CNB

RESEARCH
DEVELOPMENT
INNOVATION
2013/2014 REPORT

CSIC

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EXCELENCIA
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OCHOA

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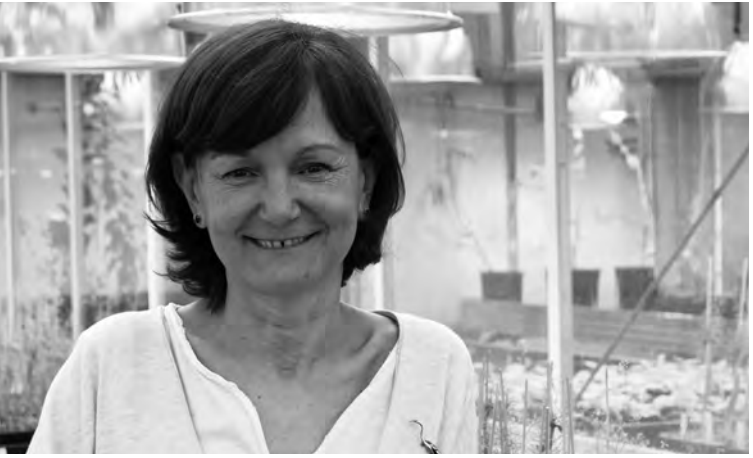

Welcome to the CNB

Carmen Castresana
DIRECTOR

In the past two years we have faced a number of important ups and downs. In 2014, we lamented the passing away of Juan Pablo Albar, in whom we lost a great colleague and the scientist who put our centre on the international map of proteomics research.

To some extent, Juan Pablo's widely known capacity to pursue his goals with unswerving determination also represents the attitude our centre has had to adopt in the face of an unprecedented economic crisis; it would otherwise be difficult to explain how, despite severe cuts in research funding, the CNB has succeeded in preserving its vitality and ability to produce world class science.

When we compare our revenues to the income we had in 2010, we have withstood a net reduction of 10 million euros over the past four years. The efforts of our scientists to pursue their research with these reduced resources have nonetheless made it possible to maintain the quality of our centre's work. For the biennium 2013-2014, the Scopus database registered the exceptional number of 528 scientific publications signed by CNB scientists, 489 in JCR-indexed journals. This represents both a quantitative and qualitative increase. In 2014, 44% of these publications corresponded to the top 10% of high-impact journals in their respective fields of knowledge, which exceeds the centre's five-year average by 8 percentage points.

Highlights such as the ERC Advanced Grants to Víctor de Lorenzo and Víctor Muñoz, the inclusion of Roberto Solano in Thompson Reuter's 2014 ranking of the top 1% of the world's most highly-cited scientists, or the award of the Mexico Prize for Science and Technology 2014 to Carlos Martínez-A, further reflect the scientific leadership of our researchers.

It is also a great satisfaction to see our in-house technology transfer office, established in 2010, has begun to yield returns, evidenced by the filing of eight new patents and the signing of eight license agreements in the past two years. In addition, three young CNB scientists put their business ideas into practice. Antonio Ramos founded Proteobotics, a proteomics data analysis company, distinguished with the 2014 Innovation Award by the Alberto Elzaburu Foundation. Carlos Óscar Sorzano and Lucas Sánchez initiated their entrepreneurial activities as founders of the consulting firm KineStat Pharma and the science promotion and publicity agency Scienseed, respectively.

The organisation of 288 seminars, 38 international conferences and workshops, as well as a 4-week practical summer course for undergraduate students, training of more than 200 master's degree and PhD students from 24 different countries, and the defence of 84 doctoral theses over the last two years are only a few of the numbers that illustrate the rich academic life at the CNB. We encourage the inquisitiveness of the younger members of our society by participating in the annual Spanish Science Week as well as other science fairs and events. Through our programme of guided visits to our laboratories by high schools and other groups, we promote scientific literacy among young people and foster the interest of the next generation of scientists.

We can also be very proud of our accreditation in 2014 as one of the eight Severo Ochoa Centres of Excellence in life sciences and medicine. This distinction recognises the contributions of hundreds of present and past CNB members that shaped the CNB over more than two decades. It is the acknowledgement of the collective effort of the current team of more than 600 people, who depend critically on the quality of the work carried out by each of the others in their research

groups and scientific services, in management, administration, technical support, maintenance and other services.

The benefits of the Severo Ochoa award go well beyond its immediate economic impact. In addition to a concession of 4 million euros, we qualified for two specific calls that grant nine PhD fellowships, six financed by the Spanish government and three by the La Caixa Foundation. In a pilot project, the Women for Africa Foundation selected our centre as one of several prominent institutions that will support two 6-month sabbatical stays of female African scientists. The flexibility of the Severo Ochoa grant has also enabled us to provide the necessary co-financing quota to obtain funding from the European Regional Development Fund, which we will use for the urgent renewal and update of the centre's scientific equipment, for a total value of 3.3 million euros.

There is no doubt that the Severo Ochoa award is associated with many very welcome benefits, but they can only mitigate the effects of years of severe cuts in research funding. A particular challenge for our centre will be the renewal of our scientific staff. The mean age of our group leaders is currently 53 years, and some of our most prominent scientists will retire in the next couple of years. In addition, the severely reduced public employment offer has not allowed career consolidation for many scientists who have been doing excellent research at the CNB for years.

I hope that the joint effort of the 20 Severo Ochoa Centres of Excellence, eight of which form part of the CSIC, can convince our administration and our government that to be internationally competitive, apart from a substantial increase in research funding and provision for human resources, we need to put an end to our struggle against the windmills of excessive bureaucracy. Far from the aims of a scientist's activity, these obstacles jeopardise our efforts with incomprehensible hiring restrictions, unpredictable career tracks, and lack of continuity and long-term vision in research funding.

Although this foreword should limit itself to events at our centre in 2013 and 2014, it is impossible to conclude without expressing, in the name of all of us at the CNB, our deepest appreciation and gratitude to everyone whose exemplary commitment helped to avoid the potentially disastrous consequences of the fire that destroyed our low voltage power plant on 14 February 2015, in which we came very close to losing the invaluable results of many years of research. Our aim for the near future is to implement additional prevention and security protocols, and to obtain the personnel needed to safeguard our scientific patrimony.

It is important that we transmit to our authorities the need to strengthen our institutions and our scientific system. Achieving this goal will very much depend on the support we receive from our administration, our government, and our society, necessary for our efforts to improve health, agriculture and the environment.

Enjoy browsing through the pages of the CNB 2013-2014 report!

Note: The technical approach of this scientific report might be complex for non-specialists. For this reason, we have published a small booklet in Spanish and in English that explains our work at the CNB, why it is important for society and is worth funding. If you visit us, ask for a copy, or read it online at the hyperlinks below.

CNB: Investigar para mejorar nuestra vida
http://www.cnb.csic.es/images/divulg/FolletoCNB2014.pdf

CNB: Research to improve life:
http://www.cnb.csic.es/images/divulg/LeafletCNB2014.pdf

1 / Plant Molecular Genetics

Research in the Plant Molecular Genetics Department studies the molecular basis of the regulatory pathways that control plant development, environmental adaptation and protective responses to biotic and abiotic stresses. Interests of our research groups focus on the control of root architecture, shoot branching, photomorphogenesis and photoperiodism, adaptation to nutrient shortage or to toxic metals, and responses to pathogens and pests. Besides the intrinsic fundamental interest in understanding key biological processes in plants, our research seeks to derive new tools and methods to improve crop production, based on the use of natural biodiversity and on genetic engineering innovations. Biotechnological applications such as the use of plants as biopharmaceutical factories or as tools to fight environmental problems are also being studied. Our research is routinely carried out in the model species *Arabidopsis thaliana* and *Nicotiana benthamiana,* but crop species such as tomato, potato and Prunus are also major subjects of study.

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

PRINCIPAL INVESTIGATOR:
Carlos Alonso-Blanco

POSTDOCTORAL SCIENTISTS:
Belén Méndez de Vigo
Israel Ausín

PREDOCTORAL SCIENTISTS:
Marija Savic

TECHNICAL ASSISTANT:
Mercedes Ramiro

STUDENT:
Verónica Aguilar Perea

The main objective of our research is to understand the genetic and molecular mechanisms involved in plant adaptation. We are particularly interested in determining how developmental traits allow plant adaptation to different climates. To this end, we are dissecting genetic variation in the model annual plant *Arabidopsis thaliana* in nature. Our group currently focusses on two specific objectives.

On the one hand, we are performing genetic analysis of naturally-occurring variation for a key quantitative developmental trait, the timing of flowering. We have analysed the genetic basis of the variation for flowering initiation in relation to several environmental factors (Alonso-Blanco and Mendez-Vigo, 2014). Genetic mapping identified the locus *Flowering Arabidopsis* QTL 1 (FAQ1) as a major effect QTL that strongly affects the flowering response to photoperiod. Fine mapping and complementation demonstrated that FAQ1 is the gene *short vegetative phase* (SVP), a key flowering regulator that encodes a MADS transcription factor. We identified a new functional allele caused by a single amino acid substitution in the MADS domain, which produces SVP loss-of-function and early flowering. This natural allele appears to be distributed exclusively in Asia and its effect depends on genetic background (Mendez-Vigo *et al.,* 2013).

On the other hand, we developed a collection of wild *A. thaliana* genotypes from the Iberian Peninsula, which is a permanent experimental population for genetic and environmental association analyses (Fig. 1). Genetic analysis of this collection previously showed that Iberia is the world region with the largest known diversity of *A. thaliana,* which supports the hypothesis of multiple Iberian refuges during the last glaciations. To understand how *A. thaliana* has adapted to the climates of its southern limit, we have extended this collection to Northern Africa. Analyses identified a genetic lineage distributed exclusively in Iberia and Morocco, which did not contribute to the colonisation of the rest of Europe (Fig. 2). Further gene diversity analyses suggest that Morocco was a refuge for *A. thaliana* during the last glaciations and that the species colonised the Iberian Peninsula from Africa (Brennan *et al.*, 2014).

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Méndez-Vigo B, Martínez-Zapater JM, Alonso-Blanco C. The flowering repressor SVP underlies a novel *Arabidopsis thaliana* QTL interacting with the genetic background. PLoS Genet 2013; 9:e1003289

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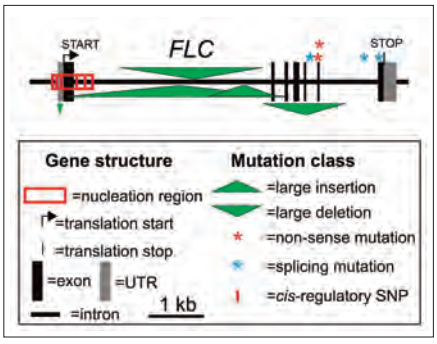

1 *Functional nucleotidic variation in the flowering repressor FLC of Arabidopsis*

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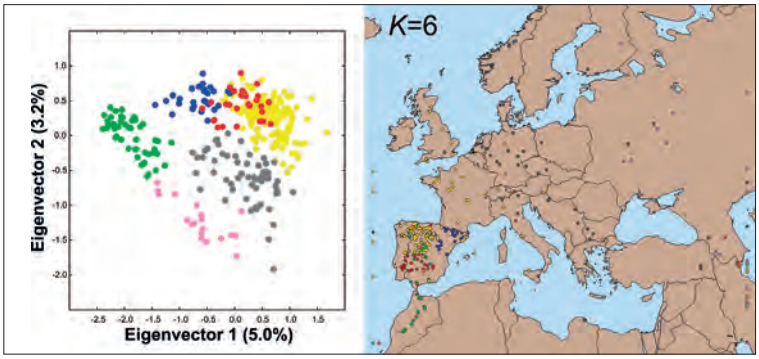

2 *Genetic and geographic structure of A. thaliana at a global scale. The six groups detected by clustering analysis are shown in different colours in a scatter plot displaying the first two eigenvectors estimated by PCA (left) and in a worldwide collection of accessions. Accessions located outside the Eurasian map are shown at their corresponding latitude on the left (American accessions) and right (Asian accessions) edges.*

Plant immunity strategies against microbial pathogen infection

PRINCIPAL INVESTIGATOR:
Carmen Castresana

POSTDOCTORAL SCIENTISTS:
Marta Martínez
Veronica Aguilera
Ruth Marcos

PREDOCTORAL SCIENTISTS:
Yovanny Izquierdo
Ada Martínez
Ruben Fernández

TECHNICIAN:
Tomas Cascón

Plant oxylipins are a class of lipid signals involved in regulating plant development and immunity. Recent studies demonstrate the importance of the 9-LOX and alpha-DOX oxylipin pathways in the defence mechanisms activated by Arabidopsis following infection by hemibiotrophic bacteria; these oxylipin pathways participate in the three layers of defence (pre-invasion, apoplastic and systemic) triggered by plants to prevent *Pseudomonas syringae* pv *tomato* DC3000 infection. Our studies also showed high 9-LOX and alpha-DOX levels activity in roots of untreated Arabidopsis plants and participation of the 9-LOX pathway in the defence mechanisms against the root pathogen *Fusarium oxysporum* (Fig. 1). In these responses, oxylipins were found to act as regulators of oxidative stress, lipid peroxidation, hormone homeostasis and cell wall integrity. Characterisation of a series of *noxy* mutants (*non-responding to oxylipins)* insensitive to the 9-LOX products 9-hydroxyoctadecatrienoic acid (9-HOT) and 9-ketooctadecatrienoic acid (9-KOT) provided further support for the role of the 9-LOX pathway in plant defence and in signalling cell wall damage. We found that the defensive responses and cell wall modifications caused by 9-LOX products are under mitochondrial retrograde control and that mitochondria have a fundamental role in innate immunity signalling (Fig. 2). Additional experiments with mutants defective in brassinosteroids (BR), a class of plant hormones necessary for normal plant growth and cell wall integrity, showed that 9-LOX-derived oxylipins activate cell wall-based defence responses such as callose deposition by inducing BR synthesis and signalling. Studies with antioxidants showed that oxylipin-dependent activation of BR signalling is limited by the lipophilic antioxidant trolox, which indicated that lipid peroxidation helps trigger BR signalling. These results show interaction between the 9-LOX and BR pathways and point to participation of both oxylipins and brassinosteroids as part of the plant response involved in controlling cell wall-based plant defence. The results of these studies support our interest in examining the action of 9-LOX and alpha-DOX oxylipins in plant defence, whose understanding will help to develop new strategies for disease control in crop plants, a major limiting factor in reducing agricultural productivity.

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Montillet JL, Leonhardt N, Mondy S, Tranchimand S, Rumeau D, Boudsocq M, Garcia AM, Douki T, Bigeard J, Laurière C, Chevalier A, Castresana C, Hirt H. Identification of an ABA-independent oxylipin pathway that controls stomatal closure and immune defense in Arabidopsis. PLoS Biol 2013; 11:e1001513

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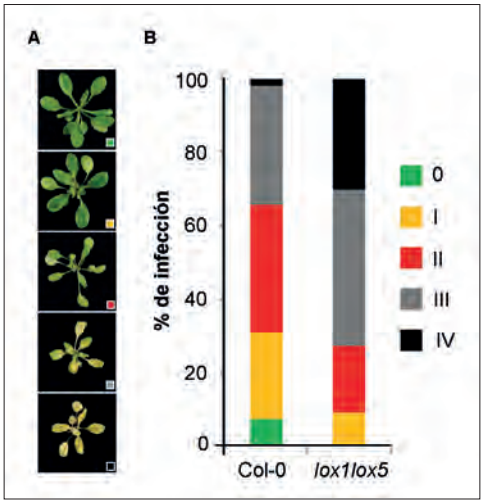
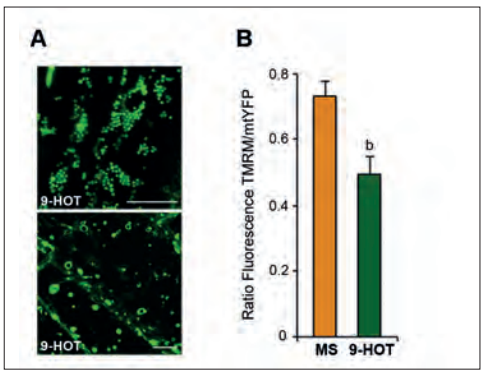


1 *The lox1lox5 mutant lacking 9-LOX activity displays enhanced susceptibility to the root pathogen Fusarium oxysporum. (A) Representative examples of infection symptoms scored on a five-point scale according to their intensity. (B) Percentage of leaves showing each of the symptoms evaluated.*

2



2 *Mitochondrial aggregation and decrease in membrane potential in response to 9-HOT. (A) Mitochondrial visualisation of 9-HOT (25 µM)-treated transgenic lines expressing 35S:Mt-YFP showed formation of aggregates (top) and annular structures (bottom). (B) TMRM (tetramethyl rhodamine methyl ester) and mt-YFP fluorescence intensities measured in untreated and 9-HOT-treated roots.*

Genetic control of shoot branching patterns in plants

PRINCIPAL INVESTIGATOR:
Pilar Cubas

POSTDOCTORAL SCIENTIST:
Michael Nicolas

PREDOCTORAL SCIENTIST:
Eduardo González Grandío

STUDENTS:
Elena Sánchez-Fontecha
Benjamin Lambour
Martín Basterrechea

TECHNICIAN:
Isabel Domínguez Cañete

Shoot branching patterns depend on a key developmental decision: whether axillary buds grow out to give a branch or remain dormant in the leaf axils. This decision is controlled by hormone-mediated endogenous and environmental stimuli. A decrease in the red to far-red light ratio (R:FR) –a sign of shading by neighbouring vegetation– triggers a set of developmental plant responses termed shade avoidance syndrome. One of these responses is suppression of axillary bud outgrowth.

The Arabidopsis gene *BRANCHED1*, which encodes a TCP transcription factor, is a point at which signals that suppress shoot branching are integrated in axillary buds. We showed that *BRC1* is necessary for branch suppression in response to shade, and *BRC1* transcription is positively regulated after exposure to low R:FR.

To understand the growth-to-dormancy transition in axillary buds, we compared transcriptomic profiles of wild-type and brc1 axillary buds, and identified sets of genes that are probably controlled by *BRC1*. We distinguished a network of upregulated abscisic acid response genes and two networks of cell cycle- and ribosome-related downregulated genes. The downregulated genes have promoters enriched in TCP binding sites, which suggests transcriptional regulation by TCP factors. We compared our transcriptomic data with two additional "active vs. dormant bud" transcriptomic data sets and found "core" coregulated gene networks closely associated to each condition.

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

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González-Grandío E, Cubas P. Identification of gene functions associated to active and dormant buds in Arabidopsis. Plant Signal Behav 2014; 9:e27994

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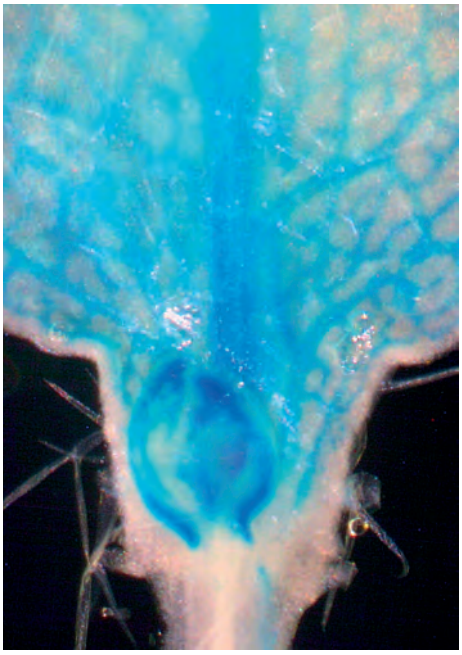

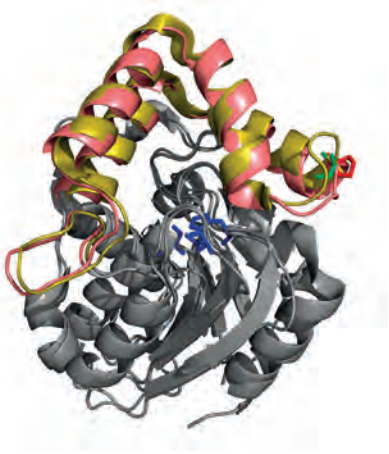

1 *GUS histochemical activity of Arabidopsis D14 promoter fused to GUS (D14pro:GUS) in an Arabidopsis transgenic plant. A detail of an Arabidopsis young axillary bud is shown*

2 *3D models of the strigolactone receptor AtD14 and karrikin receptor KAI2, based on crystallographic data (Hamiaux et al 2012; Kagiyama et al. 2013). Helical caps of D14 and KAI2 are highlighted in salmon pink and yellow, respectively. Active site residues are in blue. In red D14 Pro-169, a residue whose mutation causes a complete loss of function of the AtD14 protein.*

Plant-pathogen interaction in viral infections

PRINCIPAL INVESTIGATORS:
Juan Antonio García
Carmen Simón

POSTDOCTORAL SCIENTISTS:
Mingmin Zhao
Bernardo Rodamilans
Sandra Martínez Turiño

PREDOCTORAL SCIENTISTS:
María Calvo
José de Jesús Pérez
Fabio Pasin
Jon Ochoa
Araiz Gallo
Frida Mesel
Hongying Shan

TECHNICIAN:
Beatriz García

BIOINFORMATICIAN:
David San León

Plant viruses depend on host factors to replicate and to propagate throughout the plant and between individual plants. Plants in turn have developed antiviral defences, 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 infection by the potyvirus *Plum pox virus* (PPV), the causal agent of sharka, a damaging disease of Prunus trees.

We are interested in virus-host interactions that can help to define virus host range. We have demonstrated that single alterations in the 6K1 and CI proteins (corresponding to the cleavage site recognized by the viral protease NIa in the PPV polyprotein) are involved in alternative host adaptation of atypical PPV isolates to *Nicotiana benthamiana* and *Prunus avium*. These results suggest that fine regulation of polyprotein processing might depend on specific host factors and contribute to adaptation to specific hosts. By studying resistance of *Arabidopsis thaliana* and *Chenopodium foetidum* to cherry strain PPV isolates, we showed that defects in interactions between translation initiation factors and potyviral proteins might not only prevent infection in resistant varieties of susceptible host species, but also contribute to non-host resistance. We also demonstrated that self-cleavage of PPV P1 is negatively regulated by its highly disordered N-terminal region and relies on a specific host factor for its activation. Based on these results, we speculate that host-dependent regulation of P1/HCPro protease processing evolved to attenuate virus virulence and thus alleviate antiviral responses.

We apply the information obtained in our research to design control strategies for sharka and other viral diseases, and to develop PPV-based biotechnological tools. We have generated *N. benthamiana* transgenic lines that express different PPV-specific artificial miRNAs; they are being used to study the effectiveness and durability of antiviral resistance based on these small RNAs, and the side effects of the use of this technology on the virus evolutionary potential.

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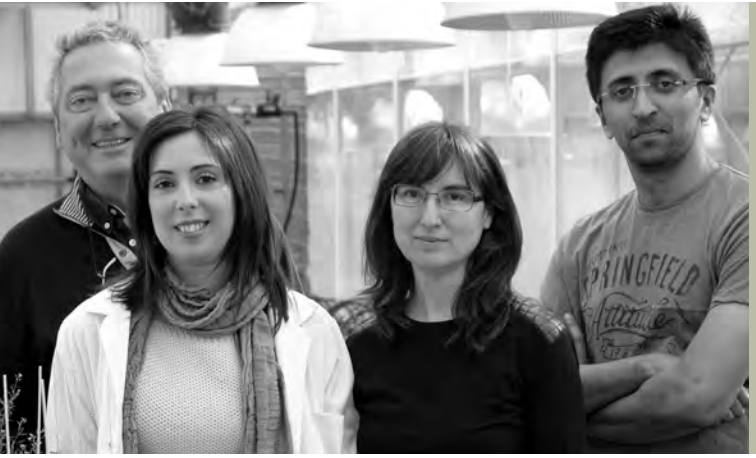
1

1 *Prunus avium (left) and Nicotiana benthamiana (right) leaves infected with Prunus- or Nicotiana-adapted Plum pox virus C isolates, respectively. The background image shows a magnification of an infected P. avium leaf displaying veinal chlorosis. (Cover, Mol Plant Microbe Interact 2014; 27:136)*

2

2 *Necrotic lesions caused by Plum pox virus (PPV) strain D (Rankovic isolate) in Chenopodium foetidum. (Cover, Mol Plant Microbe Interact. 2014; 27:1291)*



Genes involved in root architecture and in arsenic phytoremediation

PRINCIPAL INVESTIGATOR:
Antonio Leyva Tejada

POSTDOCTORAL SCIENTISTS:
Mohan TC
Cristina Navarro Solaz

TECHNICIAN:
Yolanda Leo del Puerto

In our group, we are interested in the characterisation of the molecular mechanisms involved in arsenic perception in plants. Arsenic contamination is responsible for the worst mass poisoning ever suffered by man, and is considered a silent threat to public health. Clean-up of arsenic-contaminated soils or arsenic entry into the food chain from crops irrigated with arsenic-contaminated water, of particular importance in rice, is therefore a priority concern for the World Health Organisation (Mead. *Environ Health Perspect* 2005; 113:A378). This chemical threat was particularly critical for the evolution of sessile organisms such as plants, which were forced to develop rapid tolerance responses when As(V) was present. We recently identified a QTL (quantitative trait locus) that accounts for the genetic variability for As(V) tolerance among Arabidopsis accessions worldwide. Molecular isolation and characterisation of this locus showed it encodes a plant arsenate reductase with potential applications in arsenic phytoremediation (Sanchez-Bermejo *et al.* Nat Commun 2014; 5:4617). We also identified a transcriptional repressor that modulates expression of the arsenate transporter, thus identifying the molecular basis of an alternative strategy for plant adaptation to arsenic (Castrillo *et al.* Plant Cell 2013; 25:2944).

In collaboration with other groups, we contributed to the identification of a phosphate sensor (Puga *et al.* Proc Natl Acad Sci USA 2014; 111:14947) and participated in the production of a collection of transgenic plants that conditionally express more than 600 transcription factors (Coego *et al.* Plant J 2014; 77:944). The research lines currently in progress in our laboratory will allow us to understand the mechanisms that underlie arsenic perception, which will open up new possibilities for phytoremediation of arsenic-contaminated soils and waters.

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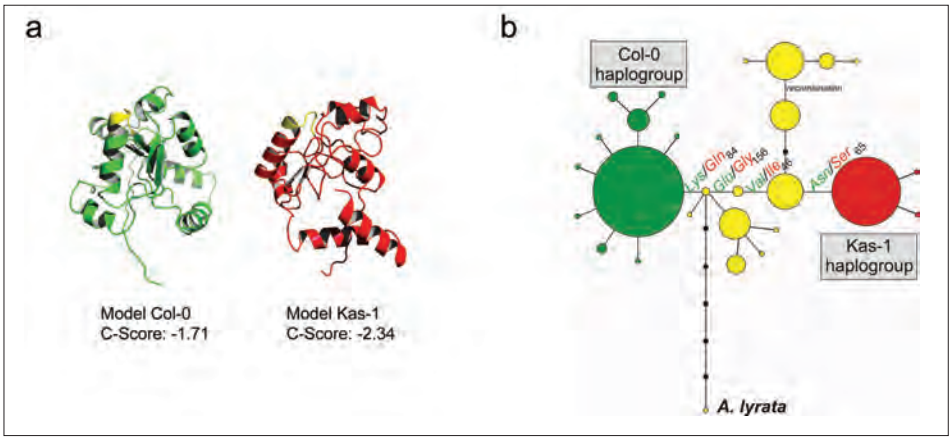
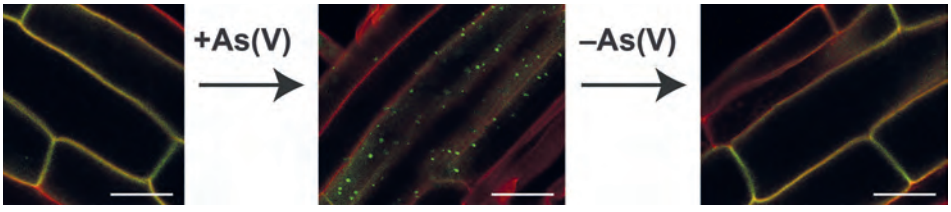


1 *Allelic variation at the arsenate reductase (AtARQ1) involves structural and regulatory polymorphisms. (a) 3D structural models of the arsenate reductase proteins from Col-0 (Green) and Kas-1 (red) predicted by I-TASSER. The catalytic sites are in yellow. (b) Haplotype network analysis using AtARQ1 proteins from the 1001 Arabidopsis genome project and Arabidopsis lyrata. Each line segment corresponds to an a.a substitution. Areas of circles are proportional to frequencies; red and green depict the two major haplogroups.*



2 *Arsenate [As(V)] delocalises and relocalises the As(V)/Phosphate transporter PHT1;1. Analysis of PHT1;1-GFP localisation after two pulses of 30 μM As(V) (±As(V)) in PHT1;1-GFP-expressing Arabidopsis root cells. Duration of each pulse and gaps between them were 1.5 h.*

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

PRINCIPAL INVESTIGATOR:
Javier Paz-Ares

POSTDOCTORAL SCIENTIST:
Mª Isabel Puga Hermida

PREDOCTORAL SCIENTISTS:
Laura Cuyàs Carrera
Charukesi Rajulu
Miguel Miñambres Martín

TECHNICIAN:
Erica Gil Velasco

Our research is focussed on the phosphate (Pi) starvation rescue system of plants, a model system for studies of gene activity, with presumed biological potential in the context of low-input agriculture. In previous years, we identified transcription factor PHR1 as a master regulator of phosphate starvation responses in plants. In the last two years, we carried out functional analysis of the two PHR1 binding sites, previously detected in a ChIP-seq assay with PHR1. We also identified a component of the Pi sensing system, SPX1, and revealed its mode of action. Finally, we began the analysis of natural variation of transcriptomic responses to Pi starvation.

To study the function of P1BSI and P1BSII, the two binding sites used by PHR1 to regulate transcription of Pi starvation-responsive genes, we prepared artificial promoters consisting of four tandem copies of P1BSI or P1BSII upstream of the -45 minimal promoter from the 35S gene of CaMV. These promoters were fused to the GUS coding region. We found that the two binding sites, which are recognised by PHR1 in dimeric and monomeric forms, respectively, are *bona fide* Pi starvation-responsive elements, with P1BSI promoting the strongest response, in line with higher affinity binding by PHR1 (Figure 1).

We also identified SPX1, whose gene is highly Pi starvation-responsive, interacting with PHR1 as a component of the Pi sensing system. Indeed, SPX1 interaction with PHR1 is Pi-dependent both *in vivo* and *in vitro,* and causes inhibition of PHR1 binding to DNA. The high SPX1 accumulation during Pi starvation is considered to provide a mechanism for rapid shutdown of Pi starvation responses once Pi is resupplied.

In a study of natural variation of molecular responses to Pi starvation, we examined the Pi starvation responsive transcriptome of four ecotypes in addition to the reference Col ecotype, and found great interecotypic differences in Pi starvation-responsive genes. The CT ecotype showed the largest differences with Col (~1000 genes differentially expressed in CT vs Col in Pi-grown plants). At present we are performing transcriptomic analysis of 100 recombinant inbred lines from a ColxCT cross to identify expression quantitative trait loci corresponding to Pi starvation-responsive genes.

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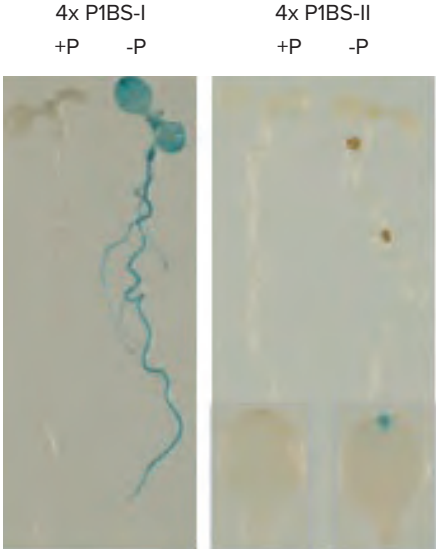
Puga MI, Mateos I, Charukesi R, Wang Z, Franco-Zorrilla JM, de Lorenzo L, Irigoyen ML, Masiero S, Bustos R, Rodríguez J, Leyva A, Rubio V, Sommer H, Paz-Ares J SPX1 is a phosphate-dependent inhibitor of phosphate starvation response 1 in Arabidopsis. Proc Natl Acad Sci USA 2014; 111:14947-14952



1 *P1BSI and P1BSII are bona fide Pi starvation response elements. Transgenic Arabidopsis plants were prepared harbouring constructs consisting of an artificial promoter with four tandem copies of P1BSI or P1BSII motifs upstream of the minimal 35S promoter and fused to the GUS coding region. Plants were grown in +P or –P conditions for 10 days before GUS staining.*

Light signalling and day length control of potato tuber formation

PRINCIPAL INVESTIGATOR:
Salomé Prat

POSTDOCTORAL SCIENTISTS:
Ana Espinosa
Cristina Martínez
Cristina Nieto
Ruth Sanz

PREDOCTORAL SCIENTISTS:
Eduard Cruz
Pilar Lasierra

TECHNICIAN:
Ana Beloso

Our group is interested in understanding the mechanisms by which hormone signalling controls plant growth and development in response to diurnal photocycles, changes in light spectra, or adverse environmental conditions. Our work focusses on gibberellins (GA), a group of hormones that control plant growth by triggering degradation of the DELLA repressors. We showed that DELLAs inhibit plant GA-regulated gene expression via interaction with PHYTOCHROME-INTERACTING FACTOR 4 and 5 (PIF4 and PIF5). DELLA interaction with the PIF bHLH domain prevents PIF binding to the promoters of their gene targets and suppresses growth. DELLAs also mediate increased tolerance to salt stress via a largely unknown pathway. In genetic screens, we identified several transcription factors whose overexpression confers increased tolerance to salt and drought stress, and which bind the DELLAs. We are currently studying

- The regulatory pathways controlled by these regulators
- The molecular mechanisms by which DELLAs modulate activity of these factors
- DELLA allelic mutations that interfere with PIF interaction but do not alter binding to these stress-related factors

Introduction of these allelic mutations into cultivated species will help to generate new cultivars more tolerant to drought and salt stress, and increase crop production in adverse climate conditions.

Our second line of research is the control of storage organ formation in the potato. We showed that potato tuberisation is triggered by a member of the potato FLOWERING LOCUS T (FT) gene family, *SP6A*. Temperatures >25ºC inhibit storage organ formation by suppressing SP6A expression; this inhibitory effect is reversed by SP6Aox, which highlights the SP6A pathway as one of the primary targets for increased potato productivity. We aim to analyse the signalling events that lead to *SP6A* suppression at warm temperatures and to identify the SP6A downstream pathway that controls tuber formation and, by extension, storage organ formation in other bulb-, rhizome- or tuberous root-forming species.

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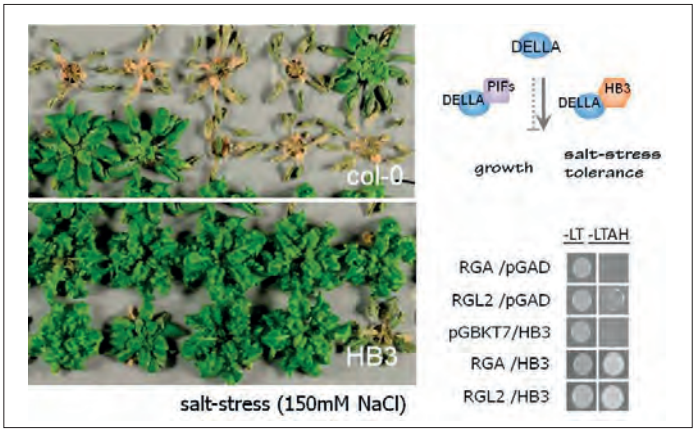
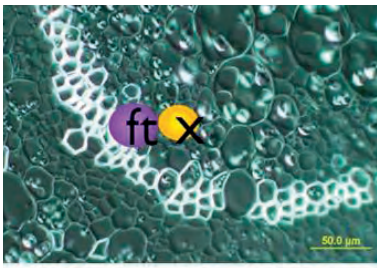
1

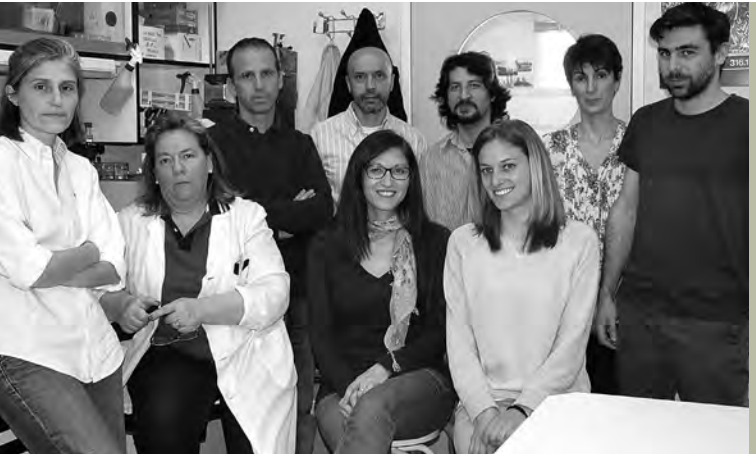


1 *The DELLA repressors confer increased tolerance to drought and salt stress. We identified several transcription factors that interact directly with DELLAs and whose overexpression increases plant survival in high salt conditions. DELLA allelic variants that interfere with PIF interaction but do not affect binding to these stress-related factors will enable us to uncouple growth inhibitory effects of these repressors from their stress tolerance function.*

2



2 *During stolon-to-tuber transition, cell division and starch accumulation is observed in cells around the vascular cambium. Identification of SP6A interactors in these cells will allow us to understand how formation of these important organs is triggered.*



Signalling networks in plant development and defence responses

PRINCIPAL INVESTIGATORS:
Jose J. Sánchez Serrano
Enrique Rojo

POSTDOCTORAL SCIENTISTS:
Maite Sanmartín
Mary Paz González

PREDOCTORAL SCIENTISTS:
Guillermo Ruano
Ramón Contreras

TECHNICIANS:
Pilar Paredes
Yolanda Fernández

VISITING SCIENTISTS:
Jan Schulze-Hüynck
(Leibniz Universität, Hannover, Germany)
Erin Johnson
(Spelman College, Atlanta GA, USA)
Francesco Stefanelli
(Università del Salento, Lecce, Italy)

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

Stem cell differentiation implies large-scale transcriptional modifications. We have identified the RNA polymerase II (Pol II) phosphatase ART and its interacting partner MINIYO (IYO) as essential factors for initiating cell differentiation in Arabidopsis. Our results suggest that coupled uptake of ART and IYO into the nucleus switches on this cell fate transition through direct regulation of Pol II gene transcription.

Reversible protein phosphorylation is a common molecular switch in signalling pathways. Protein phosphatases 2A (PP2A) dephosphorylate proteins at serine/threonine residues. PP2A is a heterotrimer consisting of a catalytic (PP2A-C), a scaffold (PP2A-A), and a regulatory (PP2A-B) subunit. The Arabidopsis genome codes for five very similar PP2A-C proteins. These are grouped in subfamily 1 (PP2A-C1, -C2, and -C5) and subfamily 2 (PP2A-C3 and -C4). Whilst single *pp2ac-3* and *pp2ac-4* mutant lines do not display any obvious phenotype, *pp2ac-3 pp2ac-4* plantlets are severely misshapen, with severe malformations of cotyledon and root primordia. 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 both affected by mutations in *PP2A-C3* and *PP2A-C4*. Our work shows 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 that entail large changes in transcriptional profiles. A major wound-signalling pathway involves jasmonic acid (JA). Large, transient increases in endogenous JA levels occur after mechanical damage, and high JA levels trigger the transcriptional activation 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 *StAOS1* and *StAOS2* must be cosuppressed simultaneously to lower plant JA levels significantly, resulting in altered cell wall structure and increased susceptibility to pathogens.

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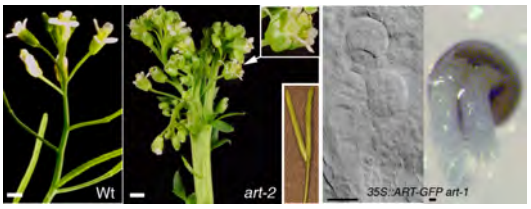
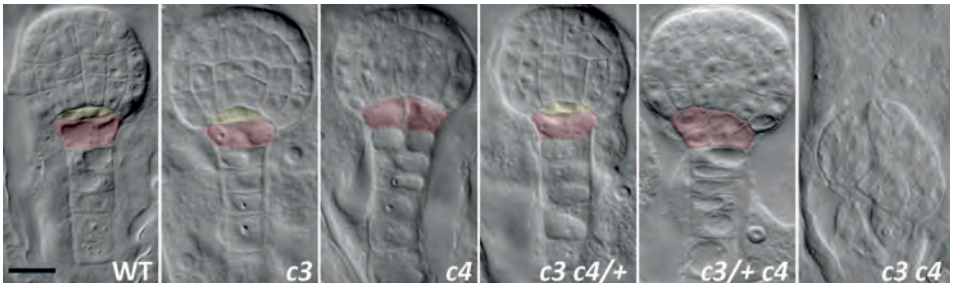


1 *RTR is necessary for cell differentiation. (Left) Primary stems from WT and rtr-2 plants. All rtr-2 plants develop fasciated meristems with split primary shoot apical meristem. Bar = 1 mm. Top inset shows enlarged image of a duplicated flower; bottom inset shows a duplicated silique from an rtr-2 plant. (Right) Twin globular embryos and twin seedlings emerging from a seed from rtr-1 plants transformed with 35S::RTR-GFP. Bar = 25 μm.*



2 *PP2A-C3 and PP2A-C4 are essential for embryo patterning. Differential interference contrast microscopy of cleared embryos at late globular stage. Genotypes are indicated at the bottom. Bar = 12.5 μm. Suspensor cells are coloured pink; lens-shaped cells produced after asymmetric division of the hypophysis are in yellow.*

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

PRINCIPAL INVESTIGATOR:
Vicente Rubio

POSTDOCTORAL SCIENTIST:
Diaa Abd El-Moneim

PREDOCTORAL SCIENTISTS:
Laura Cuyàs
Elisa Iniesto
Amr A. Nassrallah

TECHNICIANS:
Jennifer Esteban
Yolanda Fernández

VISITING SCIENTIST:
Nada Bensalem
(University of Tunis, Tunisia)

Targeted destabilisation of proteins by the ubiquitin proteasome system regulates key developmental and stress responses in plants, including their adaptation to abscisic acid (ABA)-mediated abiotic stresses such as drought, high salinity and low temperatures. 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 climatic change events. Thus, we recently showed that CULLIN4-RING E3 ubiquitin ligases (CRL4) promote ubiquitination and degradation of PYR/PYL/RCAR ABA receptors to modulate ABA signalling in Arabidopsis. For this function, CRL4 require DDA1, a type of substrate adaptor conserved in higher eukaryotes. DDA1 provides substrate specificity for CRL4 by interacting with PYL8, as well as other PYR/PYL/RCAR family members, and facilitates its proteasomal degradation. We found that DDA1 negatively regulates ABA-mediated developmental responses, including inhibition of seed germination, seedling establishment and root growth. DDA1-triggered destabilisation of PYL8 is counteracted by ABA, which protects PYL8 by limiting its polyubiquitination. In sum, our data identify a mechanism for desensitisation of ABA signalling based on the control of ABA receptor stability (Fig. 1).

We are currently characterising the protective mechanism by which ABA impedes degradation of its receptors. In addition, we are exploring the role of CRL4-DDA1 complexes in the control of chromatin dynamics, including chromatin remodelling, gene expression and DNA damage repair. For this we use various genetic, molecular and proteomic approaches. Regulatory proteins or their mutated versions of them, identified and characterised through these approaches, should help to develop modified crops with increased resistance to environmental stresses (Fig. 2). This is the case of DDA1, for which a technology to generate plants more tolerant to water stresses has been patented and licensed.

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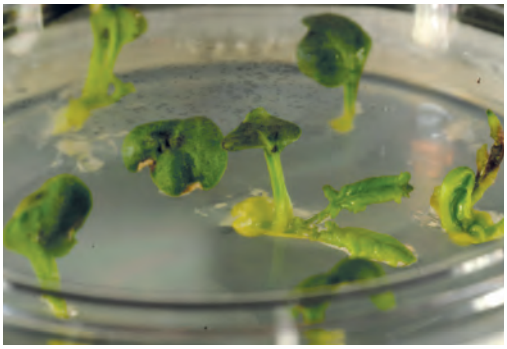
PATENT

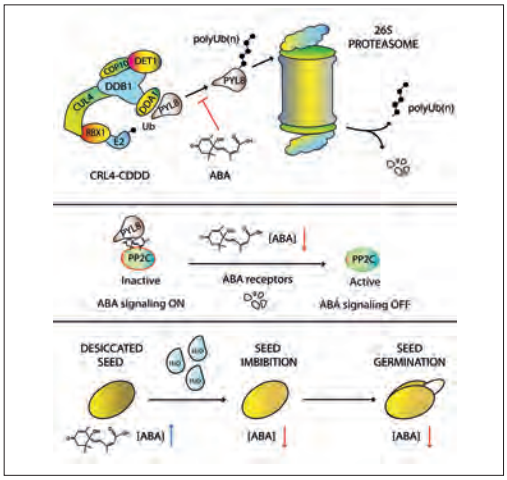
PCT/EP2014/061214. DDA1 gene for mitigating negative ABA effects on growth during abiotic stress

1

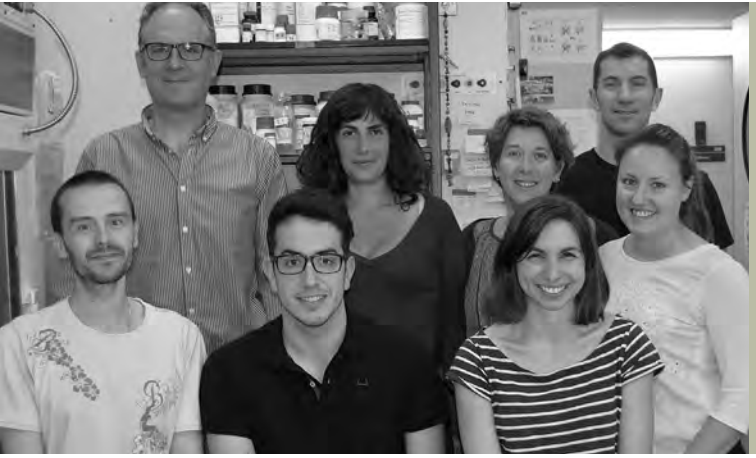


1 *Transgenic lines of colza (Brassica napus) that overexpress DDA1. Tolerance to water stresses (salinity, drought, osmotic stress) will be assayed in DDA1-overexpressing colza lines generated at the CNB's in vitro culture facility. Similar analyses will be performed in rice.*

2



2 *Model for DDA1 role in ABA desensitisation. DDA1 allows recognition and ubiquitination of ABA receptors (e.g., PYL8) by CRL4 Ub ligases. Polyubiquitinated ABA receptors are then degraded at the 26S proteasome. ABA, on the contrary, stabilises the receptors by limiting their polyubiquitination (top). Low ABA levels enable DDA1-mediated destabilisation of ABA receptors, promoting the release and activation of PP2C phosphatases, which act as negative regulators of ABA signalling (centre). In this way, DDA1 contributes to ABA desensitisation when levels of this hormone diminish; that is, when stress conditions disappear or during seed imbibition and germination (bottom).*



The jasmonate signalling pathway in Arabidopsis

PRINCIPAL INVESTIGATOR:
Roberto Solano

STAFF SCIENTIST:
Jose Manuel Chico

POSTDOCTORAL SCIENTISTS:
Andrea Chini
Selena Giménez Ibañez
Marta Boter

PREDOCTORAL SCIENTISTS:
Isabel Monte
Guillermo Fernández Jiménez
Andrés Ortigosa Urbieta

TECHNICIAN:
Gemma Fernández Barbero

Plants are able to 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 re-programming of the cell. The main focus in my lab is to understand one of the pathways involved in this network, 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 JA pathway with other pathways within the network. Understanding these molecular interactions is essential to decipher how one single hormone can activate so many different physiological responses in the plant, and how the plant is able to discriminate between different stresses and select the correct set of responses to each. This knowledge is basic for the design of biotechnological and agronomic applications.

The major achievements of our group in the last two years are:

- Identification and characterisation of the bacterial effector HopX1, which enhances plant susceptibility to biotrophic pathogens by activating the JA signalling pathway. HopX1 has a protease activity that degrades the key JA-repressors JAZ (Gimenez-Ibanez *et al.* PLoS Biol 2014)
- Design and characterisation of a potent, specific antagonist of JA perception, COR-MO, with important biotechnological potential (Monte *et al.* Nat Chem Biol 2014)
- Determination of the DNA-binding sequence specificities of over 60 plant transcription factors, through the use of a protein-binding microarray (Franco-Zorrilla *et al.* Proc Natl Acad Sci USA 2014; Boer *et al.* Cell 2014)
- Discovery of the mechanism by which canopy shade reduces JA-dependent defences (Chico *et al.* Plant Cell 2014)
- Identification and characterisation of three bHLH transcription factors that repress JA responses (Fonseca *et al.* PLoS One 2014)
- Discovery of the mechanism by which MYC transcription factors (MYC2, 3 and 4) regulate glucosinolate biosynthesis (Schweizer *et al.* Plant Cell 2013)
- Analysis of protein disorder in the *Arabidopsis thaliana* proteome (Pietrosemoli *et al.* PLoS One, 2013)

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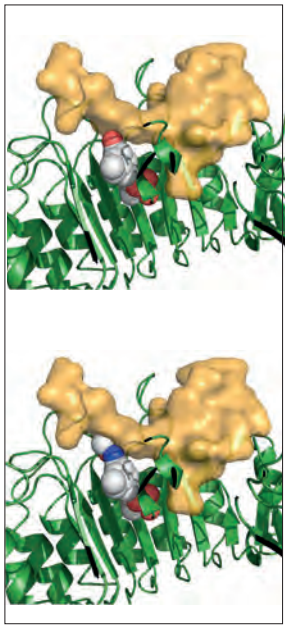
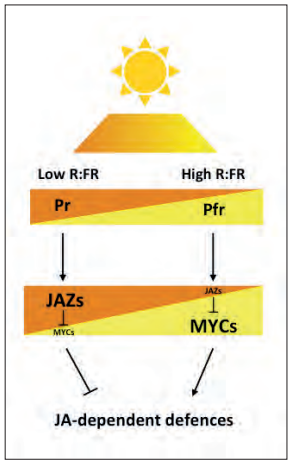
PATENT

EP 13382362.5. Novel compounds antagonizing JA-Ile perception





2 *Model of regulation of JA-mediated defences by light quality through modulation of MYC and JAZ stability. R/FR ratios determine the balance of activation/deactivation (Pfr/Pr) of phytochromes, which differentially regulate the stability of MYC TF and their JAZ repressors, and therefore, the defence output of the plant. Ambient light (high R/FR ratios) shifts the Pr/Pfr equilibrium to the active Pfr form, which enhances JA-dependent defences by mediating MYC stabilisation and allowing JA-mediated degradation of their JAZ repressors. Conversely, in shade conditions (low R/FR ratios), the phythochrome equilibrium is shifted towards the inactive Pr form, thus reducing JA-dependent defences by destabilising MYC and stabilising JAZ. The balance of active phytochromes, which depends on the R/FR ratio, thus regulates the relative amount of MYC and JAZ proteins and therefore define the JA-dependent defence output of the plant.*

1 *COR-MO is an antagonist of jasmonate perception. (Top) Molecular model of the interaction of COR with the co-receptor COI1 (green; ribbon representation) and JAZ degron (yellow; surface representation) based on crystal structure described by Sheard et al., 2010. (Bottom) The same complex with COR-MO instead of COR, showing the steric impediment of JAZ interaction.*

2 / Macromolecular Structures

The department's interests cover a broad array of biological problems, from fundamental questions associated with protein folding (Muñoz and Valpuesta) to the functional and structural characterisation of various molecular machines, especially virus structures and their components (Casasnovas, Carrascosa, Castón, Risco, San Martín and van Raaij), and DNA repair nanomachines (Moreno-Herrero). These studies are carried out using the numerous structural and biophysical techniques available in the department, including X-ray diffraction, nuclear magnetic resonance, single-molecule techniques (optical and magnetic tweezers) and various state-of-the-art spectroscopic techniques. Of special note is the development of microscopy techniques such as atomic force, optical, and X-ray microscopy, and particularly transmission electron microscopy in its distinct variants (single-particle cryoelectron microscopy and cryoelectron tomography), of which the CNB hosts one of the largest communities in the world. This work is strongly supported by continuous software development in the fields of image processing (Carazo, Fernández) and transcriptomics (Pascual-Montano), which has led to the CNB hosting of the INSTRUCT image processing centre, a pan-European research infrastructure network facility that provides expertise and access to high quality instruments. Technical developments are also pursued in the field of proteomics (Albar), which resulted in the CNB being chosen to head the Spanish proteomic facilities network (PROTEORED) and participation in the Human Proteome Project.

Functional proteomics

PRINCIPAL INVESTIGATOR:
Juan Pablo Albar

SENIOR SCIENTIST:
Alberto Paradela

POSTDOCTORAL SCIENTISTS:
Severine Gharbi
Miguel Marcilla
María del Carmen Mena
Manuel Lombardía

PREDOCTORAL SCIENTISTS:
Salvador Martínez de Bartolomé
Rosana Navajas
J. Alberto Medina
Antonio Ramos
Adán Alpízar
Carmen González
Gonzalo Martínez
Miguel A. López

ADMINISTRATION:
María Dolores Segura
Virginia Pavón

Functional proteomics draws a complete map of protein dynamics, interactions and posttranslational modifications that take place in the cell. Our group monitors proteins involved in molecular interactions and pathways relevant to pathologies in a variety of tissues, cell types and organisms following various experimental treatments/conditions. We are also incorporating the latest technologies for specific functional proteomic projects:

1. Human Proteome Project: This HUPO-sponsored project intends a systematic mapping of the human proteome by constructing a protein catalogue on a chromosome-to-chromosome basis. We coordinate a national initiative consisting of 15 proteomics laboratories that study the protein expression profile of chromosome 16 in a broad panel of human cell lines. We design experimental approaches to detect and quantify both the "conspicuous" and the "hidden" proteome. This initiative is driven by the most advanced technology using standard unbiased shotgun as well as targeted (MRM) experimental approaches.

2. Deciphering signal transduction networks by phosphoproteomic analyses. TCR signalling and the specific role of diacylglycerol is being studied using a combination of phosphopeptide enrichment and SILAC labelling, allowing accurate phosphoprotein and phosphopeptide analysis and quantitation.

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

4. Computational proteomics

• Application of probability-based methods for large-scale peptide and protein identification and quantitation from tandem mass spectrometry data

• Implementation of strategies for data mining visualisation

• Development of proteomics data analysis workflow, from data integration, validation, inspection, deposition and reporting (MIAPE Extractor tool, part of the ProteoRed MIAPE Web ToolKit)

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

6. Gluten allergen analysis. In the context of the study of coeliac diseases, our lab uses classical and proteomics experimental approaches to characterise wheat, barley and rye prolamins in foods.

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In memoriam

Juan Pablo Albar *(1953-2014), a lover of great challenges and an insatiable explorer, left us early in the morning of 19 July 2014. From the late 1990s on, he became one of the pioneers behind proteomics in Spain, and a key point of reference in leading national and international initiatives to understand human biology and the causes of illness. He was a scientific researcher for the Spanish Research Council, group leader of the Proteomics Facility of the CNB-CSIC, early leader and coordinator of the Carlos III Health Institute Networked Proteomics Platform (ProteoRed-ISCIII) as well as coordinator of the Biomolecular and Bio-computing Resources Platform PRB2-ISCIII. He was also a founder and a member of the executive board of the Spanish Society of Proteomics, a founder and coordinator of the Spanish network of Proteomics Laboratories ProteoRed-Genoma España, a driving force in founding the European Proteomics Association (EuPA), a member of the Human Proteomics Organisation (HUPO) Council, and a member of the Executive Committee of the Human Proteome Project (HPP). His work yielded over 160 scientific publications, inspiring many other researchers in the field of proteomics and biochemistry in general. In these few lines, we want to pay homage to someone who never set limits on his achievements in life and work, and who always wanted to go further.*

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

PRINCIPAL INVESTIGATOR:
José María Carazo

POSTDOCTORAL SCIENTISTS:
Carlos Oscar Sorzano Sánchez
Javier Vargas Balbuena
Joaquín Otón Pérez
Jordi Burguet Castell
Joan Segura Mora
Johan Busselez

PREDOCTORAL SCIENTISTS:
Jose Miguel de la Rosa Trevín
Airen Zaldívar Peraza
Ana Lucia Álvarez Cabrera
Vahid Abrishami

TECHNICIANS:
Jesús Cuenca
Blanca E. Benítez
Ignacio Foche
Laura del Caño
José Gutiérrez Tabuenca

VISITING SCIENTISTS:
Roberto Marabini
(Universidad Autónoma de Madrid, Spain)

The 2013-2014 biennium was particularly important for the field of cryogenic electron microscopy (cryo EM). Thanks to key technological developments in instruments and software, it became obvious in 2013 that cryo EM had indeed matured as a technique, such that we can now systematically produce quasi-atomic structural information for a wide range of very challenging macromolecular complexes. The need to re-accommodate all image processing workflows to address the new challenges in the field consequently became the target of the group. This goal fitted very well with our focus on methods and software development in our role as the image processing infrastructure providers for Instruct, the structural biology project of the European Strategic Forum for Research Infrastructures.

We have explored new areas of cryo EM image processing within our traditional software suite XMIPP (downloaded in recent years by thousands of researchers from all over the world). We addressed a number of topics central to enhanced microscope performance and optimal image processing, such as accurate, parameter-free optical characterisation, automatic analysis of micrographs, or image-ranking procedures, all critical to render cryo EM a high throughput technique for structural biology. With regard to software, we introduced the beta version of our key development, Scipion, a graphic image-processing workflow integrator that assures full tracking of all analytic results, while offering an environment that integrates most of the best known software packages in the field.

With respect to X-microscopy, using the X-ray microscope at the Spanish synchrotron ALBA, we produced the first detailed optical characterisation with this type of instrument, while advancing in automation and image processing capabilities.

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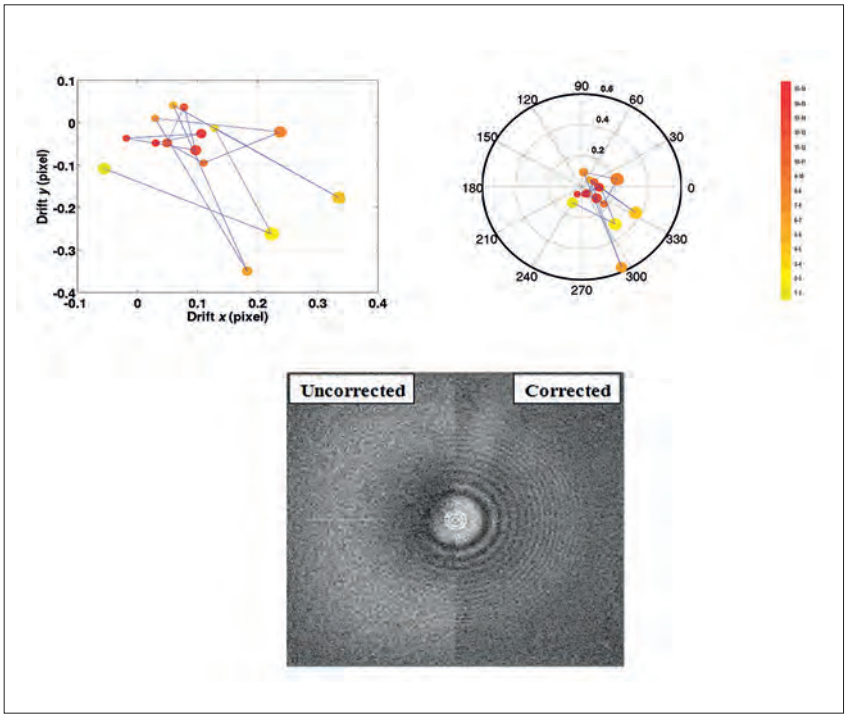

1 *Results of our 2D optical flow approach for frame alignment from movie data collected with the help of the new generation of director electron detectors. Note that 2D optical flow provides a unique characterisation of both local and global frame displacements, besides providing accurate and automatic (virtually parameter-free) image correction. We show Cartesian and polar displacement plots (left and right, respectively, top row), together with how a typical CTF (periodogram) looks before (left) and after (right) correction (bottom).*

Cell-cell and virus-cell interactions

PRINCIPAL INVESTIGATOR:
José M. Casasnovas

SENIOR SCIENTIST:
César Santiago

POSTDOCTORAL SCIENTISTS:
Rosario Recacha
Manuela Zonca
Alberto Martínez Colom

PREDOCTORAL SCIENTISTS:
Meriem Echbarthi
Gaurav Mudgal

TECHNICIAN:
Susana Rodríguez

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

Cell-cell interactions

We studied how members of the TIM (T cell/transmembrane, immunoglobulin and mucin domain) and ICAM (intercellular cell adhesion molecules) subfamilies mediate cell-cell interactions. The TIM proteins are pattern recognition receptors, specialised in recognition of the PtdSer (phosphatidylserine) cell death signal. We helped to show that TIM-1 mediates cell adhesion interaction that regulates T cell trafficking during inflammation. The ICAM proteins have long been linked to cell adhesion processes. Our recent research focussed on ICAM-5, a protein expressed exclusively in telencephalic neurons. We used X-ray crystallography to determine how ICAM-5 mediate cell adhesion among neurons. ICAM-5 has an S-shaped configuration that supports formation of a cell adhesion complex built of multiple molecules on the neuron surface (Figure 1).

Virus-receptor interactions and virus neutralisation by antibodies

To analyse virus-receptor interactions in measles virus and coronavirus, we determined crystal structures of virus-receptor complexes. These structures defined the way in which measles virus and certain coronavirus bind to cell surface proteins; we identified the principal determinants of receptor recognition in those viruses. Moreover, we are analysing how antibodies prevent and neutralise virus infections. Potent neutralising antibodies of measles virus and coronavirus target virus residues engaged in binding to cell surface receptors, indicating that prevention of virus entry into host cells is a major mechanism of virus neutralisation by the immune system (Figure 2).

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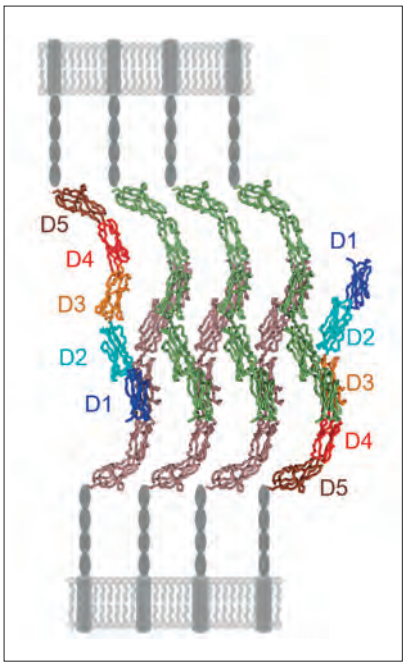

1 *Molecular model of the ICAM-5 homophilic cell adhesion complex in neurons.*

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

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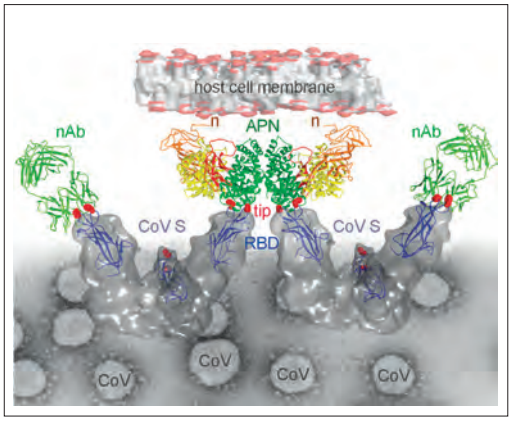

Structure of macromolecular assemblies

PRINCIPAL INVESTIGATOR:
José L. Carrascosa

SENIOR SCIENTIST:
Rocío Arranz

POSTDOCTORAL SCIENTISTS:
Francisco Javier Chichón García
Ana Cuervo Gaspar
Rebeca Bocanegra Rojo
María José Rodríguez Gómez

PREDOCTORAL SCIENTISTS:
Michele Chiappi
Verónica González García
José Javier Conesa Muñoz

TECHNICIANS:
Mar Pulido Cid
Encarna Cebrián Talaya

The study of virus assembly allows us the molecular basis of macromolecular interactions with chemical and functional properties of the complexes assembled during the morphogenetic pathway of virus construction. Our group combines cryo-electron microscopy and computer three-dimensional image processing to obtain structures, at nanometric resolution, involved in bacteriophage T7 assembly. We solved two conformations of the DNA packaging motor (T7 large terminase), as well as several structures related to the DNA ejection complex (T7 tail), to obtain a detailed description of the DNA translocation process involved in the virus life cycle. Some components of these viruses (connector, terminase) are presently being used in synthetic environments to obtain delivery vehicles with improved specificity and efficiency.

Our structural studies are complemented with single-molecule analysis methods (atomic force microscopy, optical tweezers) to determine the mechanical properties of individual viral particles and subcomplexes at the nanoscopic scale. In this way, we associated the different mechanical behaviour of T7 intermediate maturation particles with their quasi-atomic structures. We also described macromolecular properties of various viral components and assemblies (dielectric constant of viral components, force development by molecular motors, differential properties of dsDNA and RNA).

We continued to extend the use of correlative microscopy by integration of electron tomographic methods with other microscopies, in particular with soft X-ray microscopy. The cryo-tomograms of whole, unperturbed cells infected with vaccinia virus obtained by X-ray microscopy showed previously unreported quantitative aspects of viral maturation and compartmentalisation. To exploit the possibilities of combined X-ray microscopy and analysis, we studied the interaction of magnetic nanoparticles and eukaryotic cells. Development of an approach that uses X-ray spectroscopy and tomography helped us to correlate the topological studies of cellular distribution of nanoparticles with the chemical quantitative information of metal uptake by cells.

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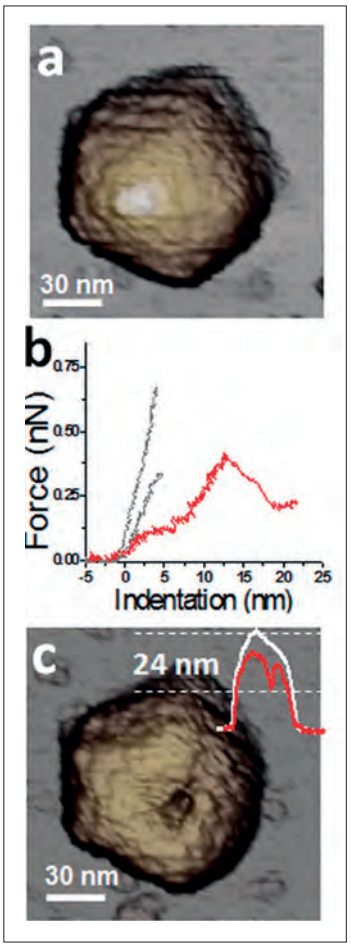

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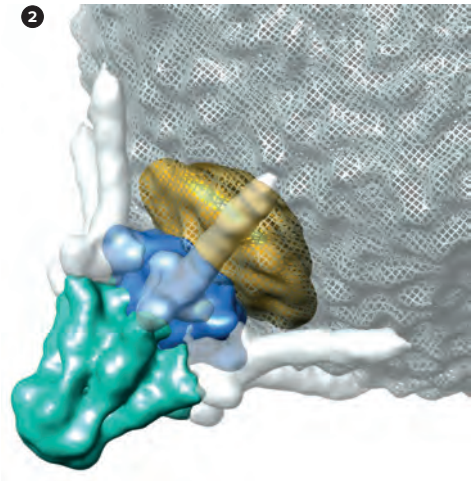

1 *Mechanical fatigue and fracture of T7 proheads. (a) AFM image before mechanical fatigue of a prohead. (b) Three nanoindentation cycles performed on (a). The red curve fractured the prohead. (c) Fractured prohead. The inset shows a comparison of the profiles along the damaged area before (white) and after (red) fracture.*

2 *Tail complex of bacteriophage T7 obtained by cryo-electron microscopy and computer reconstruction. Tail structural components are segmented in different colours. Capsid is outlined as a grey network.*

Viral molecular machines lab

PRINCIPAL INVESTIGATOR:
José R. Castón

POSTDOCTORAL SCIENTIST:
Ana Correia

PREDOCTORAL SCIENTISTS:
Elena Pacual Vega
Josué Gómez Blanco
Mariana Castrillo Briceño
Carlos Pérez Mata

VISITING SCIENTIST:
Daniel Luque Buzo
(Instituto de Salud Carlos III, Spain)
Joona Mikkilä
(Aalto University, Finland)

All live organisms assemble macromolecular nanomachines to carry out complex biological processes. These machines are proteins and nucleic acid-protein complexes that convert chemical energy into conformational changes; they are very dynamic and show conformational variability inherent to their mechanisms. Many fundamental cell biological and biochemical processes controlled by these macromolecular assemblies were discovered through the study of viruses, which alter cell functions and modulate cell behaviour.

As model systems of virus-derived molecular machines we study viral capsids, a paradigm of conformational flexibility, to analyse dynamic processes and macromolecular complexes that control fundamental processes in the virus life cycle. Three-dimensional (3D) structures of these macromolecular assemblies are crucial for advancing our understanding of key biological processes, i.e., how a molecular machine or protein complex works correctly or malfunctions.

We use a multidisciplinary approach that has led to structural analysis by 3D cryo-electron microscopy (cryo-EM) combined with atomic structures (hybrid approach). Our studies intend to establish the molecular interactions of the components in these complex assemblies, as well as the molecular basis of their functional implications. We have incorporated state-of-the-art approaches to obtain near-atomic resolution structure directly from two-dimensional micrographs. We are also using 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. Finally, virus-like particles can be used as nanocontainers to encapsulate different types of materials.

Our group studies several viral systems with varying levels of complexity, focussed on a number of double-stranded (ds)RNA viruses such as the birnavirus infectious bursal disease virus (IBDV; an avian pathogen), infectious pancreatic necrosis virus (IPNV; a fish pathogen) and several fungal viruses such as Rosellinia necatrix quadrivirus 1 (RnQV1) and Penicillium chrysogenum virus (PcV), as well as single-stranded (ss)RNA viruses such as rabbit hemorrhagic disease virus (RHDV) and human rhinovirus (HRV-2). Some of these viruses cause serious diseases, and structural characterisation of their macromolecular assemblies will offer new approaches to altering their function, as well as possible vaccination strategies. Modified capsids offer excellent opportunities in nanotechnology and nanomedicine.

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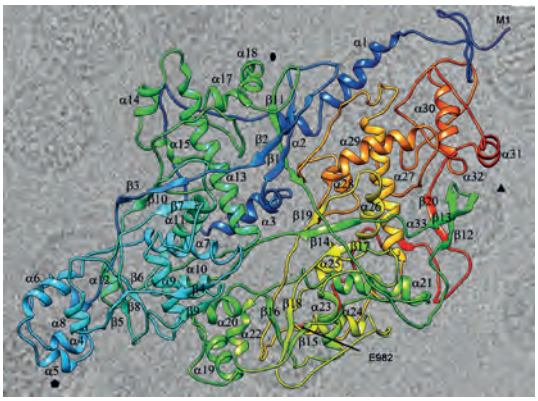

1 *Three-dimensional cryo-EM reconstruction of PcV virions at 4.1 Å resolution. Ribbon diagram of the PcV capsid protein, rainbow-coloured from blue (N terminus) to red (C terminus), the first (Met1; M1) and last (Glu982; E982) residues are shown. Symbols indicate icosahedral symmetry axis. The background image shows PcV particles frozen in vitreous ice.*

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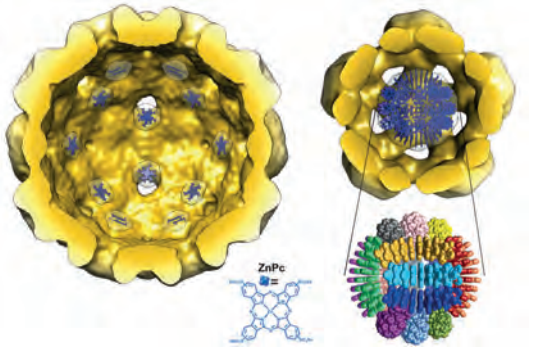

2 *Models of phtalocyanine (ZnPc) organisation within ZnPc-loaded T=3 (left) and T=1 (right) VLP of cowpea chlorotic mottle virus (CCMV). ZnPc-loaded T=3: fitting of 20 ZnPc dimers into the internal density located beneath the hexameric capsomers in 28 nm capsids. ZnPc-loaded T=1: fitting of 18 ZnPc 10-mer stacks into the internal density of 20 nm cryo-EM 3D reconstructed particles.*

Electron tomography and image processing of cell structures

PRINCIPAL INVESTIGATOR:
José Jesús Fernández

POSTDOCTORAL SCIENTISTS:
Mª del Rosario Fernández Fernández
José Ignacio Agulleiro Baldó

PREDOCTORAL SCIENTIST:
Antonio Martínez Sánchez

VISITING SCIENTISTS:
Desireé Ruiz García
Eva Martín Solana

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

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

We also work in close collaboration with Dr Sam Li (UCSF) on the structural elucidation of the microtubule-organising centre (MTOC). Dysfunction of this complex cell organelle is linked to many diseases. We also collaborate with teams at the CNB and other international groups in experimental structural studies.

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

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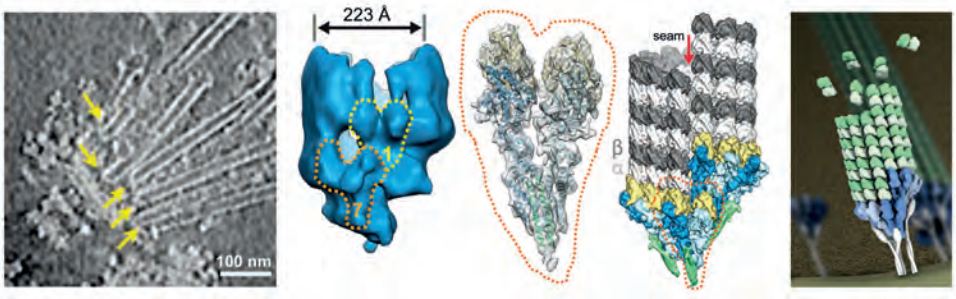

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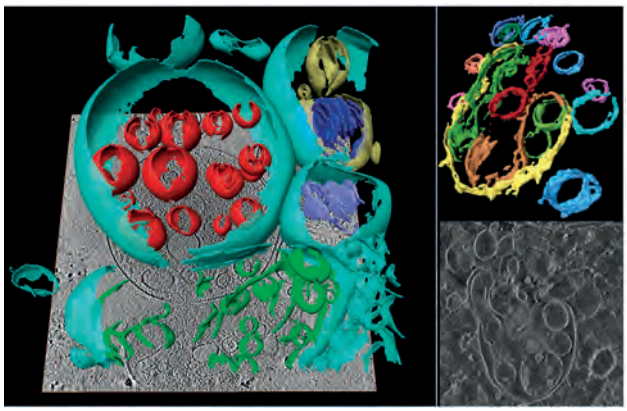

1 *Microtubule nucleation machinery as seen by electron cryomicroscopy. Electron cryotomography of isolated spindle pole bodies from yeast and subsequent subtomogram averaging revealed the in situ structure at 4 nm resolution, showing a ring of seven gTuSC subunits matching microtubule geometry. Electron cryomicroscopy and iterative helical real-space reconstruction allowed determination of the in vitro gTuSC structure at high resolution (7 Å) and modelling in pseudo-atomic detail.*

2 *Three-dimensional visualisation of neuron subcellular architecture with electron tomography and image processing techniques. Left, Electron cryotomography of cerebrocortical synaptosomes from wild type rat. Right, Multivesicular body from a wild type mouse striatal sample prepared with high-pressure freezing and freeze-substitution.*

Molecular biophysics of DNA repair nanomachines

PRINCIPAL INVESTIGATOR:
Fernando Moreno-Herrero

POSTDOCTORAL SCIENTIST:
Carolina Carrasco

PREDOCTORAL SCIENTISTS:
Benjamin Gollnick
María Eugenia Fuentes-Pérez
César López Pastrana
Julene Madariaga Marcos
Alejandro Martín González

We develop and use single-molecule techniques to study the inner workings of protein machines involved in DNA repair processes and maintenance of chromosome structure. We are also interested in the mechanical properties of nucleic acids and their interaction with proteins using single-molecule approaches.

In the last two years, we reported the first use of the magnetic tweezers approach to monitor the dynamics of double-stranded (ds)DNA break resection at single molecule resolution. We defined the functional mechanism of the *Bacillus subtilis* AddAB complex by showing that the Chi recognition domain antagonises translocation activity, causing the complex to stall transiently at Chi sequences. We proposed a simple model for failed and successful Chi recognition, in which the battle between translocation- and sequence-specific binding activities selectively filters for *bona fide* Chi sequences. We also contrasted the mechanical properties of dsRNA and dsDNA at the single-molecule level using magnetic tweezers, optical tweezers and AFM, and showed the structural differences that arise from the A- and B-form arrangements of the polymers. dsRNA has two main elasticity regimes, an entropic regime ended at the A-form contour length and an intrinsic regime ended with a low-cooperative overstretching transition that extends 1.7 times its A-form contour length. We also studied Rep protein binding to single-stranded (ss)DNA in the AT-rich region of the plasmid replication origin. We found a tripartite structure composed of Rep proteins, dsDNA containing Rep-binding sites, and a strand-specific ssDNA of the DNA unwinding element. Finally, we completed construction of an optical tweezers machine (OT) that can manipulate and apply force to single DNA molecules.

Our single-molecule technology was also used in several collaborative projects that include the characterisation of nanometre-size DNA origami structures for potential nanopore devices (with Dr Ulrich F. Keyser, Univ. Cambridge), study of the interaction between a small condensing peptide and ssDNA and dsDNA (with Dr Felix Ritort, Univ. Barcelona), and imaging of bacterial prionoid RepA-WH1 filament structures (with Drs Óscar Llorca and Rafael Giraldo, CIB-CSIC).

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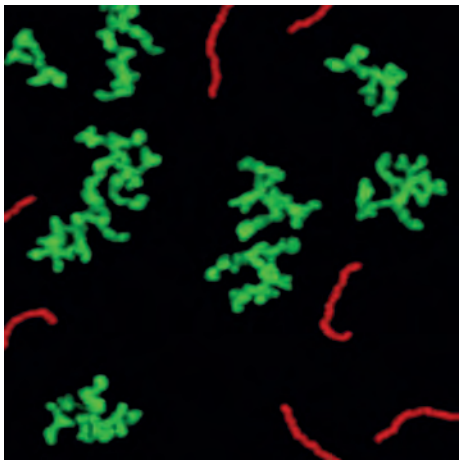

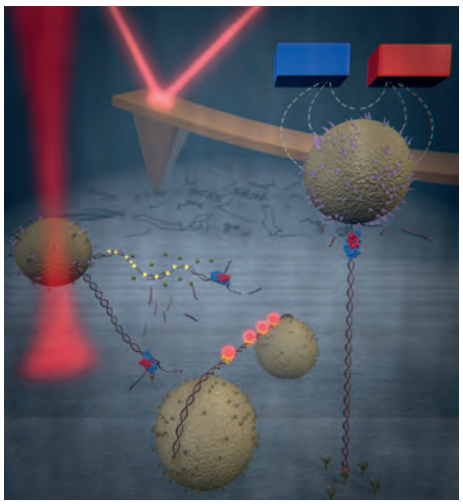

❶ *AFM imaging of single- and double-stranded DNA. DNA molecules in ss and ds form were imaged simultaneously with atomic force microscopy (AFM). The difference in their secondary structures is readily visible with AFM, and allowed us to colour each population of molecules. Using an AFM volumetric method with dsDNA fragment as a fiducial marker, we show that ssDNA molecule length can be accurately related to their AFM volume.*

❷ *Single-molecule methods for watching DNA repair one molecule at a time. AFM (background) for characterisation of SMC-like proteins involved in DNA repair. Optical tweezers (centre) assays used to monitor DNA resection. Tightrope DNA assay (front left) to monitor the dynamics of DNA excision repair proteins using quantum dots. Magnetic tweezers (front right) method to follow resection of a DNA end by the B. subtilis AddAB helicase-nuclease.*

Conformational-functional behaviour of proteins

PRINCIPAL INVESTIGATOR:
Víctor Muñoz

SENIOR SCIENTISTS:
Eva de Alba Bastarrechea
Mourad Sadqi

POSTDOCTORAL SCIENTISTS:
Luis Alberto Campos Prieto
Eduardo López Viñas
Marta Jiménez González
Nicola D'Amelio
Milagros Castellanos Molina
Agnieszka Lewandowska
Pilar López Navajas
Michele Cerminara
Clara M. Santiveri Martín-Varés
Lorenzo Sborgi

PREDOCTORAL SCIENTISTS:
Jörg Schönfelder
Rajendra Sharma
Francisco Ramos
Ravishankar Ramanathan
Celia Sánchez de Medina-Revilla
Nagalakshmi T. Sooriyanarayanan

LABORATORY MANAGER:
Ignacio Torres Rodríguez

Our group studies the conformational and functional behaviour of proteins using a multidisciplinary approach that combines physical chemistry, advanced molecular spectroscopy (ultrafast and single-molecule), structural biology, computer science, protein engineering, and molecular biology. Our efforts in the last two years have centered on three major areas -- experimental and theoretical analysis of protein folding ensembles, investigation of the molecular rheostat hypothesis, and engineering of macromolecular assemblies.

In reference to analysis of protein folding ensembles, we continued our efforts from previous years in developing a catalogue of folding archetypes corresponding to small single-domain proteins with elementary combinations of secondary structure elements.

Our second area of interest emerged from the experimental discovery of downhill folding, which led us to hypothesise that downhill folding modules can exploit their special behaviour, by which they change structure gradually rather than cooperatively, to implement molecular rheostats. Molecular rheostats are proteins able to produce analogue signals at the single-molecule level rather than the binary outputs of conventional molecular switches. These rheostats could offer a new variety of functional and regulatory properties for processes that depend on quantitative signals at the nanoscale; such devices might be used in nature. They also offer tremendous potential for nanobiotechnological applications. In this area, we recently showed proof of concept of a rheostatic biosensor based on the downhill folding module BBL. In parallel, we analyse the biological roles of conformational rheostats in coordinating protein networks via conformational selection, in the phenomenon of DNA sliding and homing-to-target by transcription factors during gene expression, and as molecular springs in macromolecular assemblies.

In a third research area, we recently embarked on a new project that involves engineering of macromolecular devices from monomeric globular proteins. This effort borrows ideas from molecular evolution to implement a design strategy that would facilitate domain swapping between otherwise monomeric proteins by engineering their folding behaviour in ways that reduce their intrinsic folding cooperativity.

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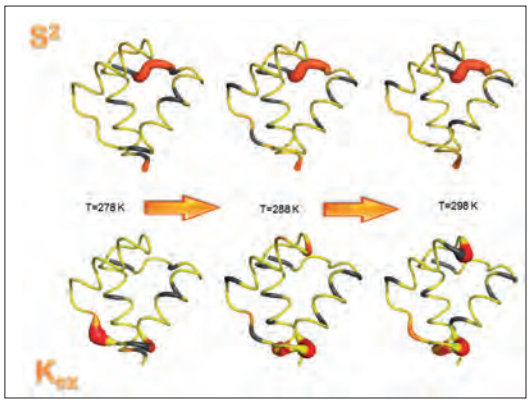

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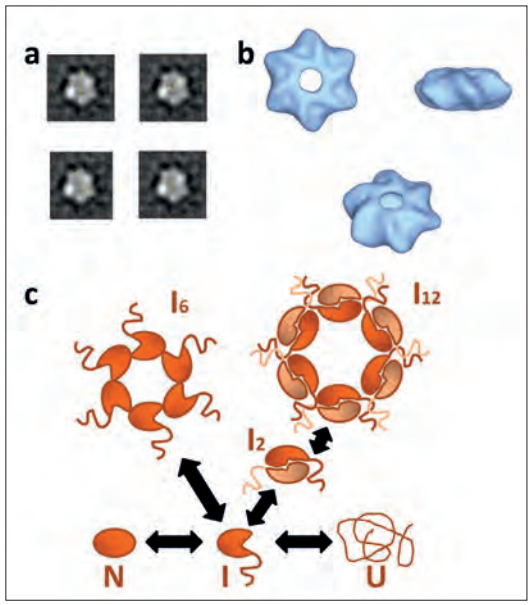

1 *Representation of amide backbone dynamics of the protein structure (pdb code 1ENH) showing mobility in the piconanosecond time scale, according to model-free Lipari Szabo analysis. Order parameter S2 and exchange constant K_{ex} are shown. Colour intensity and tube thickness are inversely proportional to the entity of the parameter in the case of S2, as a lower value indicates greater mobility. Amides for which data are absent are shown in grey.*

2 *Controlled oligomerisation of CI2 induced by mutation. (a) Electron microscopy of individual particles for the dodecamer. (b) 3D reconstruction of the dodecamer after averaging individual particles. (c) Scheme for the oligomerisation process in CI2 mutants.*

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Functional bioinformatics

PRINCIPAL INVESTIGATOR:
Alberto Pascual-Montano

POSTDOCTORAL SCIENTISTS:
Marta Martínez
Javier Setoain

PREDOCTORAL SCIENTISTS:
Daniel Tabas Madrid
Monica Franch Sarto
Dannys Jorge Martínez Herrera

To understand the biology underlying experimental settings, we develop new methods and analytical techniques to solve clearly oriented, specific biological questions. We concentrate on functional bioinformatics, on new methodologies for analysis and interpretation of biological data in two main areas, transcriptomics and functional analysis.

Our recent results are related to transcriptomics regulation. We determined potential interactions between microRNAs and their target transcripts. These interactions are predicted using our methods, then quantified using expression information from mRNA and miRNA in the same samples. We also designed techniques to compare expression profiles with public databases of experiments, allowing association of new information regarding diseases and drugs. A web-based application can be accessed at http://m3rna.cnb.csic.es

The group studies new ways of drug repurposing, using a known drug to treat conditions different from those for which it was designed. We developed NFFinder, a bioinformatics tool to identify potentially useful drugs in the context of orphan diseases. NFFinder uses transcriptomic data to find relationships between drugs, diseases and a phenotype of interest. On a dashboard, the application shows a series of graphics and tables to help researchers formulate repositioning hypotheses and identify potential biological relationships between drugs and diseases. NFFinder is freely available at http://nffinder.cnb.csic.es

Finally, we coordinated and developed dasHPPboard, a proteomics-based dashboard that collects and reports experiments from the Spanish Human Proteome Project consortium (SpHPP), following the strategy of analogue genomics projects such as the Encyclopedia of DNA Elements (ENCODE), which provides a vast amount of data on human cell line experiments. The dashboard includes results of Shotgun and Selected Reaction Monitoring proteomics experiments, post-translational modification information and proteogenomics studies. We processed transcriptomics data from the ENCODE and Human Body Map (HBM) projects for identification of specific gene expression patterns in different cell lines and tissues, with special interest in expressed genes with little available proteomic evidence (missing proteins). Peptide databases were built using single nucleotide variants and novel junctions derived from RNA-Seq data that can be used in search engines for sample-specific protein identification on the same cell lines or tissues. dasHPPboard is freely accessed at http://sphppdashboard.cnb.csic.es

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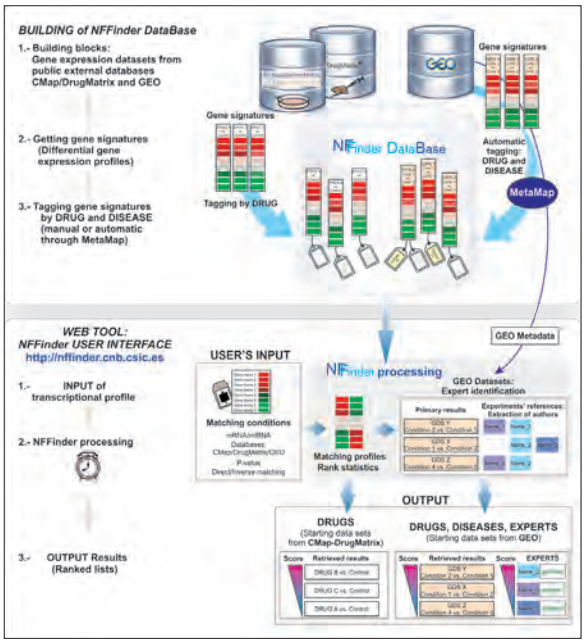

1 *NFFinder, a tool for searching similar transcriptomics experiments in the context of drug repositioning.*

Cell structure lab

PRINCIPAL INVESTIGATOR:
Cristina Risco Ortiz

POSTDOCTORAL SCIENTISTS:
Isabel Fernández de Castro Martín
Laura Sanz Sánchez
Raquel Tenorio Vela

PREDOCTORAL SCIENTIST:
Moisés García Serradilla

MASTER'S DEGREE STUDENT:
David Martínez Tobar
Monserrat Lara Rojas

UNDERGRADUATE STUDENT:
Paula Ortega González

ERASMUS STUDENT:
Juan Carlos García Torres

The project of the Cell Structure Laboratory makes use of current developments in imaging technology to study the biogenesis of viral factories and the structures that support virus replication, morphogenesis and egress. Recent developments in sample preparation and visualisation by light and electron microscopy are facilitating our understanding of the complex interactions that viruses trigger inside cells (Fernández de Castro *et al.* Cell Microbiol 2013). Among these new technologies, three-dimensional (3D) imaging is making a notable impact in the field of virology, as it helps us understand how viruses control cells (Risco *et al.* Annu Rev Virol 2014).

In the last two years, our group has characterised the architecture of the virus factory assembled by the human reovirus (Fernández de Castro *et al.* mBio 2014) and the role of the ESCRT machinery and oxysterol-binding proteins in the formation of the Tombusvirus replicase in intracellular membranes (Barajas *et al.* PLoS Pathog 2014a; Barajas *et al.* PLoS Pathog 2014b). This work was done in collaboration with Drs Terry Dermody (Valderbilt University, TN, USA) and Dr. Peter D. Nagy (Kentucky University, USA). The assembly of the Tombusvirus replicase complex was visualised by metal-tagging transmission electron microscopy, a new, highly-sensitive nanotechnology developed in our lab that reveals proteins in cells at molecular-scale resolution (Fernández de Castro *et al.* Methods Cell Biol 2014).

For the first time, we have 3D images that show how the viral replicase molecules change their organisation when incorporated into the active domains of the intracellular replication compartment. Several steps in the bunyavirus life cycle were also characterised, including the organisation of the viral ribonucleoproteins in the infectious virions (Li *et al.* Proc Natl Acad Sci USA 2013), the assembly of viral factories and replication organelles in cells (Risco & Fernández de Castro Subcell Biochem 2013; Fernández de Castro & Risco Future Virol 2014) and the structures that facilitate virus egress and propagation (Sanz-Sánchez & Risco PLoS One 2013). In 2013, our group created the microscopy consulting service BioGrid Solutions to provide expert advice to scientists and companies (http://www.cnb.csic.es/~Riscolab/).

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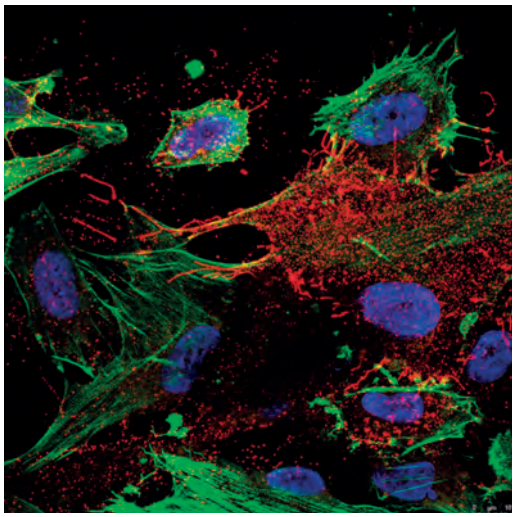

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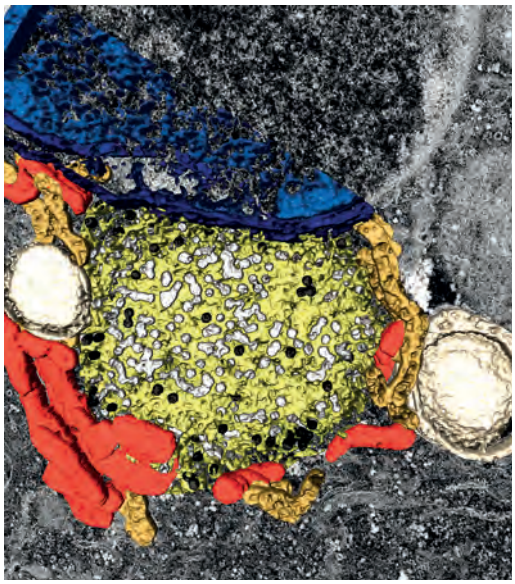

1 *Confocal microscopy of bunyavirus egress and propagation*

2 *Reovirus factory in three dimensions*

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Structural and physical determinants of adenovirus assembly

PRINCIPAL INVESTIGATOR:
Carmen San Martín

POSTDOCTORAL SCIENTISTS:
Ana J. Pérez Berná
Marta del Álamo Camuñas

PREDOCTORAL SCIENTISTS
Rosa Menéndez Conejero
Gabriela N. Condezo Castro
Álvaro Ortega Esteban

STUDENTS:
Javier Cantón Bailón
Verónica Arenas Carreño

TECHNICIAN:
María López Sanz

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 in nanobiomedicine. We approach the problem from an interdisciplinary point of view, combining biophysics, computational, structural and molecular biology techniques.

Adenoviruses (AdV) are pathogens with particular clinical relevance in the immunocompromised population. They are widely used as vectors for gene therapy, vaccination and oncolysis. Adenoviruses have one of the most complex icosahedral, non-enveloped capsids known. Their genome, a dsDNA molecule, is bound to large amounts of positively charged proteins that help condense it and form the core, which is confined within an icosahedral capsid composed of multiple copies of seven viral proteins. The final stage of adenovirus morphogenesis consists of proteolytic processing of several capsid and core proteins. The immature virus, which contains all precursor proteins, is not infectious due to an uncoating defect.

Using atomic force microscopy in physiological conditions, we showed in real time how individual AdV mature and immature particles disassemble sequentially due to mechanical fatigue. We also identified a previously unreported substrate for the viral protease, the putative scaffolding protein L1 52/55k. Cleavages in L1 52/55k disrupt its interactions with other virion components, providing a mechanism for its removal during maturation. Although more than 200 types of AdV have been isolated from a variety of vertebrates, most current knowledge of their biology is derived from a few human AdV. We provided the first molecular description for a lizard AdV with novel architecture of proteins involved in binding to the virus receptor in the cell membrane. This AdV also has favourable characteristics as an alternative therapeutic vector.

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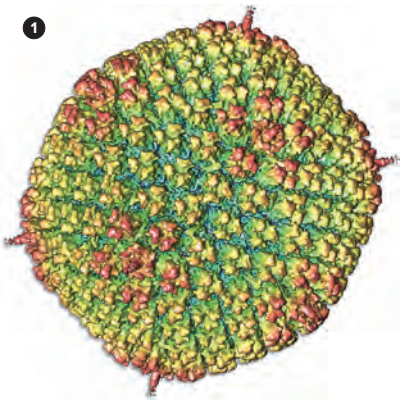

1 *General architecture of human adenovirus as determined by cryo-electron microscopy. This complex capsid has a maximum diameter of 95 nm, not including the long fibres that protrude from the vertices, whose flexibility renders them invisible in cryo-EM studies. The capsid is composed of multiple copies of seven proteins, and contains the dsDNA genome, condensed with the help of three viral proteins similar to histones. Protein L1 52/55k is needed for genome packaging but is not present in the final virion; it is released after packaging with the help of the viral protease.*

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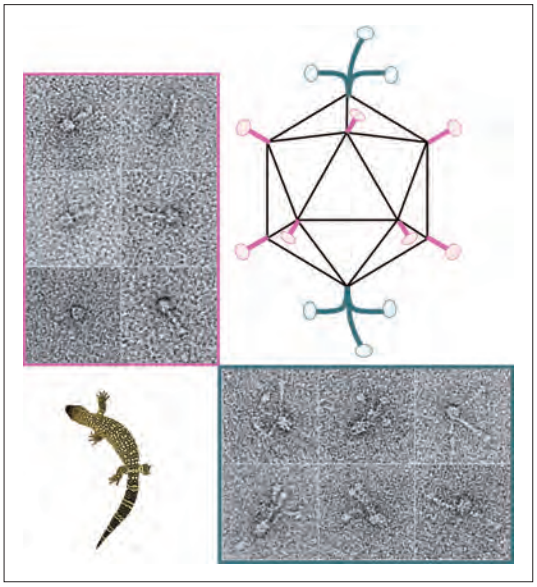

2 *The unusual vertex organisation of a lizard adenovirus. Lizard adenovirus type 2 (LAdV-2) was isolated from Heloderma horridum (bottom left). It is the first adenovirus with either one short (pink) or three long (green) fibres per vertex. Our studies indicate that only one or two vertices per particle harbour the long fibre triplet. Because fibres bind the virus receptor at the cell surface, this previously unobserved fibre arrangement suggests an alternative form of cell entry for this reptilian adenovirus.*

Structure and function of molecular chaperones

PRINCIPAL INVESTIGATOR:
José María Valpuesta

SENIOR SCIENTISTS:
Rocío Arranz
Jorge Cuéllar

POSTDOCTORAL SCIENTISTS:
Elías Herrero
Begoña Sot

PREDOCTORAL SCIENTISTS:
Sara Alvira
Srdja Drakulic
Lucía Quintana
Marina Serna
Marta Ukleja

TECHNICIANS:
Ana Beloso
Virginia Rodríguez

VISITING SCIENTISTS:
Paulina Aguilera
(Universidad de Chile)
Anna Karlowicz
(University of Gdansk, Poland)
Alina Röhl
(Munich Technical University, Germany)
Andreas Svanstrom
(University of Gothenburg, Sweden)

Molecular chaperones are proteins that assist the folding and degradation of other proteins. Our main line of work deals with the structural and functional characterisation of different molecular chaperones and their interaction in the protein folding and degradation assembly pathways. Using various techniques, principally electron microscopy and image processing, we work with chaperones like CCT, Hsp110, Hsp90, Hsp70, Hsp40, nucleoplasmin, and with some of their cochaperones, including Hop, Hip and CHIP. We have characterised several complexes formed by these chaperones and their co-chaperones that form part of the various assembly lines involved in protein homeostasis with substrates such as p53, VHL, Gb transducin. The same techniques have been used to study other proteins and macromolecular complexes such as amyloids and RNA processing proteins.

Finally, we have worked on 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 DNA polymerase from phage Φ29.

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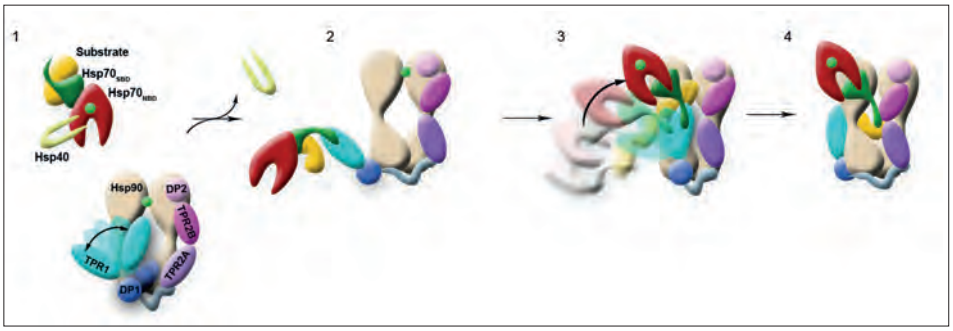

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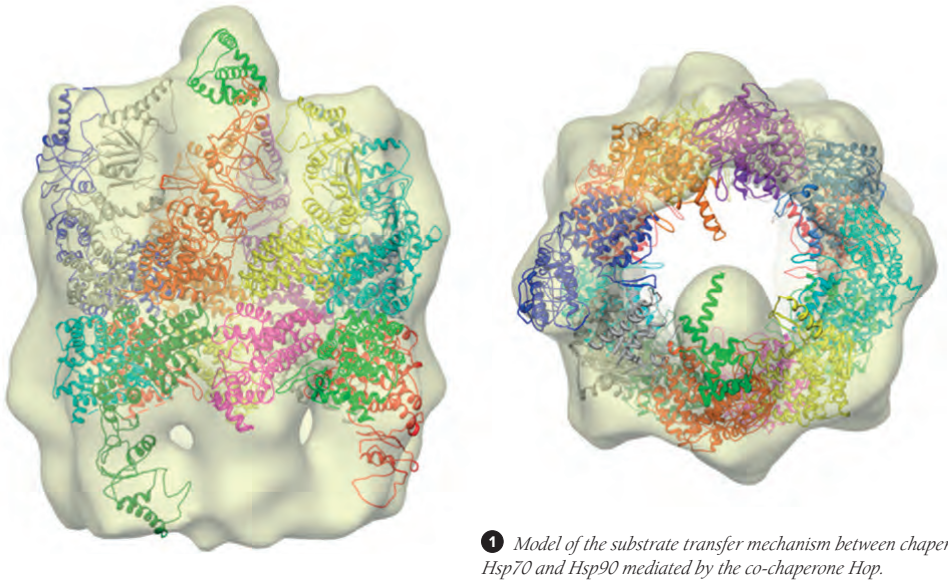

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1 *Model of the substrate transfer mechanism between chaperones Hsp70 and Hsp90 mediated by the co-chaperone Hop.*

2 *Two orthogonal views of the complex between the chaperonin CCT and the protein PDC5, with the docking of the crystal structure of the open form of CCT coloured by subunit (Protein Data Bank code 2XSM) and the atomic structure of PDCD5 in green (Protein Data Bank code 2K6B).*

Structural biology of viral fibres

PRINCIPAL INVESTIGATOR:
Mark J. van Raaij

POSTDOCTORAL SCIENTISTS:
Laura Córdoba García
Meritxell Granell Puig

PREDOCTORAL SCIENTISTS:
Carmela García Doval
Abhimanyu K. Singh
Thanh H. Nguyen
Marta Sanz Gaitero

VISITING SCIENTIST:
Monica Z. Ballmann
(Hungarian Academy of Sciences, Hungary)

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 T4, T5 and T7. These fibres all have the same basic architecture; they are trimeric and have an N-terminal virus or bacteriophage attachment domain, a long, thin but stable shaft domain, and a globular C-terminal cell attachment domain. These trimeric fibrous proteins are very resistant to temperature- and detergent- induced denaturation.

In 2013 and 2014, we determined the structures of the L-shaped tail fibre of bacteriophage T5 (the pb1 protein) with and without its C-terminal intramolecular chaperone, of the bacteriophage T4 fibre protein gp34, and of several animal adenovirus fibre proteins (snake atadenovirus 1, turkey siadenovirus 3, bovine atadenovirus 4, goose aviadenovirus 4 and mouse mastadenovirus 2).

Knowledge of the structures of viral and bacteriophage fibre proteins could lead to a variety of biotechnological applications. As adenovirus is used in experimental gene therapy, modification of its fibre should allow targeting to specific cell receptors. Modification of the bacteriophage fibre receptor binding specificities could permit improved detection and elimination of specific bacteria.

We also collaborated with other research groups in crystallisation and structure solutions for the proteins and peptides they produce, and determined structures of cyclic antibiotic peptides, bacterial dehydroquinases and shikimate kinases in complex with inhibitors, and of the bacteriophage K endolysin domain CHAPK.

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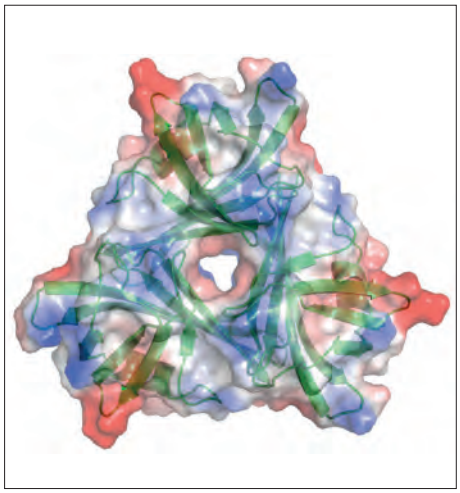

1 *Structure of the atadenovirus Snake Adenovirus 1 fibre head.*

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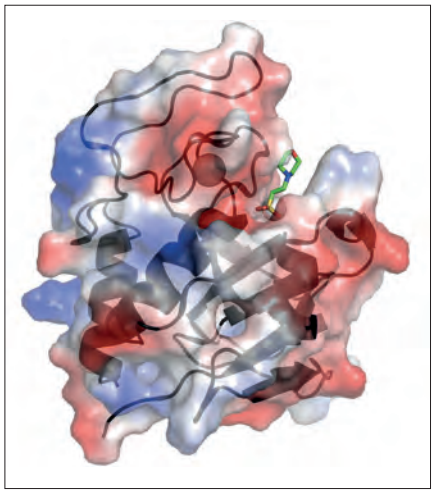

2 *Structure of the staphylococcal endolysin CHAPK domain.*

3 / Immunology and Oncology

The Department of Immunology and Oncology studies the molecular and cellular basis of immune system function and tumour development. Our intention is to generate knowledge that can help develop new and improved approaches to immune response modulation during inflammatory reactions, infection and tumour development, and to identify targets for the prevention, diagnosis and treatment of cancer.

This is an exciting time for research in immunology and oncology. In the last two years, the department's work has addressed many aspects of innate and adaptive immunity, which without doubt will have an impact on immunotherapeutic applications. We continue at the front line of immunology and oncology research, 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.

In 2013-2014, Dr Yolanda Carrasco was established as a Staff Scientist in the department, and we have welcomed Dr Antonio Bernad's group, which reinforces the department's activity in stem cell research. The molecular and cellular mechanisms that underlie the immune response and tumour development often overlap, providing many opportunities for collaboration among the groups in the department as well as with other labs in the CNB and at other institutes. An example is the nanobiomedicine initiative to develop nanotechnology-based biomedical applications.

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

PRINCIPAL INVESTIGATOR:
Carlos Ardavín

SENIOR SCIENTIST:
María López-Bravo

PREDOCTORAL SCIENTISTS:
María Minguito
Jorge Domínguez
Lidia Feo

TECHNICIAN:
Leticia González

VISITING SCIENTISTS:
Fernanda Sánchez Vallecillo
(CONICET, Argentina)
Daria Kamzol
(Wrocław University of Technology, Poland)

Our research explores the development and functional specialisation of inflammatory dendritic cells and macrophages during the immune response to bacterial (*Klebsiella pneumoniae, Citrobacter rodentium*) and fungal (*Candida albicans*) infections, allergic reactions induced by acaridae-derived allergens, and sterile inflammation caused by peritoneal injury.

The methodology designed for addressing these objectives involves the following experimental approaches:

Development of *in vitro* and *in vivo* infection models, allergic reactions and peritoneal injury in different mouse strains, including mice deficient in cytokines, cytokine/chemokine receptors, or molecules involved in dendritic cell and macrophage differentiation or activation.

Cell biology techniques designed for the purification or isolation of defined cell populations from mouse bone marrow, lung, kidney, lymph nodes, spleen and peritoneum involving magnetic bead and FACS cell separation methods.

Analysis of monocytes, dendritic cells and macrophages in cell suspensions or tissue sections, by electron microscopy or confocal microscopy after immunofluorescent staining.

Analysis of gene expression at the mRNA level by real-time quantitative PCR, whole mouse genome microarray analyses and chromatin immunoprecipitation.

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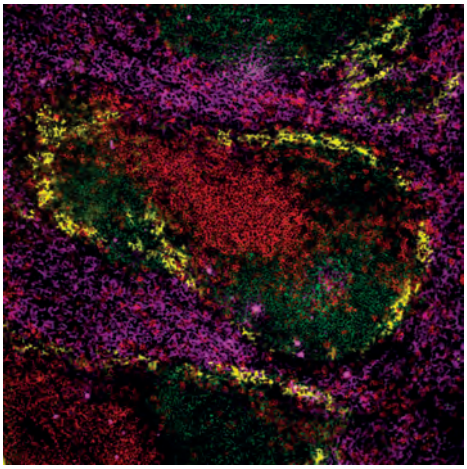

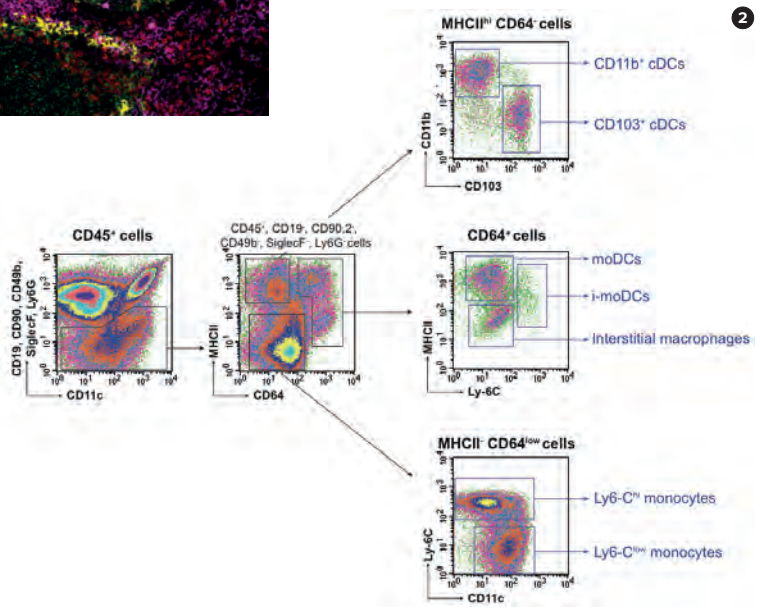

2

1 *Characterisation of spleen macrophage subpopulations during Listeria monocytogenes infection*

2 *Six-colour FACS analysis of lung dendritic cell and macrophage subsets during HDM-induced allergy*

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

PRINCIPAL INVESTIGATOR:
Dimitrios Balomenos

PREDOCTORAL SCIENTISTS:
Gorjana Rackov
Rahman Shokri
Ana Belén Saccone García
Kathrin Weber

STUDENTS:
Adrián Madrigal Avilés
Jorge de Andrés Anaya

SELECTED PUBLICATIONS

Daszkiewicz L, Vazquez-Mateo C, Rackov C, Ballesteros-Tato A, Weber K, Madrigal-Aviles A, Di Pilato M, Fotedar A, Fotedar R, Flores JM, Esteban M, Martinez-A C, Balomenos D. Distinct p21 requirements for regulating normal and self-reactive T cells through IFN-γ production. Sci Rep 2014; 5:7691

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

T cell responses are necessary to react to and eliminate external microorganisms and protect humans from infection. Genetic or environmental factors can cause aberrant responses of T cells to the organism they are meant to protect, generating autoreactive memory T cells. Compared to normal memory T cells, autoreactive T cells have unique features due to their repeated encounters with autoantigens. We work to identify differences between normal and autoimmune memory T cells.

Enhanced p21 expression suppresses autoimmune but not normal T cell memory responses

We previously identified p21 as a regulator of autoimmune manifestations. Although several later studies from our group and other laboratories addressed the effect of p21 in T cells and autoimmunity, the mechanism by which p21 directs its autoimmunity-suppressing effect has been a point of debate. We showed that p21 overexpression by T cells of autoimmune lupus-prone Fas-deficient (*lpr*) mice reduces autoimmunity independently of the p21 effect as a cell cycle inhibitor; instead, p21 limited activation of autoreactive B6-*lpr* memory T cells and their ability to produce IFN-γ. We are addressing the therapeutic potential of p21, since it reduces autoreactive T cell activation but does not affect normal protective immunity.

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 have identified a previously unreported role for Fas, and establish an attenuating role in the response of persistently activated T cells. We now focus on the mechanistic aspects of this new role of the Fas/FasL system on preactivated T cells.

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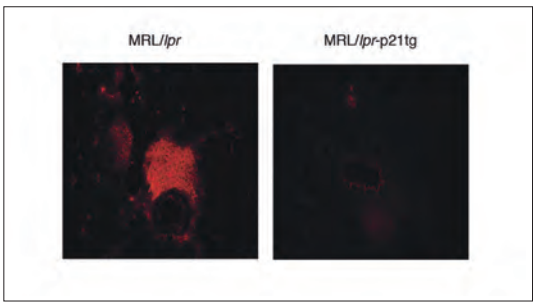

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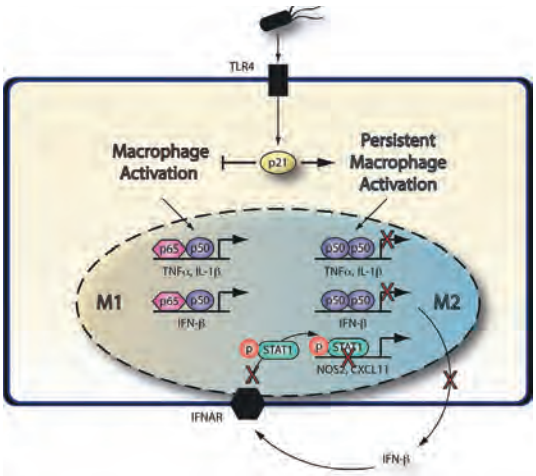

p21 regulates macrophage activation and polarisation

Independently of its cell cycle inhibitory capacity, p21 regulates macrophage activation by controlling the NF-κB pathway, and decreases sensitivity to septic shock. We also found that p21 enables polarisation of M1 to M2 macrophages by promoting p50/p50 formation, and thus inhibits IFN-β production, STAT1 phosphorylation and iNOS production after persistent inflammation. We are studying how p21 controls macrophage activation and polarisation.

1 *Decreased infiltration of destructive CD4+ T cells (red) in the kidneys of p21-overexpressing MRL/lpr-p21tg compared to MRL/lpr mice.*

2 *p21 regulates macrophage activation through NF-κB inhibition. During persistent inflammation, p21 promotes p50/p50 formation and M1 to M2 macrophage polarisation by suppressing IFN-β production and downstream events that define M1 responses.*

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

PRINCIPAL INVESTIGATOR:
Domingo F. Barber

POSTDOCTORAL SCIENTISTS:
José Manuel Rojas Carrasco
Marinn Talelli
Raquel Mejías Laguna

PREDOCTORAL SCIENTISTS:
Patricia Hernández Flores
Laura Sanz Ortega
Vladimir Mulens
Laura Morillas Navas
Roberto Spada
Teresa M. Zotes Ciprés

TECHNICIAN:
Sonia Pérez-Yagüe

TECHNOLOGY ADVISOR:
José Luis Tajada Herráiz

The group's research lines are organised around the study of the mechanisms that regulate the immune response and immune tolerance, as modulation of these mechanisms might be a treatment strategy for autoimmune diseases and in cancer immunotherapy.

We studied the role of phosphoinositol-3-kinase p110γ in the regulation of *in situ* apoptosis, macrophage proliferation and polarisation towards M1 or M2 phenotypes in atherosclerotic lesions. Atherosclerotic plaques in fat-fed LDLR$^{-/-}$p110γ$^{-/-}$ mice were smaller than controls, with less immune cell infiltration and less macrophage proliferation in atherosclerotic lesions. This was associated with higher intracellular cAMP levels, suggesting that higher cAMP levels and the ensuing inhibition of macrophage proliferation contribute to atheroprotection in LDLR-null mice lacking p110γ.

We observed increased expression of the NKG2D ligand MICA in systemic lupus erythematosus (SLE) patient kidneys but not healthy subjects. We found glomerulus-specific expression of the NKG2D ligands Rae-1 and Mult-1 in various murine SLE models, which correlated with a larger number of glomerular-infiltrating NK cells. MRL/MpJ and MRL/MpJ^{lpr} SLE-like mice showed phenotypically immature NK cells accumulate in the spleen, but not in bone marrow or kidneys of diseased mice. These findings and the presence of glomerulus-specific NKG2D ligands in lupus-prone mice identify a role for NK cells and NKG2D ligands in the lupus nephritic process.

We also develop nanoparticle-based nanomedicines that allow efficient, specific targeting of drugs, biomolecules or cell types to a desired site of action for antitumour and immunosuppressive therapies. This objective is divided into five sub-objectives:

1. Comparative analysis of approaches to efficient nanoparticle targeting in cancer therapies, and development of new-generation polymeric magnetic nanoparticles.

2. Direct specific magnetic nanoparticle-loaded cells by applying an external magnetic field to the target tissue for immunomodulatory lymphoid cell transfer therapies.

3. Development and therapeutic study of magnetic nanoparticles functionalised with RNA interference.

4. Study of the cell interactome induced by magnetic nanoparticle and metabolic degradation of iron oxide nanoparticles to reduce systemic toxicity and increase effectiveness.

5. Study of the biological effects of intracellular hyperthermia-mediated nanoparticles.

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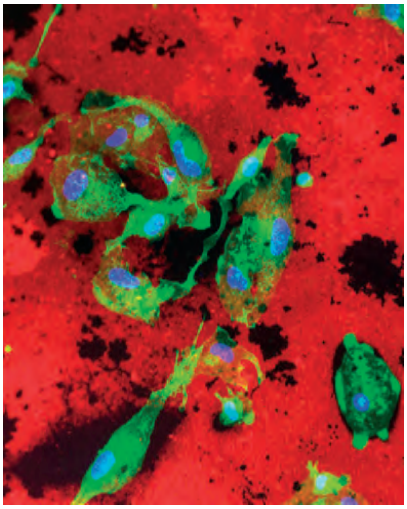
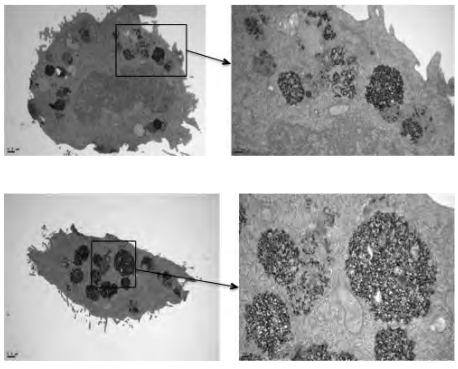
PATENT

P201431531. Dispositivo para generar hipertermia en células mediante nanopartículas magnéticas (MNPs)



1 *Matrix (red) degradation by THP-1 derived macrophages (green) associated with superparamagnetic ion oxide nanoparticles (SPIONs). (Photo: L Sanz/JM Rojas)*



2 *Uptake of magnetic nanoparticles by pancreatic adenocarcinoma (Pan02) cells, observed by transmission electron microscopy (TEM). (Photo: M Talelli)*

Cardiac stem cells

PRINCIPAL INVESTIGATOR:
Antonio Bernad Miana

POSTDOCTORAL SCIENTISTS:
José Luis Torán García
Susana Cañón Sánchez
María Tomé Pizarro

PREDOCTORAL SCIENTISTS:
Diego Herrero Alonso

TECHNICAL ASSISTANCE:
Rosa María Carmona Canorea
Susana Aguilar García

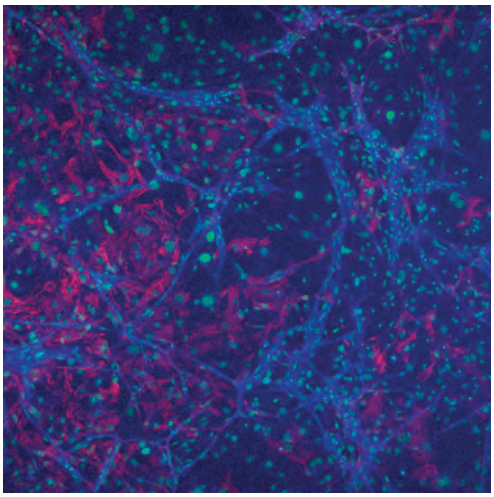
VISITOR:
Francisco Miguel Cruz Uréndez
(CNIC, Madrid)

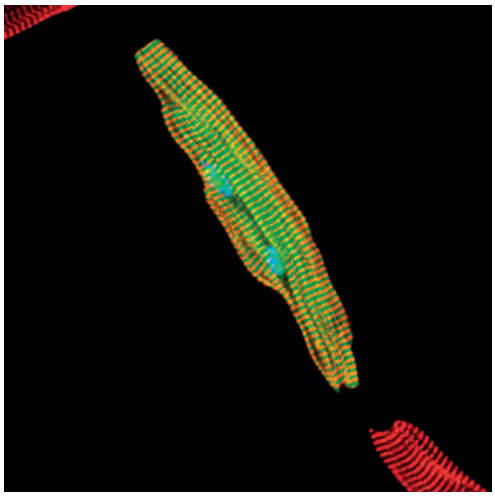
Myocardial infarction is one of the major challenges facing health care systems in developed countries. Its treatment, despite recent advances, remains a serious problem and requires multidisciplinary approaches. Adult organ function depends on the concerted, regulated action of specialised stem cells. Our group focusses on the study of multipotent cardiac progenitor cells (CPC) isolated from adult heart, although precise understanding of cardiac precursor and stem cell biology is still lacking. This is a critical step toward developing more rational strategies to fight against cardiovascular disease.

Human CPC (also mouse and pig) have been characterised as MSC (mesenchymal stem cell)-like populations. Partial hCPC membrane proteomic analysis allowed the definition of more than 30 relevant functions especially associated with adult stem cell biology. We demonstrated that podocalyxin-like protein 1 (PODXL) regulates hCPC activation, migration and differentiation, and also modulates their local immunoregulatory capacity (Moscoso *et al.,* 2013). In the last two years, we have been involved in an intensive collaborative European effort (CAREMI) for the molecular characterisation of hCPC using complementary high-throughput platforms (global proteomics, ITRAQ, RNAseq). In summary, we have identified 9,943 proteins, of which 25.3% are exclusive to or preferentially expressed in hCPC.

In 2014, an international clinical trial (Ia-IIb) was launched to evaluate the therapeutic potential of hCPC in acute myocardial infarct. At the moment, the escalation phase has concluded (6 patients) and the final phase (49 additional patients) is under way.

In mouse models, we demonstrated that the transcriptional factor Bmi-1+ (a member of the polycomb family) is an important marker of mouse CPC (mCMC). The Bmi-1+ CMC population (B-CPC) contributes to homeostatic cardiomyocyte turnover and repair after acute injury, and fulfils the criteria for denomination as long-term cardiac resident stem cells. B-CPC are distributed throughout the entire myocardium, forming small groups of cells found preferentially in perivascular locations or embedded in sarcomers. We are currently studying the distinct stimuli that affect the biological responses of B-CPC *in vivo*, as well as its importance in heart turnover and functional demonstration of candidate pathways identified by differential RNAseq analysis.





1 *Spontaneous differentiation of B-CPC in vitro. Endothelial cells (CD31, blue) and smooth muscle cells (SMA; red); nuclei (green).*

2 *Mature binucleated cardiomyocyte derived in vivo from B-CPC (YFP+). Sarcomeric α-actinin(red); nuclei (blue).*

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B cell dynamics

GROUP LEADER:
Yolanda R. Carrasco

PREDOCTORAL SCIENTISTS:
Julia Sáez de Guinoa Corral
Laura Barrio Cano

MASTER'S DEGREE STUDENTS:
Sara Violeta Merino Cortés
Sara Román García
Adrián Tirado

Lymphocyte dynamic plasticity is intrinsic to lymphocyte function and is crucial for adaptive immune protection. Better knowledge of the molecular mechanisms that govern lymphocyte behaviour will reveal essential aspects of the immune response. It will also help to understand the ability of certain cancer cells to acquire lymphocyte-like dynamics in order to migrate and invade other tissues (metastasis). Using B lymphocytes as a model system and state-of-the-art technology, we study (1) the molecular mechanisms that underlie the switch between two opposite cell behaviours (stopped/synapse vs motile/kinapse) in the presence of chemokine and antigen, and (2) the functional relevance of the synapse/kinapse status balance for B cell fate; (3) we also study the importance of pathogen-mediated effects on B cell dynamics in influencing and their influence on the synapse/kinapse balance. Our recent results can be summarised in two main points.

Molecular mechanisms involved in the regulation of B cell dynamics by antigen and CXCL13. We identified a major role for the scaffold protein vinculin in governing B cell adhesion and motility. BCR signalling triggers vinculin recruitment to the immune synapse, where vinculin co-localises with F-actin at the peripheral supramolecular activation cluster (pSMAC). Lack of vinculin at the synapse impairs appropriate pSMAC assembly, enabling B cells to move in response to CXCL13 while displacing the antigen cluster to the cell uropod. Syk and actomyosin regulate vinculin recruitment and stability at the synapse. Vinculin activation and translocation from cytosol to adhesion sites requires its interaction with the lipid PIP2. Accordingly, PIP2 and the enzyme that produces it, PIPKIgamma, localise at the immune synapse pSMAC, and vinculin recruitment coincides with a PIPKIgamma-mediated PIP2 wave at early stages of synapse formation.

Modulation of B cell dynamics by innate signals. We found that TLR4 stimulation by lipopolysaccharide (LPS; a cell wall component of gram-negative bacteria) modulates B cell behaviour in dose- and exposure time-dependent manners. TLR4-stimulated B cells have enhanced cell polarisation and migration, as well as increased directionality in their movement compared to unstimulated B cells; the MyD88-dependent signalling cascade and Rac GTPases are involved in these changes.

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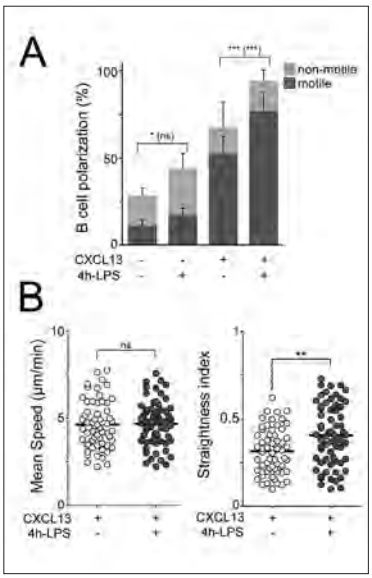

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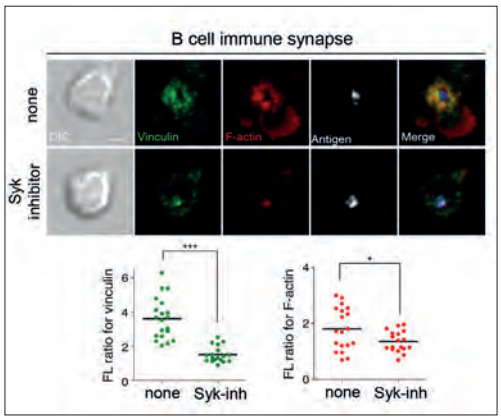

1 *TLR4 stimulation enhances B cell polarisation, migration and straightness*

2 *Syk regulates vinculin recruitment to the B cell immune synapse*

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

PRINCIPAL INVESTIGATOR:
Ana C. Carrera

POSTDOCTORAL SCIENTISTS:
Ana María González-García
Ariel E. Cariaga Martínez
Rosa Varona Valle
Raúl Jiménez Pérez
Javier Redondo Muñoz

PREDOCTORAL SCIENTISTS:
Jesús Vallejo Díaz
Vicente Pérez García

TECHNICIANS:
Carmen Hernández Agüero
Lorena Sanz González

We focus on the molecular mechanisms by which kinases control cell behaviour and, when altered, human disease. In recent years, we have concentrated especially on phosphoinositide 3-kinase (PI3-kinase), an enzyme that generates the PIP3 product, which is increased in cancer and autoimmunity as it triggers cell survival, invasion and division.

PI3-kinase mouse models and biochemical approach

PI3-kinases are heterodimers composed of a p110 catalytic and a p85 regulatory subunit. p110alpha and beta, and associated p85alpha and p85beta, are ubiquitous and are altered in cancer, whereas p110delta and the closely-related p110gamma isoform are more abundant in haematopoietic cells; when deregulated, they are involved in development of chronic inflammation/autoimmunity. In animal models, we study the physiological function of PI3K and the consequences of its deregulation in cancer and autoimmunity.

Mechanism of PI3-kinase beta action on DNA/chromatin remodelling

The PI3-kinase pathway is frequently altered in cancer. Many laboratories and companies have focussed on PI3-kinase alpha; we study the less well-known PI3-kinase beta isoform. We showed that PI3-kinase beta (p110beta) localises to the nucleus and regulates DNA replication, segregation and repair. We aim to determine the molecular basis of p110beta action in DNA homeostasis and chromatin remodelling in cancer and stem cells to understand why certain tumour types increase p110beta expression.

Alternative cancer treatment based on interfering molecules

Metastasis remains the leading cause of death from many tumours. The PIP3 product enables cell survival; for this reason, cells with altered regulation of PI3-kinase alpha or beta, or with mutations in the phosphatase PTEN (which reduces PIP3 levels), are present in ~50% of human tumours, most often at metastatic phases.

PI3-kinase is a key target in cancer, but only broad-spectrum inhibitors or p110alpha inhibitors are being tested clinically. Our aim is to develop a strategy for the treatment of tumours accessible by endoscopy (or orally), based on delivery of interfering molecules (RNA and peptides). The first target selected is p85beta. p85 subunits regulate the activity and localisation of this enzyme. Normal cells express mainly p85alpha, while some tumour types show predominant p85beta expression that correlates with metastasis. In parallel to defining p85beta mechanism of action in metastasis, we study the effect of interfering with p85beta action in an established tumour in the mouse.

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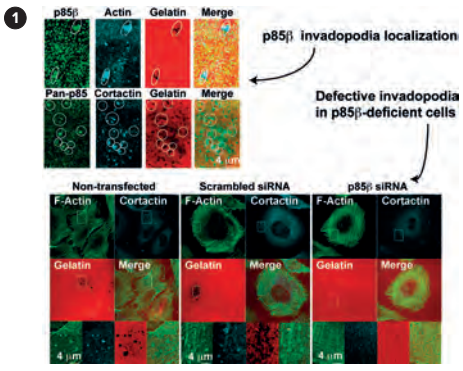
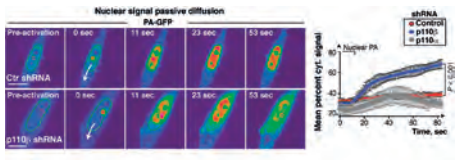




1 *Localisation of p85beta (green) at invadopodia identified by costaining of actin or cortactin (blue) and by its capacity to degrade gelatin (red). Depletion of p85beta with interfering RNA impairs cell invasion analysed as the degradation of gelatin (red) in controls but not in cells lacking p85beta expression (bottom).*

2 *Passive diffusion of GFP outside the nucleus, enhanced after p110beta depletion.*

Role of stress-activated protein kinase p38MAPK in human diseases

PRINCIPAL INVESTIGATOR:
Ana Cuenda

ASSOCIATE SCIENTIST:
Lourdes Planelles

POSTDOCTORAL SCIENTISTS:
Ana Risco

PREDOCTORAL SCIENTISTS:
Rafal Zur
Dayanira Alsina-Beauchamp
Alejandra Escós

TECHNICIAN:
Ruth Gómez-Caro
Carla Argüello Pilco

MASTER'S DEGREE STUDENTS:
Angel Nuñez-Buiza
Laura García-Ibañez
Esther Blanco

VISITING SCIENTISTS:
Noelia Aparicio
(Universidad Francisco de Vitoria, Spain),
Carmen Vela
(Universidad Europea de Madrid, Spain)

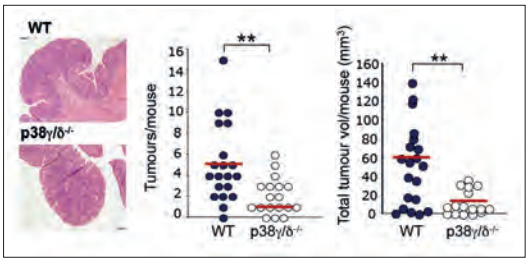
Our group studies the physiological and pathological functions of the p38MAPK family of mitogen-activated protein kinases. Our research focusses on

• the discovery of new substrates, interacting proteins and inhibitors for these kinases, as well as study of their physiological roles using mice transgenic for distinct p38 isoforms, and

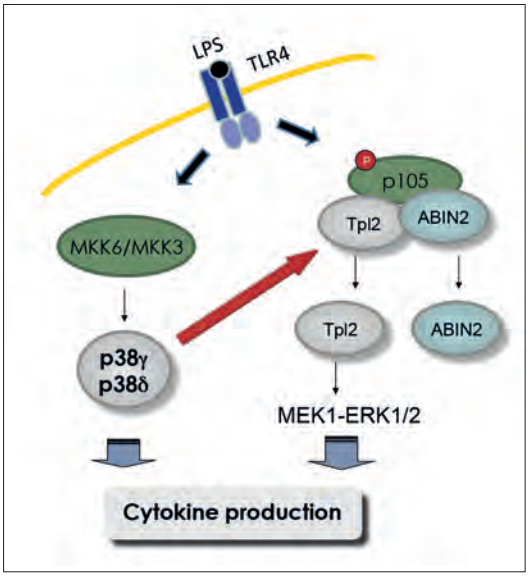
• the study of p38MAPK as a link between chronic inflammation and cancer, and as mediators of chronic inflammatory diseases.

In the 2013-2014 period, we analysed the role of p38γ and p38δ, two of the less well-known p38MAPK family members, in chronic inflammation and in cancer, using several mouse models. In the collagen-induced arthritis (CIA) model of chronic inflammation, we showed that combined p38γ and p38δ deficiency markedly reduced arthritis severity and suppressed clinical disease, synovial inflammation and bone destruction compared with that in WT (wild-type) mice. Reduced disease severity in p38γ/δ-null mice was associated with lower cytokine production, T cell proliferation and anti-collagen antibody responses than in controls, indicating that p38γ and p38δ are crucial regulators of inflammatory joint destruction in CIA. We proposed p38γ and p38δ as potential therapeutic targets in complex diseases, such as rheumatoid arthritis, that involve innate and adaptive immune responses.

Chronic inflammation is a known risk factor for tumourigenesis. We analysed the role of p38γ and p38δ in colitis-associated colon cancer using the azoxymethane/dextran sodium sulphate colitis-associated colorectal cancer model. We found that p38γ/δ deficiency decreased tumour formation significantly, in parallel with decreased production of proinflammatory cytokines and chemokines. Analysis of leukocyte populations in p38γ/δ-null mouse colon showed less macrophage and neutrophil recruitment than in WT mice. WT chimaeric mice with transplanted p38γ/δ$^{-/-}$ bone marrow (BM) had fewer tumours than WT mice transplanted with WT BM, whereas tumour number increased significantly in p38γ/δ$^{-/-}$ chimaeric mice with WT BM compared to p38γ/δ$^{-/-}$ mice that received p38γ/δ$^{-/-}$ BM. Our results establish that p38γ and p38δ are central to colon inflammation-induced tumour formation by regulating haematopoietic cell response to injury, and validate p38γ and p38δ as potential targets for cancer therapy.



1 *Reduced incidence of colitis-associated tumours in p38γ/δ-deficient mice*



2 *New p38γ/p38δ-dependent pathway of cytokine production in response to bacteria lipopolysaccharide (LPS)*

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Cancer epigenetics

PRINCIPAL INVESTIGATOR:
Mario Fernández Fraga

POSTDOCTORAL SCIENTISTS:
Agustín Fernández Fernández
Rocío González Urdinguio
Gustavo Fernández Bayón
Sandra Rodríguez Rodero

PREDOCTORAL SCIENTISTS:
Estela García Toraño
María García García
Antonella Carrella

STUDENTS:
Álvaro del Real Bolt
Thalia Belmonte García

TECHNICIANS:
Cristina Mangas Alonso
Cristina Bravo Mendoza

The cancer epigenetics laboratory is interested in the study of epigenetic mechanisms during cell development and differentiation as well as their alterations in ageing and cancer. To address these issues, we carried out genome-wide analyses of DNA methylation and histone modification in adult stem and terminally differentiated cells and in different tumour types. Using these approaches, we identified a chromatin signature (H3K4me1) associated with DNA hypomethylation in human ageing. We also identified DNA methylation patterns associated with specific thyroid cancer subtypes and with the expression of glycine N-methyltransferase in human hepatocellular carcinoma.

Using autologous and heterologous experimental systems in mice, we demonstrated that the aberrant DNA demethylation-dependent expression of the cytokine GM-CSF and its receptor by colon cancer cells has strong an Antitumoural activity of cytokine GM-CSF in colon cancer ti-tumour effects and is an independent marker of good prognosis in patients with colorectal tumours. Our laboratory also had the opportunity to collaborate in a study that, using a genetic progression model of Braf(V600E), identified new targets for therapeutic intervention in intestinal cancers. We also contributed to the first reconstruction of the Denisovan and the Neanderthal DNA methylomes.

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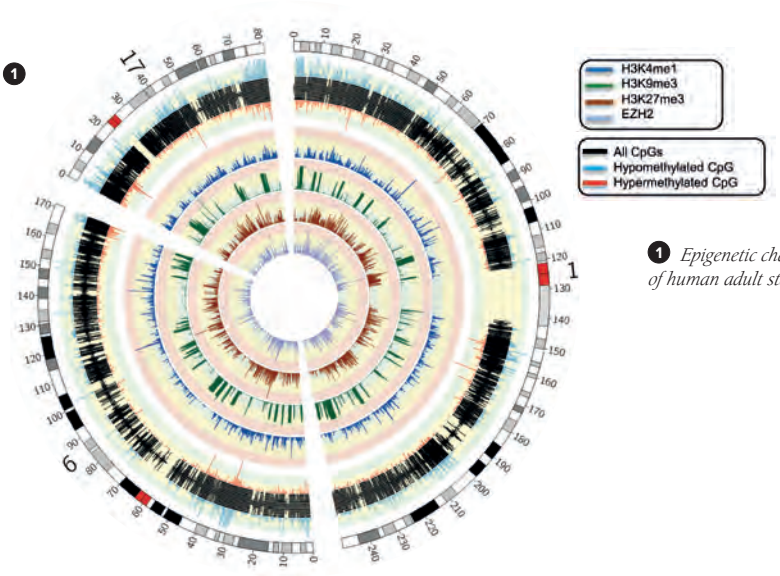

1 *Epigenetic changes during ageing of human adult stem cells.*

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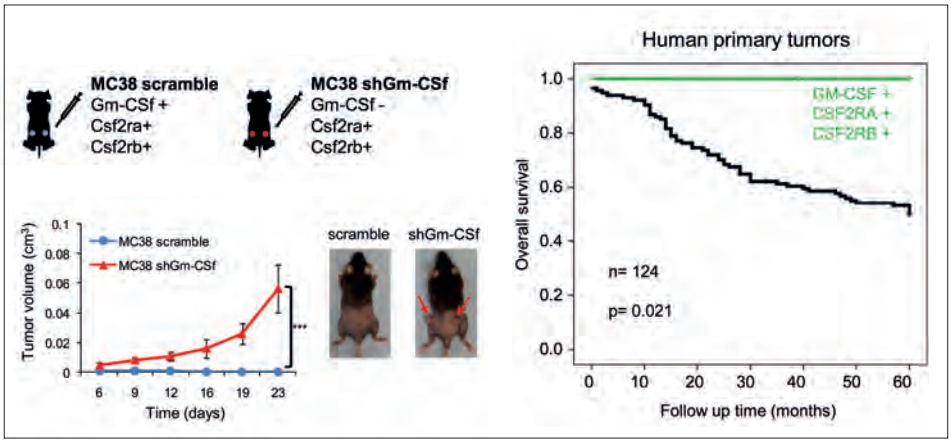

2 *Antitumour activity of cytokine GM-CSF in colon cancer.*

Anti-tumour activity of chemokine receptor-specific antibodies

PRINCIPAL INVESTIGATOR:
Leonor Kremer

POSTDOCTORAL SCIENTIST:
Sonia Chamorro

PREDOCTORAL SCIENTISTS:
María Vela
Mercedes Llorente

TECHNICIAN:
María Lozano

Chemokines, small cytokines that direct cell movement, have a central role in the maintenance of innate and acquired immunity. These proteins interact with specific G protein-coupled receptors and participate in the pathogenesis of inflammatory and infectious diseases as well as in cancer. Chemokines can help limit tumour development by increasing leukocyte migration to a tumour site and by inducing long-term antitumour immunity. In contrast, they can also facilitate tumour cell survival, proliferation, and metastatic potential. Expression of a chemokine receptor by a tumour preferentially directs metastasis to the organs in which the chemokine ligand is secreted, suggesting chemokine receptors as promising therapeutic targets.

Our main research interest is to determine how chemokines participate in the control of tumour growth and progression, and to evaluate their potential as antitumour targets. The use of specific monoclonal antibodies for chemokine receptor targeting allows us to block signalling by preventing ligand binding to its receptor, and also to tag the tumour cells and trigger a host immune response to them.

The chemokine receptor CCR9 is expressed primarily on thymocytes and a small subset of intraepithelial lymphocytes. Its overexpression increases the migratory and invasive capacity of prostate cancer cells, directs ovarian cancer and melanoma metastases to the small intestine, and activates anti-apoptotic pathways that lead to survival and increased proliferation of leukaemia cell lines. We recently generated and characterised mouse anti-human CCR9 monoclonal antibodies that reduced human T lymphoblastic cell tumours transplanted into mice by >85%. Tumour size reduction was concomitant with an increase in the fraction of apoptotic tumour cells and in tumour necrotic areas, as well as a decrease in the fraction of proliferating cells and in tumour vascularisation. Our results suggest that CCR9-expressing tumours such as acute and chronic T cell lineage leukaemia, prostate cancer, breast cancer and melanoma might be targeted with these antibodies.

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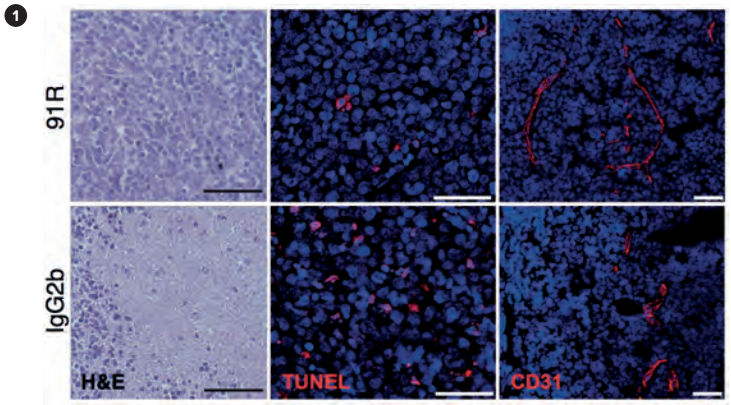
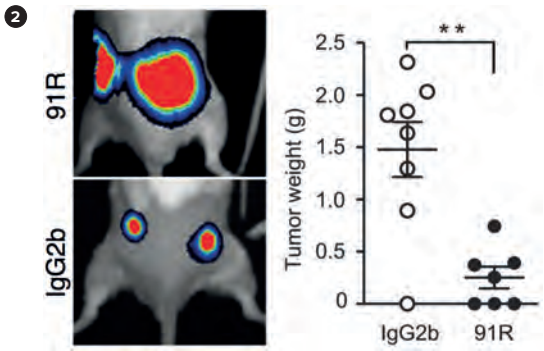
PATENT

PCT/EP2014/075578. Anticuerpos frente a CCR9 y sus aplicaciones



1 *Effects of the treatment with the anti-CCR9 mAb 91R on xenograft MOLT-4 tumours. 91R promotes apoptosis and necrosis and reduces cell proliferation and angiogenesis in CCR9+ tumour xenografts. (Left) Necrosis and apoptosis was detected by haematoxylin/eosin staining of tissue sections (Bar = 25 μm). (Centre) Apoptosis level of tumour cells was analysed by TUNEL assays (Bar = 50 μm). (Right) Blood vessels were detected by CD31 staining (Bar = 50 μm).*



2 *91R-induced reduction of leukaemia xenograft growth. Luminescent MOLT-4 cells were inoculated subcutaneously into each flank of BALB/c Rag2$^{-/-}$ mice on day 0. Experimental groups received intraperitoneal inoculations of 91R or control IgG2b mAb on days 1 and 6. (Left) Luminescence image of a representative mouse from each group, at day 19 post-cell inoculation. (Right) Tumour weights per mouse at experiment endpoint (day 62), after sacrifice and tumour removal.*

Signalling networks in inflammation and cancer

GROUP LEADER:
Santos Mañes

SENIOR SCIENTISTS:
Emilia Mira
Rosa Ana Lacalle

POSTDOCTORAL SCIENTIST:
Manuel Tardáguila

PREDOCTORAL SCIENTISTS:
Juan Carlos de Karam
Lorena Carmona-Rodríguez
Jesús Ogando
Ana Martín Leal

TECHNICIAN:
Rosa M. Peregil

VISITING STUDENTS:
Verena Haage
(Konstanz University, Germany)
Jez Marston
(Yale University, New Haven CT, USA)
Alexandros Karabatzakis
(University of Thessaly, Greece)
Álvaro San Hipólito Luengo
(Univ Europea de Madrid, Spain)
Toni Bauzá Massanet
(Universidad de Lleida, Spain)
Gabriel García Molina
(Univ de Alcalá de Henares, Spain)

Recent clinical and experimental evidence indicates that solid tumours exacerbate inflammation to favour their own progression. This leads to a tumour microenvironment orchestrated largely by inflammatory cells, which alters the metabolic needs of the tissue, fostering proliferation, survival, mutagenesis, migration and metastasis of malignant cells. In addition, tumour-induced inflammation usually leads to angiogenesis (the process by which new blood vessels are formed from pre-existing vasculature) and immunosuppression, which impedes immune-mediated tumour clearance. The immune system is nonetheless able to identify and delete neoplastic cells, and clinical practice now indicates that reprogramming of immune cells is a therapeutically relevant strategy for certain tumours. Successful restriction of the progression or even the eradication of advanced neoplasias using passive immunotherapy with antibodies that block immune checkpoints (CTLA4, PD1/PD-L1/2) or adoptive transfer of T cells genetically engineered to express chimaeric antigen receptors has had such an extraordinary impact on clinical oncology that the journal *Science* chose cancer immunotherapy as the major scientific breakthrough in 2013.

Harnessing the maximum therapeutic potential of the immune system requires detailed comprehension of the cellular and molecular networks in the tumour microenvironment, as well as of the mechanisms that regulate immune responses. We hope that knowledge 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 our group in these two years include:

I. Identification of the pro-inflammatory chemokine CX3CL1 (fractalkine) as a tumour promoter of ErbB2$^+$ breast carcinomas through its crosstalk with the ErbB signalling pathway.

II. Determination of the genetic program elicited by lovastatin (a mevalonate pathway inhibitor) in the tumour microenvironment, which leads to normalisation of tumour-associated vasculature and anti-tumour polarisation of innate immune cells.

III. Characterisation of the interaction between the chemokine receptor CXCR4, which is implicated in tumour metastasis, with the actin-binding protein filamin-A, and the consequences of this bond in receptor signalling and internalisation.

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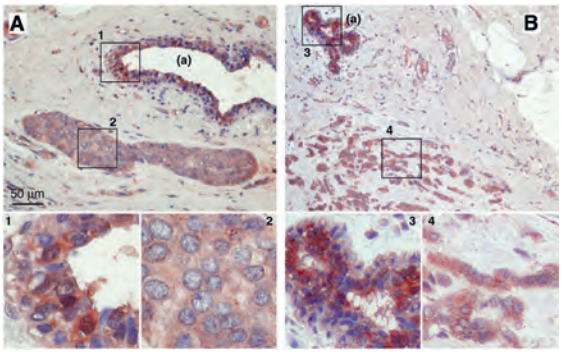
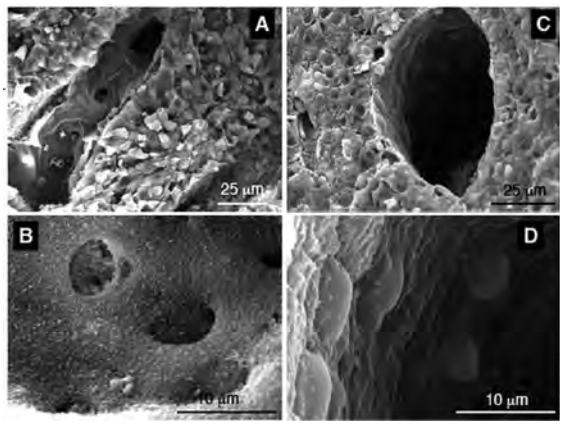


1 *CX3CL1 staining of in situ (A) and invasive (B) human ductal carcinomas; adjacent non-tumour breast tissue (a) is also indicated. Higher magnification insets are also shown (see numbers). Although CX3CL1 expression is downregulated in tumour tissues, CX3CL1 paradoxically promotes the growth of new neoplastic lesions.*



2 *Scanning electron micrographs showing the ultrastructure of blood vessels of sporadic breast tumours in mice treated with vehicle (A,B) or lovastatin (C,D). Note the irregular borders and discontinuities or gaps in the vessels of vehicle-treated mice, which suggest endothelial cell hyperactivity. This contrasts with the regular, continuous, tightly-packed endothelium in lovastatin-treated tumours, which lends an appearance of "smoothness" to the vessel lumen.*

Stem cells and immunity

PRINCIPAL INVESTIGATOR:
Carlos Martínez-A

SENIOR SCIENTIST:
Karel van Wely

POSTDOCTORAL SCIENTISTS:
Thierry Fischer
Cristina Pacios Bras
Ricardo Villares
Jesús de Celis
Julio Gutiérrez Hernández
María Ángeles Garcia

PREDOCTORAL SCIENTIST:
Amaia Talavera

TECHNICIANS
Ainhoa Sánchez
Agnes Fütterer
Astrid Alonso Guerrero

We study the connections between embryonic stem cell (ESC) differentiation and chromosome instability. Most sporadic tumours have a combination of genetic defects termed chromosomal instability (CIN), found in ~85% of non-hereditary carcinomas. We study aneuploidy (whole-chromosome CIN) and a combination of translocations, duplications and deletions (structural CIN).

We focus on the Dido locus, a gene complex strongly expressed in ESC, that encodes three splice variants (Dido1, Dido2 and Dido3). Whereas Dido2 and Dido3 have the PHD, TF2SM and SPOC protein domains, Dido1 only encompasses PHD. Dido3, the largest, most broadly expressed isoform, is a nuclear protein that interacts with centrosomes and the synaptonemal complex in somatic and germ cells, respectively.

N-terminal truncation of Dido3 (Dido3ΔNT) provokes centrosome amplification and CIN. Dido3 interacts with several proteins, including histone deacetylase 6 (HDAC6). We showed that Dido3-dependent targeting of HDAC6 is a key determinant of cilium size in growth-arrested cells. Dido3 availability at the centrosome governs ciliary HDAC6 levels, and redistribution of the two proteins controls tubulin acetylation. Increased Dido3ΔNT expression and interaction with HDCA6 results in ciliopathies (Figure 1). We used this mutation to interpret classical features of CIN, including frequency of chromosome breakage during cell growth. We consider that, by generating substrates for the non-homologous end-joining system, CIN-associated centromere fission helps to initiate breakage-fusion-bridge cycles and acquisition of additional copies of chromothriptic chromosomes.

C-terminal truncation of the *Dido3DCT* gene causes early embryonic lethality. ESC derived from blastocysts of Dido3ΔCT mutants do not differentiate *in vitro*, in contrast to wild-type cells. Differentiation is restored by ectopic expression of wild-type Dido3 and constructs bearing different Dido domains, including the PHD domain. We showed that Dido3 PHD binding to histone H3K4me3 is disrupted by mitotic phosphorylation of an adjacent histone threonine, which triggers Dido3 translocation from chromatin to spindle microtubules. Our crystal structure of Dido3 PHD in complex with H3K4me3 revealed an aromatic-cage-like binding site. Biochemical, structural, and mutational analyses of the binding mechanism identified specificity and affinity determinants, and explained the affinity of other PHD domains for H3K4me3 (Figure 2A). Our findings link transcriptional control in embryonic development and regulation of cell division (Figure 2B).

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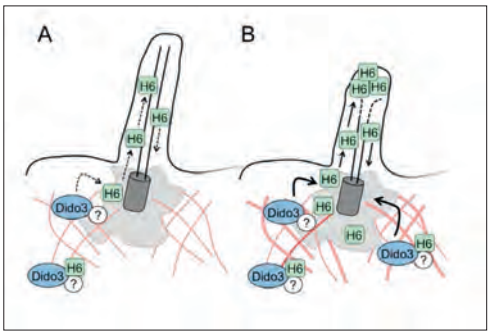

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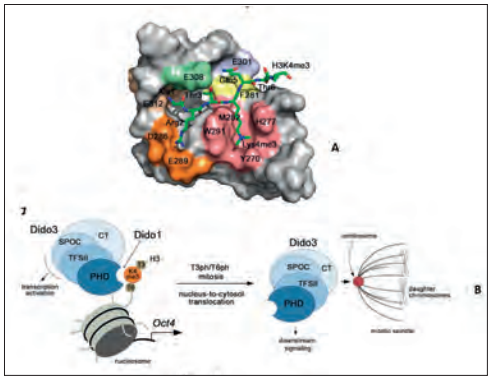

1 *Working model of Dido-dependent histone deacetylase 6 (HDAC6) targeting. (A) Basal levels of Dido (blue) and HDAC6 (green) maintain a steady state in which cilium growth and resorption are in equilibrium. (B) Increased Dido recruitment to the basal body, through build-up of actin fibres (red) or liberation from the nucleus, for example, results in higher local HDAC6 concentrations. Subsequent intraciliary transport directs HDAC6 to the apical tip, where tubulin deacetylation promotes gradual reduction in cilium size.*

2 *The crystal structure of the PHD finger of Dido in complex with the H3K4me3 peptide and a model of the biological effects. (A) The Dido PHD finger is depicted as a solid surface with the peptide shown in a stick model. Protein residues involved in interactions with peptide Ala1 (wheat), Arg2 (orange), Thr3 (light green), Lys4me3 (salmon), Gln5 (light blue), and Thr6 (yellow). (B) A model for Dido3 translocation from chromatin to the mitotic spindle in mitosis.*

Chemokine receptors: new targets for therapeutic intervention

PRINCIPAL INVESTIGATOR:
Mario Mellado

SENIOR SCIENTIST:
José Miguel Rodríguez Frade

POSTDOCTORAL SCIENTISTS:
Ricardo Villares
Laura Martínez Muñoz
Rubén Rodríguez Barroso

PREDOCTORAL SCIENTISTS:
Dimitri Kakabadse
Graciela Cascio

TECHNICIAN:
Mª Pilar Lucas

Although originally described as specific mediators of leukocyte directional movement, the chemokines are involved in a much wider variety of physiological and pathological processes including tumour cell growth and metastasis, angiogenesis, chronic inflammatory diseases and HIV-1 infection. Chemokine biology is 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.

Using fluorescence resonance energy transfer (FRET) techniques, we have confirmed the functional relevance and regulation of homo- and heterodimeric receptor complexes. HIV-1 entry into a target cell requires interaction between viral envelope glycoprotein gp120 and host cell membrane chemokine receptors CXCR4 or CCR5 in combination with CD4. We observed oligomerisation between membrane receptors involved in HIV-1 infection, and demonstrate that CD4/CCR5/CXCR4 complexes modify the gp120 binding, actin polymerisation and cytoskeleton rearrangement necessary for HIV-1 entry.

We also studied the functional relevance of several chemokine-triggered signalling events, and reported that the JANUS kinases (JAK) participate in chemokine-induced integrin activation. We showed that reduced JAK1 and JAK2 expression impair naïve T cell migration in response to gradients of the chemokines CXCL12 and CCL21, and that the *in vivo* homing of these naïve T cells to lymph nodes is decreased. In addition, we found that the JAK pathway is activated following CXCL12 stimulation in neural precursor cells (NPC), but that JAK activity is not necessary for NPC migration. Immature interneuron migration to the cerebral cortex is nonetheless dependent on CXCL12-mediated PI3K p110β activation.

As chemokines play an essential role in inflammatory and autoimmune disease, we also initiated the study of a murine model of type 1 diabetes, a T cell-mediated autoimmune disease characterised by immune cell infiltration of pancreatic islets and destruction of insulin-producing β-cells. We observed that sustained growth hormone expression reduced prodromal disease symptoms and eliminated progression to overt diabetes. The effect involves several GH-mediated mechanisms; GH altered the cytokine environment, triggered anti-inflammatory macrophage (M2) polarisation, maintained activity of the suppressor T cell population, and limited Th17 cell plasticity.

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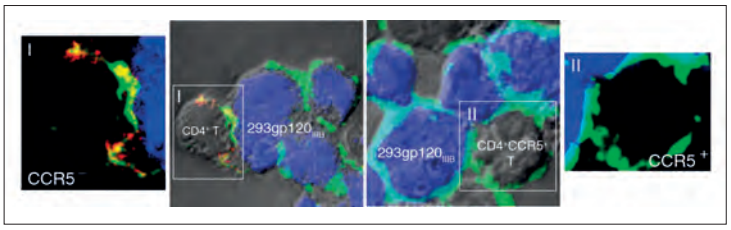

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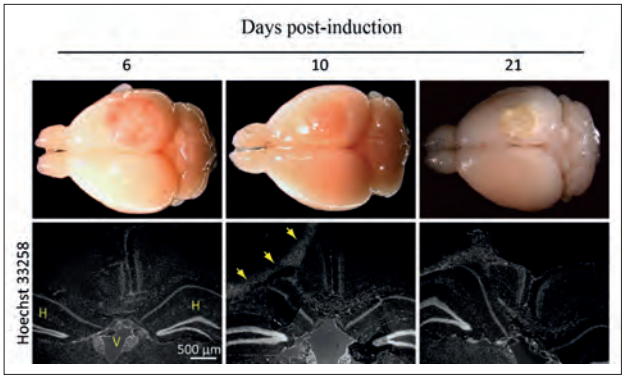

1 *CCR5 expression on CD4+ T cells inhibits formation of the X4 gp120-induced virological synapse by blocking F-actin recruitment (green) and ERM phosphorylation (red).*

2 *Ischaemic stroke model in mice. Representative images of murine brains isolated at various days post-induction (dpi) of the lesion (6, 10 and 21 days; top panel). Nuclear staining of coronal brain sections from mice with ischemia at distinct dpi (bottom). Images show sections of equivalent position on the axial axis. The hippocampus (H) and third ventricle (V) are labelled. Arrows indicate the glial scar, dashed line marks the damaged area.*

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

PRINCIPAL INVESTIGATOR:
Isabel Mérida

POSTDOCTORAL SCIENTISTS:
Juana Antonia Ávila
Pedro Torres

PREDOCTORAL SCIENTISTS:
Denise Soutar
Elena Andrada
María Tello
Gonzalo Martínez

STUDENTS:
Manuel Daza
Martí Duran

TECHNICIANS:
Raquel Arcos
Rosa Liébana

Highly transformed solid tumours acquire invasive features and the ability to escape from antitumour immunity. The therapies that help to overcome immune evasion by tumours are an important new strategy for cancer treatment. We study the molecular mechanisms by which tumours couple the development of malignant traits to the impairment of T cell responses. We showed that diacylglycerol metabolism by two enzymes of the diacylglycerol kinase (DGK) family limit T cell responses. We are delimiting the contribution of diacylglycerol kinases to T cell functions and oncogenic transformation. Targeting these enzymes, alone or in combination with other inhibitors in wide clinical use, could help in the treatment of aggressive forms of cancer.

We work in two different albeit complementary areas:

1. DGKα and ζ as negative regulators of the adaptive immune response

The DGK are a conserved family of lipid kinases that phosphorylate diacylglycerol (DAG), catalysing its conversion into phosphatidic acid (PA). In T lymphocytes, DGKalpha and zeta limit Ras guanyl-releasing protein (RasGRP1)-dependent activation of Ras and activation of certain PKC isoforms. DGKalpha is abundantly expressed in quiescent T lymphocytes and we have demonstrated its transcriptional repression by antigens and IL2-mediated activation of the PI3K/AKT/FoxO axis. This negative regulation is necessary for adequate T cell functions, and its failure results in induction of a non-responsive state known as anergy. DGKalpha expression is elevated in tumour-infiltrating T cells, and we are developing genetic and chemical tools that help us to better understand the mechanisms by which tumour cells induce upregulation of DGKalpha in infiltrating T cells. DGKzeta is also expressed in T lymphocytes and we have shown its interaction with sorting nexin 27 (SNX27).

2. DGKα and ζ: lipid restraint on tumour metabolism

DGKalpha sustains tumour cell survival, migration and invasion, and its pharmacological targeting abolishes tumour growth, with no effect on untransformed cell survival, suggesting its potential as a cancer-specific target. We described DGKalpha upregulation in tumour 3D cultures as part of the transcriptional program that helps sustain Src activation. Src regulation by DGKalpha limits the effect of Src inhibitors, and *DGKA* transcriptional upregulation in response to PI3K/Akt inhibitors contributes to development of pharmacological resistance.

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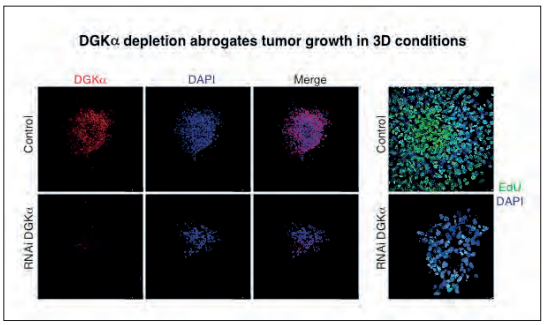

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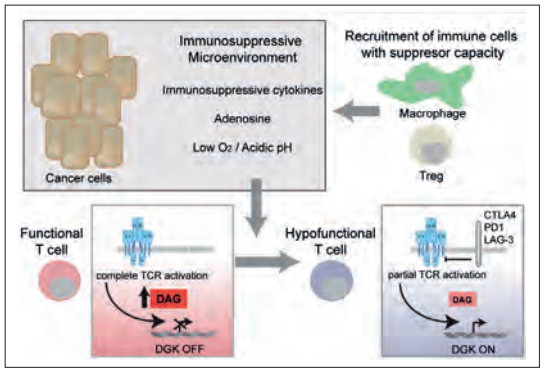
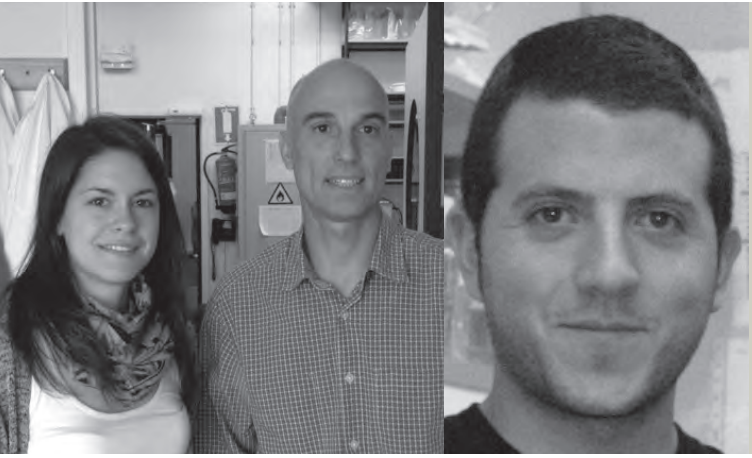


1 *DGKα is necessary for 3D tumour growth. Left panels show 3D cultures of control or DGKα -depleted colon cancer cells stained with a fluorescent DNA marker (4',6-diamidino-2-phenylindole; DAPI) for total cell analysis and for DGK expression. Right panels show control and DGKα -silenced colon cancer cells stained with DAPI and proliferation inferred by staining with EdU (5-ethynyl-2'-deoxyuridine).*

2 *DGK upregulation contributes to tumour immune evasion. Enhanced tumour metabolism generates a hypoxic, acidic microenvironment, and tumour-secreted cytokines recruit cells with immunosuppressive functions (e.g., Treg cells). This microenvironment facilitates tumour-infiltrating lymphocyte switching from an active to a hypofunctional state. Increased expression of negative receptors (such as CTLA4, PD-1 or LAG-3) and negative regulators such as DGK attenuates TCR signalling and fosters tumour immune evasion.*



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

PRINCIPAL INVESTIGATOR:
Ignacio Moreno de Alborán

POSTDOCTORAL SCIENTIST:
David Fernández Antorán

STUDENTS:
Antonio Navarro
Mercedes Pérez Olivares

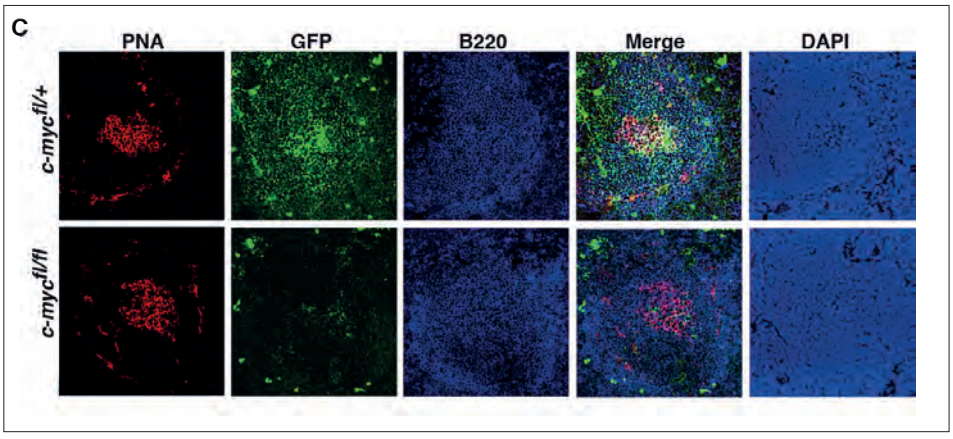
SELECTED PUBLICATIONS

Lee YY, Moujalled D, Doerflinger, M, Gangoda L, Weston R, Rahimi A, Moreno de Alboran I, Herold M, Bouillet P, Xu Q. CREB-binding protein (CBP) regulates beta-adrenoceptor (beta-AR)-mediated apoptosis. Cell Death Differ 2013; 20:941-952

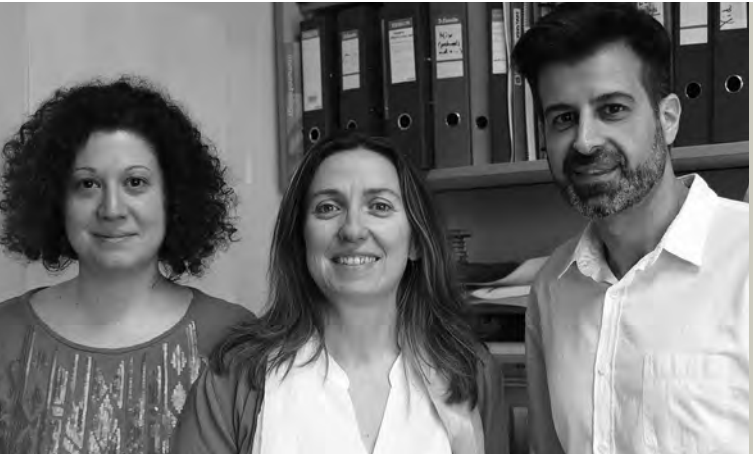
Fernandez D, Ortiz M, Rodriguez L, Garcia A, Martinez D, Moreno de Alboran I. The Proto-Oncogene c-myc Regulates Antibody Secretion and Ig Class Switch Recombination. J Immunol 2013; 190:6135-6144

A potent, specific immune response to an antigen involves the generation of antibodies by B lymphocytes. These cells must undergo a process called terminal differentiation, which leads to the generation of at least two cell populations, antibody-secreting and memory B cells. This process is linked to a transcriptional program characterised by the activation of Blimp-1, a transcriptional repressor that inhibits expression of c-Myc. C-Myc is a transcription factor involved in many biological functions and is a key player in the regulation of cell proliferation and apoptosis. Given the large number of genes regulated by c-Myc, we tested whether its function in terminal B cell differentiation was restricted to the regulation of cell proliferation or whether it had additional functions.

Our results indicated that c-Myc regulates three basic functions in terminal B cell differentiation: cell proliferation, IgM secretion and regulation of immunoglobulin class switch recombination (CSR). We observed that c-Myc-deficient B lymphocytes hypersecreted IgM and were unable to undergo CSR. In addition, we found that c-Myc transcriptionally regulated activation-induced deaminase (AID), a protein essential for this process. We thus propose that c-Myc acts as a link between CSR and cell proliferation. We also studied the function of c-Myc during an *in vivo* immune response. Our studies showed that c-Myc is necessary for differentiation of the antibody-secreting and memory B cells that are a hallmark of an immune response. These results position c-Myc as a key regulator in the process of terminal B lymphocyte differentiation and an essential factor for a competent, robust immune response.



1 *Immunofluorescence of spleen sections from immunised homozygous and heterozygous mice. PNA lectin was used to identify germinal centre B cells and anti-GFP to detect c-Myc-deficient cells. B220 identifies B cells and DAPI labels DNA.*



APRIL signalling pathway regulation and function

PRINCIPAL INVESTIGATOR:
Lourdes Planelles

POSTDOCTORAL SCIENTIST:
Manuela Zonca

STUDENT:
Fernando Gutiérrez del Burgo

SELECTED PUBLICATION

García-Castro A, Zonca M, Florindo-Pinheiro D, Carvalho-Pinto CE, Cordero A, Gutiérrez Del Burgo B, García-Grande A, Mañes S, Hahne M, González-Suárez E, Planelles L. APRIL promotes breast tumor growth and metastasis and is associated with aggressive basal breast cancer. Carcinogenesis 2015; doi: 10.1093/carcin/bgv020

APRIL (a proliferation-inducing ligand) is a cytokine of the tumour necrosis factor family described to be involved in B cell survival, proliferation and antibody production. APRIL transgenic (Tg) mice show an expanded peritoneal B-1 B cell population, increased natural antibody levels, and extended T-independent humoural responses compare to controls. In our laboratory, we are studying the molecular programmes activated by the APRIL pathway on B cells, as well as its potential clinical use.

The APRIL pathway is also associated with haematologic malignancies. In addition, APRIL is overexpressed in breast carcinoma tissue lesions, although neither its role in breast tumourigenesis nor the underlying molecular mechanism is known. To study the participation of the APRIL pathway in breast carcinoma, we have addressed this question using cell lines, mouse tumour models and primary samples. We observed that several breast cancer cell lines express APRIL and both of its receptors, BCMA (B cell maturation antigen) and TACI (transmembrane activator and CAML-interactor), independently of luminal or basal tumour cell phenotype. We found that the mitogen-activated protein kinases p38, ERK1/2 and JNK1/2 are activated in response to APRIL. Silencing experiments decreased cell proliferation, demonstrating that APRIL is a critical autocrine factor for breast tumour growth. Studies of 4T1 orthotopic breast tumours in APRIL-Tg mice showed that an APRIL-enriched environment increased tumour growth and promoted lung metastasis associated with enhanced tumour cell proliferation; BCMA and TACI expression suggests that both participate in these processes. We also detected APRIL, BCMA and TACI in luminal, TNBC and HER2 primary breast carcinomas, with higher levels in more aggressive basal tumours. APRIL was observed near Ki67^{+} nuclei and was distributed heterogeneously in the cancer cells, in the leukocyte infiltrate, and in the myoepithelial layer adjacent to the tumour area; these results imply that APRIL provides proliferation signals to tumour cells through paracrine and autocrine signalling. Our study identifies the participation of APRIL signalling in breast cancer promotion. We propose impairment of this pathway as a potential therapeutic strategy.

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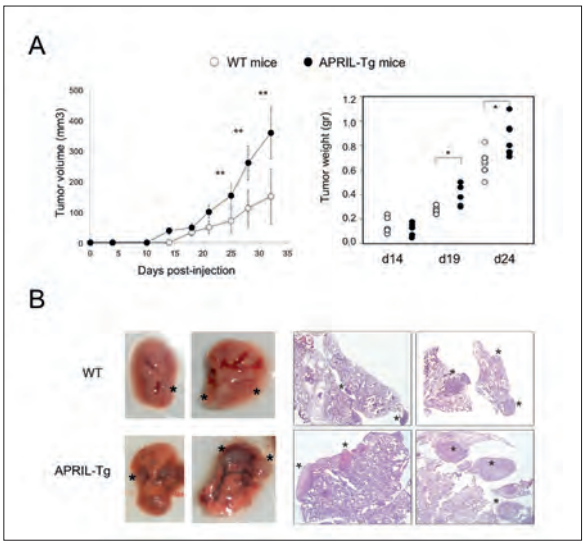

1 *4T1 tumour growth and lung metastasis are enhanced in an APRIL-enriched environment. 4T1 cells were transplanted into the mammary fat pad of syngeneic WT and APRIL-Tg female mice. (A) Mean tumour volume ± SD measured twice weekly (left) and individual weight at different times post-sacrifice (right) of 4T1 tumours from WT (white circles) and APRIL-Tg (black circles) mice; n = 6/group. (B) Representative images of lung macrometastases (day 39) in WT and APRIL-Tg mice. Asterisks mark some metastases. One representative experiment of three is shown.*

2 *Human basal breast carcinomas express APRIL protein. Immunohistochemistry analysis of APRIL expression in three paraffin-embedded basal invasive ductal breast carcinomas. Representative images of APRIL staining (brown) are shown. Squared areas indicate APRIL positive cells in b) "normal" ducts near tumour cells and c) tumour cell cytoplasm.*

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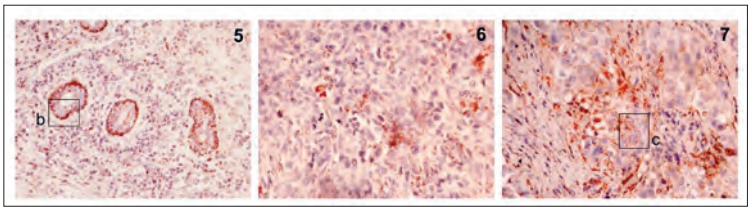

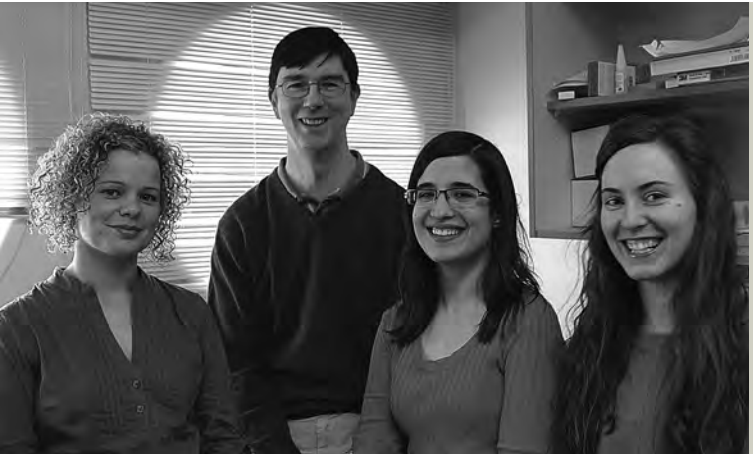

Receptor ligand interactions in immune responses to cancer and viruses

PRINCIPAL INVESTIGATOR:
Hugh Reyburn

PREDOCTORAL SCIENTISTS:
Gema Romera Cardenas
Daniela Dukovska
Miriam Agúndez Llaca
Alfonso Blázquez Moreno

TECHNICIAN:
Ruth Gómez-Caro Gil

We study the interactions between pathogens and cells of the immune system to gain insight into functionally important features of the immune system, with the objective of applying this knowledge to studies of human disease.

The main research project is the identification of genes expressed by tumour cells and cells infected by viruses whose expression is associated with activation of NK cell cytotoxicity and cytokine secretion. We also study NK receptors that modulate lymphocyte activation, in particular the cell biology of these receptors, to understand their regulated expression and function. Recently we began to study patients with primary T cell immunodeficiencies and have identified defects in NK cell development and function as well as aberrant expression of NK receptors on the defective T cells of these patients.

1. We showed that the shedding of molecules such as MICA and ICAM-1 is increased after HCMV infection both *in vitro* and *in vivo*. This phenomenon depends on increased 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 may be of use as a biomarker in patients at risk of developing CMV disease.

2. We discovered that innate immune recognition of double-stranded RNA produced during viral infection plays a key role in the induction of NKG2D ligand expression. 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 virus and influenza virus whose expression markedly reduces the induction of both IFN-β and NKG2D ligands after infection.

3. Our recent analyses of patients with primary immunodeficiency diseases (PID) characterised by T cell lymphopaenia suggest defects other than simply the absence of T cell function. Specifically, the NK cells of these patients, although normal in number, were immature. These observations raise the exciting hypothesis that disruption of the normal crosstalk between immune cells can negatively affect the differentiation and function of the remaining cells of the immune system, and so contribute to the spectrum of pathology in these patients.

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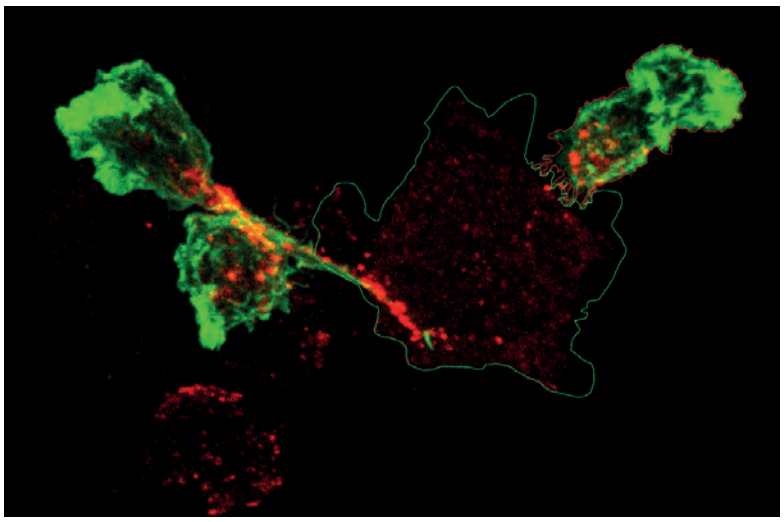
Ashiru O, López-Cobo S, Fernández-Messina L, Pontes-Quero S, Pandolfi R, Reyburn HT, Valés-Gómez M. A GPI anchor explains the unique biological features of the common NKG2D-ligand allele MICA*008. Biochem J 2013; 454:295-302



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

T cell signalling in autoimmune diseases and cancer

PRINCIPAL INVESTIGATOR:
Jesús María Salvador

POSTDOCTORAL SCIENTISTS:
Heiko Johnen
María Salvador Bernáldez

PREDOCTORAL SCIENTISTS:
Laura González Silva
Umberto Rosato

TECHNICIANS:
Vanesa Cano Daganzo
Carmen Mireya Martínez García

SELECTED PUBLICATIONS

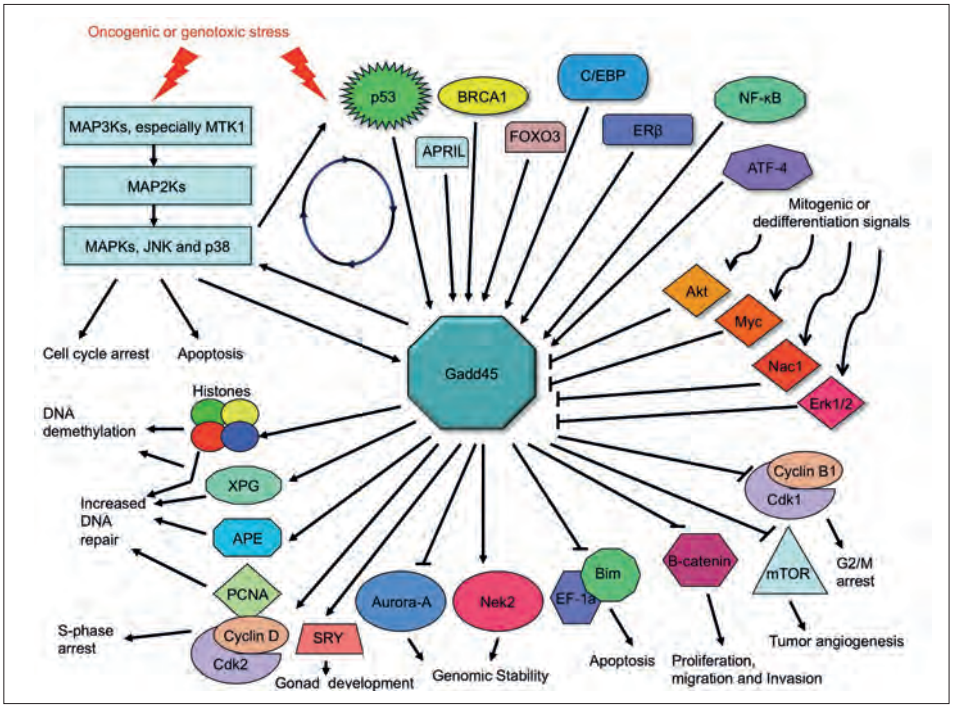
Salvador JM, Brown-Clay JD, Fornace AJ. Gadd45 in stress signalling, cell cycle control and apoptosis. Adv Exp Med Biol 2013; 793:1-19

Johnen H, González-Silva L, Carramolino L, Flores JM, Torres M, Salvador JM. Gadd45g is essential for primary sex determination, male fertility and testis development. PLoS One 2013; 8:e58751

Our group studies the biological functions of the Gadd45 and p38 MAPK (mitogen-activated protein kinase) families in the suppression and development of autoimmunity and cancer. Gadd45 proteins are characterised mainly as classical tumour suppressors that induce cell cycle arrest and apoptosis in response to DNA damage or oncogenic stimuli. They play key roles in a range of other physiological processes, including DNA demethylation and repair, maintenance of genomic stability through mitosis and immunological regulation and activation, although the molecular mechanisms involved in these functions are still under study.

We found an important role for Gadd45a in suppression of autoimmunity through regulation of CD4+ T cell functions. Whereas Gadd45 is typically associated with growth arrest in most cell types, p38 activation has a key stimulatory role in lymphocytes. p38 is necessary for T cell activation and Gadd45a is a major modulator of p38 in this process. Gadd45 acts as an autoimmune suppressor *in vivo* by negatively regulating T cell proliferation in response to TCR activation. Unlike Gadd45a, the Gadd45b and Gadd45g isoforms potentiate p38 signalling in Th1 and CD8+ cytotoxic T cells, which is necessary for full effector function. Gadd45b is necessary for full expression of the Th1 lineage-inducing protein T-bet. Gadd45 family members thus appear to work synergistically to promote full maturation and function of Th1 and CD8+ cells.

In addition to the role in suppression of autoimmunity, we identified an unanticipated function for Gadd45g, but not Gadd45a or Gadd45b, during embryonic development. Gadd45g expression is central to male fertility, testis development and sex determination. Gadd45g-deficient mice showed an unexpected male-to-female sex reversal phenotype. We found that Gadd45g is necessary for SRY expression and that lack of Gadd45g blocks SOX9, resulting in ovary development. The genetic basis of human male-to-female sex reversal remains unexplained in the majority of cases. Our results identify Gadd45g as a candidate gene in human non-syndromic male infertility and in partial or complete male-to-female primary sex reversal in 46, XY individuals.



1 *Upstream regulators of Gadd45 and its downstream inducers. Gadd45 controls crucial functions, including proliferation, apoptosis, cell cycle arrest, tumour angiogenesis, genomic stability and gonad development.*

Tumour immune activation and evasion

PRINCIPAL INVESTIGATOR:
Mar Valés Gómez

POSTDOCTORAL SCIENTIST:
Gloria Esteso Tornero

PREDOCTORAL SCIENTISTS:
Eva M. García Cuesta
Sheila López Cobo
Daniela Dukovska

VISITORS:
José Ramón Vidal Castiñeira
Daria Gajewska
Myriam Oliveira Rodríguez

Our laboratory studies different aspects of the cell biology of various ligands for the activating receptor NKG2D. In addition to the importance of this receptor-ligand system for immune response activation, the release of NKG2D ligands by tumour cells modulates receptor expression on effector T and NK cells and thus impairs cytotoxicity. Moreover, elevated serum levels of NKG2D ligands correlate with a poorer prognosis in cancer patients. The apparent complexity of the regulation of NKG2D ligand cell surface expression and shedding impedes understanding of the interactions between NKG2D-expressing immune effectors and target cells. The existence of two ligand families encoded in distinct regions of chromosome 6 was initially thought to be responsible for this biological diversity, but we have shown that the NKG2D ligands share properties that do not fit this simple genetic classification.

MICA and MICB (major histocompatibility complex class I-related chain A/B) are two very polymorphic genes that make up one of the two human NKG2D ligand families. We reported that the human MICA allele, MICA*008, is attached to the membrane through a GPI anchor. The importance of this unanticipated finding resides in the fact that the MICA*008 allele shows the highest frequency worldwide, and is not affected by various viral immune evasion mechanisms that target other MICA alleles. We also found that, at difference from other MICA alleles, MICA*008 is released in exosomes (Ashiru *et al.* Cancer Res 2010; 70:481). That both NKG2D ligand families have transmembrane and GPI-anchored members explains why pathogen immunoevasins are able to recognise some but not all MICA alleles, and suggests that this structural dichotomy permits, in evolutionary terms, the pathogen-driven blockade of cellular pathways.

Our research aims to better understand the association of these molecules with cancer progression.

SELECTED PUBLICATIONS

Esteso G, Luzón E, Sarmiento E, Gómez-Caro R, Steinle A, Murphy G, Carbone J, Valés-Gómez M, Reyburn HT. Altered micro-RNA expression after infection with human cytomegalovirus leads to TIMP3 downregulation and increased shedding of metalloprotease substrates, including MICA. J Immunol 2014; 193:1344-1352

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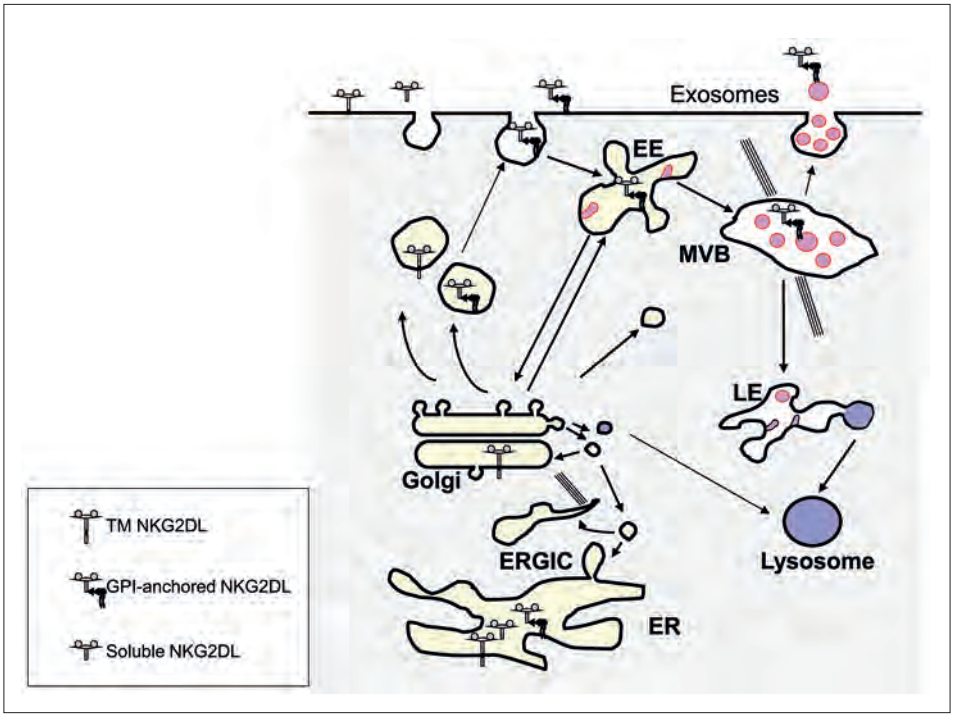

1 *Possibilities for NKG2D ligand processing*

4 / Molecular and Cellular Biology

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

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

PRINCIPAL INVESTIGATOR:
Inés M. Antón

POSTDOCTORAL SCIENTISTS:
Ricardo Gargini
Laura Mateos

PREDOCTORAL SCIENTIST:
Esther García

SELECTED PUBLICATIONS

Bañón-Rodríguez I, Sáez de Guinoa J, Bernardini A, Ragazzini C, Fernández E, Carrasco YR, Jones GE, Wandosell F, Anton IM. WIP regulates persistence of cell migration and ruffle formation in both mesenchymal and amoeboid modes of motility. PLoS One 2013; 8:e007036.

Franco-Villanueva A, Fernández-López E, Gabandé-Rodríguez E, Bañón-Rodríguez I, Esteban JA, Anton IM, MD Ledesma. WIP modulates dendritic spine actin cytoskeleton by transcriptional control of lipid metabolic enzymes. Human Mol Genet 2014; 23:4383-4395

García E, Machesky LM, Jones GE, Anton IM. WIP is necessary for matrix invasion by breast cancer cells. Eur J Cell Biol 2014; 93:413- 423

Vijayakumara V, Monypenny J, Machesky L, Lilla S, Thrasher AJ, Antón IM, Calle Y, Jones GE. Tyrosine phosphorylation of WIP releases bound WASP and impairs podosome assembly in macrophages. J Cell Sci 2014; doi: 10.1242/jcs.154880

Our aim is to determine the mechanisms that regulate cytoskeletal dynamics in essential actin-mediated cell functions such as migration, invasion, and neuronal differentiation. We study actin-binding proteins such as (N)WASP (neural Wiskott-Aldrich syndrome protein), WIP (WASP-interacting protein) and WIRE (WIP-related) to understand the molecular mechanisms that underlie inflammation-mediated conditions, tumour invasion and neurological diseases.

Using animal models and recombinant lentivirus, we identified an essential role for WIP in persistence during amoeboid (B lymphocyte) and mesenchymal (fibroblast) migration as well as in fibroblast chemotaxis. Advanced imaging techniques and biochemical approaches allowed us to elucidate the role of WIP in the formation of actin-rich invasive structures, podosomes and invadopodia. We determined how Btk-mediated tyrosine phosphorylation of WIP triggers WASP release from the WIP-WASP complex to regulate podosome lifetime. Using 2D and 3D culture systems, we demonstrated that WIP is necessary for invadopodium formation and matrix degradation by basal breast cancer cells. Finally, we identified WIP as a component of neuronal synapses whose absence increases dendritic spine size and filamentous actin levels in a RhoA/ROCK/profilinIIa-dependent manner. These effects depend on the reduction of membrane sphingomyelin due to transcriptional upregulation of neutral sphingomyelinase through active RhoA; this enhances RhoA binding to the membrane in steady state but prevents changes in response to stimulus. Sphingomyelinase inhibition or sphingomyelin addition reverses the RhoA-dependent increase in filamentous actin, as well as functional anomalies in WIP-deficient synapses. Our findings characterise WIP as a link between membrane lipid composition and actin cytoskeleton at dendritic spines. They also help to explain cognitive deficits shared by individuals bearing mutations in the region assigned to the gene that encodes WIP.

Our goal is to understand the molecular basis of the mechanism that regulates actin polymerisation, a process that underlies numerous essential cell functions whose deregulation leads to serious human diseases. We thus hope to provide new diagnostic, prognostic and/or therapeutic tools for neurological disorders, inflammation-mediated affections, tumour initiation and metastasis.

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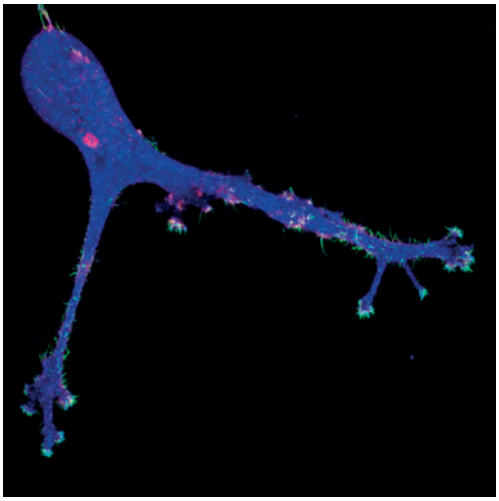

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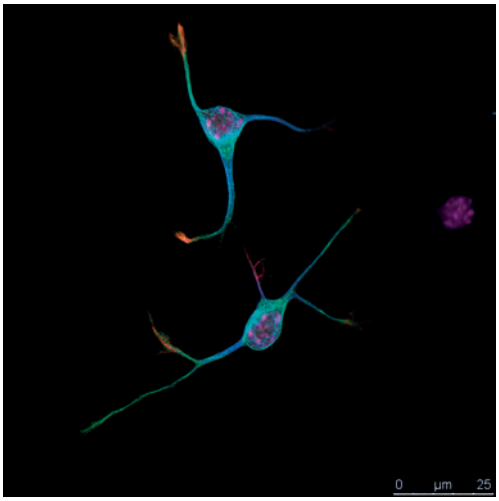

1 *WIP localises at invasive protrusions developed in 3D matrices. Immunofluorescence of invasive MDA-MB-231 cells grown on Matrigel, fixed and stained for WIP (green), N-WASP (blue) and actin (red).*

2 *N-WASP is expressed in the cytoplasm of primary murine neurons. Immunofluorescence of murine neurons grown on coverslips for one day, fixed and stained for N-WASP (green), tyrosinated alpha-tubulin (blue), actin (red) and nuclei (pink).*

Coronavirus: replication, virus-host interactions, and protection

PRINCIPAL INVESTIGATOR:
Luis Enjuanes

SENIOR SCIENTISTS:
Isabel Sola
Fernando Almazán

POSTDOCTORAL SCIENTISTS:
Sonia Zúñiga
José Luis Nieto
Marta López de Diego

PREDOCTORAL SCIENTISTS:
José Manuel Jiménez
Martina Bécares
José Ángel Regla
Lucía Morales
Carlos Castaño
Alejandro Pascual
Javier Gutiérrez
Javier Cantón
Silvia Márquez Jurado

TECHNICIANS:
Carlos M. Sánchez
Raúl Fernández
Margarita González

Human infections of the lower respiratory tract are a growing health problem. The annual number of hospital admissions due to pneumonia and acute respiratory disease syndrome was estimated during 2010 at 11.9 million worldwide. The problem is even greater when we consider the elderly population, which responds to vaccination significantly less effectively.

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

The main aims of our research are:

• To identify CoV genes responsible for virus virulence, to delete or modify them to develop attenuated viruses that are good vaccine candidates, and to determine their effectiveness in animal model systems.

• To identify cell signalling pathways needed for CoV replication, to select antiviral drugs that inhibit these pathways and interfere with virus replication without affecting cell viability. These studies include analysis of the cell phosphorylation networks that affect viral proteins and contribute to virus virulence, to select the corresponding antivirals.

• To determine how CoV influence innate immune responses, to regulate the magnitude and class of response to control CoV-induced pathogenesis. Special attention is provided to the induction of inflammation and to inflammasome activation, as overstimulation of these pathways seems to be responsible for an increase in fatalities during SARS-CoV and MERS-CoV epidemics.

• To study the RNA epigenetic control of innate immune responses to CoV, to promote the expression of small or long non-coding RNAs that favour the innate immune response or counteract the production of RNAs that inhibit this response.

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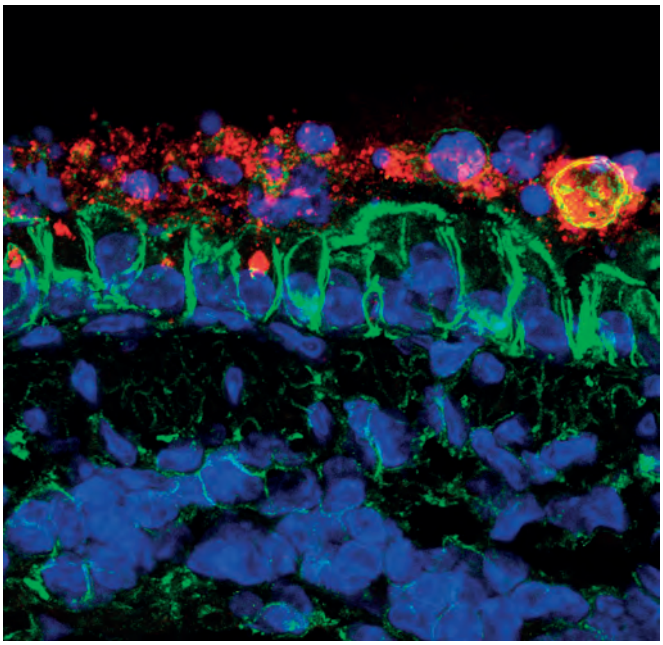
Mateos-Gomez PA, Morales L, Zuñiga S, Enjuanes L, Sola I. Long-distance RNA-RNA interactions in the coronavirus genome form high-order structures promoting discontinuous RNA synthesis during transcription. J Virol 2013; 87:177-186

Almazan F, DeDiego ML, Sola I, Zuñiga S, Nieto-Torres JL, Marquez-Jurado S, Andres G, Enjuanes L. Engineering a replication-competent, propagation-defective Middle East respiratory syndrome coronavirus as a vaccine candidate. mBio 2013; 4:e00650-00613

DeDiego ML, Nieto-Torres JL, Regla-Nava JA, Jimenez-Guardeno JM, Fernandez-Delgado R, Fett C, Castano-Rodriguez C, Perlman S, Enjuanes L. Inhibition of NF-kappaB mediated inflammation in severe acute respiratory syndrome coronavirus-infected mice increases survival. J Virol 2014; 88:913-924

Nieto-Torres JL, Dediego ML, Verdia-Baguena C, Jimenez-Guardeno JM, Regla-Nava JA, Fernandez-Delgado R, Castano-Rodriguez C, Alcaraz A, Torres J, Aguilella VM, Enjuanes L. Severe acute respiratory syndrome coronavirus envelope protein ion channel activity promotes virus fitness and pathogenesis. PLoS Pathog 2014; 10:e1004077

Jimenez-Guardeno JM, Nieto-Torres JL, DeDiego ML, Regla-Nava JA, Fernandez-Delgado R, Castano-Rodriguez C, Enjuanes L. The PDZ-binding motif of severe acute respiratory syndrome coronavirus envelope protein is a determinant of viral pathogenesis. PLoS Pathog 2014; 10:e1004320



1 *Pulmonary epithelium disassembly induced by SARS-CoV infection in mice. The external layer of the epithelium (green) has been destroyed as a consequence of SARS-CoV infection (red).*

Poxvirus and vaccines

PRINCIPAL INVESTIGATOR:
Mariano Esteban

POSTDOCTORAL SCIENTISTS:
Liliana Carreño
Carmen E. Gómez
Beatriz Perdiguero
Juan F. García
Aneesh Vijayan
Ana Cáceres
Lucas Sánchez
Victoria Cepeda

PREDOCTORAL SCIENTISTS:
Patricia Pérez
María Quirós
Mauro di Pilato
Ernesto Mejías
Suresh Chithathur

TECHNICIANS:
María Victoria Jiménez
Cristina Sánchez

The main objectives of our laboratory are geared to understanding the molecular basis of the pathogenesis of infectious agents and their interaction with the host, and to use this knowledge to develop effective vaccines against human diseases like HIV/AIDS, hepatitis C, chikungunya, malaria, leishmaniasis, and prostate cancer. As a model system of an infectious agent and as a delivery vector to express genes of interest, we use vaccinia virus (VV), a member of the poxvirus family.

The research areas in our lab are a) vaccinia virus structure, b) the mechanism of interferon antiviral and antitumour action, c) virus-host cell interaction, and d) development of vaccines for prevalent human diseases.

Our main achievements in 2013-2014 include

1. The first therapeutic phase I clinical trial in Spain with the HIV/AIDS vaccine candidate MVA-B developed by our group, in HIV-infected individuals under HAART (highly active antiretroviral therapy). The results showed a good safety profile and immunogenicity, particularly for specific activation of CD4+ T cells and induction of anti-V1/V2 antibodies, a marker of protection. In addition, the vaccine appeared to reduce viral load after antiretroviral interruption.

2. We developed a vaccine against chikungunya virus, an RNA virus that causes severe articular pains and is spreading worldwide via the tiger mosquito *Aedes albopictus.*

3. We developed vectors based on the attenuated VV strain NYVAC expressing HIV-1 clade C trimeric gp140 and Gag-Pol-Nef, which showed excellent immune behaviour in preclinical trials. These vectors are being tested for safety and immunogenicity in phase I clinical trials.

4. We genetically modified the poxvirus-based vaccine candidates MVA and NYVAC by selective deletion of viral immunomodulatory genes, and showed optimised immunological behaviour against HIV.

5. We identified a mechanism of HIV immune activation through NYVAC modulation of the functional switch in neutrophils.

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García-Arriaza J, Gómez CE, Sorzano COS, Esteban M. Deletion of the vaccinia virus N2L gene encoding an inhibitor of IRF3 improves the immunogenicity of MVA expressing HIV-1 antigens. J Virol 2014; 88:3392-3410

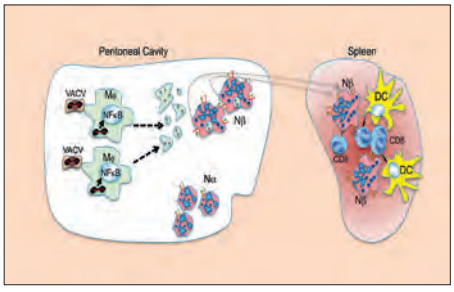
Perdiguero B, Gómez CE, Cepeda V, Sánchez-Sampedro L, García-Arriaza J, Mejías-Pérez E, Jiménez V, Sánchez C, Sorzano CÓ, Oliveros JC, Delaloye J, Roger T, Calandra T, Asbach B, Wagner R, Kibler KV, Jacobs BL, Pantaleo G, Esteban M. Virological and Immunological Characterisation of Novel NYVAC-Based HIV/AIDS Vaccine Candidates Expressing Clade C Trimeric Soluble gp140(ZM96) and Gag(ZM96)-Pol-Nef(CN54) as Virus-Like Particles. J Virol 2014; doi: 10.1128/JVI.02469-14

PATENTS

PCT/ES2014/070246. MVA-HCV as a vaccine against hepatitis C

PCT/EP2014/076310. MVA-CHIKV as a vaccine against Chikungunya virus

1



1 *Vaccine candidate MVA-CHIKV expressing the structural antigens of chikungunya virus triggers neutralising antibodies and fully protects after challenge with the virus.*

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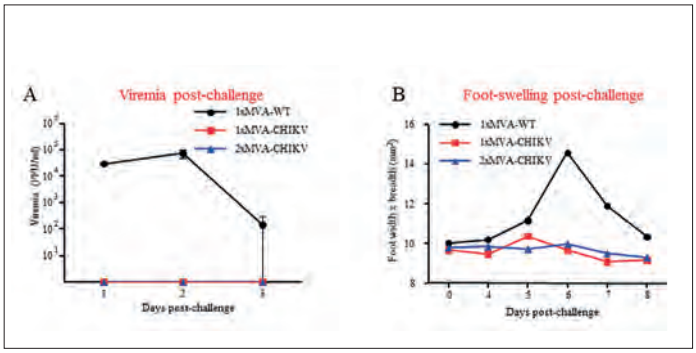
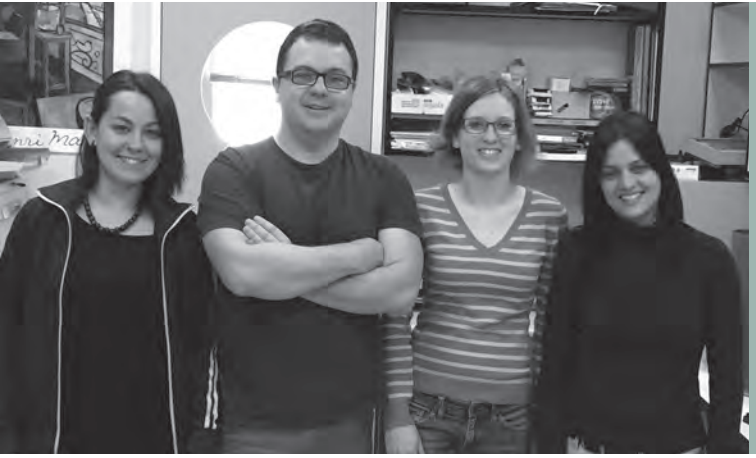


2 *HIV vaccine candidate activates NF-κB to trigger specific T cell immune responses through neutrophil recruitment.*



Infection by hepatitis C viruses

PRINCIPAL INVESTIGATOR:
Pablo Gastaminza Landart

PREDOCTORAL SCIENTISTS:
Lidia Mingorance Pérez
Claudia Vasallo Vega

STUDENTS:
Maria Chiara Sorbo
Victoria Castro Illana

TECHNICIAN:
Martina Friesland

Hepatitis C virus (HCV) is a pathogen that infects 3% of the human population worldwide. Despite great efforts to control this pandemic, 3 to 4 million people become infected and about 350,000 individuals die of HCV-related diseases every year. 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 identified a host factor, the sigma-1 receptor (S1R), with a specific role at the onset of the HCV life cycle. This cell 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. Mechanistic studies indicated that early steps in viral RNA replication downstream of translation of the incoming viral genomes are rate-limited by cellular S1R levels. These findings raise the possibility that HCV uses MAM as a gateway to the cell machinery necessary for efficient viral replication. We are currently determining the molecular mechanisms by which HCV uses S1R and the potential pathological consequences, using broad approaches that involve determination of alterations in the proteomic composition of S1R-containing macromolecular complexes during HCV infection.

In addition to these basic studies, we sought new molecules with antiviral potential against HCV. Using a screening system designed in-house, we interrogated a chemical library of 281 compounds approved for use in clinical practice for non-HCV applications. The rationale is that prior knowledge of the molecular mechanisms of their pharmacological action as well as their cell targets, toxicity, bioavailability and pharmacokinetics could expedite translation to the clinic. In this study, we identified a set of 12 compounds, of which two (hydroxyzine and benztropine) were selected for further characterisation. At micromolar concentrations, these compounds selectively blocked HCV entry; hydroxyzine was antiviral at clinically achievable doses, preferentially for genotype 2 viruses.

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Carnero E, Díez J, Fortes P, Gastaminza P, Majano P, Martínez MA, Pérez-del-Pulgar S, Quer J, López-Labrador FX; Grupo de Estudio de Hepatitis Víricas de la Sociedad Española de Virología. Gastroenterol Hepatol 2013; 3610:641-646

Larrea E, Riezu-Boj JI, Aldabe R, Guembe L, Echeverria I, Balasiddaiah A, Gastaminza P, Civeira MP, Sarobe P, Prieto J. Dysregulation of interferon regulatory factors impairs the expression of immunostimulatory molecules in hepatitis C virus genotype 1-infected hepatocytes. Gut 2014; 63:665-673

Friesland M, Mingorance L, Chung J, Chisari FV, Gastaminza P. Sigma-1 receptor regulates early steps of viral RNA replication at the onset of hepatitis C virus infection. J Virol 2013; 87:6377-6390

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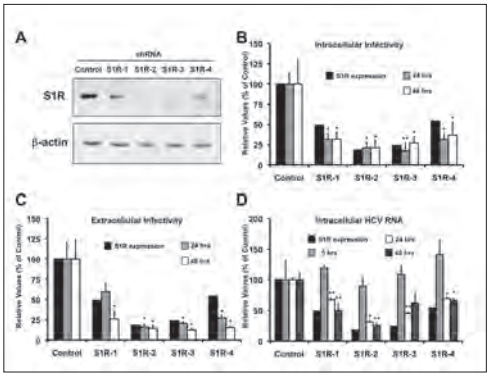
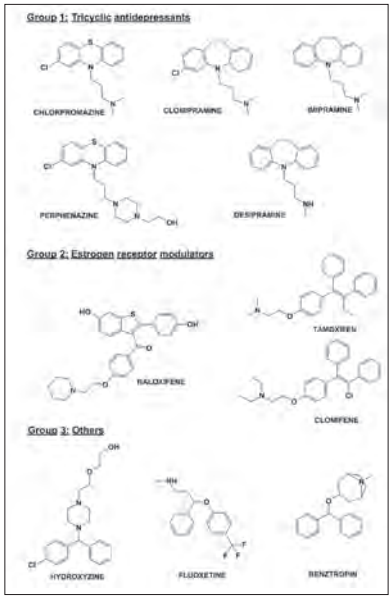


1 *Cellular S1R levels are limiting for HCV infection. Huh-7 cells were transduced with lentiviral vectors expressing an irrelevant sequence or shRNA targeting S1R mRNA, and infected with the cell-culture-adapted HCV D183v at a multiplicity of infection (m.o.i.) of 10. A) Western blot analysis of total cell extracts showing reduced S1R expression at the time of inoculation (six days post-transduction) in shRNA-expressing cell lines and a loading control (β–actin). B) Intracellular and C) extracellular infectivity titres (ffu/ml) in HCV-infected cells expressed as mean ± SD (n = 3). D) Normalised HCV RNA levels were determined at 5, 24 and 48 hours post-infection by RT-qPCR. Normalised S1R expression in panels B and C was quantified from panel A. Statistical significance of the differences with the control dataset was determined using Student's t-test (* p<0.05; ** p<0.01).*

2



2 *Chemical structure of the primary screening hits. These include previously identified anti-HCV compound family members such as tricyclic antidepressants and synthetic oestrogen receptor modulators. Hydroxyzine and benztropine were further characterised.*

Biological noise

PRINCIPAL INVESTIGATOR:
Francisco J. Iborra

PREDOCTORAL STUDENT:
Teresa Trigueros

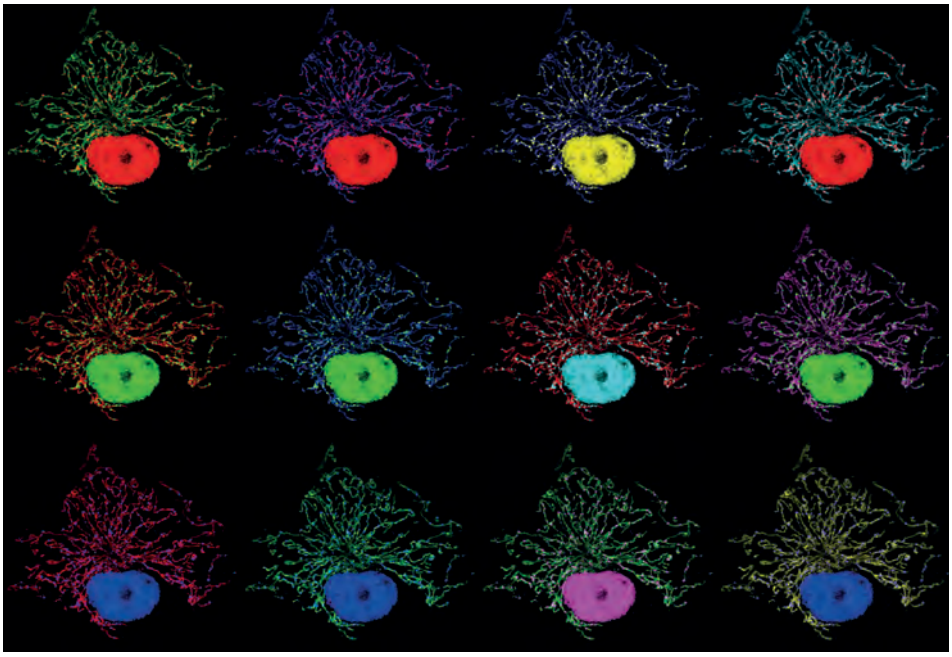
SELECTED PUBLICATIONS

Romero-Moya D, Montes R, Navarro-Montero O, Iborra FJ, Martin M, Bueno C & Menendez P. Cord blood CD34+ haematopoietic cells with low levels of mitochondrial mass are enriched in haematopoietic repopulating stem cell function. Haematologica 2013; 98:1022-1029

Our lab is interested in the origin of the phenotypic variability between genetically identical individuals. The reason we pursue 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 as intrinsic or extrinsic. Intrinsic variability, or intrinsic noise, is due to differences in the expression patterns of specific genes and depends on levels of the factors that control expression of such genes. Extrinsic variability (extrinsic noise) affects many genes within a single cell. We approach this question using a combination of experimental and computational techniques.

Our group demonstrated that one factor that contributes to extrinsic noise is the difference in the mitochondrial content in clonal cell populations. This is because the activity of RNA polymerase II is very sensitive to changes in cellular ATP, which is derived from mitochondria. 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.

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 they contribute to disease. We have shown that variability in mitochondria content also affects mRNA content and affects mRNA species differently, as we hypothesised. One unexpected effect of this mitochondrial variability is the acute change in alternative splicing, which can be attributed to the effect of mitochondria on RNA pol II (Guantes *et al.* Genome Res 2015; 25:633).



Animal models by genetic manipulation

PRINCIPAL INVESTIGATOR:
Lluís Montoliu

POSTDOCTORAL SCIENTISTS:
Almudena Fernández López
Mónica Martínez García
Davide Seruggia

PREDOCTORAL SCIENTISTS:
Santiago Josa de Ramos
Julia Fernández Punzano

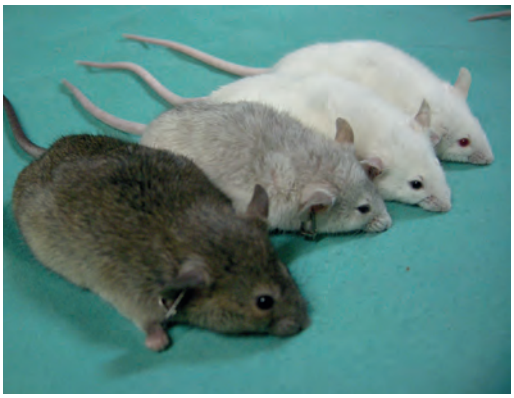
STUDENT:
Irene Robles

TECHNICIAN:
Marta Cantero

We are interested in understanding the function of regulatory elements necessary to identify gene expression domains in mammalian genomes and that help to specify gene expression patterns in space and time. Our basic aim is to improve adequate interpretation of the genome, especially the function of intergenic sequences, where most regulatory elements are located, and to improve the design of gene transfer strategies applied in animal transgenesis and gene therapy.

One of the experimental models in the lab is the tyrosinase gene, which encodes an enzyme that activates synthesis of the pigment melanin, which is regulated throughout development and is tissue-specific (expressed only in melanocytes and retinal pigment epithelium cells). This locus served to identify genome boundaries or insulators, which protect the tyrosinase gene from surrounding genes. In transgenic zebrafish and mice, we use different types of gene constructs based on plasmids and artificial chromosomes, which we modify by homologous recombination to study the relevance of specific sequences. Functional analysis of regulatory elements within the intergenic sequences can be now addressed more efficiently, thanks to the new CRISPR-Cas9 gene modification system that we have implemented successfully in our laboratory.

In addition, we generated and analysed new animal models to study visual abnormalities, including anomalies that affect retina development associated with albinism, as well as other retinopathies such as achromatopsia. In transgenic mice, we identified deficiency in L-DOPA (one of the intermediate metabolites in the melanin synthesis pathway) as the principal cause of the visual alterations in albinism. Within our participation in the CIBERER-ISCIII, we developed various additional animal models (transgenic and knockout) for the study of rare human diseases. In collaboration with Ángel Carracedo (Universidad de Santiago de Compostela) and Carmen Ayuso (Fundación Jiménez Díaz), we have established an experimental approach for the universal genetic diagnosis of all known mutations in the various albinism genes; in cooperation with ALBA, the Spanish association in support of people with albinism, we are already applying this approach to people with albinism and their relatives.



1 *Child with oculocutaneous albinism type I (OCA1) (Photo: Ana Yturralde)*

2 *Mouse models to study tyrosinase gene expression and albinism (Photo: Davide Seruggia)*

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Harms DW, Quadros RM, Seruggia D, Ohtsuka M, Takahashi G, Montoliu L, Gurumurthy CB. Mouse Genome Editing Using the CRISPR/Cas System. Curr Protoc Hum Genet 2014; 83:15.7.1-15.7.27

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Court F, Camprubi C, Garcia CV, Guillaumet-Adkins A, Sparago A, Seruggia D, Sandoval J, Esteller M, Martin-Trujillo A, Riccio A, Montoliu L, Monk D. The PEG13-DMR and brain-specific enhancers dictate imprinted expression within the 8q24 intellectual disability risk locus. Epigenetics Chromatin 2014; 7:5

Montoliu L, Grønskov K, Wei AH, Martínez-García M, Fernández A, Arveiler B, Morice-Picard F, Riazuddin S, Suzuki T, Ahmed ZM, Rosenberg T, Li W. Increasing the complexity: new genes and new types of albinism. Pigment Cell Melanoma Res 2014; 27:11-18

Functional analysis of the transcriptional repressor DREAM

PRINCIPAL INVESTIGATOR:
José Ramón Naranjo

SENIOR SCIENTISTS:
Britt Mellström
Alberto Rábano

POSTDOCTORAL SCIENTIST:
Juan Casado

PREDOCTORAL SCIENTISTS:
Elena Higueras
David Martínez-Alcocer
Javier Morón Oset

TECHNICIANS:
M. Paz González
Xosé Manuel Dopazo

We study the nuclear components of activity- and Ca^{2+}-dependent transcriptional responses in neurons and immune cells, to understand the molecular determinants of downstream events responsible for plastic changes in synaptic function. We also develop tools with which to intervene in physiological output processes including learning and memory, pain sensitisation and neurodegeneration.

Altered neuronal calcium homeostasis and early compensatory changes in transcription programmes 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 transcription 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 several mouse models of NDD, including AD, DS and HD. Genetic experiments show that this could be part of a neuroprotective mechanism.

We hypothesise the Ca^{2+}-dependent transcriptional repressor DREAM as an active/central component of several nucleoprotein complexes that specifically mediate various transcriptional cascades triggered by membrane depolarisation in neurons, which are essential in neuronal plasticity and synaptic dysfunction. Work in progress analyses the role of DREAM in regulating transcription in cell and animal models of NDD. Through these studies, we hope to better understand early changes in the transcriptome and epigenome and to explore new avenues for therapeutic intervention to boost early endogenous neuroprotective mechanisms.

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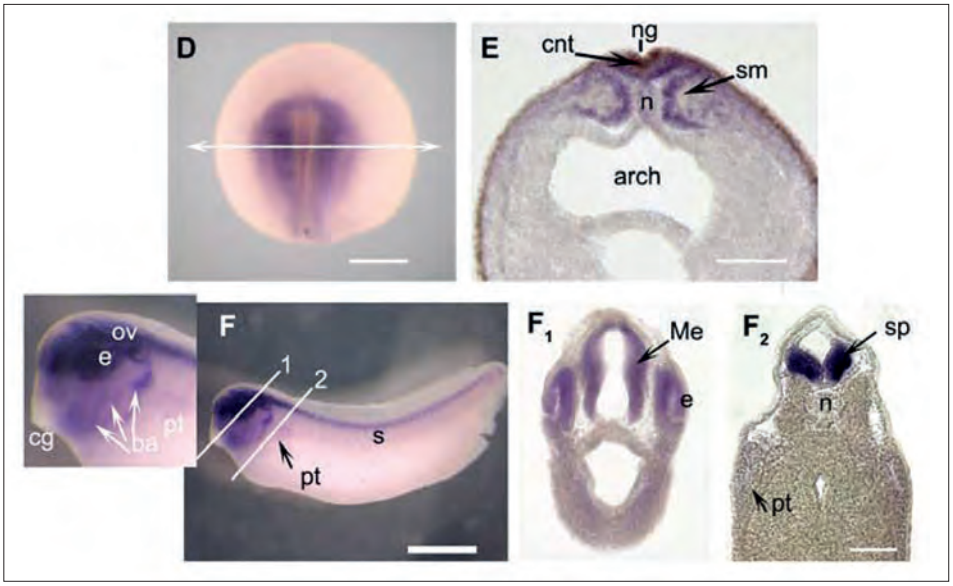
P9312EP00. Methods for the prognosis and diagnosis of neurodegenerative diseases

P201431898. Compuestos moduladores del sensor neuronal de calcio DREAM y sus usos terapéuticos



1 *Kchip1 mRNA distribution during Xenopus embryonic development. (D) KChIP1 is strongly expressed in the entire neural plate. (E) Detail showing the expression in the neural groove (ng), the closing neural tube (cnt) and somitic mesoderm (sm). Arch, archeteron, n, notochord. (F) In stage 33/34 larvae, Kchip1 is expressed strongly in central nervous system structures (forebrain, midbrain, rhombomeres), spinal cord (s) and the eyes (e). Bar = 1 mm. F1 and F2 transverse sections at the indicated levels visualise Kchip1 expression in the diencephalon, spinal cord (sp), pronephric tubules (pt), and notochord (n). Bar F1 and F2 = 0.2 mm.*

Cerebral cortical development

PRINCIPAL INVESTIGATOR:
Marta Nieto López

POSTDOCTORAL SCIENTIST:
Linnea Weiss

PREDOCTORAL SCIENTISTS:
Carlos García Briz
María Fernanda Rodríguez Tornos

STUDENTS:
Alba Fernández Rodrigo
Noelía Sofía de León

In our studies, we 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, responsible for most aspects of cognition and behaviour, is the most recently evolved structure in the human brain. A large number of functionally and morphologically distinct neuronal types specify brain cortical areas and control cerebral functions. We help 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 is expanded in humans, probably contributing to the increased cognitive capacity of the mammalian brain. It is the last to appear during development and in evolution. Our research showed that the transcription factors Cux1 and Cux2 are responsible for the extremely high degree of connectivity of these neurons and their participation in intra-cortical circuits that control higher brain functions. In our ongoing work, we dissect the neuronal characteristics modified by these genes to generate specialised neurons. We analyse how these are coordinated with experience and plasticity to generate the stereotyped networks of the human brain.

We also identified molecular mechanisms of axonal modelling and plasticity linked to these neurons, which participate in formation and physiology of brain circuits. In collaboration with other CNB groups, we study models of cell migration. Our research provides basic knowledge of the mechanisms of neural specification and circuit formation, the potential programs of reprogramming neurons, and the specific advantages and plasticity of the human brain. These data have broad, direct implications for understanding the specific functions of the cortex in intellectual processing. They might also explain underlying mechanisms of brain diseases that originate in childhood, and those of neurodegeneration, which is increasingly reported as plasticity-related.

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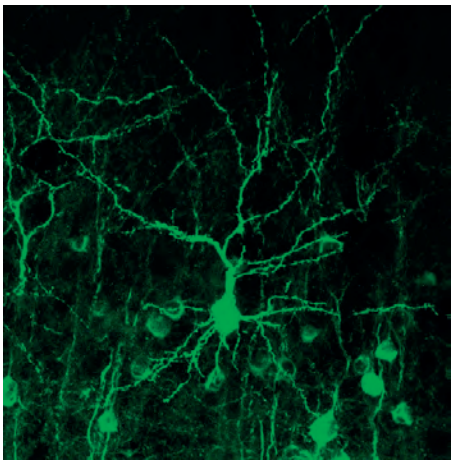
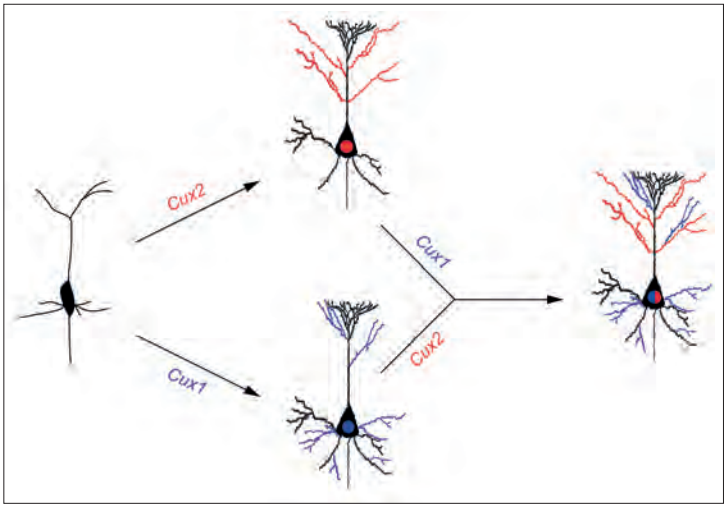
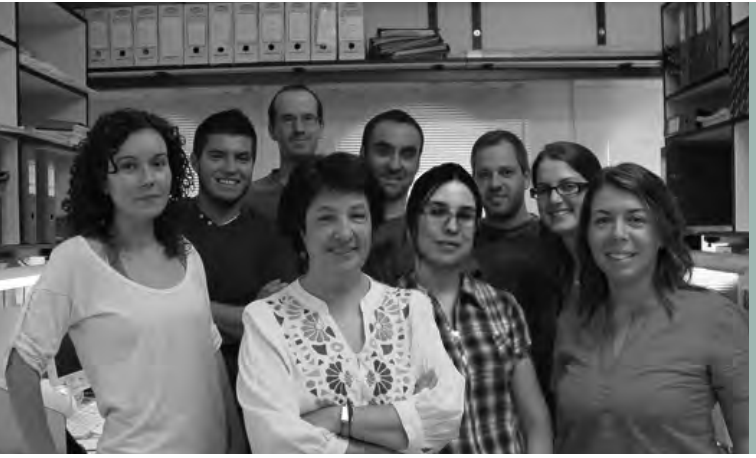


1 *GFP reveals the morphology of a layer II-III neuron. Representative confocal micrograph showing GFP-expressing neurons in the somatosensory cortex in mice. Neuron morphology was analysed after in utero electroporation of embryonic day 15 cortical precursors with the CAG-GFP vector.*

2



2 *Cux proteins selectively target apical and basal dendritic domains of layer II-III cortical neurons. Scheme summarising the additive and complementary functions of Cux1 and Cux2. During brain development, Cux1 expression promotes the development of basal processes while Cux2 contributes to apical dendrite differentiation. Co-expression of the two genes determines the final dendritic arbor of layer II-III neurons.*



Mechanisms of interaction between the influenza virus and the infected cell

PRINCIPAL INVESTIGATOR:
Amelia Nieto

POSTDOCTORAL SCIENTISTS:
Ana Falcón
Laura Marcos-Villar

PREDOCTORAL SCIENTISTS:
Pablo Chavez
Paloma Rodríguez
Alejandra Pazo

TECHNICIAN:
Noelia Zamarreño

Influenza virus polymerase establishes productive interactions with host-cell factors, including components of the cellular transcription and translation apparatus. We study the role of host factors that modulate both positive and negatively influenza virus replication and characterise the endogenous functions of these factors. hCLE and CHD6 are some of these viral polymerase-interacting proteins. hCLE, a shuttling protein that associates with common interacting proteins in the nucleus and the cytosol, is a positive modulator of influenza virus replication. Its nuclear import requires active transcription, which suggests a prominent role in nuclear and cytoplasmic RNA function. The chromatin remodeller CHD6 is a negative modulator of influenza virus replication that controls cell proliferation by promoting DNA synthesis.

Viral pathogenicity mediated by influenza virus polymerase has also been studied. We associated individual changes in PA and PB2 polymerase subunits with increased pathogenicity in a mouse model. In addition, a human influenza virus isolated from a fatal case showed individual changes in PA and PB2 polymerase subunits, which supports the role of viral polymerase as a pathogenicity factor. We also studied the role of human host factors, which might increase the fatality rate in influenza infection and could constitute high risk factors. The CCR5 chemokine receptor has a crucial role in this process; its loss of function increases the fatality rate several-fold. CCR5 deletion or loss of function is thus a high risk factor in man.

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Yángüez E, García-Culebras A, Frau A, Llompart C, Knobeloch KP, Gutierrez-Erlandsson S, García-Sastre S, Esteban M, Nieto A, Guerra S. ISG15 regulates peritoneal macrophages functionality against viral infection. PLoS Pathog 2013;9:e1003632

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Pérez-González A, Pazo A, Navajas R, Ciordia S, Rodríguez-Frandsen A Nieto A. hCLE/C14orf166 associates with DDX1-HSPC117-FAM98B in a novel transcription-dependent shuttling RNA-transporting complex. PLoS One 2014; 9:e90957

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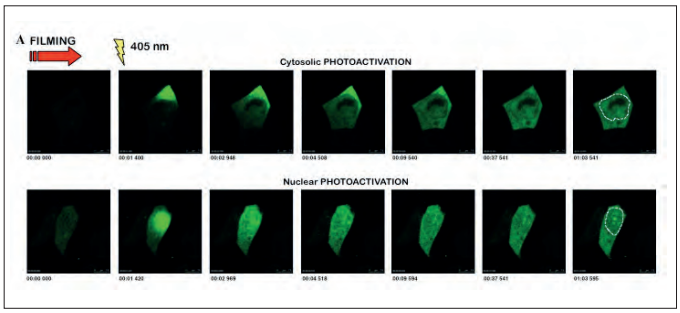

1 *hCLE shuttles in and out of the nucleus. Cultured HEK293T cells were transfected with phCLE-PAGFP (photoactivatable GFP) plasmid and were used for live cell microscopy at 24 h post-transfection. Photoactivation was applied in the cytosol (top panel) to visualise hCLE import. Photoactivation was applied in the nucleus (bottom) to visualise hCLE export. Numbers beneath the figures indicate minutes, seconds and milliseconds post-photoactivation. A dotted line marks the nuclear boundary.*

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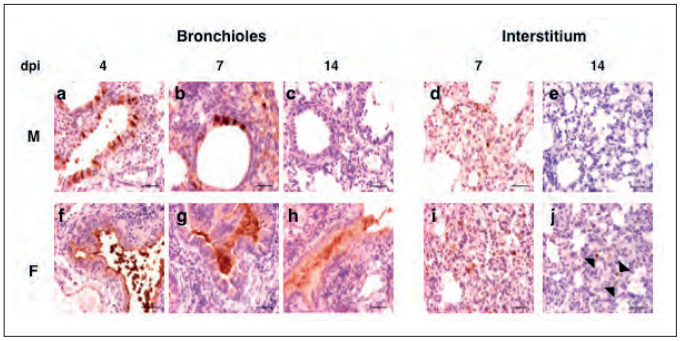

2 *Histopathology of lungs of mice inoculated with M (mild) virus (a-d) or F (fatal) virus (e-h). Haematoxylin/eosin staining; bar = 50 μm. Lung of mouse inoculated with M virus. a) 4 dpi, Mild congestion and diffuse lymphoid and phagocytic cells infiltrate the interstitium. b) 7 dpi, Moderate thickening of interalveolar walls. c) 14 dpi, Interstitial pneumonia with moderate proliferation of pneumocytes type II. d) 7 dpi, Interstitial pneumonia with thickened interalveolar walls. Lung of mouse inoculated with F virus, f) 4 dpi, Thickened interalveolar walls with consolidation of the pulmonary parenchyma (interstitial pneumonia). g) 7 dpi, Moderate increase in interstitial macrophages. h) 14 dpi, Increase in macrophages and syncytial cell formation. i) 7 dpi, Severe hyperplasia of phagocytic cells in the interstitium. Arrowheads show examples of the lesion in j.*

Transcription and replication of influenza virus RNA

PRINCIPAL INVESTIGATOR:
Juan Ortín

POSTDOCTORAL SCIENTISTS:
Susana de Lucas
Lorena Ver
Rocío Coloma

PREDOCTORAL SCIENTISTS:
Maite Pérez-Cidoncha
Sara Landeras-Bueno
Joan Peredo

TECHNICIANS:
Yolanda Fernández
Noelia Zamarreño

In the years 2013-2014, our group studied influenza virus interactions with the host cell during virus replication, as well as the cellular role of some of the virus-interacting factors.

The mechanism of action of the human Staufen1 protein (hStau1), which participates in the virus infection cycle, was elucidated by a combination of affinity purification, mutation and deep sequencing of hStau1 intracellular complexes. Results showed that hStau1 interacts with a specific sequence signature present in a subset of cellular mRNAs and determines hStau1-dependent translation. In addition, we identified a set of miRNAs specifically associated with hStau1 and showed that miR124 is specifically relevant. Downregulation of hStau1 thus affects the process of dendritic arborisation, although it does not alter maintenance of the differentiated state in cultured neuroblastoma cells.

The NS1 protein is a key player in the influenza virus-host interaction; it counteracts the activation of the cellular innate immune response. We determined specific protein sites involved in this NS1 function by random mutation and phenotype screening using an IFN-dependent GFP-expressing cell line. In addition, we carried out an unbiased screen of virus populations after replication in IFN-defective cells and used deep sequencing to show that essentially all virus genes are constrained by the IFN response. Mutations in genes not known to be involved in IFN counteraction thus lead to IFN-hyperinducing viruses.

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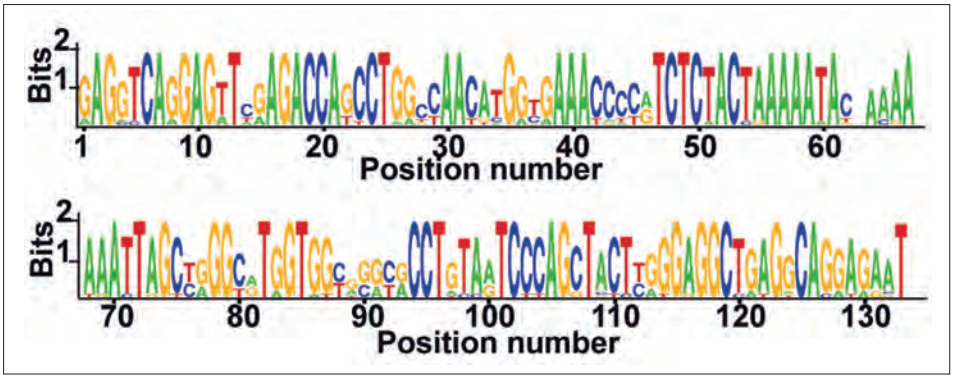

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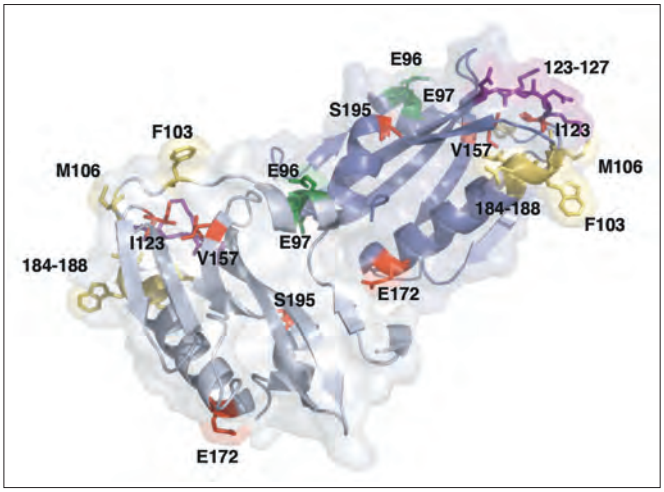


1 *Comparative sequence analysis of the mRNAs specifically associated to wt-hStau1 complexes. Sequence motif found by alignment of the protected sequences present in the mRNAs preferentially associated to wt-hStau1 complexes (de Lucas et al., Nucleic Acids Res 2014).*

2 *Atomic structure of the NS1 effector domain dimer showing the amino acids responsible for TRIM25 binding (E96, E97, green), PKR binding (123-127, purple), CPSF binding (103, 106, 184-188, yellow) and those identified as present in IFN-inducing viruses (I123, V157, E172 and S195, red) (Pérez-Cidoncha et al., PLoS One 2014).*

Molecular characterisation and epidemiology of torovirus

PRINCIPAL INVESTIGATOR:
Dolores Rodríguez

PREDOCTORAL SCIENTISTS:
Ginés Francisco Ávila Pérez
Gliselle Nieves Molina
Liliana Lilibeth Cubas Gaona

TECHNICIAN:
Susana Plazuelo Calvo

SELECTED PUBLICATIONS

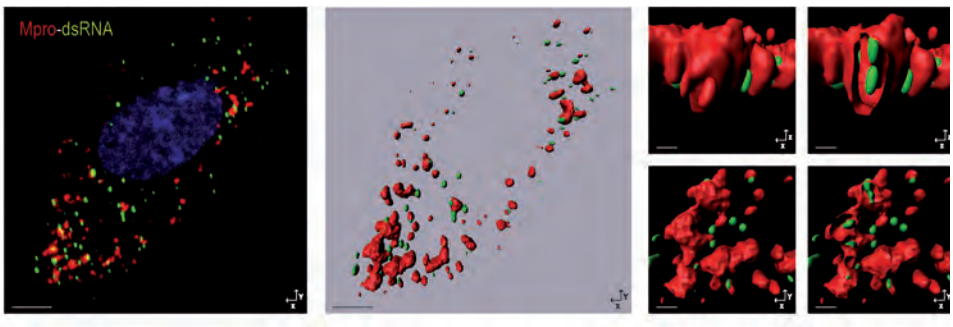
Pignatelli J, Alonso-Padilla J, Rodríguez D. Lineage specific antigenic differences in porcine torovirus hemagglutinin-esterase (PToV-HE) protein. Vet Res 2013; 44:126

Toroviruses (ToV) are enveloped, positive single-stranded RNA viruses of cattle, horses, pigs and humans. They are associated with enteric infections and diarrhoea, especially in young animals and children, and are considered a potential zoonotic threat. The Torovirinae subfamily of the Coronaviridae family (order Nidovirales) comprises four species: equine (EToV), bovine (BToV), porcine (PToV) and human torovirus (HToV). Information gathered from different epidemiological studies, including ours, indicates that ToV are widely prevalent in porcine and bovine livestock. Nonetheless, these viruses have been poorly studied to date.

To determine the prevalence of PToV in Spanish herds, we used serological and virological analyses of samples from adult and young animals from 100 farms distributed throughout Spain, and identified virus strains of the two defined PToV lineages. We found that the HE protein, which forms small spikes on the surface of the virions, shows distinct lineage-associated antigenic characteristics. Our results showed that PToV is endemic in Spain, and indicated that the HE protein has an important role in PToV-host interactions. The implications of this protein in immune protection could explain the chronic infection/re-infection cycle in the Spanish pig population.

The complex interactions between ToV and host defence mechanisms determines the outcome of the ToV-caused disease, but can also influence the immune response to a concurrent or subsequent infection by an unrelated pathogen. One of our main goals is thus to understand ToV-host cell interactions. We are currently studying the ability of the virus to counteract the innate immune response mediated by type I interferon (IFN). Our findings indicate that the virus can block both IFN secretion and expression of IFN-stimulated genes. At least two viral proteins are involved in IFN antagonism.

RNA viruses form their replication/transcription complexes in association with cell membranes. To characterise these complexes in ToV-infected cells, we are using various imaging techniques in combination with a panel of antibodies to distinct viral proteins involved in these processes.



1 *Localisation of torovirus replication/transcription complexes. Confocal microscopy image showing the localisation of the dsRNA replication intermediate (green) and the viral protease Mpro (red)(left panel). Three-dimensional reconstruction from the confocal image: top view (centre), front view and view of a vertical section that shows the dsRNA inside the structure labelled with the Mpro antibodies (top right), and top view and view of a cross section (bottom right).*

Molecular biology of birnavirus

PRINCIPAL INVESTIGATOR:
José F. Rodríguez

POSTDOCTORAL SCIENTIST:
Rosa Mª Moreno

PREDOCTORAL SCIENTISTS:
Idoia Busnadiego
Diego M. Ferrero
Fernando Méndez
Gemma Millán
Tomás de Garay

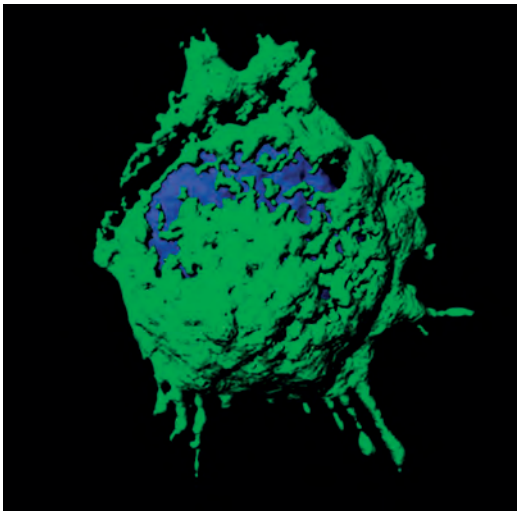
TECHNICIAN:
Antonio Varas

VISITING SCIENTISTS:
Laura Delgui
(IHEM-CONICET, Argentina)
Yaser Almeida
(Centro de Inmunología Molecular, Cuba)
Rubén Sánchez-Eugenia
(Unidad de Biofísica UPV-CSIC, Spain)
Ainhoa Erce
Yanaisis Alvarez
(Inst. Química Física Rocasolano-CSIC, Spain)
Javier Hervás
Natalia Comino
(Unidad de Biofísica UPV-CSIC, Spain)

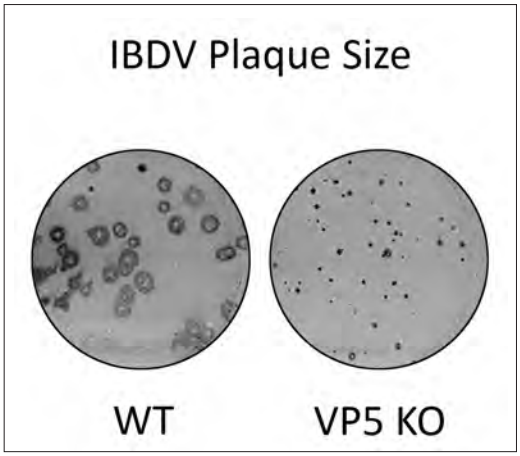
The Birnaviridae family comprises naked icosahedral viruses with bipartite dsRNA genomes that infect a wide variety of animal species including insects, aquatic fauna and birds. Despite its socioeconomic importance, critical aspects of birnavirus molecular biology are poorly characterised. Our main virus model, infectious bursal disease virus (IBDV), is the aetiological agent of an acute immunosuppressive disease that affects juvenile domestic chickens and causes heavy economic losses to the poultry industry world-wide (http://www.oie.int/eng/maladies/en_classification2007.htm?e1d7). The main goal of our laboratory is to better understand the virus-host interactions that underlie birnavirus pathogenesis, and to use this knowledge to develop sustainable strategies for birnavirus-borne disease control.

Our group currently focusses on unravelling the strategies the virus uses to evade host innate immune responses and on characterising virus egress mechanism(s) and their relation to virus virulence.

We recently showed that VP3 polypeptide, a dsRNA binding protein, acts as an efficient sheltering device that prevents detection of virus replication complexes by specialised cell sensors, thus preventing the onset of specific antiviral responses. By mapping the VP3-dsRNA binding domain, we determined that single mutations that affect a critical lysine residue within this domain are sufficient to completely abrogate virus replication.



The release of IBDV particles is customarily viewed as a process directly linked to the destruction of infected cells. Recent data from our laboratory nonetheless strongly suggest that IBDV also uses an alternative non-lytic cell-to-cell spreading mechanism. This mechanism appears to be strictly dependent on expression of VP5, a small, non-structural virus polypeptide that specifically binds monophosphorylated phosphoinositide lipids found at the cytosolic face of distinct cell membranes. Characterisation of this alternative egress mechanism could offer new prospects for efficient control of IBDV dissemination.



1 *3D reconstruction of the IBDV VP5 polypeptide subcellular distribution. Cells were processed for immunofluorescence using rabbit anti-VP5 serum followed by incubation with goat anti-rabbit coupled to Alexa-488 (green). Nuclei were stained with DAPI (blue).*

2 *Abrogation of VP5 expression thwarts IBDV cell-to-cell dissemination. The image shows the result of parallel plaque assays performed with IBDV wild-type (WT) and a VP5 knockout mutant deficient for VP5 expression (VP5 KO) on QM7 cells.*

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Dalton RM, Rodríguez JF. Rescue of infectious birnavirus from recombinant ribonucleoprotein complexes. PLoS One 2014; 9:e87790

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PATENT

P9571EP00. VLP, obtention methods and applications thereof.

Embryonic development and differentiation in vertebrates

PRINCIPAL INVESTIGATOR:
Juan José Sanz-Ezquerro

PREDOCTORAL SCIENTIST:
Verónica Uribe

STUDENTS:
Rocío Brea
Laura Higuera
Álvaro Rubio

TECHNICIAN:
Paloma Vaquero

SELECTED PUBLICATIONS

Uribe V, Badia-Careaga C, Casanova JC, Domínguez JN, de la Pompa JL, Sanz-Ezquerro JJ. Arid3b is essential for second heart field cell deployment and heart patterning. Development 2014; 141:4168-4181

Our group is interested in understanding the molecular and cellular basis of organ formation during embryonic development. This knowledge is important for identifying the origin of congenital malformations and for the design of therapies for human diseases with alterations in developmental genes and signalling pathways. We study heart and digit formation in animal models (mouse and chicken). We have made the following recent contributions.

The tip of digits is a structure characterised by specific gene expression

Formation of the last phalanx of the digits occurs by a special mechanism, different from that of proximal phalanges. We showed that Sp8 and Bambi are expressed in the digit tips and that this expression can be used to distinguish a true tip from that in a truncated digit (Casanova *et al.* PloS One 2012; 7:e52781). We also found that application of Fgf8 to digit primordia can elongate digits in some but not all cases, which suggests different digit morphogenesis programmes. Characterisation of these specific mechanisms could lead to understanding of the evolution of limbs, one of the most diversified anatomical structures. The study of these mechanisms is also important for understanding the unique capacity of digit tips to regenerate, which could have biomedical applications. We will continue this research line, analysing the regenerative capacity of digits in animals with a reduced inflammatory response, to study the relationship between regeneration and inflammation.

The gene Arid3b is necessary for heart development

Arid3b is a transcription factor whose functions are poorly characterised. We determined that Arid3b is expressed in the primitive heart and that it is necessary for heart development (Uribe *et al.* Development 2014). *Arid3b*-deficient embryos die early in development due to defects in the addition of cardiac progenitors to the heart tube. Arid3b appears to control cell motility and expression of cardiac factors, at least through the regulation of other genes such as *Lims2* and *Bhlhb2*. This result extends our knowledge of *Arid3b* during embryo development, suggesting a general role in cell movement, and possible involvement in cancer.

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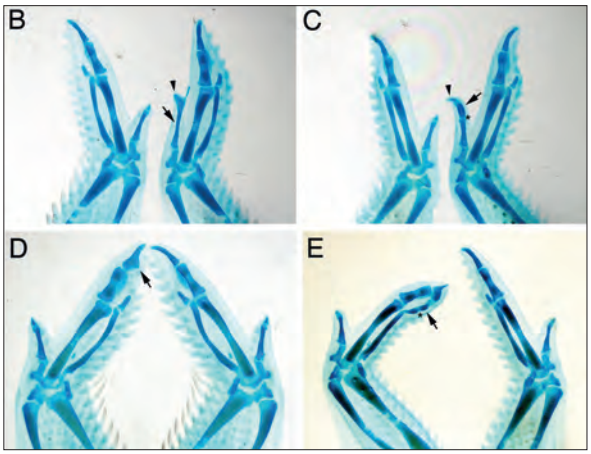

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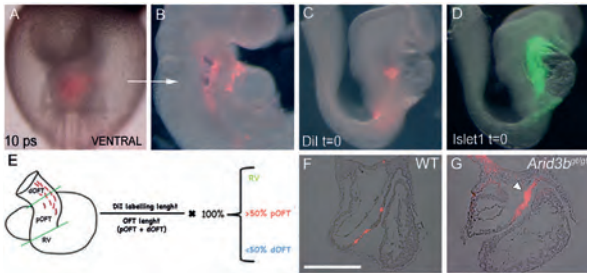
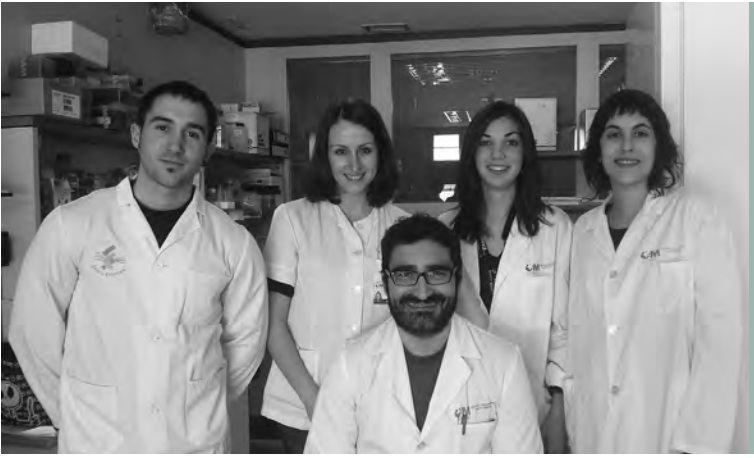


1 *Fgf8 induces elongation and extra phalanges in digits 1 and 3, but not digit 2 of the chick wing. Beads soaked in Fgf8 were applied to the first (B,C) or second (D,E) interdigital spaces at the time of initial digit condensation. Five days after the operation, embryos were collected and stained with alcian green to reveal skeletal elements. Controls are shown next to experimental limbs. Fgf8 induced elongation of digit 1 (B, C, arrow) and 3 (E, arrow), but did not induce elongation of digit 2 (D, arrow).*

2 *Addition of progenitors to the developing heart is altered in Arid3b-deficient embryos. (A) Ventral view of DiI labelling (red, to label progenitor cells) in a representative embryo at t=0. (B) Lateral view after 24 h in vitro culture. (C,D) DiI labelling is in an Islet1-positive region (D, immunostaining with Islet1 antibody). (E) Scheme illustrating the three regions into which the heart was subdivided for data analysis. (F,G) Cryosections of wild-type and mutant embryos 24 h after labelling; note accumulation of DiI-labelled cells at the entrance of the heart in the mutant (arrowhead). Cells do not enter the heart as efficiently as in the wt embryo.*



Cellular immunobiology and microbiology

PRINCIPAL INVESTIGATOR:
Esteban Veiga Chacón

POSTDOCTORAL SCIENTIST:
Aránzazu Cruz Adalia

PREDOCTORAL SCIENTIST:
Guillermo Ramírez Santiago

TECHNICIAN:
Mónica Torres Torresano

VISITING SCIENTISTS:
Raquel García Ferreras
(Universidad de Lleida, Spain)
Adrián Izquierdo Martínez
(Universidad de Murcia, Spain)
Isabel Fernández Fernández
(Universidad de Oviedo, Spain)
Samatha Kao
(University of New Mexico, USA)

During 2013-2014, we were involved in studying the interactions between pathogenic bacteria and cells of the immune system, and identified a way that T cells capture bacteria, that is, bacterial transinfection. Some pathogenic bacteria (*Listeria monocytogenes*, *Salmonella enterica* and *Shigella flexneri*) are able to invade T lymphocytes *in vivo* and modify their behaviour. We found that T cells capture bacteria by transinfection from previously infected dendritic cells (DC). This process requires direct contact between the two cells and is enhanced by antigen presentation. It is an extremely powerful T cell mechanism for bacterial capture and is T cell-driven, as non-pathogenic bacteria are also captured. Some viruses such as HIV use a similar transinfection mechanism to reach CD4+ T cells through infected DC. Transinfected T cells killed the captured bacteria within the first hours post-infection, more efficiently than professional phagocytes.

These results show that T lymphocytes, cells of adaptive immunity, can capture and kill bacteria in a manner thought to be exclusive to cells of innate immunity. Moreover, transinfected T cells secrete large amounts of proinflammatory cytokines (IL-6, interferon-γ, TNFα) with important roles in bacterial clearance and protection from *L. monocytogenes* infection.

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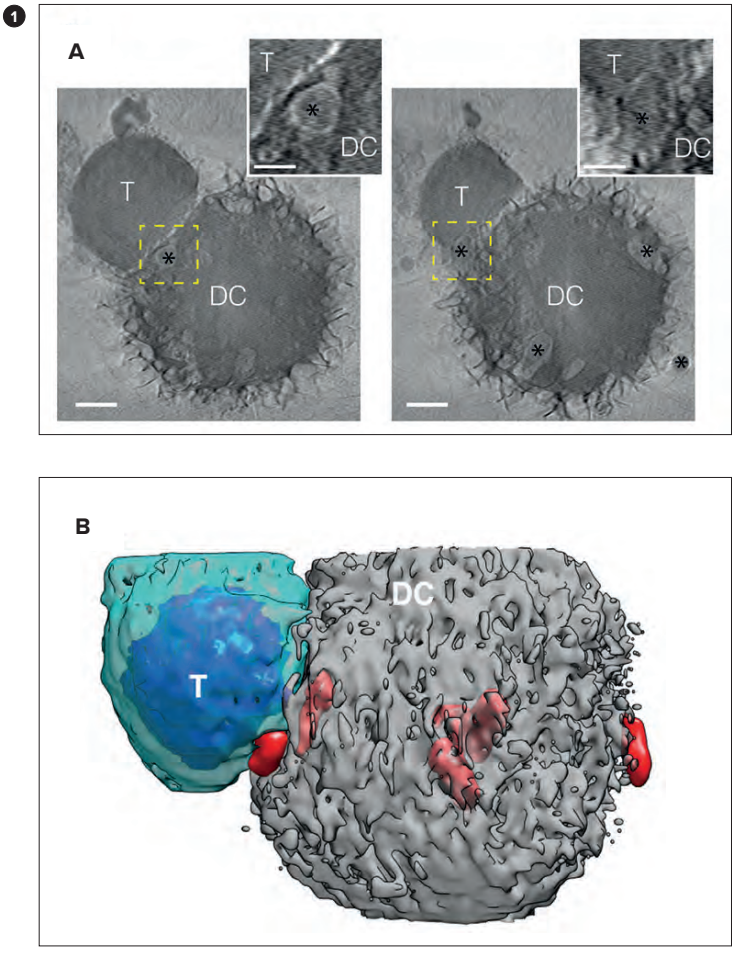

1 *(A) Cryo soft X-ray tomogram of transinfection. (B) Volumetric representation of the tomogram in A. Bacteria are shown in red, T cells, cyan, and DC, gray. The T cell nucleus is shown in blue (Cruz-Adalia et al. Cell Host Microbe 2014).*

5 / Microbial Biotechnology

Research in the Department of Microbial Biotechnology focusses on microbes of environmental, industrial or clinical relevance. Our work includes several approaches based on molecular genetics, systems and synthetic biology, genomics, proteomics and metagenomics. The scientific objectives of the department include five complementary aspects of microbial biology:

• Environmental microbiology. We study the regulatory mechanisms that degrade organic pollutants by analysing global regulation networks that control the hierarchical assimilation of nutrients in complex environments. Understanding the overall regulation of bacterial metabolism will allow us to optimise bioremediation strategies and industrially important biotransformation processes. We use metagenomic approaches to evaluate the effect of toxic compounds, including biocides and herbicides, on natural microbial communities.

• Microbial pathogens. We direct our efforts to host-pathogen interactions in infections caused by intracellular and opportunistic bacterial pathogens. In addition, we study basic processes of microbial physiology, such as cell division, that are relevant in infection and to define antimicrobial targets.

• Microbial resistance to antibiotics and the search for new antimicrobials. We work to understand the mechanisms of bacterial resistance to antibiotics and to analyse the complex responses elicited upon exposure of microbes to sub-lethal concentrations of antibiotics. In addition, we search for new targets as a way to develop new antimicrobials.

• Microbial responses to hostile environments. The purpose is to determine bacterial responses to stressful environments, including general stress responses and specific responses to agents that cause DNA damage. We study how bacterial viruses and yeasts replicate their DNA and how bacteria repair DNA damage and promote segregation to improve genome stability.

• Microbial engineering. The goal is to generate bacterial strains optimised to obtain products of interest (recombinant antibodies, hydrolytic enzymes), or to detect and degrade pollutants. We engineer bacterial strains that attach to specific surfaces such as antigen-expressing human cells, including tumour cells, which often express proteins abnormally on the plasma membrane.

Genetic stability

PRINCIPAL INVESTIGATOR:
Juan C. Alonso

POSTDOCTORAL SCIENTISTS:
Verónica Barca
Begoña Carrasco
S.M. Ahasanul Kobir
Violeta Rodríguez
Daniel Sánchez

PREDOCTORAL SCIENTISTS:
Carolina Gándara
Héctor Romero
Ester Serrano
Mariangela Tabone
Andrea Volante
Tribhuwan Yadav

TECHNICIAN:
Chiara Marchisone

Our research centres on the study of the molecular mechanisms that bacteria of the Firmicutes phylum use to maintain genomic stability, promote horizontal gene transfer and achieve accurate plasmid segregation. Using *Bacillus subtilis* as a model, we showed that the DNA damage response recruits various complex molecular machineries depending on the type of DNA damage, double-strand breaks (DSB) or single-strand gaps (SSG). We found that RecN, in concert with PNPase, promotes dynamic recruitment of DNA ends onto a repair centre. The AddAB or the RecJ-RecQ(RecS)-SsbA complex creates a 3'-ssDNA tailed duplex at the breaks, and RecN recruits RecA mediators (SsbA, RecOR) and modulators (RecF, RecX, RecU, RecD) to initiate recombinational repair (Fig. 1). In addition, we observed that DisA, in concert with RadA/Sms and c-di-AMP, recognise recombination intermediates and modulate SSG repair. More than 150 genes are switched on during natural competence, with DprA, SsbB, SsbA, RecO(R), RecX, and CoiA contributing to RecA activation to increase genetic diversity. By studying the segregation machineries, we showed that i. low copy number plasmids require homodimeric ParA-like (δ2), ParB-like (ω_2) and *parS* regions for stable inheritance at cell division (Fig. 2); ii. δ_2-ATP, which associates dynamically to the nucleoid, captures and tethers the plasmid-bound ω_2-*parS* complex to the nucleoid; iii. at stoichiometric δ_2 and ω_2 concentrations, the latter facilitates ATP hydrolysis, creating a gradient of nucleoid-bound self-organising δ_2 clouds; and iv. dynamic assembly/disassembly of the nsDNA-δ_2-ω_2-*parS* complex moves the plasmid molecule towards the cell poles to guarantee faithful segregation.

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PATENT

20140308246. A method of making biological containment factors for use in selectively killing target bacteria such as *Bacillus anthracis*, *Clostridium botulinum*, *Clostridium perfringens*, and other select agents

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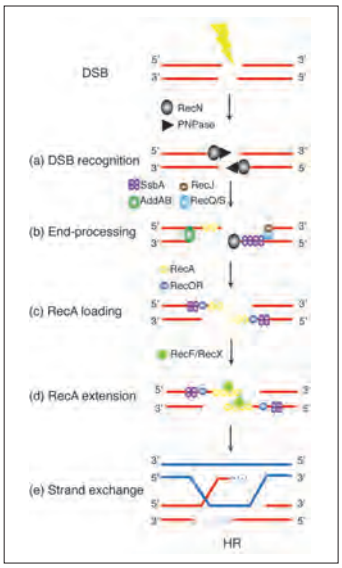

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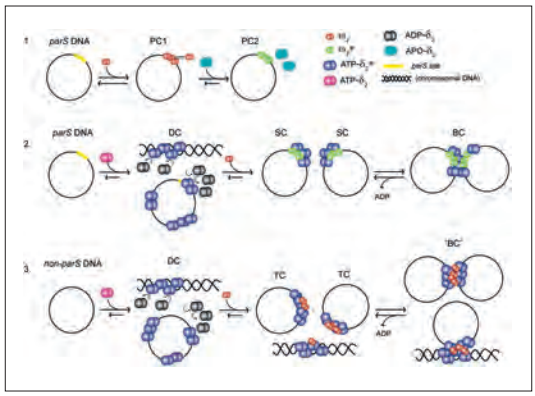
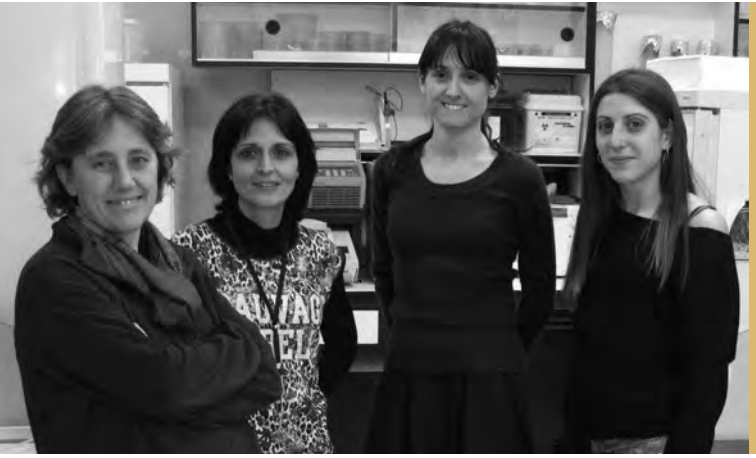


1 *Early stages in DNA repair by homologous recombination in B. subtilis. RecN, in concert with PNPase and SsbA, binds ssDNA regions at broken DNA ends (DSB recognition). Long-range end processing catalysed by the AddAB or the RecJ-RecQ(RecS)-SsbA complex generates a 3'-ssDNA tail coated by SsbA (end-processing). RecN might recruit the RecO-RecR complex there. RecO promotes SsbA disassembly and loads RecA onto ssDNA (RecA loading). A RecA filament at one of the processed ends promotes invasion of an intact homologous DNA to form the displacement loop (D-loop) recombination intermediate modulated by RecF and RecX.*

2 *Dynamic assembly of different types of protein-DNA complexes. 1. Protein ω_2 binds parS DNA to form an unstable partition complex PC1, but after interaction with Apo-δ_2, it undergoes a conformational transition leading to ω_2* and stable PC2. 2. δ_2 interaction with any DNA induces conformational changes in the protein (ATP-δ_2*). Protein δ_2 binds DNA and forms dynamic complexes (DC). Protein ω_2* at the stable PC2 promotes δ_2 redistribution to the segregosome (SC). Two or more SC produce a large higher-order complex (BC). δ_2 at parS regulates SC and bridging complex (BC) formation. 3. Interaction of δ_2 at the DC with ω_2 facilitates TC formation and accumulation of "BC".*



Recombination-dependent DNA replication

PRINCIPAL INVESTIGATOR:
Silvia Ayora Hirsch

SENIOR SCIENTIST:
Elena M. Seco

PREDOCTORAL SCIENTIST:
Ambra Lo Piano

STUDENTS:
Verónica Freire
Verónica Alonso
Ignacio Baquedano

TECHNICIAN:
María López Sanz

VISITING SCIENTIST:
Ana Valero
(INRA, France)

Our research centres on the mechanisms that cells use to continue DNA replication when this process encounters impediments that can 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). We use a simple model system, *Bacillus subtilis* and its bacteriophage SPP1, and several biophysical, structural and molecular biology techniques to study the recombination mechanisms that lead to replication restart.

A central step in the recombination reaction is the resolution of the recombination intermediate. Using single molecule analysis, we studied the cleavage preference of the RecU Holliday junction-resolving enzyme, and how it is modulated by other proteins such as the branch migration helicases RecG and RuvAB (Figure 1). Our results can be extended to other systems and the method developed should be applicable for the study of mechanisms used by other junction-binding enzymes involved in branch migration and junction resolution of the recombination intermediate.

We have reconstituted the replisomes of phage SPP1 and its host *B. subtilis in vitro*, and analysed the effect of recombination proteins in DNA replication *in vitro* (Figure 2). RecA slightly affects DNA replication *in vitro,* and RecO addition facilitates RecA-mediated inhibition of DNA synthesis. These results will be further analysed in the next few years. They suggest that RecA might prevent potentially dangerous forms of DNA repair that occur during replication.

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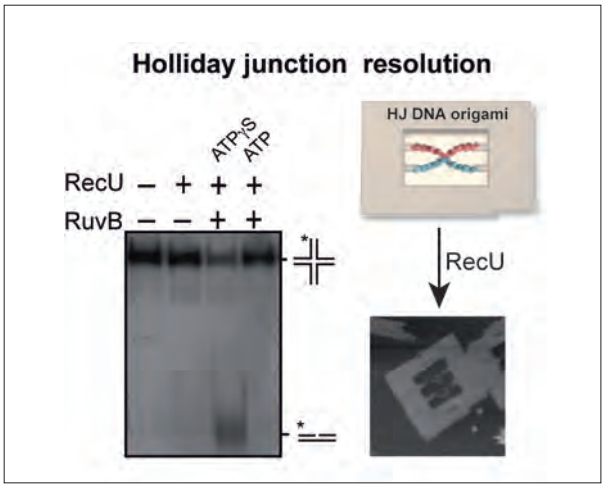

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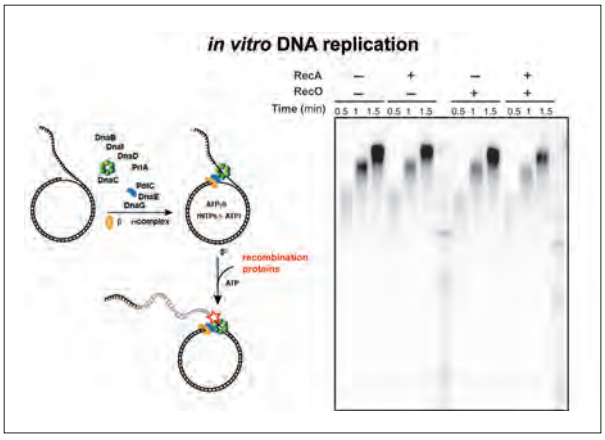


1 *Resolution of the Holliday junction recombination intermediate is modulated by the RuvB protein (left) and can be analysed with the DNA origami technique (right).*

2 *Scheme of the in vitro DNA replication system used (left) and the effect of RecA and RecO recombination proteins on DNA replication (right). Reactions were performed with 13 purified replication proteins from B. subtilis. Purified RecA and RecO recombination proteins inhibited the reaction.*

Stress and bacterial evolution

PRINCIPAL INVESTIGATOR:
Jesús Blázquez

POSTDOCTORAL SCIENTISTS:
Alexandro Rodríguez Rojas
Alejandro Couce
Noelia Alonso
Ynés Valencia

PREDOCTORAL SCIENTISTS:
Thuy Do Thi
Jerónimo Rodriguez Beltrán
Claudia Ibacache
Jasmina Vasilijevi
Elva Yubero

TECHNICIANS:
Coloma Costas
Noelia Sánchez

The major interest of our group is to understand the bacterial responses to stress. We specifically study hypermutation and hyper-recombination as "bacterial strategies" to speed adaptation to environmental stress. One model used is antibiotic stress and the development of antibiotic resistance. Our work focusses on stable and inducible hypermutation/hyper-recombination in *Escherichia coli, Pseudomonas aeruginosa* and *Mycobacterium smegmatis/tuberculosis*.

We currently study

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.

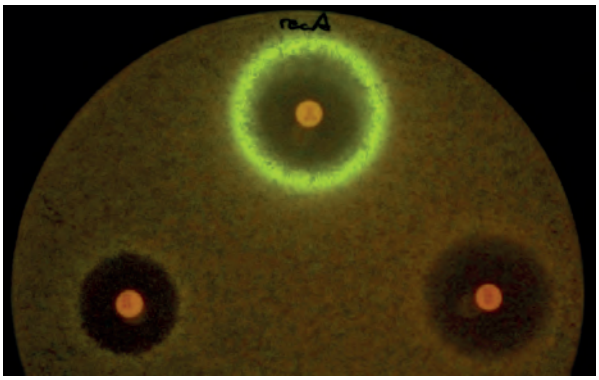
2. Regulation of stress responses and inducible hypermutation.

- Environmental regulation of mutagenesis
- Transcriptional regulation of specialised DNA polymerases (of the SOS regulon)
- Effect of antibiotics on mutation and recombination: do antibiotics promote 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 beta-lactamase inhibitors.

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



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Cell cycle, DNA replication and genome stability in eukaryotes

PRINCIPAL INVESTIGATOR:
Arturo Calzada

PREDOCTORAL SCIENTISTS:
Fernando Devesa Geanini
Fábia Araújo Gomes

STUDENTS:
María Luisa Macías Muñoz
Héctor Sánchez San Román
Azmane Mohamed
Marta Martínez-Lage García
María López Chiloeches

TECHNICIAN:
Ángel Valera

We aim to understand the factors and mechanisms by which the cell division programme maintains stable eukaryotic genomes across generations. The complex structure, size, and fragmentation of eukaryotic genomes, and the strict synchrony between termination of DNA replication and initiation of chromosome segregation during cell cycles complicate the transmission of exact genomes to progeny. This complexity and the vast numbers of divisions necessary to reach and maintain cell populations provide ample opportunities for DNA errors. Abortive cell division or aberrant genomes are very infrequent in normal cells; this indicates the robustness of the mechanisms and regulation of cell division programmes, which are yet not fully understood. Although they are rare, undetected errors ensue spontaneously in progeny and can threaten genome stability and health of the organism. Deregulation of proliferation control during the G1 phase, abnormalities during DNA replication, basal activation of DNA damage repair pathways, and unscheduled chromosome segregation are frequently associated with elevated genome instability, appear in most oncogenic cell cycles, and are hallmarks of cancer. The mechanics of genome instability acquisition in deregulated cell cycles at the molecular level is nonetheless poorly understood.

We studied the regulation of pausing of replication forks during DNA replication at fork-pausing sites. This pausing depends on the fork protection complex proteins Tof1/Csm3, which bind to the replisome. We found that Tof1 is regulated during normal S phases by phosphorylation dependent on the essential S phase kinase Cdc7/Dbf4 and on the Mec1 kinase (Figure 1), revealing active control of fork-pausing during DNA replication. We also studied the molecular abnormalities of DNA replication that potentially contribute to genome instability in cells deregulated in the G1 phase. We demonstrated that the CDK inactivators Cdh1-APC/C and Sic1 cooperate to promote efficient activity of DNA replication origins and optimal distribution of replication initiation events on the chromosomes (Figure 2). We also found that this control is essential to maintain low rates of genome instability.

Our experience in analysing chromosome integrity also allowed us to assist other groups in characterising telomere stability and DNA damage repair during DNA replication.

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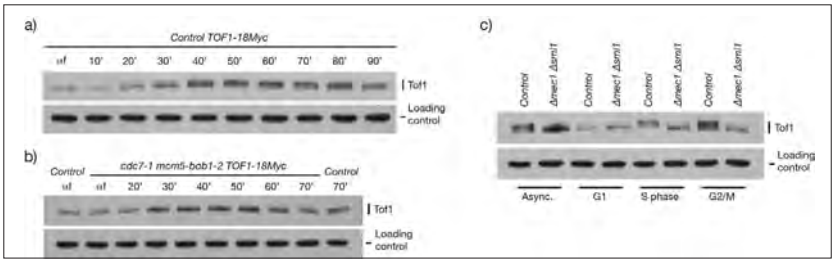

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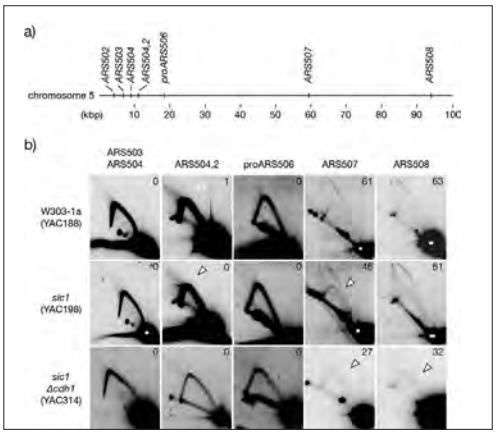

1 *Phosphorylation status of Tof1 during the cell cycle in Saccharomyces cerevisiae cells. a) Control cells. b) Cells lacking Cdc/Dbf4 kinase activity. c) Cells lacking Mec1.*

2 *Two-dimensional electrophoresis of DNA replication intermediates shows that deregulated G1 phase proliferation control impairs the activity of DNA replication origins (arrowheads, decrease in replication bubbles) on a chromosome arm.*

Biotechnology of protein secretion systems in *Escherichia coli*

PRINCIPAL INVESTIGATOR:
Luis Ángel Fernández

POSTDOCTORAL SCIENTIST:
Beatriz Álvarez González

PREDOCTORAL SCIENTISTS:
David Ruano-Gallego
Carlos Piñero Lambea
Valencio Salema
Massiel Cepeda Molero
Carmen Mañas Torres

Our work focusses on engineering *Escherichia coli* bacteria and their protein secretion systems for biomedical applications. We study protein secretion systems found in enteropathogenic (EPEC), enterohemorrhagic (EHEC) and uropathogenic (UPEC) *E. coli* strains and engineer them to develop new protein expression tools that can be applied for selection, production and *in vivo* delivery of therapeutic proteins, such as recombinant antibodies, by non-pathogenic *E. coli* strains. We work with single-domain antibodies (sdAb) or nanobodies, the smallest antibody fragments with full antigen-binding capacity known to date. Nanobodies are based on single VH domains obtained from heavy chain-only antibodies found in camelids (*e.g.*, dromedaries, llamas). Nanobodies have high affinity and specificity for their antigens and closely resemble human VH sequences.

In the last two years, our studies have concentrated on

1) Developing *E. coli* display technology based on type V secretion systems (T5SS). T5SS members are proteins with the capacity for "self-translocation" across the bacterial outer membrane, like the intimin-invasin family and the classical autotransporters. We have engineered the T5SS translocator domains to display nanobodies on the surface of *E. coli* and selected high-affinity binders of antigens relevant to human disease.

2) Re-programming *E. coli* adhesion to tumours with synthetic adhesins. Nanobody display on the *E. coli* allowed us to generate "synthetic adhesins" that can drive attachment of bacteria to target antigenic surfaces, including tumour cells that express cell surface antigens. We demonstrated that specific tumours can be targeted and colonised efficiently *in vivo* by low doses of engineered *E. coli* strains expressing synthetic adhesins that bind antigens expressed on the tumour cell surface. We intend to develop engineered *E. coli* strains with synthetic adhesins for the early diagnosis and therapy of specific human tumours.

3) Injection of nanobodies from *E. coli* into human cells. We are exploiting the type III protein secretion system (T3SS) from EPEC and EHEC *E. coli* strains for direct delivery of therapeutic proteins and nanobodies from *E. coli* cells into the cytosol of human cells.

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PATENT

US 8,623349 B2. System methods and microorganisms for antibody production with type III secretion system

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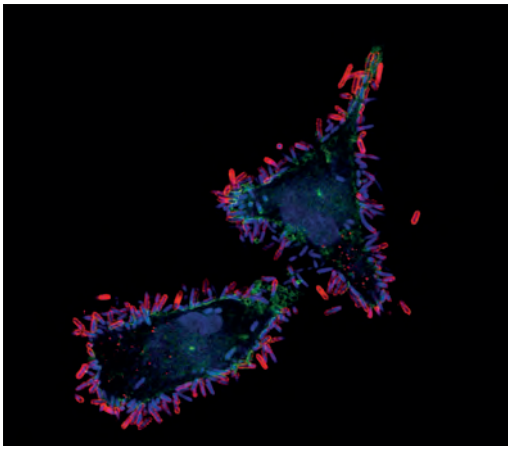

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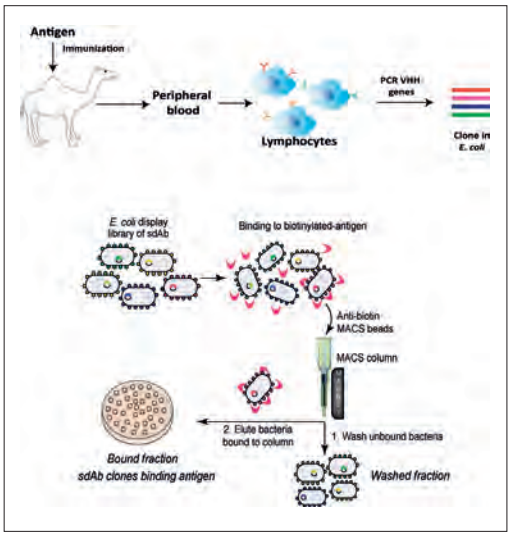

1 *Fluorescence confocal microscopy image showing E. coli bacteria (red) with synthetic adhesins targeting an antigen (green) expressed on the surface of human tumour cells (nuclei and bacterial DNA stained in blue). Synthetic adhesins can drive adhesion of E. coli bacteria to specific cells and surfaces.*

2 *The process of selection of single-domain antibodies (sdAb) by E. coli display. The VHHs gene segments are amplified by the lymphocytes of an immunised dromedary and cloned in a vector with the T5SS translocator domain for expression on the E. coli bacterial cell surface. These bacteria are incubated with the biotin-labelled target antigen, followed by anti-biotin magnetic beads. Antigen-binding clones are captured in magnetic cell sorting (MACS) columns and plated for amplification.*

Laboratory of intracellular bacterial pathogens

PRINCIPAL INVESTIGATOR:
Francisco García del Portillo

SENIOR SCIENTIST:
María Graciela Pucciarelli

POSTDOCTORAL SCIENTISTS:
Noelia López-Montero
Daniela Gargano
Juan J. Quereda
Álvaro D. Ortega
Alberto Tiérrez

PREDOCTORAL SCIENTISTS:
Gadea Rico-Pérez
Estel Ramos
Virginia Figueroa

TECHNICIANS:
Diana Barroso
Pablo García

Our group studies two "model" intracellular bacterial pathogens important in human and animal health, *Listeria monocytogenes* and *Salmonella enterica* serovar Typhimurium. These bacteria have different cell wall architecture and adopt distinct lifestyles (cytosol versus intra-vacuolar) within the eukaryotic cell. The common aim of our studies is to identify envelope changes during host cell colonisation and to understand how these changes shape the course of infection.

Listeria monocytogenes. This pathogen produces a large number of surface proteins, many exclusive to this bacterium and with unknown function. A family of surface proteins of particular interest to us is that composed of proteins covalently bound to the peptidoglycan after processing of their LPXTG-sorting motif. We have linked the biological function of two *L. monocytogenes* LPXTG surface proteins, Lmo0412 and Lmo1413, to bacteria interaction with the host. Lack of either protein alters virulence in the mouse model. In addition, we dissected the mechanism used by intracellular *L. monocytogenes* to upregulate the LPXTG protein Lmo0514 inside host cells. This mechanism involves the regulatory small RNA (sRNA) Rli27 and a long 5'-UTR present in a defined *lmo0514* mRNA transcript isoform. We are currently pursuing the biological role of Lmo0514 inside host cells.

S. enterica serovar Typhimurium. This pathogen establishes persistent infections in which bacteria reside in a "dormant-like" state within eukaryotic cells. The molecular basis of this phenomenon is poorly understood. Using the fibroblast *S. enterica* Typhimurium infection model, we characterised the expression of numerous regulatory sRNAs in non-proliferating intracellular bacteria, and identified an sRNA species induced in these conditions. We also examined the role of toxin-antitoxin (TA) modules, which respond to many stresses, in establishing persistent infections inside eukaryotic cells. These studies showed an unexpectedly large number of TA modules in *S. enterica* Typhimurium and the selective use of some in distinct host cell types. We continued our studies on the enzymatic machinery that supports peptidoglycan metabolism in intracellular bacteria and those focussed on peptidoglycan sensing during persistent infection. Lastly, we have linked *S.* Typhimurium persistence to a type of autophagy not previously reported in bacterial infections.

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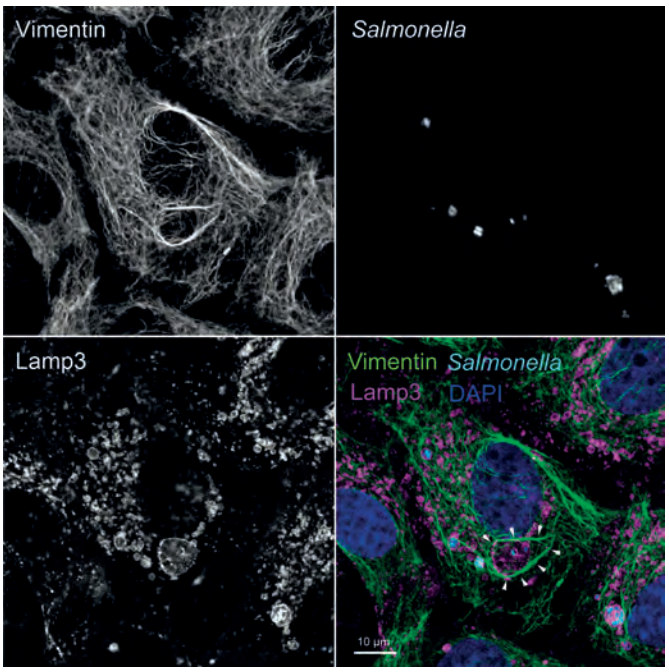
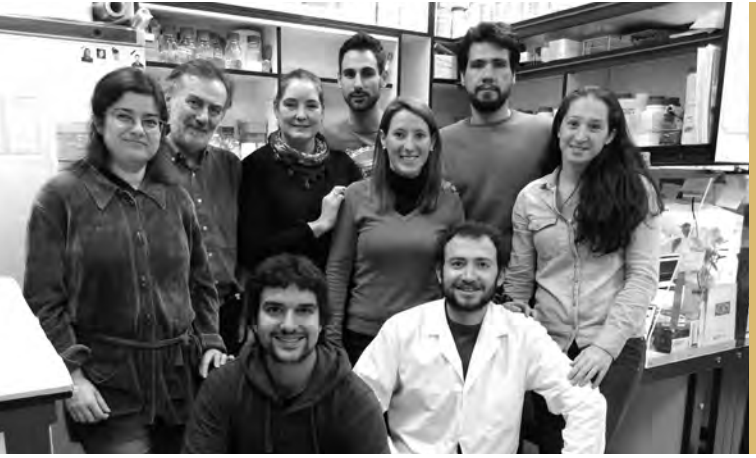


1 *Persistent infection of fibroblasts by S. Typhimurium correlates with an autophagy process that eliminates part of the intracellular bacterial population. NRK-49F rat fibroblasts infected with S. Typhimurium. Note the vimentin cage that segregates the autophagosome that is digesting some bacteria from the remaining cell contents (arrowheads). Other intracellular bacteria persist outside the autophagosome within Lamp3-enriched vacuolar compartments. Vimentin (green), S. Typhimurium (Salmonella, cyan), lysosomal membrane glycoprotein Lamp3 (magenta), nuclei (DAPI, blue). Bar = 10 µm.*



Opportunistic pathogens

PRINCIPAL INVESTIGATOR:
José Luis Martínez

POSTDOCTORAL SCIENTISTS:
Blanca Sánchez
Sara Hernando-Amado
Alejandra Bernardini

PREDOCTORAL SCIENTISTS:
Felipe Lira
Manuel Alcalde
Fernando Corona

STUDENT:
Paula Blanco

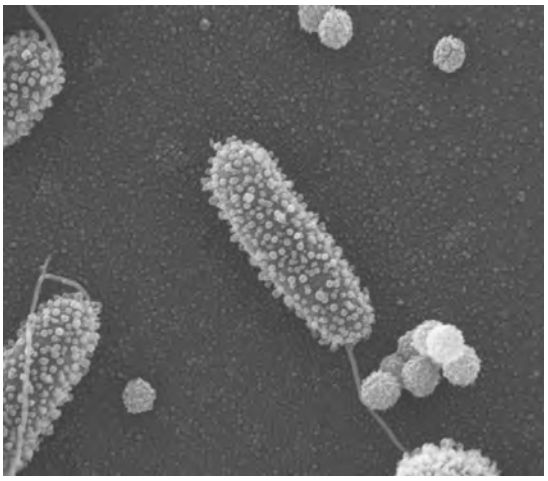
TECHNICIAN:
Trinidad Cuesta

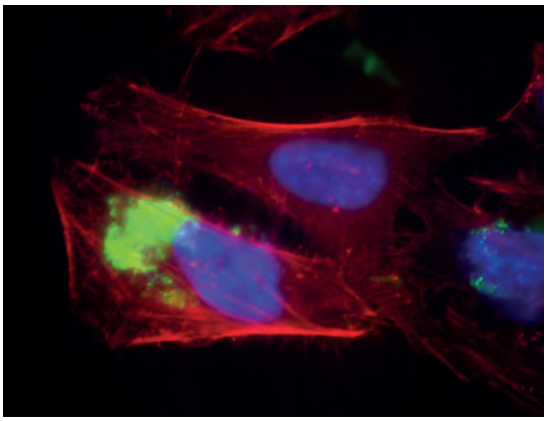
Nosocomial infections due to opportunistic pathogens are a serious health problem. As models for understanding the pathogenetic mechanisms of these microorganisms, we use *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*, free-living bacteria with a characteristic phenotype of intrinsic resistance to antibiotics. Acquisition of resistance in these organisms is the result 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 study the biology of opportunistic pathogens, focussing on the networks and the evolutionary processes that connect resistance and virulence. This includes analysis of the role of non-clinical natural ecosystems in acquisition and evolution of antibiotic resistance and virulence, and the host responses triggered by these pathogens. We are examining the intrinsic resistome, that is, the set of genes that contributes to the characteristic phenotype of susceptibility to antibiotics of a given bacterial species. Our work could provide information on potential drug targets for use in reducing antibiotic resistance.

We are especially interested in multidrug efflux pumps. These elements are found in all living beings and contribute to many processes, including resistance to anticancer chemotherapy in humans and antibiotic resistance in bacteria. Whereas these pumps can expel these drugs, however, they have different original functions in nature. We recently showed that the efflux pump SmeDEF in *S. maltophilia* is major determinant of resistance to quinolones (a family of synthetic antimicrobials), whose function is colonisation of roots in plants.

Acquisition of resistance can have a fitness cost, reflected as less competitivity of resistant compared with susceptible bacteria. We showed that fitness costs are gene-specific and that the effect on bacterial physiology of acquiring resistance is also specific, rather than a general metabolic burden. We found that fitness costs can be compensated, with no need for secondary mutation, by metabolic rewiring that adjusts bacterial physiology to the changes produced by resistance acquisition.





1 *Scanning electron microscopy of P. aeruginosa cells growing in conditions that stimulate type three secretion. The small spheres are extracellular vesicles.*

2 *Intracellular accumulation of ExoS toxin (green) secreted by P. aeruginosa through its type three secretion system.*

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

PRINCIPAL INVESTIGATOR:
Rafael P. Mellado

POSTDOCTORAL SCIENTIST:
Sonia Gullón

PREDOCTORAL SCIENTIST:
Rebeca López Vicente

TECHNICIAN:
Silvia Marín

SELECTED PUBLICATIONS

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Pérez Mellado R. Biotecnología: bioseguridad y biocustodia en la investigación. ARBOR 2014; 190:a150

The group continued to focus its research on the physiological and molecular characterisation of the main protein secretion mechanism (Sec system) of the soil Gram-positive bacteria *Streptomyces lividans,* widely used in industry as an efficient producer of extracellular hydrolytic enzymes and other compounds of industrial interest. Secretory protein overproduction triggers a secretion stress response, eliciting the synthesis of three specific proteases that degrade misfolded proteins, and a stringent response in *Streptomyces.*

Bacterial lipoproteins are a specialised class of membrane proteins reported to play a pivotal role in bacterial cell physiology, including protein folding. Absence of functional type II signal peptidase (Lsp), which cleaves the lipoprotein signal peptide, apparently produces a translocase blockage, as determined by the amount of the overproduced alpha-amylase that clearly accumulates on the internal surface of the membrane. As a result of the translocase blockage, Lsp deficiency reduces synthesis of secretory proteins in *S. lividans*, as is the case when the cell is deficient in the translocase complex (SecG mutant strain) or the major type I signal peptidase (SipY mutant strain). The *lsp* mutation also triggers a stringent response, as the absence of functional SBP lipoproteins causes non-sensing of solutes (nutrients) in the culture medium. These findings are of particular relevance with respect to characterising potential bottlenecks in *S. lividans* secretion and optimising *S. lividans* for the overproduction of secretory proteins of industrial application.

For a number of years, we have monitored the rhizobacterial communities of transgenic maize tolerant to glyphosate. In a new research line, we identified the most attractive method-combination workflow to analyse next generation sequencing results from rhizobacterial community experimental data, depending on sequence variability number and length. The worldwide increase in glyphosate-resistant weed populations led to new cultivation strategies based on combinations of pre- and post-emergence herbicides. Over a one-year cultivation cycle, we evaluated the impact of several herbicide combinations on the rhizobacterial community of glyphosate-tolerant Bt-maize and compared them to those of untreated or glyphosate-treated soils, and analysed the resilience of the microbial communities by comparing their relative composition at the end of the cultivation cycle.

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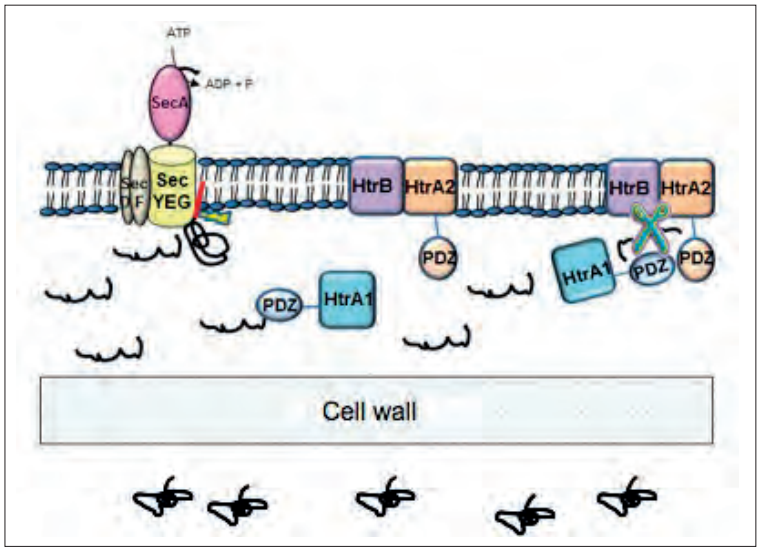

1 *Proposed model of protease action when secretory proteins are overproduced. Soluble HtrA1 recognises the misfolded proteins on the outer cytoplasmic membrane, while HtrB and HtrA2 remain in the membrane, forming heterodimers. The HtrA1-protein complex interacts with the HtrB-HtrA2 heterodimer via the PDZ domains, creating a structure able to cleave the misfolded proteins.*

Regulation of the metabolism of hydrocarbons in bacteria

PRINCIPAL INVESTIGATOR:
Fernando Rojo

POSTDOCTORAL SCIENTISTS:
Renata Moreno
Emma Sevilla

PREDOCTORAL SCIENTISTS:
Sofía Hernández
Ruggero La Rosa
Dione Sánchez

TECHNICIAN:
Luis Yuste

VISITING SCIENTIST:
Elva Quiroz
(U. Autónoma de México, Mexico)

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

Our aim is to characterise the overall regulation networks responsible for catabolite repression, identify their components, the signals to which they respond, the molecular mechanisms by which they regulate gene expression, and determine how they modulate metabolism. The regulatory proteins involved in these networks differ among 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 currently focus on two catabolite repression networks. One relies on the Crc and Hfq proteins, which ultimately inhibit translation of mRNA with the specific CA sequence motif within their translation initiation region. Two small RNAs, the levels of which vary greatly depending on growth conditions, antagonise the inhibitory effect of Hfq and Crc. 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 at some genes, but not at others. This could be relevant for several biotechnological applications.

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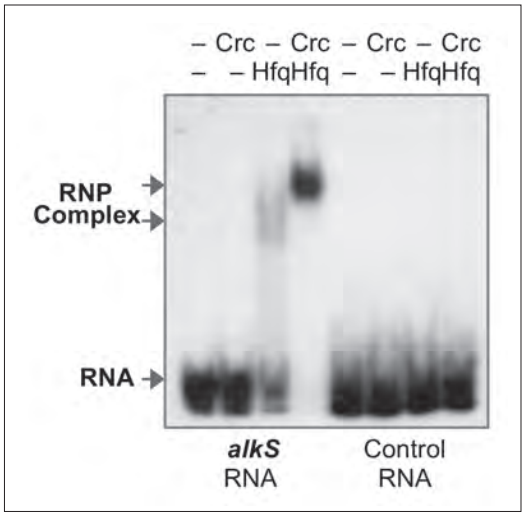

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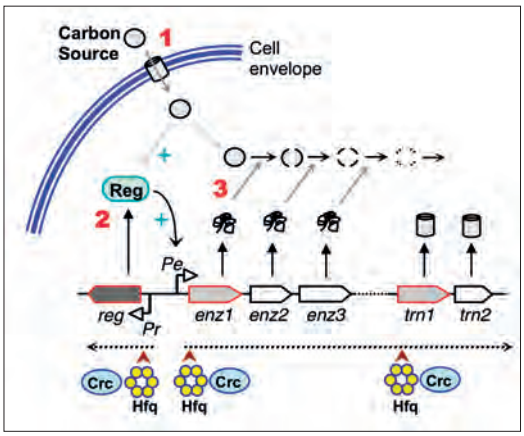

1 *Effect of Crc on Hfq protein binding to RNA oligonucleotides bearing (alkS RNA) or lacking (control RNA), a "catabolite activity" motif.*

2 *Crc and Hfq proteins can modulate the activity of catabolic pathways for carbon sources by controlling the expression of genes involved in uptake (trn) and metabolism (enz1, enz2, etc.) of the carbon source or in the regulation (reg) of catabolic genes.*

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Genetic control of the cell cycle

PRINCIPAL INVESTIGATOR:
Miguel Vicente

POSTDOCTORAL SCIENTISTS:
Ana Isabel Rico
Paolo Natale
Susanne Gola
Alicia Sánchez Gorostiaga

PREDOCTORAL SCIENTISTS:
Manuel Pazos
Marcin Krupka
Cristina Ortiz
Laura Cueto

TECHNICIANS:
Pilar Palacios
Mercedes Casanova

PROJECT MANAGER:
Moira Torrent

VISITING SCIENTIST:
Daniela Musu
(Università degli Studi di Cagliari, Italy)

Our research aims to partially reproduce in the test tube the assembly and functions of the divisome components, a dedicated set of proteins that gather at midcell and divide a bacteria into two equal daughters. Division is accurately regulated in the cell, both in time and space, ensuring that no division occurs unless all the components of the mother have been duplicated and distributed equally to each daughter. A good number of the divisome elements and a few of their functions are known, and their assembly sequence and location in the cell have been determined. This knowledge facilitates a synthetic biology approach in which a discrete number of different divisome components can be assembled and tested in artificial containers.

We use two types of containers, vesicles and maxicells. Vesicles are cell-free systems in which the desired divisome component or a mixture of them can be encapsulated and their function tested under controlled biochemical conditions. FtsZ, a tubulin analogue with a crucial role in divisome function, can be placed inside vesicles. In permeable vesicles, FtsZ can be induced to form polymers by addition of GTP, the nucleotide that supplies energy to this process in bacteria. We anchored the FtsZ protein to the inner face of the vesicles using ZipA, one of the proteins that naturally tethers it to the cytoplasmic membrane of *Escherichia coli*. Addition of GTP results in the shrinking of these vesicles.

Maxicells are *E. coli* cells in which the nucleoid is degraded by controlled irradiation with ultraviolet light. They contain no chromosome but can express those genes that have been previously introduced in plasmids that, due to their small size, escape irradiation damage. In normal cells, the nucleoid associated with the protein SlmA prevents assembly of a divisome at midcell by occlusion. Besides the nucleoid, maxicells also lack SlmA and thus do not have this occlusion mechanism. They nonetheless retain an active alternative system, a set of three Min proteins that oscillate from pole to pole, preventing FtsZ polymerisation at sites other than the centre.

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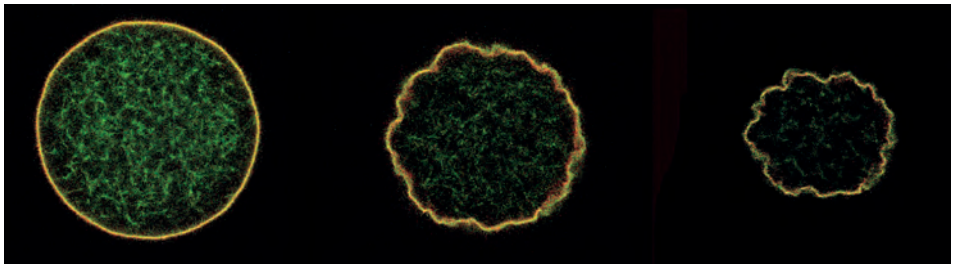

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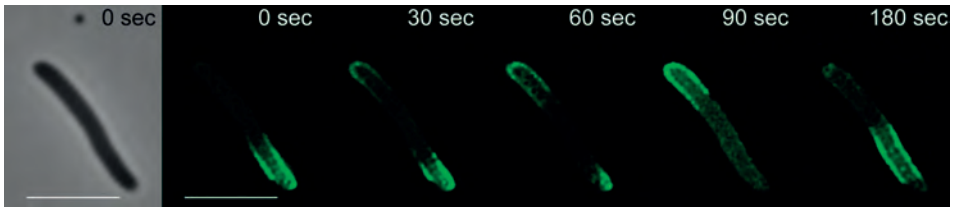

1 *Shrinkage of a vesicle with ZipA inserted into its membrane and FtsZ encased in the lumen. ZipA was labelled with a red fluorescent dye, whereas FtsZ was labelled in green. Yellow fluorescence is observed where the two proteins coincide. To initiate the experiment, GMP-CPP, a slowly hydrolysable GTP analogue, is added (left). This leads to a slow polymerisation of FtsZ, followed by progressive shrinkage of the vesicle (centre and right). Reproduced from Cabré et al. J Biol Chem 2013.*

2 *The MinD protein explores the bacterial length. A green fluorescent variant of MinD oscillates from pole to pole. MinD guides MinC, a protein able to block cell division. As both proteins travel from one pole to the other, the only site at which they are absent for a longer period is the centre. This mechanism ensures that the bacterium divides only through the middle. The image at the left shows the full image of the bacteria as seen by phase contrast microscopy. Bar = 5 μm. Modified from Pazos et al., 2014; PLoS One 9:e91984.*

6 / Systems Biology

Although molecular biology is considered to have been founded by physicists, this circumstance did not result in a quantitative culture and an accurate, standardised descriptive language characteristic of the hard sciences. On the contrary, with very few exceptions, the biosciences that developed since that time seldom took the opportunity to formalise the mechanisms and functions of living systems with accurate languages and codes, with a nearly complete disregard for metrology (the theory and practice of quantitative measurement). Systems Biology occupies this niche by analysing biological entities as comprehensible physicochemical objects with a functioning logic that can be modelled, understood and reshaped. By the same token, synthetic biology is not just a contemporary update of the recombinant DNA technologies of the past 30 years, along with a descriptive language imported from electrical and industrial engineering. It is also a new interpretative key for living systems as well as a declaration of intent on the use and reprogramming of biological objects for human benefit. In the same way that scientific chemistry as initiated by Lavoisier evolved into the chemical engineering that is the basis of our industrial society, biology has acquired a transforming potential that could lead to a type of industry and economy very different from the current paradigm. The CNB SysBio Program figures in the contemporary landscape by developing research lines in environmental genomics, network biology, systemic computation and metabolic engineering.

Molecular environmental microbiology laboratory

PRINCIPAL INVESTIGATOR:
Víctor de Lorenzo

POSTDOCTORAL SCIENTISTS:
Ozlem Akkaya
Yamal Al-Ramahí
Ilaria Benedetti
Belén Calles
David Fernández
Angel Goñi
Juhyun Kim
Esteban Martínez
Pablo I. Nikel

PREDOCTORAL SCIENTISTS:
Mª de los Angeles Hueso
Alberto Sánchez- Pascuala

TECHNICIANS:
Sofia Fraile
Tomás Aparicio

ADMINISTRATIVE ASSISTANT:
Inés Merino

VISITING SCIENTIST:
Ana Zuñiga
(Univ. Adolfo Ibáñez, Chile)

The longstanding aim of our team is to produce biological agents for biosensing, remediation and (where possible) transformation of urban and industrial chemical waste that is otherwise dumped into the environment. To this end, we explore and capitalise on the interface between environmental microbiology and synthetic biology. Our workhorse is the soil bacterium *Pseudomonas putida*, which combines the ease of genetic programming typical of *Escherichia coli* with the safety, robustness and metabolic abilities required of whole-cell catalysts for applications in harsh biotechnological settings. Specific activities include [i] development of *P. putida* as a reliable chassis for implantation of genetic and metabolic circuits. This involves editing the extant genome of this microorganism to enhance desirable properties and reduce drawbacks. We also use surface-display systems to design complex catalytic properties completely separate from the cell metabolism, as well as artificial communities by expression of ectopic adhesins. [ii] Use of camel antibodies as tools for metabolic and regulatory engineering of *P. putida*. Various expression systems allow the targeting of camelid VHH fragments to the intracellular compartment, the periplasm or the external medium of the cells. This allows selective perturbations of chosen metabolic routes to enhance desired metabolic and regulatory qualities of cells. [iii] Genetic tools for deep refactoring of *P. putida* metabolic properties. The new assets we are developing include a large collection of standardised plasmid and transposon vectors, as well as dedicated reporter systems for parameterisation of the gene expression flow and for switching entire metabolic regimes. [iv] The TOL system borne by plasmid pWW0 as a reference for metabolic circuit implantation. The two operons for toluene and *m*-xylene biodegradation encoded in pWW0 offer a natural case of expansion of the metabolic repertoire of environmental bacteria through acquisition of new genes. [v] Deep metabolic engineering of *P. putida*. Current efforts attempt to develop strains programmed to adopt an entirely anoxic metabolism and/or a genuine glycolytic mode of glucose use. This involves the re-engineering of dozens of genes and metabolic modules.

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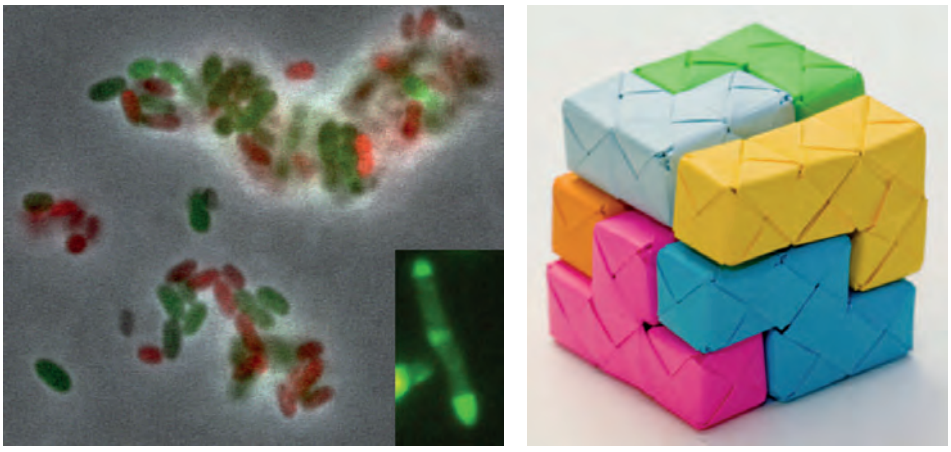

1 *Environmental bacteria offer an outstanding opportunity in synthetic biology for engineering new-to-nature properties in specific members of the community; this includes the possibility of designing consortia with predetermined catalytic activities (which we term "catalytic origami"). The genome of selected strains of P. putida must be edited to generate a smooth cell surface, on top of which artificial adhesins can be programmed genetically. Communication between cells of such consortia can also be engineered by repurposing pathway components for the degradation of recalcitrant compounds.*

Evolutionary systems

PRINCIPAL INVESTIGATOR:
Susanna Manrubia

POSTDOCTORAL SCIENTISTS:
Jacobo Aguirre
José A. Capitán

Our main interest is theoretical investigation of evolutionary systems of different kinds. We develop models inspired by the phenomena observed in natural systems, chiefly populations of RNA sequences and RNA viruses. Our approach addresses the study of general properties of evolving and adapting systems or, at a more specific level, tries to reproduce and predict the response of such populations to environmental changes. We study the properties of the genotype-phenotype map through models such as the folded state of RNA sequences, focusing on the topological structure of neutral networks of genotypes and its relevance in adaptation and molecular innovation. To understand the limitations of simple genotype-phenotype maps, we recently introduced toyLIFE, a multilevel computational model that proceeds simply but realistically from gene sequence to emerging metabolic network. In a broader scenario, we are also interested in modelling the interaction between agents organised in networks that vie for resources such as food or mates, as competitive interactions that represent one of the driving forces behind evolution and natural selection in biological systems. Finally, we explore the application of complex systems to biotechnology through development of analysis techniques with environmental and health purposes. We apply graph theory to antibody microarrays to improve the characterisation of experimental samples, with direct application to allergy control, toxin detection in fresh water ecosystems and planetary sciences. Our studies of viral response to antiviral treatments have determined optimal modes of drug administration to minimise viral load and mutant escape.

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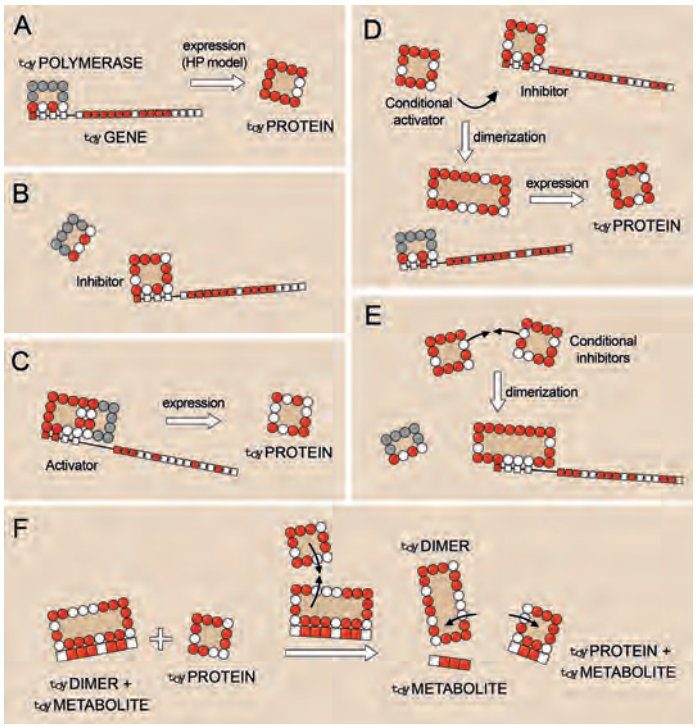
Arias CF, Catalán P, Manrubia S, Cuesta JA. toyLIFE: a computational framework to study the multi-level organisation of the genotype-phenotype map. Sci Rep 2014; 4:7549



1 *ToyLIFE is a simple molecular universe in which genes formed by chains of toyNucleotides are translated into toyProteins. The latter are folded in two dimensions following simple rules of interaction between hydrophobic and hydrophilic toyAminoacids. The same interaction rules are applied between any pair of elements in toyLIFE leading to the emergence of new molecular properties, Boolean networks of interactions between genes, or toyMetabolite catabolism. More information in Arias et al. (Sci Rep 2014; 4:7549).*

Computational systems biology

PRINCIPAL INVESTIGATOR:
Florencio Pazos Cabaleiro

POSTDOCTORAL SCIENTIST:
Mónica Chagoyen Quiles

PREDOCTORAL SCIENTISTS:
Daniel López López
David Ochoa García
Javier López-Ibañez Infante
Guillermo García Martín
Sara Fernández Novo

VISITING SCIENTIST:
David San León Granado
(CSIC, Spain)

In the last two years, we continued work on various aspects of our three main research lines. For prediction of protein-protein interactions, we improved our MIRRORTREE system for co-evolution-based prediction of protein interaction partners in a number of ways. These include the introduction of an estimator of the statistical significance of an observed co-evolutionary score and incorporation of predicted solvent accessibility. For the functional study of biological networks, we continued to improve our pioneering MBROLE system for the functional analysis of metabolomic data, with an exhaustive study of the role of intrinsic protein disorder in shaping molecular networks of *Arabidopsis thaliana*. For the prediction of protein functional sites, we developed a new system for the concomitant prediction of fold, molecular function, and functional sites for structural domains, which is publicly available through the COPRED web server (http://csbg.cnb.csic.es/copred/). In addition, we continued to collaborate with experimental groups, helping with their bioinformatics needs, applying the above methodologies and tools.

Detailed information can be found at: http://csbg.cnb.csic.es/

SELECTED PUBLICATIONS

Espaillat A, Carrasco-Lopez C, Bernardo-Garcia N, Pietrosemoli N, Otero LH, Alvarez L, de Pedro MA, Pazos F, Davis BM, Waldor MKI, Hermoso JA, Cava F. Structural basis for the broad specificity of a new family of amino-acid racemases. Acta Crystallogr D 2014; D70: 79-90

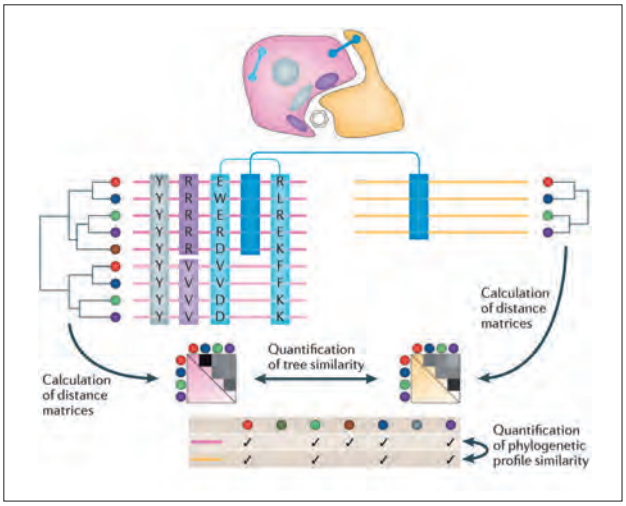
Lopez D, Pazos F. COPRED: prediction of fold, GO molecular function and functional residues at the domain level. Bioinformatics 2013; 29: 1811-1812

Juan D, Pazos F, Valencia A. Emerging methods in protein co-evolution. Nat Rev Genet 2013; 14: 249-261

Pietrosemoli N, García-Martín JA, Solano R, Pazos F. Genome-Wide Analysis of Protein Disorder in *Arabidopsis thaliana*: Implications for Plant Environmental Adaptation. PLoS One 2013; 8: e55524.

Chagoyen M, Pazos F. Tools for the functional interpretation of metabolomic experiments. Brief Bioinform 2013; 14: 737-744

1



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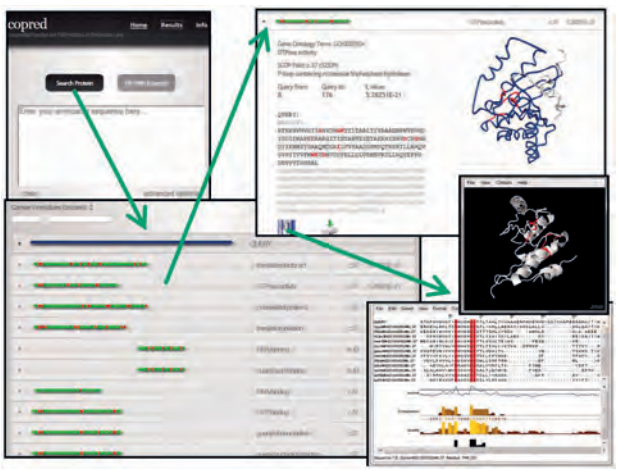
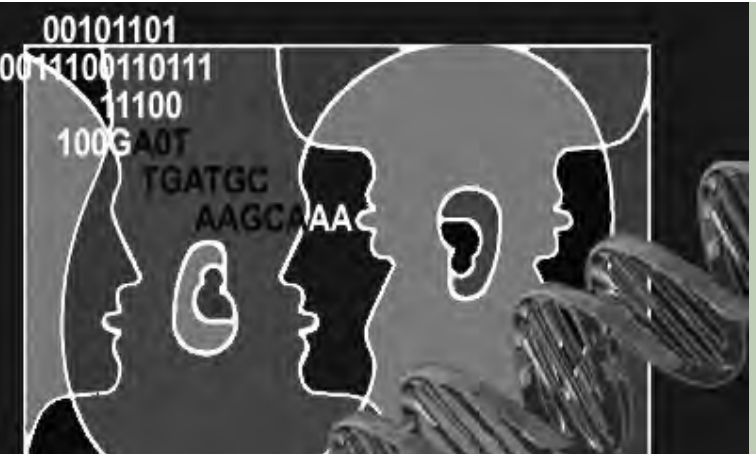


1 *Co-evolutionary information extracted from protein multiple sequence alignments indicates protein features related to structure, function and interactions.*

2 *User interface of the COPRED system for the concomitant prediction of protein function and functional sites.*



Logic of genomic systems

PRINCIPAL INVESTIGATOR:
Juan F. Poyatos

PREDOCTORAL SCIENTISTS:
Clara Moreno
Djordje Bajic

VISITING SCIENTIST:
Guillermo Rodrigo
(Universitat Politècnica de València, Spain)

SELECTED PUBLICATIONS

Rodrigo G, Bajic D, Elola I, Poyatos JF. Antagonistic autogenous control of gene expression enhances the dynamic response of a resistance phenotype. bioRxiv 2014; doi: 10.1101/008169

Bajic D, Moreno C, Poyatos JF. Rewiring of genetic networks in response to modification of genetic background. Genome Biol Evol 2014; 6:3267

Cavaliere M, Poyatos JF. Plasticity facilitates sustainable growth in the commons. J Royal Soc Interface 2013; 10:20121006

Two main problems were the centre of our research over the last two years. The first refers broadly to the limits of our present representation of biological systems as circuits and networks. The second problem deals with how the behaviour of individual cells determines the collective dynamics of cell populations.

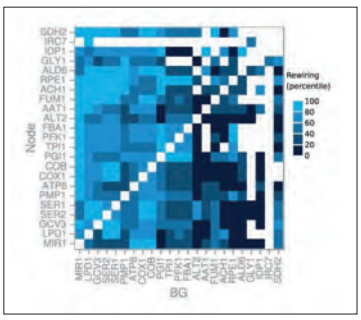
The representation of biological complexity

We first analysed the stability of genetic networks. These networks help to map the molecular circuitry within cells by establishing genetic interactions between proteins (two proteins are "genetically" connected when the phenotype of the double mutant differs from that predicted for the combination of single mutations alone). The architecture of genetic networks can be modified with variation of genetic context (other mutations), which we studied by *in silico* modelling and by analysis of high-throughput data. Stability appeared to be closely connected to the mechanistic causes of robustness in the system under study. The second question refers to the assembly of transcriptional networks from its basic building blocks, or network motifs. We examined how the combination of functionally conflicting motifs could lead to trade-offs, and how such trade-offs can be resolved. We combined quantitative experiments and mathematical modelling in a bacterial model system with antagonistic autogenous control, the multiple antibiotic resistance regulatory module in *Escherichia coli*.

Individual behaviour and collective dynamics of cells

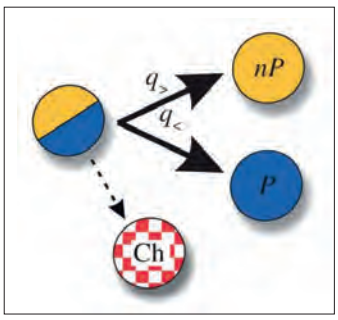
Cells commonly cooperate among themselves by the secretion of molecules used by other cells ("public goods"). These interactions help establish cellular communities that are naturally unstable due to the rise of mutant individuals. These individuals avoid the costs associated with production of public goods, which can collapse the community. We analysed the persistence of these systems by examining a situation in which production of public goods by individual cells followed different strategies –constitutive, stochastic or plastic. We developed a mathematical model to quantify how each strategy would function in the presence of the non-contributing mutants, in a model that also controlled for a number of structural parameters. This analysis currently serves for the design and construction of robust bacterial communities in the lab.

1



1 *Rewiring of hubs in response to hub deletions. The only hub with a purely biosynthetic function (IRC7) deviates most from this general pattern.*

2



2 *Individual cells as plastic producers. A plastic producer can contribute to the public good (producer state, P) or not (non-producer state, nP). Cheaters (Ch), that is, agents that are permanently in the nP state, arise from plastic producers by mutation.*

Microbial community modelling

PRINCIPAL INVESTIGATOR:
Javier Tamames de la Huerta

SENIOR SCIENTIST:
Carlos Pedrós-Alió

TECHNICIAN:
Pablo David Sánchez

VISITING SCIENTISTS:
Ana Suárez-Suárez
(Newcastle University, UK)
Cristina García
(Universidad de Barcelona, Spain)
Elena Toril
(Centro de Astrobiología, INTA-CSIC, Spain)
Laura Sala
(Universidad de Barcelona, Spain)
Tomeu Viver
(IMEDEA-CSIC, Spain)

In these two years, our group has established a solid methodology for the analysis of genomes, metagenomes and metatranscriptomes that has been applied successfully in several instances. We are particularly interested in ecological scenarios such as the detoxification of polluted environments (Guazzaroni *et al.,* 2013), function of hypersaline environments (López-López *et al.,* 2013), and responses to temperature gradients in thermophilic microbial mats (in collaboration with the Pontificia Universidad Católica de Chile, 2014). We also collaborate with several laboratories on the clinical importance of genomes and microbiomes, such as the group of Dr F. Baquero (Hospital Ramón y Cajal, Madrid) in cystic fibrosis, and that of Dr I. Tomás (Universidad de Santiago de Compostela, Spain) in the role of oral microbiomes in periodontal health.

We also study the rules that govern assembly of natural bacterial communities, by defining the relationships between environmental factors and species presence or co-ocurrence. This led us to define patterns of functional redundancy in metagenomes, that is, in which cases particular species can be replaced by counterparts with no influence on the overall performance of the microbiome. We also developed predictive systems to determine environmental preferences of individual species based on their genomic compositions. We are now working to extend these tools to predict possible compatibilities and co-ocurrences between species in given settings. In the future, we expect to be able to accurately predict the effect of specific perturbations in the performance of the microbiome, and therefore to propose optimal combinations of species for living in defined conditions or fulfilling given roles.

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 2013; 7:122-136

López-López A, Richter M, Peña A, Tamames J, Rosselló-Móra R. New insights into the archaeal diversity of a hypersaline microbial mat obtained by a metagenomic approach. Syst Appl Microbiol 2013; 36:205-214

Tamames J, Durante-Rodríguez G. Taxonomy becoming a driving force in genome sequencing projects. Syst Appl Microbiol 2013; 36:215-217

Jiménez G, Blanch AR, Tamames J, Rosselló-Mora R. Complete Genome Sequence of *Bacillus toyonensis* BCT-7112T, the Active Ingredient of the Feed Additive Preparation Toyocerin. Genome Announc 2013; 1:1

Pascual-García A, Tamames J, Bastolla U. Bacteria dialog with Santa Rosalia: Are aggregations of cosmopolitan bacteria mainly explained by habitat filtering or by ecological interactions? BMC Microbiol 2014; 14:284

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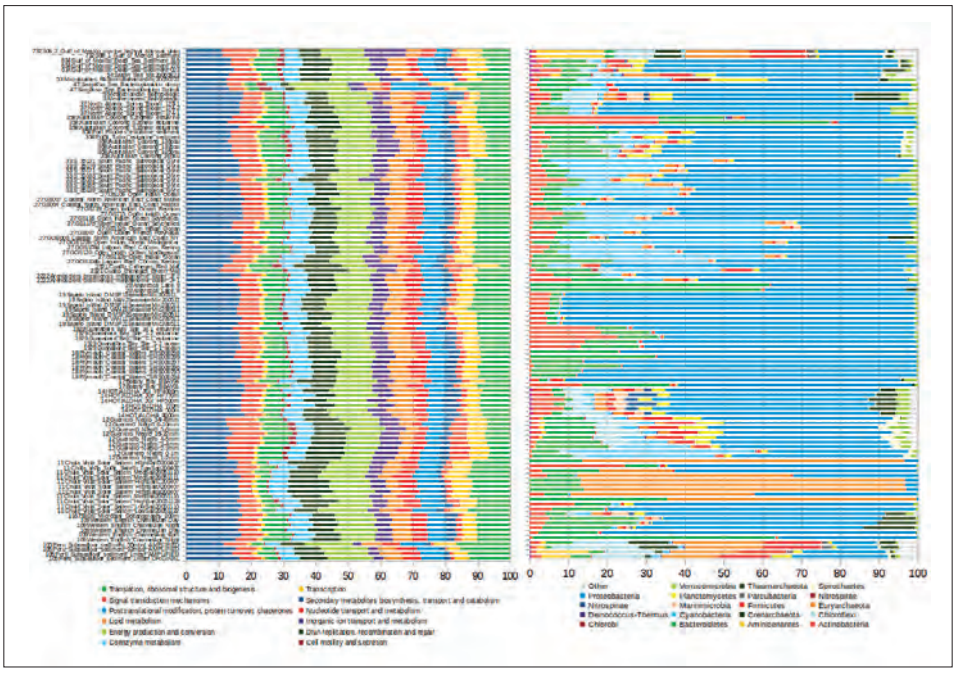


1 *Comparison of 100 marine metagenomes showing how functional uniformity (left) corresponds to rather different taxonomic compositions (right). This indicates a high degree of functional redundancy, in which community members can be replaced with no perturbation of the functional profile of the microbiome.*

7 / 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 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.

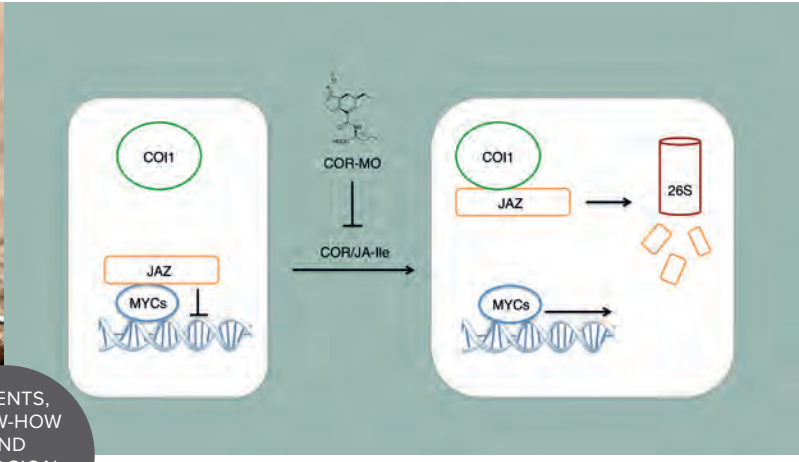
The CNB is one of the CSIC research institutes with its own Technology Transfer Department, which works in close collaboration with the CSIC Deputy Vice-presidency for Knowledge Transfer.

The Technology Transfer Department promotes the use of our research results for society's benefit, to potentiate the biotechnology sector as well as basic and clinical research.

TECHNOLOGY TRANSFER MANAGER
Ana Sanz Herrero

Contact Information:

asanz@cnb.csic.es
Tel. [+ 34] 91 585 4306



PATENTS, KNOW-HOW AND BIOLOGICAL MATERIALS LICENSED

DDA1 gene for mitigating negative ABA effects on growth during abiotic stress

Improved germination, seedling establishment, root growth and enhanced crop growth under stress conditions

Dr Vicente Rubio's research group found that PYL8, a receptor for the water-stress hormone ABA, is specifically targeted for ubiquitin-mediated degradation by DDA1 protein, which previously had no known function. The inventors showed that DDA1 binds to PYL8 *in vivo* and mediates its degradation through the proteasome, and that DDA1 overexpression in transgenic plants reduces plant sensitivity to ABA. The commonly observed ABA repression of seed germination, seedling establishment and root growth is much reduced in DDA1-overexpressing plants. These plants are also less sensitive to NaCl- or mannitol-mediated inhibition of seed germination compared to wild type. DDA1 is highly conserved in vascular plants, including all important crop species.

RESEARCH GROUP:
Dr Vicente Rubio

APPLICATION NUMBER AND PRIORITY DATE:
European patent application EP13382197.5, 29/05/2013

INTERNATIONAL PCT APPLICATION:
PCT/EP2014/061214, 29/05/2014 (published as WO/2014/191539)

PRESENT SITUATION:
Exclusive license agreement to Plant Bioscience Limited (PBL) (11/01/2013) but sublicensing rights are available from PBL

REFERENCE:
Irigoyen *et al.* Targeted degradation of abscisic acid receptor PYL8 is mediated by ubiquitin ligase substrate adaptor DDA1 in Arabidopsis. Plant Cell 2014; 26:712-28

PATENTS LICENSED IN 2013

Plants that overexpress DDA1 gene are more resistant to abscisic acid-mediated abiotic stress such as drought.

Control of plant pathogens using novel coronatine derivatives

Modifications in coronatine confer potency and specificity for protecting plants against bacterial pathogens and enhance plant defences

Dr Roberto Solano's research group, with the company Lipidox, has designed, synthesised and characterised a novel, potent, highly specific antagonist of jasmonic acid perception. These coronatine derivative compounds competitively inhibit binding of JA-Ile to its co-receptor and thereby provide a tool to potentiate crop defences against biotrophic and hemi-biotrophic pathogens.

RESEARCH GROUP:
Dr Roberto Solano

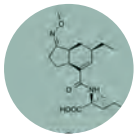
APPLICATION NUMBER AND PRIORITY DATE:
European patent application EP13382362.5, 18/09/2013

INTERNATIONAL PCT APPLICATION:
PCT/EP2014/069796, 17/09/2014. The International Patent Application has been published as WO2015/040061

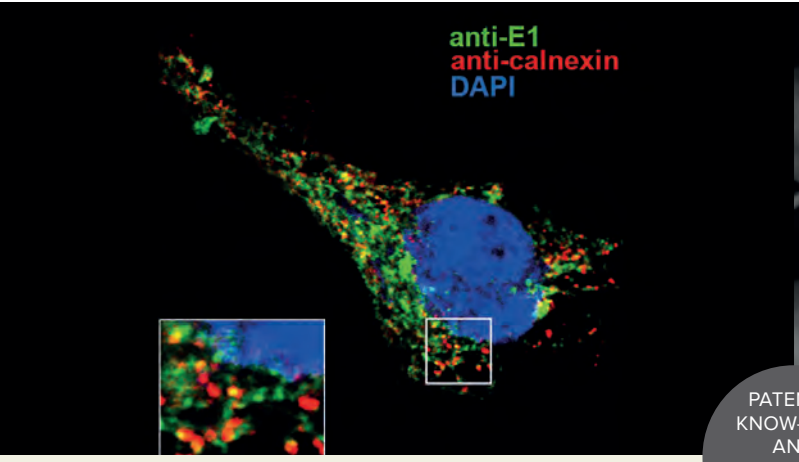
PRESENT SITUATION:
Exclusive license agreement to Plant Bioscience Limited (PBL) (31/05/2013) but sublicensing rights are available from PBL

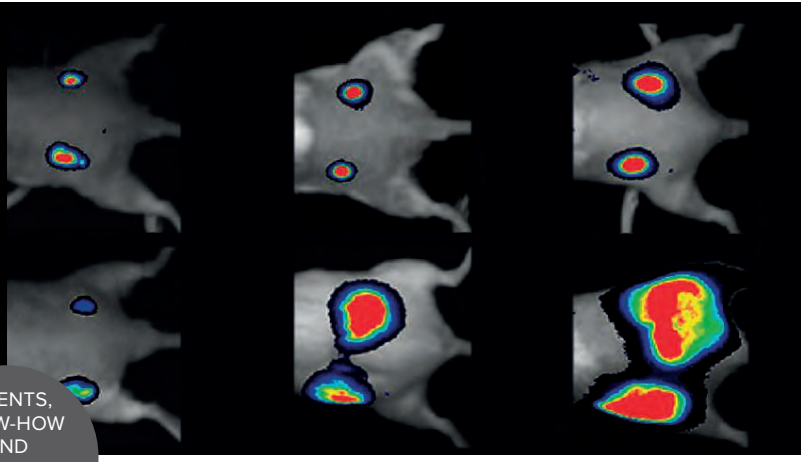
REFERENCE:
Monte *et al.* Rational design of a ligand-based antagonist of jasmonate perception. Nat Chem Biol 2014; 10:671-6

PATENTS LICENSED IN 2013



Coronatine derivative (COR-MO) efficiently antagonises JA-Ile perception by the COI1-JAZ co-receptor interaction and blocks jasmonate signaling. (+)-7-iso-jasmonoyl-L-isoleucine (JA-Ile) is a p1lant hormone involved in plant development and stress response that signals through a COI1–JAZ co-receptor complex.





PATENTS, KNOW-HOW AND BIOLOGICAL MATERIALS LICENSED

Hepatitis C virus (HCV) vaccine candidate based on recombinant modified Ankara virus (MVA) expressing the near full-length HCV genome

Dr Mariano Esteban's research group has developed a vaccine prototype for hepatitis C virus (HCV) based on an attenuated vaccinia vector (MVA) that expresses all HCV proteins except the C-terminal domain of NS5B protein. The main advantage of this MVA-HCV vector is that in a single product it is possible to amplify the *in vivo* immune response to all HCV antigens. In two murine models (BALB/c and humanised HLA-A2), vaccination with MVA-HCV vector mainly induces activation of $CD8^+$ T lymphocytes, although $CD4^+$ T cells are also produced. The antigenic response is produced to all HCV proteins, but unlike other viral vectors, MVA-HCV vaccination preferentially activates p7 and NS2 antigen responses. Moreover, MVA-HCV induces a broad response to HCV antigens that is polyfunctional (activates various cytokines) and durable.

RESEARCH GROUP:
Dr Mariano Esteban

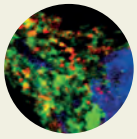
APPLICATION NUMBER AND PRIORITY DATE:
P201330467, 02/04/2013

INTERNATIONAL PCT APPLICATION:
PCT/ES2014/070246, 31/03/2014. 09/10/2014 published as WO 2014

PRESENT SITUATION:
Exclusive license agreement to Plant Bioscience Limited (PBL) (6/05/2014) but sublicensing rights are available from PBL

REFERENCE:
Gómez *et al.* High, broad, polyfunctional, and durable T cell immune responses induced in mice by a novel hepatitis C virus (HCV) vaccine candidate (MVA-HCV) based on modified vaccinia virus Ankara expressing the nearly full-length HCV genome. J Virol 2013; 87:7282-300

PATENTS LICENSED IN 2014



Expression of hepatitis C (HCV) protein E1 by the vaccine candidate MVA-HCV in human HeLa cells as revealed by confocal microscopy. E1 protein (green), endoplasmic reticulum (red) and DNA (blue).

Human CCR9 monoclonal antibodies for diagnosis and therapy of cancer and other diseases

Dr Leonor Kremer's research group, in collaboration with scientists from the Centro de Investigaciones Biológicas (CIB-CSIC), developed monoclonal antibodies that bind specifically to the human CCR9 chemokine receptor. These antibodies inhibit CCR9+ tumour growth in an *in vivo* mouse xenograft model. The results show the potential of the 91R monoclonal antibody as a therapeutic agent for treatment of CCR9-expressing tumours.

RESEARCH GROUP:
Dr Leonor Kremer

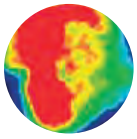
APPLICATION NUMBER AND PRIORITY DATE:
EP13382469.8, 25/11/2013

INTERNATIONAL PCT APPLICATION:
PCT/EP2014/075578, 25/11/ 2014

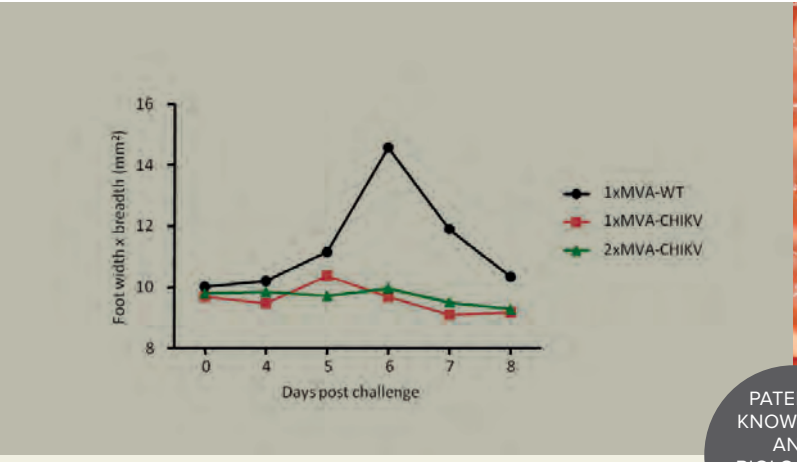
PRESENT SITUATION:
Exclusive license agreement to SunRock Biopharma SL, 30/09/2014

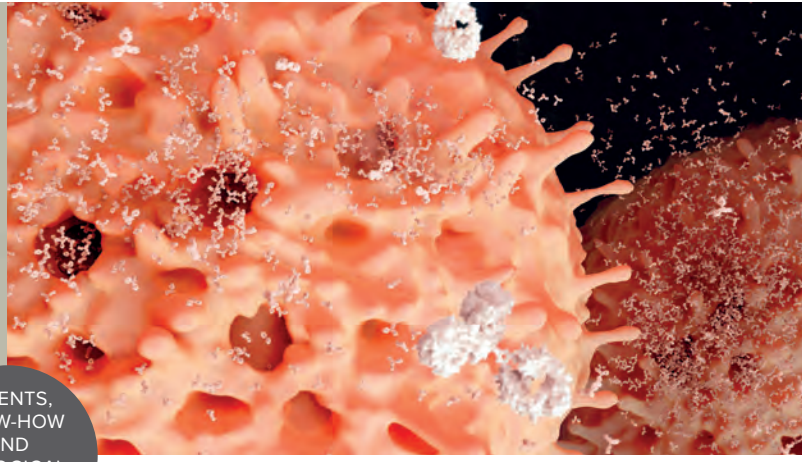
REFERENCE:
Chamorro *et al.* Antitumor effects of a monoclonal antibody to human CCR9 in leukemia cell xenografts. mAb. 2014; 6:1000-12

PATENTS LICENSED IN 2014



Bioluminescence image showing growth of tumours derived from human T cell acute lymphoblastic leukaemia cells implanted in immunodeficient mice, in response to treatment with 2an anti-human CCR9 mAb (top) or a control mAb (bottom)





PATENTS, KNOW-HOW AND BIOLOGICAL MATERIALS LICENSED

Chikungunya vaccine candidate based on recombinant modified Ankara virus (MVA)

Dr Mariano Esteban's research group, in collaboration with Dr Peter Liljeström from the Karolinska Institute, has developed a vaccine candidate to chikungunya virus (CHIKV), an emerging pandemic that affects approximately 5 million people, based on an attenuated poxvirus vector expressing the CHIKV structural genes. Immunisation with MVA-CHIKV induces strong, broad, polyfunctional adaptive CHIKV-specific T cell immune responses in mice. Immunisation with MVA-CHIKV induces neutralising antibodies to CHIKV. MVA-CHIKV is highly immunogenic and effective, as a single dose protects mice against chikungunya infection. MVA-CHIKV triggers an innate immune response in human macrophages and dendritic cells, inducing type I IFN, proinflammatory cytokines, and chemokine expression.

RESEARCH GROUP:
Dr Mariano Esteban

APPLICATION NUMBER AND PRIORITY DATE:
PCT/EP2014/076310, 02/12/2014

PRESENT SITUATION:
Exclusive license agreement to Plant Bioscience Limited (PBL) (14/07/2014) but sublicensing rights are available from PBL

REFERENCE:
García-Arriaza *et al.* A novel poxvirus-based vaccine, MVA-CHIKV, is highly immunogenic and protects mice against chikungunya infection. J Virol 2014; 88:3527-47

PATENTS LICENSED IN 2014

Immunisation with MVA-CHIKV protects mice against CHIKV infection. Foot swelling in animals immunised with one dose of MVA-WT or one or two doses of MVA-CHIKV and challenged with a total of 10^6 PFU of CHIKV subcutaneously in both feet.

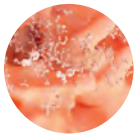
Cell lines producing monoclonal antibodies against the CCR2 human receptor

RESEARCH GROUP:
Dr José Miguel Rodriguez Frade and Mario Mellado

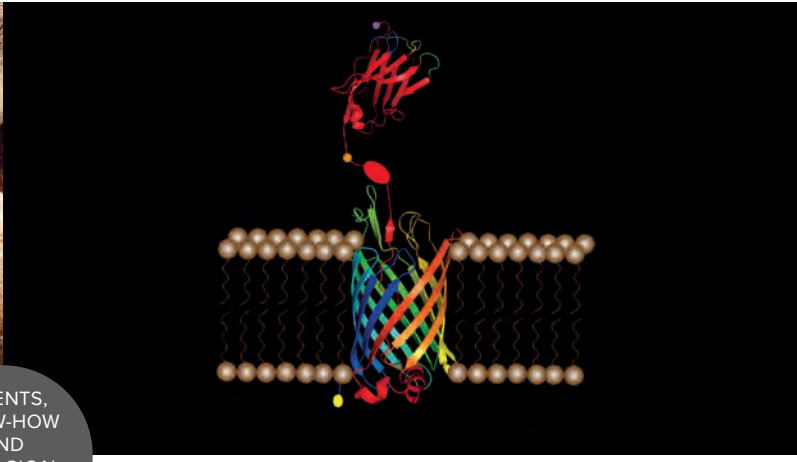
PRESENT SITUATION:
Exclusive license agreement to a company (06/05/2013)

REFERENCE:
Frade *et al.* Characterisation of the CCR2 chemokine receptor: functional CCR2 receptor expression in B cells. J Immunol 1997; 159:5576-5584

BIOLOGICAL MATERIALS LICENSED IN 2013



Monoclonal antibodies against CCR2



PATENTS, KNOW-HOW AND BIOLOGICAL MATERIALS LICENSED

R5 monoclonal antibody to gliadin for gluten determination through a flow-through kit

RESEARCH GROUP:
Dr Enrique Méndez

PRESENT SITUATION:
Exclusive license agreement to two companies (Inmunología y Genética Aplicada SA and Institute for Environmental Health, Inc.)

REFERENCE:
Valdés I, García E, Llorente M, Méndez E. Simple sandwich ELISA based on the use of a single monoclonal antibody (RE) as the coating and means of detection. A quantitative cocktail gluten-extraction procedure for heat-processed foods was also tested. Eur J Gastroenterol Hepatol 2003; 5:465-74

KNOW-HOW AND BIOLOGICAL MATERIALS LICENSED IN 2014

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

Know-how and biological materials related to methods for recombinant antibody expression, selection and purification

Dr Luis Ángel Fernández's research group has know-how and biological material related to the expression of recombinant antibodies on the *Escherichia coli* cell surface (bacterial display), know-how related to the selection methods from an antibody library of recombinant antibodies able to bind specifically to an antigen (bacterial display, magnetic cell sorting (MACS), or fluorescent cell sorting (FACS)), as well as know-how and materials for the purification of recombinant antibodies from bacterial cells.

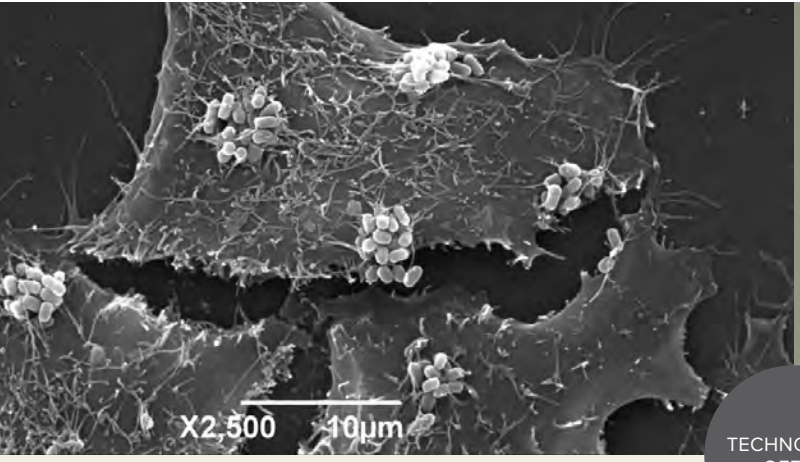
RESEARCH GROUP:
Dr Luis Ángel Fernández Herrero

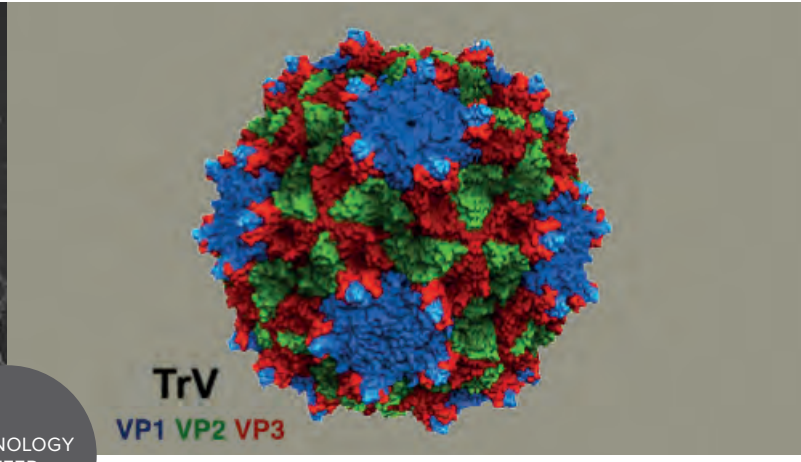
PRESENT SITUATION:
License agreement to Bacmine SL

REFERENCES:
• Salema *et al.* Selection of single domain antibodies from immune libraries displayed on the surface of *E. coli* cells with two beta-domains of opposite topologies. PLoS One 2013; 8:e75126
• Salema & Fernández. High yield purification of nanobodies from the periplasm of *E. coli* as fusions with the maltose-binding protein. Protein Expr Purif 2013; 91:42-8

KNOW-HOW LICENSED IN 2014

Surface display of a nanobody on the outer membrane of E. coli anchored to the intimin beta-barrel.





TECHNOLOGY OFFER

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

Dr Luis Ángel Fernández's research group has 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 function by 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:
Dr Luis Angel Fernández Herrero

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

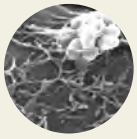
INTERNATIONAL PCT APPLICATION:
PCT/ES08/070045

PRESENT SITUATION:
Spanish patent has been granted (12/03/2007). US Patent has been granted and published as US 8,623,349 B2 (Jan 7, 2014)

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 to transfer the protein-encoding gene by viral infection or transfection

REFERENCE:
Blanco Toribio A. *et al.* Direct injection of functional single-domain antibodies from *E. coli* into human cells. PLoS One. 2010:e15227

PATENTS AVAILABLE FOR LICENSING



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.

Recombinant triatoma virus (TrV): a new platform for the development of chimaeric VLP vaccines and a potential insect biocide

Dr Francisco Rodríguez's research group, in collaboration with the Biophysics Foundation of Bizkaia and University of the Basque Country, has developed virus-like particles (VLP) derived from triatoma virus (TrV). These VLP can be used as adjuvant or epitope carrier for vaccine design. The present invention also relates to a process of obtaining an infectious TrV as a biological agent to control Chagas disease vectors.

RESEARCH GROUP:
Dr José Francisco Rodríguez

APPLICATION NUMBER AND PRIORITY DATE:
EP14382001.7, 03/01/2014

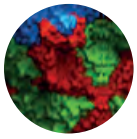
INTERNATIONAL PCT APPLICATION:
PCT/EP2015/050054, 05/01/2015

PRESENT SITUATION:
Companies interested in a patent license are being sought

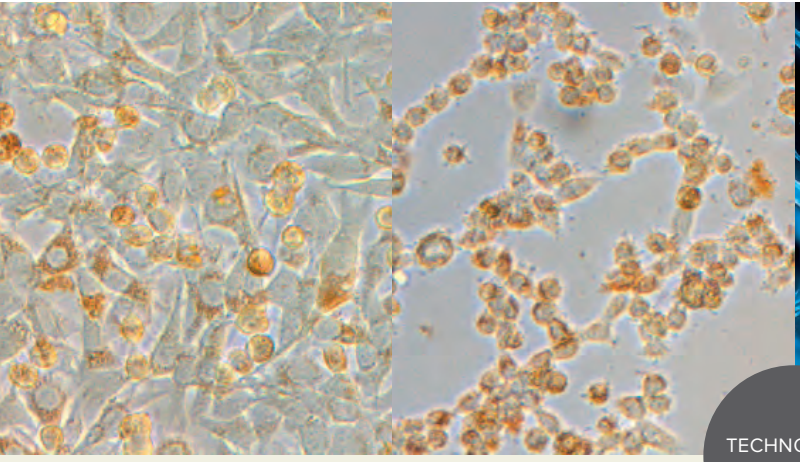
MAIN INNOVATIONS AND ADVANTAGES:
- The TrV-VLP allow epitope exposure on the internal or external surface of the VLP by insertion or substitution of amino acids, facilitating their recognition by the immune system and/or increasing their immunogenicity
- TrV capsids are stable over a very wide acidic pH range and after lyophilisation
- Production of TrV-VLPS bearing different epitopes through expression in insect cells by infection of recombinant baculoviruses
- Recombinant TrV could also be used as biological agent to control Chagas disease vectors

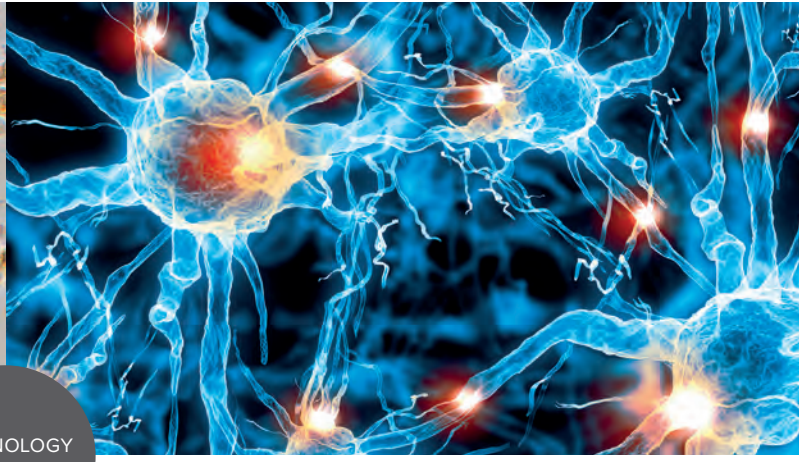
REFERENCE:
Sánchez-Eugenia R *et al. Triatoma virus* structural polyprotein expression, processing and assembly into virus-like particles. J Gen Virol. 2015 Jan;96(Pt 1):64-73.

PATENTS AVAILABLE FOR LICENSING



Crystallographic structure of triatoma virus. The capsid structure of TrV is icosahedral of about 30 nm in diameter and is made of three structural proteins (ranging from 28 to 37 kDa) folded with a standard jelly-roll topology.





TECHNOLOGY OFFER

Device to induce hyperthermia in cells through magnetic nanoparticles

Dr Domingo Barber's research group has developed an instrument that generates a controlled alternating magnetic field to induce hyperthermia in cells through magnetic nanoparticles.

RESEARCH GROUP:
Dr Domingo Barber

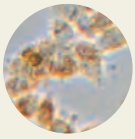
APPLICATION NUMBER AND PRIORITY DATE:
P201431531, 17/10/2014

INTERNATIONAL PCT APPLICATION:
Pending

PRESENT SITUATION:
Companies interested in a patent license are being sought

MAIN INNOVATIONS AND ADVANTAGES:
• A unique "non-contact"-based method for precise measurement of the temperature reached by the nanoparticles inside cells (with precision up to 0.01ºC)
• Frequency and amplitude of the magnetic field can be programmed automatically. It allows changes in parameters such as frequency, amplitude, pulse code modulation, or a combination of these, all digitally controlled in real time
• User-friendly software for data capture, representation and analysis to control magnetic field parameters and nanoparticle temperature
• Equipment is compact and user-friendly, and requires no parts exchange when working in a wide frequency/potency range, making it ideal for hyperthermia research
• Can be used in materials sciences to analyse the heating properties of materials and nanoparticles (SAR curves).

PATENTS AVAILABLE FOR LICENSING



Microscopic images showing Panc02 murine pancreatic adenocarcinoma cells treated by magnetic nanoparticles (left) or by magnetic nanoparticles together with alternating magnetic field(right), generated by the patented device for 60 min., with frequency of 250 kHz (right). Pictures were taken 3 h post-treatment.

Novel compounds to modulate DREAM activity

Dr José Ramón Naranjo's research group, in collaboration with scientists from the Institute for Chemical Medicine (IQM-CSIC), have developed compounds with a nuclear structure derived from phenylacetamide, able to modulate the calcium neuronal sensor DREAM; they can be preventive or therapeutic compounds for treatment of diseases with altered DREAM expression.

RESEARCH GROUP:
Dr José Ramón Naranjo

APPLICATION NUMBER AND PRIORITY DATE:
P201431898, 22 Dec 2014

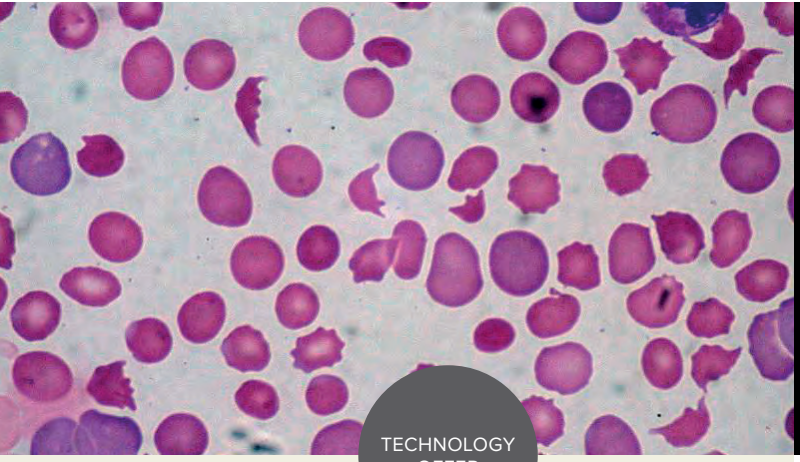
INTERNATIONAL PCT APPLICATION:
Pending

PRESENT SITUATION:
Companies interested in a patent license are being sought

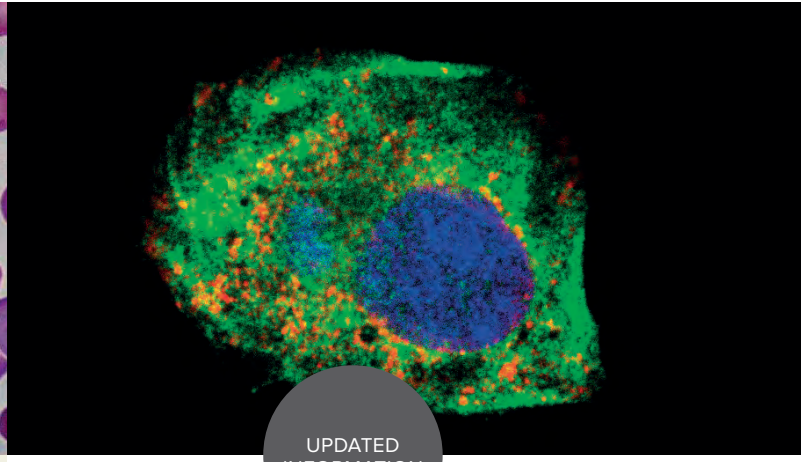
MAIN INNOVATIONS AND ADVANTAGES:
The concept of DREAM as a therapeutic target is novel and the development of various compounds able to bind to and modify DREAM activity could open new avenues for the treatment of DREAM-related neurodegenerative disorders.

PATENTS AVAILABLE FOR LICENSING

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



TECHNOLOGY OFFER



UPDATED INFORMATION

Nanosensor and nanobody to detect fibrinogen in blood

Abnormally high fibrinogen levels in plasma are associated with cardiovascular diseases, whereas abnormally low concentrations are linked to risk of bleeding. Dr Luis Ángel Fernández's research group developed a nanobody (single-domain antibody) to detect fibrinogen specifically in plasma. In collaboration with scientists from the Universidad Complutense of Madrid, they developed a fibrinogen-immunosensing device based on this nanobody and the use of magnetic beads. In this biosensor, free fibrinogen in solution and immobilised fibrinogen compete for binding to a fixed amount of specific biotinylated nanobody. Captured biotinylated nanobody is labelled with streptavidin-horseradish peroxidase. The magnetic beads are then captured by a magnet on the surface of screen-printed carbon electrodes, followed by amperometric detection at -0.20V by measuring the catalytic current generated by H_2O_2 addition, using hydroquinone as a redox mediator.

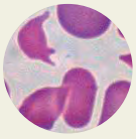
RESEARCH GROUP:
Luis Ángel Fernández Herrero

PRESENT SITUATION:
Companies interested in licensing the nanobody and/or the immunosensor are being sought

MAIN INNOVATIONS AND ADVANTAGES:
• The nanobody is produced in bacteria at lower cost than conventional monoclonal antibodies
• The assay can be performed in diluted plasma samples, with a total analysis time of 90 min and a detection limit of 0.044 mg ml^{-1} fibrinogen. Only 0.01 ml plasma is needed.
• The nanobody could be used to develop other diagnostic materials such as ELISA kits

REFERENCE:
Campuzano *et al.* Disposable amperometric magnetoimmunosensors using nanobodies as biorecognition element. Determination of fibrinogen in plasma. Biosens Bioelectron 2014; 52:255-60

BIOLOGICAL MATERIALS AVAILABLE FOR LICENSING



Detection of human fibrinogen concentration in plasma by an ELISA-based method.

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 clinical trials are complete and Phase II clinical trials are expected to begin in 2016.

RESEARCH GROUP:
Dr Mariano Esteban

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

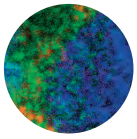
INTERNATIONAL PCT APPLICATION:
PCT/US10/032966

COUNTRIES SELECTED IN NATIONAL PHASE:
EU, US, CA

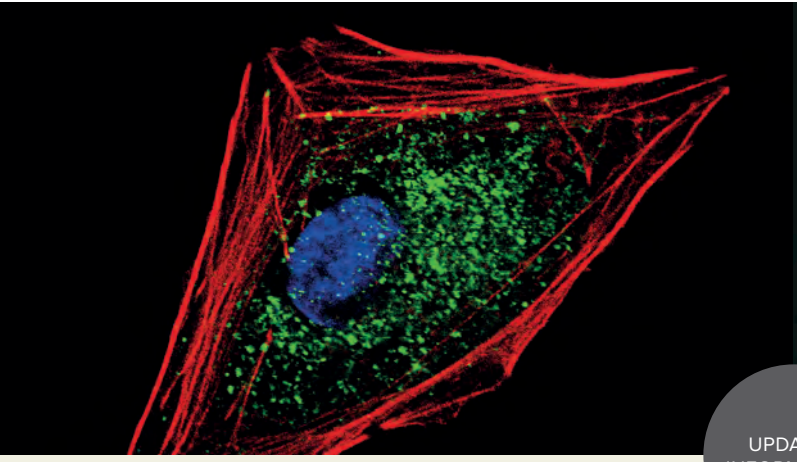
PRESENT SITUATION:
Patent examination in each country. Patent compromised to Sanofi Pasteur Ltd.

REFERENCE:
Perdiguero *et al.* Virological and immunological characterisation of novel NYVAC-based HIV/AIDS vaccine candidates expressing clade C trimeric soluble gp140(ZM96) and Gag(ZM96)-Pol-Nef(CN54) as virus-like particles. J Virol 2015; 89:970-88

PATENTS LICENSED BEFORE 2013



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.



UPDATED INFORMATION

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

The research group lead by Dr Mariano Esteban has developed a prototype vaccine against HIV based on modified Ankara virus (MVA-B) expressing four antigens (Env, Gag, Pol, Nef) of HIV-1, clades B and C. During a phase I clinical trial in 30 healthy individuals, 90% of the volunteers developed an immune response against the virus that was maintained after 1 year. A phase I clinical trial with HIV-infected volunteers showed an increase in Gag-specific T cell responses, but it had no major impact on the latent reservoir or the rebound of plasma viral load after interruption of HIV therapy. MVA-C is in phase I clinical trials by the UK-HVC group.

RESEARCH GROUP:
Mariano Esteban Rodríguez

APPLICATION NUMBER AND PRIORITY DATE:
P200501841, 27/07/2005 and P200600762 (24.03.2006)

INTERNATIONAL PCT APPLICATION: PCT/ES06/070114

COUNTRIES SELECTED IN NATIONAL PHASE: EU, US

PRESENT SITUATION: Exclusive license agreement to Laboratorios del Dr. Esteve S.A. y la Fundación Irsi-CAIXA. (30/06/2012). EU Patent has been granted (EP1921146 B1) and validated in 22 countries. US Patent has been granted (US 8,871,219 B2, 28/10/2014).

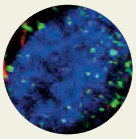
REFERENCE:

• García F, *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; 29:8309-16.

• Gómez CE *et al.* The HIV/AIDS vaccine candidate MVA-B administered as a single immunogen in humans triggers robust, polyfunctional, and selective effector memory T cell responses to HIV-1 antigens. J Virol 2011; 85:11468-78.

• Mothe B *et al.* Safety and immunogenicity of a modified vaccinia Ankara-based HIV-1 vaccine (MVA-B) in HIV-1-infected patients alone or in combination with a drug to reactivate latent HIV-1. J Antimicrob Chemother 2015; 70:1833-42.

PATENTS LICENSED BEFORE 2013



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 a good immunogenicity profile against HIV antigens in phase I clinical trials. In red, phalloidin staining of the cytoskeleton. The cell nucleus appears in blue.

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

The research group led by Dr Pilar Cubas has discovered the BRC1-like genes that control shoot branching in potato and tomato.

RESEARCH GROUP:
Dr 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. China patent granted (No 200880158052)

PRESENT SITUATION:
Exclusive license to NINSAR (18/05/2011). The contract with NINSAR finalised in 2014 and a new licensing agreement was signed with Semillas Fitó SAU and BHN Seed.

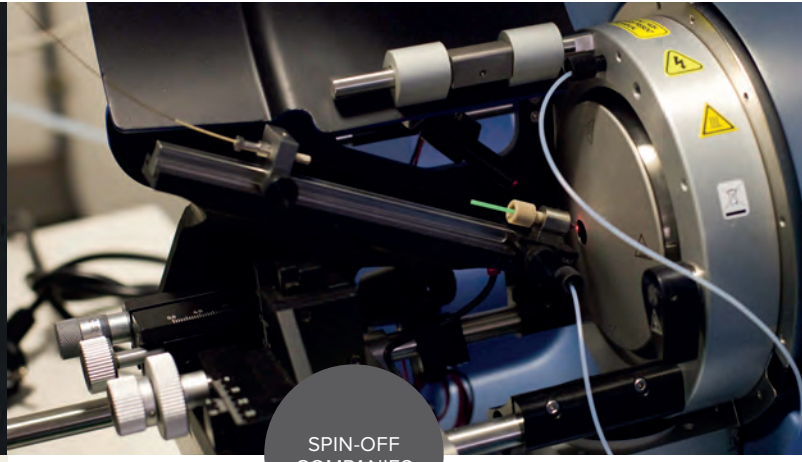
REFERENCE:

Martin-Trillo M *et al.* Role of tomato BRANCHED1-like genes in the control of shoot branching. Plant J. 2011; 67:701-14

PATENTS LICENSED BEFORE 2013

The SlBRC1b gene from Solanum lycopersicum suppresses the formation of basal branches in tomato. Centre: Control plants. Left and right: Tomato plants with the SlBRC1b gene partially inactivated..

UPDATED INFORMATION



SPIN-OFF COMPANIES

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

The research group led by Dr Pilar Cubas discovered that the *Solanum pennellii* BRANCHED1-like gene causes reduced basal shoot branching when introgressed into *S. lycopersicum*.

RESEARCH GROUP:
Dr 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 license to NINSAR (22/02/2012). The contract with NINSAR finalised in 2014 and a new licensing agreement was signed with Semillas Fitó SAU and BHN Seed.

REFERENCE:
Martin-Trillo M *et al.* Role of tomato BRANCHED1-like genes in the control of shoot branching. Plant J. 2011; 67:701-14

PATENTS LICENSED BEFORE 2013

The SpBRC1a gene from S. pennellii suppresses the elongation of branches in tomato. Left: Control plant. The red arrow indicates an incipient branch.

Proteobotics

Contact: Antonio Ramos. proteobotics@gmail.com

Proteobotics was created in 2013 by Antonio Ramos, a scientist in Dr Juan Pablo Albar's group. It specialises in identifying peptides and proteins using mass spectrometry data. The company received a 2014 Innovation Award from the Fundación Alberto Elzaburu.

In large-scale proteomics projects, millions of peptide ion collision spectra (MS/MS) are generated that must be matched to theoretical spectrum models inferred from known peptide sequences to identify proteins. A number of database search engines using different scoring systems have been and are being developed to this end. CNB scientists 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, increases remarkably the number of proteins identified. The process can be extended by integration of additional sources of information besides primary search engine results.

MAIN INNOVATIONS AND ADVANTAGES:
- It is extremely flexible and greatly increases the number of proteins identified
- Great accuracy in terms of error rate control

CNB-CSIC COLLABORATION:
Proteobotics signed an option to license a CSIC patent for a method to identify peptides and proteins from mass spectrometry data (P200930402, 01/07/2009). The patent was granted in Europe and is under examination in the US. The company has also signed a collaboration agreement with the CNB-CSIC.

Method for identifying peptides and proteins from mass spectrometry data. High throughput, proteome-wide identification of proteins using tandem mass spectrometry requires computational methods to interpret and filter large sets of peptide ion collision data.

8 / Scientific Services

One of the most important assets of the CNB is its platform of scientific-technical services. They provide leading-edge technology in the fields of structural biology and image processing, cell biology, genetically modified mouse and plant models, genomics and proteomics, as well as bioinformatics and computational biology. The centre also stands out for large research installations, such as its animal facility, greenhouse and one of the few high-level biocontainment (BSL-3) laboratories currently operative in Spain.

Electron microscopy

HEAD OF SERVICE:
Cristina Patiño Martín

PERSONNEL:
Rocío San Andrés Cervilla
Ana Beloso Quiñones
Javier Bueno Chamorro

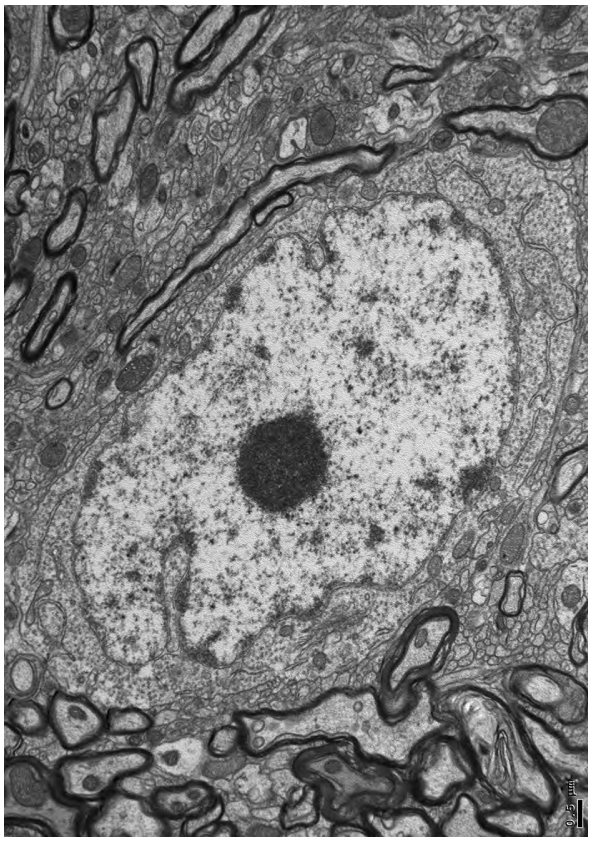
The electron microscopy service offers a variety of equipment and techniques for the preparation, processing and analysis of biological samples (cell and bacterial cultures, cell fractions, proteins, viruses, animal and plant tissues) by transmission electron microscopy.

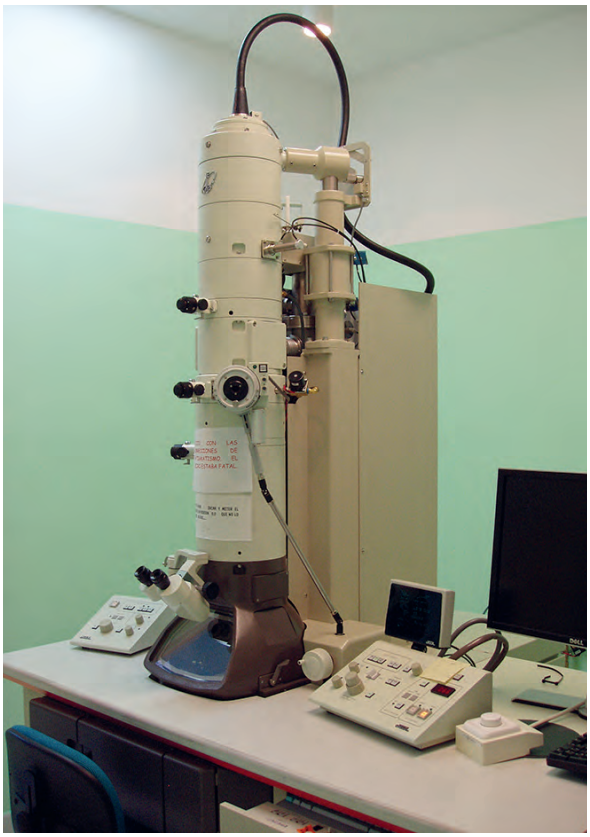
The technical staff provides support to users in the correct use of equipment and methodologies. We offer regular training in the techniques and methods available. We also take care of the sample preparation, if required, and image acquisition and provide support for data interpretation.

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, immunolabelling, *in situ* hybridisation, conventional transmission electron and low dose electron microscopy.

Specialised equipment

- JEOL JEM 1011 transmission electron microscope with Gatan ES1000W camera
- Leica Ultracut UC6 cryo-ultramicrotome
- Reichert Ultracut E ultramicrotome
- Automatic cryosubstitution system Leica EM AFS2
- High-pressure vitrification system Leica EM PACT2
- Sample trimmer Leica EM TRIM
- Knifemaker Reichert
- Carbon coating system Leica EM MED020



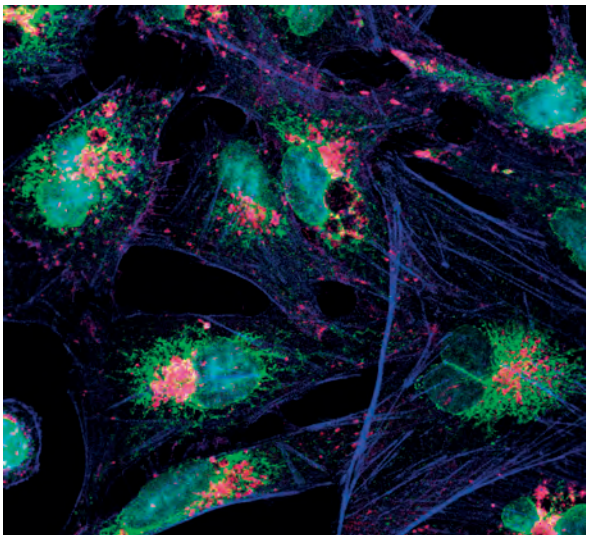


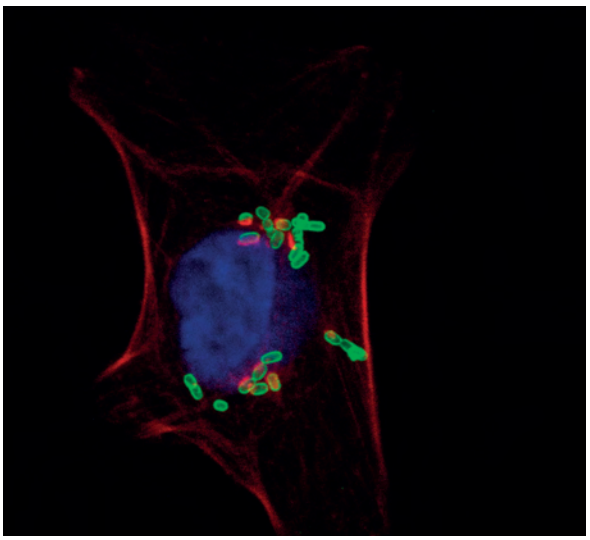
Confocal microscopy

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, ImageJ, 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

Macromolecular X-ray crystallography

LEAD SCIENTIST:
César Santiago

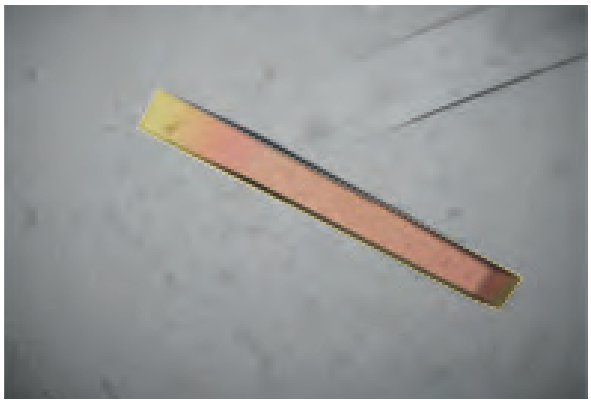
Protein X-ray crystallography is a high-resolution technique that allows us to study protein structure at atomic level. This method provides a detailed view of protein function, ligand and protein interactions, supramolecular organisation and mutants related to human diseases. Great improvements both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

The macromolecular X-ray crystallography facility provides the following techniques:

- Advice and supervision of protein production from cloning to expression in bacterial, yeast and eukaryotic systems
- Support and training in protein purification to obtain crystal-grade protein
- Automated macromolecular crystallisation
- Optimisation of crystallisation conditions 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 visualisation of crystallisation plates
- CrystalTrak database suite for crystallisation screening and improvement of positive trials



Proteomics facility

LEAD SCIENTISTS:
Juan Pablo Albar
Alberto Paradela

PERSONNEL:
Gema Bravo
Sergio Ciordia
Manuel Lombardía
Alberto Medina
Mari Carmen Mena
Rosana Navajas

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). For differential proteomics (quantitative proteomics), we use metabolic or chemical stable isotope labelling (ICPL, SILAC, iTRAQ,TMT) combined with LC-MS/MS analysis. We also offer targeted and, in combination with AQUA peptides, absolute quantitative protein analysis by selected/multiple reaction monitoring (S/MRM-MS). Phosphorylation analysis is performed through specific phosphopeptide enrichment procedures followed by LC-MS/MS analysis, using CID (collision-induced dissociation) or ETD (electron transfer dissociation) fragmentation methods. Prolamin detection and characterisation by ELISA 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 CNB Proteomics Facility is a member of the Proteored-ISCIII Platform (Plataforma en Red de Proteómica-Carlos III).

Services:

- Two-dimensional gel electrophoresis
- Protein identification and characterisation by MALDI-TOF/TOF, ESI Q-TOF, ESI QQQ and ESI ion trap mass spectrometry
- Protein quantitation by metabolic and chemical stable isotopic labelling (SILAC, ICPL, iTRAQ, TMT)
- Selected/multiple reaction monitoring (S/MRM-MS)
- Identification and characterisation of post-translational modifications
- Peptide synthesis and membrane-bound peptide array design
- Gluten analysis by ELISA and mass spectrometry

Protein tools unit

LEAD SCIENTIST:
Leonor Kremer

SCIENTISTS:
María Teresa Martín (*Molecular Interactions*)
Mónica García-Gallo (*Immunobiology*)
Mercedes Llorente (*Immunochemistry*)

TECHNICIANS:
Tamara Rueda
Laura Martín
María Lozano

The Protein Tools Unit focusses on production and characterisation of custom monoclonal antibodies, immune response studies, design and development of immunoassays, protein labelling and molecular interaction analysis. The Unit is a founder member of the EuroMAbNet, the first European non-profit organisation of multidisciplinary academic laboratories specialised in mAb production, which provides researchers working in the field with a framework for exchange of knowledge, methods and materials.

Research tools and services are provided to scientists from the CNB, other CSIC institutes, universities, public research organisations and private companies. The laboratory offers expertise, technical assistance, advices with data analysis and interpretation, user training and introduction of new methods. The core facility also organises theoretical and practical courses and assists with preparation of manuscripts and oral presentations.

The facility is equipped with a surface plasmon resonance biosensor (Biacore 3000), which allows sensitive, reliable characterisation of biomolecular interactions and provides information such as kinetics and thermodynamic parameters of the binding events. The biosensor has been used for applications such as protein-protein interaction (a bacterial toxin-antitoxin system), antibody-antigen (monoclonal antibodies, nanobodies, trimerbodies), dsRNA-protein (RNA-VP3), lipid-protein (FAK-liposomes) and small molecule-protein (DREAM).

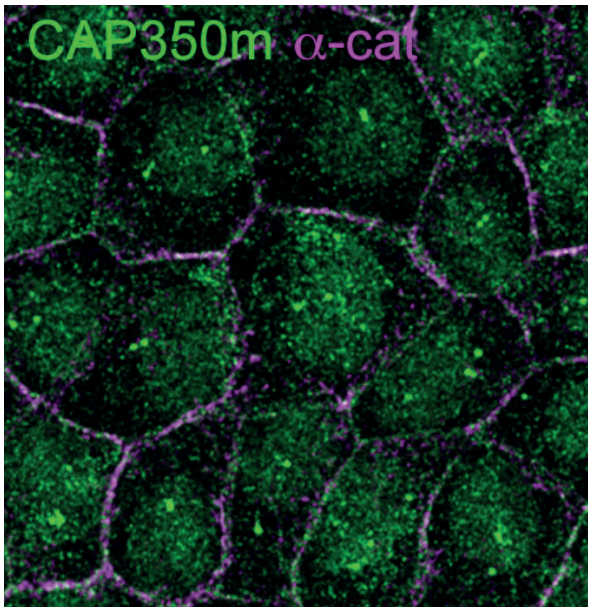
In this period, new monoclonal antibodies were raised and characterised against viral proteins (SARS-E, gp120), immunoglobulins (canine IgE), blood proteins (coagulation Factor V, CD5L), neurodegenerative disease-related proteins (DREAM, TAU, beta amyloid peptides), centrosomal proteins (CAP350), mitochondrial proteins (SCAM3), and chemokine receptors (CCR9).

Specialised equipment:

- ÄKTAprime plus chromatography system (GE Healthcare)
- SPR Biacore 3000 (GE Healthcare)
- EnVision 2104 Multilabel Reader (Perkin Elmer)
- Other equipment: biological safety cabinets, CO_2 incubators, centrifuges, microfuges, inverted fluorescence microscope (Zeiss Axiovert 40 CFL), thermal cycler, microplate reader, protein gel electrophoresis systems, Western blot systems and electrophoresis power supply units

Website: www.cnb.csic.es/index.php/ProteinTools

1



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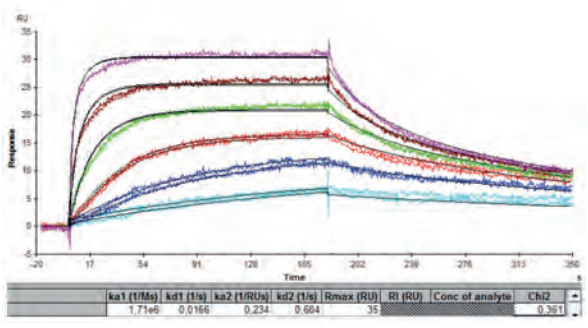


1 *Merged immunofluorescence image of MDCKII cells double-stained with α-catenin rabbit polyclonal and CAP350 mAb (figure kindly provided by María P. Gavilán and Rosa M. Ríos, CSIC-CABIMER)*

2 *SPR analysis of an antibody-peptide interaction using a biosensor Biacore 3000. The antibody was used at concentrations from 0.41 nM to 100 nM, at 25ºC, with a flow rate of 30 µl/min. Data were collected for 180 s of the association phase and 180 s of the dissociation phase. Sensorgrams for different concentrations of analyte were overlaid, aligned and analysed with BIAevaluation Software 4.1*

Genomics unit

LEAD SCIENTIST:
José Manuel Franco Zorrilla

PERSONNEL:
Gloria García Casado
Irene López-Vidriero
Marta Godoy
Luis Almonacid
Beatriz Martín
Iria Calvete

The Genomics Unit at the CNB focusses on the analysis of gene expression from biological samples using microarrays, interrogating the activity of complete genomes in a single experiment, and helping to elucidate the genetic basis of biological processes. We routinely hybridise and analyse one- and two-channel microarrays, including Agilent, Affymetrix, and custom microarrays.

Our services include microarray printing, analysis of RNA integrity and microarray hybridisations. Raw data are analysed statistically using state-of-the-art algorithms, and filtered results are supplied to scientists in an easy-to-use, web-based tool developed by the Unit. We offer advice and support in the use of several bioinformatic tools for functional analysis of genes and genomes, helping researchers with the biological interpretation of the results. Finally, we also offer the possibility of validating gene expression data by real-time qPCR analysis. Through the Genomics Unit, the CNB participates in the Parque Científico de Madrid (CSIC-PCM) Ultrasequencing Platform, located physically at the CSIC-PCM installations. This platform has the capacity for massive sequencing experiments of complete genomes, transcriptomes, small RNAs or DNA/RNA-proteins interactions.

Research projects are constantly being developed by our personnel, who implement new services and technologies for our users. These include new microarray-based technologies such as a new DNA chip for studying DNA-protein interactions.

Tiller service:

• Leaded by Dr Carlos Alonso Blanco, the TILLer Service is an international public service to search for EMS-induced mutants in the model plant *Arabidopsis thaliana.*

• 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 has identified more than 500 mutants in those genes.

Computational genomics

LEAD SCIENTIST:
Juan Carlos Oliveros Collazos

Current advances in genomics-related technologies such as DNA microarrays and more recent 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 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

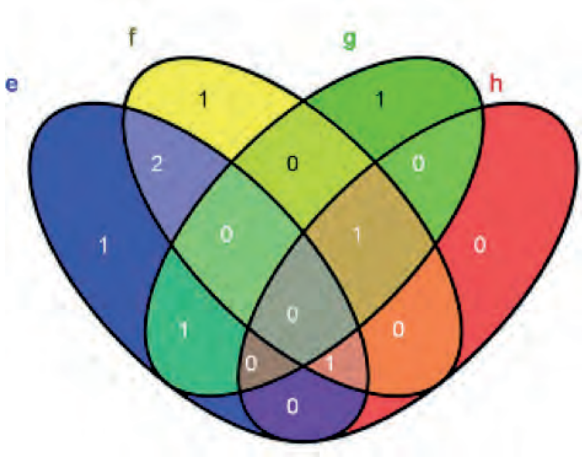
• Biostatistical support for the correct interpretation of genomics-related results

• 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

LEAD SCIENTIST:
José Ramón Valverde Carrillo

The Scientific Computing Service provides advanced support for scientific data analysis in bioinformatics and biocomputing through close collaboration and organisation of international courses (in-house and abroad).

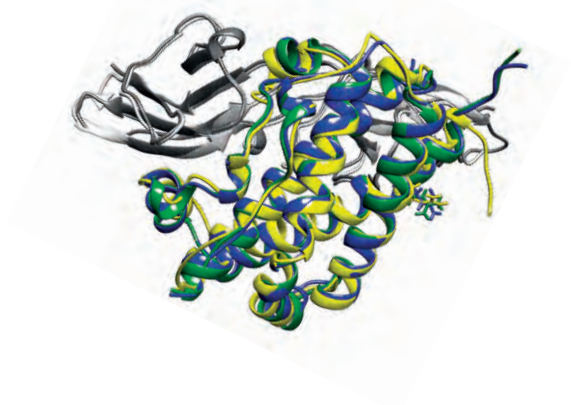
Our main areas of work include modern bioinformatics next-generation sequencing (NGS) data analyses, metagenomics and *de novo* genome sequencing for topics such as the effect of pesticides on the rhizosphere, sequencing novel *Escherichia coli* mutant strains, as well as offering courses on phylogeny analysis and metagenomics.

Computational biology includes binding pocket identification, docking, drug screening, *in silico* mutagenesis, molecular and quantum dynamics, QM and QM/MM (quantum mechanics/molecular mechanics) models and reaction modelling, which we use to study human growth hormone mutants, select natural compounds against cancer stem cells, resolve the reactions in the bacterial ε-ζ complex; we also offer courses on biocomputing.

Advanced biostatistical analyses include treatment of complex experimental setups related to new therapies, proteomics, non-linear longitudinal analyses, and an international course on biostatistics.

We provide support for many computer languages to produce dedicated software, and organise an international course on the Python programming language.

We coordinated the Iberoamerican Network on FLOSS for Biomedicine (CYTED 510RT0391), in collaboration with pioneering NGS groups, through COST action BM1006 (SEQAHEAD). We participate in EU CBRN CoE P35 and in EMBnet; with numerous external institutions including the University of Alcalá de Henares, we coordinate the Master in Bioinformatics of the University of Colombo (Sri Lanka), the Advanced Course in Genomics organised by ILRI in Nairobi, (Kenya), and many other courses in Europe, Latin America, Asia and Africa.



Sequence analysis and structure prediction

LEAD SCIENTIST:
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:

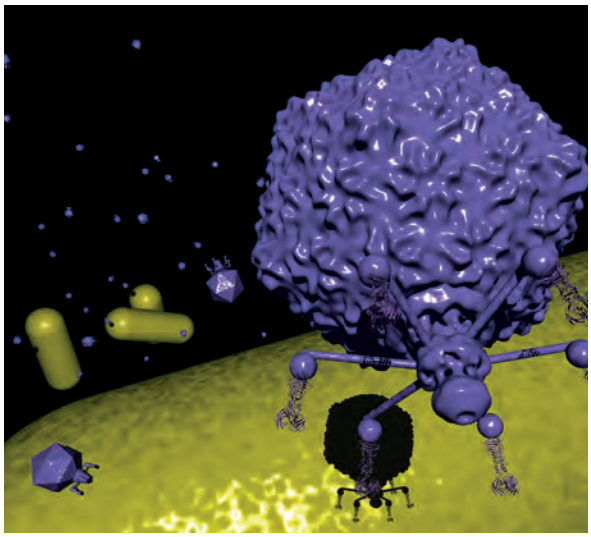
- Predict protein structure
- Search for homologous proteins
- Generate multiple sequence alignments
- Produce structural organisation drafts
- Study relevant residues for protein structure/function
- Extract sequence features from full proteomes

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.



Flow cytometry

LEAD SCIENTIST:
Mª del Carmen Moreno-Ortiz Navarro

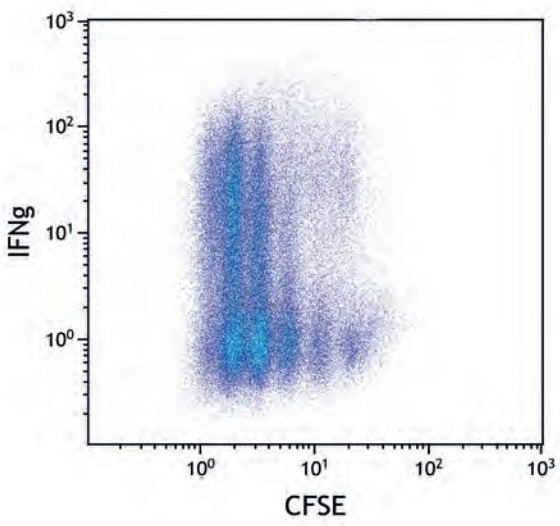
PERSONNEL:
Sara Escudero García

Flow cytometry is a technology of multiparametric cell analysis for detection and identification of molecules and cell structures using fluorescent markers and conjugated antibodies. Flow sorting is an extension of this technology, by which any cell or object measured can be separated selectively from the suspension based on properties measured in the flow stream. Flow cytometry techniques have undergone significant development in recent years in their ability to analyse thousands of cells per second and provide statistical information instantly.

The Flow Cytometry Unit provides scientific and technological support to CNB research groups, as well as to researchers from the public and private sectors.

The unit offers:

- Training and advice on flow cytometry principles and applications
- Development of new applications and experimental design, incorporating new technologies and reagents
- Quantification of secreted cytokines by multiplex assays
- Professional analysis with specific software
- Cellular isolation by cell sorting
- Maintenance of stock of antibodies and commonly used reagents



Equipment:

- BD FACSCalibur Analyzer: 4 colours, 2 laser (488 nm and 633 nm)
- Beckman Coulter EPICS XL-MCL Analyzer: 4 colours, 1 laser (488 nm)
- Beckman Coulter CYTOMICS FC 500 Analyzer: 5 colours, 2 laser (488 nm and 633 nm)
- Beckman Coulter CYTOMICS FC 500 Analyzer: 5 colours, 1 laser (488 nm)
- BD LSRII Analyzer: 8 colors, 3 laser (488 nm, 633 nm and 405 nm)
- Beckman Coulter GALLIOS Analyzer: 10 colours, 3 laser (488 nm, 633 nm and 405 nm)
- Luminex 100 IS Multiparametric Analyzer: A system that can be used to quantify multiple cytokines (up to 100) or any other soluble molecule from a single sample
- Cell Sorter Beckman Coulter Moflow XDP: 10 colours, 3 laser (488 nm, 633 nm and 405 nm)

The unit also provides 3 PC platforms for analysis with specific software: WindMDI, CXP, MultiTime, MultiCycle, DIVA, FlowJo, Summit, and Kaluza

Common applications performed routinely:

- Cell viability (IP, 7AAD, DAPI)
- Viability fixed cells; cell cycle and ploidy (IP, DAPI)
- Studies of mitotic populations (G2/M) with phosphohistone 3
- Ploidy levels in plants (IP)
- Proliferation assessment with BrdU or EdU
- Cell proliferation (CFSE, CellTrace)
- Apoptosis (SubG0/G1, annexin V, TUNEL)
- Gene expression using fluorescent proteins
- Intracellular signalling (phosphoproteins)
- Cell migration studies
- Intracellular Ca^{2+} mobilisation
- Immunophenophenotyping using multiparametric analysis up to 10 colours
- Study of T regulatory cells; intracellular cytokines
- Quantitation of soluble molecules, cytokines using multiplex assays
- Cell sorting

website: www.cnb.csic.es/~citometria

Greenhouse

HEAD OF SERVICE:
Tomás Heras Gamo

PERSONNEL:
Alejandro Barrasa Fustes
Esperanza Parrilla Carrillo

The Greenhouse Service takes care of the following facilities:

• A standard greenhouse with 8 cabinets (total growth surface: 180 m^2)

• A P2 safety level greenhouse with 4 cabinets (total growth surface: 83 m^2)

• 16 climate chambers

The Greenhouse Service 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

Animal facility

HEAD OF SERVICE:
Angel Naranjo

RESEARCH TECHNICIAN:
Javier Martín Torre

SHIPMENT COORDINATOR AND ADMINISTRATION:
Alberto García

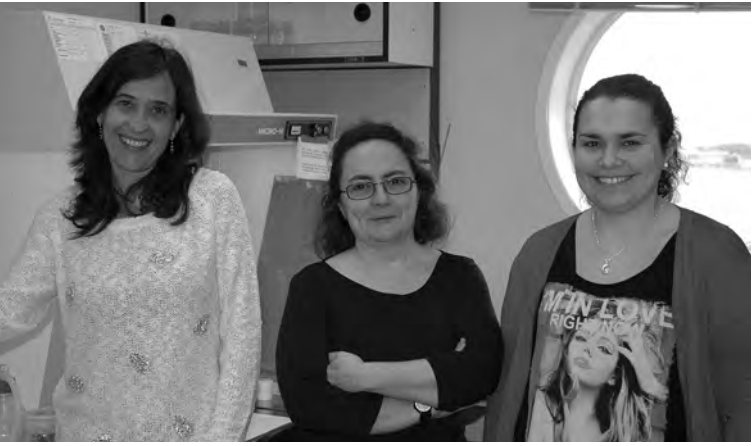
AREA AND COLONY MANAGERS:
Antonio Morales
Raquel Gutiérrez
Eladio Martínez

ANIMAL TECHNICIANS:
Angel Moreno
Israel López
Rebeca Acuña
Sergio Magallón
Alicia González
Rubí Jaramillo
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, aiding in 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 provides services for various techniques used in mouse research models. Veterinary staff gives research assistance in surgical techniques, selection of animal models, animal health surveillance, laboratory animal care, and animal well-being. We also organise courses to obtain accreditation for working with animals and manage colonies of genetically modified animals.

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



In vitro plant culture facility

HEAD OF SERVICE:
Raquel Piqueras Martín

PERSONNEL:
María Luisa Peinado Vallejo
Beatriz Casal López

The CNB *In vitro* Plant Culture Facility offers technical expertise and maintains a variety of equipment necessary for:

• sowing and growth of cells, tissues and seedlings of many plant species, including Arabidopsis, *Nicotiana sp,* tomato, potato and *Brassica sp*

• their maintenance and propagation

• generation of plant protoplast and cell cultures

We also assist in obtaining genetically modified plants by

• stable transformation of plants: using *Agrobacterium tumefaciens* as a transgene carrier

• transient transformation of plants using the particle delivery system

The *In vitro* Plant Culture Facility provides service to any researcher at the CNB and works very closely with members of the Plant Molecular Genetics Department. With appropriate authorisation, our services are also available to researchers from other institutions and companies.

Histology facility

LEAD SCIENTIST:
Lluís Montoliu

PERSONNEL:
Soledad Montalbán
Óscar Sánchez

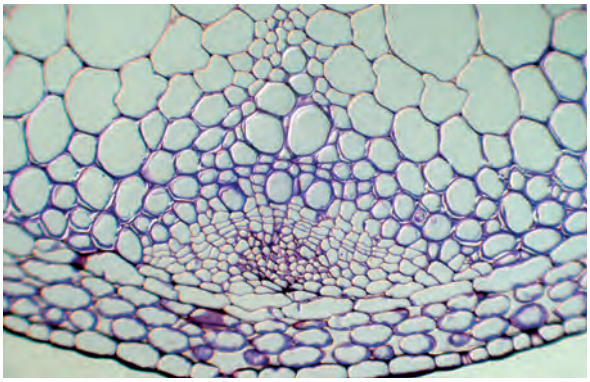
The CNB Histology Facility offers the preparation of animal and plant biological samples for histological analysis. All requests are processed electronically, through the facility's website, in Spanish and in English. Available methods include the preparation of wax and plastic (resin) blocks and the preparation of histological sections with an automated microtome. We also offer preparation and cryostat sectioning of frozen blocks. All sections can be counterstained with any of the various staining procedures available (including haematoxylin/eosin, crystal violet, PAS, Mason's trichrome, elastin fibres/Van Gieson) or can be used for immunohistochemistry. The Facility can implement new staining procedures or additional histological methods, according to the demand and user/researcher needs. The CNB Histology Facility has ample experience in processing a large variety of animal and plant tissues and organs.

The CNB Histology Facility is integrated within the INNOTEK technological platform of the Campus of Excellence UAM+CSIC, and is associated with the IIBm-UAM/CSIC Histology Facility, coordinated by the CNB Histology Facility, to offer CNB and IIBm researchers a larger processing capacity for histological samples. The CNB Histology Facility has joined the SEFALER technological platform of the CIBERER/ISCIII.

Publication

Chevalier F, Montalbán-Iglesias S, Sánchez OJ, Montoliu L, Cubas P. Plastic Embedding of Arabidopsis Stem Sections. Bio-protocol 2014; 4:e1261

Website: www.cnb.csic.es/~histocnb/



Mouse embryo cryopreservation facility

LEAD SCIENTIST:
Lluís Montoliu

PERSONNEL:
Julia Fernández Punzano
María Jesús del Hierro Sánchez
Marta Castrillo Labrado
Isabel Martín-Dorado Caballero

The CNB Mouse Embryo Cryopreservation Facility offers researchers the possibility of freezing and maintaining transgenic and knockout mouse lines in the form of embryos and/or sperm. Additional services available include the thawing of sperm and/or embryos and revitalisation of cryopreserved mouse lines, *in vitro* fertilisation, assessment and/or logistical support for importing/exporting frozen or refrigerated embryos or sperm to and from the CNB, quality controls and genotyping procedures. Cryopreservation of mouse lines is a highly recommended process for archiving animal models used in biology, biomedicine and biotechnology for long periods of time, The conditions are stable and safe, and dispense with the need to maintain mouse lines alive and breeding, therefore saving space, funds and reducing the number of animals in experiments, according to current animal welfare regulations. The National Centre for Biotechnology (CNB-CSIC) hosts the Spanish node of the European Project INFRAFRONTIER-EMMA, whose objective is the cryopreservation, organised archiving and coordinated distribution of mouse lines of interest for the scientific community in Biomedicine.

The CNB-CSIC and CNIO signed a scientific cooperation agreement to allow archiving and distribution of mutant mouse lines of interest in biomedical research, generated by CNIO researchers, through the INFRAFRONTIER-EMMA project. The CNB Mouse Embryo Cryopreservation Facility is integrated in the scientific-technological platforms INNOTEK, from the Campus of Excellence UAM+CSIC, and SEFALER of the CIBERER/ISCIII. The CSIC and the IRDA-University of Kumamoto signed an academic cooperation agreement to promote the exchange of knowledge, personnel and information on the mouse embryos and sperm archiving and cryopreservation activities undertaken at the Spanish node of INFRAFRONTIER-EMMA at the CNB-CSIC and at the CARD archive, coordinated by Dr Lluís Montoliu and Prof Naomi Nakagata, respectively. The first initiative derived from this cooperation took place in October 2013 with the International CARD-CNB Mouse Embryo and Sperm Cryopreservation Course, organised at the CNB by Naomi Nakagata and Lluís Montoliu.

Publication:

INFRAFRONTIER-providing mutant mouse resources as research tools for the international scientific community. INFRAFRONTIER Consortium. Nucleic Acids Res 2015; 43(Database issue):D1171-5

Website: www.cnb.csic.es/~criocnb/

Transgenesis

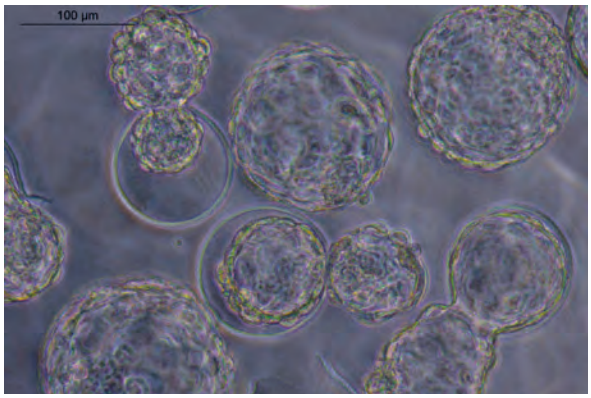
LEAD INVESTIGATOR:
Mª Belén Pintado Sanjuanbenito

PERSONNEL:
Alfredo Serrano Montalbo
Marta García Flores
Verónica Domínguez Plaza

The CNB-CBMSO Transgenesis Unit provides support to researchers linked to the CSIC-UAM Platform in the generation, establishment and interchange of genetically modified mouse models. The unit offers technical and scientific advice on the best strategy to achieve the desired model 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.

The unit provides the following services:

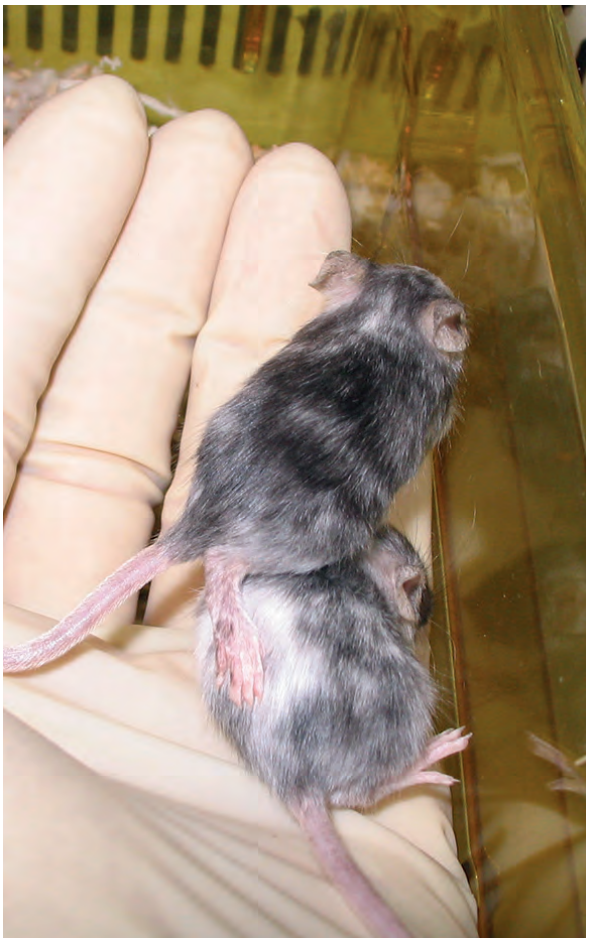
- Advice on the design of target vectors or constructs for microinjection
- Pronuclear microinjection of plasmid, BAC or YAC DNA
- Vector electroporation in R1 or G4 ES cell lines
- Zinc finger nuclease injection
- Handling of ES cells from international consortia
- ES cell injection or aggregation to 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 femtojet 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



Radiation protection & biological safety

HEAD OF SERVICE:
Fernando Usera Mena

SUPERVISOR:
Sonia Calvo Ladrero

PERSONNEL:
Jessica Gaspar Navarro
Aránzazu de la Encina Valencia

Tasks and services:

• Preliminary evaluation and periodic control of biological, chemical and radiological risk

• Management of radioisotope acquisition and the means, equipment and instruments for prevention and protection

• Design of biosafe labs and other facilities

• Issuing the CNB Basic Guide, the Safety & Health CNB Manual, and specific associated procedures

• Handling and administration of reports and documents related to start-up procedures or legally required operating conditions

• Safety and health seminars for personnel exposed to potential risk agents

• Classification and signalling of risk areas and laboratories, and control of compliance with safety and health rules

• Cooperation in the management of medical and dosimetric surveillance of exposed personnel. Maintenance of medical and dosimetric records of exposed personnel

• Action in incidents, accidents and emergency situations following previously established procedures

• Managing the production and conditioning of biosanitary, toxic and radioactive waste at the sources, internal handling and storage until transfer to an authorised waste management facility

Facilities

The service surveys the operational risks in all CNB labs and facilities, and directly operates the gamma irradiator, the radioactive facility central premises, and the biosafety level 3 laboratory.

Radioactive facility

The CNB radioactive facility is a Category 2, non-encapsulated source type and is equipped with all required means of shielding, containment and detection of ionising radiation. It also has the following equipment:

Two cabins for radioisotopes

• One biosafety class II cabin

• One CO_2 incubator

• Ultracentrifuge, centrifuges and microfuges

• One speed vac

• One hybridisation oven

Biosafety level 3 laboratory

The lab has three *in vitro* cell culture sublaboratories with all necessary equipment for safe handling of biological agents included in Risk Group 3 and to perform activities with genetically modified organisms that require such a degree of confinement. It is equipped with:

Three biosafety class II cabins

• Two cell culture incubators

• One microbiological culture incubator

• One double door steam steriliser

• One SAS for biological inactivation of small items

• One SAS for biological inactivation of large items

• One refrigerated ultracentrifuge

• Three portable refrigerated centrifuges

• Three refrigerated microcentrifuges

• Three inverted optics microscopes

• One liquid nitrogen tank

• Three ultrafreezers (-80ºC)

• Data transmission network (computers and telephone)

Several alarm systems to alert of incidents/accidents or malfunctions

Website: www.cnb.csic.es/index.php/biosafety

Tissue culture, washing and sterilisation

HEAD OF SERVICE:
Rosa María Bravo

MEDIA PREPARATION:
Ana Montero
Concepción Cobeña Chivato

CELL CULTURES:
Sonia Rodríguez Murcia

WASHING AND STERILISATION:
Enrique Méndez
Anunciación Romero
Margarita Felipe Hombrados
Josefa Pérez Alfaro
Carlos Enríquez Casas
Arancha Rodríguez Martínez
Carmen Berdeal Mera
Ángeles Sánchez Pérez
Ana Isabel Nieto Jiménez
Rosa Ramos Hernández
Ángel Valera López

9 / Direction and Management

Director

Carmen Castresana

Vice-director

Fernando Rojo

Vice-director

Isabel Mérida

Scientific officer

Peter Klatt

Outreach manager

Alfonso Mora

Outreach adviser

Miguel Vicente

Technology transfer manager

Ana Sanz Herrero

Assistant to the director

Yolanda García

General manager

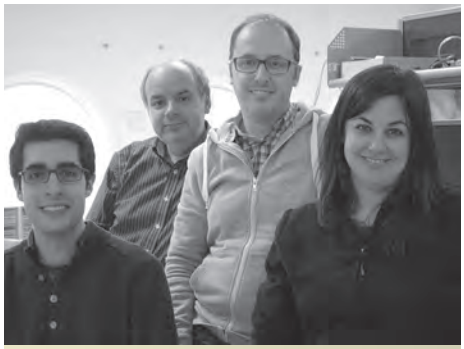
Miguel Anchuelo

Librarian

Mª Dolores Aparicio Trujillo

Photographer

Inés Poveda



Information technologies

HEAD OF SERVICE:
Sonia de Diego

PERSONNEL:
Íñigo Oficialdegui
Alberto Sánchez
Julio César Aldariz
Óscar Bodas

SERVICE:
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 and backup servers
Registry of DNS equipment
Internet protocol telephony
(shared with General Services)
Development of applications and databases

Project management

HEAD OF SERVICE:
Soraya Olmedilla María

PERSONNEL:
Aurora Cabrerizo Alonso
Daniel Martín Hernando
Diana Gloria Pastor Calero
Pilar Ara Laúna

Human resources

HEAD OF SERVICE:
Marina Hernando Bellido

PERSONNEL:
Javier Tortosa Nieto
Lourdes Triana Iznaola
Susana Pena González.
Mª Jesús Torrado Macias

Economic management

HEAD OF SERVICE:
Mariano Muñoz Jiménez

PERSONNEL:
Rufino Fernández Senso
Rafael López Laso
Isabel Guerra Álvarez
María José Gregorio Usano
María del Carmen Berreiros Cano
Francisco Luis Aparicio Reyes
Carmen Vaz Pereña

DIO management

PERSONNEL:
Antonio Rodríguez Terán
Catherine Mark
Coral Bastos

Purchasing and supplies

HEAD OF SERVICE:
Ramón Serrano Coronado

PERSONNEL:
María José Caballero Martín
Gloria del Sastre Martín
Julio Díez Álvarez
Antonio Pastor Encabo
Juan Carlos Bermudo Zamora

Security

HEAD OF SERVICE:
Sócrates Gutiérrez Monreal

PERSONNEL:
Jesús Payán
Fernando Albarrán
Marcos Fuentes
Abderramim Asgou
Yolanda Cantos

Maintenance

HEAD OF SERVICE:
Antonio Dueñas

PERSONNEL:
Enrique Mejías
Conrado Pedraza
Jesús González
Juan Carlos Cuenca
Alfonso García

Instrumentation

HEAD OF SERVICE:
Ismael Gómez López

PERSONNEL:
Carlos González Redondo
Juan Ignacio Golpe de la Fuente

Workshop

Daniel Pastora

Construction and infrastructure planning

Javier Zarco

General services

HEAD OF SERVICE:
Gabriel Sánchez de Lamadrid

PERSONNEL:
Julián Grande Palomino
Manuel Grande Palomino
David Suárez
José Miguel de la Hoz
Paloma González
Carolina Nogales
Santa López
Aileen Bonsol
Pilar Cutillas
Lourdes Sánchez
Adela García
Socorro Muñóz
Elga Joana Villamizar
Beyca López
Juan Pablo Illescas
Beatriz García
Ana María Puerto

10 / Annexes

Scientific meetings

In 2013 and 2014, a total of 288 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.

15 February 2013

On Robustness and Evolvability

Víctor de Lorenzo

27-31 May 2013

Programming Python: a practical course

José Ramón Valverde

7 June 2013

Division from A to Z

Miguel Vicente
Moira Torrent

24 June-5 July 2013

Synthetic Biology in action!

Víctor de Lorenzo

1-26 July 2013

1st CNB Course on Introduction to Research

Carmen Castresana
Alfonso Mora

23-26 September 2013

Genética y gestión de colonias: animales de experimentación

Ángel Naranjo

15-16 October 2013

Descifrando el desarrollo del cerebro: aproximaciones interdisciplinares hacia la comprensión y tratamiento de sus patologías

Marta Nieto
Paola Bovolenta

29 October 2013

II Jornada de desarrollo preclínico: Medicamentos de terapias avanzadas

Vivotecnia

12 November 2013

Encuentro investigación-empresa: Aplicaciones biotecnológicas en salud humana y animal

Ana Sanz

11-15 November 2013

Técnicas inmunoquímicas: caracterización y cuantificación de proteínas mediante anticuerpos

Leonor Kremer

17-19 November 2013

XV Congreso de la Sociedad Española de Biología Celular

Inés M. Antón
Rosa Rios

27-28 November 2013

From inflammation to cell plasticity: the new hallmarks of cancer

Santos Mañes
Isabel Mérida

28-30 November 2013

Stem Cells Workshop

Francisco J. Iborra (Scientific Committee)

29 November 2013

In vitro reconstitution of the 3R (replication, repair, recombination)

Silvia Ayora

17-18 December 2013

XXI CNB Scientific Workshop

Alfonso Mora

19 December 2013

XXI Workshop Avances en Biología Molecular por Jóvenes Investigadores en el Extranjero

Domingo F. Barber
Lourdes Planelles
Yolanda R. Carrasco
Alfonso Mora

2014

3-15 January 2014

Hands-on training school on three-dimensional EM of macromolecular complexes

José María Carazo

15-17 January 2014

Hands-on training school on soft X-ray cryo-tomography reconstruction of cells

José María Carazo

24 January 2014.

Colloquium on cellular decision making

Víctor de Lorenzo

26-29 January 2014

FEBS-ASM Workshop on Biology on RNA in host-pathogen interactions

Francisco García-del Portillo
Cecilia M. Arraiano

28 February 2014

Cell Division Reconstruction workshop

Miguel Vicente

3-13 March 2014

Curso para el personal de animalario que lleva a cabo los procedimientos: modulo de categoría B

Ángel Naranjo
Elena Hevia

2-4 April 2014

VIII Reunión de la Red de Estructura y Función de Proteínas

José María Valpuesta
José L. Carrascosa

5-6 April 2014

2nd European Days of Albinism

Lluís Montoliu (Scientific Committee)

28-29 April 2014

3rd Madrid Meeting on Dendritic Cells and Macrophages

Carlos Ardavín
Ángel Corbí
David Sancho
Antonio Castrillo

27 May 2014

Genome editing: A new tool for generating genetically modified animal models

Belén Pintado
Paloma Arias

1-6 June 2014

XIII International Nidovirus Symposium

Luis Enjuanes

9-20 June 2014

Atelier *Pseudomonas putida* 2014

Víctor de Lorenzo

16-18 June 2014

The Protein Multiverse Workshop

Víctor Muñoz

27 June 2014

Control Sanitario: del animalario al laboratorio tras las nuevas recomendaciones FELASA

Ángel Naranjo
Belén Pintado

30 June - 25 July 2014

2nd CNB Course on Introduction to Research

Carmen Castresana
Alfonso Mora

1-4 July 2014

XIV Congreso de la Sociedad Española de Biotecnología

José Luis Martínez (Scientific Committee)

29 September - 2 October 2014

Curso de genética y gestión de colonias de animales de experimentación

Ángel Naranjo

5-8 October 2014

HUPO 13th Annual World Congress

Juan Pablo Albar
Concha Gil
Juanjo Calvete
Fernando J. Corrales

10 October 2014

1er Meeting de la Iniciativa en NanoBiomedicina del CNB

Domingo F. Barber
José L. Carrascosa

20-23 October 2014

Curso sobre células madre e inmunidad

Carlos Martínez-A
Juan Carlos Izpisua Belmonte

16-17 December 2014

XXII CNB Scientific Workshop

Alfonso Mora

22 December 2014

XXII Workshop Avances en Biología Molecular por Jóvenes Investigadores en el Extranjero

Domingo F. Barber
Yolanda R. Carrasco
Alfonso Mora

Doctoral theses

In 2013 and 2014, 84 students obtained the PhD degree under the supervision of CNB researchers

Sara Alvira de Celis

Caracterización estructural del complejo de plegamiento formado por las chaperonas Hsp90 y Hsp70 y su cochaperona Hop (José María Valpuesta)

Laura Barrio Cano

Regulación de la dinámica de las células B por estímulos de tipo innato: efectos de TLR4 y su ligando LPS (Yolanda R. Carrasco)

Ilaria Benedetti

Design of standardised molecular tools to analyse regulatory properties and biotechnological applications of the soil bacterium *Pseudomonas putida* (Víctor de Lorenzo)

Alejandra Bernardini

Papel de la respuesta a choque térmico en la resistencia a quinolonas de *Stenotrophomonas maltophilia* (José Luis Martínez & María Blanca Sánchez)

Stella Bernardo García

El factor PIF4 es un integrador de las señales de brasinosteroides (BR) y giberelinas (GA) (Salomé Prat)

Ana Cáceres Núñez

Papel de la fosfatasa celular DUSP-1 en la infección por el virus vaccinia (Mariano Esteban)

María Calvo del Castillo

Identification of pathogenicity determinants involved in the adaptation of Plum pox virus strain C to *Prunus avium* and herbaceous hosts (Juan Antonio García)

Mariana Castrillo Briceño

Ensamblaje y maduración de la cápsida del virus de la bursitis infecciosa: Estructura y función de la proteasa viral VP4 (José Ruiz Castón)

Michele Chiappi

Multimodal approach to the interaction of OD15 nanoparticles and MCF-7 cancer cells (José L. Carrascosa & Francisco Javier Chichón)

Laura Cuyàs Carrera

tALIX, a component of the endomembrane system involved in phosphate starvation signalling (Javier Paz-Ares & Vicente Rubio)

Daniela Dukovska

Immune response in infection: role of human NKG2H receptor and study of lymphocytes (Mar Valés & Hugh Reyburn)

Srdja Drakulic

Structural characterisation of *Saccharomyces cerevisiae* transcription machinery (José María Valpuesta & Jorge Cuellar)

Isabel Fernández de Castro

Caracterización de las factorías virales y complejos replicativos ensamblados por virus ARN (Cristina Risco)

Estefanía Fernández López

Influencia de WIP en el citoesqueleto de actina y la composición lipídica de las espinas dendríticas (Inés M. Antón & Dolores Ledesma)

María Eugenia Fuentes Pérez

Caracterización de proteínas, ácidos nucleicos e interacciones proteína: proteína y mediante microscopía de fuerzas atómicas (Fernando Moreno-Herrero)

Carmela García Doval

Estructura de fibras de bacteriófagos (Mark J. van Raaij)

Beatriz García García

Comparación de diferentes sistemas de expresión para la producción en plantas de proteínas del coronavirus del SARS y análisis de la estabilidad genómica de los vectores basados en el virus de la Sharka (Juan Antonio García)

Esther García González

Roles of WIP and WICH/WIRE proteins in formation and maturation of invasive protrusions in human breast cancer cells (Inés M. Antón)

Guillermo García León

Interconexión entre la resistencia intrínseca y adquirida a quinolonas *en Stenotrophomonas maltophilia* (José Luis Martínez & María Blanca Sánchez)

Josué Gómez Blanco

Análisis estructural de los chrysovirus mediante criomicroscopía electrónica tridimensional: estructura a resolución cuasi-atómica del virus de *Penicillium chrysogenum* (José Ruiz Castón & Daniel Luque)

Guadalupe Gómez Mauricio

Caracterización y evaluación del potencial terapéutico de células madre mesenquimales obtenidas de tejido adiposos en el tratamiento del infarto agudo de miocardio en modelo porcino (Antonio Bernad)

Verónica González García
Mecanismos moleculares implicados en la infección del bacteriófago T7 (José L. Carrascosa & Ana Cuervo)

Laura González Silva
Función de Gadd45g en el sistema inmunológico y desarrollo embrionario (Jesús María Salvador)

Julia Sáez de Guinoa Corral
Modulación del comportamiento de las células B en respuesta a estimulación por quimioquina y antígeno: mecanismos moleculares implicados (Yolanda R. Carrasco)

Sofía Hernández Arranz
Regulación del metabolismo del carbono en *Pseudomonas putida*: estrategia del sistema regulador formado por la proteína Crc y los sRNAs CrcZ y CrcY (Fernando Rojo & Renata Moreno)

María Ibarra Daudén
Caracterización de la terminasa mayor del bacteriófago T7 (José L. Carrascosa & Jaime Martín-Benito)

Alina Elena Ionel
Cambios estructurales implicados en la maduración de la cápsida del bacteriófago T7 (José L. Carrascosa & Jaime Martín-Benito)

Dimitri Kakabadse Schmidt
Actividad de la hormona de crecimiento en el desarrollo de enfermedades autoinmunes (Mario Mellado & Ricardo Villares)

Juhyun Kim
Transcription dynamics in TOL plasmid pWW0 of the soil bacterium *Pseudomonas putida* mt-2 (Víctor de Lorenzo)

Abhimanyu Kumar Singh
Structural and functional characterisation of animal adenovirus capsid proteins (Mark J. van Raaij)

Marcin Krupka
A structural switch involved in the bidirectional polymerization and membrane attachment of the streptococcal FtsA division protein (Miguel Vicente & Ana Isabel Rico)

Sara Landeras Bueno
Interacciones del virus de la gripe con la célula hospedadora: identificación de dianas celulares con potencial para la inhibición del virus (Juan Ortín)

Elena Liarte Marín
Estudio del origen y la diferenciación de las células dendríticas inflamatorias durante la infección por *Leishmania major* (Carlos Ardavín)

Daniel López López
Nuevas aproximaciones computacionales para el estudio y la predicción funcional de dominios de proteínas (Florencio Pazos)

Noelia López Montero
Replicación y ensamblaje del *Arbovirus bunyamwera* en células del insecto vector *Aedes albopictus* (Cristina Risco Ortiz)

Vadir López Salmerón
ELF3 regulates cotyledon expansion and hypocotyl growth rhythmicity (Salomé Prat)

Rebeca López Vicente
Identificación de un nuevo sistema de dos componentes y tres proteasas implicados en la respuesta al estrés por secreción en *Streptomyces lividans* (Rafael Pérez Mellado & Sonia Gullón Blanco)

Ambra Lo Piano
Análisis de los requerimientos de la replicación dependiente de recombinación en el bacteriófago SPP1 Juan C. Alonso (Silvia Ayora)

Ruth Marcos Corrales
Papel de las 9-lipooxigenasas de *Arabidopsis thaliana* en respuesta al daño en la pared celular (Carmen Castresana)

Salvador Martínez de Bartolomé Izquierdo
Métodos de validación de identificaciones a gran escala de proteínas y desarrollo e implementación de estándares en proteómica (Juan Pablo Albar & Jesús Vázquez)

Antonio Martínez Sánchez
Computational techniques based on differential geometry for segmentation in electron tomography (José Jesús Fernández)

Alberto Medina Auñón
Procesamiento automatizado de datos proteómicos: desde la espectrometría de masas al conocimiento biológico (Juan Pablo Albar & José María Carazo)

Rosa Menéndez Conejero
Structural studies on adenovirus: the human adenovirus packaging motor, and characterisation of two reptilian adenoviruses (Carmen San Martín)

Pablo Mesa García
Caracterización del mecanismo de acción de la helicasa replicativa G40P y su cargador G39P en la replicación del bacteriófago SPP1 de *Bacillus subtilis* (Juan Carlos Alonso)

José Alberto Morín Lantero
Estudio a nivel de moléculas individuales de la actividad de replicación del ADN de la polimerasa del bacteriófago Phi29 (José L. Carrascosa & Borja Ibarra)

Gaurav Mudgal
Structural insights into coronavirus binding to host aminopeptidase N and interaction dynamics (José María Casasnovas)

Miguel Ángel Muñoz Alía
Neutralización de genotipos del virus del sarampión: identificación y caracterización en la hemaglutinina viral de epitopos neutralizantes de amplio espectro e inmunodominantes en la infección natural (Rafael Fernández Muñoz & Mariano Esteban)

José Luis Nieto Torres
Características y relevancia en patogénesis de la actividad canal iónico de la proteína E del coronavirus causante del síndrome respiratorio agudo y grave (Luis Enjuanes & Marta López de Diego)

David Ochoa García
Improving co-evolution-based methods for protein-protein interaction prediction (Florencio Pazos)

Jorge Andrés Olivares Pacheco

Cambios en la fisiología de *Pseudomonas aeruginosa* causados por la sobre-expresión del sistema múltiple de bombeo MexEF-OprN (José Luis Martínez & Carolina Álvarez Ortega)

Elena Pascual Vega

Bases estructurales de la cápsida del virus de la bursitis infecciosa para el desarrollo de futuras aplicaciones biotecnológicas (José L. Carrascosa & José Ruiz Castón)

Manuel Pazos Don Pedro

Stability, placement and interactions of the divisome components in nucleoid-deprived *Escherichia coli* cells (Miguel Vicente & Paolo Natale)

Joan Peredo Hernández

Análisis del papel de la proteína hSTAUFEN1 en procesos de regulación de la traducción, diferenciación neuronal e infección por el virus de la gripe (Juan Ortín & Susana de Lucas)

María Ángeles Pérez Calvo

Caracterización estructural y funcional del complejo formado por DnaK y GrpE de *Escherichia coli* (José María Valpuesta & Óscar Llorca)

Maite Pérez Cidoncha

Análisis de la interacción entre el virus de la gripe y la respuesta celular innata (Juan Ortín)

Vicente Pérez García

Papel de la oligomerización de las PI3K de Clase IA p110α y p110β en la regulación de la actividad de PTEN (Ana Clara Carrera)

José de Jesús Pérez Martínez

Caracterización de la modificación por O-GlcNAc de la proteína de la cápside del potyvirus Plum pox virus y su relevancia para la infección viral (Juan Antonio García)

Carlos Piñero Lambea

Design and construction of synthetic adhesins driving the specific attachment of *Escherichia coli* to target surfaces, cells and tumours (Luis Ángel Fernández & Gustavo Bodelón)

Charukesi Rajulu

Control of phosphate starvation responses in *Arabidopsis thaliana* (Javier Paz-Ares)

Rubén Rodríguez Barroso

Heterodímeros de receptores de quimioquinas, nuevas unidades funcionales que contribuyen a la plasticidad de la respuesta celular (Mario Mellado & Laura Martínez Muñoz)

María Fernanda Rodríguez Tornos

Función de los factores de transcripción Cux1 y Cux2 en la actividad neuronal y en la conectividad a través del cuerpo calloso (Marta Nieto)

Gema Romera Cárdenas

Desarrollo de un sistema para estudiar la pérdida de función en las células NK (Hugh Reyburn)

David Ruano Gallego

Engineering *Escherichia coli* K-12 for the secretion of single domain antibodies against attaching and effacing bacterial pathogens and for the injection of proteins of therapeutic potential into human cells (Luis Ángel Fernández)

Valencio Salema

Development of *Escherichia coli* cell surface display for selection of single domain antibodies from immune libraries (Luis Ángel Fernández)

Verónica Sánchez Alonso

Función de RalGDS en cáncer asociado a colitis y en el desarrollo de la célula T (Ana González García)

Diego Sebastián Ferrero

Caracterización estructural y funcional de la ARN polimerasa dependiente de ARN del virus de *Thosea asigna* (Francisco Rodríguez-Aguirre & Núria Verdaguer)

Marina Serna Gil

Caracterización estructural de las chaperonas TBCE y TBCB, involucradas en la homeostasis de tubulina α (José María Valpuesta & Jaime Martín-Benito)

Davide Seruggia

Structural and functional description of the mouse Tyr locus (Lluis Montoliu)

Nagalakshmi Tiruvarur Sooriyanarayanan

Buttressing a new paradigm in protein folding: experimental tools to distinguish between downhill and multi-state folding mechanisms (Víctor Muñoz & Mourad Sadqi)

Denise Soutar Moroni

Papel de las diacilglicerol quinasas en la regulación de la respuesta inmune (Isabel Mérida)

Roberto Spada

Characterisation of NK cells in mouse models of systemic lupus erythematosus and of the role of the p85β subunit in NKG2D signalling in NK cells (Domingo F. Barber)

Mohan TC

Molecular mechanisms involved in arsenic perception and tolerance in *Arabidopsis thaliana* (Antonio Leyva & Gabriel Castrillo)

Thi Thuy Do

Mutagenic effect of antibiotics on *Escherichia coli* and new genes of antibiotic resistance in *Mycobacterium smegmatis* (Jesús Blázquez & Alfredo Castañeda García)

María Tomé Pizarro

Papel de los microRNAs en las propiedades terapéuticas de las células madre mesenquimales humanas (Antonio Bernad & Manuel Ángel González de la Peña)

Marta Ukleja

Architecture of the CCR4-NOT complex from *Schizosaccharomyces pombe,* a multifunctional cellular machine (Jorge Cuéllar)

Verónica Uribe

Analysis of the role of Arid3b in cardiac development (Juan José Sanz Ezquerro)

Íñigo Valiente Alandí

Papel del gen Polycomb Bmi1 en la biología de las células madre cardíacas residentes en mamíferos (Antonio Bernad)

Cristina Vicente García

Identification and functional validation of genomic boundaries in mammals (Lluís Montoliu)

Aneesh Vijayan

Effect of vaccinia virus 14K protein: a case study with malaria vaccine based on circumsporozoite protein (Mariano Esteban)

Andrea Volante

pSM19035: dissection of the plasmid partitioning machinery (Juan Carlos Alonso & Silvia Ayora)

Tribhuwan Yadav

Assembly of RecA onto single-stranded DNA: Distinct role of mediator proteins in DNA repair and genetic recombination in *Bacillus subtilis* (Juan Carlos Alonso)

Manuela Zonca

Insights into the cytokine APRIL in B cell trafficking and in adipose-derived mesenchymal stem cells (Lourdes Planelles)

María Teresa Zotes Ciprés

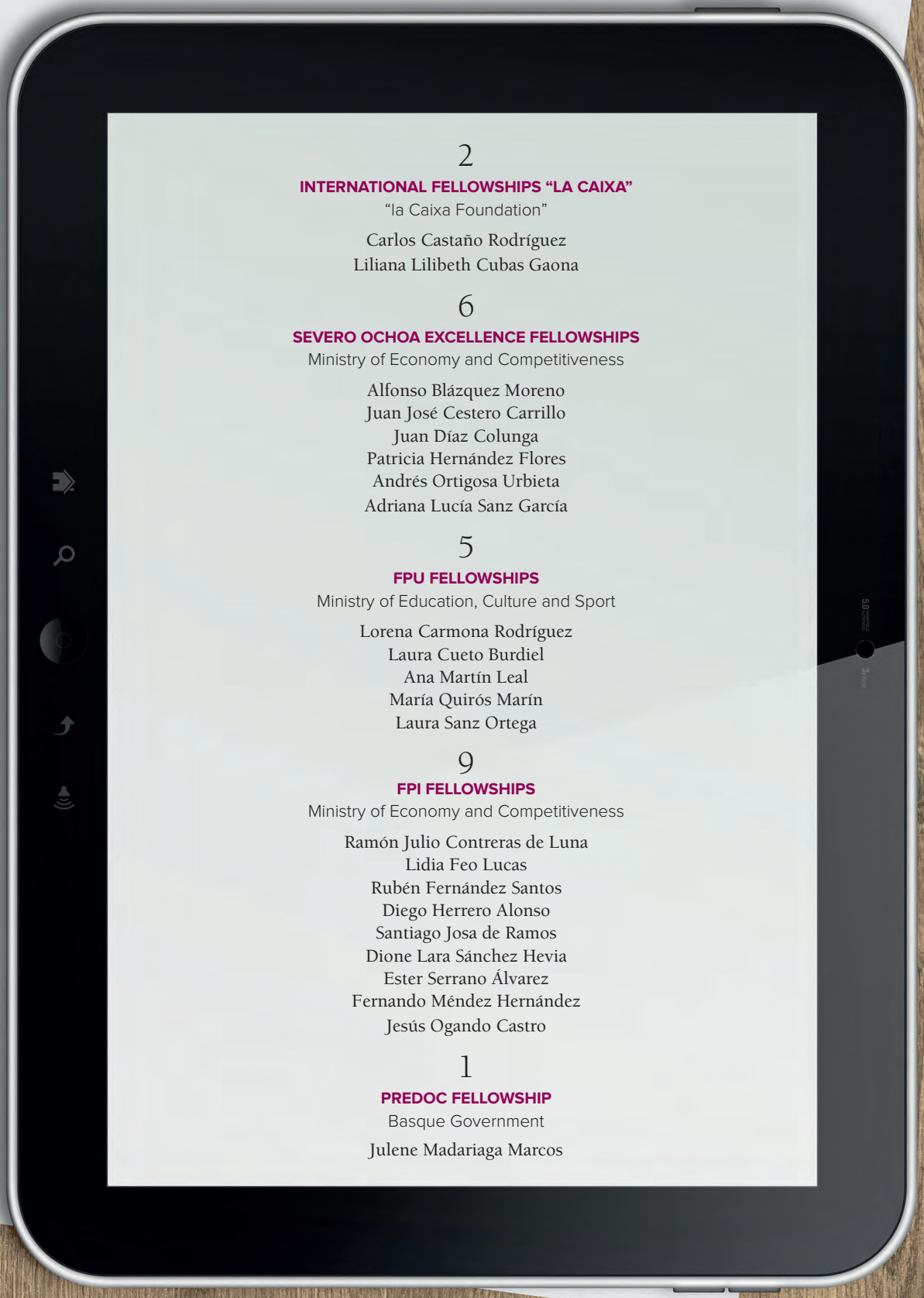
Implicación de p110delta PI3K en la distribución de las células estromales de los órganos linfoides secundarios y de p110gamma PI3K en la proliferación de los macrófagos en las lesiones ateroscleróticas (Domingo F. Barber)

Rafal Zur

Alternative p38 mitogen-activated protein kinases: important players in skin inflammation and skin tumourigenesis (Ana Cuenda)

PhD fellows

In 2013 and 2014, 23 students obtained PhD fellowships



2

INTERNATIONAL FELLOWSHIPS "LA CAIXA"

"la Caixa Foundation"

Carlos Castaño Rodríguez
Liliana Lilibeth Cubas Gaona

6

SEVERO OCHOA EXCELLENCE FELLOWSHIPS

Ministry of Economy and Competitiveness

Alfonso Blázquez Moreno
Juan José Cestero Carrillo
Juan Díaz Colunga
Patricia Hernández Flores
Andrés Ortigosa Urbieta
Adriana Lucía Sanz García

5

FPU FELLOWSHIPS

Ministry of Education, Culture and Sport

Lorena Carmona Rodríguez
Laura Cueto Burdiel
Ana Martín Leal
María Quirós Marín
Laura Sanz Ortega

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FPI FELLOWSHIPS

Ministry of Economy and Competitiveness

Ramón Julio Contreras de Luna
Lidia Feo Lucas
Rubén Fernández Santos
Diego Herrero Alonso
Santiago Josa de Ramos
Dione Lara Sánchez Hevia
Ester Serrano Álvarez
Fernando Méndez Hernández
Jesús Ogando Castro

1

PREDOC FELLOWSHIP

Basque Government

Julene Madariaga Marcos

Scientific Advisory Board

Our external Scientific Advisory Board closely monitors the Centre's scientific activities through periodic evaluations and acts as advisory organ to the Director of the CNB.

Wolfgang Baumeister

Director of the Molecular Structural Biology Department at the Max Planck Institute for Biochemistry, Martinsried, Germany.

Pioneer in the development of cryoelectron microscopy and in the elucidation of the structure of the proteosome. EMBO member and Foreign Honorary Member of the American Academy of Arts and Sciences. Max Planck Research Prize 2000, Luis Jeantet Prize for Medicine 2003, Schleiden Medal 2005, Harvey Prize in Science and Technology 2005.

Maarten Koornneef

Director of the Plant Breeding and Genetics Department at the Max Planck Institute for Plant Breeding Research, Cologne, Germany.

World leading expert in the use of Arabidopsis natural variation for the genetic dissection of complex traits as a source of novel tools for plant breeding. Elected foreign member of the National Academy of Sciences of the USA and the European Academy of Science (Academia Europaea). Silver Medal Award of the International Plant Growth Substances Association.

Juan Luis Ramos

Director of the Biotechnology Area at Abengoa Research, Seville, Spain and Full Professor for Molecular and Cellular Biology at the CSIC research centre Estación Experimental del Zaidín, Granada, Spain.

Internationally recognised expert in the genetics and molecular biology of microbes and their biotechnological use for environmental bioremediation and restoration. Member of the American Society for Microbiology and the European Academy of Microbiology. Rey Jaime I Prize for Environmental Protection 2012.

Anne Ridley

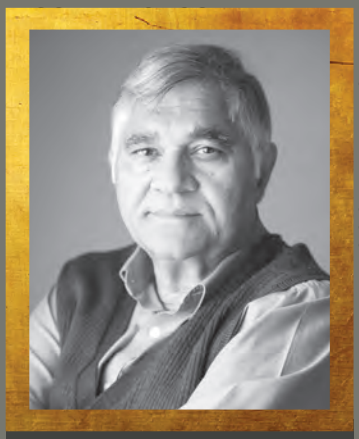
Professor for Cell Biology at King's College London, United Kingdom.

Internationally recognised for her seminal work on signalling in cell migration and how this relates to cancer progression and inflammation. Member of EMBO 2003, Fellow of the Academy of Medical Sciences UK 2012, Chair of the ERC Advanced Grant Panel 2013 for Cellular and Developmental Biology. Hooke Medal 2000 of the British Society for Cell Biology and Liliane Bettencourt Prize for Life Sciences 2004.

Anna Tramontano

Professor of Biochemistry at the University of Rome "La Sapienza", Italy.

Pioneer in the development of computational biology methods for protein structure analysis and their application to problems of biomedical interest. Member of EMBO, the Scientific Council of the ERC, and the European Academy of Science (Academia Europaea). KAUST Global Research Partnership Award 2008 and Tartufari prize 2010 of the Accademia dei Lincei.



Inder Verma

Professor of Genetics, Irwin and Joan Jacobs Chair in Exemplary Life Sciences and American Cancer Society Professor for Molecular Biology at the Salk Institute for Biological Studies, La Jolla (CA), USA.

World leading authority on the development of genetically engineered viruses for gene therapy vectors. Member of the National Academy of Science and the American Philosophical Society. Vilcek Foundation Prize 2008, AAISCR Lifetime Achievement Award 2008, Robert J. and Claire Pasarow Foundation Medical Research Award 2010.

Grants awarded

NATIONAL FUNDING

2013	783,290 €	Spanish Government: **783,290 €** Regional Governments: **0 €** Foundations: **0 €**
2014	12,157,104 €	Spanish Government: **11,896,198 €** Regional Governments: **110,906 €** Foundations: **150,000 €**

INTERNATIONAL FUNDING

2013	3,974,946 €	European Commission: **3,685,955 €** Governmental Agencies: **102,273 €** Foundations: **186,718 €**
2014	492,176 €	European Commission: **0 €** Governmental Agencies: **492,176 €** Foundations: **0 €**

2013
4,758,236 €

2014
12,649,280 €

2013 **5.6**

2014 **6.3**

JCR-INDEXED JOURNALS
489

2013 **266**
2014 **223**

FIRST QUARTILE (Q1) JOURNALS
396 *(81%)*

2013 **209** *(79%)*
2014 **187** *(84%)*

FIRST DECILE (D1) JOURNALS
188 *(38%)*

2013 **89** *(33%)*
2014 **99** *(44%)*

INTERNATIONAL COLLABORATION
281 *(57%)*

2013 **148** *(56%)*
2014 **133** *(60%)*

WITH CNB SCIENTIST AS SENIOR AUTHOR
255 *(52%)*

2013 **142** *(53%)*
2014 **113** *(51%)*

PUBLICATIONS

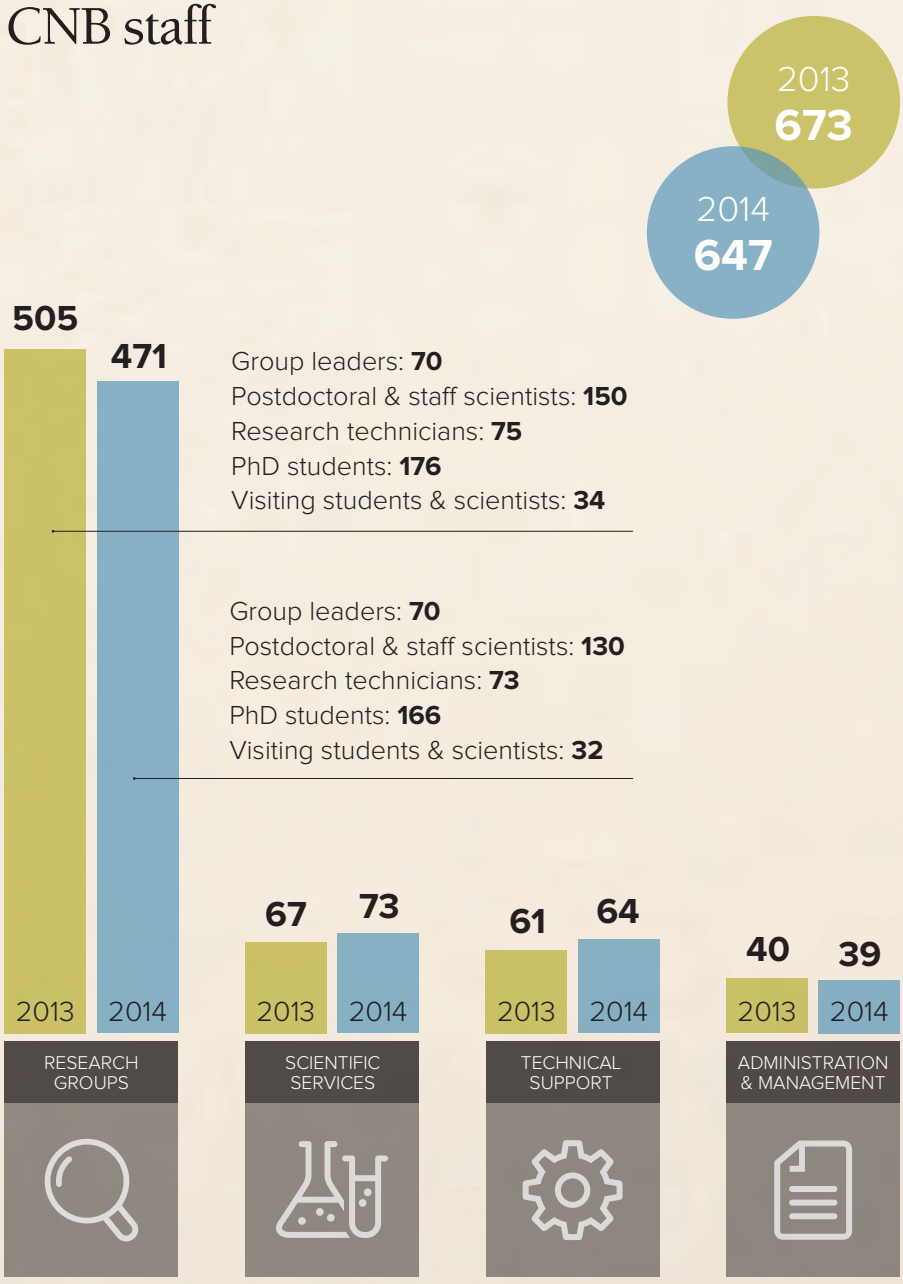
FEMALE	
2013	2014
271 (54%)	**255** (54%)

MALE	
2013	2014
234 (46%)	**216** (46%)

SPANISH	
2013	2014
387 (77%)	**378** (80%)

FOREIGNERS	
2013	2014
118 (23%)	**94** (20%)

CNB staff


2013
673
2014
647
505
471
Group leaders: 70
Postdoctoral & staff scientists: 150
Research technicians: 75
PhD students: 176
Visiting students & scientists: 34
Group leaders: 70
Postdoctoral & staff scientists: 130
Research technicians: 73
PhD students: 166
Visiting students & scientists: 32
67
73
61
64
40
39
2013
2014
RESEARCH GROUPS
SCIENTIFIC SERVICES
TECHNICAL SUPPORT
ADMINISTRATION & MANAGEMENT

Promotion of scientific literacy

GUIDED VISITS

2013	14	462 students
2014	12	367 students

EXTERNAL EVENTS

2013	2	Jornadas de Orientación UAM: 45 students Liceo Europeo: 90 students
2014	3	MediaLab Prado: 54 students Colegio Zurbarán: 60 students Finde Científico: 320 visitors

PRESS RELEASES

2013	13	117 mentions
2014	28	131 mentions

REPORT COORDINATORS:
Alfonso Mora
Yolanda García
Catherine Mark
Peter Klatt
Carmen Castresana

SCIENTIFIC PHOTOGRAPY:
Inés Poveda

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INNOVATION
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Centro Nacional de Biotecnología
Campus de Cantoblanco
Darwin 3, Madrid 28049, Spain
Tel.: [+ 34] 91 585 4500 / Fax: [+ 34] 91 585 4506
www.cnb.csic.es

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