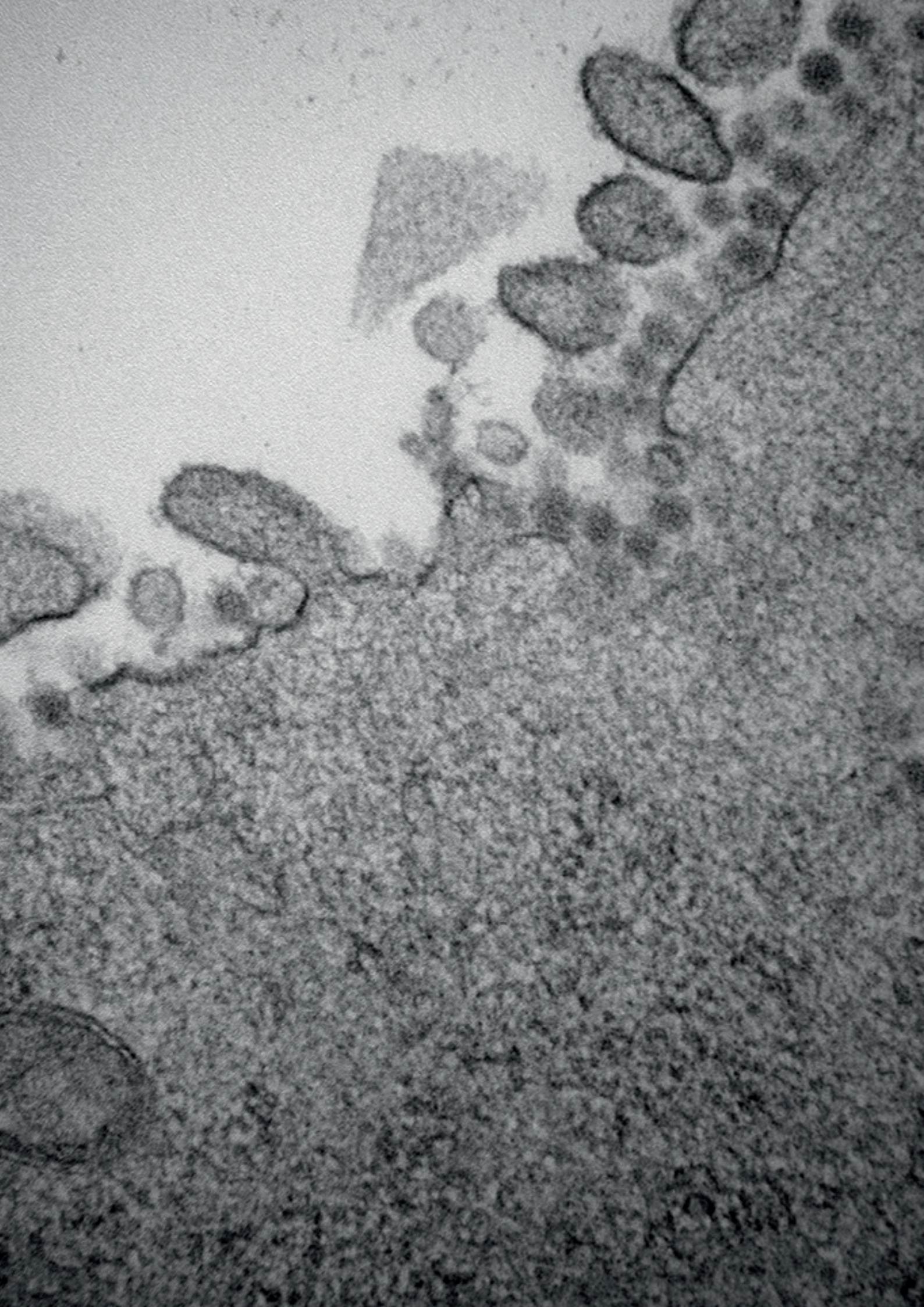

2

MOLECULAR AND CELLULAR BIOLOGY

The Department of Molecular and Cellular Biology hosts 14 independent research groups working in two broad, closely interwoven research areas, with the goal of identifying specific therapeutic targets for use in disease prevention and control. The first area focuses on dissecting viral replication mechanisms and on 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 characterizing 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 help to provide essential scientific background for the development of new biotechnological tools.

During this period our department has undergone some changes. Last year saw the retirement of prominent virologist Juan Ortín; a few months later, the young virologist Urtzi Garaigorta joined our department.

HEAD OF DEPARTMENT

Marta Nieto

OUR RESEARCH GROUPS

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

Non-lytic IBDV release. Image courtesy of José F. Rodríguez

Molecular basis of actin cytoskeleton reorganization in neuritogenesis, cell motility, tumour generation and invasiveness

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Alterations in the PI3K-Akt-GSK3/mTORC pathways are the basis of aging-related pathologies, such as neurodegenerative disorders (Alzheimer's disease) and cancer. Our research interests centre on the role of actin-binding elements in the regulation of Akt-mediated signalling routes that control motility, invasiveness and survival, and how they contribute to neuron differentiation/degeneration or tumourigenesis. Our model actin-binding proteins are (N)WASP (neural Wiskott-Aldrich syndrome protein), WIP (WASP-interacting protein) and WIRE (WIP-related).

Using animal models, recombinant lentivirus, and advanced imaging techniques (two- and three-dimensional cultures) in combination with biochemical and proteomic approaches, we have described a role for WIP in neuron differentiation through the regulation of survival pathways (PI3K-Akt and mTORC1); in addition, we showed how Akt/FoxO/bim contribute to tumourigenesis. Most tumours are initiated and maintained from a subpopulation of migratory and invasive mesenchymal cancer stem cells (CSC), responsible for acquisition of aggressive tumour phenotypes resistant to many therapeutic treatments and responsible for tumour recurrence. These mesenchymal CSC are thus attractive targets for novel therapeutic approaches.

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

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Franco A, Wandosell F, IM Anton. Neuritic complexity of hippocampal neurons depends on WIP-mediated mTORC1 and Abl activities. Brain Behav 2015; 5 (11) e00359

García E, Ragazzini, C, Yu X, Cuesta-García E, Zech T, Sarrio D, Machesky LM, IM Anton. WIP and WICH/WIRE co-ordinately control invadopodium formation and maturation in human breast cancer cell invasión. Sci Rep 2016; 6: 23590

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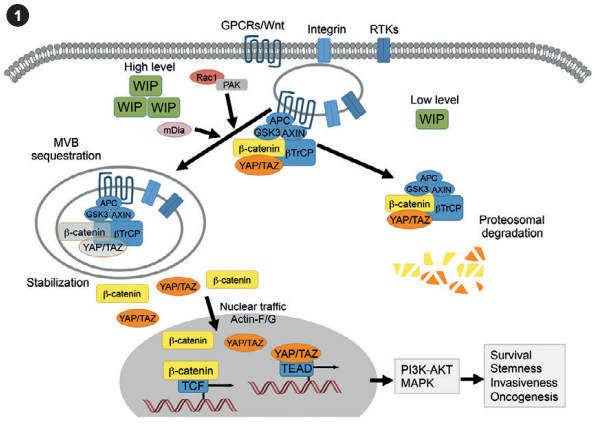
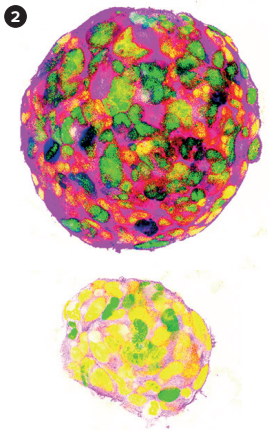
Vijayakumara V, Monypenny J, Machesky L, Lilla S, Thrasher AJ, Antón IM, Calle Y, Jones GE. Tyrosine phosphorylation of WIP releases bound WASP and impairs podosome assembly in macrophages. J Cell Sci. 2015; 128: 251-65





1 WIP drives tumourigenesis by regulating the endocytic system to promote YAP/TAZ and beta-catenin stability.

2 WIP expression is increased in highly proliferative glioblastomas. Confocal images of WIP (pink) and Ki67 (green) expression and DAPI (yellow) nuclear staining in tumour spheres from high WIP-expressing (top) and low WIP-expressing (bottom) human glioblastomas.

Coronavirus: replication, virus-host interactions and protection

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(CRCM-INSERM, Marseille, France)
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SELECTED PUBLICATIONS

Regla-Nava JA, Nieto-Torres JL,..., Enjuanes L, DeDiego ML. SARS CoV with mutations in E protein are attenuated and promising vaccine candidates. J Virol 2015; 89: 3870-3887

Sola I, Almazán F, Zúñiga S, Enjuanes L. Continuous and discontinuous RNA synthesis in CoV. Annu Rev Virol 2015; 2: 265-288

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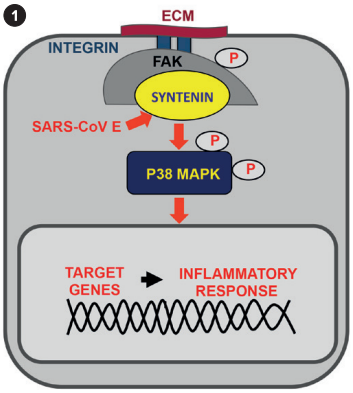
Human infections of the lower respiratory tract are a growing health problem. The annual number of hospital admissions due to pneumonia and acute respiratory distress syndrome was estimated during 2010 at 11.9 million worldwide. The problem is even greater in the elderly population, which responds to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

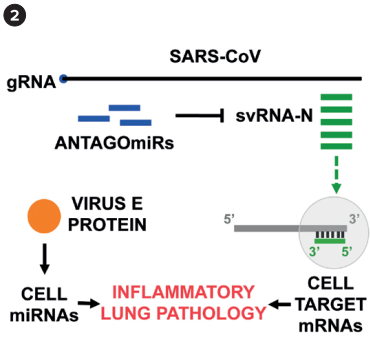
Viruses are responsible for most respiratory infections; among them, human coronaviruses (CoV) are the cause of up to 15% of all respiratory problems. Six human CoVs have been described, HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, SARS-CoV, and MERS-CoV, the last two of which cause deadly infections.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

The main aims of our research are to:

• Identify CoV genes responsible for virus virulence, in order to delete or modify them using reverse genetics, to develop attenuated viruses that are promising vaccine candidates, and to determine their effectiveness in animal model systems.

• Identify cell-signalling pathways involved in CoV replication and pathology, and to select antiviral drugs that inhibit these pathways and interfere with virus replication. In particular, we study cell phosphorylation networks that affect viral proteins and contribute to virus virulence.





• Determine how CoVs influence the magnitude and class of the innate immune response to induce pathogenesis. Special attention is paid to induction of inflammation and to inflammasome activation, since overstimulation of these pathways appears to be responsible for an increase in fatalities during SARS-CoV and MERS-CoV epidemics.

• Study the RNA epigenetic regulation of the innate immune response in CoV infection by determining the contribution of non-coding host miRNAs and virus-derived small RNAs to the inflammatory lung pathology, to identify small non-coding RNAs as antiviral targets.

1

In infections by SARS-CoV, its envelope (E) protein is expressed. Virus virulence is conditioned by this protein through interaction with syntenin, a cell protein that promotes p38 MAPK phosphorylation and NF-kappaB activation; in mice, this leads to lung inflammation, oedema and death, which resembles the case of SARS-CoV-infected human patients.

2

Contribution of host and viral small non-coding RNAs to SARS-CoV lung pathology. Host microRNAs (miRNAs) expressed during infection by a virulent virus encoding the E protein were significantly enriched for cytokine-mediated inflammatory pathways compared with attenuated SARS-CoV-ΔE, suggesting that E protein-induced inflammatory response is in part coordinated by miRNAs. The discovery of small viral RNAs (svRNAs) derived from the SARS-CoV genome reveals a novel aspect in coronavirus-host interaction. During infection, a svRNA derived from virus N gene (svRNA-N) contributes to the enhancement of SARS-CoV-induced lung inflammatory pathology. Antisense svRNA-N inhibitors (ANTAGOmiR) significantly reduced pulmonary inflammation *in vivo* infection.

Poxvirus and vaccines

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SELECTED PUBLICATIONS

Di Pilato M, Mejías-Pérez E,... Esteban M. NFκB activation by modified vaccinia virus as a novel strategy to enhance neutrophil migration and HIV-specific T-cell responses. Proc Natl Acad Sci U S A. 2015; 112: E1333-42

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The main objectives of our laboratory are to understand the molecular basis of the pathogenesis of infectious agents and their interaction with the host, and to use this knowledge to develop effective vaccines for human diseases such as HIV/AIDS, hepatitis C, chikungunya, ebola, malaria, leishmaniasis, and cancer. As a model system of infectious agent and as a delivery vector for expression of genes of interest, we used vaccinia virus (VACV), a member of the poxvirus family.

Our aim is to translate basic science into preclinical and clinical assays, optimizing the immunogenicity of the poxvirus vectors MVA and NYVAC, identifying immune mechanisms and correlates of protection, leading to vaccine candidates for prevalent human diseases.

We maintain a fruitful collaboration with HIV vaccine experts in the USA and Europe through CAVD projects financed by Bill and Melinda Gates Foundation and the EU H2020 programmes, as well as with other EU colleagues developing ebola and chikungunya vaccines.

Main achievements in 2015-2016:

1. We performed the first therapeutic phase I clinical trial in Spain with the HIV/AIDS vaccine candidate MVA-B developed by our group. The vaccine activates CD4+ T cell responses and induced a trend to reduce viral load after antiretroviral interruption.
2. We developed a vaccine candidate (MVA-CHIKV) that fully protects mice and monkeys against chikungunya virus (CHIKV), an RNA virus that causes severe articular pain and is spreading worldwide through the bite of the tiger mosquito *Aedes albopictus*.
3. We developed a more potent HIV vaccine candidate vector than that used in the prophylactic phase III clinical trial (RV144) in Thailand.
4. We optimized the ability of the NYVAC vector to induce relevant HIV-specific immune responses by deleting type I and II interferon immmunomodulatory viral genes.
5. We identified a mechanism of HIV immune activation through modulation of the functional switch of neutrophils.

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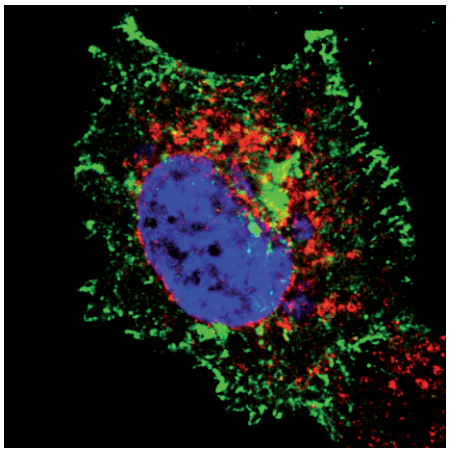

1 The chikungunya recombinant vaccine candidate MVA-CHIKV expressing E2 virus protein (green) during virus infection. Red, endoplasmic reticulum; blue, nuclei

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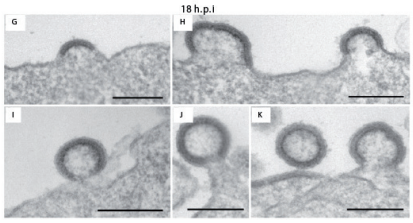

2 The HIV-1 recombinant vaccine candidate NYVAC-GPN producing VLP during infection

Hepatitis C virus infection

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SELECTED PUBLICATIONS

Benedicto I, Gondar V, Molina-Jiménez F, García-Buey L, López-Cabrera M, Gastaminza P, Majano PL. Clathrin mediates infectious hepatitis C virus particle egress. J Virol 2015; 89: 4180-90

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Pérez-Berná AJ, Rodríguez MJ, Chichón FJ, Friesland MF, Sorrentino A, Carrascosa JL, Pereiro E, Gastaminza P. Structural Changes In Cells Imaged by Soft X-ray Cryo-Tomography During Hepatitis C Virus Infection. ACS Nano 2016; 10: 6597-611

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

Our laboratory studies pathogenic human viral infections, and centres on understanding the molecular basis of viral pathogenesis and identifying new molecular targets for antiviral therapy. Our final aim is to propose new therapeutic approaches for antiviral treatment and for reversion of virus-induced pathogenesis. We consider that determining the cellular and molecular mechanisms by which the virus replicates will provide new opportunities in the fight against clinically relevant human pathogens.

To achieve these general aims, we use cell culture models of infection, in which pharmacological and genetic manipulation of the system enables study of the fundamental aspects of virus-host interactions. We currently concentrate on the basic aspects of hepatitis C virus infection (HCV). HCV establishes chronic infections in humans, which are for the most part subclinical, but can lead to development of life-threatening pathologies such as cirrhosis and hepatocellular carcinoma (HCC). HCV is the leading cause of the need for liver transplantation worldwide. HCV interference with cell homeostasis, in particular with lipid metabolism, is emerging as a key determinant of disease progression. HCV infection also causes profound stress to the infected cells, which are exploited by the virus to co-opt stress responses and build up the viral machinery and to promote infected cell survival. HCV infection thus constitutes a good model of subversion of cell metabolism by viruses that promote survival of rogue cells as a strategy for persistence. Cell culture models of persistent HCV infection also provide an excellent opportunity to study various aspects of chronic ER and oxidative stress responses, mechanisms that are deregulated in apparently distant human pathologies such as neurodegenerative diseases or cancer. We thus think that the HCV capacity to enable cell survival while inflicting severe damage on the cell can be used as a biological probe to unravel molecular mechanisms shared with other pathologies.

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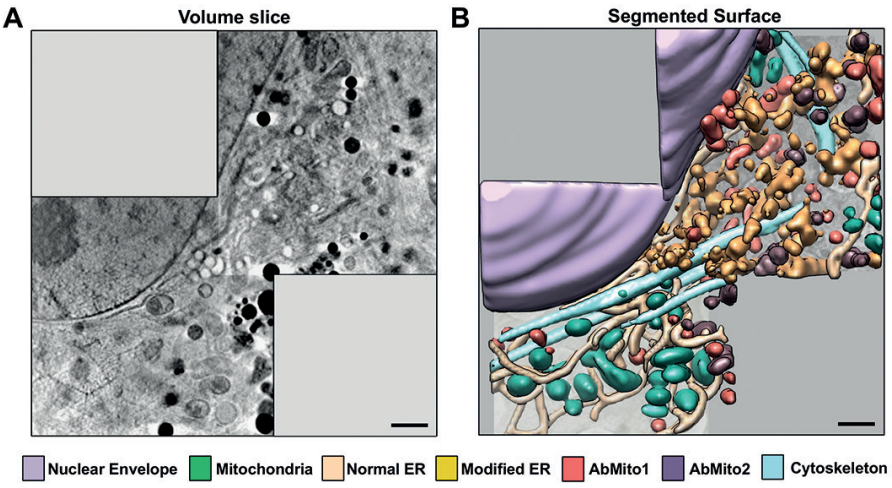
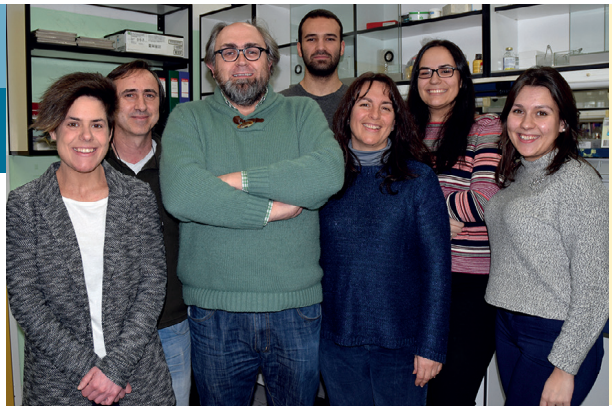


1

Modifications of HCV-infected cytoplasm at early HCV infection stages. Human hepatoma (Huh-7) cells were infected with trans-complemented HCV virions at low multiplicity of infection. Infected cells were identified using a reporter system at very early stages of the virus replication cycle (~16 hours post-infection). Native cells were analysed by soft X-ray tomography to obtain 3D information on whole cell architecture. The figure shows two merged tomograms of adjacent areas of interest in a cell at early stages of infection, where normal and adjacent virus-transformed regions are observed. Volume slice (A) and manually segmented surface representation of selected areas (B) are shown. AbMito1 and AbMito2: anormal mitochondria. Scale bar, 2 µm (from Pérez-Berná *et al.*, 2016).



Biological noise and its physiopathological implications

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SELECTED PUBLICATIONS

Guantes R, Rastrojo A, das Neves R, Lima A, Aguado B, Iborra F. Global variability in gene expression and alternative splicing is modulated by mitochondrial content. Genome Res 2015; 25: 633-644

Sola I, Almazán F, Zuñiga S, Enjuanes L. Continuous and discontinuous RNA synthesis in coronaviruses. Annu Rev Virol 2015; 2: 13.1-13.24

Marquez-Jurado S, Nogales A, Zuñiga S, Enjuanes L, Almazán F. Identification of a gamma interferon-activated inhibitor of translation-like RNA motif at the 3' end of the transmissible gastroenteritis coronavirus genome modulating innate immune response. MBio 2015; 6: e00105-15

Almazán F, Marquez-Jurado S, Nogales A, Enjuanes L. Engineering infectious cDNAs of coronavirus as bacterial artificial chromosomes. Methods Mol Biol 2015; 1282:135-52

Guantes R, Diaz-Colunga J, Iborra F. Mitochondria and the non-genetic origins of cell-to-cell variability: more is different. Bioessays 2016; 38: 64-76

Cells in a clonal population can vary considerably in size, morphology, molecular components and activity. This non-genic heterogeneity (phenotypic variability) is due mainly to differential gene expression and plays an essential role in many important biological processes such as development, cell differentiation, apoptosis, cancer and viral infection. Our group is interested in the study of the origin and consequences of this phenotypic variability.

With regard to the origin of phenotypic variability, we found that mitochondrial content is a global factor that modulates mRNA and protein abundance as well as alternative splicing. With respect to its consequences, we are interested in studying the effects of mitochondrial heterogeneity in important biological processes such as apoptosis, cell differentiation, cancer and viral infection. We provided evidence of a dependency of the completion of the apoptotic program on mitochondrial content. This finding could have great impact on the understanding of tumour relapse and partial response to chemotherapy. Due to the importance of mitochondria in energy generation, metabolism and innate immune signalling, another major research interest of our group is to study the effect of mitochondria variability on virus infections, and how viruses affect mitochondrial metabolism and dynamics, using influenza and Zika viruses as models. These studies will improve our understanding of virus-mitochondria interactions and facilitate identification of new targets for antiviral therapy.

In addition to these basic studies, another aspect of our research interests is the potential translational value of our findings. To this end, we have undertaken a collaboration with several pathologists to study the diagnostic and prognostic value of our discoveries on the role of mitochondria on phenotypic variability.

To achieve these objectives, we have assembled a multidisciplinary research team with experts in the fields of molecular and cellular biology, computational and systems biology and virology.

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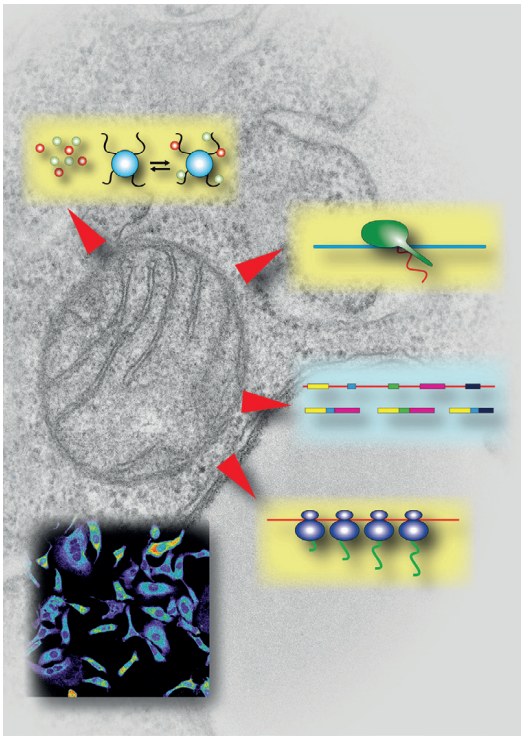

1 Electron micrograph of one mitochondrion with radiating arrows to the steps of gene expression where mitochondria play a role. Modified from cover Bioessays 1/2016

Generating animal models by genetic manipulation

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

We have generated and analysed new animal models to study visual abnormalities that affect retina development associated with albinism and other retinopathies, such as achromatopsia. Using mouse models, we have explored the use of small molecules as potential therapeutic candidates for albinism. In collaboration with Angel Carracedo (University of Santiago de Compostela) and Carmen Ayuso (University Hospital Jiménez Díaz Foundation), within the CIBERER-ISCIII, we devised a proposal for the universal genetic diagnosis of all known mutations in albinism, which we are applying in cooperation with ALBA, the Spanish association in support of people with albinism. We also launched the Albino Day initiative for the first time in Spain, a date in hospital when patients with albinism are explored by experts specialized in this rare genetic condition.

We are also interested in understanding the function of regulatory elements necessary to identify gene expression domains in mammalian genomes and that contribute to specify their expression pattern in space and time. The mouse tyrosinase locus, used as experimental model, has helped us identify genome boundaries or insulators that protect it from surrounding genes. In transgenic animals –zebrafish and mice— we use different types of gene constructs to study the relevance of specific sequences. The functional analysis of regulatory elements within the intergenic sequences can be now addressed more efficiently, thanks to the new CRISPR-Cas9 gene modification system, whose application in mice we pioneered in Spain and implemented successfully in our laboratory.

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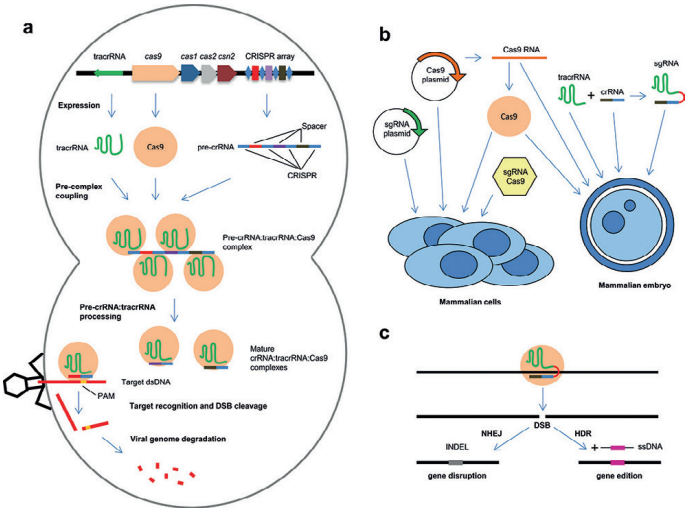

1

The natural bacterial CRISPR-Cas system for defence against viruses (a) and derived genome editing tools in mammalian cells (b, c) (from Mojica FJM and Montoliu L, 2016).

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Seruggia D, *et al.* Functional validation of mouse tyrosinase non-coding regulatory DNA elements by CRISPR-Cas9-mediated mutagenesis. Nucleic Acids Res 2015; 43: 4855-67

Scavizzi F, *et al.*Blastocyst genotyping for quality control of mouse mutant archives: an ethical and economical approach. Transgenic Res 2015; 24:921-7

Wang J, *et al.* MIR retrotransposon sequences provide insulators to the human genome. Proc Natl Acad Sci USA 2015; 112: E4428-37

Oliveros JC, *et al.* Breaking-Cas-interactive design of guide RNAs for CRISPR-Cas experiments for ENSEMBL genomes. Nucleic Acids Res 2016; 44: W267-71

Mojica FJ, Montoliu L. On the Origin of CRISPR-Cas Technology: From Prokaryotes to Mammals. Trends Microbiol 2016; 24: 811-20

Functional analysis of the transcriptional repressor DREAM

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Our major research focus is on the multifunctional protein DREAM and its role in the control of calcium homeostasis in health and disease.

DREAM (downstream regulatory element antagonist modulator), also known as calsenilin or KChIP3, is a Ca^{2+}-binding protein of the neuronal calcium sensor (NCS) superfamily that interacts with specific sites in DNA to repress transcription of target genes in a Ca^{2+}-dependent manner. In addition, DREAM interacts with specific proteins to exert various specialized functions in different subcellular compartments. By controlling activity-dependent gene expression and specific protein-protein interactions, DREAM participates in many physiological processes in- and outside the central nervous system. Work from our group and others has shown important regulatory roles for DREAM in learning and memory in the hippocampus, in pain control in the spinal cord, as well as in the immune response, in inflammation, in the thyroid gland and in the placenta. Recent studies also show DREAM involvement in several neurodegenerative disorders including Huntington disease and Alzheimer disease.

DREAM was originally associated with Alzheimer disease because of its interaction with presenilins. Altered neuronal calcium homeostasis and early compensatory changes in transcriptional programmes are nonetheless common features of many neurodegenerative disorders, which offers the opportunity to explore a role for DREAM in these pathologies.

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

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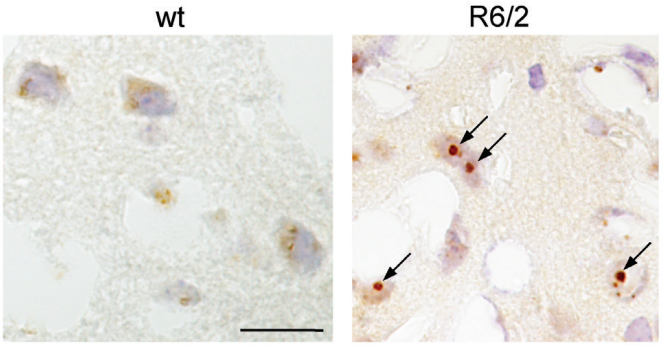

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Immunohistochemistry for ATF6alpha in striatal neurons from wild type (wt) and R6/2 mice. R6/2 is a mouse model for Huntington's disease in which deficient ATF6 processing leads to the appearance of dense intracellular aggregates (arrows). Chronic treatment with repaglinide reduces disease symptoms and the number of ATF6 aggregates. Repaglinide activates ATF6 processing by blocking the interaction between DREAM and ATF6 (from Naranjo , 2016).

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Ruiz-DeDiego I, Naranjo JR, Hervé D, Moratalla R. Dopaminergic regulation of olfactory type G-protein α subunit expression in the striatum. Mov Disord 2015; 30: 1039-49

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Ruiz-DeDiego I, Mellstrom B, Vallejo M, Naranjo JR, Moratalla R. Activation of DREAM, a calcium-binding protein, reduces L-DOPA-induced dyskinesias in mice. Biol Psychiatry 2015; 77: 95-105

Mellström B, Kastanauskaite A, Knafo S, Gonzalez P, Dopazo XM, Ruiz-Nuño A, Jefferys JG, Zhuo M, Bliss TV, Naranjo JR, DeFelipe J. Specific cytoarchitectural changes in hippocampal subareas in daDREAM mice. Mol Brain 2016; 9: 22

Naranjo JR, Zhang H, Villar D, González P, Dopazo XM, Morón-Oset J, Higueras E, Oliveros JC, Arrabal MD, Prieto A, Cercós P, González T, De la Cruz A, Casado-Vela J, Rábano A, Valenzuela C, Gutierrez-Rodriguez M, Li JY, Mellström B. Activating transcription factor 6 derepression mediates neuroprotection in Huntington's disease. J Clin Invest 2016; 126: 627-38

Cerebral cortical development

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SELECTED PUBLICATIONS

Cubelos B, Briz CG, Esteban-Ortega GM, Nieto M. Cux1 and Cux2 selectively target basal and apical dendritic compartments of layer II-III cortical neurons. Dev Neurobiol 2015; 75: 163-72

Rodríguez-Tornos FM*, Briz CG*, Weiss LA, Sebastián-Serrano A, Ares S, Navarrete M, Frangeul L, Galazo M, Jabaudon D, Esteban JA, Nieto M. (*equal contribution). Cux1 enables inter-hemispheric connections of layer II-III neurons by regulating Kv1-dependent firing. Neuron 2016; 89: 494-506

Doan RN, Bae B, Johnson MB, Cubelos B, Chang C, Hossain AA, Al-Saad S, Mukaddes NM, Oner O, Al-Saffar M, Balkhy S, Gascon GG, The Homozygosity Mapping Consortium for Autism, Nieto M, Walsh CA. Mutations in human accelerated regions disrupt cognition and social behavior. Cell 2016; 167: 341-354.e12

Our investigations aim at defining the cellular and molecular mechanisms that govern the development of neuronal networks in the cerebral cortex. The cerebral cortex is the most evolved region of the mammalian brain and one of the most complex biological structures known. It is responsible for most aspects of cognition and behaviour. We aim to understand its normal function as well as the mechanisms that fail in disease. A large number of functionally and morphologically different neuron types specify brain cortical areas and coordinately control cerebral functions. We focus on studying how transcription factors (TFs) direct the generation, specification and wiring of these neurons. We aim (1) to understand the intrinsic mechanisms that regulate the progressive specification and proliferation of progenitor cells into an ordered pattern that produces exact numbers of each neuronal type. Once generated, these neurons select their connections precisely and in tight coordination with activity. Shaping the brain in coordination with experience optimizes the functional circuit to respond to the external world. This also occurs in a manner that creates common behaviour between individuals. We are therefore interested in (2) determining how intrinsic TFs translate neuronal identity into aspects of connectivity; (3) studying TF regulation of developmental neuroplasticity in conjunction with activity, and understanding how the inter-related brain circuits (visual, sensory, auditory, etc.) assemble in such a coordinated manner that creates a perfectly functional human brain. Defects in neuron proliferation, specification and connectivity are the cause of different cognitive defects including human retardation, bipolar disorders or schizophrenia. Related mechanisms are involved in neurodegeneration. We thus exploit our basic studies to analyse specific aspects of cortical alterations linked to developmental defects (4). Moreover, we are interested in exploring the limits of plasticity, the capacity for reprogramming and possible boosting strategies to repair the brain (5).

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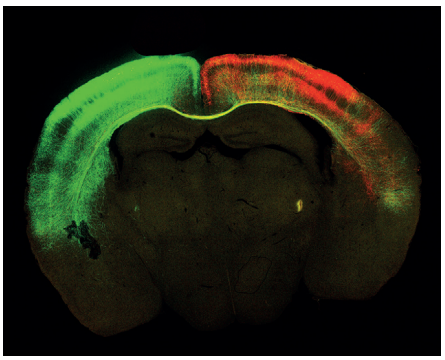
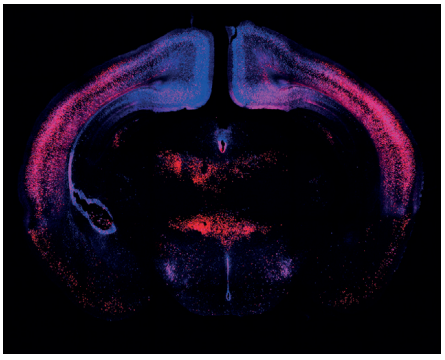


1 Red and green fluorescence labelling of connections of cortical pyramidal neurons in left and right cerebral hemispheres by *in utero* electroporation in mice. Image of a coronal section of postnatal (P) day 16 brain.

2



2 Red and blue fluorescent labelling of connections of the cerebral cortex using axonal tracers.

Mechanisms of interaction between influenza virus and the infected cell

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SELECTED PUBLICATIONS

Falcon A, Cuevas MT, Rodriguez-Frandsen A, Reyes N, Pozo F, Moreno S, Ledesma J, Martínez-Alarcón J, Nieto A, Casas I. CCR5 deficiency predispose to fatal outcome in influenza virus infection. J Gen Virol 2015; 96: 2074-8

Ver LS, Marcos-Villar L, Landeras-Bueno S, Nieto A, Ortín J. The cellular factor NXP2/MORC3 is a positive regulator for influenza virus multiplication. J Virol 2015; 89: 10023-30

Rodríguez A, de Lucas S, Pérez-González A, Pérez-Cidoncha M, Roldan-Gomendio A, Pazo A, Landeras-Bueno S, Marcos-Villar, L Ortín J, Nieto A. hCLE/C14orf166, a cellular protein required for viral replication, is incorporated into influenza virus particles. Sci Rep 2016; 6: 20744

Marcos-Villar L, Pazo A, Nieto A. Influenza virus and the chromatin: Role of CHD1 chromatin remodeler on virus life cycle. J Virol 2016; 90: 3694-707

Rodriguez P, Pérez-Morgado MI, Gonzalez VM, Martín ME, Nieto A. Inhibition of influenza virus replication bt DNA aptamers targeting a cellular component of translation initiation. Mol Ther Nucl Acids 2016; 5: e308

During the past two years we studied the interactions between the influenza virus polymerase and the infected cell, as well as the epigenetic changes induced by the infection. We have made major achievements in two main areas:

1. Cellular proteins that interact with influenza virus polymerase proteins

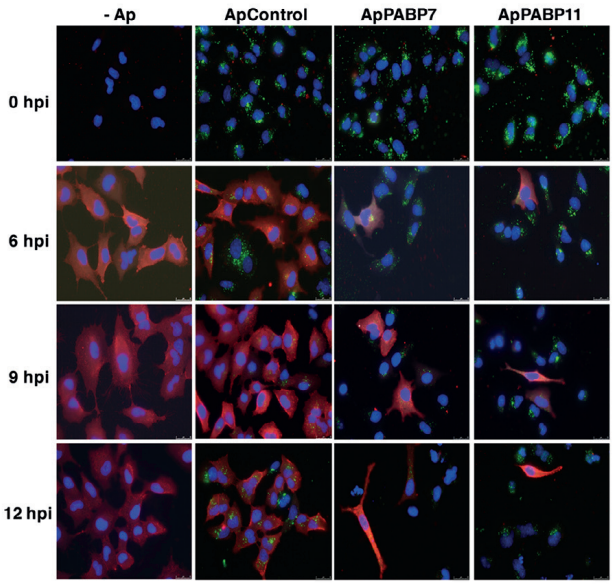
We characterized the interaction of hCLE, a positive modulator of the RNAP II, with viral polymerase complex, its function as a positive modulator of influenza virus replication, and its incorporation in viral particles. With regard to hCLE endogenous function, we previously characterized its activity as a positive modulator of RNAP II, and found that hCLE forms a shuttling complex with DDX1-HSPC117-FAM98B proteins involved in local translation.

Viral polymerase also interacts with the chromatin remodeller CHD1. Once viral transcription no longer requires cellular transcription, CHD1 is degraded in parallel with the cellular RNAP II in a process triggered by the viral polymerase.

We previously reported the interaction between viral NS1 protein and viral polymerase with components of the cellular translation machinery. Now we used DNA aptamers that impair this association; their use decreases viral replication and they might be useful as potential antiviral compounds.

2. Effect of influenza virus infection on chromatin remodellers and epigenetic changes induced in the infected cell

We studied the epigenetic changes of the cellular chromatin that take place during infection. DNA methylation is not modified, but histone modifications are altered. We observed a general decrease in histone acetylation, together with an increase in H3K79 methylation. By inhibiting the specific methylase of this residue, we found that it controls the antiviral response and, hence, influenza virus replication.



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1 Effect of PABP1 aptamers on influenza virus protein and distribution. A549 cells were untransfected or transfected with indicated Alexa 488-labelled aptamers. At 12 hours post-transfection, aptamers were washed and cells were infected with influenza virus at different times post-infection (hpi); immunofluorescence assays were performed using antibodies to HA protein. Alexa 488, green; HA protein, red.

Influenza virus transcription and replication

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SELECTED PUBLICATIONS

Ortín J, Martín-Benito J. The RNA synthesis machinery of negative-stranded RNA viruses. Virology 2015; 479-480: 532-44

Ver LS, Marcos-Villar L, Landeras-Bueno S, Nieto A, Ortín J. The cellular factor NXP2/MORC3 is a positive regulator for influenza virus multiplication. J Virol 2015; 89: 10023-30

Landeras-Bueno S, Ortín J. Regulation of influenza virus infection by long non-coding RNAs. Virus Res 2016; 212: 78-84

Rodriguez-Frandsen A, de Lucas S, Pérez-González A, Pérez-Cidoncha M, Roldan-Gomendio A, Pazo A, Marcos-Villar L, Landeras-Bueno S, Ortín J, Nieto A. hCLE/C14orf166, a cellular protein required for viral replication, is incorporated into influenza virus particles. Sci Rep 2016; 6: 20744

Landeras-Bueno S, Fernández Y, Falcón A, Oliveros JC, Ortín J. Chemical genomics identifies the PERK-mediated unfolded protein stress as a cellular target for influenza virus inhibition. MBio 2016; 7: e00085-16

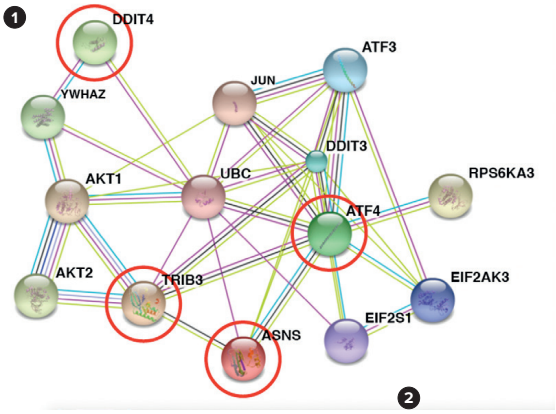
Valcárcel J, Ortín J. Viral cell biology: Influenza raids the splicing store. Nat Microbiol 2016; 1: 16100

In the years 2015-2016, our group continued studies on the interactions of influenza virus with the host cell, analysing the cellular roles of some of the virus polymerase-interacting factors.

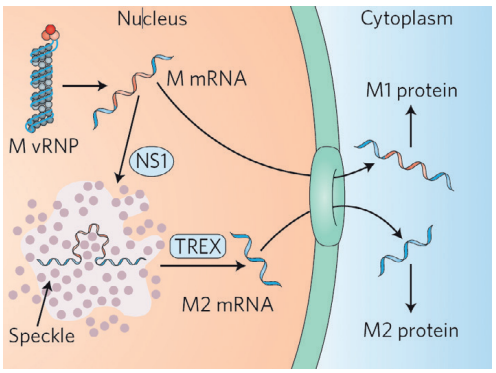
In a previous proteomic analysis of the virus polymerase interaction with host cellular factors, we identified the protein NXP2/MORC3 as potentially relevant, and have now shown that NXP2/MORC3, a member of the nuclear matrix proteins, is important for virus transcription but is not involved in viral RNA replication. In addition, we verified that the cellular transcription factor hCLE/C14orf166 is incorporated into mature virus particles.

As an alternative for identifying cellular genes important for influenza virus replication, we also used a chemical genomics approach. A collection of drugs, previously approved for use in humans, was tested for their capacity to inhibit a GFP-based replicon system adapted for medium-throughput screening. Of more than 700 drugs tested, only montelukast (MK) showed consistent inhibition of all virus strains used. To identify the cellular pathway altered by MK treatment, we used RNA-seq analysis of cells infected or mock infected, untreated or treated with MK. We found that virus infection stimulates the PERK-mediated arm of the UPR pathway, whereas MK can restore this pathway to normal levels, thereby inhibiting virus multiplication.

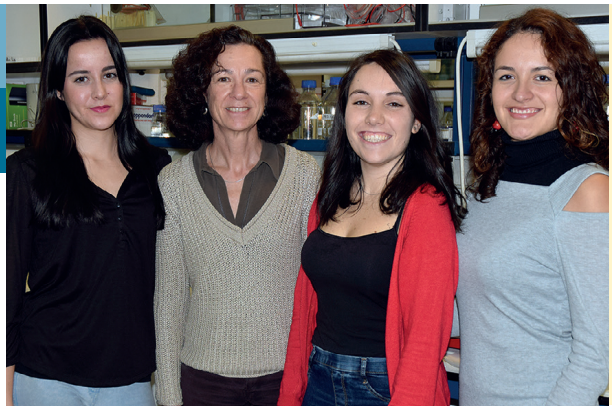
During these years, we also published reviews on the virus RNA replication machinery and virus interaction with the cellular splicing system and with lnc-RNAs.



1 Protein-protein interaction network as visualized by the STRING database. The nodes represent proteins. Red circles show most of the proteins differentially expressed in influenza virus-infected cells following MK treatment.



2 Localization of influenza M mRNA splicing in speckles. When viral M mRNA is transported to the speckled compartment by the viral NS1 protein, it is spliced to generate M2 mRNA, which produces the viral M2 ion-channel protein; otherwise it is translated directly to M1 protein.



Molecular characterization and epidemiology of torovirus

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Toroviruses are emerging viruses that cause enteric diseases in various animal species and in man, and yet remain largely neglected. Previous studies from our laboratory revealed high incidence of porcine torovirus in Spanish livestock. These results, which coincide with those obtained in other countries, reinforce the idea of high worldwide prevalence of toroviruses.

Our research group is interested in understanding the basis of torovirus pathogenesis, for which we have analysed the interaction between the virus and the cell defence mechanisms during torovirus infection. Our recent results show that the equine torovirus (BEV) and its structural proteins, when expressed individually, cause apoptosis of infected cells through the cell death receptor and the mitochondrial pathways. Using different approaches, we demonstrated that the cellular eIF2α phosphorylation kinase PKR plays an essential role in torovirus-induced apoptosis. Inhibition of the PERK kinase nevertheless enhanced the apoptosis produced by BEV and its structural proteins, which indicates that this kinase contributes to survival under the cellular stress caused by viral infection. Intestinal cell death by apoptosis is probably the cause of the diarrhoea produced by toroviruses. We are also studying the antagonist activity of several viral proteins that allow the virus to evade IFN (interferon) antiviral action before progeny viruses are produced. We showed that the BEV M and N proteins suppress IFN α/β expression by acting at different levels of the IFN pathway.

In addition, we used a variety of imaging techniques, including confocal and electron microscopy and electron tomography, to characterize the replication complexes (RTC) built by toroviruses. We identified double membrane vesicles (DMV) in the cytoplasm of BEV-infected cells and found a close relationship between the RTC and the DMV of BEV. Our goal is to characterize the mechanisms that lead to membrane proliferation and vesicle accumulation.

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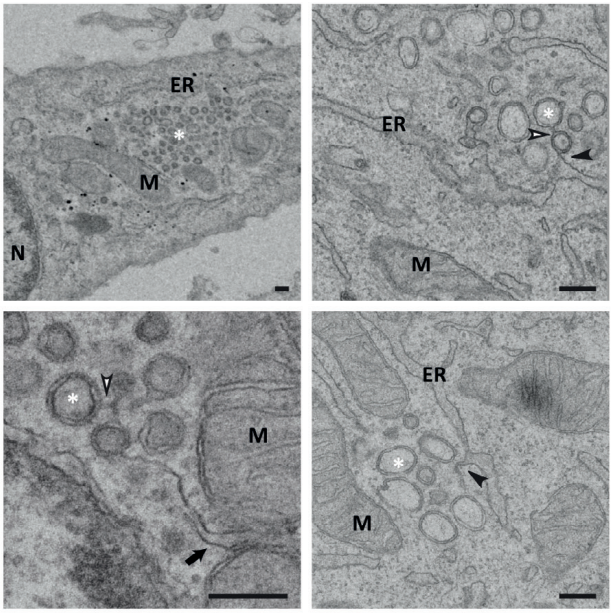

1 Identification of double membrane vesicles (DMV) in cells infected with the equine torovirus Berne virus or BEV. DMV are arranged in clusters in specific areas of the cytoplasm (asterisk), surrounded by ER membranes and mitochondria (M). Connections between DMV (white arrowhead) and between DMV and the ER (black arrowhead) are frequently observed. Seeming continuity between DMV and mitochondrial-associated ER membranes (MAM) was also observed occasionally (black arrow).

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Fernández-Escobar M, Nájera JL, Baldanta S, Rodriguez D, Way M, Esteban M, Guerra S. Suppression of NYVAC infection in HeLa cells requires RNAse L but is independent of PKR activity. PLoS One 2015 10: e0123470

Ávila-Pérez GF, Rejas MT, Rodríguez D. Ultrastructural characterization of membranous torovirus replication factories. Cell Microbiol 2016; 18: 1691–1708

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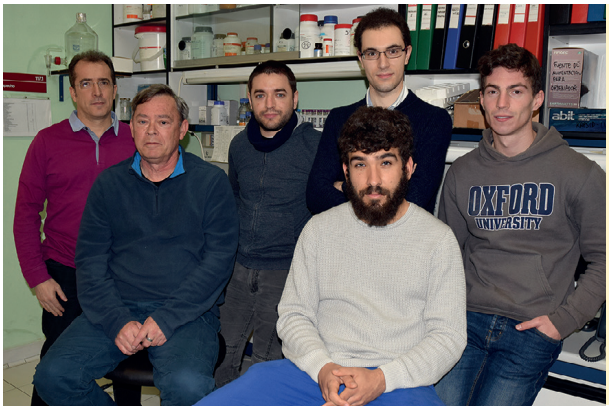

Molecular biology of birnaviruses

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SELECTED PUBLICATIONS

Ferrero DS, *et al.* The structure of the RNA-dependent RNA polymerase of a permutotetravirus suggests a link between primer-dependent and primer-independent polymerases. PLoS Pathog 2015; 11: e1005265

Méndez F, *et al.* Infectious bursal disease virus VP5 polypeptide: a phosphoinositide-binding protein required for efficient cell-to-cell virus dissemination. PLoS One 2015; 10: e0123470

Ferrero D, *et al.* Infectious bursal disease virus VP3 upregulates VP1-mediated RNA-dependent RNA replication. J Virol 2015; 89: 11165-8

Landajuela A, *et al.* Lipid geometry and bilayer curvature modulate LC3/GABARAP-mediated model autophagosomal elongation. Biophys J 2016; 110: 411-22

Lago M, *et al.* Aquabirnavirus polyploidy: A new strategy to modulate virulence? J Gen Virol 2016; 97: 1168-1177

Birnaviruses are unconventional double-stranded (dsRNA) RNA viruses characterized by the lack of an inner transcriptional core, a subviral structure responsible for both RNA metabolism and control of antiviral innate host responses, which are strictly conserved in all other dsRNA viruses. Indeed, Birnaviruses use unique molecular strategies to ensure genome expression/replication and to evade innate antiviral responses. Our main virus model is the infectious bursal disease virus (IBDV), the aetiological agent of an acute immunosuppressive disease that affects domestic animals, causing major losses to the poultry industry worldwide. For many years, our group focused mainly on the functional and structural characterization of the virus as well as on virus particle assembly. Our current work centres on the detection and molecular characterization of mechanisms that allow IBDV to evade and/or counteract host innate immune responses, and to unravel the molecular basis for IBDV virulence. In addition to our IBDV work, we are collaborating with other laboratories that work on different virology and structural biology topics.

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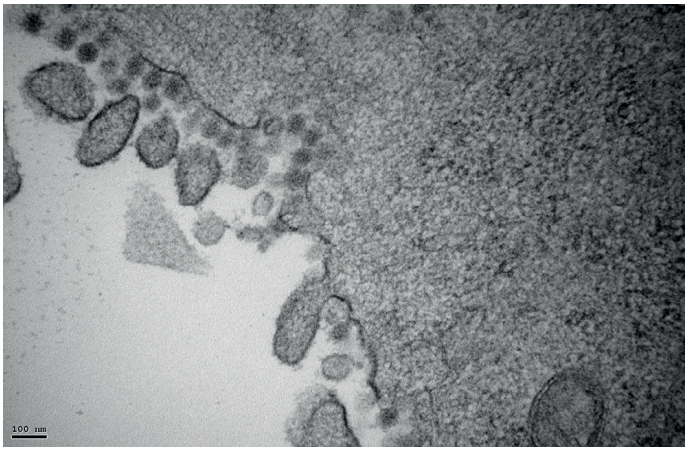
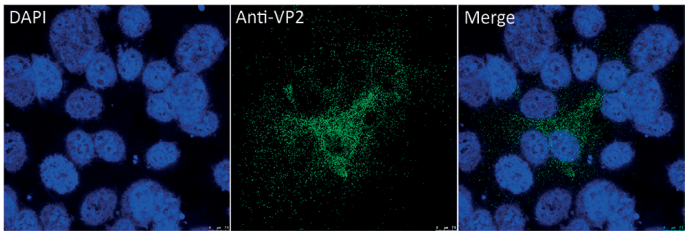
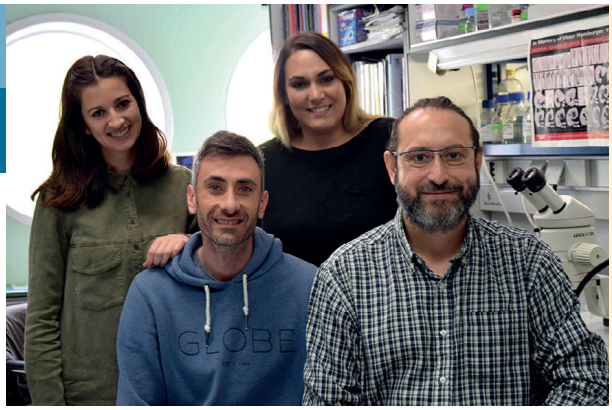


1 **Non-lytic IBDV release.** Transmission electron microscopy (TEM) image of a QM7 cell and IBDV-infected cell, showing newly egressed virus particles attached to the outer plasma membrane leaflet. The infected culture was fixed at 16 h post-infection and processed for TEM analysis. Bar, 100 nm.

2



2 **Non-lytic IBDV release.** Confocal microscopy images from a QM7 cell culture infected with IBDV showing the release of virus particles. The infected culture was fixed at 16 h post-infection and processed for immunostaining with anti-VP2 serum (green). Nuclei were labelled with DAPI (blue). The right panel shows the overlay of both fluorescence signals.



Embryonic development and differentiation in vertebrates

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Ana García

Our group is interested in understanding the molecular and cellular basis of organ formation during embryonic development. This knowledge is important for identifying the origin of congenital malformations and to understand the basis of evolution and regeneration. We use animal models (mouse and chicken embryos) to address several biological questions such as heart and limb development (Casanova et al., Development, 2011; Uribe et al., Development, 2014).

During the years 2015-16, we focused on digit development and regeneration. One aspect that interests us is the digit tip. The last phalanx of the digits is formed via a special mechanism different from that of proximal phalanges (Casanova et al., Dev. Growth Diff., 2007). We showed that application of Fgf8 to digit primordia can elongate the digits but not in all cases, implying the existence of different programmes for digit morphogenesis (Casanova et al., Plos One, 2012). We studied the potential for digit elongation by Fgf signalling and explored the possibility of experimental induction of hyperphalangy (presence of extra phalanges, such as in cetacean limbs) using magnetic nanoparticles. We also analysed the basis for avian wing digit truncation and the possible role of MKP3 in a negative feedback loop that regulates Fgf signalling in this context.

We studied a possible relationship between inflammation and organ regeneration. Regeneration ability in higher animals is reduced (with the digit tip being one of the few examples), and the role of inflammation in this process is debated; inflammation was shown to improve organ and tissue regeneration in some cases, but in others it was detrimental. We want to understand the role of inflammation in several models of regeneration, such as digit tip regeneration after amputation, muscle regeneration or intestinal epithelial regeneration. In particular, we are studying the role of inflammatory processes regulated by the p38 signalling pathway. The study of these mechanisms is needed to understand the unique capacity of digit tips to regenerate, which could have biomedical applications.

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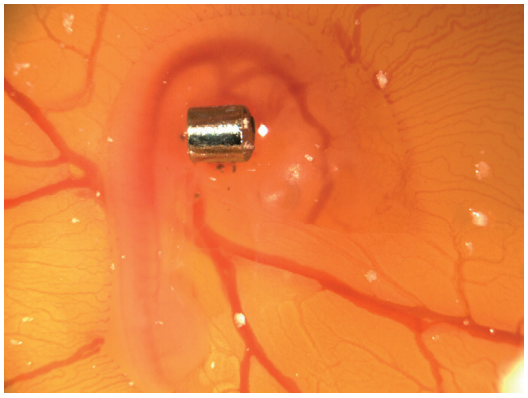
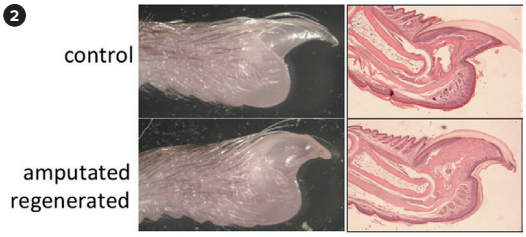


1

Nanoparticles can be used as a delivery method for bioactive substances *in ovo*. The image shows application of growth factor-coated nanoparticles to a chicken embryo and localization to a specific region (distal limb bud) using a magnet.

2



2

Regeneration of the digit tip is observed after amputation at a distal plane. A normal tip is regenerated as seen by gross anatomy (left) and histological analysis (haematoxylin and eosin staining, right).

Cellular immunobiology and microbiology

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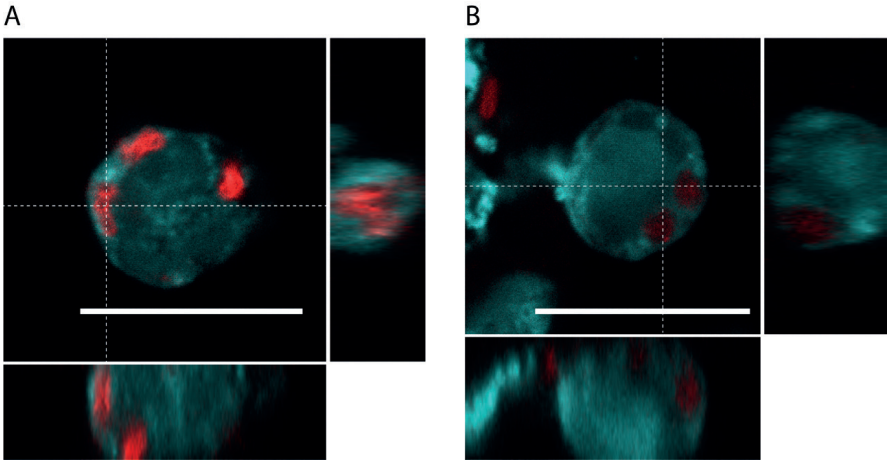
We previously showed that conventional αβCD4⁺ T cells, the paradigm of adaptive immunity, can capture bacteria efficiently from dendritic cells (DC) through a process called transinfection (Cruz-Adalia *et al.* in Cell Host Microbe. 2014; 15:611). As this process is driven by T cells and not by bacteria, it would be more appropriate to term it transphagocytosis. Transphagocytic (ti) CD4⁺ T cells kill internalized bacteria in a manner reminiscent of innate immune cells. Moreover, tiCD4⁺ T secrete large amounts of pro-inflammatory cytokines, contributing to the early immune response. These findings expand our current knowledge of CD4⁺ T functions and blur the separation between innate and adaptive immunity.

The main research line of our laboratory is the characterization of the novel abilities of tiCD4⁺ T cells and their possible use as therapeutic tools.

1



1 Orthogonal views from confocal images of CD4+ T cells (cyan) capturing (A) and destroying (B) bacteria (*Listeria monocytogenes*; red). Bar = 10 µ

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