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

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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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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

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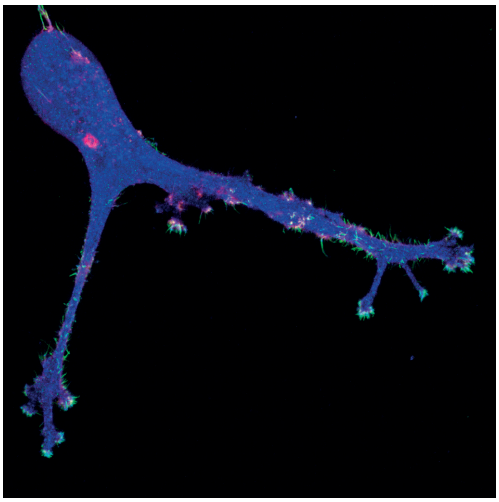

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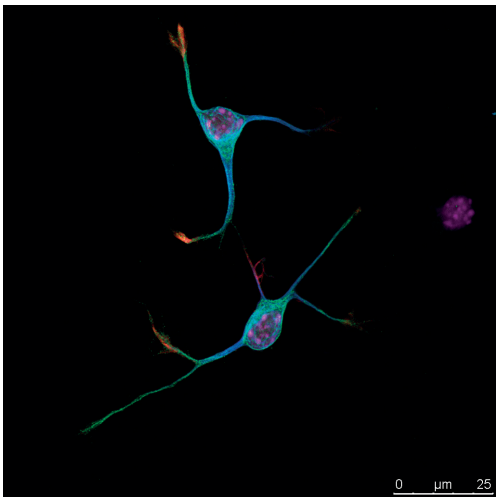

1 *WIP localises at invasive protrusions developed in 3D matrices. Immunofluorescence of invasive MDA-MB-231 cells grown on Matrigel, fixed and stained for WIP (green), N-WASP (blue) and actin (red).*

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

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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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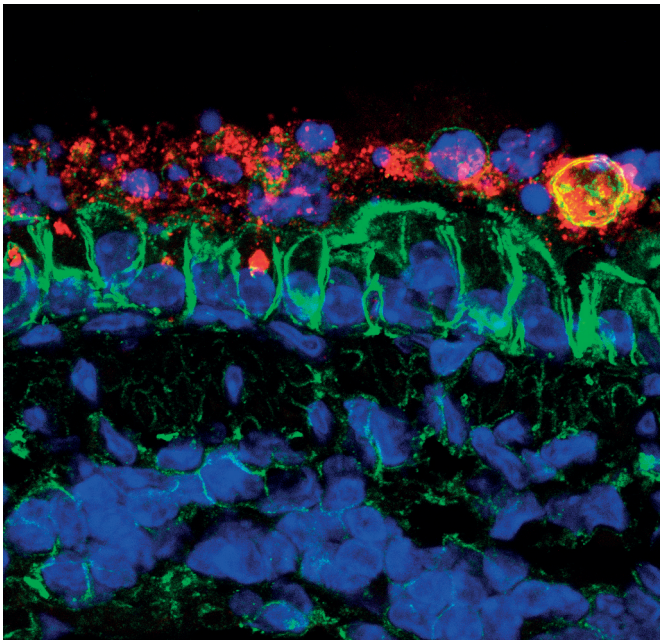

1 *Pulmonary epithelium disassembly induced by SARS-CoV infection in mice. The external layer of the epithelium (green) has been destroyed as a consequence of SARS-CoV infection (red).*

Poxvirus and vaccines

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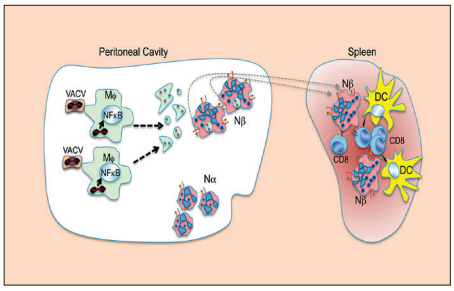
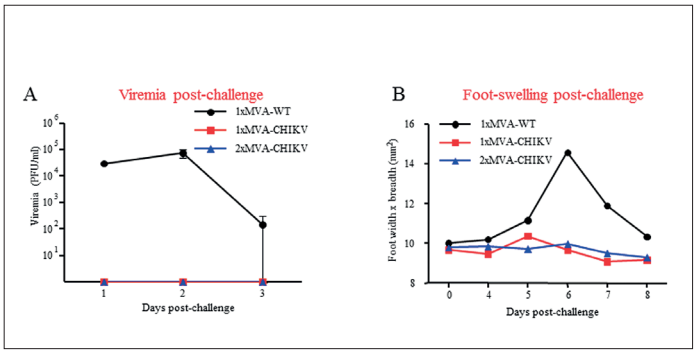
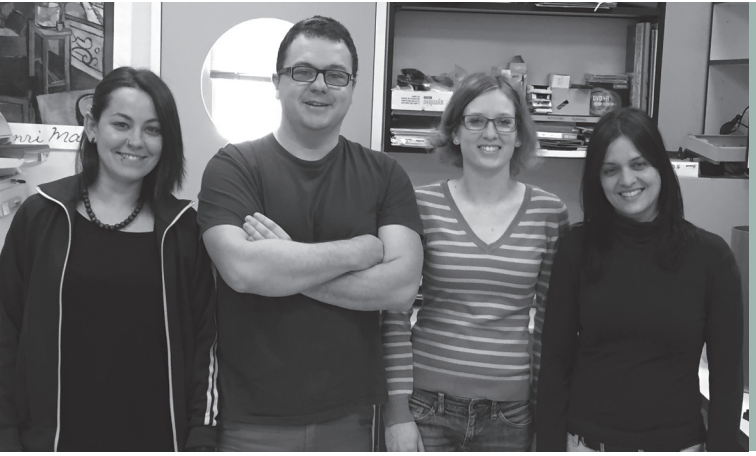
PCT/EP2014/076310. MVA-CHIKV as a vaccine against Chikungunya virus



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



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



Infection by hepatitis C viruses

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STUDENTS:
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Victoria Castro Illana

TECHNICIAN:
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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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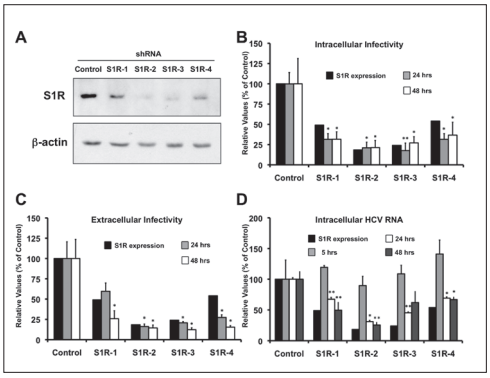

1 *Cellular S1R levels are limiting for HCV infection. Huh-7 cells were transduced with lentiviral vectors expressing an irrelevant sequence or shRNA targeting S1R mRNA, and infected with the cell-culture-adapted HCV D183v at a multiplicity of infection (m.o.i.) of 10. A) Western blot analysis of total 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).*

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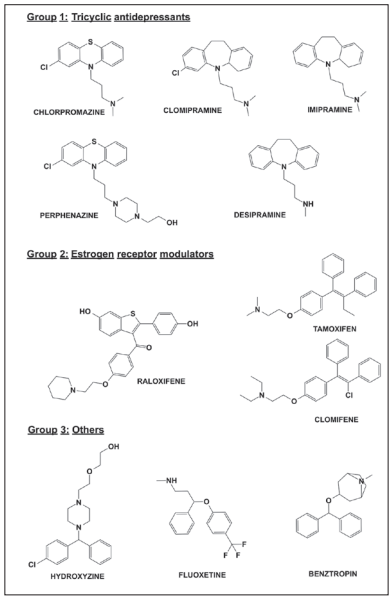

2 *Chemical structure of the primary screening hits. These include previously identified anti-HCV compound family members such as tricyclic antidepressants and synthetic oestrogen receptor modulators. Hydroxyzine and benztropine were further characterised.*

Biological noise

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PREDOCTORAL STUDENT:
Teresa Trigueros

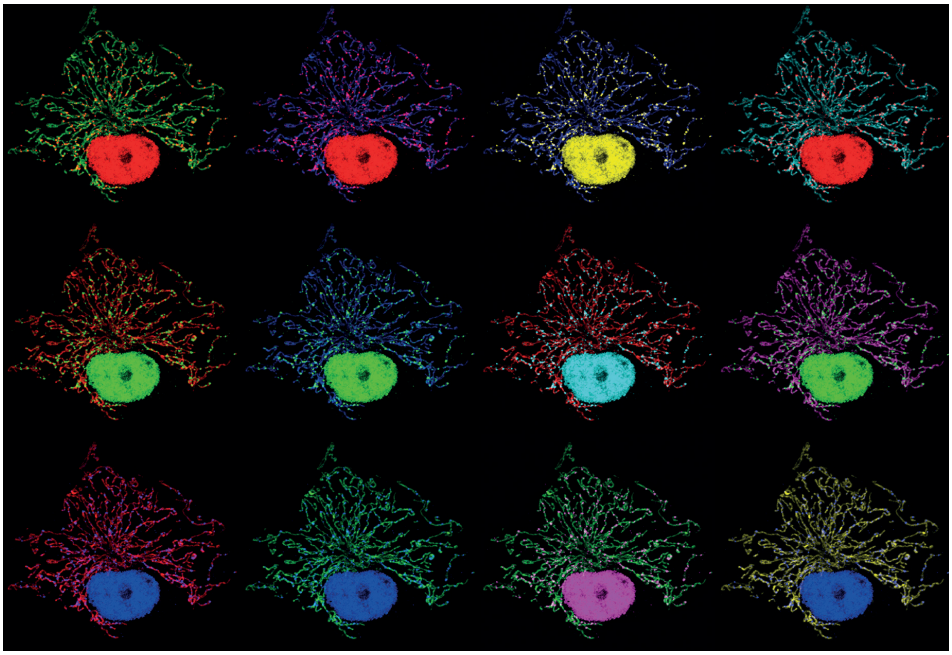
SELECTED PUBLICATIONS

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

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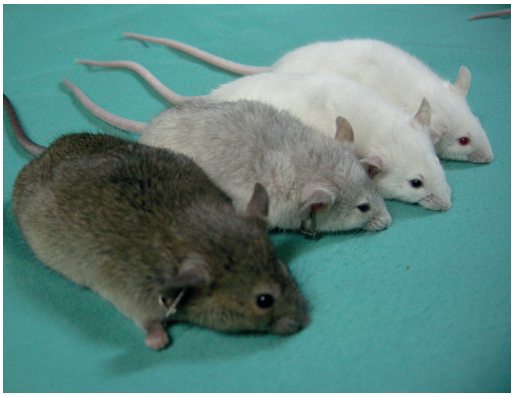
We are interested in understanding the function of regulatory elements necessary to identify gene expression domains in mammalian genomes and that help to specify gene expression patterns in space and time. Our basic aim is to improve adequate interpretation of the genome, especially the function of 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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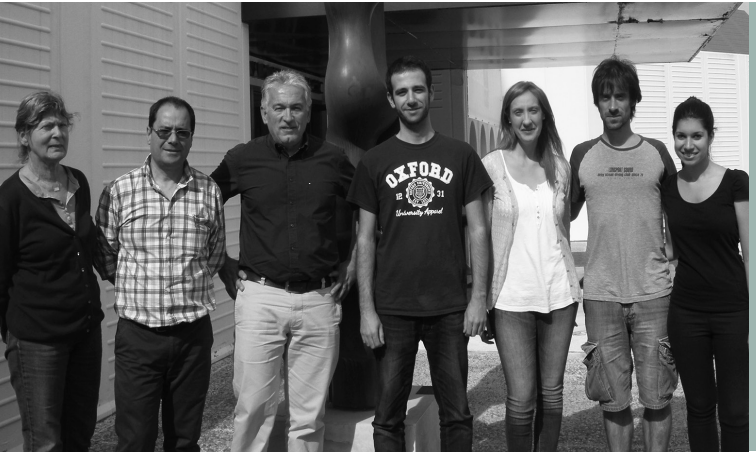

Functional analysis of the transcriptional repressor DREAM

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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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PATENTS

P9312EP00. Methods for the prognosis and diagnosis of neurodegenerative diseases

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

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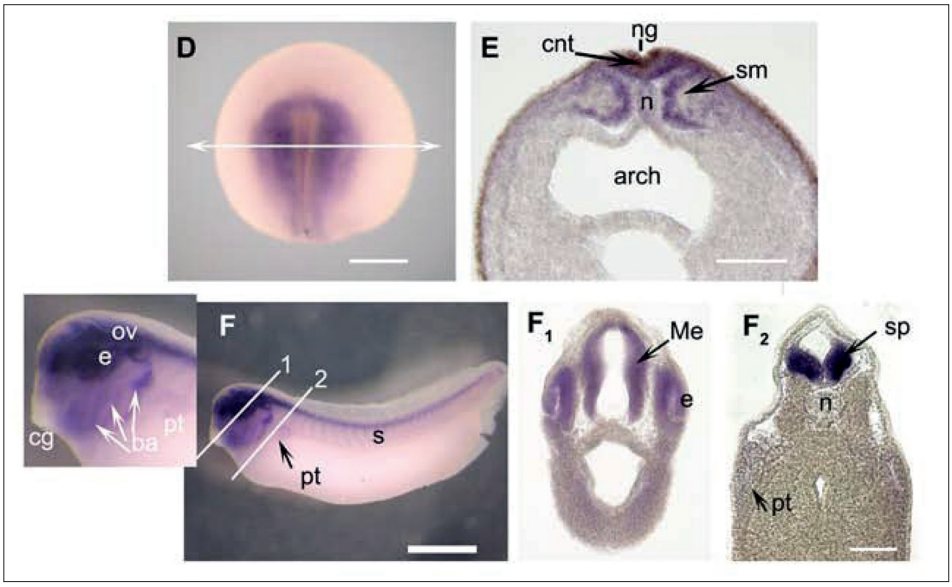

1 *Kchip1 mRNA distribution during Xenopus embryonic development. (D) KChIP1 is strongly expressed in the entire neural plate. (E) Detail showing the expression in the neural groove (ng), the closing neural tube (cnt) and somitic mesoderm (sm). Arch, archeteron, n, notochord. (F) In stage 33/34 larvae, 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

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

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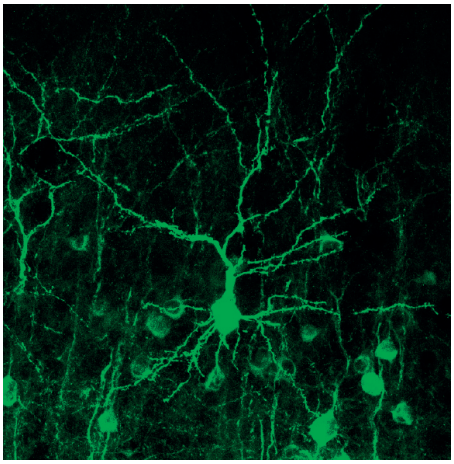
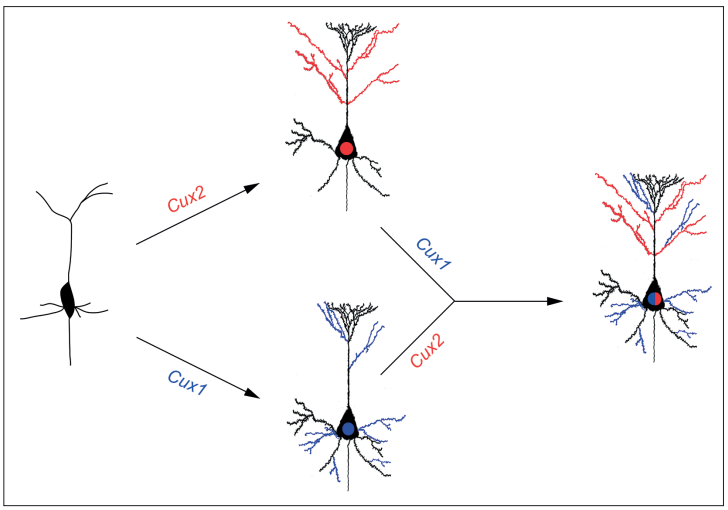
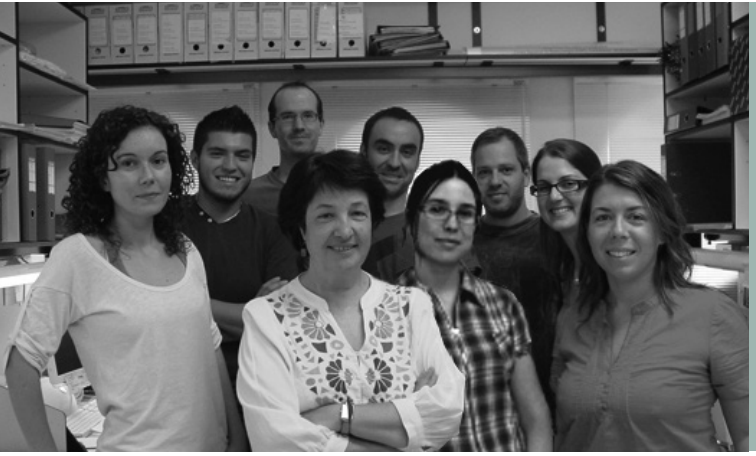


1 GFP reveals the morphology of a layer II-III neuron. Representative confocal micrograph showing GFP-expressing neurons in the somatosensory cortex in mice. Neuron morphology was analysed after in utero electroporation of embryonic day 15 cortical precursors with the CAG-GFP vector.

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

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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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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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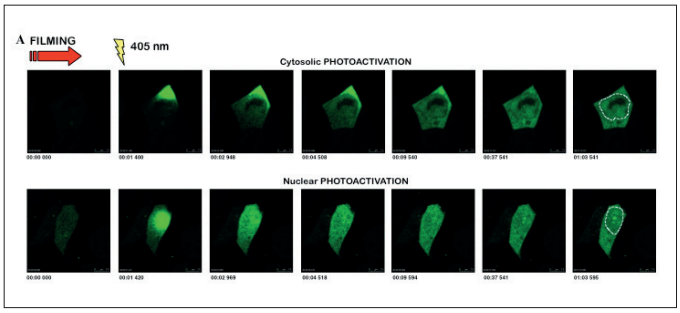

1 *hCLE shuttles in and out of the nucleus. Cultured HEK293T cells were transfected with phCLE-PAGFP (photoactivatable GFP) plasmid and were used for live cell microscopy at 24 h post-transfection. Photoactivation was applied in the cytosol (top panel) to visualise hCLE import. Photoactivation was applied 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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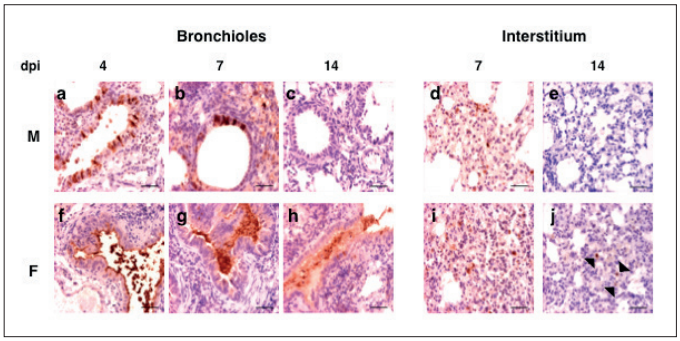

2 *Histopathology of lungs of mice inoculated with M (mild) virus (a-d) or F (fatal) virus (e-h). Haematoxylin/eosin staining; bar = 50 μm. Lung of mouse inoculated with M virus. a) 4 dpi, Mild congestion and diffuse lymphoid and phagocytic cells infiltrate the interstitium. b) 7 dpi, Moderate thickening 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

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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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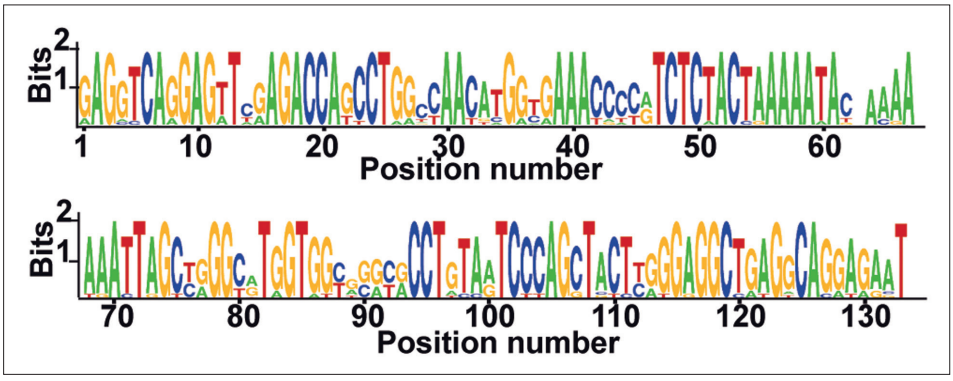

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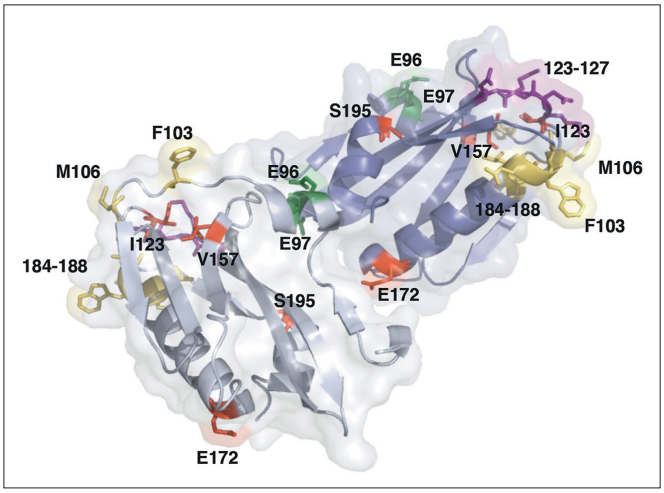

1 *Comparative sequence analysis of the mRNAs specifically associated to wt-hStau1 complexes. Sequence motif found by alignment of the protected sequences present in the mRNAs preferentially associated to wt-hStau1 complexes (de Lucas et al., Nucleic Acids Res 2014).*

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

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SELECTED PUBLICATIONS

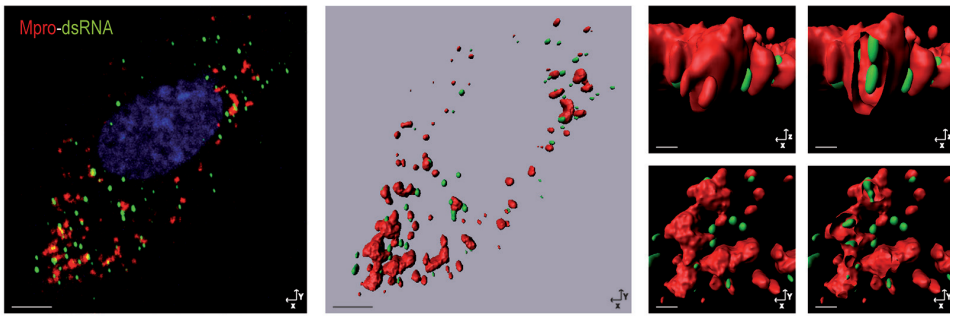
Pignatelli J, Alonso-Padilla J, Rodríguez D. Lineage specific antigenic differences in porcine torovirus hemagglutinin-esterase (PToV-HE) protein. Vet Res 2013; 44:126

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

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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.

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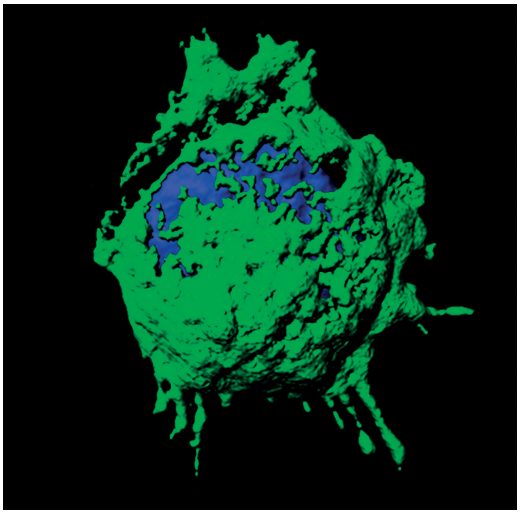
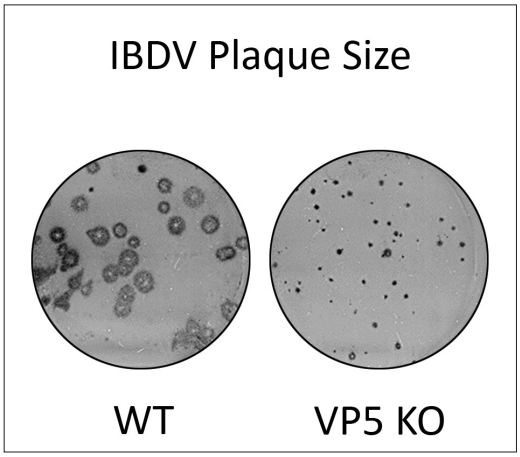


The release of IBDV particles is customarily viewed as a process directly linked to the destruction of infected cells. Recent data from our laboratory nonetheless strongly suggest that IBDV also uses an alternative non-lytic cell-to-cell spreading mechanism. This mechanism appears to be strictly dependent 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.

2



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

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

PATENT

P9571EP00. VLP, obtention methods and applications thereof.

Embryonic development and differentiation in vertebrates

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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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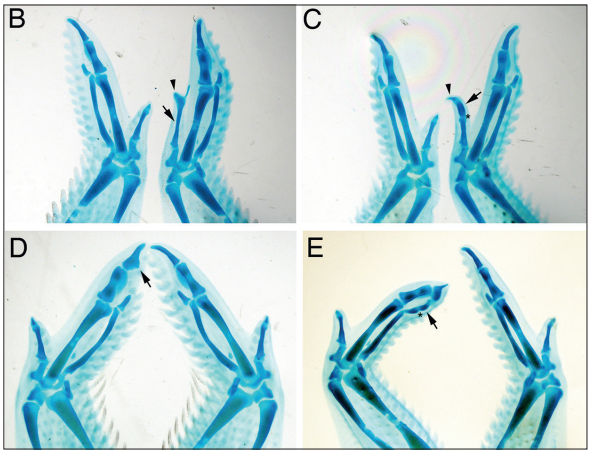

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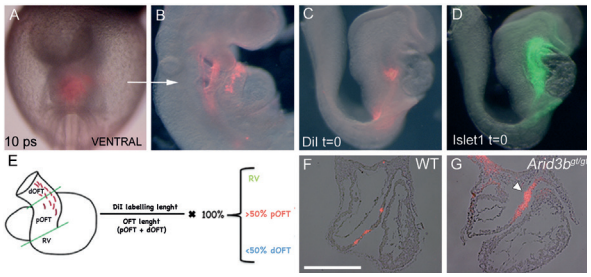
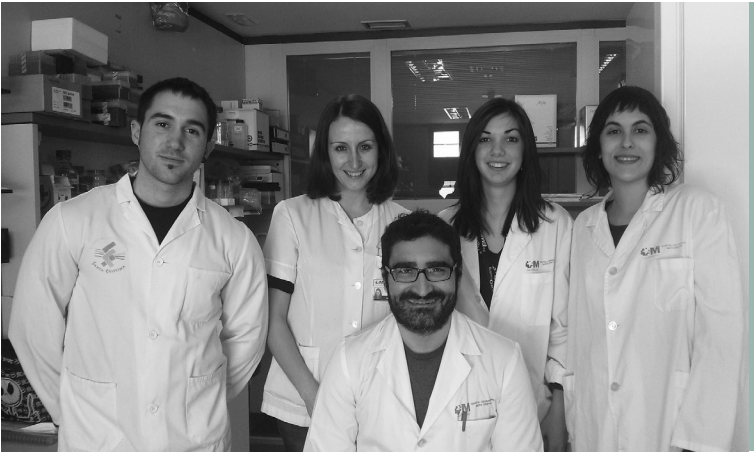


1 *Fgf8 induces elongation and extra phalanges in digits 1 and 3, but not digit 2 of the chick wing. Beads soaked in Fgf8 were applied to the first (B,C) or second (D,E) interdigital spaces at the time of initial digit condensation. Five days after the operation, embryos were collected and stained with 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

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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.

SELECTED PUBLICATIONS

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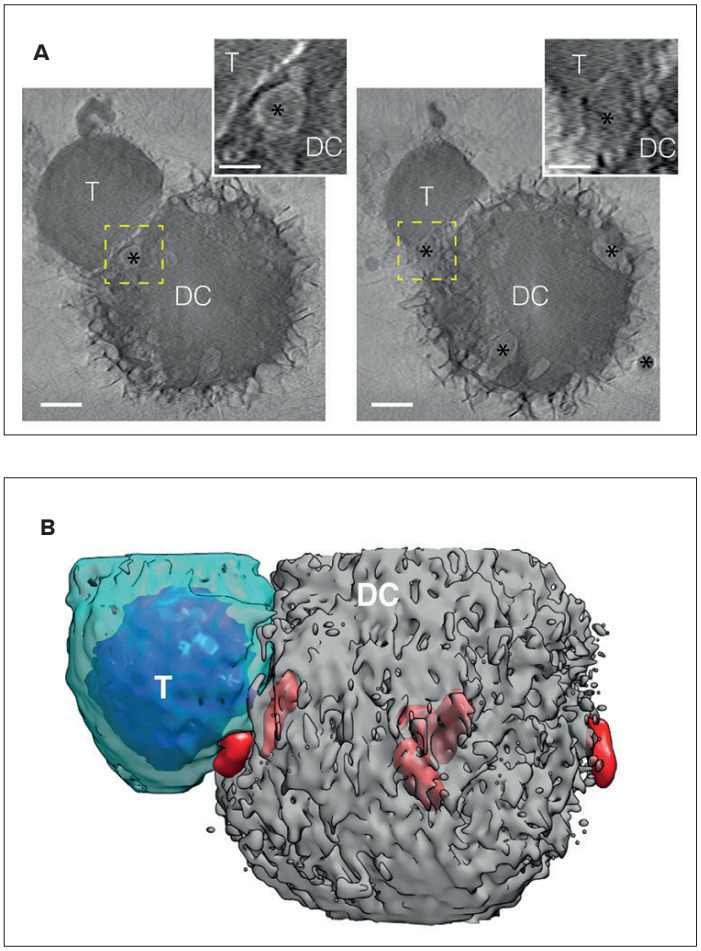

1 *(A) Cryo soft X-ray tomogram of transinfection. (B) Volumetric representation of the tomogram in A. Bacteria are shown in red, T cells, cyan, and DC, gray. The T cell nucleus is shown in blue (Cruz-Adalia et al. Cell Host Microbe 2014).*