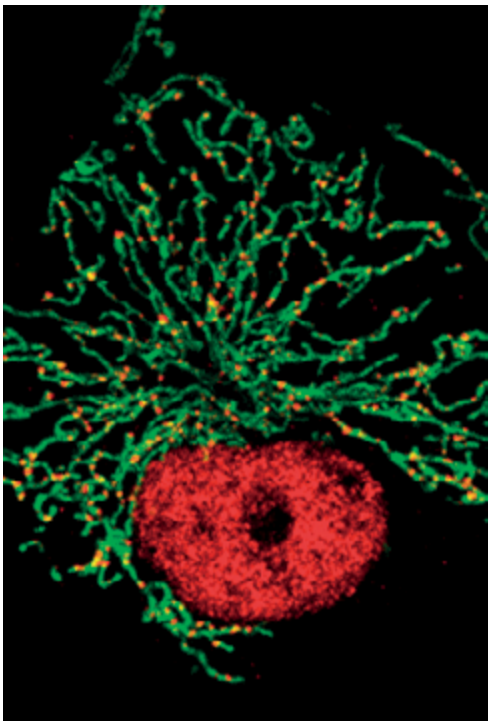
Our lab is interested in the origin of the phenotypic variability between genetically identical individuals. The reason we pursuit this endeavour is that non-genic variability is the basis of many pathophysiological processes such as cell differentiation, cellular responses to drugs, and even the execution of apoptotic programmes.

Non-genetic phenotypic variability can be classified in two types, intrinsic and extrinsic. Intrinsic variability or noise is due to differences in the expression patterns of specific genes and depends on the levels of the factors that control the expression of such genes. On the other hand, extrinsic variability (or extrinsic noise) affects many genes inside a single cell.

Our group demonstrated that one of the factors that contributes to extrinsic noise is the difference in the mitochondria content in clonal populations of cells. This is due to the fact that the activity of RNA pol II is very sensitive to changes in cellular ATP, which is derived from mitochondria (das Neves, *et al.,* 2010). To understand the implications of the heterogeneous distribution of mitochondria, we have modelled how differences in mitochondria between individual cells can be responsible for extrinsic noise in gene expression or noise in cell cycle length and cell differentiation (Johnston, *et al.,* 2012). We found that human umbilical cord haematopoietic stem cells have fewer mitochondria than those committed to differentiation programs (Romero-Moya, *et al.,* 2013). Our aim is characterise how mitochondria influence gene expression and study how mitochondria can contribute to disease.







❶ *Cellular cosmos. Chromatin spread.This image shows DNA in blue, RNA polymerase II in red and nascent transcripts (Br-RNA) in green.*

❷ *Cell with green mitochondria and red transcription. Transcription visualised as incorporation of BrdU into nascent RNA shows transcription activity in mitochondria and nucleus.*

❸ *Nuclear compartmentalisation. Human lymphocyte showing DNA in red and stable RNA in green.*

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SELECTED PUBLICATIONS

Zurita E, Chagoyen M, Cantero M, Alonso R, González-Neira A, López-Jiménez A, López-Moreno JA, Landel CP, Benítez J, Pazos F, Montoliu L. Genetic polymorphisms among C57BL/6 mouse inbred strains. Transgenic Res. 2011 Jun;20(3):481-9

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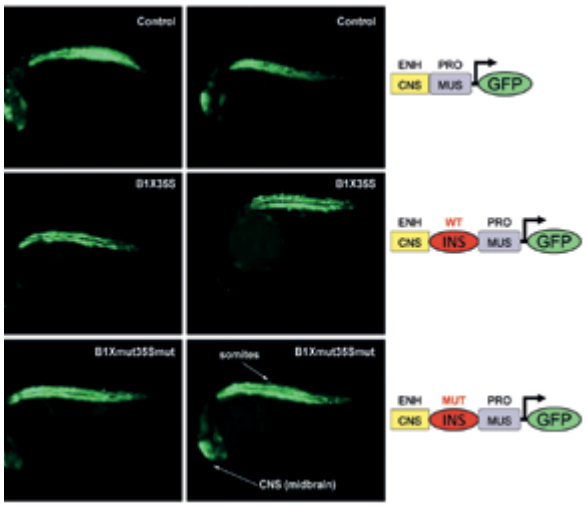
PATENT

P201231296 New animal model for acromatopsia.

Animal models by genetic manipulation

In our laboratory, we want to understand how mammalian expression domains work and how they are organised within genomes. We focus our interest on the identification and characterisation of genomic boundaries or insulators. Insulators can be used effectively in biotechnological applications as spacers, as boundaries, in any gene expression construct for gene transfer experiments. They prevent inappropriate expression patterns of transgenes or gene therapy constructs, by insulating them from neighbouring sequences at the insertion site in the host genome. We are searching for new insulator sequences in vertebrate genomes. We initiate our experiments through *in silico* predictions; insulator candidates are subsequently validated functionally *in vitro* using cells and the enhancer blocking assay. Finally, we carry out *in vivo* studies in transgenic animals, with zebrafish (in collaboration with J.L. Gómez-Skarmeta, Centro Andaluz de Biología de Desarrollo, Seville) and mice bearing appropriate informative constructs. In the last two years, in collaboration with a number of national and international research groups, we described and functionally validated several new types of boundary elements. Our laboratory also generates and analyses new mouse models to study alterations in vision associated with albinism, a rare disease studied in the scope of the CIBERER-ISCIII centre (www.ciberer.es). We also collaborate with Spanish and French associations in support of people with albinism, ALBA (www.albinismo.es) and GENESPOIR (www.genespoir.org). With the group of A. Carracedo (Univ. Santiago de Compostela), we are developing a universal genetic diagnosis for all known albinism-associated genetic mutations (>600).

Our expertise in mouse embryo and sperm cryopreservation enabled participation in the EU FP7 Projects in mouse functional genomics, including INFRAFRONTIER and EMMA, the European Mouse Mutant Archive, whose Spanish node at the CNB is coordinated by Lluís Montoliu. Finally, through collaborations, we have generated a number of additional transgenic mouse models to study human diseases, including Alzheimer's. For this work, we exploited our yeast artificial chromosome (YAC) transgene technology, which has been instrumental in scientific contracts with biotechnological companies.



1 *Functional validation and mechanism of the SINEB1 element X35S working as a boundary in transgenic zebrafish expressing GFP. See Roman et al. (2011) for additional information.*

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TECHNICIANSD
Mª Paz González
Xosé Manuel Dopazo

Functional analysis of the transcriptional repressor DREAM

We study the nuclear components of activity- and Ca^{2+}-dependent transcriptional responses in neurons and immune cells to

i) understand the molecular determinants of downstream events responsible for plastic changes in synaptic function, and

ii) develop tools to intervene in physiological output processes including learning and memory, pain sensitisation and neurodegeneration.

Altered neuronal calcium homeostasis and early compensatory changes in transcriptional programs are common features of many neurodegenerative pathologies including Alzheimer's (AD), Down syndrome (DS) and Huntington's disease (HD). DREAM (DRE antagonist modulator), a Ca^{2+}-dependent transcriptional repressor also known as calsenilin, has an important role in neurodegenerative diseases (NDD) through the control of Ca^{2+} homeostasis. Changes in DREAM levels are found in mouse models of several NDD, including AD, DS and HD. Genetic experiments show that this could form part of a neuroprotective mechanism.

We anticipate that DREAM is an active/central component of several nucleoprotein complexes that specifically mediate the various transcriptional cascades triggered by neuron membrane depolarisation essential for neuronal plasticity and synaptic dysfunction. Our work in progress analyses the role of DREAM in the regulation of transcription in cell and animal NDD models, to better understand early changes in the transcriptome and epigenome and to explore new targets for therapeutic intervention to boost early endogenous neuroprotective mechanisms.

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Dierssen M, Fedrizzi L, Gomez-Villafuertes R, de Lagran MM, Gutierrez-Adan A, Sahún I, Pintado B, Oliveros JC, Dopazo XM, Gonzalez P, Brini M, Mellström B, Carafoli E, Naranjo JR. Reduced Mid1 expression and delayed neuromotor development in daDREAM transgenic mice. Front Mol Neurosci. 2012;5:58

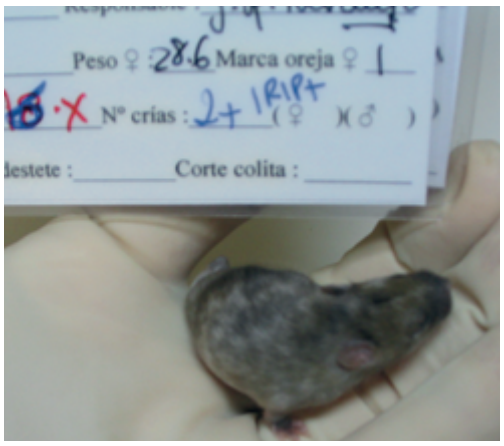
Calì T, Fedrizzi L, Ottolini D, Gomez-Villafuertes R, Mellström B, Naranjo JR, Carafoli E, Brini M. Ca^{2+}-activated nucleotidase 1, a novel target gene for the transcriptional repressor DREAM (Downstream Regulatory Element Antagonist Modulator), is involved in protein folding and degradation. J Biol Chem. 2012 May 25;287(22):18478-91

Naranjo JR, Mellström B. Ca^{2+}-dependent transcriptional control of Ca^{2+} homeostasis. J Biol Chem. 2012 Sep 14;287(38):31674-80

PATENT

PCT/ES12/070020. Compounds for the treatment of neurodegenerative diseases.

❶



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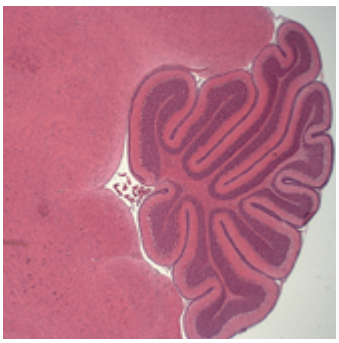

❶ *Double DREAM knock-in mouse carrying three mutations in the DREAM gene. This mouse chimaera was prepared in collaboration with the Transgenesis Service at CNB using consecutively two different zinc finger nucleases (ZFN) that targets exon II and exon VII of the DREAM gene.*

❷ *DREAM regulates early cerebellar development by controlling the expression of the /midline 1/ gene (Mid 1). A sagittal section of the cerebellum of a 15-day-old daDREAM transgenic mouse is shown.*

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Beatriz Cubelos Álvarez

SELECTED PUBLICATIONS

Rodríguez-Tornos FM, San Aniceto I, Cubelos B, Nieto M. Enrichment of conserved synaptic activity-responsive element in neuronal genes predicts a coordinated response of MEF2, CREB and SRF. PLoS One. 2013;8(1):e53848

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Aviles-Espinosa R, Andilla J, Porcar-Guezenec R, Olarte OE, Nieto M, Levecq X, Artigas D, Loza-Alvarez P. Measurement and correction of *in vivo* sample aberrations employing a nonlinear guide-star in two-photon excited fluorescence microscopy. Biomed Opt Express. 2011 Nov 1;2(11):3135-49

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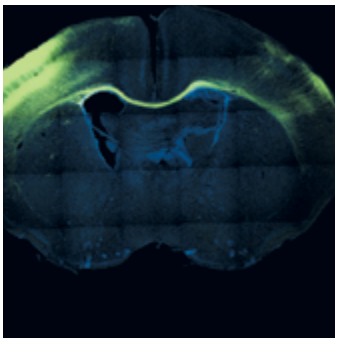
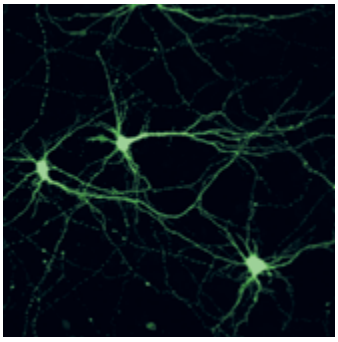
Cerebral cortical development

Our studies aim to define the cellular and molecular mechanisms that govern the generation of neurons and circuits of the mammalian cerebral cortex. The mammalian cerebral cortex is responsible for most aspects of cognition and behaviour, and it is the most evolved structure in the human brain. A large number of functionally and morphologically distinct neurone types specify brain cortical areas and coordinately control cerebral functions. We helped to understand the programmes that specify the identity of the neurons in the upper layers of the cerebral cortex. This subpopulation of pyramidal neurons characterises higher mammals and it is expanded in humans, probably contributing of the increased cognitive capacity of the mammalian brain. It is the last to appear both during development and in evolution. Our research showed that the transcription factors Cux1 and Cux2 determine the extremely high degree of connectivity of these neurons and their participation in intra-cortical circuits responsible for higher brain functions (Cubelos *et al.*, Neuron 2010). In our ongoing work, we are dissecting the neuronal aspects modified by these genes and how Cux programs coordinated with experience and plasticity to generate stereotyped networks.

We also identified molecular mechanisms of axon modelling and plasticity acting on these neurons, which participate in the formation and physiology of brain circuits (Sebastián-Serrano *et al.*, PLoS ONE 7(2):e31590; Rodríguez-Tornos *et al.*, PLoS ONE 8(1):e53515). In collaboration with other CNB groups, we study their modes of migration (Franco *et al.*, Cer Cortex 22(5):1191-202). Our research provides basic knowledge of the mechanisms of neural specification and circuit formation, the potential programmes of reprogramming neurons, and the specific advantages and plasticity of the human brain. They have broad direct implication for understanding the specific functions of the cortex in intellectual processing and the underlying mechanisms of brain diseases, particularly those that originate in childhood, as well as for neurodegeneration, which is increasingly reported to be related to plasticity.





❶ *Connectivity of the corpus callosum visualised with GFP. Confocal images obtained from a cortical section of the telencephalon of a P16 WT mice electroporated with green fluorescence protein (GFP) at embryonic day 15.5. Electroporated cells located in the SS cortex extend their axons, cross the midline and invade the contralateral SS cortex. Colateral axonal projections form synapses in layer II-III and V of both ipsilateral and contralateral hemispheres.*

❷ *Morphological analysis of neuronal networks. Pyramidal cortical neurons obtained from an 18.5-day embryo transfected with a GFP plasmid.*

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SELECTED PUBLICATIONS

Yángüez E, Castello A, Welnowska E, Carrasco L, Goodfellow I, Nieto A. Functional impairment of eIF4A and eIF4G factors correlates with inhibition of influenza virus mRNA translation. Virology. 2011 Apr 25;413(1):93-102

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Yángüez E, Rodriguez P, Goodfellow I, Nieto A. Influenza virus polymerase confers independence of the cellular cap-binding factor eIF4E for viral mRNA translation. Virology. 2012 Jan 20;422(2):297-307

Rodriguez A, Falcon A, Cuevas MT, Pozo F, Guerra S, García-Barreno B, Martinez-Orellana P, Pérez-Breña P, Montoya M, Melero JA, Pizarro M, Ortin J, Casas I, Nieto A. Characterisation *in vitro* and *in vivo* of a pandemic H1N1 influenza virus from a fatal case. PLoS One. 2013;8(1):e53515

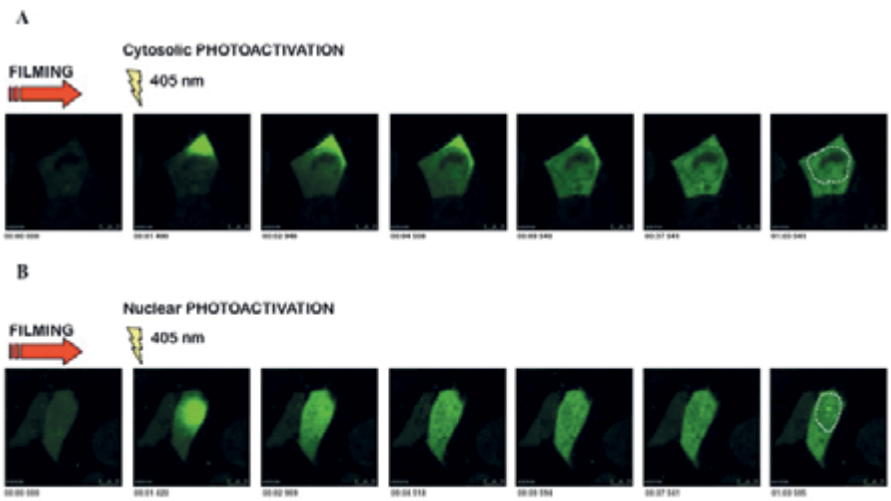
PATENT

P201130879, PCT/ES12/070388
An improved method to produce influenza vaccine for humans in cell culture.

Mechanisms of interaction between the influenza virus and the infected cell

Influenza virus employs an unusual RNA transcription mechanism that uses as primers short-capped oligonucleotides scavenged from newly synthesised RNAP II transcripts. This fact entirely determines its life cycle, since it requires functional coupling between viral and cellular transcription machineries. The viral polymerase has a key role in establishing productive interactions with host cell factors involved in cellular transcription and translation.

Among the cell factors that interact with viral polymerase, we have characterised two transcription-related factors, hCLE, a positive modulator of RNA polymerase II and CHD6, a chromatin remodeller. Whereas hCLE also positively modulates influenza virus replication, CHD6 is a negative modulator that relocates from active to inactive chromatin during infection. Although viral and cellular transcriptions are functionally coupled, degradation of cellular RNA polymerase II occurs once synthesis of viral mRNA is completed, probably to avoid competition. This degradation appears to be a virulence marker. Reconstituted viral polymerase from its cDNAs causes RNAP II degradation, and PA and PB2 polymerase subunits contribute individually. We have characterised the specific residues in PA and PB2 that are involved in the RNAP II degradation process. In addition, CHD6 is also degraded after infection, and its proteolysis kinetically parallels that of RNAP II.



1 *The positive modulator of viral replication, hCLE, is a shuttling protein. Cultured HEK293T cells were transfected with a plasmid that expresses a recombinant hCLE-PAGFP (photoactivatable GFP) protein and 24h post-transfection they were used for live cell microscopy. (A) Photoactivation was applied in the cytosol to visualise hCLE import. (B) Photoactivation was applied in the nucleus to visualise hCLE export. A dotted line marking the boundary of the nucleus is included in the last panels.*

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SELECTED PUBLICATIONS

Resa-Infante P, Jorba N, Coloma R, Ortin J. The influenza RNA synthesis machine: Advances in its structure and function. RNA Biol. 2011 Mar-Apr;8(2):207-15

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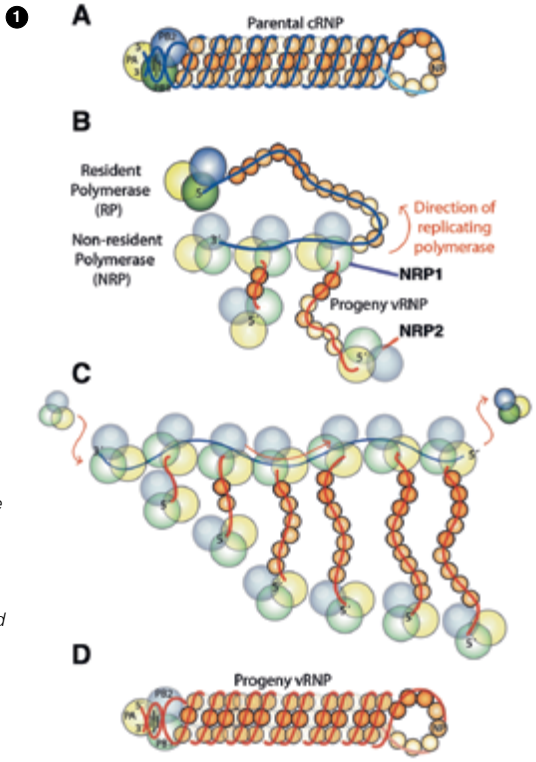
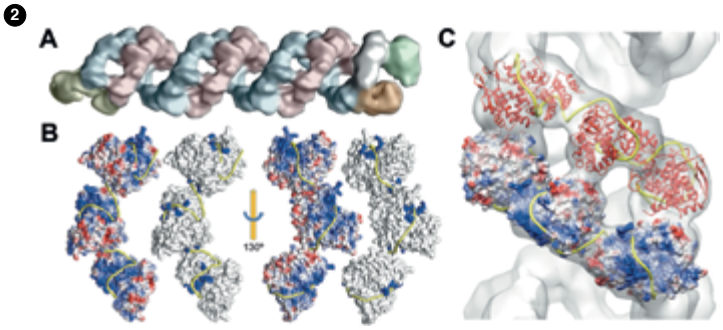
Transcription and replication of influenza virus RNA

In the years 2011-2012, our group studied the structure of influenza virus ribonucleoprotein complexes (RNPs) and the RNA polymerase, as well as their interactions with the host cell during virus replication. The structure of the polymerase isolated from a recombinant RNP and the polymerase associated to a short template RNA have been analysed by electron microscopy and 3D reconstruction from stained or frozen samples. In addition, the 3D structure of native virion RNPs was determined by a combination of cryo-electron microscopy and cryo-electron tomography. The results showed that the RNP has a double helical structure with two opposite polarity NP chains that interact with one another in a closed structure; one of the ends shows a small loop containing 3 NP monomers and the other contains the polymerase complex. This structure has allowed us to propose intra- and inter-strand NP-NP interactions as well as the localisation of the template RNA, and to infer potential mechanisms for viral transcription, replication and encapsidation.

The role of the host proline- and glutamine-rich splicing factor (SFPQ) in influenza virus infection has been studied by gene silencing. Downregulation of SFPQ led to strong reductions in influenza virus yield, but did not affect the multiplication of unrelated viruses. In SFPQ silencing conditions, virus transcription, RNA replication and gene expression were severely affected and *in vitro* studies showed that SFPQ plays a role in the polyadenylation of influenza virus mRNA.





1 *A trans model for the second step in influenza RNA replication. The diagram presents the proposed process for generation of progeny vRNP. (A) cRNP template. (B) Multiple initiation events by a non-resident polymerase and assembly of progeny vRNP. (C) Displacement of resident polymerase by the replication complex. (D) Structure of the progeny vRNP.*

2 *Model for the structure of an influenza virus helical RNP. (A) Composite volume generated by combining the central portion of an RNP and the RNP ends, showing the polymerase (green and brown) and the terminal NP loop (yellow). (B) Model for localisation of the viral RNA (yellow thread) in one NP strand, represented as surface potential or showing the residues whose mutation reduces NP-RNA interaction (highlighted in blue). (C) Model for localisation of the template RNA (yellow thread) in the double-helical structure of an RNP.*

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SELECTED PUBLICATIONS

Marcos-Villar L, Gallego P, Muñoz-Fontela C, de la Cruz-Herrera CF, Campagna M, González D, Lopitz-Otsoa F, Rodríguez MS, Rivas C. Kaposi's sarcoma-associated herpesvirus lana2 protein interacts with the pocket proteins and inhibits their sumoylation. Oncogene. 2013 Jan 14. doi: 10.1038/onc.2012.603

Campagna M, Marcos-Villar L, Arnoldi F, de la Cruz-Herrera CF, Gallego P, González-Santamaría J, González D, Lopitz-Otsoa F, Rodriguez MS, Burrone OR, Rivas C. Rotavirus viroplasm proteins interact with the cellular SUMOylation system: implications for viroplasm-like structure formation. J Virol. 2013 Jan;87(2):807-17

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González-Santamaría J, Campagna M, García MA, Marcos-Villar L, González D, Gallego P, Lopitz-Otsoa F, Guerra S, Rodríguez MS, Esteban M, Rivas C. Regulation of vaccinia virus E3 protein by small ubiquitin-like modifier proteins. J Virol. 2011 Dec;85(24):12890-900

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Virus and cancer

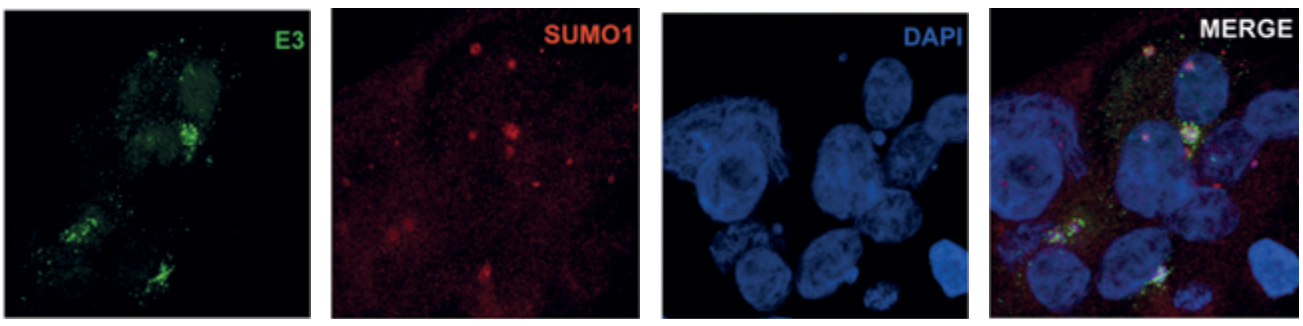
Our group is interested in the relationship between viruses and cancer. Tumour viruses induce oncogenesis by manipulating an array of cellular pathways, some of which are extensively regulated by the small ubiquitin-like protein SUMO. SUMOylation is a reversible post-translational modification by which SUMO is covalently attached to a target protein and changes its activity, subcellular localisation and/or interaction with other macromolecules. Modification by SUMO is involved in many biological functions, and there is some evidence that implicates misregulated SUMOylation in tumourigenesis. The relevance of SUMO conjugation in virus replication is illustrated by the finding that many host proteins involved in innate and intrinsic immunity are regulated by SUMOylation, and that SUMO is a contributor to the regulatory process that governs the initiation of the type I interferon (IFN) response. The importance of SUMO is exemplified by the fact that viruses have evolved means to take advantage of the conserved host cell SUMOylation machinery, either by modulating essential components or as targets of this post-translational modification themselves. Based on these data, we are interested in

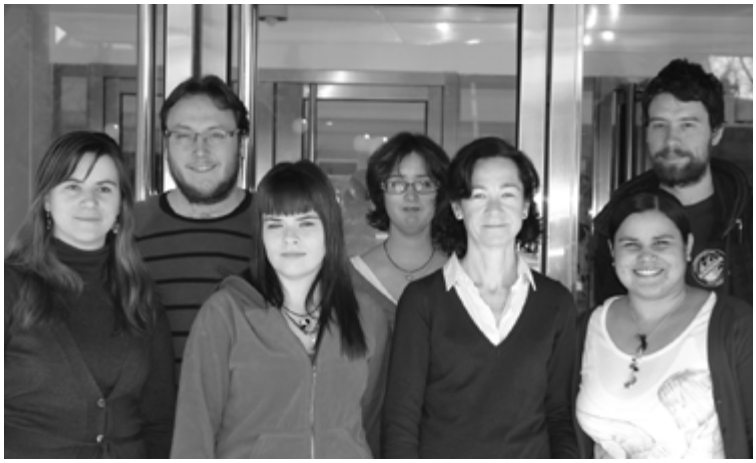
1. identifying how viruses and their regulatory proteins exploit the host cell SUMO modification system
2. evaluating the role of SUMO modification in virus replication, and
3. determining how alteration of the SUMOylation pathways by tumour viruses might affect cell transformation

As a result of our studies on the interplay between virus and SUMO over the last two years, we have

- demonstrated that the tumour suppressor PTEN is modified by SUMO, and that SUMOylation contributes to the control of virus infection by PTEN
- demonstrated that the pocket proteins p107 and p130 are SUMOylated, and identified LANA2 as the first example of a KSHV protein that can inhibit their conjugation to SUMO
- demonstrated that rotaviruses exploit the SUMOylation machinery of the cell to improve their replication
- demonstrated the important roles of both SUMO and ubiquitin in the regulation of the vaccinia virus E3 protein
- identified p53 acetylation as an indispensable event that enables the p53-mediated antiviral response

1 *Vaccinia virus E3 protein colocalines with SUMO1 in de viral factories.*





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SELECTED PUBLICATIONS

Maestre AM, Garzón A, Rodríguez D. Equine torovirus (BEV) induces caspase-mediated apoptosis in infected cells. PLoS One. 2011;6(6):e20972.

Guerra S, Abaitua F, Martínez-Sobrido L, Esteban M, García-Sastre A, Rodríguez D. Host-range restriction of vaccinia virus E3L deletion mutant can be overcome *in vitro*, but not *in vivo*, by expression of the influenza virus NS1 protein. PLoS One. 2011;6(12):e28677

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Molecular characterisation and epidemiology of torovirus

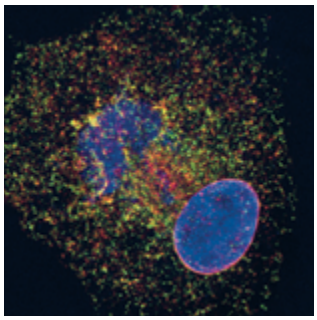
Toroviruses (family *Coronaviridae*, order *Nidovirales*) are emergent viruses with a potential of zoonotic transmission that can cause enteric disease and diarrhoea in various animal species and in humans. Four torovirus species or genotypes have been recognised thus far, on the basis of their hosts: human (HToV), bovine (BToV), porcine (PToV) and equine (EToV). The equine torovirus, known as BEV, is the only one that has been adapted to grow in cultured cells, and is therefore the model used to study different aspects of torovirus biology.

One of our research interests focusses on virus-host interactions. We showed that BEV-infected cells undergo apoptosis, and that both the extrinsic and intrinsic pathways are involved. The double-stranded RNA-dependent protein kinase PKR appears to be a major determinant in apoptosis induction. The contribution of other factors related to cell stress is nonetheless under investigation.

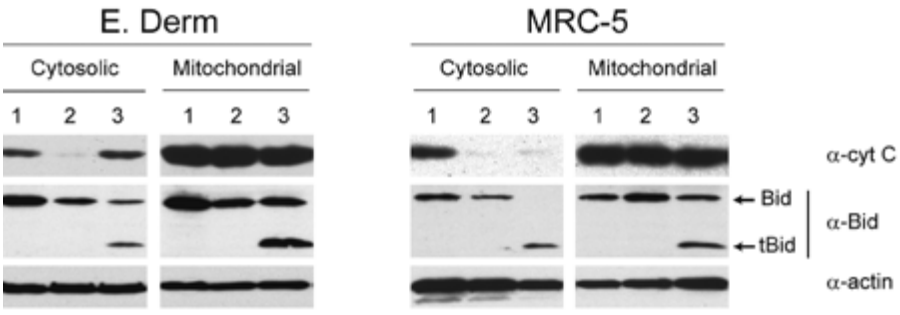
Examination of BEV-infected cells by electron and confocal microscopy suggests that, as for other RNA viruses, torovirus replication/transcription complexes are built in association with cell membranes. To characterise these complexes morphologically and functionally, we generated a panel of antibodies to several viral proteins involved in these processes. To study the biogenesis of these structures, we use these antibodies in combination with approaches involving gene silencing and treatment with various drugs to block different cellular pathways in BEV-infected cells.

We are also interested in defining the epidemiological situation of toroviruses in Spain, for which we developed specific molecular and serological diagnostic assays. We carried out a large torovirus seroepidemiological survey that included adult and young animals from 100 farms distributed throughout Spain. The results showed that this virus is endemic in pig herds in Spain. The overall serological pattern indicates continuous spread of the virus, suggesting that chronically infected adult animals could act as reservoirs. The impact of this high PToV seroprevalence in pig production remains unknown.

In the course of our epidemiological studies, we identified virus strains of the two defined PToV lineages. The antigenic variations among these strains and the relationship between their genetic and antigenic properties are being studied.



1 *Subcellular localisation of the haemagglutinin-esterase (HE) protein from porcine torovirus analysed by confocal microscopy. Cells infected with a recombinant vaccinia virus expressing HE were labelled with anti-HE antibodies (red), antibodies against calnexin (green) and the DNA stain DAPI (blue).*



2 *Apoptosis caused by the equine torovirus BEV triggers cytochrome c release from mitochondria and cleavage of Bid. Cytosolic and mitochondrial fractions from E. Derm and MRC-5 cells mock-infected (lanes 2), treated with staurosporine (lanes 1) or infected with BEV for 24 h (lanes 3) were analysed by Western blot with antibodies to cytochrome c and Bid proteins. Reactivity with anti-actin antibody was used as a loading control.*

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SELECTED PUBLICATIONS

Busnadiego I, Maestre AM, Rodríguez D, Rodríguez JF. The infectious bursal disease virus RNA-binding VP3 polypeptide inhibits PKR-mediated apoptosis. PLoS One. 2012;7(10):e46768

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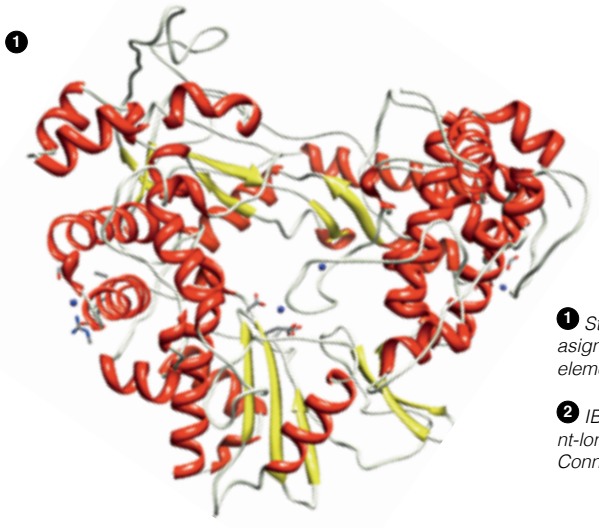
Molecular biology of birnavirus

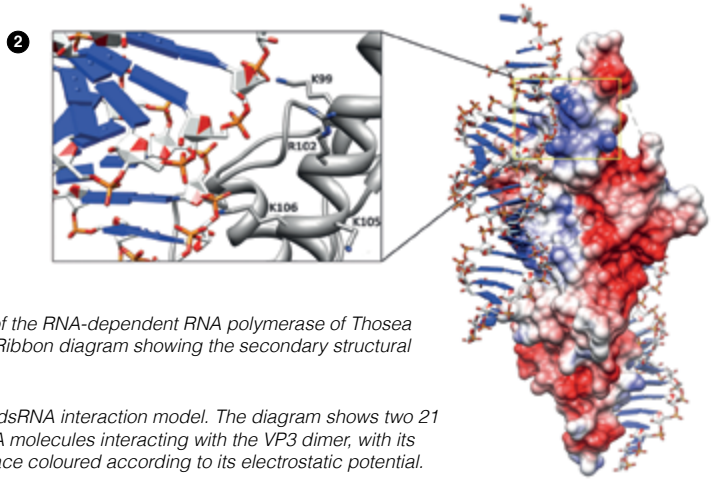
The *Birnaviridae* family groups icosahedral naked viruses with bipartite dsRNA genomes. Members of this family infect a wide variety of animal species including insects, aquatic fauna and birds. Our main virus model is the infectious bursal disease virus (IBDV), the aetiological agent of an acute immunosuppressive disease that affects domestic chickens, causing huge economic losses to the poultry industry world-wide. Our laboratory focusses primarily on three major topics, virus structure and assembly, the molecular basis for IBDV pathogenesis and virulence, and birnavirus evolution.

Our previous work showed that IBDV capsid assembly only requires a concerted interaction between trimers of the pVP2 (the precursor of the VP2 capsid polypeptide) and VP3, a multifunctional protein that acts as a scaffolding element during particle assembly. These two proteins are released from a large polyprotein following a series of stepwise proteolytic processing events that involve the participation of a polyprotein-embedded protease (VP4) and able to assemble self-cleavage of pVP2. We recently showed that the generation of a pVP2 molecular form able to assemble into the trimers required for the formation of particle pentamers is strictly dependent on the cleavage of the pVP2 precursor by a cell protease, the puromycin-sensitive aminopeptidase. This finding provides the final piece in the complex polyprotein proteolytic processing cascade, and opens an as yet unexplored path to for understanding IBDV tissue tropism.

Regarding the molecular basis of IBDV pathogenesis, we found that the VP3 polypeptide has a critical role in blocking different arms of the innate host response, *e.g.,* dsRNA-induced gene silencing and PKR activation.

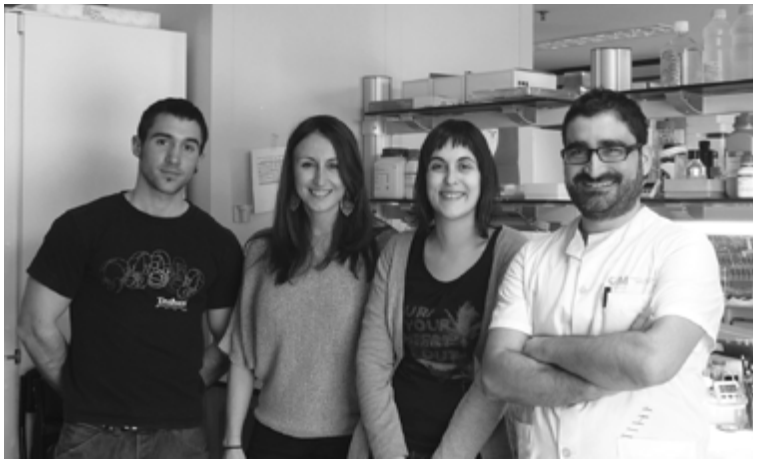
Birnaviruses exhibit a number of features that greatly resemble those of ssRNA viruses of the *Noda-* and *Tetraviridae* families, suggesting that birnaviruses might represent an evolutionary link that connects dsRNA and ssRNA viruses. To analyse this hypothesis, we are comparing the structure and function of the RNA-dependent RNA polymerases (RdRp) from members of these three virus families. As part of this project, we recently solved the crystal structure of the tetravirus Thosea asigna virus.





❶ *Structure of the RNA-dependent RNA polymerase of Thosea asigna virus. Ribbon diagram showing the secondary structural elements.*

❷ *IBDV VP3/dsRNA interaction model. The diagram shows two 21 nt-long dsRNA molecules interacting with the VP3 dimer, with its Connolly surface coloured according to its electrostatic potential.*



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Calabia-Linares C, Robles-Valero J, Perez-Martinez M, Martín-Cofreces N, Alfonso-Pérez M, Gutierrez-Vázquez C, Mittelbrunn M, Ibiza S, Urbano-Olmos FR, Aguado-Ballano C, Sánchez-Sorzano CO, Sanchez-Madrid F, Veiga E. Endosomal clathrin drives actin accumulation at the immunological synapse. J Cell Sci 24(Pt 5):820-30. 2011

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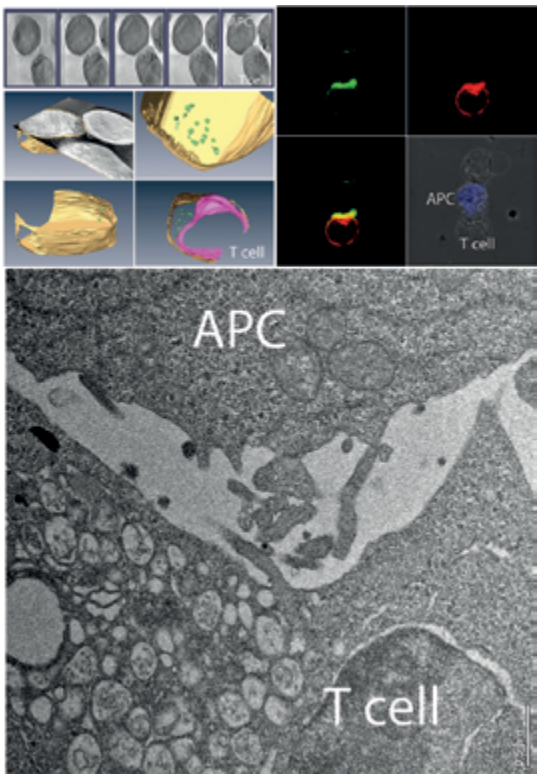
Cellular immunobiology and microbiology

We are interested in the molecular mechanisms that drive T cell activation. Antigen-specific cognate interaction of T lymphocytes with antigen-presenting cells (APC) drives major morphological and functional changes in T cells, including actin rearrangements at the immune synapse (IS) formed at the cell-cell contact area. We found that clathrin, a protein involved in endocytic processes, drives actin accumulation at the IS. Clathrin is recruited to the IS with kinetics that parallel that of actin. Knockdown of clathrin prevents accumulation at the IS of actin and proteins involved in actin polymerisation, such as dynamin-2, the Arp2/3 complex and CD2AP. The clathrin pool involved in actin accumulation at the IS is linked to multivesicular bodies that polarise to the cell-cell contact zone, but not to plasma membrane or the Golgi complex. These data underscore the role of clathrin in polymerisation at the interface of T cells and APC. We are also trying to determine the ultrastructure of the IS to define the relation of the cytoskeleton with the subcellular organelles (i.e., multivesicular bodies; MVB) that polarise to and direct massive actin accumulation at the IS. To determine its structure precisely, we are analysing the IS by cryo-X-ray tomography and cryo-electron tomography. We will integrate the 3D spatial information from each experiment at different resolutions (4D) and time stages of synapse formation (5D). We are also interested in the interactions between pathogenic bacteria and cells of the immune system. We are studying the ways that bacteria spread through the infected host. Finally, we will explore the capacity of different bacteria to modify immune responses.



1 *Different resolution views of the IS: fluorescence, X-ray tomography and electron microscopy*

04 Microbial Biotechnology

The Department of Microbial Biotechnology integrates research to gain knowledge of key aspects of microbial biology with environmental, clinical or biotechnological relevance. The department hosts ten groups that use several complementary aspects of microbial biology, with approaches that include molecular genetics, genomics, proteomics and metagenomics. The subjects studied include:

Environmental microbiology. We aim to understand how microorganisms degrade organic compounds that generate environmental problems, as well as why a microorganism that can efficiently degrade a compound of interest in laboratory conditions does not usually perform so well in natural conditions. We also study the rhizobial communities of transgenic plants and the effect of herbicides on these communities.

Microbial responses to hostile environments. The focus is to understand bacterial responses to stressful environments, including the reaction to host defence responses and to agents that cause DNA damage. We study how microbial pathogens avoid host defence mechanisms, and how bacteria and fungi replicate DNA and repair DNA damage to improve genome stability.

Microbial pathogens. Efforts are directed to understand how microbial pathogens infect eukaryotic hosts, with emphasis on host-pathogen interactions in infections caused by intracellular bacteria and by opportunistic pathogens. This will facilitate discovery of new targets to combat infections.

Microbial resistance to antibiotics and search for new antimicrobials. We focus on mechanisms of bacterial resistance to antibiotics and analyse the complex responses elicited by exposure of microbes to sublethal concentrations of antibiotics. In addition, we search for novel targets in essential functions to develop drugs to fight pathogens.

Microbial engineering. The purpose is to generate bacterial strains optimised to obtain products of interest, such as recombinant antibodies or hydrolytic enzymes, or to detect and degrade pollutants. We also work to understand the mechanisms bacteria use to export and secrete proteins, which could help in the development of bacterial recombinant strains to be used as delivery systems in the treatment of human diseases.

These topics are highly interconnected. While most established pathogens normally cope with stress conditions by developing efficient adaptive responses, the opportunistic pathogens are metabolically very versatile, making them efficient biodegraders of pollutants. Fighting against pathogenic microorganisms requires deep understanding of their behaviour during infection and of how resistance develops as pathogens are challenged by antibiotics.

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Soberón NE, Lioy VS, Pratto F, Volante A, Alonso JC. Molecular anatomy of the Streptococcus pyogenes pSM19035 partition and segrosome complexes. Nucleic Acids Res. 2011. 39(7):2624-37

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Genetic stability

Our research focusses on the study of the mechanisms used by bacteria of the phylum Firmicutes to secure the structural and segregational stability of genetic information. *Bacillus subtilis* cells are used to study repair by recombination and plasmid segregational stability during vegetative growth, and genetic recombination during natural competence. Using DNA repair by recombination, we have shown that:

1. the DNA damage response recruits complex molecular machinery; among the first responders is the RecN protein, which in concert with PNPase promotes the dynamic recruitment of DNA ends

2. AddAB or RecJ, together with the RecQ or RecS helicase and SsbA, create a 3' single-stranded (ss) DNA tailed duplex at the DNA breaks, and RecN recruits recombination proteins to form a repair centre

3. different accessory proteins (*e.g.,* RecFOR, SsbA) regulate RecA filament formation to repair the DNA breaks and the branch migration helicase RuvAB, in concert with the resolvase (RecU), processes the recombination intermediates.

Using genetic recombination, we have shown that:

1. different mediators (*e.g.,* RecO, DprA) regulate RecA nucleation onto the taken up ssDNA, coated by SsbA and/or SsbB during chromosomal transformation

2. RecO- or DprA-mediated strand annealing contributes to circularisation of the incoming plasmid ssDNA during plasmid transformation

3. different RecA modulators (*e.g.,* RecF, RecX, RecU) shut off RecA-mediated DNA strand exchange.

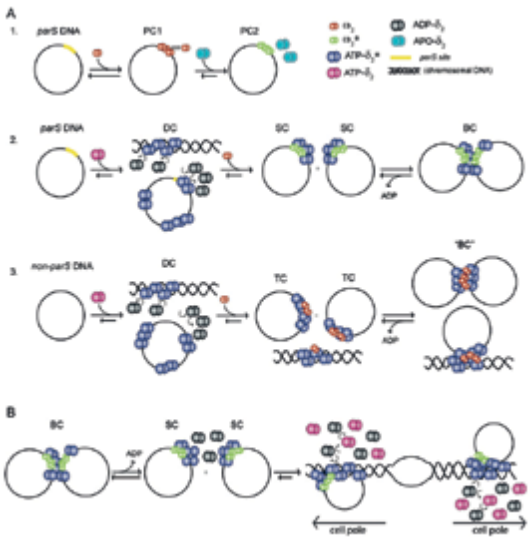
Using the segregation model, we have shown that:

1. the pSM19035 global regulator ω (ω2) controls the fine-tuning of plasmid copy number, plasmid segregation, and the toxin-antitoxin (TA) system

2. the partitioning protein ω2 binds to a centromeric-like region (PC1 and PC2) and the dimeric δ (δ2) ATPase facilitates ω2 recruitment onto parS DNA, leading to a segrosome complex (SC), and this complex facilitates the formation of bridging complexes (BC) and δ2-mediated hydrolysis of ATP

3. the ATP hydrolysis leads to δ2 disassembly from DNA, and the dynamic assembly/disassembly moves the plasmid molecule towards the cell poles to guarantee faithful segregation

4. when partition fails, the TA system halts proliferation of plasmid-free cells, with subsequent overgrowth of plasmid-bearing cells.



1 *Dynamic assembly of different types of protein·DNA complexes*

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Ayora S, Carrasco B, Cárdenas PP, César CE, Cañas C, Yadav T, Marchisone C, Alonso JC. Double-strand break repair in bacteria: a view from *Bacillus subtilis*. FEMS Microbiol Rev. 2011 Nov;35(6):1055-81

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Kidane D, Ayora S, Sweasy JB, Graumann PL, Alonso JC. The cell pole: the site of cross talk between the DNA uptake and genetic recombination machinery. Crit Rev Biochem Mol Biol. 2012 Nov-Dec;47(6):531-55

Recombination-dependent DNA replication

Our research focusses on the mechanisms that cells use to continue replication progress when this process encounters impediments to DNA replication, which may eventually collapse the fork, producing a broken DNA end. Replication restart is then mediated by proteins that were initially identified by their roles in homologous recombination and repair of DNA double-strand breaks (DSB). Accurate repair of DSBs is essential to life. Study of the recombination mechanisms has shown the complexity of the recombination process, due to the large numbers of proteins involved. We thus use a simple model system, *Bacillus subtilis* and its bacteriophage SPP1, and several biophysics, structural and molecular biology techniques to decipher these complex mechanisms (Ayora *et al.*, 2011; Lo Piano *et al.,* 2011).

One process we study in depth is the resolution of a central homologous recombination intermediate, the Holliday junction structure. In the last two years, we explored the role of the stalk region in the RecU Holliday junction resolvase, and show that it is essential for interaction with RecA and for recognition and specific binding to a Holliday junction structure (Cañas *et al.*, 2011 J. Mol. Biol., 410:34-49). Resolution of a Holliday junction is also an essential step during the shift from theta to concatemeric replication in the phage SPP1. We characterised the SPP1-encoded Holliday junction resolvase. Our analyses showed that G44P, which has limited similarity to other Holliday junction resolvases, cleaves Holliday junctions and replicated D-loops, and might participate in recombination-dependent DNA replication (Zecchi *et al.*, 2012 PLoS ONE, 7, e48440).

One goal of our group is the *in vitro* reconstitution, using purified SPP1 replication and recombination proteins, of the mechanisms that operate at a stalled replication fork and lead to replication restart.

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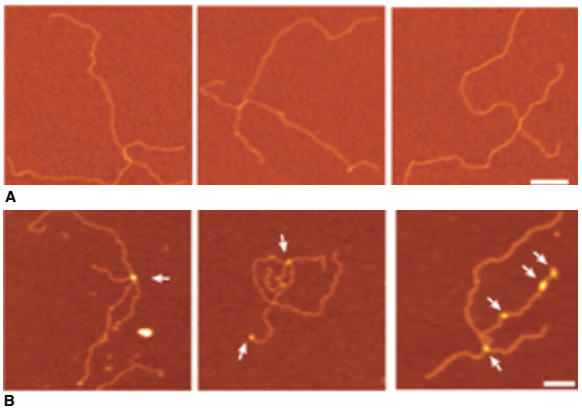

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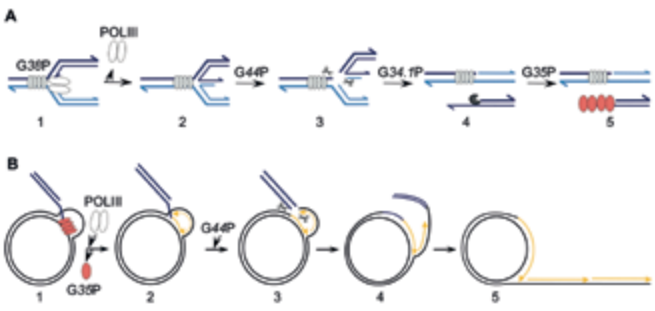

1 *Visualisation by atomic force microscopy of the Holliday junction structure (A) and binding of the G44P protein to this recombination intermediate (B). G44P bound to the junction as well as to the dsDNA arms. Arrows denote protein-DNA complexes. Scale bar = 100 nm.*

2 *Recombination-dependent DNA replication (RDR) in the SPP1 virus generates concatemeric linear DNA. (A) The generation of a double-strand break (DSB) triggers RDR (B).*

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SELECTED PUBLICATIONS

Thi TD, López E, Rodríguez-Rojas A, Rodríguez-Beltrán J, Couce A, Guelfo JR, Castañeda-García A, Blázquez J. The effect of recA inactivation on mutagenesis of *Escherichia coli* exposed to sub-lethal concentrations of antimicrobials. J Antimicrob Chemother. 2011 Mar;66(3):531-8

Rodríguez-Rojas A, Oliver A, Blázquez J. Intrinsic and environmental mutagenesis are key components of Pseudomonas aeruginosa chronic lung infections. J Infect Dis. 2012;205 (1):121-7

Rodríguez-Beltrán J, Rodríguez-Rojas A, Guelfo JR, Couce A, Blázquez J. The *Escherichia coli* SOS gene dinF protects against oxidative stress and bile salts. PLoS One. 2012;7(4):e34791

Blázquez J, Couce A, Rodríguez-Beltrán J, Rodríguez-Rojas A. Antimicrobials as promoters of genetic variation. Curr Opin Microbiol. 2012 Oct;15(5):561-9

Couce A, Guelfo JR, Blázquez J. Mutational spectrum drives the rise of mutator bacteria. PLoS Genet. 2013 Jan;9(1):e1003167

Stress and bacterial evolution

The major interest of the group is to understand the bacterial responses to stress. We specifically study hypermutation and hyper-recombination as "bacterial strategies" to speed adaptation to environmental stresses. One of the models used here is antibiotic stress and the development of antibiotic resistance. Our work is focussed on both stable and inducible hypermutation/hyper-recombination in *E. coli*, *P. aeruginosa* and *M. smegmatis/tuberculosis*.

We are currently studying:

1. Compensation of stable hypermutation. Once adapted, hypermutable bacteria must decrease (compensate) the high mutation rate to avoid accumulation of deleterious mutations. Our study aims to determine the molecular mechanisms involved in this compensation.

2. Regulation of stress responses and inducible hypermutation

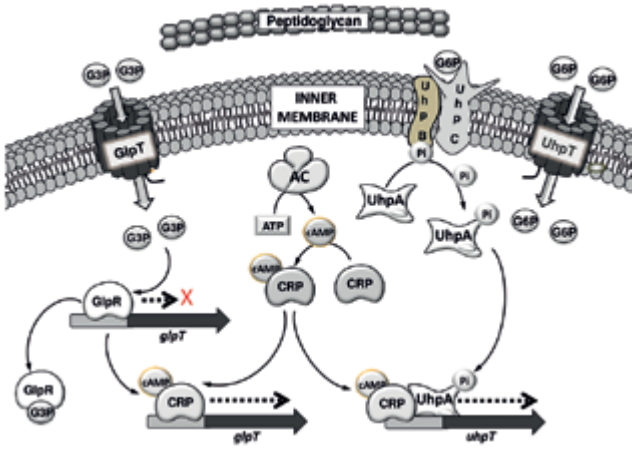
- Environmental regulation of mutagenesis
- Transcriptional regulation of specialised DNA polymerases (belonging to the SOS regulon)
- Effect of antibiotics on mutation and recombination: are antibiotics promoters of antibiotic resistance?

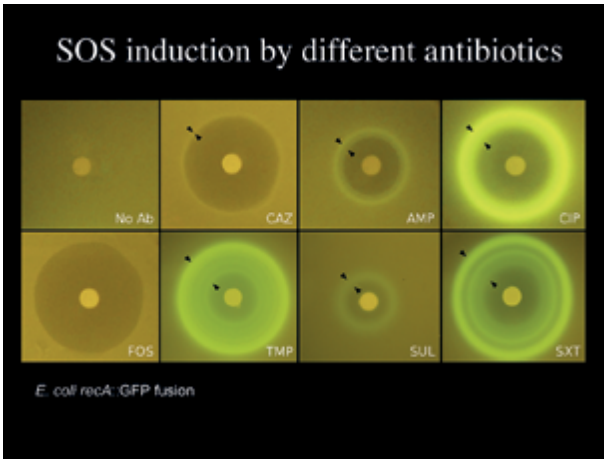
3. Hypermutation in bacteria lacking a DNA mismatch repair system (MMR) such as Mycobacterium and Streptomyces. This will allow the use of hypermutant/hyper-recombinant bacteria of industrial interest as biotechnological tools to produce modified biosynthetic pathways.

4. Evolution of resistance to beta-lactam antibiotics and the development of new inhibitors of beta-lactamases

5 The molecular basis of bacterial evolution. Combatting antibiotic resistance by preventing evolution (mutation, recombination and horizontal transfer).

1 *Regulation of GlpT and UhpT.*





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SELECTED PUBLICATIONS

Merchan S, Pedelini L, Hueso G, Calzada A, Serrano R, Yenush L. Genetic alterations leading to increases in internal potassium concentrations are detrimental for DNA integrity in *Saccharomyces cerevisiae*. Genes Cells (2011) vol. 16 (2) pp. 152-65

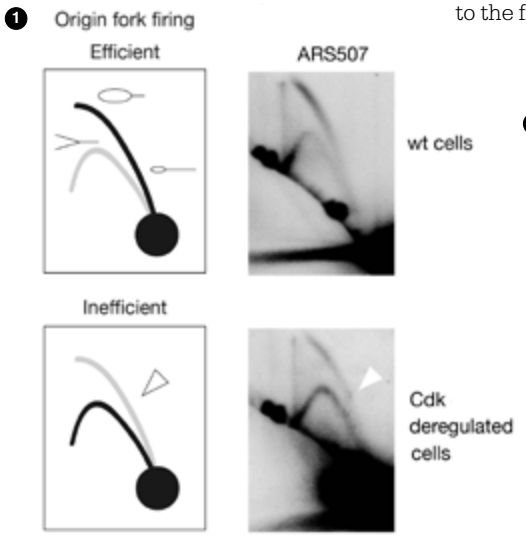
Cell cycle, DNA replication and genome stability in eukaryotes

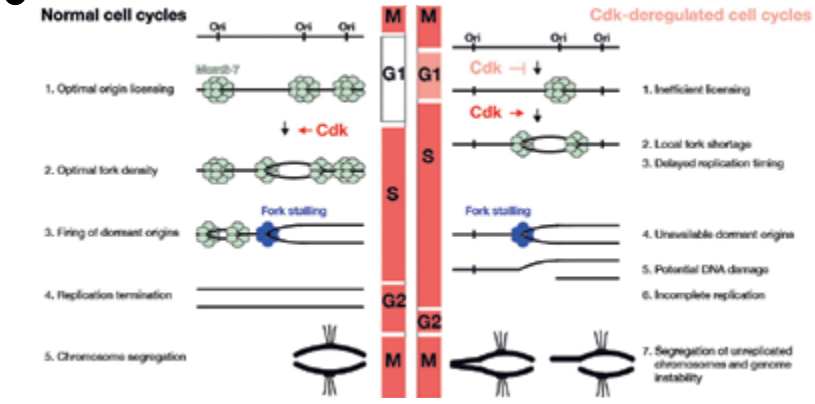
We study the regulation of chromosomal replication, the process that duplicates the genome during each cell division, by the cell cycle machinery that ensures correct division of the mother cell into two daughter cells. Both processes must be synchronised for stable genome transmission to progeny, and deregulation during DNA replication or other errors are mutagenic and have disease-causing potential. We use the model eukaryotic organism *Saccharomyces cerevisiae*, which shows conservation of numerous cell biology processes throughout eukaryotic evolution.

How replication is regulated to terminate correctly, and what molecular aberrations occur during abnormal, disease-causing cell cycles is largely unknown. Both questions are the focus of our research. Cells prepare DNA replication during the preceding G1 phase, in which they test the environmental conditions and enter the cell cycle or remain arrested. Impairing this schedule is deleterious for cells and increases genome instability, thought to be mediated by S phase abnormalities. We found that altering potassium homeostasis impairs normal cell cycle progression in G1, reduces the efficiency of DNA replication, requires the DNA damage response pathway, and is detrimental to genome integrity. This also applies during tumourigenic cell cycles. Cyclin-dependent kinases (Cdk) regulate progression through G1 and are frequently deregulated in cancer cells. These cells show chromosome instability and unscheduled G1/S transition and S phase progression, but the molecular aberrations during replication and how they contribute to instability is little known. We studied these questions in Cdk-deregulated yeast cells and found decreased distribution and density of origins at a chromosome arm. Origins should be widespread in large numbers along chromosomes for complete, stress-resistant DNA replication and prevention of genome instability; consistent with this, we found that the decreased origin density correlates directly with chromosome instability in the region. Origin activity is also altered in p27$^{-/-}$ MEF (murine embryonic fibroblasts). Finally, in both eukaryotes, origins are differentially compromised by Cdk deregulation, which supports non-uniform chromosome instability during tumorigenesis. Origin shortage might thus contribute to the fragility of certain chromosome regions frequently observed during oncogenesis.

1 *Two-dimensional DNA (2D) gels of replication intermediates to measure the activity and efficiency of DNA.*

2 *Schedule of cell cycle progression, and dynamics of replication and segregation of a given chromosome region in normal and Cdk-deregulated cells, as suggested by our work.*





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SELECTED PUBLICATIONS

Palomino C, Marín E, Fernández LÁ. The fimbrial usher FimD follows the SurA-BamB pathway for its assembly in the outer membrane of *Escherichia* coli. J Bacteriol. 2011 Oct;193(19):5222-30

Fernández LÁ, Muyldermans S. Recent developments in engineering and delivery of protein and antibody therapeutics. Curr Opin Biotechnol. 2011 Dec;22(6):839-42

Jurado P, Fernández LA, de Lorenzo V. Production and characterisation of a recombinant single-chain antibody (scFv) for tracing the sigma 54 factor of *Pseudomonas putida*. J Biotechnol. 2012 Jul 31;160(1-2):33-41

Bodelón G, Palomino C, Fernández LÁ. Immunoglobulin domains in *E. coli* and other enterobacteria: from pathogenesis to applications in antibody technologies FEMS Microbiol Rev. 2012 Mar;37(2):204-50

Protein secretion and antibody expression

Our group conducts both basic and biotech-oriented research to understand and exploit protein secretion in *E. coli* and other Gram-negative bacteria (*proteobacteria*). Our basic research centres on the molecular mechanisms that bacterial pathogens use to secrete proteins (cytotoxins, proteases, adhesins) and to assemble cell surface organelles (fimbriae) that participate in bacterial virulence. We focus especially on those proteins and surface organelles secreted and assembled by (EPEC), enterohemorragic (EHEC) and uropathogenic (UPEC) *E. coli* strains. The biotechnological projects exploit these protein secretion systems to develop novel expression and selection technologies for recombinant antibodies in non-pathogenic commensal and laboratory *E. coli* strains. Among the recombinant antibody formats available (single-chain Fv, Fab, Fc-fusions, etc.), we focus on single-domain antibodies (sdAb) or nanobodies, the smallest antibody fragment known to date (~15 kDa) with full antigen-binding capacity. Nanobodies are based on a single VH domain obtained by recombinant DNA technology from heavy-chain-only antibodies from camelids (dromedaries, llamas). Despite the lack of a paired VL domain, nanobodies show high affinity and specificity for their cognate antigens. In addition, they are very similar to human VH3 sequences, making them excellent candidates for many applications, including therapy.

In the last few years, we demonstrated the capacity of the bacterial type V secretion system (T5SS) for bacterial display of single-domain antibodies, and are exploiting their translocator domains for selection of nanobodies to antigens of interest in biomedicine. We have also studied *E. coli* type 1 fimbriae by analysing the biogenesis of the fimbrial usher FimD, its interaction with periplasmic chaperones, and the Bam complex and its mechanism of activation by FimH. Finally, we continued our studies using the type III protein secretion system (T3SS) from EPEC strains to deliver single-domain antibodies from *E. coli* cells into the cytosol of human cells.

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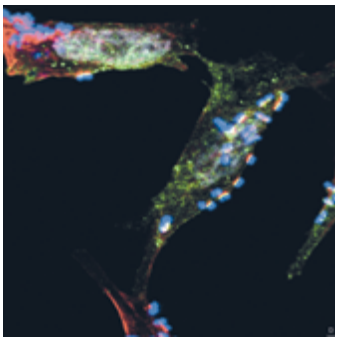

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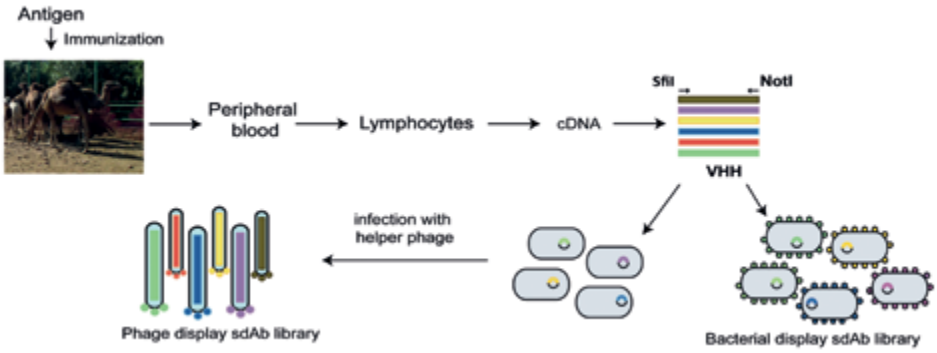

1 *Confocal microscopy of human HeLa cells cultured in vitro and infected with EHEC (anti-O157 monoclonal antibody, blue). Extracellular EHEC bacteria inject the translocated intimin receptor (Tir) into the HeLa cell cytoplasm, triggering actin pedestal formation beneath the adhered bacteria. Tir is stained green with anti-Tir rabbit polyclonal antibody and actin is stained red with phalloidin; yellow indicates Tir and actin colocalisation*

2 *Cloning of single-domain antibodies from immunised camelids and their expression on the E. coli cell surface (bacterial display) or filamentous bacteriophages (phage display) for screening and isolation of specific binders of an antigen of interest.*

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SELECTED PUBLICATIONS

Ortega A, Gonzalo-Asensio J, García-Del Portillo F. Dynamics of *Salmonella* small RNA expression in non-growing bacteria located inside eukaryotic cells. RNA Biol. 2012 Apr;9(4):469-88

García-del Portillo F, Cossart P. A new view to intracellular pathogens and host responses in the South of Spain. EMBO Mol Med. 2012 Mar;4(3):160-4

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Paradela A, Mariscotti JF, Navajas R, Ramos-Fernández A, Albar JP, García-del Portillo F. Inverse regulation in the metabolic genes pckA and metE revealed by proteomic analysis of the *Salmonella* RcsCDB regulon. J Proteome Res. 2011 Aug 5;10(8):3386-98

Intracellular bacterial pathogens

Our group is interested in deciphering the cues that sustain the ability of certain bacterial pathogens to successfully colonise the intracellular niche of eukaryotic cells. As models, we use two intracellular bacterial pathogens with marked differences in their envelope structure, the Gram-negative bacterium *Salmonella enterica* serovar Typhimurium and the Gram-positive bacterium *Listeria monocytogenes*.

A largely unexplored area of the biology of *S. enterica* serovar Typhimurium involves the mechanisms it uses to establish a dormant non-growing state within the eukaryotic cell. *In vivo* and *in vitro* experiments support the idea that *S. enterica* serovar Typhimurium has evolved to spend most of its "intracellular life" in a state of "limited proliferation". Our studies have uncovered a set of small regulatory RNA (sRNA) that are upregulated by dormant intracellular *Salmonella* upon entry into the host cell. Transcriptomic analyses also showed a profound change in the expression prolife of numerous pathogen genes, including marked upregulation of genes that map to the virulence plasmid pSLT. Most of these plasmid genes were known to be silent in laboratory conditions. In dormant non-growing intracellular bacteria, we are currently analysing the mechanisms that activate important virulence regulators such as the PhoP-PhoQ system and the *Salmonella* pathogenicity islands 1 (SPI-1) and 2 (SPI-2). Our future efforts will focus on understanding how the bacterial envelope is modified when the pathogens adapts to this unique non-growing lifestyle within the eukaryotic cell.

In the research devoted to *Listeria monocytogenes*, we have progressed in the elucidation of changes in the cell wall when this pathogen infects epithelial cells. Proteomic studies identified the surface proteins bearing the C-terminal sorting motif LPXTG that are synthesised by the pathogen during the proliferation phase in the eukaryotic cytosol. We also characterised a unique mode of association of the ActA surface protein to the peptidoglycan when the pathogen adapts to the intracellular lifestyle. Our ongoing investigation focusses on new *L. monocytogenes* LPXTG family surface proteins of unknown function that might have a role in stabilisation of the cell wall architecture and in host cell invasion.

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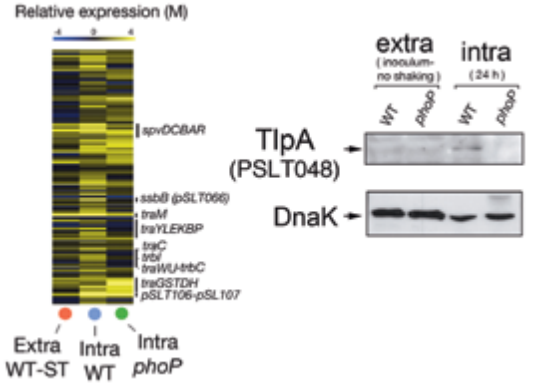
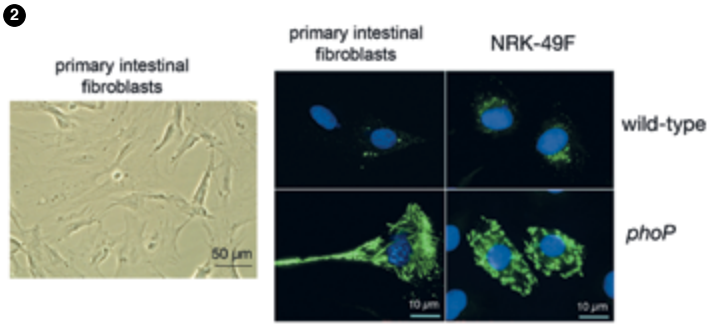


1 *Expression profiles in extracellular and intracellular bacteria of Salmonella enterica serovar Typhimurium genes that map to the virulence plasmid pSLT*

2



2 *Demonstration of the phenotype similarity of S. enterica serovar Typhimurium in cultured fibroblasts (rat cell line NRK) and in primary fibroblasts isolated from mouse intestine*

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SELECTED PUBLICATIONS

Olivares J, Alvarez-Ortega C, Linares JF, Rojo F, Köhler T, Martínez JL. Overproduction of the multidrug efflux pump MexEF-OprN does not impair *Pseudomonas aeruginosa* fitness in competition tests, but produces specific changes in bacterial regulatory networks. Environ Microbiol. 2012 Aug;14(8):1968-81

Sánchez MB, Martínez JL. Differential epigenetic compatibility of qnr antibiotic resistance determinants with the chromosome of *Escherichia* coli. PLoS One. 2012;7(5):e35149. doi

Martínez JL, Baquero F, Andersson DI. Beyond serial passages: new methods for predicting the emergence of resistance to novel antibiotics. Curr Opin Pharmacol. 2011 Oct;11(5):439-45

Hernández A, Ruiz FM, Romero A, Martínez JL. The binding of triclosan to SmeT, the repressor of the multidrug efflux pump SmeDEF, induces antibiotic resistance in *Stenotrophomonas maltophilia*. PLoS Pathog. 2011 Jun;7(6):e1002103

Martínez JL, Rojo F. Metabolic regulation of antibiotic resistance. FEMS Microbiol Rev. 2011 Sep;35(5):768-89

Opportunistic pathogens

Nosocomial infections due to opportunistic pathogens constitute a relevant health problem. As models for understanding the mechanisms involved in the pathogenic process of these microorganisms, we are using *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*. These organisms are free-living bacteria with a characteristic phenotype of intrinsic resistance to antibiotics. Acquisition of resistance is the consequence of millions of years of evolution in natural, non-clinical ecosystems, long before humans began to use antibiotics for therapy. We recently expanded our interest to the study of *Klebsiella pneumoniae*. We aim to understand the biology of opportunistic pathogens, focussing on the networks and the evolutionary processes that connect resistance and virulence. This includes understanding the role of non-clinical natural ecosystems in the acquisition and evolution of antibiotic resistance and virulence, as well as how bacteria evolve during infection (mainly in chronic infection) and the differential host responses triggered by these pathogens. Our work might also provide information on novel targets in the search for drugs of use for reducing resistance to antibiotics.

Among these potential targets, we are especially interested in studying multidrug efflux pumps. These resistance elements are present in all live beings and contribute to many processes, including resistance to anticancer chemotherapy in humans and antibiotic resistance in bacteria. Nevertheless, whilst these pumps can expel these drugs, they have a different original function in nature. We found that the substrate of the MexEF-OprN *P. aeruginosa* efflux pump is L-kynurenine, an intermediate in the biosynthesis of the quorum-sensing molecule PQS. Expression of efflux pumps is usually downregulated; these elements can nonetheless be overexpressed in the presence of an effector. This is the case of the *S. maltophilia* efflux pump SmeDEF, whose expression is triggered by the biocide triclosan, suggesting that commonly used biocides might select for antibiotic-resistant microorganisms.

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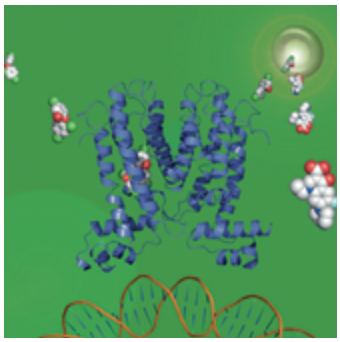

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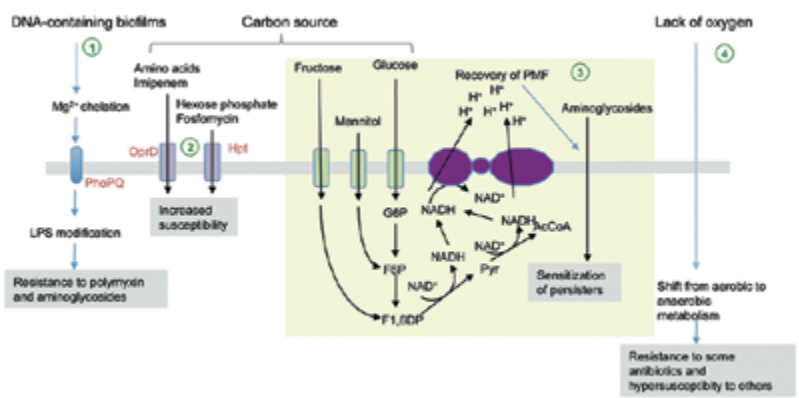
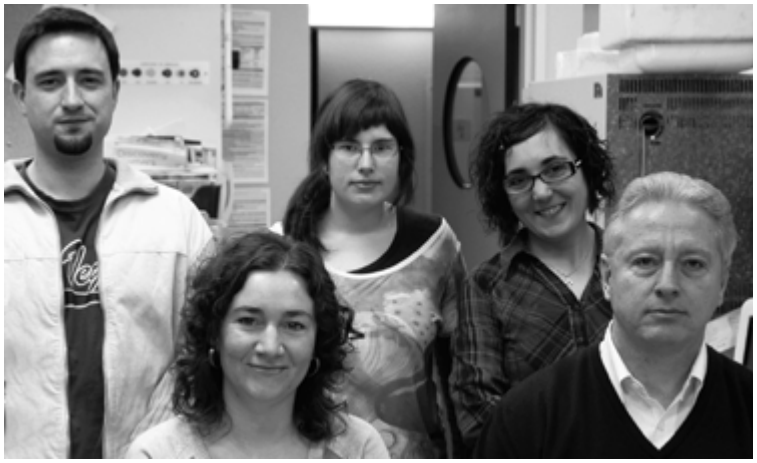


1 *Overexpression of efflux pumps can be achieved when an effector binds the negative transcriptional regulator of the system, which is released from its operator. As a consequence, the pump is overexpressed and extrudes different compounds, including antibiotics.*

2 *The phenotype of susceptibility to antibiotics can change under different conditions: 1. The presence of extracellular DNA at biofilms chelates cations and induce the pathway of resistance to antimicrobial peptides. 2. The presence of a given carbon source may induce the expression of its transporter. If the transporter is used as well by an antibiotic, its induction makes bacteria hypersusceptible. 3. The use of specific carbon sources can rescue bacteria from an antibiotic-resistant persistent phenotype. 4. Hypoxia, which is a common situation at the deepest zones of bacterial biofilms, alters the susceptibiity to antibiotics (From FEMS Microbiol Rev (2011) 35: 768).*



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SELECTED PUBLICATIONS

Barriuso J, Marín S, Mellado RP. Potential accumulative effect of the herbicide glyphosate on glyphosate-tolerant maize rhizobacterial communities over a three-year cultivation period. PLoS One. 2011;6(11):e27558

Barriuso J, Valverde JR, Mellado RP. Estimation of bacterial diversity using next generation sequencing of 16S rDNA: a comparison of different workflows. BMC Bioinformatics. 2011 Dec 14;12:473

Gullón S, Palomino C, Navajas R, Paradela A, Mellado RP. Translocase and major signal peptidase malfunctions cause a bald phenotype in *Streptomyces* lividans and a decrease in secretory protein production. J Biotechnol. 2012 Aug 31;160(3-4):112-22

Rozas D, Gullón S, Mellado RP. A novel two-component system involved in the transition to secondary metabolism in *Streptomyces coelicolor*. PLoS One. 2012;7(2):e31760

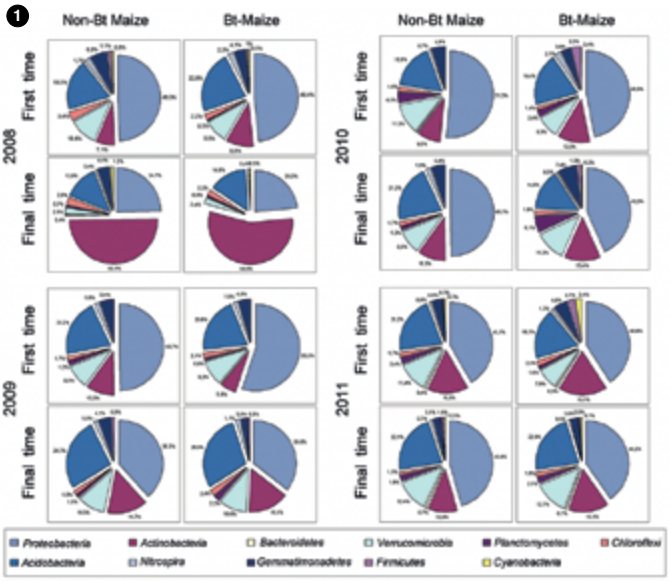
Barriuso J, Valverde JR, Mellado RP. Effect of Cry1Ab protein on rhizobacterial communities of Bt-maize over a four-year cultivation period. PLoS One. 2012;7(4):e35481

Heterologous gene expression and secretion in Gram-positive bacteria of industrial application

We traditionally focus our research on the physiological and molecular characterisation of the main protein secretion mechanism (Sec system) of soil Gram-positive bacteria of the *Streptomyces* genus, namely *S. lividans* and *S. coelicolor*. These are widely used in industry as efficient producers of extracellular hydrolytic enzymes and other compounds of industrial interest.

Deficiency in the translocase complex (SecG mutant strain) or the major type I signal peptidase (SipY mutant strain) function in *S. lividans* results in a group of genes seemingly regulated in the same way, including the absence of secretory protein production in both cases. These genes can be linked directly or indirectly to the *bld* cascade, suggesting its involvement in the cell response to the secretory defect of both mutant strains. Proteomic and transcriptomic analyses have determined that a newly identified *S. coelicolor* two-component system influences various processes characterised by the transition from primary to secondary metabolism, eliciting a partial stringent response and altered patterns of secretory proteins and antibiotics. Overproduction of alpha-amylase in *S. lividans* causes secretion stress and permitted identification of a two-component CssRS-like system that regulates three HtrA-like proteases, which appear to be involved in the degradation of misfolded secretory proteins.

We recently compared the effect of glyphosate (RoundupPlus), a post-emergency applied herbicide, and Harness GTZ, a pre-emergency applied herbicide, on the rhizobacterial communities of genetically modified NK603 glyphosate-tolerant maize. The effect was monitored by high throughput DNA pyrosequencing (Next Generation Sequencing, NGS) of the bacterial DNA coding for the 16S rRNA hypervariable V6 region. The results strongly suggest that glyphosate is environmentally less aggressive over long-term continuous cultivation periods. The rhizobacterial communities of transgenic maize engineered to express the *Bacillus thuringensis* Cry toxin (Bt maize) were equally monitored for a number of years. We tested several simple and complex workflows to analyse NGS from rhizobacterial community experimental data, using a variety of available tools, and determined their accuracy and efficiency under various conditions. We have identified which method-combination workflow is more attractive depending on sequence variability number and length.



1 *Taxonomic breakdown of certain rhizobacterial community phyla in cultures of Bt-maize over a four-year period. The percentages of Proteobacteria, Actinobacteria, Bacteroidetes, Verrucomicrobia, Planctomycetes, Chloroflexi, Acidobacteria, Nitrospira, Gemmatimonadetes, Firmicutes and Cyanobacteria are indicated and do not include the unassigned sequences. Unclassified sequences were not included, as they were of no taxonomic use.*

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SELECTED PUBLICATIONS

Fonseca P, Moreno R, Rojo F. Growth of *Pseudomonas putida* at low temperature: global transcriptomic and proteomic analyses. Environ. Microbiol. Rep. 2011, 3: 329-339

Martínez JL, Rojo F. Metabolic regulation of antibiotic resistance. FEMS Microbiol Rev. 2011 Sep;35(5):768-789

Moreno R, Fonseca P, Rojo F. Two small RNAs, CrcY and CrcZ, act in concert to sequester the Crc global regulator in *Pseudomonas putida*, modulating catabolite repression. Mol Microbiol. 2012 Jan;83(1):24-40

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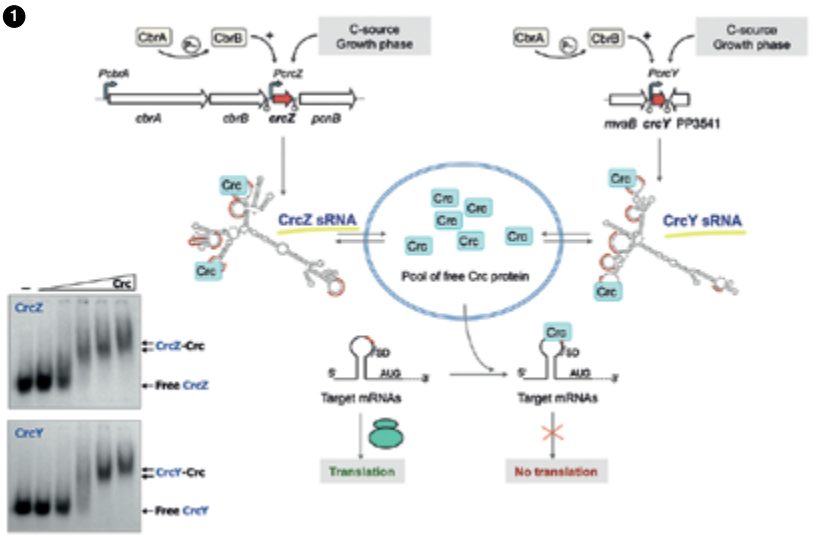
Regulation of the metabolism of hydrocarbons in bacteria

To be competitive in the environments they colonise, bacteria should optimise metabolism to attain maximum gain from the available nutrients at a 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 use one of them preferentially, leaving other non-preferred compounds aside until the preferred one is consumed. This selection implies a complex regulatory process termed catabolite repression control. Unravelling the molecular mechanisms that underlie these regulatory events helps to understand how bacteria coordinate their metabolism and their gene expression programs, to optimise growth. It also helps to design and optimise biotechnological processes and is important for understanding 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 particularly relevant example of this kind of non-preferred compounds. The influence of catabolite repression goes beyond the optimisation of metabolism, since it also affects virulence and antibiotic resistance in pathogenic bacteria.

Our aim is to characterise the global regulation networks responsible for catabolite repression, identifying their components, the signals to which they respond, and the molecular mechanisms by which they regulate gene expression. The regulatory proteins involved in these networks are different in distinct microorganisms. We use *Pseudomonas putida* as an experimental model because it has a versatile metabolism, it colonises very diverse habitats and is widely used in biotechnology. We are currently focussed on two catabolite repression networks.

One relies on the Crc protein; our work has shown that Crc binds to an unpaired A-rich sequence located at the translation initiation region of some mRNAs, thereby inhibiting their translation. Crc availability is controlled by two small RNAs, the levels of which vary sharply depending on growth conditions. The other regulatory network under study receives signals from the electron transport chain, thereby coordinating respiration with metabolic needs. Finally, we found that growth temperature influences catabolite repression. At low growth temperatures, repression is relieved in some genes, but not in others. This can be relevant for a number of biotechnological applications.



1 *The CrcZ and CrcY sRNAs control the availability of free Crc protein in P. putida. Crc inhibits the translation of many genes in response to diverse signals.*

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SELECTED PUBLICATIONS

Jiménez M, Martos A, Vicente M, Rivas G. Reconstitution and organization of *Escherichia coli* proto-ring elements (FtsZ and FtsA) inside giant unilamellar vesicles obtained from bacterial inner membranes. J Biol Chem. 2011 Apr 1;286(13):11236-41

Krupka M, Rivas G, Rico AI, Vicente M. Key role of two terminal domains in the bidirectional polymerization of FtsA protein. J Biol Chem. 2012 Mar 2;287(10):7756-65

Mateos-Gil P, Paez A, Hörger I, Rivas G, Vicente M, Tarazona P, Vélez M. Depolymerization dynamics of individual FtsZ filaments. Proc Natl Acad Sci USA. 2012 May 22;109(21):8133-8

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Karch H, Denamur E, Dobrindt U, Finlay BB, Hengge R, Johannes L, Ron EZ, Tønjum T, Sansonetti PJ, Vicente M. The enemy within us: lessons from the 2011 European *Escherichia coli* O104:H4 outbreak. EMBO Mol Med. 2012 Sep;4(9):841-8

Genetic control of the cell cycle

What do Luciano Pavarotti and Freddy Mercury have in common? The answer is that although they suffered from serious diseases, pancreatic cancer and AIDS, respectively, both ultimately died of pneumonia, an infectious disease produced by the bacterium *Streptococcus pneumoniae*. Bacterial infections are not a thing of the past, as we were sorely reminded by the 2011 *Escherichia coli* outbreak in Germany, in which over 50 deaths were registered. Another infectious disease that produces a great deal of human suffering and is a major cause of death in poor countries is tuberculosis, a consequence of *Mycobacterium tuberculosis* infection. This microbe infects one-third of the world population and is often found latent in the infected persons.

A common feature of all infections is that antibiotics, the medicines that have been used to treat them, are losing their effectiveness as microbes gradually acquire resistance to those already in use. To maintain our health, new drugs to block the proliferation of bacteria are needed urgently. To find them we study those proteins that most microbes need to multiply.

We have identified details as to how the *E. coli* protein FtsZ, also needed by *S. pneumoniae*, *M. tuberculosis* and many other pathogens, works in the test tube. We then try test new compounds predicted to block its activity, to select those that can be used as antimicrobials. We have also analysed FtsA, another protein needed for microbial proliferation, which we purified from *S. pneumoniae*. We study WhiB5, a *M. tuberculosis* protein implicated in the virulence of this pathogen and in its exit from latency. This could help to develop strategies to avoid reactivation of latent infection.

Together with a third protein called ZipA, FtsZ and FtsA assemble to form the initial machinery needed for *E. coli* multiplication. An important part of our recent and future work is to obtain knowledge that will allow test tube reconstruction of this machinery, called the proto-ring because it first assembles to form a ring at mid-cell. This synthetic biology approach will yield powerful tools to develop and test new antibiotics.

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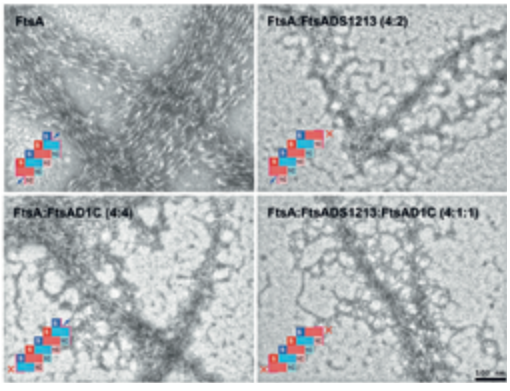
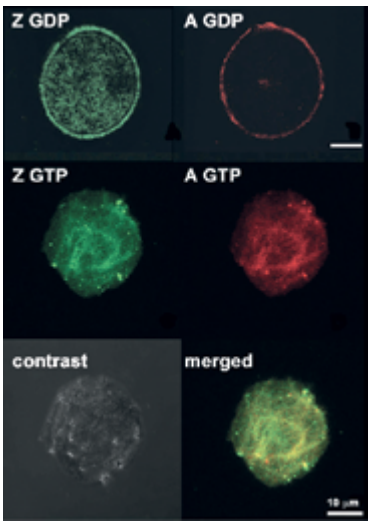


1 *Electron microscopy images of polymers formed by mixtures of streptococcal FtsA protein containing FtsA variants in different proportions*

2 *Polymerisation of FtsZ (green) triggered by GTP, detaches FtsA (red) from its location at the inner side of Escherichia coli membrane vesicles, pulling it to the vesicle lumen where both proteins subsequently colocalise*

2



05 Plant Molecular Genetics

Research is conducted by the Plant Molecular Genetics Department to uncover the signalling pathways involved in the main growth and adaptive responses of plants to environmental changes and pathogenic diseases. The department includes groups whose research focusses on the control of root architecture, shoot branching, response to light quality or duration of day length, innate immune responses to pathogens and viruses, and adaptive responses to nutrient shortage or the presence of toxic metals. Besides the intrinsic fundamental interest in understanding key biological processes in plants, our research will allow the development of new tools and methods to improve crop production and quality, selection of new varieties more resistant to pathogens or their modification to reduce fertiliser needs. Biotechnological applications such as the use of plants as biopharmaceutical factories or as tools to fight environmental problems arising from spillages and/or accumulation of toxic substances are also being studied. Research is carried out mainly in the model species *Arabidopsis thaliana* and *Nicotiana benthamiana*, but crop species such as tomato, potato and Prunus are also studied.

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SELECTED PUBLICATIONS

Sánchez-Bermejo E, Méndez-Vigo B, Picó FX, Martínez-Zapater JM, Alonso-Blanco C. Novel natural alleles at FLC and LVR loci account for enhanced vernalization responses in *Arabidopsis thaliana*. Plant Cell Environ. 2012 Sep;35(9):1672-84

Gujas B, Alonso-Blanco C, Hardtke CS. Natural *Arabidopsis* brx loss-of-function alleles confer root adaptation to acidic soil. Curr Biol. 2012 Oct 23;22(20):1962-8

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Gomaa NH, Montesinos-Navarro A, Alonso-Blanco C, Picó FX. Temporal variation in genetic diversity and effective population size of Mediterranean and subalpine *Arabidopsis thaliana* populations. Mol Ecol. 2011 Sep;20(17):3540-54

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Genetic and molecular basis of naturally-occurring variation in plant development

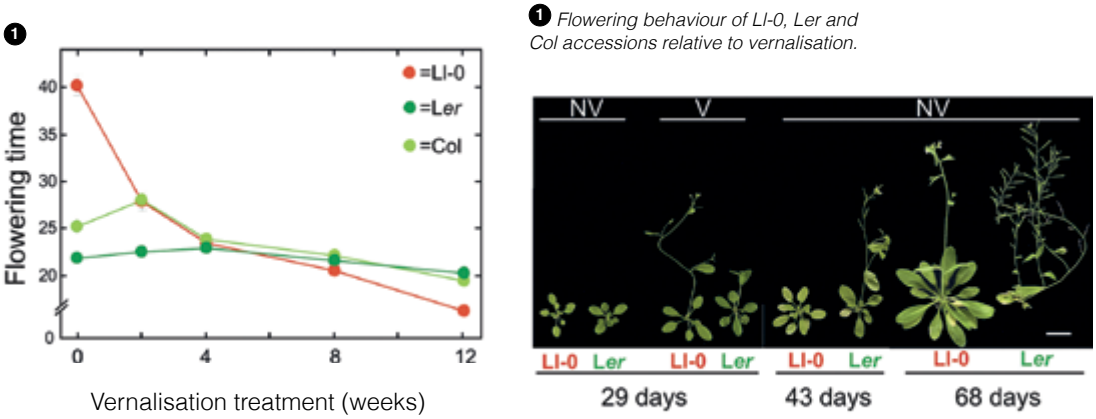
The main objective of our research is to understand the genetic and molecular mechanisms involved in plant adaptation. We are dissecting existing genetic variation in the model annual plant *Arabidopsis thaliana* in nature. Similar to many other plant species, individuals and populations of *A. thaliana* living in distinct geographical regions differ in many developmental traits that are presumed to reflect adaptations to different environments. To exploit this genetic variation for understanding plant adaptation, our group currently focusses on two specific objectives.

We are carrying out genetic analysis of naturally occurring variation for a key quantitative developmental trait, the timing of flowering. We analysed the genetic basis of the variation for flowering initiation relative to vernalisation, the induction of flowering by low winter temperatures. For these studies, we developed a new RIL population of 139 lines derived from the cross *LerxLl-0* and have grown them with different vernalisation treatments at 4°C. The study of this population by QTL (quantitative trait locus) mapping and by expression and association analyses led to

1. identification of small effect loci termed *Llagostera vernalisation response (LVR)*

2. identification of a cis-regulatory polymorphism in the *FLC* gene that might confer climatic adaptation by increasing vernalisation sensitivity (Sanchez-Bermejo *et al.*, 2012).

In addition, we developed a collection of wild *A. thaliana* genotypes from the Iberian Peninsula, which serves as a permanent experimental population for genetic and environmental association analyses. As a first step in exploiting this population, we characterised 182 Iberian genotypes for flowering behaviour and sequenced four flowering genes, *FRI, FLC, PhyC and CRY2*, involved in the vernalisation and photoperiod pathways. We found a new natural allelic series of *FRI* and *FLC* genes by association mapping. In addition, geographic and climatic association analyses showed that frequent Iberian alleles in *FLC* and *PhyC* are probably involved in climatic adaptation (Mendez-Vigo *et al.*, 2011).



1 *Flowering behaviour of Ll-0, Ler and Col accessions relative to vernalisation.*

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SELECTED PUBLICATIONS

López MA, Vicente J, Kulasekaran S, Vellosillo T, Martínez M, Irigoyen ML, Cascón T, Bannenberg G, Hamberg M, Castresana C. Antagonistic role of 9-lipoxygenase-derived oxylipins and ethylene in the control of oxidative stress, lipid peroxidation and plant defence. Plant J. 2011 Aug;67(3):447-58

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Vellosillo T, Aguilera V, Marcos R, Bartsch M, Vicente J, Cascón T, Hamberg M, Castresana C. Defense activated by 9-lipoxygenase-derived oxylipins requires specific mitochondrial proteins. Plant Physiol. 2013 Feb;161(2):617-27

Plant immunity strategies against microbial pathogen infection

Plant oxylipins are a class of lipid signalling molecules with a critical role in protecting plants against pathogen attack. Recent studies demonstrate the participation of the 9-LOX and alpha-DOX oxylipin pathways in the defence mechanisms activated by *Arabidopsis* following infection by hemibiotrophic bacteria, in which these enzymes collaborate to achieve full resistance against virulent strains. We showed that these oxylipin pathways participate in the three layers of defence –pre-invasion, apoplastic and systemic defence– triggered by plants to prevent *Pseudomonas syringae pv tomato* DC3000 infection. In these responses, oxylipins were found to act as regulators of oxidative stress, lipid peroxidation and hormone homeostasis. Our studies also showed high 9-LOX and alpha-DOX levels activity in roots of untreated *Arabidopsis* plants, suggesting that these oxylipin pathways participate in plant defence against root pathogens, a process that remains poorly understood.

Studies to characterise non-response to oxylipins *(noxy)*, a series of *Arabidopsis* mutants insensitive to the 9-LOX product 9-hydroxy-10,12,15-octadecatrienoic acid (9-HOT), demonstrated the importance of cell wall modifications as a component of 9-LOX-induced defence. We found that a majority (71%) of 41 *noxy* mutants studied had added insensitivity to isoxaben, an herbicide that inhibits cellulose synthesis and alters the cell wall. The specific mutants *noxy2, noxy15,* and *noxy38*, insensitive to both 9-HOT and isoxaben, showed enhanced susceptibility to *Pseudomonas syringae* DC3000, as well as reduced activation of salicylic acid-responding genes. Moreover, map-based cloning, fluorescence microscopy and molecular analyses of the three *noxy* mutants showed that the defence activated by 9-lipoxygenase requires specific mitochondrial proteins. Our results demonstrated that the defensive responses and cell wall modifications caused by 9-HOT are under mitochondrial retrograde control, and that mitochondria have a fundamental role in innate immunity signalling. These findings support our interest in examining 9-LOX and alpha-DOX oxylipin pathway functions in plant defence, as well as in identifying the molecular components that mediate their activity. Our studies could provide knowledge to help develop alternative strategies for disease control in crop plants, a major limiting factor that reduces agricultural productivity.

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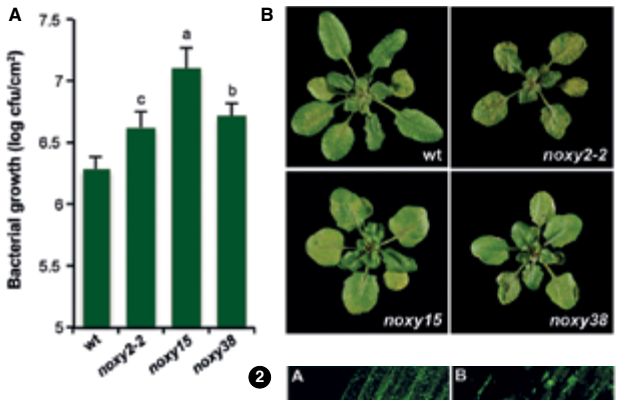

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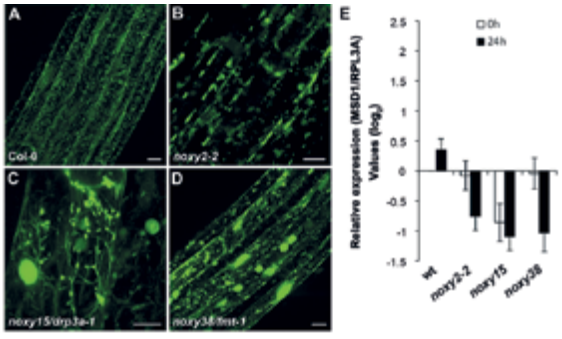

1 *The mutants noxy2, noxy15, and noxy38, showed enhanced susceptibility to Pseudomonas syringae DC3000 infection. Bacterial growth (A) and lesions (B).*

2 *Images of mitochondria (green) in roots of Col-0 (A), noxy2-2 (B), noxy15/drp3a-1 (C) and noxy38/fmt-1 (D). Expression of mitochondrial manganese superoxide dismutase (MSD1) in wild-type and noxy mutants (E).*

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SELECTED PUBLICATIONS

Preston JC, Hileman LC, Cubas P. Reduce, reuse, and recycle: Developmental evolution of trait diversification. Am J Bot. 2011 Mar;98(3):397-403

Martín-Trillo M, Grandío EG, Serra F, Marcel F, Rodríguez-Buey ML, Schmitz G, Theres K, Bendahmane A, Dopazo H, Cubas P. Role of tomato BRANCHED1-like genes in the control of shoot branching. Plant J. 2011 Aug;67(4):701-714

PATENTS

EP11166057.7, PCT/2012/058892: *SpBRANCHED1a* of *Solanum pennellii* and tomato plants with reduced branching comprising this heterologous *SpBRANCHED1a* gene

P20090008,P201030915 DIV, PCT/ES09/070538. Genes regulating plant branching, promoters, genetic constructs containing same and uses thereof

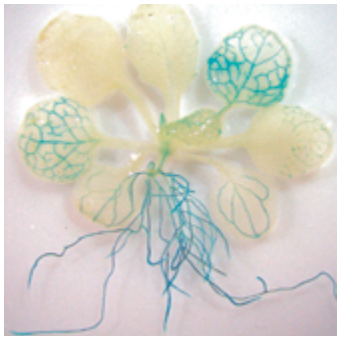
Genetic analysis of axillary meristem development

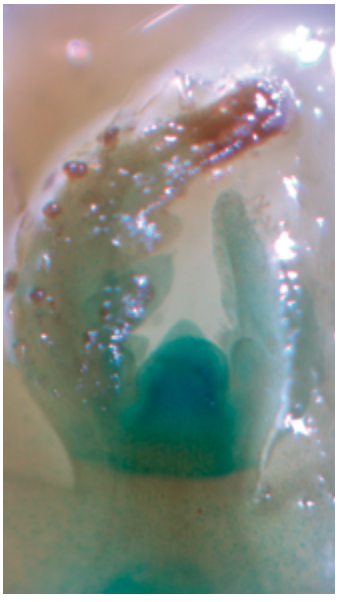
We are studying the genetic basis of the control of axillary bud development in the model system *Arabidopsis*, and in the crop species tomato and potato, in which control of lateral shoot branching is of great agronomic interest. We have characterised the *Arabidopsis BRANCHED1 (BRC1)* gene, which acts as a central switch of axillary bud development and outgrowth. We are now expanding our knowledge of the genetic networks involving *BRC1* in *Arabidopsis*.

The *Arabidopsis thaliana* gene *BRANCHED1 (BRC1)*, expressed in axillary buds, is necessary for branch suppression in response to shade. *BRC1* is negatively controlled by phyB. Transcriptional profiling of wild-type and *brc1* buds of plants treated with simulated shade revealed a group of genes whose mRNA levels are dependent on BRC1. Among them there is a set of upregulated ABA-response genes, and a network of cell cycle- and ribosome-related downregulated genes. The downregulated genes have promoters enriched in TCP binding sites, suggesting that they are transcriptionally controlled by TCP factors. We are now testing whether they are *BRC1* direct targets.

We also used two mutageneses to identify new components of the pathways that control branching and identified a number of so-called *seto* mutants (bushy mutants in shade) and *sud* mutants (*SUppressors of BRANCHED1)*; we are now in the process of cloning and analysing them.

Solanaceae is a family including a large number of species in which the control of branch outgrowth is of great agronomic interest, and for which understanding the function of some of the key players will help optimise plant architecture and yield. Our work has shown that in tomato and potato, species with branching patterns different from those of *Arabidopsis*, two *BRC1*-like paralogues (*BRC1a* and *BRC1b)* are coexpressed in axillary buds. Reverse genetic analyses confirmed that tomato *SlBRC1b* has a role in the promotion of axillary bud arrest. In contrast, *SlBRC1a*, which encodes a divergent protein with a novel C-t domain, has a still unclear role in this process. Evolution rate studies indicate that whereas *BRC1b* evolved under strong purifying selection in the clade comprising *S. lycopersicum*, *BRC1a* from other closely related wild tomato species and potato evolved at a faster rate under positive selection.





❷ *Axillary bud of a tomato transgenic plant carrying an Arabidopsis BRANCHED1 promoter fused to beta-glucuronidase (GUS). GUS expression is detected by a histochemical staining that gives a blue product in the presence of GUS.*

❶ *SETO5 gene expression in Arabidopsis visualised by a promoter:beta-glucuronidase (GUS) fusion. GUS expression is detected by histochemical staining that gives a blue product in the presence of GUS.*

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SELECTED PUBLICATIONS

Valli A, Oliveros JC, Molnar A, Baulcombe D, García JA. The specific binding to 21-nt double-stranded RNAs is crucial for the anti-silencing activity of Cucumber vein yellowing virus P1b and perturbs endogenous small RNA populations. RNA. 2011 Jun;17(6):1148-58.

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Maliogka VI, Salvador B, Carbonell A, Sáenz P, León DS, Oliveros JC, Delgadillo MO, García JA, Simón-Mateo C. Virus variants with differences in the P1 protein coexist in a Plum pox virus population and display particular host-dependent pathogenicity features. Mol Plant Pathol. 2012 Oct;13(8):877-86

Valli A, Busnadiego I, Maliogka V, Ferrero D, Castón JR, Rodríguez JF, García JA. The VP3 factor from viruses of Birnaviridae family suppresses RNA silencing by binding both long and small RNA duplexes. PLoS One. 2012;7(9):e45957

Plant-pathogen interaction in viral infections

Plant viruses depend largely on host factors to replicate within the cell and to propagate throughout the plant and between individual plants. Plants have in turn developed antiviral defence mechanisms, which must be counteracted by viral factors. These viral factors appear to be preferred targets for alternative plant defences. In our laboratory, we try to understand this complex interplay, mainly in the infection of the potyvirus Plum pox virus (PPV), the causal agent of sharka, a damaging disease of Prunus trees.

We are interested in defence responses related to RNA silencing and its viral suppressors, and especially in distinguishing common and virus-specific features of these silencing suppression proteins in potyviral infection. Our results demonstrate that although potyviruses can exploit different sources of anti-silencing activity, their own silencing suppressors can help to define the specific host range of the virus. We also showed that single amino acid changes at the N-terminal region of the capsid protein (CP) control specific PPV adaptation to *Prunus persica* and *Nicotiana* species. Our findings suggest that species-specific interactions of the CP N-terminal region with host factors are important in viral long distance movement, and that an unknown resistance mechanism interferes with these interactions in *Nicotiana* species. We are characterising the host counterparts and the molecular pathways involved.

Another main priority is to apply the information obtained in our research to designing control strategies for sharka and other viral diseases.

We are currently trying to establish principles for the design of effective, durable anti-viral resistance based on artificial miRNA, to obtain *N. benthamiana* and *P. persica* plants resistant to PPV infection. Finally, another target of interest is the development of PPV-based plant expression vectors. To broaden the range of plants susceptible to PPV-based vectors, we developed an infectious cDNA clone of a PPV isolate of strain C, the only one that infects cherry trees in nature. Biological features of this cloned isolate are at present being analysed.

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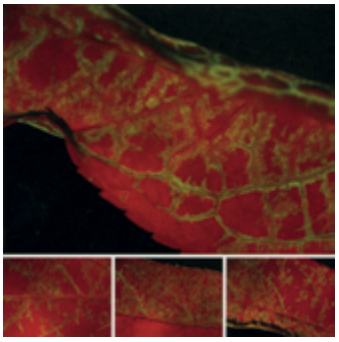

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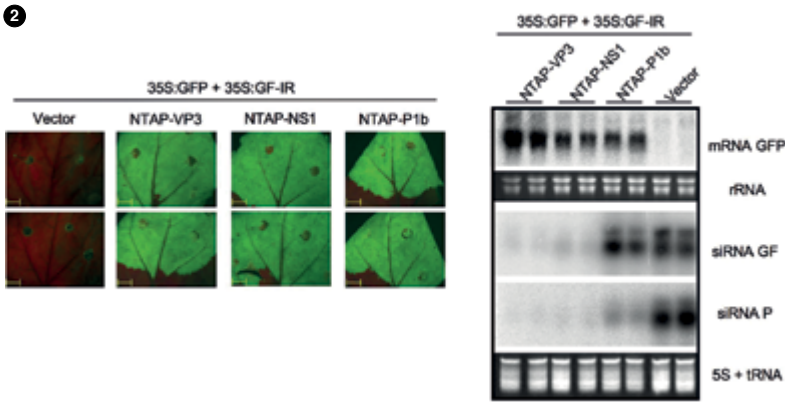

1 *Infection of GF-305 peach seedlings by a Plum pox virus (PPV)-derived virus expressing Cucumber vein yellowing virus (CVYV) P1b*

2 *Proteins from animal and plant viruses suppress RNA silencing. GFP fluorescence pictures and Northern blot analyses of GFP mRNA and GFP- derived siRNAs (right panels) in agroinfiltrated leaf patches.*

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Lanza M, Garcia-Ponce B, Castrillo G, Catarecha P, Sauer M, Rodriguez-Serrano M, Páez-García A, Sánchez-Bermejo E, T C M, Leo del Puerto Y, Sandalio LM, Paz-Ares J, Leyva A. Role of actin cytoskeleton in brassinosteroid signaling and in its integration with the auxin response in plants. *Dev Cell*. 2012 Jun 12;22(6):1275-85

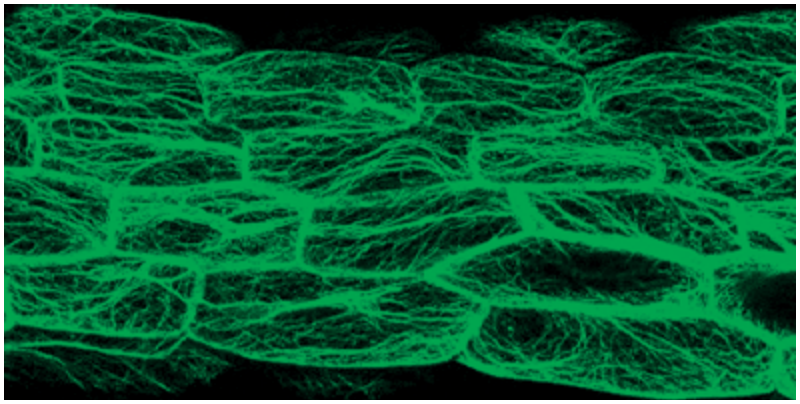
Molecular mechanisms underlying root architecture and arsenic phytoremediation

Our group has two research lines; in the first, we study the molecular mechanisms that underlie arsenic perception and in the second, we study mechanisms involved in root architecture.

For root architecture studies, we performed a mutant screening in *Arabidopsis* to identify mutants altered in the spatial distribution of roots. We identified several mutants altered in root architecture, including actin2-4, a mutant allele of the ACTIN2 gene. This mutant has increased actin dynamics, leading to enhancement of tropic responses and auxin transcriptional responsiveness, and thus mimics the auxin/brassinosteroid synergistic response.

In the context of our research on the mechanisms underlying arsenic perception, using a transgenic plant that expresses the phosphate (Pi) transporter promoter fused to the luciferase gene, we identified a set of genes inducible by Pi starvation that are repressed by arsenate (As(V)) with extraordinary speed (30 min), while it takes 36 hours to be repressed by Pi.

The use of the transgenic line PHT1;1:LUC allows identification of mutants with kinetic altered by As(V). This is a new approach to the characterisation of the As(V) signalling pathway and its possible crosstalk with that of Pi. In the last year, we identified and characterised several transcription factors involved in arsenic perception. Moreover, we analysed the natural variability of As(V) tolerance in a large collection of *Arabidopsis* ecotypes; we identified a quantitative trait locus that we are currently cloning.



1 *Confocal analysis of actin filament configuration in 5-day-old seedlings of Col-0 Arabidopsis hypocotyls grown on vertical MS plates.*

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SELECTED PUBLICATIONS

Fernández P, Chini A, Fernández-Barbero G, Chico JM, Gimenez-Ibanez S, Geerinck J, Schweizer F, Godoy M, Franco-Zorrilla JM, Pawels L, Puga MI, Paz-Ares J, Goosens A, Reymond P, de Jaeger G, Solano R. (2011) The bHLH transcription factors MYC3 and MYC4 are targets of JAZ repressors and act additively with MYC2 in the activation of JA responses. *Plant Cell*. 23, 701-715.

Bayle V, Arrighi JF, Creff A, Nespoulous C, Vialaret J, Rossignol M, Gonzalez E, Paz-Ares J, Nussaume L. (2011) *Arabidopsis thaliana* high-affinity phosphate transporters exhibit multiple levels of post-translational regulation. *Plant Cell*, 23, 701.

Castrillo G, Turck F, Leveugle M, Lecharny A, Carbonero P, Coupland G, Paz-Ares J, Oñate-Sánchez L. (2011) Speeding cis-trans regulation discovery by phylogenomic analyses coupled with screenings of an arrayed library of *Arabidopsis* transcription factors. PLoS One. 6:e21524.

Lanza M, Garcia-Ponce B, Castrillo G, Catarecha P, Sauer M, Rodriguez-Serrano M, Páez-García A, Sánchez-Bermejo E, Tc M, Leo Del Puerto Y, Sandalio LM, Paz-Ares J, Leyva A. (2012) Role of actin cytoskeleton in brassinosteroid signaling and in its integration with the auxin response in plants. *Dev Cell*. 22, 1275-1285.

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

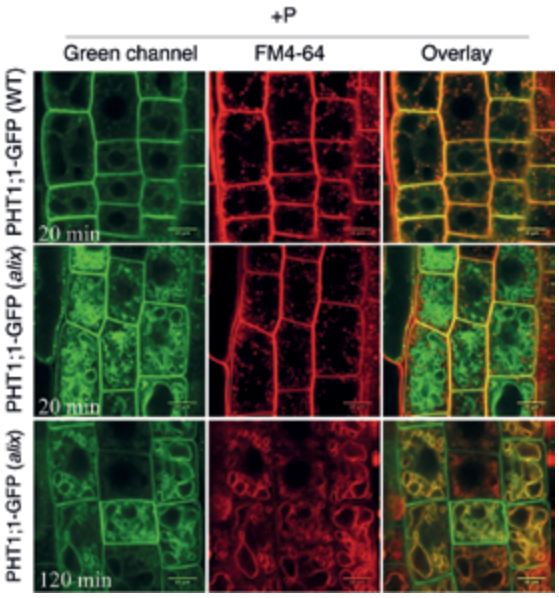
Our research is focussed on the phosphate (Pi) starvation rescue system of plants, a model system for studies of gene activity, and with presumed biological potential in the context of low input agriculture. In previous years, we identified the transcription factor PHR1 as a master regulator of Pi starvation responses in plants. In the last two years, we advanced our knowledge of additional transcription factors and other regulators of this response and performed a genome-wide analysis of PHR1 targets *in vivo*:

1. Additional transcription factors that affect Pi starvation responses were sought in a library of transgenic plants conditionally expressing 1000 different transcription factors; these were generated in the context of the TRANPLANTA Project (CONSOLIDER INGENIO program), which we coordinated. We initiated the ionomic analysis of these lines by ICP-OES and have now identified four transcription factors whose overexpression results in altered Pi content.

2. Additional regulators of Pi starvation responses were identified from a suppressor screen of the *phr1* mutant, leading to the isolation of AtALIX, a scaffold protein of the endomembrane system. Cell biological analysis of AtALIX showed that it alters the recycling of Pi transporters.

3. Genome-wide analysis of PHR1 targets. Using RNA-seq, we identified approximately 2400 targets of PHR1. Analysis of the bound region showed that PHR1 binds its targets via two sites (PHR1 binding site I and II; P1BSI and P1BSII), which are bound by PHR1 using two different modes, dimer and monomer. P1BSI- and P1BSII-containing targets show differential enrichment in ontology classes and in P1BS-unrelated motifs. This study allowed us to conclude that PHR1-based control uses a dual regulatory logic and that the underlying P1BSI and P1BSII motifs have different evolutionary constraints.

1



1 *AtALIX regulates Pi transporter recycling. Root epidermal cells of 5-day-old seedlings of wild type (WT) and Atalix (alix) mutants overexpressing PHT1;1-GFP grown in Pi-rich conditions were observed by confocal laser scanning. Seedlings were treated with 2 μM FM4-64 for 5 minutes, and observations made after 20 and 120 minutes. PHT1;1-GFP in WT is located in plasma membrane and in sorting endosomes, as shown by PHT1;1-GFP and FM4-64 colocalisation in both compartments after 20 minutes. PHT1;1-GFP in the Atalix background is also found in tonoplasts, as shown by colocalisation of PHT1;1-GFP fluorescence with FM4-64 after 120 minutes, indicating that AtALIX function is needed for correct Pi transporter recycling.*

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SELECTED PUBLICATIONS

Kloosterman B, Abelenda JA, Gomez M del M, Oortwijn M, de Boer JM, Kowitwanich K, Horvath BM, van Eck HJ, Smaczniak C, Prat S, Visser RG, Bachem CW. (2013) Naturally occurring allele diversity allows potato cultivation in northern latitudes. Nature. 495(7440):246-50

Soy J, Leivar P, González-Schain N, Sentandreu M, Prat S, Quail PH, Monte E. (2012) Phytochrome-imposed oscillations in PIF3 protein abundance regulate hypocotyl growth under diurnal light/dark conditions in *Arabidopsis*. Plant J. 71(3):390-401.

Bou-Torrent J, Martínez-García JF, García-Martínez JL, Prat S. (2011) Gibberellin A1 metabolism contributes to the control of photoperiod-mediated tuberization in potato. (2011) PLoS One. 6(9):e24458.

Navarro C, Abelenda JA, Cruz-Oró E, Cuéllar CA, Tamaki S, Silva J, Shimamoto K, Prat S. (2011) Control of flowering and storage organ formation in potato by FLOWERING LOCUS T. Nature. 478(7367):119-22.

Abelenda JA, Navarro C, Prat S. (2011) From the model to the crop: genes controlling tuber formation in potato. Curr Opin Biotechnol. 22(2):287-92.

PATENT

PCT/ES2010/070265. Genes homologous to the FLOWERING LOCUS T (FT) gene and the use thereof for modulating tuberization. Extended to USA, EU, Brazil, India and Australia.

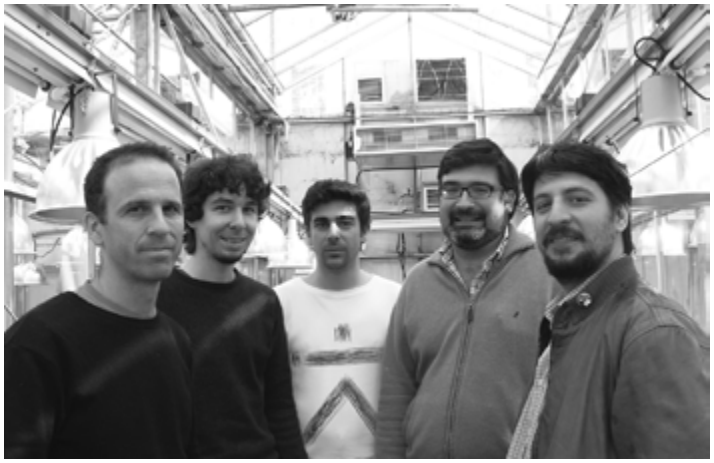
Hormonal control of light signalling

By perceiving changes in the quality of incident light or oscillations in diurnal light hours, plants are able to sense the surrounding environment and recognise year season progression. Signalling pathways implicated in response to shade proved to be closely related to those that control seedling de-etiolation; the phytochrome-interacting factor (PIF) family of bHLH factors has an important function in these responses. These factors accumulate in the nucleus in the dark or in FR spectrum-enriched light, but in red light they are rapidly destabilised by the light receptor phytochrome. PIF-regulated expression, on the other hand, is tightly regulated by the endogenous developmental programmes, which allows plants to adjust growth and architecture to variable light conditions and preclude excessive elongation. The gibberellin (GA) and brassinosteroid (BR) hormones are essential in regulating this crosstalk.

In previous studies, we showed that the DELLA repressors, which are central to GA signalling, repress cell elongation by sequestering PIF in an inactive complex unable to bind DNA. We also provided evidence that BR signalling is necessary for GA-induced growth, and that GA and BR pathways interact downstream of DELLAs. We uncovered a role of BRs in promoting PIF4 accumulation by inhibiting phosphorylation of this factor by the GSK3 kinase BIN2, with a negative role in BR signalling. Our results demonstrate that PIF4 and the BR signalling factors BES1/BZR1 are obligate partners for activation of cell elongation genes, the PIF/BES1/ BZR1 activation complex thus providing a robust mechanism for cell elongation control in response to light, GA, and BR.

In studies of the photoperiodic control of potato storage organ formation, we have shown that a conserved CONSTANS-FT module mediates SD-dependent tuberisation. Potato CONSTANS modulates expression of a FT homologue (SP5G), which acts as a tuberisation repressor by inhibiting activation of the *SP6A* gene or mobile tuberisation signal. Elevated temperatures, which inhibit tuber formation, block *SP6A* activation, and overexpression of this gene preserves high tuber yields in heat stress conditions. These transgenic lines are suitable for cultivation in tropical regions.

1 *Increased yield in tubers of potato FT plants under heat-stress conditions*



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SELECTED PUBLICATIONS

Sanmartín, M. Sauer, M., Muñoz, A., Rojo E. (2012). MINIYO and transcriptional elongation: lifting the roadblock to differentiation. Transcription 3: 1-4.

Sanmartín, M. Sauer, M., Muñoz, A., Jan Zouhar, J., Ordóñez, A., van de Ven, W. Caro, E., Sánchez, M., Raikhel N.V., Gutiérrez, C., Sánchez-Serrano, J.J., Rojo E. (2011). A molecular switch for initiating cell differentiation in *Arabidopsis*. Curr. Biol. 21, 999-1008.

Rosado, A., Hicks, G.R., Norambuena, L., Rogachev, I., Meir, S., Pourcel, L., Zouhar, J., Brown, M.Q., Boirsdore, M.P., Puckrin, R.S., Cutler, S., Rojo, E., Aharoni, A., Raikhel, N.V. (2011). Sortin1-hypersensitive mutants link vacuolar-trafficking defects and flavonoid metabolism in *Arabidopsis* vegetative tissues. Chemistry & Biology 18, 187-197.

PATENT

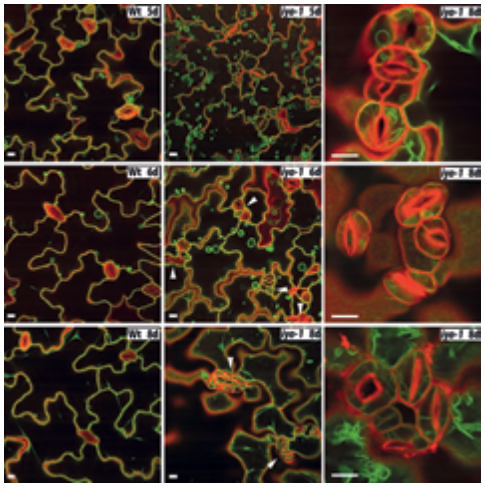
PCT/EP12/059312 Procedure to modify plant architecture and improve the crops yield through the control of entering into cell differentiation

Intracellular trafficking in plants

The subcellular localisation of proteins is a key parameter in defining their function. We are interested in studying how proteins are targeted and transported to their cellular destinations in plants, focusing on trafficking processes that are of particular relevance to these organisms.

A conspicuous peculiarity of the plant endomembrane system is the presence of very large vacuoles, which in most cells occupy the majority of the cell volume. The enlarged vacuoles of plants are necessary adaptations to autotrophy and immobility, as they provide an energy-cheap organ expansion mechanism for exploring the surroundings and a buffering organelle to maintain cell homeostasis. In addition, plants can have different types of vacuoles in a single cell. They develop specialised vacuoles in certain cells, such as the protein storage vacuoles in seed tissues, which constitute the main source of proteins for human and livestock nutrition. The molecular machinery responsible for the delivery of proteins and membranes to vacuoles is nonetheless largely uncharacterised. To define the mechanisms of vacuolar trafficking in plants, we designed a genetic screen in *Arabidopsis* for mutants with impaired vacuolar transport. In this way, we identified and characterised several factors involved in trafficking to the vacuole, including sorting receptors, SNARE and SM proteins, and phospholipid-modifying enzymes. We recently characterised an ENTH protein and an ARF GAP protein that act as key effectors for clathrin-coated vesicle-mediated trafficking of vacuolar proteins from the trans Golgi network to the late endosome in plants.

We also initiated a new line of research to study the mechanisms of nuclear-cytoplasmic partitioning of the IYO/ART protein complex. This dual localisation functions as a binary molecular switch that initiates cell differentiation when the IYO/ART complex is translocated to the nucleus. We are also studying the molecular activities of the IYO/ART complex and the structural basis of these activities. Our results suggest that, in the nucleus, the IYO/ART complex interacts with RNA polymerase II and activates productive transcriptional elongation of developmental regulators, triggering differentiation.



1 *Epidermal development in wild type (Wt) and iyo-1 cotyledons. Protoderm differentiation is delayed in iyo-1 plants and as a result, stomatal patterning is disrupted. The plants express a tonoplast GFP marker (green) and are stained with propidium iodide (red).*

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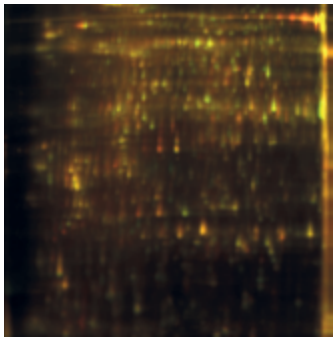
SELECTED PUBLICATIONS

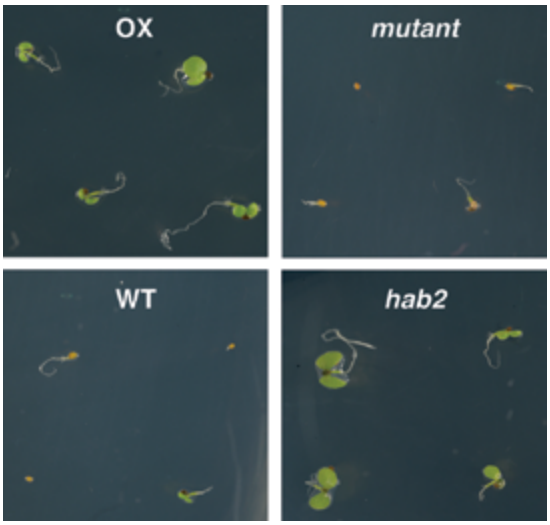
Rojas-Triana M, Bustos R, Espinosa-Ruiz A, Prat S, Paz-Ares J, Rubio V. Roles of ubiquitination in the control of phosphate starvation responses in plants. J Integr Plant Biol. 2013 Jan;55(1):40-53

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

Ubiquitin conjugation to proteins is mediated by an enzymatic cascade in which E3 ubiquitin ligase enzymes provide substrate specificity. Proteins labelled with ubiquitin chains usually undergo degradation via the proteasome, although ubiquitination can also modify protein function by altering their subcellular localisation, assembly into complexes or enzyme activity. The potential regulatory relevance of the ubiquitin pathway in plants can be understood by the fact that proteins in the ubiquitination machinery comprise 5% of the *Arabidopsis* proteome. Protein ubiquitination affects many key aspects of plant biology, including phytohormone synthesis and signalling, floral organ formation and transition, defence against pathogens, and adaptive responses to numerous abiotic stresses. In accordance with this regulatory potential, the number of E3 ubiquitin ligases and targets reported in the control of specific plant biological processes is rapidly increasing. This trend parallels that observed in other eukaryotic organisms in which ubiquitination is conserved. Our knowledge of the molecular mechanisms that regulate ubiquitination machinery function and allow a coordinated response to environmental stimuli and stress conditions remains limited, especially in plant systems. Several mechanisms that control E3 activity have nonetheless been reported, many of them conserved in eukaryotes, such as control of the assembly of Cullin-RING E3 ligases by neddylation/deneddylation cycles or regulation of the subcellular location of E3 components such as DDB1. The latter forms part of protein complexes involved in chromatin signalling and DNA damage repair, including several CUL4-RING ubiquitin ligases and ubiquitination-associated complexes.

The aim of our research is to characterise the molecular mechanisms that regulate the ubiquitination machinery in the control of plant development and stress responses associated with climate change events such as drought, high temperatures, salinity and UV radiation, using genetic, molecular and proteomic approaches. Regulatory proteins or their mutated versions identified by these approaches should help to develop modified crops with increased resistance to environmental stress.





1 *Genetic, molecular and proteomic approaches are used in our laboratory to characterise the function of specific components of the ubiquitin-proteasome system in the control of plant growth and stress tolerance.*

2 *Arabidopsis plants with gain (OX) or loss of function (mutant) for specific CUL4-RING ubiquitin ligases have opposite phenotypes for tolerance to salinity (100 mM NaCl in growth media) during seedling establishment. Wild type (WT) plants and tolerant mutants to salinity (hab2) are used as controls.*

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SELECTED PUBLICATIONS

Sanmartín, M., Sauer, M., Muñoz, A., Zouhar, J., Ordóñez, A., van de Ven, WTG., Caro, E., Sánchez, MP., Raikhel, NV., Gutiérrez, C., Sánchez-Serrano, JJ., and Rojo, E. (2011) A molecular switch for initiating cell differentiation in *Arabidopsis*. Current Biology 21, 999-1008

Sanmartín M, Sauer M, Muñoz A, Rojo E. (2012) MINIYO and transcriptional elongation: lifting the roadblock to differentiation. Transcription 3, 25-28.

PATENT

PCT/EP12/059312. Procedure to modify plant architecture and improve the crops yield through the control of entering into cell differentiation.
M. Sanmartín, Jose J. Sanchez Serrano and E. Rojo

Signalling networks in plant development and defence responses

Plants adjust their development to accommodate cell differentiation and growth to fluctuating, often detrimental environmental conditions. Intricate signalling networks translate environmental cues into reprogramming of gene expression as a major adaptive response to biotic and abiotic stresses.

Reversible protein phosphorylation is a common mechanism in transduction pathways that connect external and/or internal signals to a given cell response. A phosphorylation switch consists of i) a target protein whose intrinsic characteristics are altered by its phosphorylation status, ii) a protein kinase, and iii) a protein phosphatase, which respectively phosphorylate/dephosphorylate their target in specific circumstances. Protein phosphatases of the subgroup 2A (PP2A) dephosphorylate proteins at serine/threonine residues. PP2A is a heterotrimer that consists of a catalytic subunit (PP2A-C) whose specific activity is regulated by the binding of A (PP2A-A) and B (PP2A-B) regulatory subunits. The *Arabidopsis* genome encodes five very similar PP2A-C, grouped in subfamily 1 (*PP2A-C1, -C2*, and *-C5*) and subfamily 2 (*PP2A-C3* and -C4). The level of amino acid identity within the two subfamilies suggests redundancy in their roles. Single *pp2ac-3* and *pp2ac-4* mutant lines do not show any obvious phenotype, suggesting they indeed fulfil largely redundant functions. *pp2ac-3 pp2ac-4* plantlets are severely misshapen, however, with strong cotyledon and root primordia malformations. Our results indicate that *PP2A-C3* and *PP2A-C4* have redundant functions in controlling embryo patterning and root development, processes that depend on auxin fluxes. Moreover, polarity of the auxin efflux carrier PIN1 and auxin distribution are affected by mutations in *PP2A-C3* and *PP2A-C4*. Our work indicates functional specialisation of subfamily 2 in the regulation of PIN protein polarity and hence, of auxin fluxes and plant patterning.

Following attack by pests and/or pathogens, plants turn on inducible defence responses. A major wound-signalling pathway involves the plant hormone jasmonic acid (JA). Large, transient increases in endogenous levels of JA after mechanical damage trigger the transcriptional activation of an array of defence genes. In potato, two genes (*StAOS1* and *StAOS2*) encode the putative 13-AOS, which catalyses the first committed step in JA biosynthesis. We showed that simultaneous cosuppression of *StAOS1* and *StAOS2* is necessary to lower the JA content of the plant significantly, resulting in altered responses to wounding and increased susceptibility to plant pathogens.

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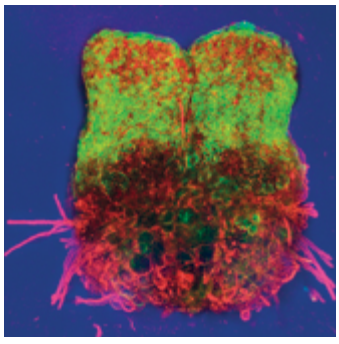

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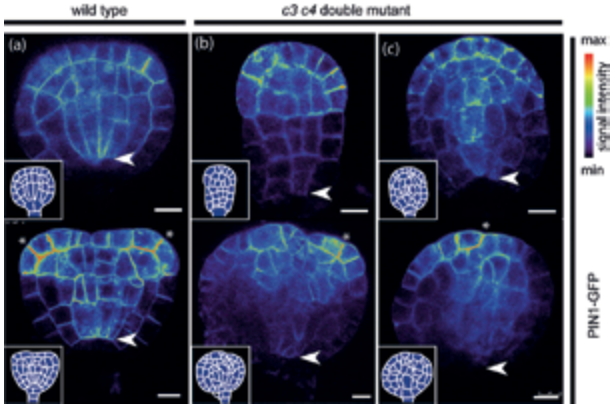
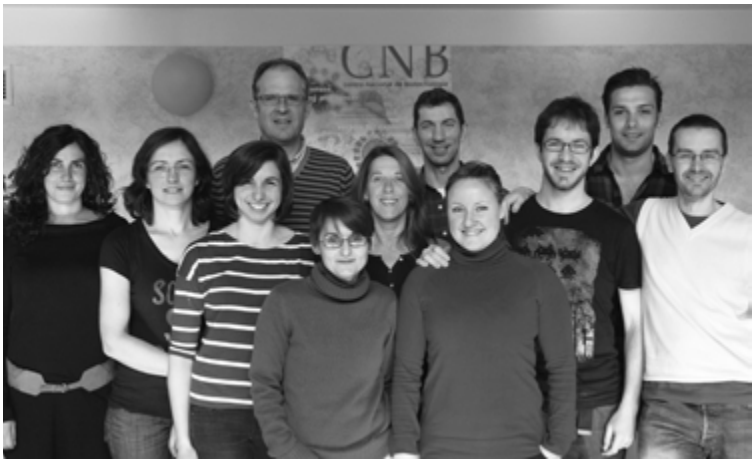


1 *DR5pro:GFP fluorescence in c3c4 seedlings. Confocal microscopy image of a c3c4 seedling expressing the auxin reporter DR5pro:GFP. GFP fluorescence (green) essentially concentrates at the cotyledons (upper side) while propidium iodide staining and chlorophyll fluorescence (red) are mostly visualised in the hypocotyl and the defective root region (lower side).*

2 *PIN1-GFP in wild type and c3c4 embryos. PIN1-GFP fluorescence i n wild type (a) and c3c4 double mutant embryos (b and c). Early embryonic stages on top, later stages at the bottom. Arrowheads indicate the border between embryo proper and the incipient root pole, asterisks mark PIN1-GFP accumulation in the protodermis at incipient cotyledon primordia. False colour coded with red for maximum, dark blue for minimum signal intensity. All bars are 10 µm.*



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SELECTED PUBLICATIONS

Godoy M, Franco-Zorrilla JM, Pérez-Pérez J, Oliveros JC, Lorenzo O, Solano R. Improved protein-binding microarrays for the identification of DNA-binding specificities of transcription factors. Plant J. 2011 May;66(4):700-11

Fernández-Calvo P, Chini A, Fernández-Barbero G, Chico JM, Gimenez-Ibanez S, Geerinck J, Eeckhout D, Schweizer F, Godoy M, Franco-Zorrilla JM, Pauwels L, Witters E, Puga MI, Paz-Ares J, Goossens A, Reymond P, De Jaeger G, Solano R. The *Arabidopsis* bHLH transcription factors MYC3 and MYC4 are targets of JAZ repressors and act additively with MYC2 in the activation of jasmonate responses. Plant Cell. 2011 Feb;23(2):701-15

Hornitschek P, Kohnen MV, Lorrain S, Rougemont J, Ljung K, López-Vidriero I, Franco-Zorrilla JM, Solano R, Trevisan M, Pradervand S, Xenarios I, Fankhauser C. Phytochrome interacting factors 4 and 5 control seedling growth in changing light conditions by directly controlling auxin signaling. Plant J. 2012 Sep;71(5):699-711

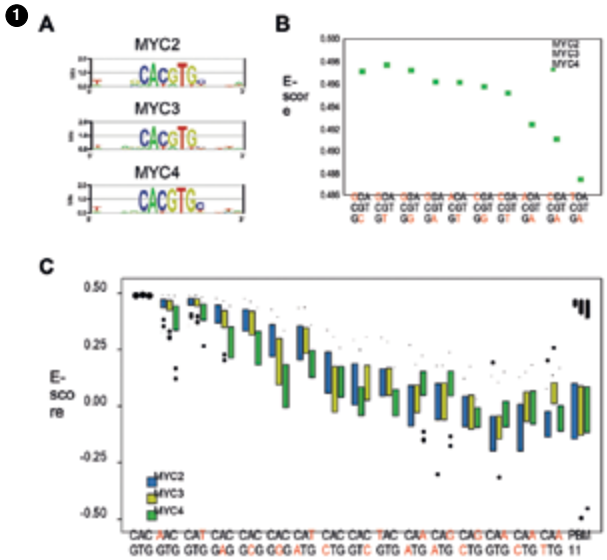
Llave C, Franco-Zorrilla JM, Solano R, Barajas D. Target validation of plant microRNAs. Methods Mol Biol. 2011;732:187-208

The jasmonate signalling pathway in Arabidopsis

We are interested in understanding how plants perceive changes in their environment and integrate stress signals with their internal developmental programs to induce adaptive responses and survive in nature. This integration depends on complex signalling networks that regulate the genetic reprogramming of the cell. The main focus of my lab is the jasmonate (JA) signalling pathway in *Arabidopsis thaliana*. JA are fatty acid-derived signalling molecules essential for plant survival in nature, since they are important activators of stress responses and developmental programmes. We aim to identify the components of this pathway and understand how these components explain the molecular interactions of the jasmonate pathway with other pathways within the network. Understanding these molecular interactions is essential to decipher how a single hormone can activate so many physiological responses in the plant, and how the plant is able to discriminate between different stress types (*e.g.,* pathogens and wounding) and select the correct set of responses to each.

We are using genomic, genetic, biochemical and molecular tools to dissect the JA signalling pathway and characterise its components. The major achievements of our group so far are:

- Identification of the first transcription factors (TF) that regulate JA responses (MYC2, MYC3, MYC4 and ERF1; Fernández Calvo *et al.*, 2011). They are essential for understanding the specificity of plant responses to the hormone.
- Discovery of the JAZ family of nuclear repressors of these TF. The discovery of the JAZ family of repressors linked the previous steps in the pathway (SCFCOI1 and the TF) and facilitated an integrated view of the core JA signalling module composed by SCFCOI1-JAZs-MYC2.
- Discovery of the active form of the hormone. Since its discovery about four decades ago, jasmonic acid was assumed to be the bioactive hormone. A combination of genetic and biochemical analyses allowed us to demonstrate that the real bioactive form of the hormone is (+)-7-iso-jasmonoyl-isoleucine.
- Discovery of SGT1b/JAI4, a regulator of SCF (Skip-Cullin-Fbox) E3 ubiquitin ligase complexes, including the JA-signalling component SCFCOI.
- Identification of the mechanism of TF repression by JAZ proteins (recruitment of the general co-repressor TOPLESS by the adaptor protein NINJA; Pauwels *et al.*, 2010).



1 *Identification of MYC2, MYC3 and MYC4 DNA-binding motifs in vitro. (A) Position weight matrix representation of the top scoring 8-mers corresponding to MYC2, MYC3 and MYC4. All three proteins showed highest binding affinity to a canonical G-box (CACGTG). (B) Enrichment scores (E-scores) of all the possible G-box-containing 8mers for the three MYC proteins tested, showing similar binding preferences of the three proteins for nucleotides at 5´ . (C) Box-plot of E-scores of G-box variants including both single-site mutations and E-boxes (CANNTG). Boxes represent quartiles 25% to 75%, and black line within represents the median of the distribution (quartile 50%). Bars indicate quartiles 1 to 25% (above) and 75 to 100% (below), and dots denote outliers of the distribution. Boxes in blue correspond to MYC2, green boxes represent MYC3 and yellow ones correspond to data from MYC4.*

06 Systems Biology

Systems Biology is a conceptual framework for studying living systems that departs from the reductionism of molecular biology; it pursues the quantitative understanding of complete biological entities rather than the mere comprehension of their parts. Needless to say, the problem at stake is complexity.

This term asks for an agenda of quantitative study of complex interactions in biological systems using an integrative perspective. One of the key goals of Systems Biology is to reveal the properties embodied in the inner organisation of complete biological objects. To this end, any Systems Biology approach is organised in three stages: description of the system, deconstruction of the system into its components and reconstruction of the system with the same or with other properties. Note that the term deconstruction has two meanings, both incorporated into the Systems Biology jargon. First, it means the stepwise dismantlement of the components of an object for reuse, recycling, or management. But deconstruction also denotes the uncovering of an implicit or hidden significance in a text or in an object, which is not apparent from a superficial description.

Finally, reconstruction of a system is the ultimate proof of understanding, echoing the celebrated remark by the 1965 Nobel laureate in Physics, Richard Feynman "…what I cannot create, I do not understand". Synthetic Biology takes this last aspect further, to the point of proposing the design of non-natural biological systems following a rational blueprint, which result in properties *á la carte* for both fundamental and biotechnological applications.

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SELECTED PUBLICATIONS

de Las Heras A, Fraile S, de Lorenzo V. Increasing signal specificity of the TOL network of *Pseudomonas putida* mt-2 by rewiring the connectivity of the master regulator XylR. PLoS Genet. 2012;8(10):e1002963

Valls M, Silva-Rocha R, Cases I, Muñoz A, de Lorenzo V. Functional analysis of the integration host factor site of the σ54 Pu promoter of *Pseudomonas putida* by *in vivo* UV imprinting. Mol Microbiol. 2011 Nov;82(3):591-601

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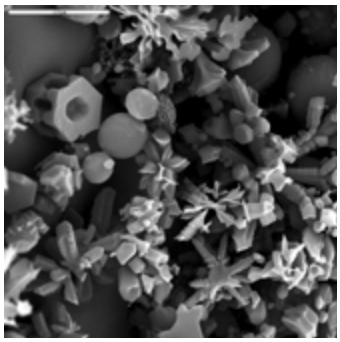
Chavarría M, Santiago C, Platero R, Krell T, Casasnovas JM, de Lorenzo V. Fructose 1-phosphate is the preferred effector of the metabolic regulator Cra of *Pseudomonas putida*. J Biol Chem. 2011 Mar 18;286(11):9351-9

Molecular environmental microbiology

Our research efforts attempt to understand how bacteria that inhabit natural niches sense and process multiple environmental signals into distinct transcriptional and post-transcriptional responses –both at the level of single cells and as a community. Unlike laboratory settings, in which growth conditions can be controlled and changed one at a time, bacteria in the environment must perpetually make gene expression decisions between activating metabolic genes for available, frequently mixed carbon sources, and those for escaping or adapting to physico-chemical stress. Our preferred experimental system involves the KT2440 strain of the soil and plant root coloniser *Pseudomonas putida* bearing the plasmid pWW0, which allows growth on toluene and m-xylene as the only C and energy source. The biotechnological side of this biological question is the possibility of programming bacteria for deliberate environmental release, aimed at biodegradation of toxic pollutants or as biosensors to monitor the presence of given chemicals. Apart from understanding and developing such sensor or catalytic bacteria, their release requires the GMO to be endowed with a high degree of containment and predictability. Our research is committed to developing novel molecular tools for the genetic analysis and construction of soil microorganisms destined for the environment or as catalysts for selected biotransformations. We have increasingly adopted conceptual and material tools that stem from contemporary systems and synthetic biology to address outstanding environmental pollution problems. During the last two years, we have made considerable progress toward establishing rules for the physical assembly of biological devices to be implanted in bacteria that are then deployed in environmental and industrial settings. The use of standard descriptive languages and genetic tools has enabled us to model regulatory networks of *P. putida* as sets of digital, Boolean logic gates similar to those employed in electric engineering. These models reveal the inner *raison d'être* of extant network architectures. In a subsequent step, we were able to redesign such logic circuits to increase their sensitivity and specificity to a suite of chemical effectors, for example, to improve the performance of biosensors. These developments pave the way toward engineering complex circuits that program bacteria to display new-to-nature properties.

1



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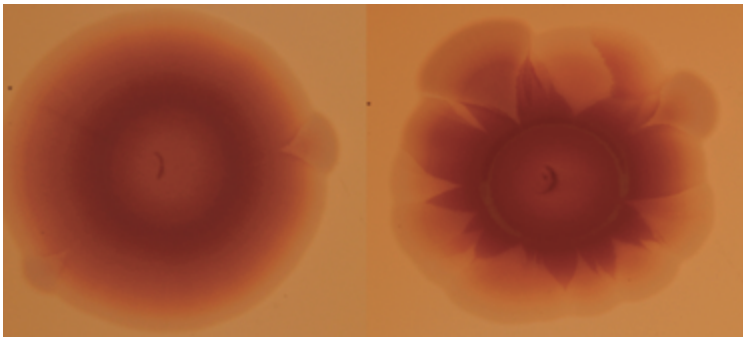
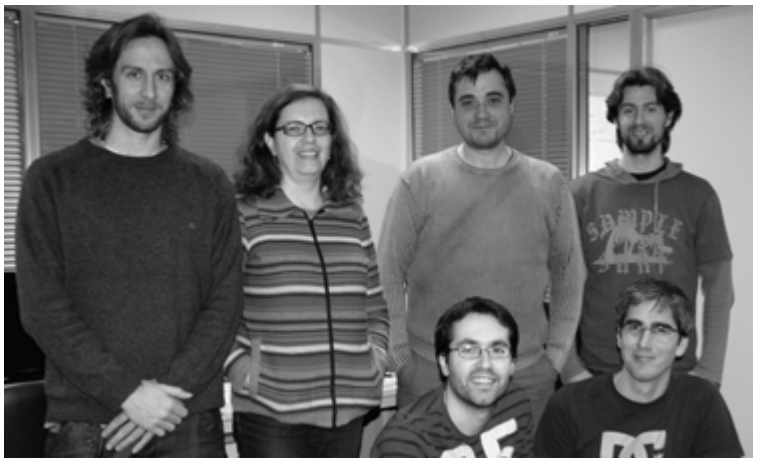


1 *Electron microscopy of frozen culture media with P. putida*

2 *Production of external polymeric substances by P. putida (wt, left) lacking the flagella (right)*



GROUP LEADER:
Florencio Pazos Cabaleiro

SENIOR SCIENTIST:
Mónica Chagoyen Quiles

PHD STUDENTS:
Daniel López López
David Ochoa García
Natalia Pietrosemol

TECHNICIAN:
Juan Carlos Sánchez Ferrero

VISITING SCIENTIST:
David San León Granado

SELECTED PUBLICATIONS

Muth T, García-Martín JA, Rausell A, Juan D, Valencia A, Pazos F. *JDet*: interactive calculation and visualization of function-related conservation patterns in multiple sequence alignments and structures. Bioinformatics. 2012 Feb 15;28(4):584-6

Chagoyen M, Pazos F. Tools for the functional interpretation of metabolomic experiments. Brief Bioinform. 2012 Oct 14

Herman D, Ochoa D, Juan D, Lopez D, Valencia A, Pazos F. Selection of organisms for the co-evolution-based study of protein interactions. BMC Bioinformatics. 2011 Sep 12;12:363

Wass MN, Fuentes G, Pons C, Pazos F, Valencia A. Towards the prediction of protein interaction partners using physical docking. Mol Syst Biol. 2011 Feb 15;7:469

Chagoyen M, Pazos F. MBRole: enrichment analysis of metabolomic data. Bioinformatics. 2011 Mar 1;27(5):730-1

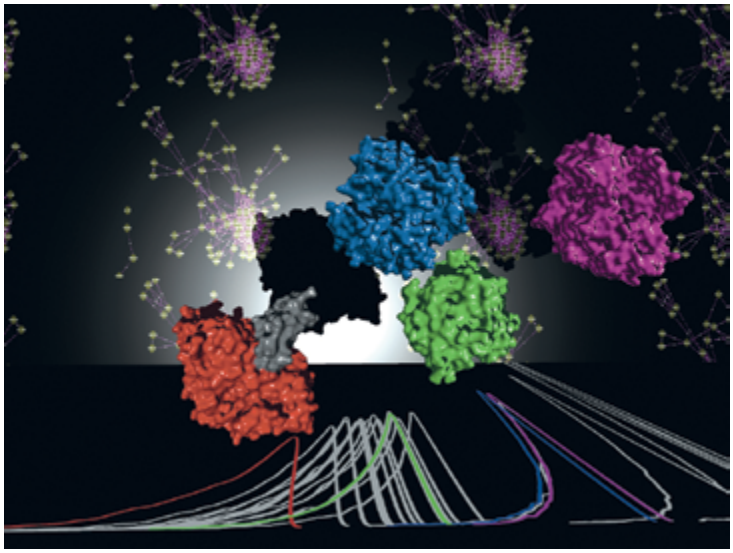
Computational systems biology

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 that complements *in vivo* and *in vitro* methodologies of biology. In addition to our lines of research, we collaborate with experimental groups by providing bioinformatics support for their specific needs, and participate in several teaching projects.

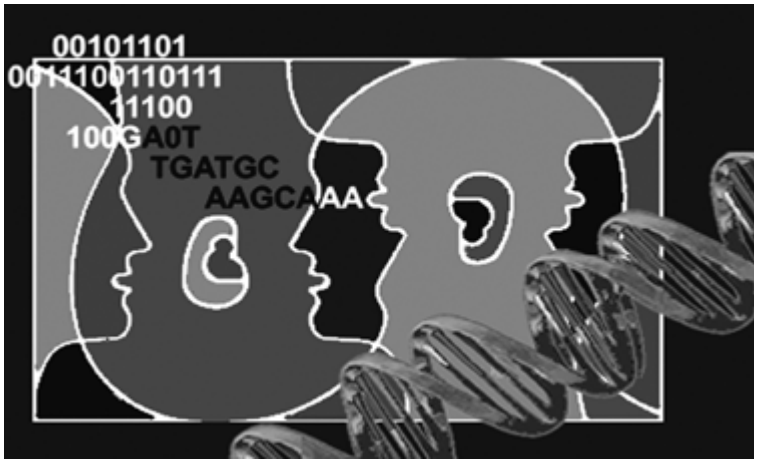
During the last two years, we have continued to develop an evolution-based method for predicting sites with functional importance in protein sequences and structures. We have developed the *JDet* package, which allows non-expert users to access those methods through an interactive graphic interface.

We also continued to develop evolution-based methods for predicting interaction partners. These methods are based mainly on the hypothesis that interacting or functionally related proteins adapt to each other during the evolutionary process (co-evolution). This can be detected as greater similarity than would be predicted between phylogenetic trees. A recent contribution was our exhaustive study on the performance of these approaches; we showed the effect of the set of organisms used for building these phylogenetic trees (Herman *et al.,* 2011). We also performed a pioneering study showing that physical docking programs, designed to predict the arrangement of two protein structures known to interact, can be also used to detect interaction partners (Wass *et al.,* 2011).

The study of living systems from a network perspective provides new biological knowledge that cannot be obtained by studying individual components (genes, proteins, etc.), however detailed. We study metabolic networks and protein interaction networks from this "top-down" approach, and have developed one of the first systems for "enrichment analysis" (widely used in transcriptomics) of large metabolomics datasets, which is accessible through a web interface (Chagoyen & Pazos, 2011; Chagoyen & Pazos, 2012).



1 *Physical docking for predicting interaction partners. The distributions of docking scores (bottom) can be used to distinguish the correct interaction partner (in red) of the grey protein from alternative non-interacting structures (blue, green and purple).*



GROUP LEADER:
Juan F. Poyatos

POSTDOCTORAL FELLOWS:
Matteo Cavaliere
Saúl Ares

PHD STUDENTS:
Djordje Bajić
Clara Moreno

SELECTED PUBLICATIONS

Bajić D, Poyatos JF. Balancing noise and plasticity in eukaryotic gene expression. BMC Genomics. 2012 Jul 27;13:343

Poyatos JF. On the search for design principles in biological systems. Adv Exp Med Biol. 2012;751:183-93

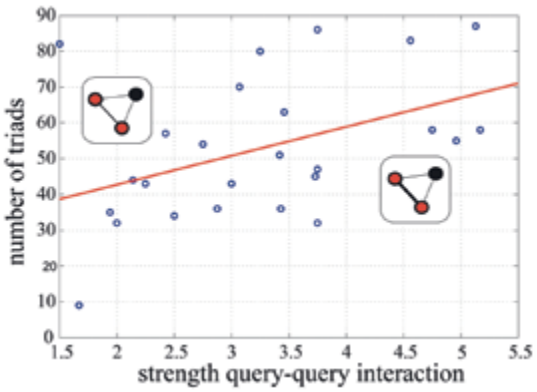
Poyatos JF. The balance of weak and strong interactions in *genetic networks*. PLoS One. 2011 Feb 10;6(2):e14598

Logic of genomic systems

In living systems, two main characteristics can influence how a given genotype expresses a phenotype; *i.e.,* the genotype-to-phenotype map. The first describes the interaction between genetic changes; specifically, the interaction between mutations. A second feature is how a given genotype gives rise to a number of phenotypes, in many cases linked to an alteration in the environment. In the last few years, we have addressed these two questions in genotype-to-phenotype mapping by studying the following problems:

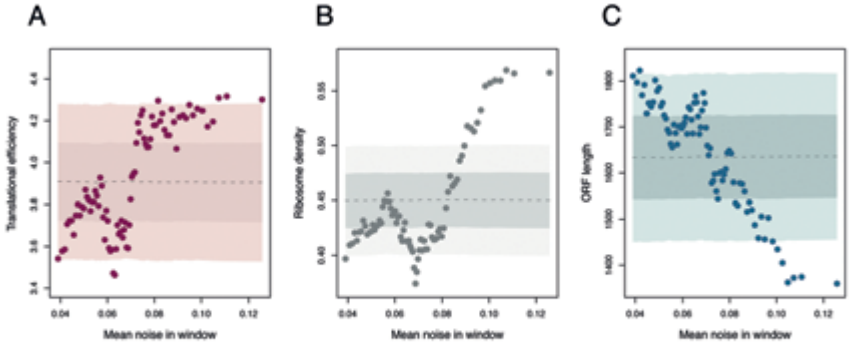
Structure and stability of genetic networks

Genetic interactions can be identified in terms of the disparity in fitness that a specific double (gene) mutant presents relative to the (expected) multiplicative effect obtained from the fitness value of the associated single mutations. These interactions are being characterised quantitatively in a comprehensive way in many model organisms and can be represented as *genetic networks*. We analysed how the strengths of these interactions are distributed in the network and what type of functional architecture (of the underlying molecular system) these patterns reveal. We are currently examining several questions regarding the conservation of the structure of these networks; for instance, we are characterising how a change in genetic context influences network structure and the appearance or disappearance of specific interactions. Knowledge of the structure and dynamics of these networks should help us understand how to predict variation in phenotypic traits within a population.



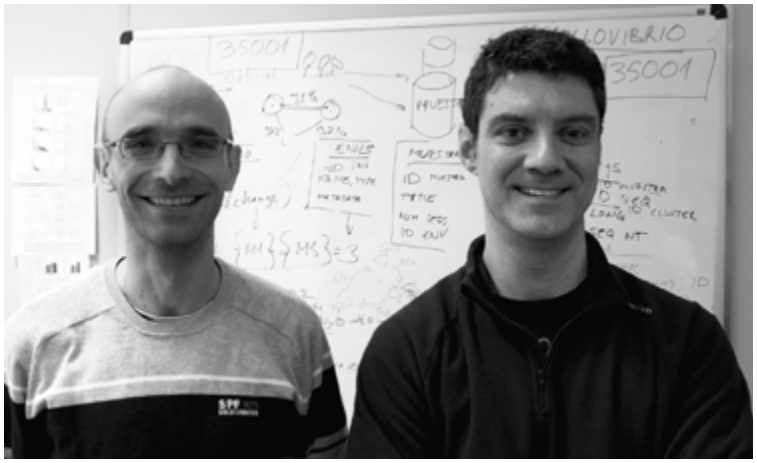
Plasticity in gene expression

Plastic variation in gene expression can also lead to different phenotypes and facilitate the adaptation of an organism to new environments with no associated genetic change. This was recently correlated to a number of molecular determinants such as strong chromatin regulation (i.e., epigenetics). These plasticity mechanisms can also be associated to the generation of expression noise (i.e., variation in gene expression in a fixed environment). We analysed the type of molecular architecture that balances noise and plasticity, and outlined two broad strategies at the transcriptional and translational level that adjust this balance. The need for this modulation appears to be a potential shaping force of genome regulation and organisation.



❶ *The number of triads established by pairs of interacting (query) signalling genes in Caenorhabditis elegans correlates with the strength of this interaction.*

❷ *Noise determinants in low plasticity genes*



GROUP LEADER:
Javier Tamames

TECHNICIANS:
Santiago de la Peña
Pablo David Sánchez

VISITING SCIENTIST:
Ana Suárez

SELECTED PUBLICATIONS

Guazzaroni ME, Herbst FA, Lores I, Tamames J, Peláez AI, López-Cortés N, Alcaide M, Del Pozo MV, Vieites JM, von Bergen M, Gallego JL, Bargiela R, López-López A, Pieper DH, Rosselló-Móra R, Sánchez J, Seifert J, Ferrer M. Metaproteogenomic insights beyond bacterial response to naphthalene exposure and bio-stimulation. ISME J. 2012 Jul;7(1):122-36

Silva-Rocha R, de Jong H, Tamames J, de Lorenzo V. The logic layout of the TOL network of *Pseudomonas putida* pWW0 plasmid stems from a metabolic amplifier motif (MAM) that optimizes biodegradation of m-xylene. BMC Syst Biol. 2011 Nov 11;5:191

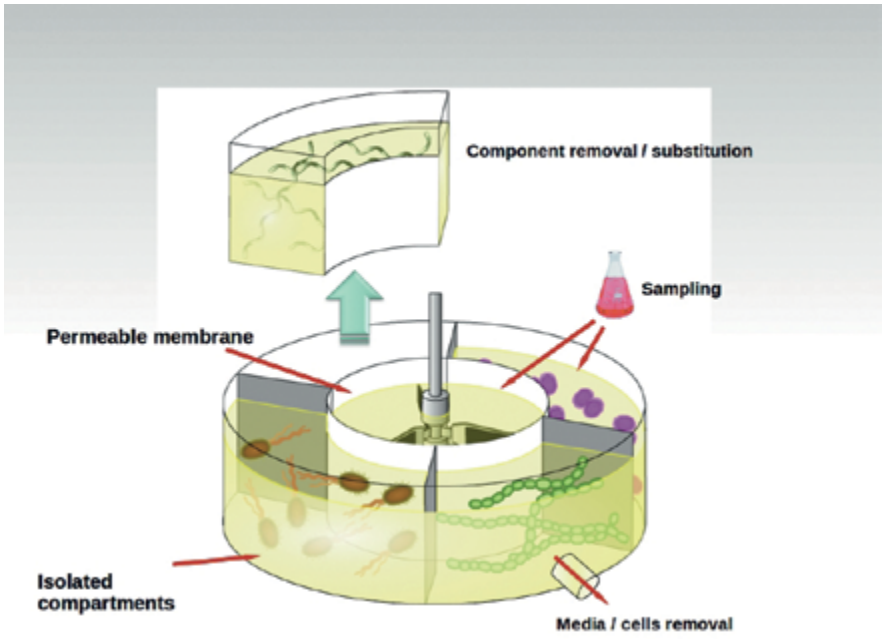
Tamames J, de la Peña S, de Lorenzo V. A priori estimation of coverage for metagenomic sequencing. Env Microbiol Reports. 2012 4, 335-41

Silva-Rocha R, Tamames J, dos Santos VM, de Lorenzo V. The logicome of environmental bacteria: merging catabolic and regulatory events with Boolean formalisms. Environ Microbiol. 2011 Sep;13(9):2389-402

Tamames J, Rosselló-Móra R. On the fitness of microbial taxonomy. Trends Microbiol. 2012 Nov;20(11):514-6

Microbial community modelling

The main motivation of our research is to study the rules that structure natural bacterial communities, to be able to replicate these communities in artificial environments or even to create new, *ad hoc* synthetic communities. We explore the patterns of interactions (mainly metabolic) between species to predict the ability of different species to form a consortium and to determine the capabilities of such an assemblage. We use computational models of the bacterial metabolism to address these issues, and will extend our activities to an experimental setting as well. We have special interest in studying metagenomic samples, to relate the functional profiles of microbia with different environmental conditions, and thus obtain information on the mechanisms of bacterial adaptation to distinct habitats.



1 *Construction of an experimental system for growing microbial communites*

07

Innovation

The Centro Nacional de Biotecnología (CNB-CSIC) has traditionally been involved in transferring the knowledge generated through its basic research to society. In the past two years, CNB scientists have applied for several patents, some of which have already been licensed. In addition, the centre has a number of biological materials (such as antibodies and proteins) that, although not protected by patent, have been commercialised to companies through licensing agreements.

The CNB is one of the CSIC research institutes with its own Technology Transfer Department, which works in close collaboration with the CSIC Vicepresidencia Adjunta de Transferencia de Conocimiento (VATC).

The Technology Transfer Department promotes the exploitation of research results obtained at the CNB for society's benefit and to potentiate the biotechnology sector and basic research.

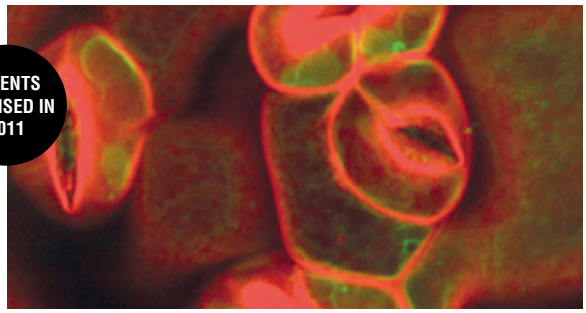
TECHNOLOGY TRANSFER MANAGER

Ana Sanz Herrero

Ana did her PhD research at the CNB, studying plant-pathogen interactions at Dr. Carmen Castresana's group. She has nine years of working experience as a research scientist in biotechnology companies in Spain and in the US, specialised in development of innovative molecular and cell biology products, and two years experience as an independent consultant for biotech companies. Ana Sanz has managed the CNB Technology Transfer Department since September 2012.

Patents and biological materials licensed

PATENTS LICENSED IN 2011



PATENTS LICENSED IN 2011

Procedure to modify plant architecture and improve the crops yield through the control of entering into cell differentiation

Dr Enrique Rojo and colleagues at the CSIC-CNB discovered and characterised the IYO and ART (or AtRTR1) genes in *Arabidopsis*, which they have demonstrated to cooperate as positive regulators of transcriptional elongation, sufficient to initiate the process of cell differentiation. IYO and ART overexpression induce early cell differentiation. The decreased rates of cell proliferation in loss-of-function IYO and ART mutants indicate that these genes are also important in undifferentiated cells to promote cell division. The inventors have transferred *Arabidopsis* IYO to transgenic tomato and shown that plant architecture can be modified, with development of increased lateral branches. They are also characterising an interesting double-embryo phenotype that could have practical applications for increasing crop plant density or affecting overall seed composition (*e.g.*, oil content).

RESEARCH GROUP:
Enrique Rojo de la Viesca

APPLICATION NUMBER AND PRIORITY DATE:
P201130812, 19/05/2011

INTERNATIONAL PCT APPLICATION:
PCT/EP12/059312, PCT/GB12/051146

COUNTRIES SELECTED IN NATIONAL PHASE:
Pending

PRESENT SITUATION:
Exclusive licence to a company (10/11/2011). The application for mammalian cells, PCT/GB12/051146, is in co-titularity with the CNIO (Manuel Serrano's Group) and is available for licensing.

Epidermal development in wild type (Wt) and iyo-1 cotyledons. Protoderm differentiation is delayed in iyo-1 plants and as a result, stomatal patterning is disrupted. The plants express a tonoplast GFP marker (green signal) and are stained with propidium iodide (red signal).

Genes regulating plant branching, promotors, genetic constructs containing same and uses thereof

The research group lead by Pilar Cubas at CNB-CSIC has discovered the BRC1-like genes which control shoot branching in potato and tomato.

RESEARCH GROUP:
Pilar Cubas Domínguez

APPLICATION NUMBER AND PRIORITY DATE:
P200900088, 13/01/2009 and its divisional P201030915 DIV, 14/06/2010

INTERNATIONAL PCT APPLICATION:
PCT/ES09/070538, PCT/ES2010/070538

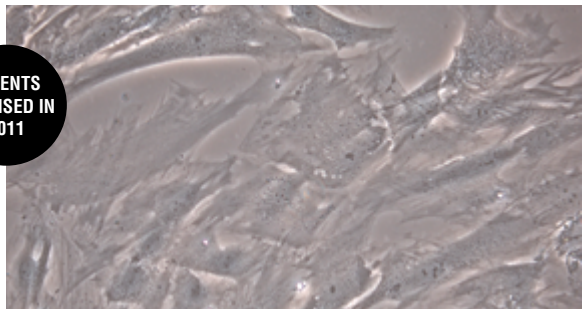
COUNTRIES SELECTED IN NATIONAL PHASE:
EU, US, CN, IN

PRESENT SITUATION:
Exclusive licence to a company (18/05/2011). The licensing agreement also includes a R&D Contract between the company and the same research group

The SIBRC1b gene from Solanum lycopersicum suppresses the formation of basal branches in tomato. Center: Control plants. Left and right: Tomato plants with the SIBRC1b gene partially inactivated.

Patents and biological materials licensed

PATENTS LICENSED IN 2011



PATENTS LICENSED IN 2011

Stem cell culture media and methods

The group led by Lourdes Planelles at the CNB-CSIC has participated in the discovery of new culture media and methods for pluripotent stem cells, which provide significant advantages over known culture media and methods. The invention also provides related culture medium supplements, compositions and uses. There is great interest in culture media and methods for expanding populations of pluripotent stem cells, particularly adipose-derived mesenchymal stem cells (ASC cells). Clinical applications of pluripotent stem cells require reproducible cell culture methods to provide adequate numbers of cells of suitable quality. Although numerous different culture media and methods have been tested for pluripotent stem cells, a great need remains for further improvements in ASC culture media and methods.

An advantage of the invention is that it can be used to culture pluripotent stem cells at a high proliferation rate, whilst maintaining their undifferentiated phenotype.

RESEARCH GROUP:
Lourdes Planelles

APPLICATION NUMBER AND PRIORITY DATE:
EP10382244, 10/08/2011 (Europe Patent as Priority Patent). Co-titularity with Cellerix (now TiGenix NV)

INTERNATIONAL PCT APPLICATION:
PCT/EP11/065540

COUNTRIES SELECTED IN NATIONAL PHASE:
Pending

PRESENT SITUATION:
Exclusive licence agreement to a company (10/08/2011) R&D Contract between the company and the same research group

Method for extracting gluten contained in heat-processed and non-heat-processed foodstuffs, compatible with an enzyme-linked immunosorbent assay, composition and kits comprising said composition

The method can be used to extract quantitatively the gluten contained in a heat-processed or non-heat-processed food sample before quantification of the gluten by ELISA. Said method is suitable for food analyses, in particular, for foodstuffs intended for coeliac sufferers.

RESEARCH GROUP:
Enrique Méndez Corman

APPLICATION NUMBER AND PRIORITY DATE:
P200101098, 14/05/2001

INTERNATIONAL PCT APPLICATION:
PCT/ES02/00208

COUNTRIES SELECTED IN NATIONAL PHASE:
EU, US, CA

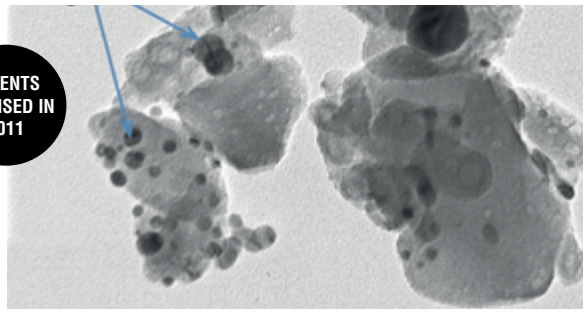
PRESENT SITUATION:
Exclusive licence to a company (28/08/2011)

Adipose-derived mesenchymal stem cells

Coeliac disease is caused by a reaction to gliadin, a prolamin (gluten protein) found in wheat, and similar proteins found in the crops of the tribe Triticeae (which includes other common grains such as barley and rye).

PATENTS LICENSED IN 2011

PATENTS LICENSED IN 2011



Nanostructure calcium silver phosphate composite powder, method for obtaining same, and bactericidal and fungicidal uses thereof

Scientists at the CNB-CSIC participated in the discovery of nanostructured powders formed by a calcium phosphate, having a particle size, and silver nanoparticles adhered to its surface that can be used as universal disinfectants. One advantage provided by the present nanocomposite powders is that nanoparticle agglomeration is avoided because the nanoparticles can be adhered to the substrate surface. A second advantage is its bactericidal and fungicidal efficiency. A third advantage is its low toxicity (far below that of commercial products, very far below toxic levels). Applications include the surgical implants sector, public facilities (toilets and hospitals, transport, etc.), air conditioning equipment, food, dentistry, paints, clothes and packaging (food, domestic, pharmaceutical, medical devices, etc.).

RESEARCH GROUP:
Francisco Malpartida Romero
This scientist is now retired

APPLICATION NUMBER AND PRIORITY DATE:
P200803695, 24-12-2008. Co-titularity with Instituto de Ciencias Materiales (ICMM-CSIC), Nanomaterials and Nanotechnology Research Center (CINN-CSIC), Instituto de Cerámica y Vídrio (ICV-CSIC)

INTERNATIONAL PCT APPLICATION:
PCT/ES09/070628

COUNTRIES SELECTED IN NATIONAL PHASE:
US, CN, EU, JP

PRESENT SITUATION
Licensed to a company, 02/12/2011. Spanish patent has been granted

Nanostructure calcium silver phosphate composite powder

Powder of vitreous composition having biocidal activity

Scientists at CNB have developed a magnetic nanoparticle which can be injected intravenously and targeted to a region of interest using an external magnetic field. It allows obtaining the optimal concentration of an active ingredient in a tumour region, with a reduction of the side effects.

RESEARCH GROUP:
Francisco Malpartida Romero
This scientist is now retired

APPLICATION NUMBER AND PRIORITY DATE:
P200931137, 09/12/2009. Co-titularity with Instituto de Ciencias Materiales (ICMM-CSIC), Nanomaterials and Nanotechnology Research Center (CINN-CSIC), Instituto de Cerámica y Vídrio (ICV-CSIC)

INTERNATIONAL PCT APPLICATION:
PCT/ES2010/070810

COUNTRIES SELECTED IN NATIONAL PHASE:
US, CN, EU, JP

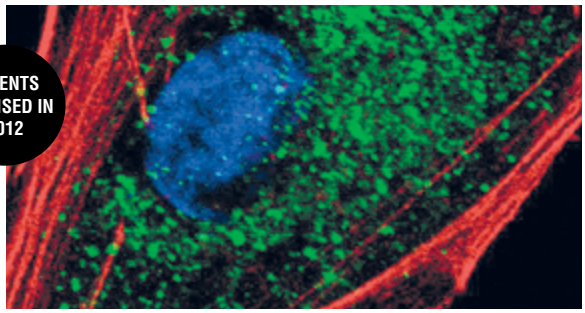
PRESENT SITUATION
Licensed to the same company as the previous patent, 02/12/2011.

Powder of vitreous having biocidal activity

Patents and biological materials licensed

PATENTS LICENSED IN 2012

PATENTS LICENSED IN 2012



SpBRANCHED1a of Solanum pennellii and tomato plants with reduced branching comprising this heterologous SpBRANCHED1a gene

The research group lead by Pilar Cubas at the CNB-CSIC discovered that the *Solanum pennellii* BRANCHED1-like gene causes reduced basal shoot branching when introgressed into *Solanum lycopersicum*.

This discovery is of great interest to generate improved tomato lines with reduced basal branching, a trait highly desired to avoid pruning of the plants during the time of flowering and fruit production.

RESEARCH GROUP:
Pilar Cubas Domínguez

APPLICATION NUMBER AND PRIORITY DATE:
EP11166057.7, 13/05/2011

INTERNATIONAL PCT APPLICATION:
PCT/EP2012/058892

COUNTRIES SELECTED IN NATIONAL PHASE:
EU

PRESENT SITUATION:
Exclusive licence to a company (22/02/2012)

The SpBRC1a gene from Solanum pennellii suppresses the elongation of branches in tomato. Left: Control plant. The red arrow indicates an incipient branch. Centre: Plant without lateral branches that carries a genomic region from Solanum pennellii including its SpBRC1a gene. Right: A Detail of plant in the center.

Recombinant vectors based on Ankara modified virus (MVA) as preventive and therapeutic vaccines against HIV

The research group lead by Dr. Mariano Esteban at the CNB has developed a prototype vaccine against HIV based on Modified Ankara Virus (MVA-B). MVA-B efficiently protects mice and macaques against simian immunodeficiency virus (SIV). MVA-B entered phase I clinical trials in 30 healthy individuals in the Gregorio Marañon (Madrid) and Clinic (Barcelona) Hospitals. In this study, 90% of the volunteers developed an immune response against the HIV virus that was maintained after 1 year in at least 85% of the individuals. Phase I clinical trials with HIV-infected volunteers (20 receive the MVA-B vaccine and 10, a placebo) were initiated in 2012 in the Gregorio Marañón, Clinic and IrsiCaixa Hospitals; results will be known in the second half of 2013.

RESEARCH GROUP:
Mariano Esteban Rodríguez

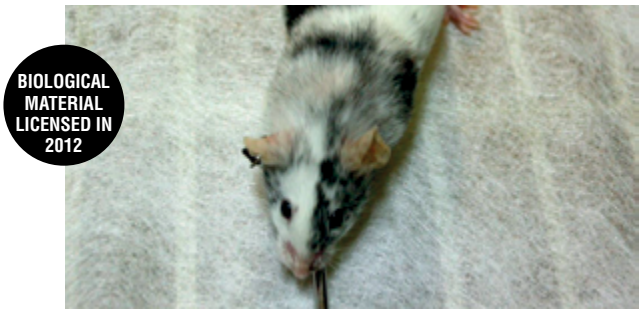
APPLICATION NUMBER AND PRIORITY DATE:
P200501841, 27/07/2005

INTERNATIONAL PCT APPLICATION:
PCT/ES06/070114

COUNTRIES SELECTED IN NATIONAL PHASE:
EU, US

PRESENT SITUATION:
Exclusive licence agreement to a company (30/06/2012)

Confocal microscopy showing expression of the cytoplasmic HIV-1 Env protein (in green) induced by the candidate HIV/AIDS vaccine vector MVA-B. This vaccine has shown good immunogenicity profile against HIV antigens in phase I clinical trials. In red, phalloidin staining of the cytoskeleton. The cell nucleus appeared in blue.



R5 monoclonal antibody against gliadin for gluten determination

RESEARCH GROUP:
Enrique Méndez Corman

PRESENT SITUATION:
Non-exclusive licence agreement to a company (08/09/2011)

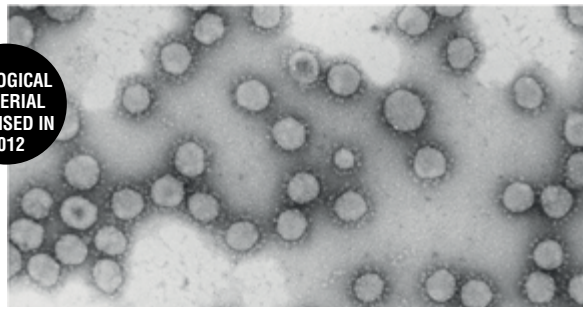
Gliadin is one of the gluten proteins found in wheat and other cereals.

Transgenic mice for use as a model for neurodegenerative diseases

RESEARCH GROUP:
Lluís Montoliú José

PRESENT SITUATION:
Exclusive licence agreement to a company (13/04/2012)

Chimaeric mouse used to generate a new transgenic animal model for sporadic Alzheimer diseases

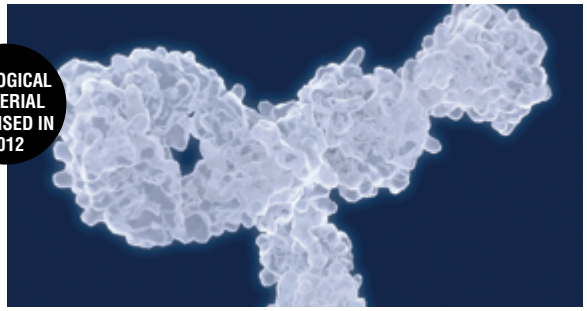


Cell lines producing monoclonal antibodies, against gastroenteritis porcine transmissible virus (GPT) and related coronavirus

RESEARCH GROUP:
Luis Enjuanes Sánchez

PRESENT SITUATION:
Exclusive licence agreement to a company (28/05/2012)

Purified enteric TGEV virus



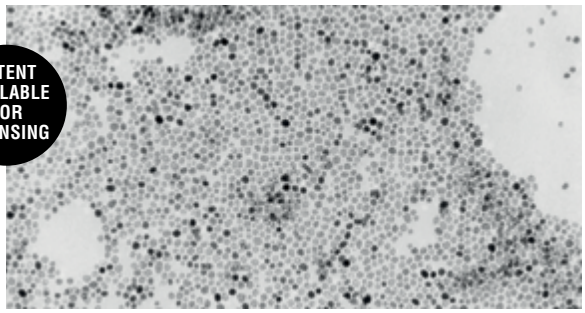
Cell lines producing monoclonal antibodies against IGF-1

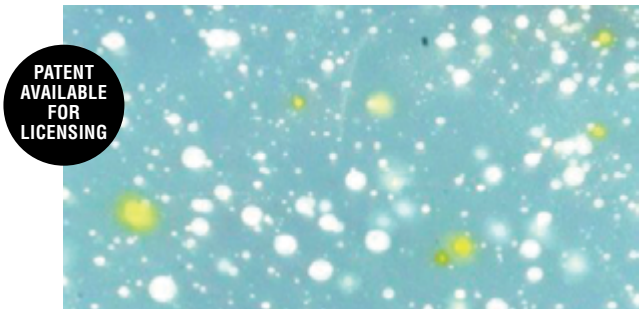
RESEARCH GROUP:
Santos Mañes Brotón

PRESENT SITUATION:
Non-exclusive licence agreement to a company (06/11/2012). They are available for other licensing agreements.

Monoclonal antibodies against IGF-1

Technology offer





Magnetic nanoparticles for use in a pharmaceutical composition

Scientists at the CNB have developed a magnetic nanoparticle which can be injected intravenously and targeted to a region of interest using an external magnetic field. It allows obtaining the optimal concentration of an active ingredient in a tumour region, with a reduction of the side effects.

RESEARCH GROUP:
Domingo Francisco Barber Castaño

APPLICATION NUMBER AND PRIORITY DATE:
P201030138, 02/02/2010

INTERNATIONAL PCT APPLICATION:
PCT/ES11/070056

PRESENT SITUATION:
Patent applications have been abandoned but the know-how is available for licensing and new nanoparticles are under development

MAIN INNOVATIONS AND ADVANTAGES:
This new nanoparticle solves the problems encountered with other therapeutic systems:
Important side effects with the systemic administration of cytokines. Moreover, when using systemic administration, the level of cytokines at the site of action is much lower than the therapeutic concentration needed, and the increase in the level is transitory.
Great therapeutic diversity in the treatments with genetic vectors injected directly into the tumour mass, or with implantation in the tumour of cells genetically modified to produce cytokines.
Significant side effects similar to the systemic administration with the injection of modified tumour cells or irradiated tumour cells as vaccines.

TEM image of magnetite nanoparticles prepared by decomposition of a metal-organic precursor in solution

Bacterial biofilm combined with liquid crystal for the preparation of an electro-optical devices

Scientists at the National Center for Biotechnology in collaboration with the Institute of Materials Sciencie of the CSIC have developed a liquid crystal type that is patterned on the structure of a bacterial biofilm, which has a high degree of porosity and displays electro-optical propierties. The production method and the use of this material for the production of devices with controllable transparency to visible light is described.

RESEARCH GROUP:
Víctor de Lorenzo

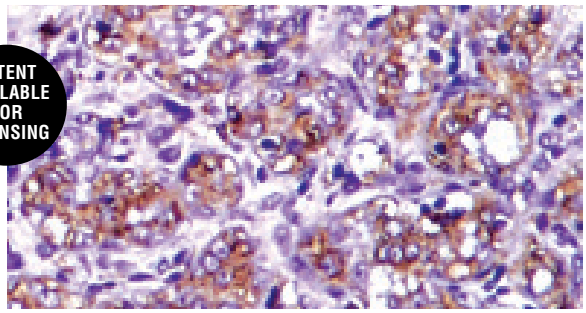
APPLICATION NUMBER AND PRIORITY DATE:
P201030295, 01.03.2010

INTERNATIONAL PCT APPLICATION:
PCT/ES11/070129

PRESENT SITUATION:
Spanish patent has been granted

Environmental bacteria like those shown in the figure (Pseudomonas putida) are prone to form biofilms that provide a complex molecular frame for developing materials with new physical and optical properties

PATENT AVAILABLE FOR LICENSING



Biomarker for cancer diagnosis, prognosis and follow-up, and a cancer diagnosis method based on its quantification in a biological sample

Scientists at the CNB-CSIC have discovered the protein p85ß, a subunit of phosphoinositide 3-kinase, as a protein marker useful for cancer diagnosis, prognosis and follow-up of tumour progression. In colon cancer samples, it was demonstrated that high p85ß protein levels correlate with an advanced cancer grade, whereas in breast cancer samples they correlate with invasive and metastatic tumours. The invention also refers to a method for cancer diagnosis, prognosis and follow-up based on the quantification of p85ß in biological samples. Industrial partners focussed on developing cancer molecular diagnostics are being sought to licence the technology.

RESEARCH GROUP:
Ana Clara Carrera

APPLICATION NUMBER AND PRIORITY DATE:
P201031137, 22/07/2010

INTERNATIONAL PCT APPLICATION:
PCT/ES2011/070451

COUNTRIES SELECTED IN NATIONAL PHASE:
US

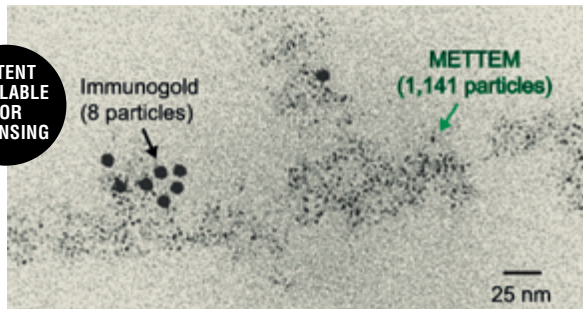
PRESENT SITUATION:
Initiating examination in US.

MAIN INNOVATIONS AND ADVANTAGES:
The PI3KR2 gene expression can be analysed by measuring the level of the mRNA encoding protein p85ß or the level of p85ß protein itself.
In human breast cancer, the p85ß marker allows discrimination between localised and metastatic cancer.
In human colon cancer, the p85ß marker is predictive of tumour grade.

REF. :
CSIC/AH_003

Confirmation of PI3K pathway activation by immunohistochemical analysis of S6 phosphorylation (red) in breast cancer tumour tissue.

PATENT AVAILABLE FOR LICENSING



Method for protein detection by electron and correlative microscopy using a clonable tag based on the protein metallothionein

A group at the National Center of Biotechnology (CNB-CSIC) has developed a method for protein detection by electron microscopy using a clonable marker based on metallothionein (MT) protein. MT is a protein that binds metal ions, such as gold. This method can be applied for protein detection in live cells, both prokaryotic and eukaryotic, in cell organelles, and in virus particles. Moreover, it allows the visualisation of the proteins in their native environment, and it is independent of antigen-antibody recognition.

RESEARCH GROUP:
Cristina Risco

APPLICATION NUMBER AND PRIORITY DATE:
P201031880, 17/12/2010

INTERNATIONAL PCT APPLICATION:
PCT/ES11/070869, PCT/ES2012/070864 (claiming priority the new material introduced in the first PCT Application).

PRESENT SITUATION:
National phase application pending. Spanish patent application has been abandoned.

MAIN INNOVATIONS AND ADVANTAGES:
It allows for *in vivo* protein detection inside cells.
It can be used for protein detection in prokaryotic and eukaryotic cells.
It can be used to detect proteins in cell organelles and in viral particles.
It allows simultaneous detection of several proteins
It is not necessary to have primary antibodies that recognise the proteins of interest.
It is independent of antigen-antibody recognition.
Sensitivity is at least two orders of magnitude higher than immunogold detection.

REF. :
CSIC/AH_004

Immunogold (antibody based method) versus METTEM (metal-tagging transmission electron microscopy).

Technology offer

PATENT AVAILABLE FOR LICENSING

PATENT AVAILABLE FOR LICENSING

Nucleic acids enconding PRRSV GP5-ecto domain and M protein

A group at the CNB-CSIC has developed a vaccine candidate to prevent porcine reproductive and respiratory syndrome virus (PRRSV), based on a recombinant transmissible gastroenteritis virus (TGEV) vector expressing selected PRRSV antigens.

RESEARCH GROUP:
Luis Enjuanes Sánchez

APPLICATION NUMBER AND PRIORITY DATE:
EP10192693, 26-11-2010. Co-titularity with Fort Dodge Veterinaria S.A. (It has been acquired by PFIZER OLOT USL)

INTERNATIONAL PCT APPLICATION:
PCT/EP11/071044

COUNTRIES SELECTED IN NATIONAL PHASE:
Pending

PRESENT SITUATION:
Patent applications have been published

The most relevant porcine virus is PRRSV

Compounds for the treatment of neurodegenerative diseases

Researchers from the CSIC, CIBERNED, CRG and Fundación CIEN have observed that changes in the expression of the protein DREAM (also known as KChIP-3 or calsenilin) in cerebral tissue precedes the onset of pathological symptoms related to Huntington's disease (HD), Alzheimer's disease (AD) and Down's syndrome (DS). These results suggest that DREAM could be an early biomarker as well as a possible therapeutic target in HD, AD and cognitive impairments related to DS. Researchers have identified two series of compounds that bind DREAM in a Ca^{2+}–dependent manner *in vitro*, and inhibit its activity. Chronic administration of these compounds in murine models of HD shows that it delays the appearance of locomotor symptoms and increases the life expectancy of the animals.

RESEARCH GROUP:
José Ramón Naranjo

APPLICATION NUMBER AND PRIORITY DATE:
P201130033, 13/01/2011

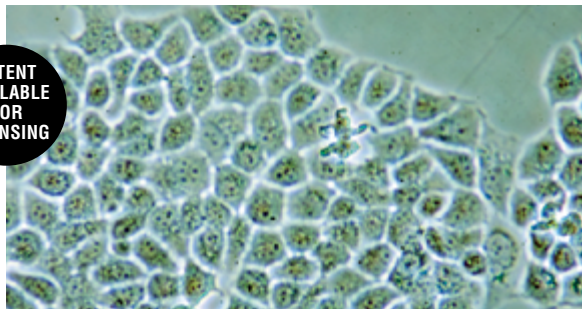
INTERNATIONAL PCT APPLICATION:
PCT/ES12/070020

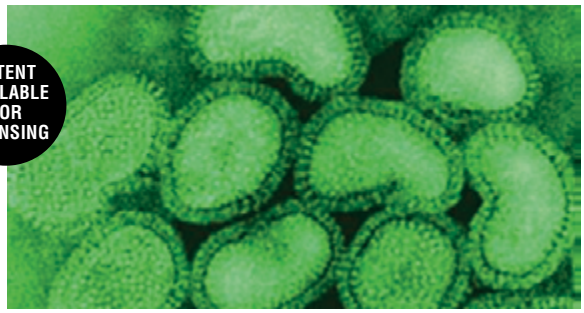
PRESENT SITUATION:
National phases application pending

MAIN INNOVATIONS AND ADVANTAGES:
Identification of DREAM protein as a novel biomarker and target related to neurodegenerative diseases
Description of a family of compounds that inhibits the target
Tested *in vivo* and *in vitro*

REF.:
CSIC/AH_005

Targeting neurodegenerative diseases from Down syndrome to Alzheimer's disease by stimulating protective mechanisms





Test for the determination of the response to treatment with 5-FU of patients with colon or breast cancer

PATENT AVAILABLE FOR LICENSING

Scientists at the National Center for Biotechnology (CNB-CSIC), the Servicio Andaluz de Salud and the Universidad de Granada have identified the RNA-dependent protein-kinase PKR as a marker to assess the response to the treatment of cancer with 5-fluorouracil (5-FU). Identification of mutations in PKR, changes in its activity, or insufficient expression, allows predicting the response to 5-FU alone or combined with other drugs or cytokines, particularly in patients with colon and breast cancer. Industrial partners are sought interested in a patent licence.

RESEARCH GROUP:
Mariano Esteban

APPLICATION NUMBER AND PRIORITY DATE:
P201130247, 24/02/2011

INTERNATIONAL PCT APPLICATION:
PCT/ES12/070115

PRESENT SITUATION:
National phases application pending

MAIN INNOVATIONS AND ADVANTAGES:
Previous studies have tried to analyse the relationship between the status of various enzymes involved in DNA replication with the response to 5-FU. In most cases, the conclusion is unclear, and the analysis was proposed of different markers to predict correctly the response to chemotherapy.
Other studies have analysed the tumour suppressor protein p53 as a marker. However, more than 50% of tumours have mutations or lack p53, which has an essential role in apoptosis and in control of the cell cycle. In these patients, it was demonstrated that functional PKR has a key role, as it is the only known alternative to p53 able to induce cell death. It is thus essential in these cases to determine PKR status to predict the effectiveness of 5-FU.

REF.:
CSIC/AH_012

Breast tumour cell

An improved method to produce influenza vaccine for humans in cell culture

PATENT AVAILABLE FOR LICENSING

Scientists at the National Center for Biotechnology (CNB-CSIC) have generated a new cell line with ten-fold higher yield of influenza virus particle production. The cell line of the invention has silent the CHD6 cellular protein, that acts as viral negative modulator. The use of this cell line would reduce vaccine production costs and would improve the capacity of massive vaccine production in case of pandemic flu. In addition, it would cooperate to change the current manufacture of vaccines in chicken embryonated eggs to efficient cell culture methods, as the former has a number of disadvantages, especially for egg-allergic individuals. Industrial partners interested in a patent licence are being sought.

RESEARCH GROUP:
Amelia Nieto Martín

APPLICATION NUMBER AND PRIORITY DATE:
P201130879, 27/05/2011

INTERNATIONAL PCT APPLICATION:
PCT/ES12/070388

PRESENT SITUATION:
National phases application pending. Spanish patent application has been abandoned.

MAIN INNOVATIONS AND ADVANTAGES:
The cell lines of the invention improve the replication of viral RNA and produce from 5 to 10 times more viral particles per infected cell.
The use of these cell lines reduces the cost of cell culture flu vaccine production and offer the possibility of massive production in case of pandemic flu.

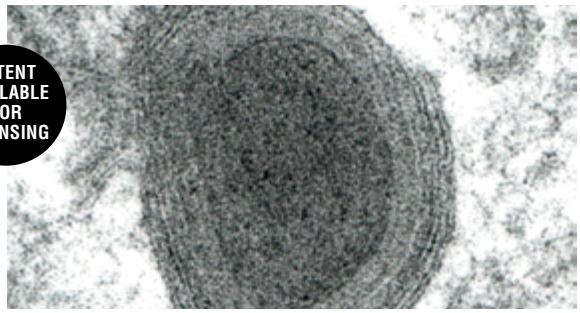
REF.:
CSIC/AH_007

Electron micrograph of influenza virus particles

Technology offer

PATENT AVAILABLE FOR LICENSING

PATENT AVAILABLE FOR LICENSING



Procedure to obtain monoclonal antibodies from complex samples of antigens

Scientists at the CNB-CSIC have discovered a method to select recombinant antibodies from camelids (camelbodies) that were obtained from complex samples of antigens.

RESEARCH GROUP:
Víctor de Lorenzo

APPLICATION NUMBER AND PRIORITY DATE:
P201131152, 06/07/2011

PRESENT SITUATION:
Spanish patent pending

The technology allows massive production of antibodies against mixtures of complex antigens (e.g., a whole cell extract) and their sorting by direct panning of the phage display library on a denaturing 1-D or 2D gels.

Recombinant vectors based on Ankara modified virus (MVA), with a deletion in the 6CL gene, as vaccines against HIV and other diseases

A group at the National Center of Biotechnology (CNB-CSIC) has developed recombinant viruses based on modified Ankara virus (MVA a highly attenuated poxvirus strain) with deletion in the 6CL gene as vaccines against HIV and other diseases such as malaria, leishmaniosis, hepatitis C and prostate cancer. This vector represents an attractive alternative for improving the immunogenicity of prototype vaccine candidates based on MVA. Industrial partners are sought interested in a patent licence.

RESEARCH GROUP:
Mariano Esteban

APPLICATION NUMBER AND PRIORITY DATE:
P201131230, 19/07/2011

INTERNATIONAL PCT APPLICATION:
PCT/ES12/070521

PRESENT SITUATION:
National phases application pending

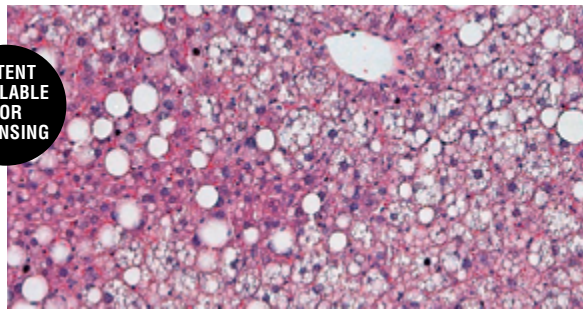
MAIN INNOVATIONS AND ADVANTAGES:
The vector of the invention replicates at the same level as the parental virus and induces innate immune responses, increasing the expression of IFNβ and the genes that are inducible by IFNα/β in human cells.
In mouse, deletion of the C6L gene produces an increase in the humoral and cellular immune responses to HIV antigens.
Deletion of the C6L gene could improve the immunogenicity of other recombinant vectors based on MVA that express other heterologous antigens (from malaria, leishmania, hepatitis C and prostate cancer) to be used as vaccines against these diseases.

REF.:
CSIC/AH_008

Modified Ankara virus (MVA) in CEF cells

PATENT AVAILABLE FOR LICENSING

PATENT AVAILABLE FOR LICENSING



Adjuvant effect of A27 protein from vaccinia virus (14K) and its applications for vaccines

A group at the CNB-CSIC has found the adjuvant-like effect of the immunogenic vaccinia virus protein 14K when fused with the circumsporozoite (CS) protein of *Plasmodium*. The chimaeric protein CS-14K has a pronounced tendency to form oligomers/aggregates, which in turn enhanced the host immune response profile in prime/boost protocols, leading to protection against malaria. The adjuvant effect of the 14K protein was shown in the design of a new vaccine against malaria, but could be used to generate similar fusion proteins as immunogens for other diseases such as HIV, hepatitis C, leishmaniasis and cancer. Industrial partners are being sought interested in a patent licence.

RESEARCH GROUP:
Mariano Esteban

APPLICATION NUMBER AND PRIORITY DATE:
P201131854, 17/11/2011

INTERNATIONAL PCT APPLICATION:
PCT/ES2012/070794

PRESENT SITUATION:
National phases application pending. Spanish patent application has been abandoned

MAIN INNOVATIONS AND ADVANTAGES:
Chimaeric protein of 14K protein improves the overall immunogenicity of the fused antigen, such as *plasmodium* CS, in the absence of any adjuvant.
The activation of type I IFN signalling by 14K fusion protein further validates its use as priming agent.
The use of proteins as priming agents is gaining acceptance due to their better efficiency and safety than DNA vectors.

REF.:.
CSIC/AH_009

Vaccine against malaria

p38 MAPK gamma and delta for use as biomarkers of NAFLD

The present invention provides a precise, non-invasive method to obtain a prognosis for and to diagnose non-alcoholic fatty liver disease. The invention corresponds to a method that analyses the levels of different kinases in blood samples. As such, it can be considered a non-invasive test to diagnose fatty liver disease, which could clearly lead to an improvement in early diagnosis, the ability to follow the disease, and in patient quality of life.

RESEARCH GROUP:
Guadalupe Sabio
This group has moved to the CNIC.

APPLICATION NUMBER AND PRIORITY DATE:
EP12164807, 19/04/2012. European patent was presented as priority patent. Co-titularity CSIC (CNB), CNIC and Salamanca University

INTERNATIONAL PCT APPLICATION:
Pending

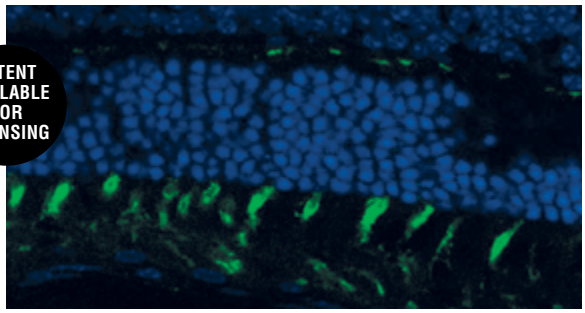
PRESENT SITUATION:
The CNIC is in charge of managing this patent .

MAIN INNOVATIONS AND ADVANTAGES:
The levels of certain kinases in the bloodstream can be used as potential biological markers of hepatic steatosis in humans, without the inconvenience and risks associated with performing liver biopsy.

Micrograph of non-alcoholic fatty liver disease.

Technology offer

PATENT AVAILABLE FOR LICENSING



PATENT AVAILABLE FOR LICENSING

New animal model for achromatopsia

Scientists at the CNB-CSIC have described a new animal model for a retinopathy, achromatopsia disease. Achromatopsia is a rare disease (affects 1-9:100,000 people) and is characterised by reduced visual acuity, nystagmus, photophobia, and total or partial loss of the ability to discriminate between colours.

RESEARCH GROUP:
Lluís Montoliu José
In co-titularity with the Centre for Biomedical Network Research on Rare Diseases (CIBERER), Alcalá de Henares University and Spanish National Cancer Research Center (CNIO)

APPLICATION NUMBER AND PRIORITY DATE:
P201231296, 13/08/2012

PRESENT SITUATION:
International PCT Application pending

MAIN INNOVATIONS AND ADVANTAGES:
This animal model will allow development and validation of new therapies for achromatopsia disease.

REF.:
CSIC/AH_014

Attenuated SARS-CoV vaccines

Scientists at the CNB-CSIC have developed a vaccine candidate to prevent severe acute respiratory syndrome (SARS) in humans. This candidate has been proved to provide one hundred percent protection in three animal model systems.

RESEARCH GROUP:
Luis Enjuanes Sánchez

APPLICATION NUMBER AND PRIORITY DATE:
EP05013727, 24/06/2005

INTERNATIONAL PCT APPLICATION:
PCT/EP06/006091

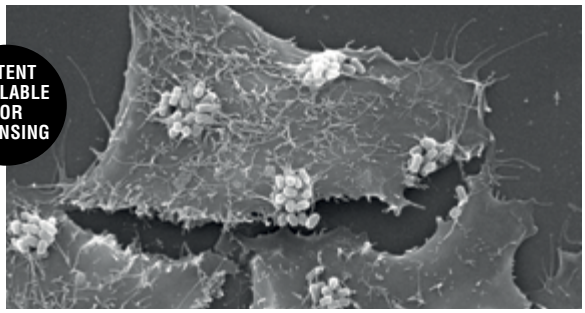
COUNTRIES SELECTED IN NATIONAL PHASE:
CN, HK

PRESENT SITUATION:
Patent examination in each country.

REF.:
CSIC/AH_016

Histological section of an adult mouse retina displaying the cone photoreceptor cells (green) and the nuclei of all cells (blue)

BSL3 containment laboratory at the CNB-CSIC



An engineered bacteria to deliver intracellular single domain antibodies into human cells

Scientists at the CNB-CSIC have developed non-invasive *Escherichia coli* bacteria bearing functional molecular syringes assembled by a Type III protein secretion system (T3SS). These bacteria can secrete and translocate single-domain antibody (sdAb) fragments with full capacity to bind to their cognate antigens to the cytoplasm of human cells. They have shown their functionality by the formation of antigen-sdAb complexes in the cytoplasm of infected cells. The use of live bacteria has great potential for *in vivo* delivery of therapeutic proteins.

RESEARCH GROUP:
Luis Angel Fernández Herrero

APPLICATION NUMBER AND PRIORITY DATE:
P200700644, 12/03/2007

INTERNATIONAL PCT APPLICATION:
PCT/ES08/070045

COUNTRIES SELECTED IN NATIONAL PHASE:
EU, US. EU application has been abandoned

PRESENT SITUATION:
Spanish patent has been granted (12/03/2007). Patent examination in US

MAIN INNOVATIONS AND ADVANTAGES:
Non-invasive *E. coli* cells carrying a Type III protein secretion system remain extracellular and can inject specifically the desired single domain antibodies.
The levels of intracellular sdAb (10^5-10^6 molecules per cell) are appropriate to modulate the activity of regulatory and cell signalling proteins. Injection of sdAb does not require bacterial invasion or the transfer of genetic material, differing from other approaches that need the transfer of the protein-encoding gene by viral infection or transfection.

REF.:
CSIC/AH_015

Scanning electron micrograph of human HeLa cells infected in vitro with attenuated EPEC bacteria carrying a functional T3SS that injects sdAb into the cytoplasm of the human cell

Diagnosis and treatment procedure for diseases with alteration of p38 kinase, the elements required and its applications

Scientists at the CNB-CSIC have developed a method to assess disease activity of rheumatoid arthritis (RA) based on measurement of the phosphorylation status on p38 Tyr^{323}. They have shown that phosphorylation of p38 on Tyr^{323} was higher in T cells from patients with active RA (P = 0.008 vs. healthy controls) than in patients with RA in remission or patients with ankylosing spondylitis. Tyr^{323} p38 phosphorylation was associated with disease activity determined by the Disease Activity Score in 28 joints (DAS28) (P = 0.017).

RESEARCH GROUP:
Jesús Mª. Salvador
Carlos Martínez-A

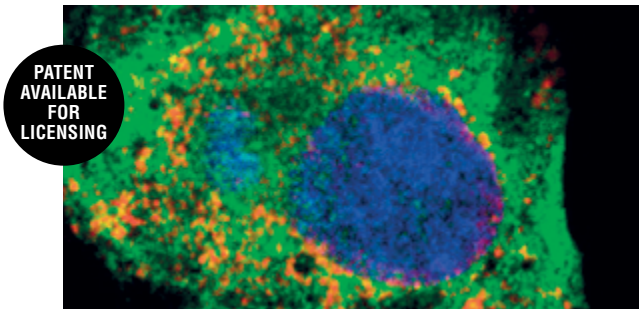
APPLICATION NUMBER AND PRIORITY DATE:
P200702770, 22/10/2007

INTERNATIONAL PCT APPLICATION:
PCT/ES08/070193

PRESENT SITUATION:
The Spanish patent application has been granted. The scientists have new biological materials and know-how regarding this technology.

REF.:
CSIC/AH_011

Rheumatoid arthritis is an autoimmune disease that attacks the joints and other body parts.



Potato plants able to tuberise under heat stress conditions and the method to produce them

A group at the National Center of Biotechnology (CNB-CSIC) has identified and sequenced the potato gene *SP6A*, orthologue to the *Arabidopsis thaliana* FLOWERING LOCUS T (FT) gene, and has demonstrated that the gene encodes a protein that acts as the tuberisation-inducing signal in potato plants. Transgenic potato plants have been generated which carry a construct consisting of a heat shock promoter from soybean, induced at temperatures higher than 35ºC, fused to the *SP6A* potato gene sequence and it has been demonstrated that they are able to tuberise under heat stress conditions (night temperatures above 25ºC).

RESEARCH GROUP:
Salomé Prat

APPLICATION NUMBER AND PRIORITY DATE:
P200930114, 29/04/2009

INTERNATIONAL PCT APPLICATION:
PCT/ES10/070265

COUNTRIES SELECTED IN NATIONAL PHASE:
EU BR, US, AU, IN

PRESENT SITUATION:
Patent examination in each country

MAIN INNOVATIONS AND ADVANTAGES:
The overexpression of the potato *SP6A* gene under the control of a heat-inducible promoter notably improves tuber production yield under heat stress conditions.
Under heat stress conditions, while control potato plants showed a 30% reduction in tuber production, transgenic plants expressing the potato *SP6A* gene showed only a slight reduction in tuber production (8% yield reduction for the transgenic line with the highest level of 6SPA gene expression).
The use of a heat-inducible promoter allows expression of the gene only under high temperature conditions that would otherwise negatively affect generation and growth.

REF.:
CSIC/AH_001

In heat conditions, control potato plants showed a 30% reduction in tuber production (left), while transgenic plants expressing the potato Sp6A gene showed only a slight reduction in tuber production (right).

Modified immunisation vectors

This invention refers to two vaccine prototypes against HIV/AIDS, referred to as NYVAC-gp140(ZM96) and NYVAC-gag(ZM96)-pol-nef (CN54), based on the attenuated modified vaccinia virus Copenhagen strain with deletion of 18 viral genes (NYVAC). The viral vectors have shown good behaviour in animal models, triggering specific immune responses to HIV antigens in preclinical trials. Phase I/II clinical trials are expected to be initiated in 2013/14.

RESEARCH GROUP:
Mariano Esteban

APPLICATION NUMBER AND PRIORITY DATE:
61/174024, 30/04/2009. Co-titularity with Arizona State University (AZ, USA), Centre Hospitalier Universitaire Vaudois (Switzerland), Leiden University Medical Center (The Netherlands), Université de Montréal (Canada), Sanofi Pasteur Ltd.

INTERNATIONAL PCT APPLICATION:
PCT/US10/032966

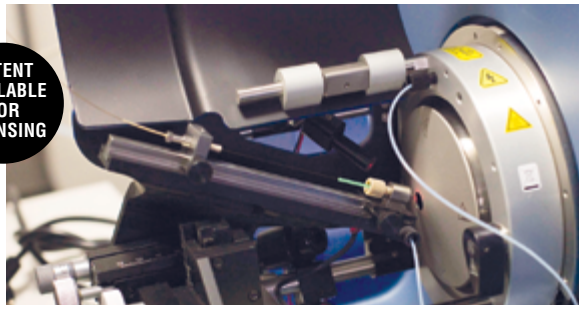
COUNTRIES SELECTED IN NATIONAL PHASE:
EU, US, CA

PRESENT SITUATION:
Patent examination in each country. Patent compromised to Sanofi Pasteur Ltd.

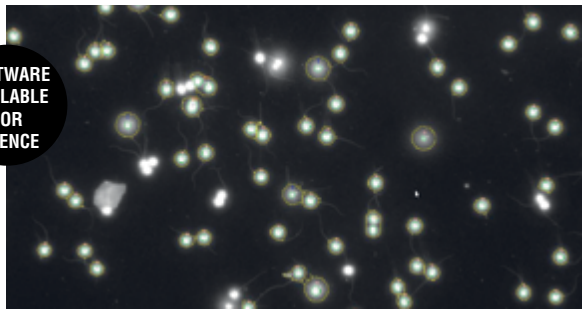
REF.:.
CSIC/AH_010

Confocal microscopy showing production of VLPs from HIV-1 expressed by the HIV/AIDS vaccine candidate NYVAC-Gag-Pol-Nef. In green are the cytoplasmic VLPs and fusion protein, in red the endoplasmic reticulum and in blue the nuclei.

PATENT AVAILABLE FOR LICENSING



SOFTWARE AVAILABLE FOR LICENCE



Method for identifying peptides and proteins from mass spectrometry data

In the course of large-scale proteomics projects, millions of peptide ion collision spectra (MS/MS) may be generated, which must be matched to theoretical spectrum models inferred from known peptide sequences in order to identify proteins

A number of database search engines using different scoring systems have been and are being developed to this end. Scientists at the National Center for Biotechnology have developed a generalised meta-search process that, by integrating partial evidence from any number and type of such database search engines into a single consensus reconstruction, remarkably increases the number of proteins identified. The process may be extended by integration of additional sources of information besides primary search engine results.

RESEARCH GROUP:
Juan Pablo Albar

APPLICATION NUMBER AND PRIORITY DATE:
P200930402, 01/07/2009

INTERNATIONAL PCT APPLICATION:
PCT/ES10/070445

COUNTRIES SELECTED IN NATIONAL PHASE:
EU, US

PRESENT SITUATION:
Patent examination in each country

MAIN INNOVATIONS AND ADVANTAGES:
It is extremely flexible and remarkably increases the number of proteins identified.
High level of accuracy in terms of error rate control.

REF.:
CSIC/AH_002

High throughput, proteome-wide identification of proteins using tandem mass spectometry requires computational methods to interpret and filter large sets of peptide ion collision data.

Automatic classification of spermatozoids from microscopic images

This is image processing software that automatically classifies the quality of a sperm sample.

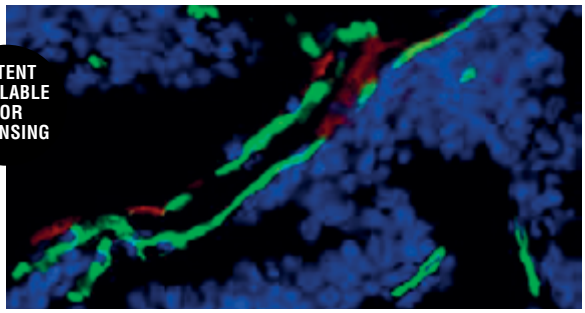
The software has been integrated into a customised microscope especially designed for high-throughput analysis of these kinds of samples.

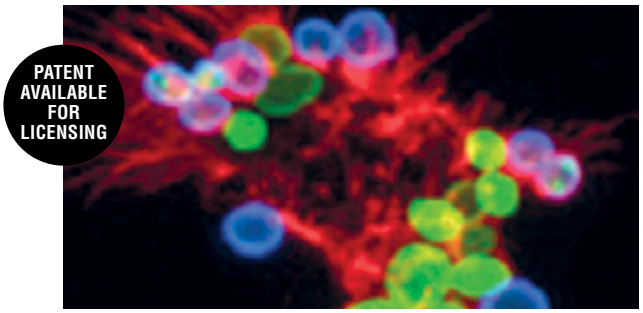
RESEARCH GROUP:
Carlos Óscar Sánchez Sorzano
In co-titularity with Roberto Marabini Ruiz (UAM) and Halotech DNA S.L.

PRESENT SITUATION:
Licence pending

Segmented sperms are highlighted in yellow with centers in green. The objective is to measure the rate of fragmented sperm cells over normal ones.

Technology offer





Use of SOD-3 for adjuvant immunotherapy of cancer

The group lead by Santos Mañes has demonstrated that the forced expression of the extracellular superoxide dismutase, also known as SOD3, in the tumour microenvironment increases the number of cytotoxic T lymphocytes infiltrating breast tumours, thus favouring the immune-based eradication of neoplastic cells. SOD3 is expressed at high levels in the mammary epithelium but its expression is drastically decreased in breast tumours. SOD3 or compounds emulating its enzymatic activity, or drugs that increase SOD3 expression in tumours might thus be useful to elaborate new pharmaceutical compositions for the prophylaxis and immunotherapy of cancer.

RESEARCH GROUP:
Santos Mañes

APPLICATION NUMBER AND PRIORITY DATE:
P201031015, 30/06/2010

PRESENT SITUATION:
Spanish patent is pending

Antibody anti-dectin-1, hybridoma producer of this antibody and its applications

The CSIC and the Biomedical Research Foundation of the La Princesa Hospital have developed an antibody to detect dectin-1 protein.

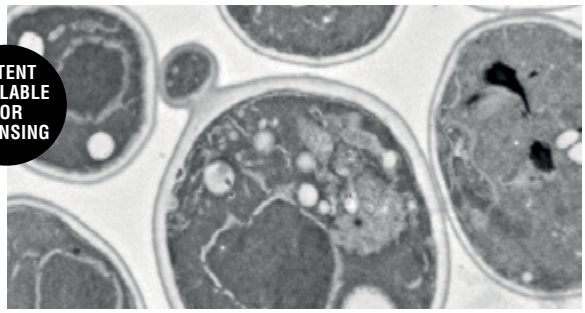
RESEARCH GROUP:
Leonor Kremer Barón

APPLICATION NUMBER AND PRIORITY DATE:
200801766, 11/06/2008

PRESENT SITUATION:
Spanish patent has been granted (07.02.2011)

SOD3 staining (red) in normal glands and breast tumors; blood vessels are stained in green.

Phagocytosis and killing of Candida albicans by human monocyte-derived dendritic cells expressing dectin-1. Confocal microscopy images showing extracellular yeast cells (blue), extracellular and internalized yeast cells (green), and actin (red).



Monoclonal antibody against the protein GAPDH from *Candida famata*

PATENT AVAILABLE FOR LICENSING

The CSIC and the Universidad Autónoma Foundation have developed a monoclonal antibody to detect GAPDH protein from *Candida famata*. This antibody could be useful for the diagnosis of infections produced by the microorganism and for its follow-up.

RESEARCH GROUP:
Leonor Kremer Barón

APPLICATION NUMBER AND PRIORITY DATE:
200930966, 06/11/2009

PRESENT SITUATION:
Spanish patent has been granted (11-06-2012)

Yeast samples were fixed and embedded in Eppon resin following standard procedures. Ultrathin sections were prepared and stained with uranyl acetate and lead citrate. Pictures were obtained in a Jeol Jem 1010 EM equipment operated at 100 kv.

Method for extracting hydrolised and native gluten

PATENT AVAILABLE FOR LICENSING

CSIC has developed a method for extracting gluten from food. The invention can be used to extract gluten from food that has been subjected to different thermal and/or hydrolytic treatments and in which the gluten-forming proteins can be hydrolysed and the structure thereof modified. The method is compatible with enzyme immunoassay systems for gluten quantification, such as competitive ELISA, as well as with other techniques used in gluten analysis.

RESEARCH GROUP:
Juan Pablo Albar Ramírez

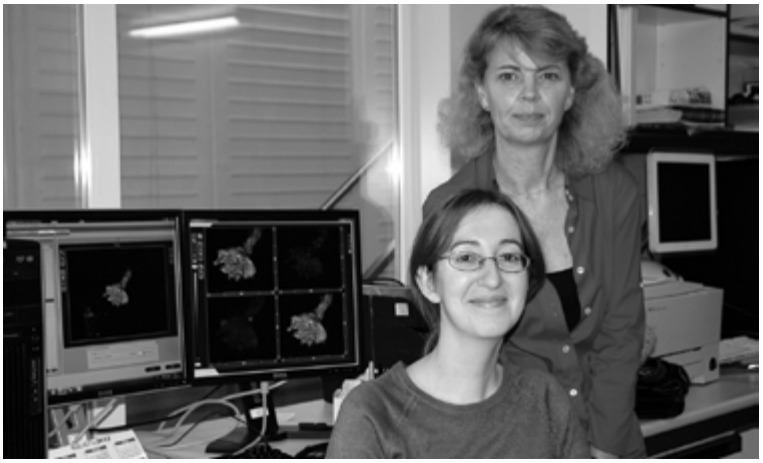
APPLICATION NUMBER AND PRIORITY DATE:
P200930445, 13.07.2009

INTERNATIONAL PCT APPLICATION:
PCT/ES2010/070487

PRESENT SITUATION:
Spanish patent has been granted (28-01-2011)

Method to extract gluten proteins from food that has been subjected to different thermal and/or hydrolytic treatments.

08 Scientific Services



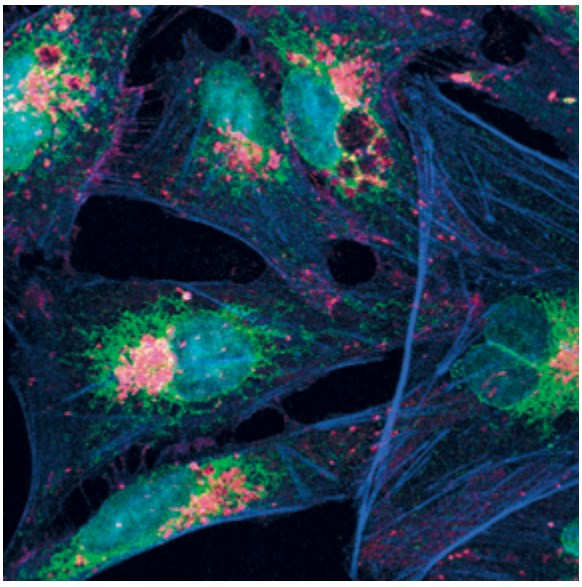
Confocal microscopy service

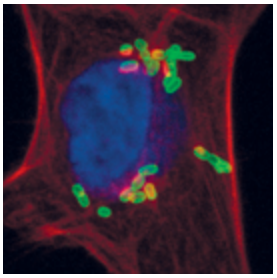
LEAD SCIENTIST:
Sylvia Gutiérrez Erlandsson

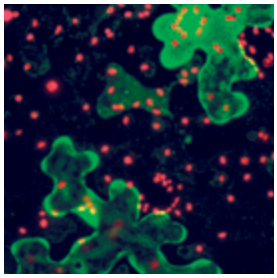
PERSONNEL:
Susana Hernández García

Confocal microscopy imaging techniques use lasers and electronic systems of digital image capture to provide optical sections of the material. 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 service provides infrastructure for fluorescence, confocal laser scanning microscopy and image processing tools, covering most light microscopy applications, with technical assistance to all its users. The equipment and services are available to all CNB personnel as well as to researchers from the public and private sectors. The technical staff offers training and advice about equipment use, available methods, and for image processing, quantification and analysis if required. Aliquots of secondary antibodies and probes with broad use in fluorescence microscopy applications are also provided.







THE FACILITY'S EQUIPMENT INCLUDES:

- Confocal multispectral Leica TCS SP5 system. Laser lines: 405, 458, 476, 488, 514, 561, 594 and 633 nm. Incubation system for *in vivo* studies
- BioRad Radiance 2100 confocal system. Laser lines: 457, 476, 488, 514, 543 and 637 nm
- Fluorescence microscope Leica DMI6000B with incubation system for *in vivo* studies and OrcaR2 monochrome digital camera for image detection
- Two epifluorescence microscopes (Leica DMRXA and Zeiss Axiophot) with colour digital cameras and one Leica stereomicroscope
- The unit also provides offline computer workstations for fluorescence and confocal image processing and analysis (LAS AF, MetaMorph, Image J, Laser Pix, Huygens, Imaris)
- Auxiliary equipment: CO_2 incubator, centrifuge, laminar flow chamber, freezer

LASER SCANNING CONFOCAL MICROSCOPY APPLICATIONS:

- Multichannel confocal imaging + transmission imaging of living cells or fixed samples (2D, 3D, 4D imaging)
- High speed confocal microscopy
- Multidimensional *in vivo* time-lapse experiments
- FRET, FRAP, photoactivation, photoswitching, lambda scan, calcium imaging
- Subcellular colocalisation studies

WIDEFIELD APPLICATIONS:

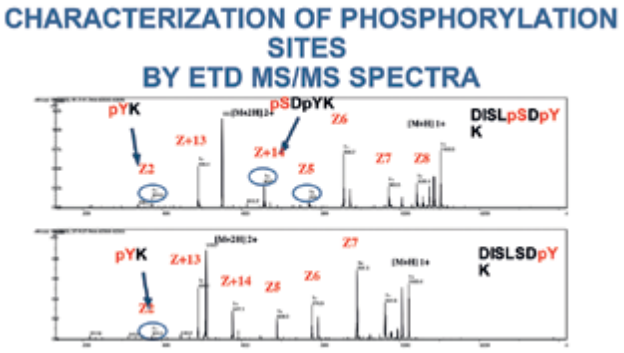
- Multichannel fluorescence imaging + transmission imaging (BF, DIC, phase contrast)
- Multidimensional *in vivo* time lapse experiments (wound healing, infection, etc.)
- Tile scan imaging

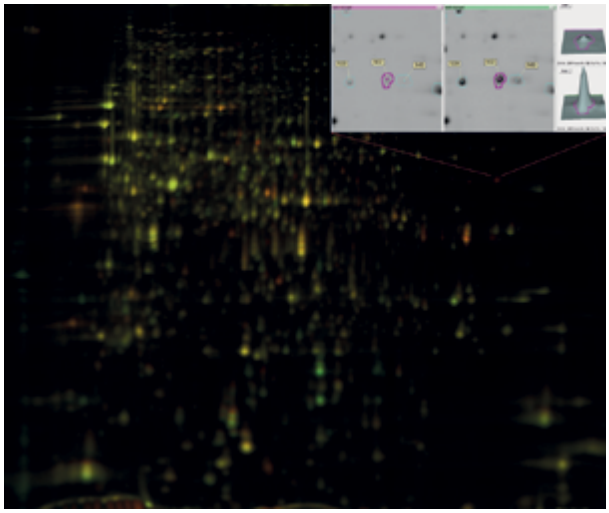
Proteomics

LEAD SCIENTIST:
Juan Pablo Albar

PERSONNEL:
Alberto Paradela
Rosana Navajas
Silvia Juarez
Sergio Ciordia
Marisol Fernández
Fernando Roncal
Manuel Lombardía
María del Carmen Mena
Alberto Medina

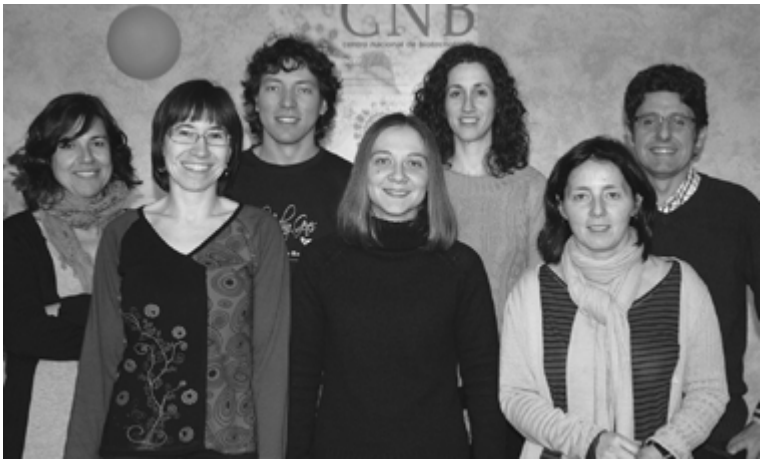
Created in 1999, the CNB Proteomics Facility maintains a technological platform suitable for large-scale protein identification and characterisation, offering its services to the CNB scientific community as well as to external researchers. Massive protein identification and characterisation is performed by multidimensional nano-HPLC chromatography coupled to a nano-electrospray ion trap mass spectrometer (MS), to a TripleQ-TOF MS, or to a MALDI TOF/TOF MS (LC-MS/MS). Differential proteomics (quantitative proteomics) is done by analysis of fluorescent-labelled samples and differential 2D-electrophoresis (2D-DIGE), as well as by stable isotope labelling (ICPL, SILAC, iTRAQ) in combination with LC-MS/MS. SELDI-ToF MS (surface enhanced laser desorption-ionisation-time of flight mass spectrometry) is used to obtain protein expression profiles. We also offer targeted and quantitative protein analysis by selected/multiple reaction monitoring (S/MRM-MS). Prolamin detection and characterisation by ELISA, quantitative PCR and mass spectrometry are also offered in our analysis portfolio. For educational purposes, we organise practical courses on topics such as quantitative proteomics and bioinformatics. The head of the CNB Proteomics Facility (Dr JP Albar) is also the General Coordinator of Proteored-ISCIII (Plataforma en Red de Proteómica-Carlos III).





SERVICES:

- Two-dimensional gel electrophoresis/differential proteomics (2D-DIGE)
- Protein identification and characterisation by MALDI-TOF/ TOF, TripleQ-TOF, ProteinChip/SELDI-TOF and ESI MS/MS mass spectrometry
- Selected/multiple reaction monitoring (S/MRM-MS)
- Protein profiling, purification and biomarker determination by SELDI-TOF MS
- Identification and characterisation of post-translational modifications
- Peptide synthesis and membrane-bound peptide array design
- Gluten analysis by ELISA, PCR and mass spectrometry



Genomics unit

LEAD INVESTIGATOR:
José Manuel Franco Zorrilla

PERSONNEL:
Irene López-Vidriero
Gloria García Casado
Marta Godoy
Juan Carlos Oliveros
Iria Calvete
Luis Almonacid
Eduardo Gil
Beatriz Martín

The Genomics Unit at the CNB focusses on gene expression analysis using microarrays (or DNA "chips"). This technology allows the study of gene expression from different biological samples, interrogating the activity of thousands of genes or complete genomes at once, which will help to elucidate the genetic basis of biological processes under study. We routinely hybridise and analyse one- and two-channel microarrays. The platforms we support now include Affymetrix, Agilent and custom microarrays.

We offer our services to CNB and external researchers; they include microarray printing, RNA integrity analysis and microarray hybridisations. We also provide statistical analysis and bioinformatic support. Raw data are statistically analysed using state-of-the-art algorithms and filtered results are supplied to customers in an easy-to-use, web-based tool developed in our unit. We offer advice and support in the use of several bioinformatic tools for functional analysis of genes and genomes, helping customers with the biological interpretation of the results.

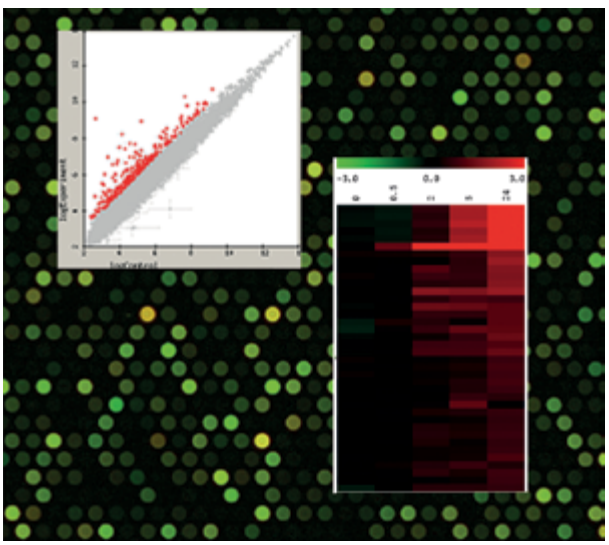
We also offer the possibility of validating gene expression data by real time qPCR analysis.

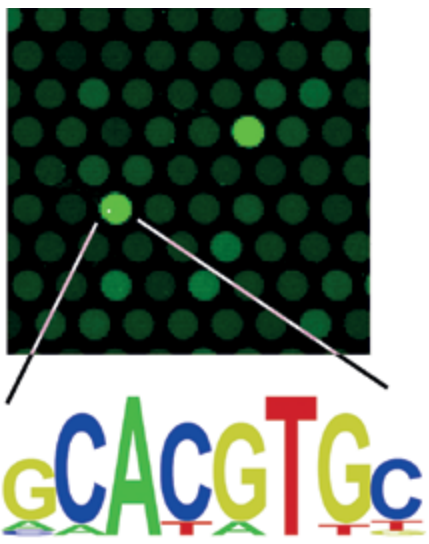
Through the Genomics Unit, the CNB participates in the CSIC-PCM Ultrasequencing Platform, physically located at the Parque Científico de Madrid installations. This platform can perform massive sequencing experiments using Genome Analyzer or Genome FLX systems, and allows the sequencing of complete genomes, transcriptomes, small RNAs or DNA/RNA-protein interactions.

Research projects are constantly being developed by our personnel to implement new services and technologies for customers. These include microarray-based technologies such as a new DNA chip for studying DNA-protein interactions, analysis of the translatome, and new strategies for analysis of the miRNA-guided degradome.

EQUIPMENT

- Complete Affymetrix platform, including fluidics station, hybridisation oven and scanner (3000 7G)
- High-resolution scanner for 1- and 2-colour microarrays (Agilent Microarray Scanner)
- Hybridisation system for NimbleGen microrarrays
- Microarray spotter MicroGrid II (Genomic Solutions)
- Bioanalyser 2100 (Agilent) for analysis of RNA/DNA sample integrity
- Automated liquid-handling workstation (Biomek 2000, Beckman Coulter)
- Laser scanner for 2-colour microarrays (Axon 4000B)
- 7900HT Fast Real-Time PCR System (Applied Biosystems)





TILLer service

LEAD INVESTIGATORS:
Carlos Alonso-Blanco
José M. Martínez-Zapater

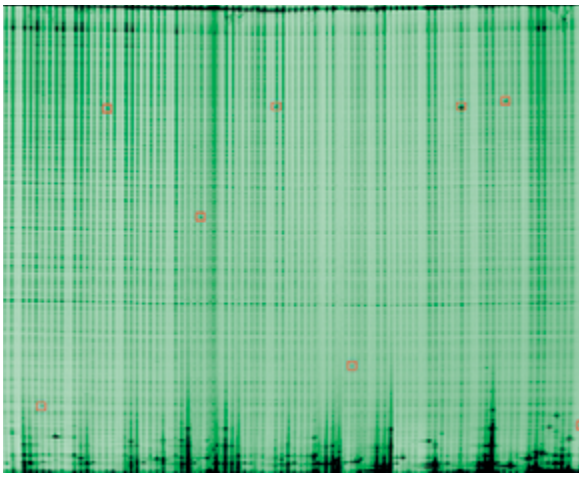
PERSONNEL:
Beatriz Martín Jouve

TILLer Service is an international public service to search for EMS-induced mutants in the model plant *Arabidopsis thaliana*. TILLer is available through the web page (http://www.cnb.csic.es/~tiller) or through the international *Arabidopsis* web page (http://www.Arabidopsis.org/).

In recent years, the TILLer Service has searched for chemically induced mutants by applying the TILLING (Targeting Induced Local Lesions in Genomes) technique in an EMS collection of 3712 mutants developed by the service for this purpose (Martin *et al.*, 2009). To date, the service has sought mutants in more than 25 genes derived from applications from several countries, and it has identified more than 500 mutants in those genes.

REFERENCE

Martín B, Ramiro M, Martínez-Zapater JM, Alonso-Blanco C (2009) A high-density collection of EMS-induced mutations for TILLING in Landsberg erecta genetic background of *Arabidopsis*. BMC Plant Biology 9:147



Detection of Arabidopsis TILLer mutant lines by LICOR 96-well denaturing gels (768 mutant lines) run after CELI digestion of heteroduplex DNA. Pools containing mutations are marked in red.

Bioinformatics initiative

LEAD INVESTIGATOR:
Alberto Pascual-Montano

PERSONNEL:
Juan Carlos Oliveros
Alberto Medina
Mónica Chagoyen Quiles
José R. Valverde
Carlos Óscar Sánchez Sorzano

In an effort to improve the visibility and coordination of the existing bioinformatics services at the CNB, the centre has promoted the creation of the Bioinformatics Initiative, whose objective is to provide coordinated analysis services to all CNB research groups. The initiative also provides a series of monthly seminars, as well as training courses in various bioinformatics topics.

THE BIOINFORMATICS INITIATIVE IS COMPOSED OF SIX SERVICES:

- Computational Genomics: This service provides bioinformatics support for the analysis, visualisation and interpretation of genomics-related projects.
- Sequence Analysis and Structure Prediction Service: Provides bioinformatics support for sequence and structural prediction topics.
- Scientific Computing Service: Covers general scientific data analysis needs of the CNB and maintenance of the national EMBnet node.
- Computational Proteomics: This service is part of the Proteomics Facility; it provides interpretation, validation and reporting of data derived from proteomics experiments.
- Functional Analysis: This service is part of the Functional Bioinformatics group and provides functional analysis of high-throughput experiments such as microarrays and next-generation sequencing studies.
- Statistical Analysis: This service provides statistical support and consultancy, from experimental design to complex statistical data analysis.

Computational genomics

LEAD SCIENTIST:
Juan Carlos Oliveros Collazos

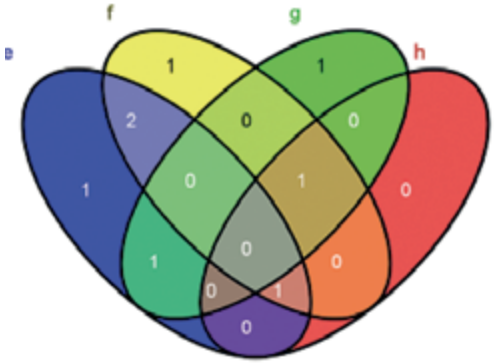
What would you do if you could sequence everything?
Avak Kahvejian, John Quackenbush & John F Thompson, 2008

Current advances in genomics-related technologies such as DNA microarrays and, more recently, ultrasequencing methods allow life science researchers to gather huge amounts of genome-wide data in little time and at a relatively low cost. Transforming these (raw) data into results, and these results into relevant biological conclusions, requires integrating specific biology and informatics skills, and the use of special software and hardware. The CNB's Computational Genomics service provides researchers with global bioinformatics support for the analysis, visualisation, and interpretation of data obtained in their genomics-related projects.

AMONG OTHER SERVICES WE OFFER:

- Assistance in experimental design for ultrasequencing and DNA microarray projects
- Biostatistical support for the correct interpretation of genomics-related results
- Genomic data viewer development and maintenance
- Development of final user interfaces for third-party bioinformatics tools
- Organisation of periodic courses and tutorials on bioinformatics and genomics

In short, in the Computational Genomics Service we try to fill the gap between the complex outcome of the many powerful biostatistical methods available and the final user's needs that require placing these heterogeneous results in the context of their research projects.



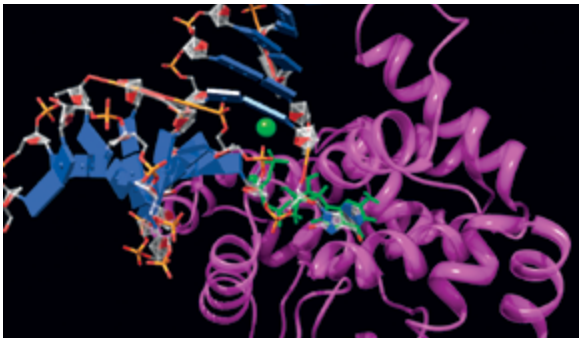
Scientific computing

GROUP LEADER:
José Ramón Valverde Carrillo

The Scientific Computing Service provides support for the solution of scientific problems that require advanced computing solutions using clusters and supercomputers located at the CNB, CSIC and CESGA, as well as distributed grid infrastructures.

CURRENTLY, OUR MAIN AREAS OF WORK ARE:

- NGS and metagenomics, with special emphasis on the analysis of biodiversity and *de novo* genome sequencing (with support from EU COST action SEQAHEAD, in which the service participates as national coordinator), pioneering the optimisation and development of analytic pipelines
- Nanotechnology and analysis of macromolecular interactions (protein-protein, protein-nucleic acids, biomolecule-inert substrates, drug-receptor) to the quantum chemical level (using techniques that include docking, molecular dynamics, quantum dynamics and quantum chemistry)
- Statistical analysis of scientific data using the programming language R
- Modelling of complex biological systems (bacterial communities, mutation dynamics, etc.) using advanced mathematical techniques
- Optimisation and reduction of research costs in the life and health sciences through the use of free software (coordinating CYTED Iberoamerican Network of Excellence 510RT0391 FreeBIT), through the organisation of meetings, courses, talks and presentations on free software, and promotion of scientific exchanges with Iberoamerica
- Delivery of web-based computing services and maintenance of various web sites (www.free-bit.org, www.es.embnet.org, sci.cnb.csic.es) specialised in scientific computing and training
- The service is available to the wider international community



Sequence analysis and structure prediction

LEAD INVESTIGATOR:
Mónica Chagoyen Quiles

PERSONNEL:
Juan Carlos Sánchez Ferrero

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

IN OUR ANALYSIS WE COMMONLY:

- 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 in the use of sequence-based methods
- Generation of high-quality protein sequence/structure images for publication

In collaboration with other CNB services, we also organise periodic courses on bioinformatic approaches for sequence analysis and protein structure prediction.

The service is offered to the CNB-CSIC as well as to other academic institutions and private organisations.

Macromolecular X-ray-crystallography

LEAD INVESTIGATOR:
César Santiago

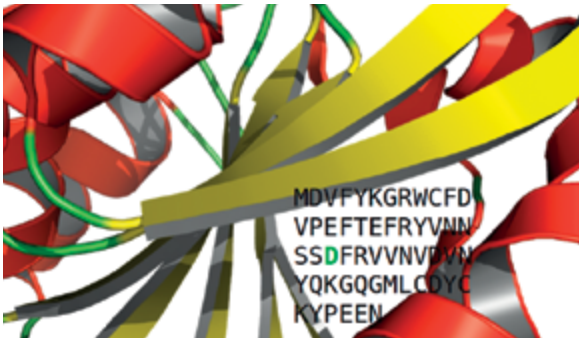
Protein X-ray crystallography is a high-resolution technique that allows us to study protein structure at the atomic level. This method provides a detailed view of protein function, ligand and protein interactions, supra molecular organisation and mutants related to human diseases. Great improvements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

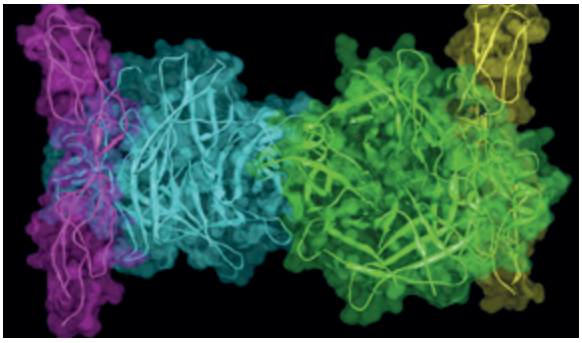
THE FACILITY PROVIDES THE FOLLOWING TECHNIQUES:

- Advice and supervision on protein production, from cloning to expression in bacterial, yeast and eukaryotic systems
- Support and training on protein purification to obtain crystal-grade protein for crystallisation
- Automated macromolecular crystallisation
- Crystallisation conditions optimisation, applying standard and in-house techniques
- Crystal mounting. Access to synchrotron beam time. X-ray diffraction data collection
- Data processing and structure resolution and analysis

SERVICE EQUIPMENT:

- Three temperature-controlled crystallisation rooms
- Genesis RSP 150 workstation (Tecan Trading AG) nanodispenser robot
- Rigaku Desktop Minstrel system for automated crystallisation plate visualisation
- CrystalTrak database suite for crystallisation screening and improvement of positive trials





CNB mouse embryo cryopreservation facility

LEAD INVESTIGATOR:
Lluís Montoliu

PERSONNEL:
Julia Fernández Punzano
María Jesús del Hierro Sánchez
Óscar Javier Sánchez Sánchez
Marta Castrillo Labrador

The CNB Mouse Embryo Cryopreservation Facility offers the possibility of cryopreserving transgenic and mutant mouse lines as frozen embryos and/or sperm. We also offer thawing of frozen mouse sperm and/or embryos and revitalisation of the cryopreserved mouse line. The cryopreservation of mouse lines is a highly recommended procedure to preserve animal models used in biology, biomedicine and biotechnology laboratories for long periods of time, safely and stably, without the need to maintain lines alive. This saves space and money and optimises the use of experimental animals, complying with current legislation on animal welfare.

We offer a variety of services and the latest methods in the field, including freezing 8-cell mouse embryos, freezing IVF-derived 2-cell mouse embryos, thawing mouse embryos and associated embryo transfer procedures to suitable pseudopregnant females for the revitalisation of mouse lines, freezing mouse sperm, thawing mouse sperm and *in vitro* fertilisation (IVF), storage of cryopreserved mouse embryos or sperm in liquid nitrogen. The CNB also hosts the Spanish node of the European EMMA project (European Mouse Mutant Archive, HYPERLINK http://www.emmanet.org), coordinated by Dr. Lluís Montoliu. The objective is the cryopreservation, organised archiving and coordinated distribution of mouse lines of interest to the biomedical research community. The current EMMA project, EMMAservice (2009-2012), funded by the European Commission (7th Framework Programme), has been extended for four additional years under the new EU Project Infrafrontier-I3 (2013-2016), approved by the EU Commission in 2012.

In 2012, the CNB-CSIC and the CNIO joined forces and signed an agreement to archive and distribute mutant mice of interest in biomedical research, generated by CNIO investigators, through the EMMA project and its Spanish node at the CNB-CSIC.

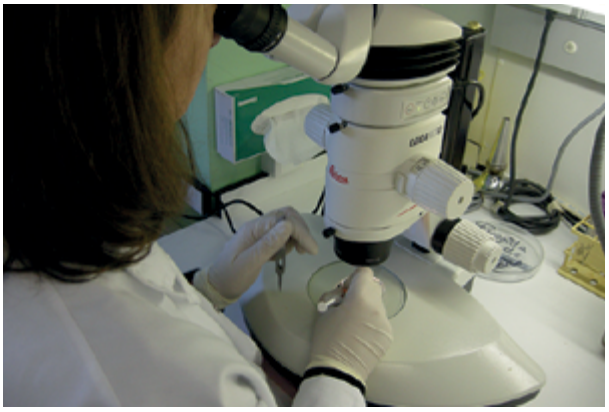
Also in 2012, the CSIC and the University of Kumamoto signed a cooperation agreement to promote exchange of knowledge, personnel and information on mouse embryo and sperm cryopreservation and archiving activities undertaken by the Spanish EMMA node at the CNB-CSIC and the CARD archive, coordinated in Japan by Prof. Naomi Nakagata. The CNB Mouse Embryo Cryopreservation Facility is integrated within the INNOTEK Scientific-Technological Platform in support of research, part of the UAM+CSIC International Campus of Excellence.

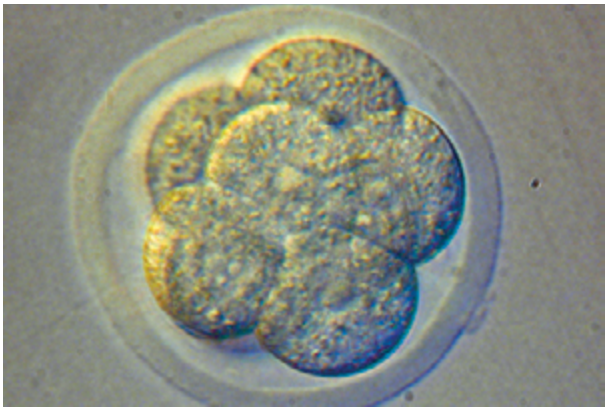
PUBLICATIONS

Kollmus H, Post R, Brielmeier M, Fernández J, Fuchs H, McKerlie C, Montoliu L, Otaegui PJ, Rebelo M, Riedesel H, Ruberte J, Sedlacek R, de Angelis MH, Schughart K. Structural and functional concepts in current mouse phenotyping and archiving facilities. J Am Assoc Lab Anim Sci. 2012 Jul;51(4):418-35

Montoliu L. Mendel: a simple excel workbook to compare the observed and expected distributions of genotypes/phenotypes in transgenic and knockout mouse crosses involving up to three unlinked loci by means of a $\chi 2$ test. Transgenic Res. 2012 Jun;21(3):677-81

Montoliu L, Whitelaw CB. Using standard nomenclature to adequately name transgenes, knockout gene alleles and any mutation associated to a genetically modified mouse strain. Transgenic Res. 2011 Apr;20(2):435-40



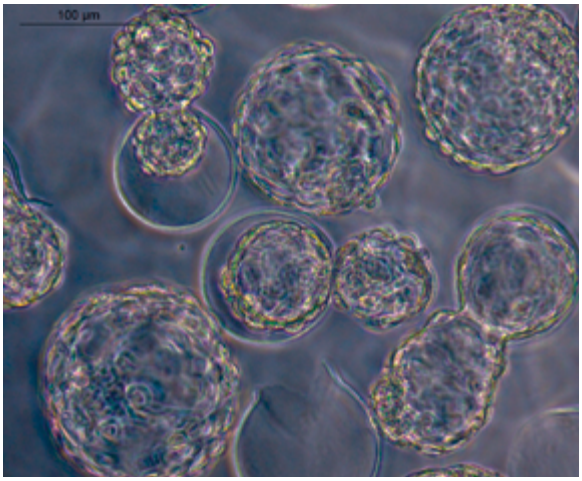


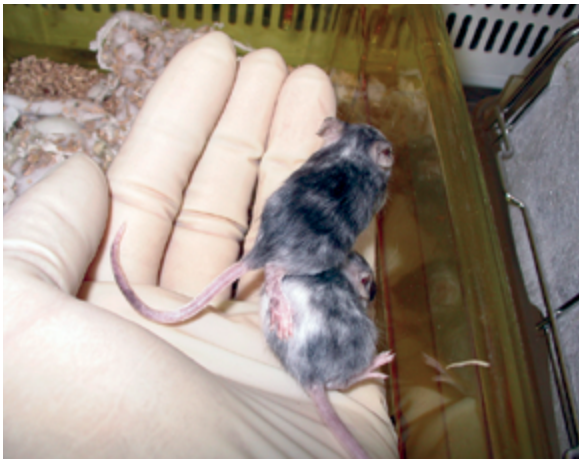
Transgenesis

LEAD INVESTIGATOR:
Mª Belén Pintado Sanjuanbenito

PERSONNEL:
Alfredo Serrano Montalbo
Marta García Flores
Verónica Domínguez Plaza

The CNB-CBMSO Transgenesis Unit provides support to researchers linked to the Platform CSIC-UAM in the creation, establishment and interchange of genetically modified mouse models. The unit offers technical and scientific advice on the best strategy to achieve the desired model, either by additive transgenesis or targeted mutagenesis (KO and KI). We also facilitate the incorporation of those models already available from international consortia or as a result of scientific interchange when the health status of the original colony does not meet the requirements of our centres. In addition, support is provided for breeding schemes to ensure the most suitable genetic background.





SERVICES:

- Advice in the design of target vectors or constructs for microinjection
- Pronuclear microinjection of plasmid, BAC or YAC DNA
- Vector electroporation in R1 or G4 ES cell lines
- Zinc finger nuclease injection
- International consortia ES cell handling
- ES cell injection or aggregation to generate chimaeras
- Embryo rederivation through IVF or embryo transfer
- DNA purification and founder identification by PCR on request
- Reproductive biotechnology to solve breeding problems of genetically modified mice
- Support in the generation, establishment and management of genetically altered mouse lines
- These activities are combined with training and education on demand, and applied research to develop and refine reproductive technologies to enhance transgenic production efficiency or colony management.

SPECIALISED EQUIPMENT:

- Two microinjection systems with hydraulic micromanipulation system and Eppendorf femptojet injector
- One electric microinjection system with piezo drill
- Dissecting microscopes
- Microforge and pipette puller
- Thermocycler and electrophoresis equipment
- Fully equipped laboratory for ES cell handling

Histology

LEAD SCIENTIST:
Lluís Montoliu

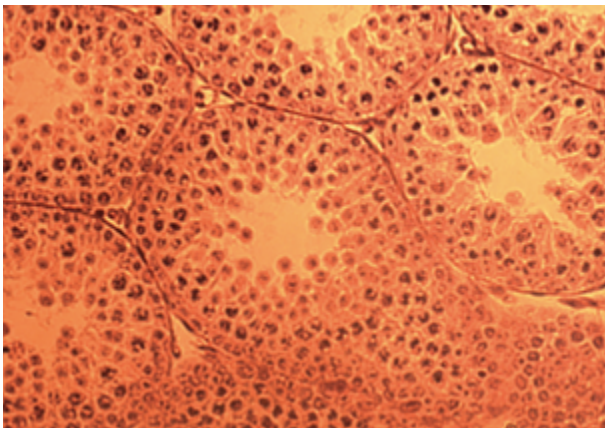
PERSONNEL:
Soledad Montalbán Iglesias
Óscar Sánchez Sánchez

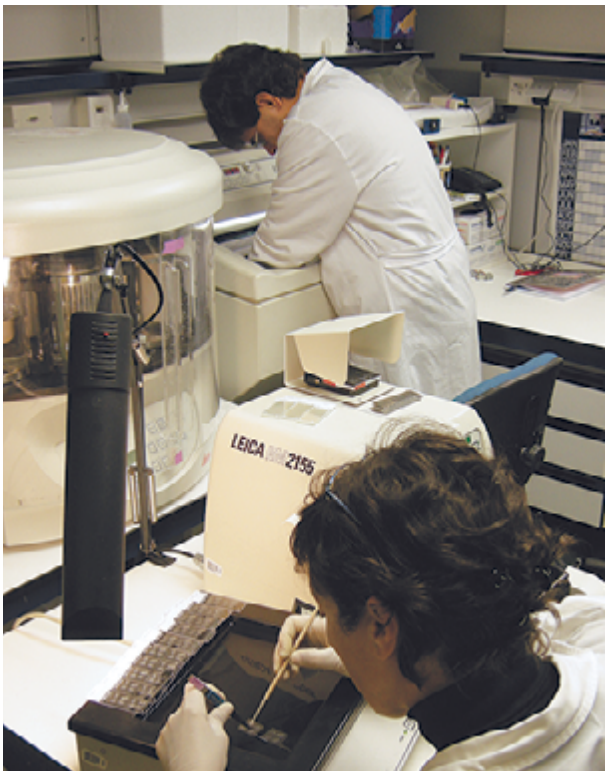
At the CNB Histology Facility, we offer methods for the histological analysis of animal and plant biological samples. Available methods include the preparation of paraffin/wax blocks and plastic (Historesin) for obtaining histological sections with the automated microtome, and the preparation of blocks for obtaining sections from frozen tissue with the cryostat. Sections can be counterstained or assigned for later analysis by immunohistochemistry.

The facility is equipped with a cryostat, two automated microtomes, a tissue processor carrousel, a paraffin/wax embedding machine, two water baths, a stereoscope, an oven, and additional small equipment to process all kinds of tissue samples. Our expertise is reflected by the large variety of tissue samples and species we have processed in the past, from animals and plants. An updated list of tissues processed by the CNB Histology Facility is available at HYPERLINK http://www.cnb.csic.es/~histocnb/tabla.html http://www.cnb.csic.es/~histocnb/tabla.html

Since 2009, the CNB Histology Facility is associated with the IIB-UAM/CSIC Histology Facility (HYPERLINK http://www.iib.uam.es/servicios/patexperimen/intro.es.html). Both centres merged the operations of their facilities under the coordination of the CNB Histology Facility, offering CNB and IIB researchers increased processing capacity of histological samples.

The CNB Histology Facility is integrated within the INNOTEK Scientific-Technological Platform in support of research, part of the UAM+CSIC International Campus of Excellence. The CNB Histology Facility web site handles all submissions electronically.



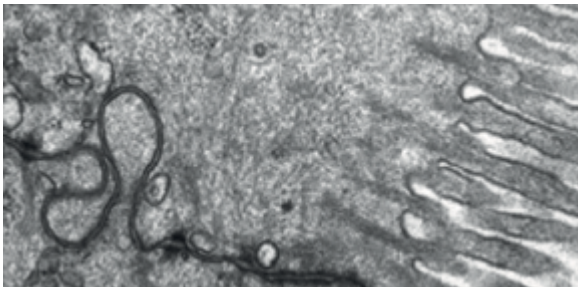


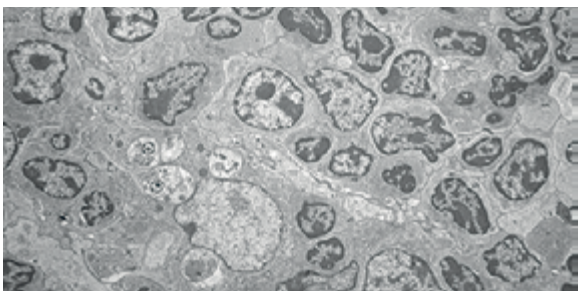
Electron microscopy

LEAD INVESTIGATOR:
Cristina Patiño Martín

PERSONNEL:
Javier Bueno Chamorro
Rocío San Andrés Cervilla

The Electron Microscopy Service offers a variety of equipment and techniques for the preparation, processing and analysis of biological samples (cell and bacterial cultures, cell fractions, proteins, viruses, animal and plant tissues) by transmission electron microscopy. The technical staff provides support to users in the correct use of equipment and methodologies. We offer regular training in the techniques and methods available. We also carry out sample preparation, if required, as well as image acquisition, and provide support for data interpretation. Techniques offered include chemical fixation and inclusion in epoxy and acrylic resins, cryofixation (plunge freezing, high pressure freezing), freeze substitution and inclusion in low temperature resins, ultramicrotomy, negative staining, immunonegative staining, immunolabeling, in situ hybridisation, conventional transmission electron and low-dose electron microscopy.





In vitro plant culture

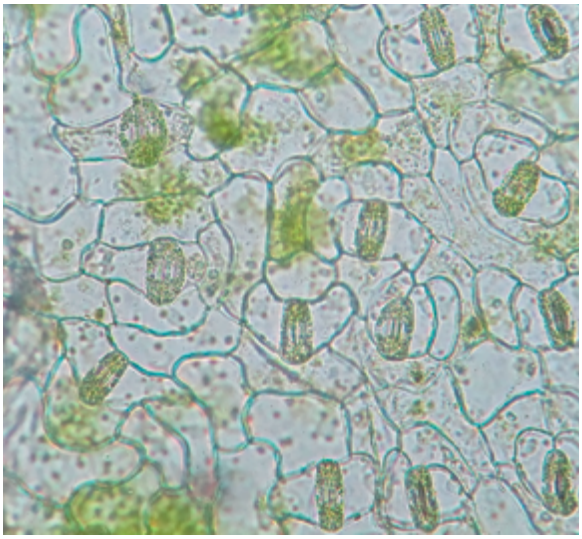
HEAD OF SERVICE:
Raquel Piqueras Martín

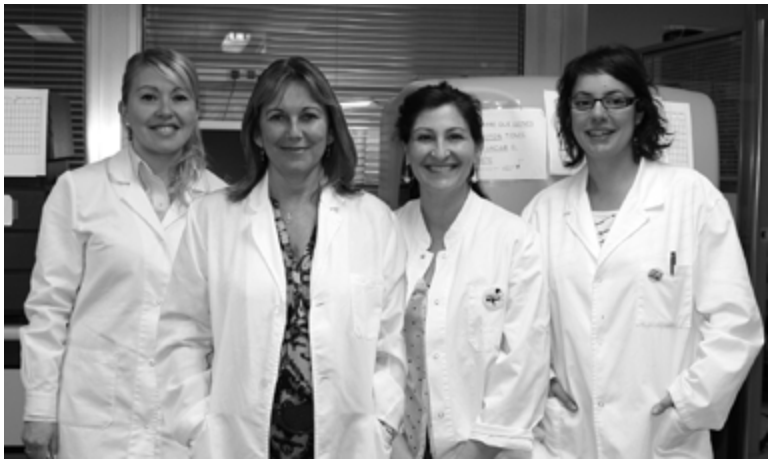
PERSONNEL:
María Luisa Peinado Vallejo
Beatriz Casal López

TASKS PERFORMED BY THE SERVICE:

- Preparation of media
- Sterilisation of seeds and seed sowing in plates
- Maintenance of plants, plant cell cultures and plant callus
- Explant propagation
- Selection of *Arabidopsis* transformants
- Transformation of *Nicotiana* spp.
- Transformation of *Solanum* spp.
- Transformation of *Lycopersicum* spp.
- Transformation of *Oryza* spp.
- Induction of *Arabidopsis* callus, and induction of plant shoots and plant roots from callus
- Storage of wild seeds of the species most frequently used at the CNB
- Mesophyll protoplast preparation

These are the routine tasks carried out in the service, although we are available for many other types of laboratory work.



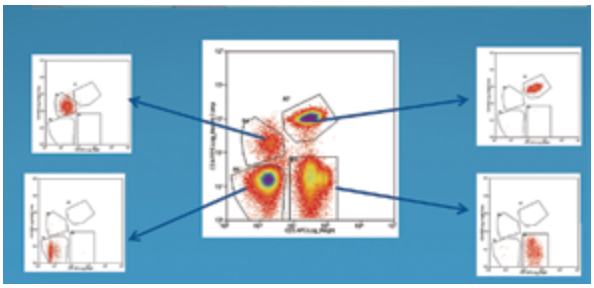


Flow cytometry

LEAD SCIENTIST:
María del Carmen Moreno-Ortiz Navarro

PERSONNEL:
Sara Escudero García
Sonia Rodríguez Murcia
Almudena González García

The facility provides scientific and technological support to CNB research groups. We have the technical and human means to guarantee maximum quality and continuous improvement of our services.

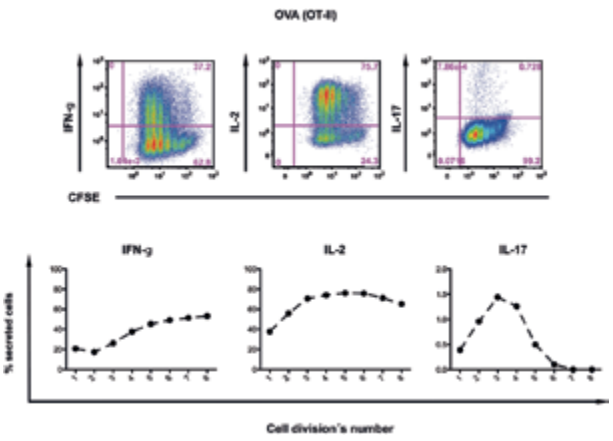


THE FACILITY OFFERS:

- Training in and advice on the principles and applications of analytical flow cytometry, to obtain maximum advantage
- Development and optimisation of applications that incorporate new technologies and reagents. In the past two years, the unit has optimised CSFE incorporation in cytokine expression assays
- Quantification of secreted cytokines by multiplexed assays
- Results analysis using specialised software
- Cell isolation by cell sorting (sorting of cell populations including cell suspensions derived from any animal organ and from cell lines)

THE FACILITY'S EQUIPMENT INCLUDES:

- BD FACSCalibur Analyser: 4 colours, 2 laser excitation (488 nm and 633 nm)
- Beckman Coulter EPICS XL-MCL Analyser: 4 colours, 1 laser excitation (488 nm)
- Beckman Coulter CYTOMICS FC 500 Analyser: 5 colours, 2 laser excitation (488 nm and 633 nm)
- Beckman Coulter CYTOMICS FC 500 Analyser: 5 colours, 1 laser excitation (488 nm)
- BD LSRII Analyser: 8 colours, 3 laser excitation (488 nm, 633 nm and 405 nm)
- Beckman Coulter GALLIOS Analyser: 10 colours, 3 laser excitation (488 nm, 633 nm and 405 nm)
- Luminex 100 IS Multiparametric Analyser: A system that can be used to quantify multiple cytokines (up to 100) or any other soluble molecule from a single sample
- Recently, the unit acquired a Cell Sorter Beckman Coulter Moflow XDP: 10 colours, 3 laser excitation (488 nm, 633 nm and 405 nm)
- The facility also provides the computer science system to analyse the results obtained: 2 PC platforms running specialised software packages (WindMDI , CXP, MultiTime, MultiCycle, DIVA, Flowjo, Summit, Kaluza



Protein tools unit

LEAD INVESTIGATOR:
Leonor Kremer

PERSONNEL:
María Teresa Martín (Molecular Interactions)
Mónica García-Gallo (Immunobiology)
Mercedes Llorente (Immunochemistry)
Tamara Rueda (Technician)
Laura Martín (Technician)
Lucio Gómez (Animal Facility)

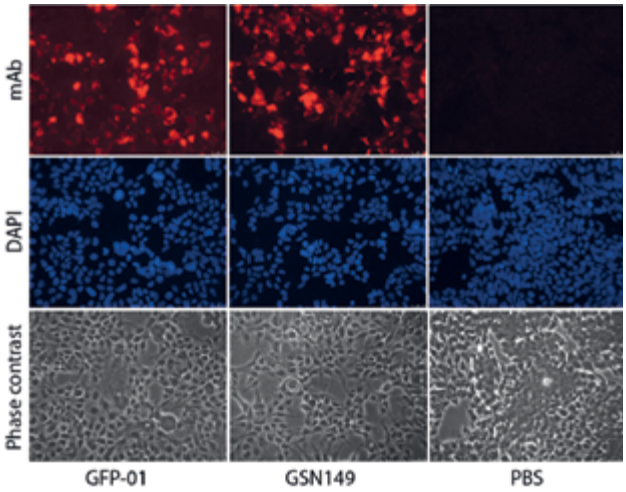
The Protein Tools Unit (PTU) is focussed on the design, production and characterisation of custom monoclonal antibodies (mAb), immune response studies, development of specific immunoassays, protein labelling and biomolecular interactions analysis.

A wide panel of mAb against different types of antigens has been developed, including fluorescent proteins (GFP), blood proteins (coagulation Factor V), neurodegenerative disease-related proteins (TAU, beta amyloid peptides), membrane raft proteins (MYADM), chemokine receptors (CCR9), FERM-containing proteins (Protein 4.1R) and nuclear proteins (Dido).

The facility has a surface plasmon resonance biosensor for the characterisation of biomolecular interactions in real time and determination of kinetic and affinity constants. This technique is applied to a wide range of samples such as proteins, antibodies, nucleic acids, carbohydrates, lipids, low molecular weight compounds, liposomes and viruses.

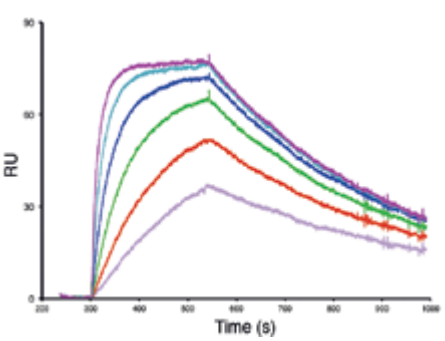
Research tools and services are provided to scientists from the CNB, other CSIC institutes, universities, public research organisations and private companies.

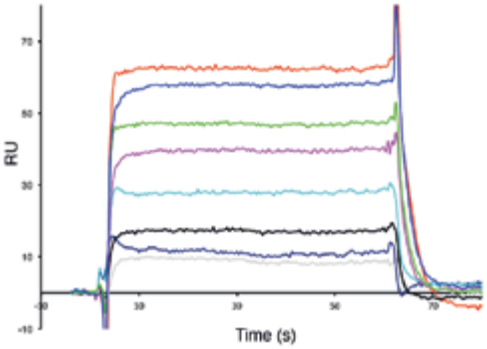
The core facility offers expertise in immunobiology and immunochemistry, technical assistance, data analysis, training in specific techniques, introduction of new methodologies and advice. In addition, the core facility organises theoretical and practical training courses.



EQUIPMENT:

- Biological safety cabinets (Nuaire 437-400E)
- Centrifuges and microfuges (Hettich)
- Inverted fluorescence microscope (Zeiss Axiovert 40 CFL).
- CO2 incubators (Thermo Steri-Cult)
- ÄKTAprime plus chromatography system (GE Healthcare)
- SPR Biacore 3000 (GE Healthcare)
- EnVision 2104 Multilabel Reader (Perkin Elmer)
- Thermal cycler (Eppendorf AG)
- Microplate reader (Bio-Rad 680)
- Protein gel electrophoresis and Western blotting systems (Mini-PROTEAN 3 and Mini Trans-blot cells)
- Electrophoresis power supply units (Bio-Rad PowerPac Basic and Universal).





Radiation protection & biological safety

HEAD OF SERVICE:
Fernando Usera Mena

SUPERVISOR:
Sonia Calvo Ladrero

TECHNICIANS:
Jessica Gaspar Navarro
Aránzazu de la Encina Valencia

FEATURES AND SERVICES:

- Risk assessment
- Acquisition of security materials
- Design of laboratories and facilities
- Acquisition and management of radioisotopes
- Editing health and safety manuals
- Processing of legal documentation for undertaking activities and operating conditions
- Training and information in chemical, biological and radiation for staff
- Classification and signposting in laboratories
- Control of compliance with health and safety norms, and of operation and use of facilities
- Control and management of staff medical and dosimetry surveillance; records maintenance
- Intervention in accidents and emergencies
- Control of production and processing of hazardous waste
- Internal transport and storage of waste for transfer to authorised or controlled disposal

FACILITIES:

The Service supervises hazardous operations in CNB laboratories and directly manages the gamma irradiator and the central radioisotopes laboratory, which has:

- 2 safety cabinets for radioisotopes
- CO_2 incubator
- Biosafety cabinet
- Ultracentrifuge, centrifuges and microcentrifuge
- Speed vac
- Hybridisation oven

The Service manages the level 3 biological containment laboratory, consisting of three sublaboratories for in vitro culture with all necessary equipment for safe handling of Risk Group 3 biological agents and contained use of genetically modified organisms.

THE LABORATORY HAS THE FOLLOWING EQUIPMENT:

- Three biosafety cabinets
- Two incubators for animal cell culture
- A tissue culture incubator
- A double-door autoclave
- A SAS for biological inactivation of small materials
- A SAS for biological inactivation of large materials
- A refrigerated ultracentrifuge
- Three refrigerated benchtop centrifuges
- Three refrigerated microcentrifuges
- Three inverted optical microscopes
- A liquid nitrogen tank
- Three ultra-freezers (-80°C)
- Communications systems: computer network and telephony
- Various alarm systems in case of malfunction, incident or accident

Animal facility

LEADING SCIENTIST:
Angel Naranjo

RESEARCH TECHNICIAN:
Javier Martín Torre

SHIPMENT COORDINATOR AND ADMINISTRATION:
Alberto García

AREA AND COLONY MANAGERS:
Antonio Morales
Raquel Gutiérrez
Eladio Martínez

ANIMAL TECHNICIANS:
Angel Moreno
Israel López
Rebeca Acuña
Sergio Magallón
Alicia González
Lola García
Susana Marcos
Patricia Sanz
Ivan Jareño
Raquel Castañera

The Laboratory Animal Facility is an area dedicated to the production and maintenance of experimental animals, supporting research, essential techniques, and legal support for this duty.

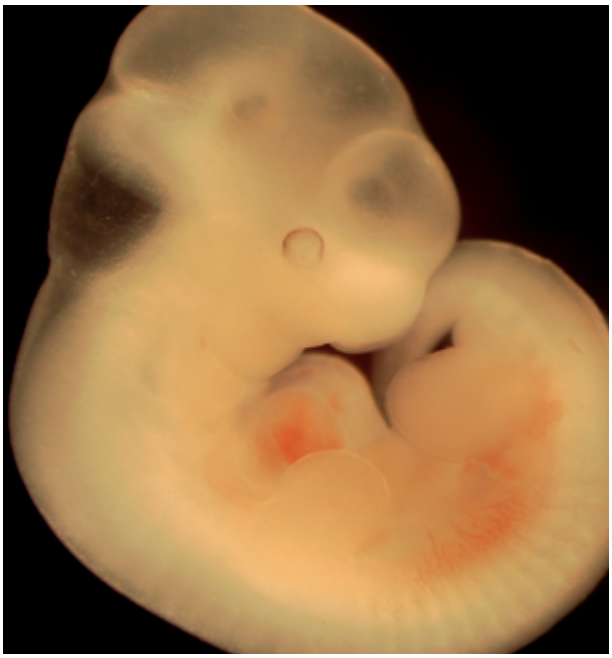
Most of the experimentation is carried out with genetically modified mice and zebra fish. Our animal facilities and equipment are specially designed for these models. The laboratory animal service provides a controlled environment for the animals, with periodic control of diet, water, temperature, air, housing and husbandry conditions.

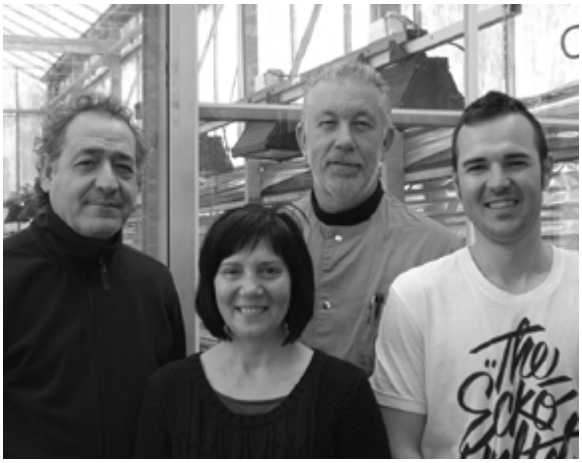
The unit is separated into several areas: quarantine, conventional, and specific pathogen-free (SPF), depending on the microbiological status of the animals; we provide special housing conditions for conventional, genetically modified, and immunodeficient animals, depending on the experimental objectives. At the same time, a totally isolated biosafety area is dedicated to *in vivo* experiments using biological agents.

The animal facility staff gives service to laboratories for obtaining commercial lines and strains of animals, shipping animals for collaboration with other institutes, and 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 provide services for various techniques used in in mouse research models. Veterinary staff give research assistance in surgical techniques, selection of animal models, animal health surveillance, laboratory animal care, and animal well-being.

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





Greenhouse

HEAD OF SERVICE:
Tomás Heras Gamo

PERSONNEL:
Alejandro Barrasa Fustes
Esperanza Parrilla Carrillo
Raúl Pedraza León

SERVICE:

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

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

DIO tissue culture

HEAD OF SERVICE:
Rosa María Bravo

MEDIA PREPARATION:
Ana Montero

CELL CULTURES:
Anunciación Romero

WASH AND STERILISATION:
Enrique Méndez

Washing & sterilisation

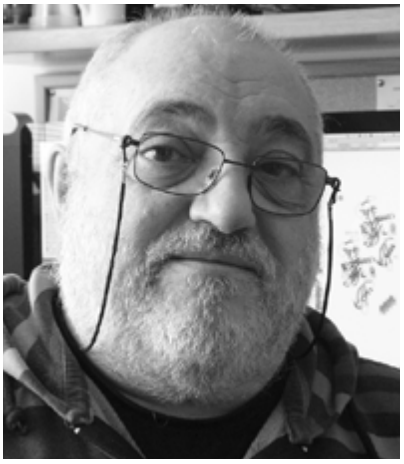
HEAD OF SERVICE:
Rosa María Bravo

PERSONNEL:
Arancha Rodríguez Martínez
Carmen Berdeal Mera
Angeles Sanchez Pérez
Rosita Ramos Hernández
Ana Isabel Nieto Jimenez
Josefa Pérez Alfaro
Carlos Enriquez Casas
Margarita Felipe Hombrados
Concepción Cobeña Chivato
Josefa Pérez Alfaro
Laura Muñoz Calvo
Margarita Felipe Hombrados
María Concepción Cobeña Chivato
María Trinidad Mba Ondo

09 Administration and Technical Services

General manager

Miguel Anchuelo



Outreach management

HEAD OF SERVICE:
Alfonso Mora

COORDINATOR:
Miguel Vicente

Through the organisation of meetings, workshops and lectures, the CNB maintains scientific contact with researchers worldwide. Our website allows us to inform other scientists as well as laypeople of our activities.

We elaborate press releases and documentation to help journalists explain the work our scientists develop at the CNB to a broader audience.

In collaboration with national education agencies, we are trying to reach high school and graduate students by organising visits, talks and activities for pupils and/or teachers. As part of our program to bring science to society, over the past two years more than 1,000 students visited the CNB.

Library

HEAD OF SERVICE:
Mª Dolores Aparicio Trujillo

SERVICES:

- Acquisition, technical processing, cataloguing and classification of monographs and serial publications
- Home-lending to personnel and laboratories
- Interlibrary loan service
- Reading room
- Access to online databases, e-journals and e-books
- Information and reference service

Human resources

HEAD OF SERVICE:
Marina Hernando Bellido

PERSONNEL:
Javier Tortosa Nieto
María Jesús Torrado Macías
Luciano López Hernández

Information technologies

HEAD OF SERVICE:
Sonia de Diego

PERSONNEL:
Óscar Bodas
Íñigo Oficialdegui
Alberto Sánchez

SERVICES:

- Microinformatics support
- Registry of equipment on the network
- E-mail accounts
- Management of distribution lists
- Management of network infrastructure (cable and wireless)
- Shared archive servers
- Information systems security
- Remote access
- Web server (not content)
- Registry of DNS equipment
- Internet protocol telephony (shared with General Services)
- Server backups

Project management

HEAD OF SERVICE:
Soraya Olmedilla María

PERSONNEL:
Aurora Cabrerizo Alonso
Pilar Ara Laúna
Daniel Martín Hernando
Moira Torrent Enjuto

Economic management

HEAD OF SERVICE:
Mariano Muñoz Jiménez

PERSONNEL:
Rafael López Laso
Elena Barreda García
Mª José Gregorio Usanos
Mª Virginia Hortal Roble
Irene Vera Jiménez
Diana Gloria Pastor Calero
Carmen Berreiros Cano
Mª Ángeles Lumbreras Carrasco
Mª Jesús Raboso Pérez

We support researchers in their administrative needs, making payments, revenue control, travel management and cost justification, etc.

Purchasing and supplies

HEAD OF SERVICE:
Ramon Serrano Coronado

PERSONNEL:
Mª José Caballero Martín
Gloria del Sastre Martín
Julio Díez Alvarez
Antonio Pastor Encabo
Juan Carlos Bermudo Zamora
Héctor Hernández Redondo

Maintenance service

HEAD OF SERVICE:
Antonio Dueñas

PERSONNEL:
Enrique Mejías
Fulgencio Moreno
Conrado Pedraza
Jesús González
Juan Carlos Cuenca
Alfonso García

Instrumentation service

HEAD OF SERVICE:
Ismael Gómez López

PERSONNEL:
Carlos González Redondo
Juan Ignacio Golpe de la Fuente

DIO management

HEAD OF SERVICE:
Antonio Rodríguez Terán

PERSONNEL:
Coral Bastos
Catherine Mark

Workshop

HEAD OF SERVICE:
Daniel Pastora

General services

HEAD OF SERVICE:
Gabriel Sanchez de Lamadrid

PERSONNEL:
Julián Grande
Manuel Grande
Vicente Martín
David Suarez

PHOTOGRAPHY:
Inés Poveda

BUILDING CONSTRUCTION AND RENOVATION:
Javier Zarco

Security

HEAD OF SERVICE:
Sócrates Gutiérrez

PERSONNEL:
Angeles Ferreiro

10 Annexes

Scientific meetings

At the CNB, we host meetings and seminars to exchange scientific results and knowledge

During the years 2011 and 2012, a total of 322 seminars were held, with speakers from outstanding national and international institutions.

We also held various workshops, meetings and courses organised with the collaboration of CNB researchers:

7-8 FEBRUARY 2011
Instruct end of preparatory phase meeting program
Jose María Carazo

4 MARCH 2011
Advances on P13K-based cancer fight
Ana Clara Carrera

11 MARCH 2011
Meeting + Bioc
Víctor de Lorenzo & Miguel Vicente

1 JUNE 2011
Desafíos en diseño e interpretación de experimentos en Biacore 3000
Leonor Kremer

14 JUNE 2011
Plataforma de secuenciación CSIC-FPCM
José Manuel Franco

2 DECEMBER 2011
Systems & synthetic biology program
Víctor de Lorenzo

19-20 DECEMBER 2011
XIX CNB scientific workshop
Alfonso Mora

21 DECEMBER 2011
Avances de biología molecular por jóvenes investigadores en el extranjero
Domingo F. Barber
Yolanda R. Carrasco
Lourdes Planelles

5 MARCH 2012
DIFHEMAT network meeting
Carlos Ardavín

27 MARCH 2012
Experimentos científicos en un entorno de cero emisiones
Juan P. Albar

13 APRIL 2012
Workshop on the study of hepatitis C virus infection
Pablo Gastaminza

28-29 MAY 2012
Madrid meeting on dentritic cells and macrophages 2012
Carlos Ardavín

2-3 JULY 2012
Workshop NGS and metagenomics
José Ramón Valverde

29-31 AUGUST 2012
15th ENPER meeting
Enrique Rojo & Roberto Solano

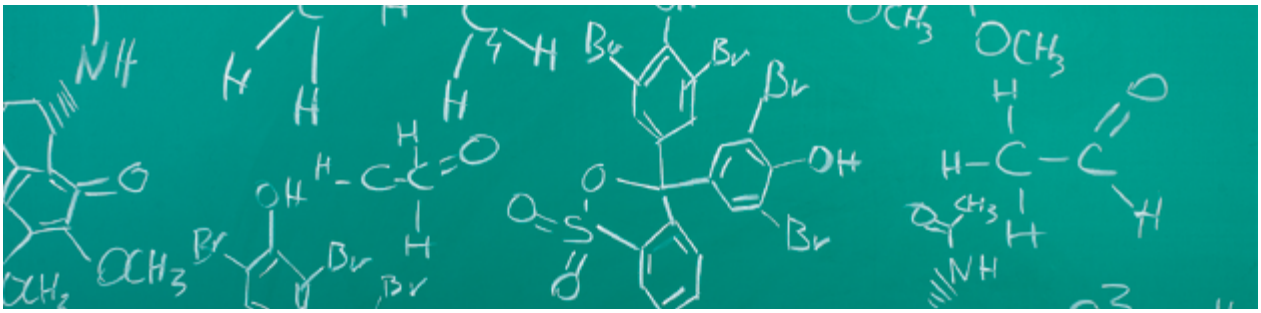
18 SEPTEMBER 2012
Workshop sobre plásmidos catabólicos
Víctor de Lorenzo

30 NOVEMBER 2012
CNB: 20 years of excellence in life sciences
José María Valpuesta

18-19 DECEMBER 2012
XX CNB Scientific Workshop
Alfonso Mora

20 DECEMBER 2012
Avances de biología molecular por jóvenes investigadores en el extranjero
Domingo F. Barber,
Yolanda R. Carrasco
Lourdes Planelles

Doctoral theses



In 2011 and 2012, 54 students obtained the PhD degree under the supervision of CNB researchers

José Ignacio Agulleiro Baldo
Reconstrucción tomográfica ultrarrápida en procesadores multicore (José Jesús Fernández)

Roberto Alfonso Dunn
Interrelación entre el virus de la gripe y la cromatina cellular: estudio del remodelador de cromatina CHD6, modulador negativo de la infección viral (Amelia Nieto Martín)

Alejandro Arce Rodríguez
Regulation of gene expression by the cAMP-Crp system in the soil bacterium *Pseudomonas putida* (Víctor de Lorenzo & Belén Calles)

Pilar Ayuda Durán
Inicio de replicación y estabilidad genómica en *Saccharomyces cerevisiae* (Arturo Calzada)

Ángela Ballesteros Morcillo
Caracterización de las proteínas TIM como receptores de fosfatidilserina (José María Casasnovas)

Carmen Calabia Linares
La sinapsis inmune: papel de clatrina como remodeladora del citoesqueleto de actina y posible vía de transmisión de infecciones bacterianas (Esteban Veiga Chacón)

Cristina Cañas Muñiz
Papel de las proteínas RuvA, RuvB y RecU en las etapas tempranas y tardías de la recombinación homóloga en *Bacillus subtilis* (Juan Carlos Alonso Navarro & Silvia Ayora Hirsch)

Ximena Cardona López
Identificación de un componente del sistema de endomembranas, AtALIX, implicado en la señalización del ayuno de fosfato en plantas (Javier Paz-Ares & Vicente Rubio)

Max Chavarría Vargas
Regulation of PTS systems and their interplay with carbon central metabolism in *Pseudomonas putida* (Víctor de Lorenzo Prieto)

María Victoria Cepeda
Caracterización de las interacciones del cytomegalovirus humano con las membranas celulares (Alberto Fraile-Ramos)

Lidia Agnieszka Daszkiewicz
Non-apoptotic Fas functions are critical in the control of T cell expansion (Dimitrios Balomenos)

Lorena Domínguez Acuña
Análisis de funciones de *Salmonella enterica* que responden al estado de no proliferación en el interior de fibroblastos (Francisco García del Portillo)

Sofía Domingo Vera
Análisis functional de DREAM en los mecanismos de excitabilidad y viabilidad neuronal (José Ramón Naranjo)

David Fernández Antorán
Función de c-Myc en la regulación transcripcional de la diferenciación de linfocitos B (Ignacio Moreno de Alborán Vierna)

Patricia Fernández Calvo
Identificación de nuevas dianas de los represores JAZ y caracterización de su papel en la activación de respuestas a la fitohormona jasmonato en *Arabidopsis thaliana* (Roberto Solano Tavira)

Dolores María Fernández Messina
Studies on the biochemistry and cell biology of the glycosylphosphatidylinositol anchored NKG2D-ligands (Mar Valés-Gómez & Hugh T. Reyburn)

Pilar Fonseca García
Búsqueda y caracterización de nuevos factores implicados en represion catabólica en *Pseudomonas putida* (Fernando Rojo de Castro & Renata Moreno Albíger)

Ana Franco Villanueva
Caracterización del papel de WIP en morfogénesis neuronal (Inés Antón Gutiérrez & Francisco Wandosell Jurado)

Pedro Gallego Jiménez
Regulación del ciclo celular y la integridad genómica por la proteína LANA2 de KSHV (Carmen Rivas Vázquez)

Esther Isabel García Arranz
Caracterización de los genes lsp y tatC implicados en la maduración y plegamiento de proteínas secretadas en *Streptomyces lividans* (Rafael Pérez Mellado)

Araceli García Castro
Caracterización de la implicación de la proteína APRIL en el cáncer de mama (Lourdes Planelles Carazo)

Esther García Tirado
In vivo and *in vitro* studies of the lrpC-topB operon of *Bacillus subtilis* (Silvia Ayora Hirsch)

Leonor Garmendia Jorge
Elementos de captura génica en ambientes naturales: Integrones (José Luis Martínez)

Doctoral theses

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El gen 7 de Alfacoronavirus 1 contrarresta las defensas del huésped y modula la virulencia del virus (Luis Enjuanes & Sonia Zúñiga)

Satish Kulasekaran
Identification of genes controlling the action of 9-LOX oxylipins in plant defense (Carmen Castresana Fernández)

Mariana Lara Neves
Minería de texto aplicada a bioinformática funcional (Alberto Pascual-Montano)

Miguel Ángel López Carrasco
Las enzimas 9-lipoxigenasas y el etileno regulan la respuesta de estrés oxidativo durante la activación de la inmunidad vegetal (Carmen Castresana Fernández)

Borja López Holgado
Estudio de los mecanismos moleculaes activados por CXCL12 en células progenitoras neurales: implicación de las distintas isoformas de fosfatidil inositol 3-quinasa de clase I en migración (Mario Mellado)

Pedro A. Mateos Gómez
Síntesis discontinua de ARN en coronavirus (Luis Enjuanes & Isabel Sola)

Raquel Mejías Laguna
Nanopartículas magnéticas como sistema de liberación localizada de interferon-gamma para inmunoterapia antitumoral (Domingo F. Barber)

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Generación de nuevos modelos animales para el estudio de la enfermedad de Alzheimer (Lluís Montoliu)

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Proteínas virales y celulares implicadas en la replicación de coronavirus (Luis Enjuanes & Fernando Almazán)

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El proto-oncogén c-myc en diferenciación terminal de linfocitos B: papel en generación de células plasmáticas y linfocitos B de memoria (Ignacio Moreno de Alborán Vierna)

Ana Páez García
Caracterización de mutantes de *Arabidopsis* alterados en la configuración espacial del sistema radicular (Antonio Leyva)

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Protein functional features extracted from primary sequences: a focus on disordered regions (Laura Segatori & Florencio Pazos)

Paloma del Reino Fernández
Implicación de p38ß y p38ß MAPKs en el desarrollo del cáncer de colon asociado a la colitis (Ana Cuenda Méndez)

María Salvador Bernáldez
Papel de Gadd45b y p38 en el desarrollo tímico y la función de células T CD4 (Jesús María Salvador)

Lucas Sánchez Sampedro
Mutantes replicativos y atenuados del virus Vaccinia como candidatos vacunales frente a la leishmaniasis (Mariano Esteban & Carmen Elena Gómez-Rodríguez)

Jesús Sánchez Ruiz
Papel de las GTPasas Ral en la function linfoide y la citotoxicidad celular (Ana María González García)

Laura Sanz Sánchez
Caracterización estructural de filamentos y estructuras membranosas inducidas por la liberación del virus Bunyamwera en células de mamífero (Cristina Risco Ortiz)

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Estudio de la regulación transcripcional de la guía axonal. Papel de los factores de transcripción Cux1, Cux2 y Pax6 (Marta Nieto López)

Carmen de Sena Tomás
Roles of the Atr1-Chk1 pathway in the phytopathogenic fungus *Ustilago maydis* (José Pérez Martín)

Virginia Silió Castrejón
Papel de PI3K IA durante la fase M (Ana Clara Carrera)

Rafael Silva Rocha
The logic of bacterial regulatory networks (Víctor de Lorenzo)

Abel Suárez Fueyo
Estudio sobre el papel de PI3K en la enfermedad del lupus eritomatoso sistémico humano y su posible aplicación terapéutica (Ana Clara Carrera & Domingo F. Barber)

Marco Taurino
Modificación genética de la biosíntesis del ácido jasmónico y efecto en la respuesta de defensa en patata (José Juan Sánchez Serrano & Maite Sanmartín Artiñano)

Pedro Torres Ayuso
Role of DGKα and DGKζ in the control of lipid metabolism in breast cancer: implications for therapeutic intervention (Isabel Mérida & Antonia Ávila Flores)

Doctoral theses

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Computacion matricial dispersa en procesadores graficos (GPUs) (José Jesús Fernández)

Cristina Vázquez Mateo
Papel de FAS y p21 en la limitación de las células T de memoria y la enfermedad autoinmune (Dimitrios Balomenos)

Beatriz Vega Blanco
Aplicación de la resonancia de plasmón superficial al estudio de la interacción CXCL12/CXCR4 (Mario Mellado)

Jorge Vicente Conde
Análisis bioquímico y funcional de oxilipinas involucradas en defensa frente a patógenos (Carmen Castresana Fernández)

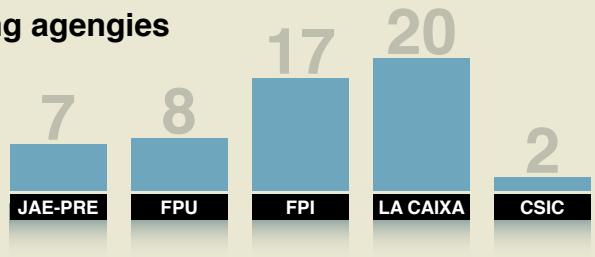
Patricio Yankilevich Farhi
Inferencia de redes génicas. Estudio del INFβen sclerosis multiple (Igor Swir)

Hugo Yébenes Revuelto
Caracterización structural de chaperoninas de tipo II mediante técnicas de criomicroscopía electronic y cristalografía de rayos X (José María Valpuesta Moralejo & Jaime Martín-Benito Romero)

María Esther Zurita Redondo
Alteraciones visuales en un modelo animal de albinismo (Lluís Montoliu)

2011 & 2012
NEW PHD STUDENTS

Funding agengies



Nationalities

TOTAL 54

Scientific advisory board

The Scientific Advisory Board, consisting of internationally recognised scientists, meets with CNB researchers to discuss the quality, significance and main focus of the research conducted at the CNB

Wolfgang P. Baumeister

Electron tomography, proteomics, proteasome structures and function

Department of Molecular Structural Biology - Max-Planck-Institut für Biochemie, Martinsried, Germany

Maarten Koornneef

Plant breeding, genetics

Plant Breeding and Genetics - Max Planck Institute for Plant Breeding, Cologne, Germany

Juan Luis Ramos

Bacterial genetics and molecular biology, biorremediation, microbial ecology

Departamento de Protección Ambiental - Estación Experimental del Zaidín, Granada, Spain

Anne Ridley

Cell signalling, cell migration in cancer and metastasis

Randall Division of Cell and Molecular Biophysics - King's College, London, UK

Anna Tramontano

Genome functional annotation, protein structure prediction, protein design, viral proteins

Department of Physics - University of Rome "La Sapienza", Rome, Italy

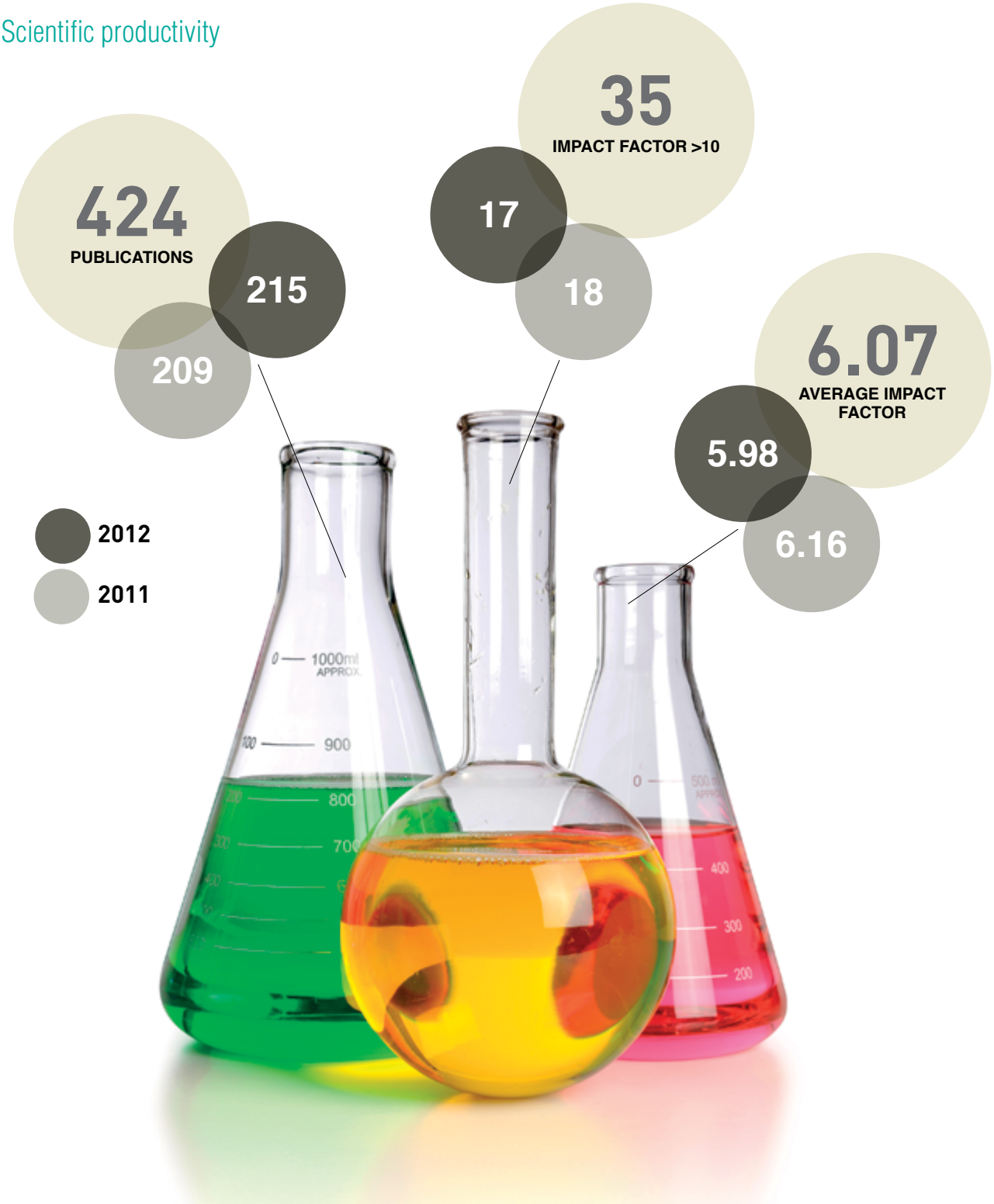
Inder Verma

Proto-oncogenes, gene therapy

Laboratory of Genetics - The Salk Institute for Biological Studies, La Jolla, CA, USA

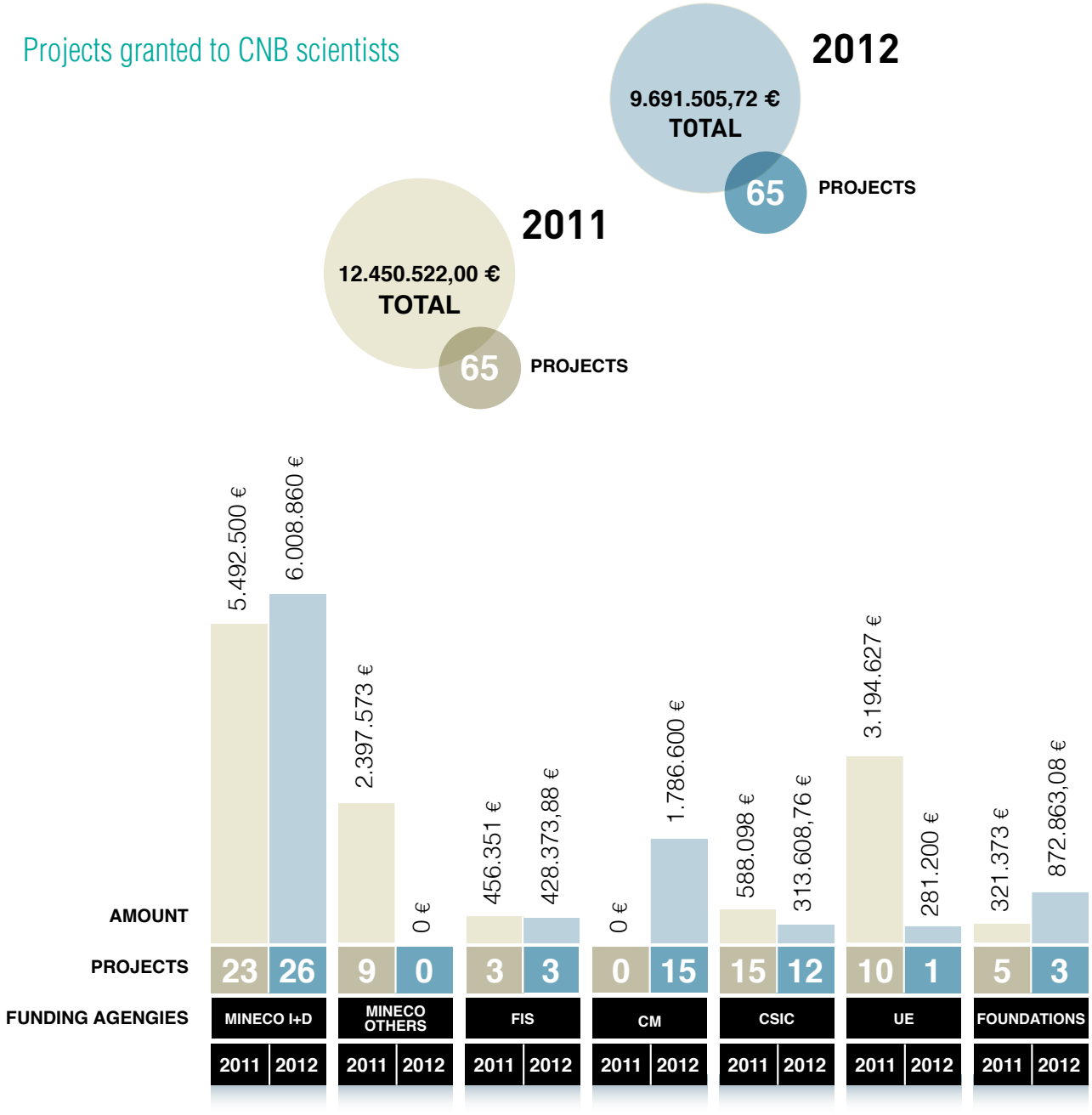
The CNB in numbers

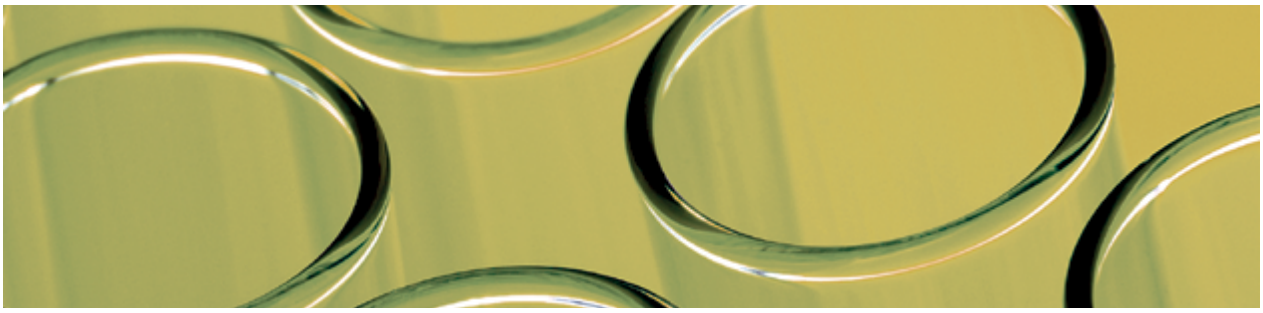
Scientific productivity



The CNB in numbers

Projects granted to CNB scientists





CNB staff

2011

586 TOTAL

407 RESEARCHERS

179 SUPPORT

142 (24%) FOREIGN STAFF (% OF RESEARCHERS)

2012

508 TOTAL

347 RESEARCHERS

161 SUPPORT

138 (39%) FOREIGN STAFF (% OF RESEARCHERS)

R+D=

2011-2012 REPORT

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2011-2012 REPORT

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