03

Cellular and Molecular Biology

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

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

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

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SELECTED PUBLICATIONS

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

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

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

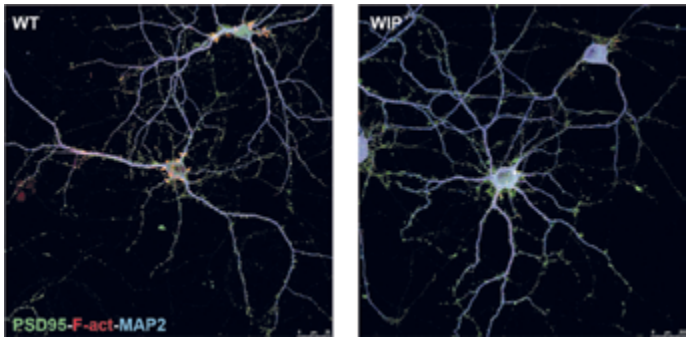
Yu X, Zech T, McDonald L, Gonzalez EG, Li A, Macpherson I, Schwarz JP, Spence H, Futó K, Timpson P, Nixon C, Ma Y, Anton IM, Visegrády B, Insall RH, Oien K, Blyth K, Norman JC, Machesky LM. N-WASP coordinates the delivery and F-actin-mediated capture of MT1-MMP at invasive pseudopods. J Cell Biol. 2012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

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

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

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

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



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

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SELECTED PUBLICATIONS

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

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

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

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

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

PATENTS

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

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

EP06762172: Attenuated SARS and use as a vaccine

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

Replication, virus-host interactions, and protection in coronavirus

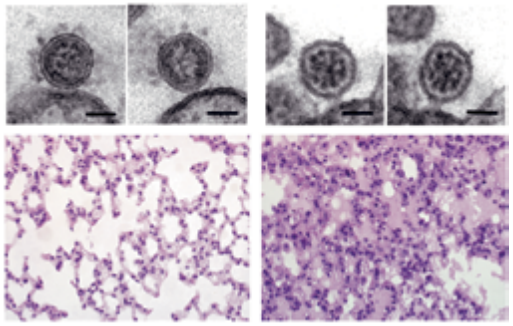
Our group is interested in the molecular basis of replication, transcription, assembly, and virus-host interactions of coronaviruses (CoV) using TGEV and SARS-CoV as models. To control disease, we focus on the impact of the host on infection by these viruses, and on the identification of signalling pathways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

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

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

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

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



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

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María Victoria Jiménez

SELECTED PUBLICATIONS

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

Gómez CE, Nájera JL, Perdiguero B, *et al.* The HIV/AIDS vaccine candidate MVA-B administered as a single immunogen in humans triggers robust, polyfunctional, and selective effector memory T cell responses to HIV-1 antigens. J Virol. 2011 Nov;85(21):11468-78

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

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

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

PATENTS

PCT/US2010/032966 Modified immunization vectors

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

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

Poxvirus and vaccines

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

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

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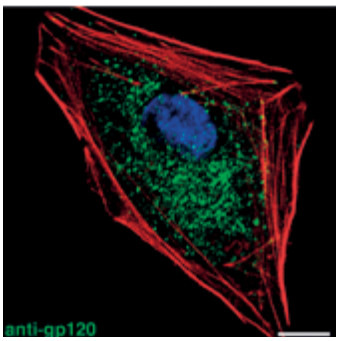

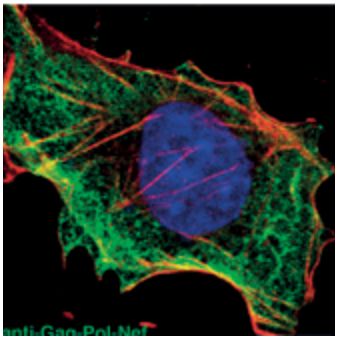

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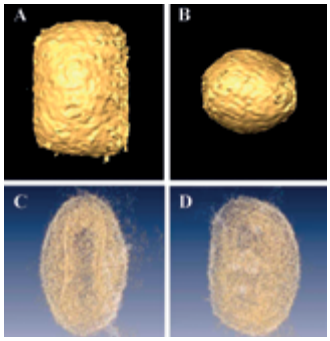

1 *Expression of the HIV antigens Env and Gag-Pol-Nef by the HIV/AIDS vaccine candidate MVA-B". Confocal microscopy of cells infected with MVA-B at 6 hours postinfection. In green, HIV antigens; in red, cytoskeletal filaments; in blue, nuclei.*

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

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SELECTED PUBLICATIONS

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

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

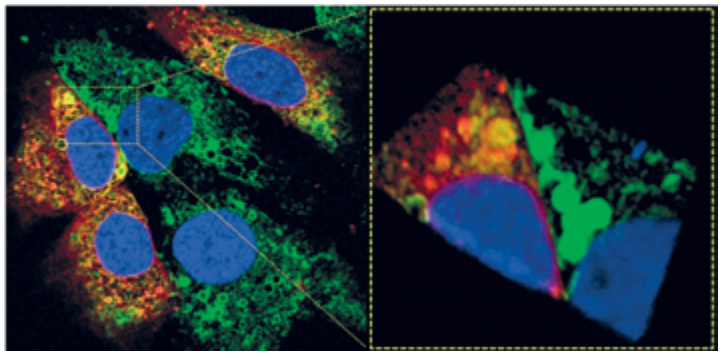
Gastaminza P, Pitram SM, Dreux M, Krasnova LB, Whitten-Bauer C, Dong J, Chung J, Fokin VV, Sharpless KB, Chisari FV. Antiviral stilbene 1,2-diamines prevent initiation of hepatitis C virus RNA replication at the outset of infection. J Virol. 2011 Jun;85(11):5513-23

Cellular factors involved in hepatitis C virus infection and pathogenesis

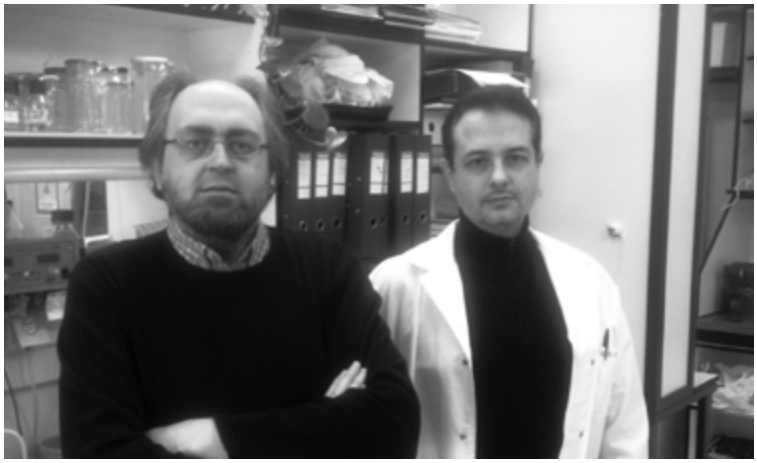
Hepatitis C virus (HCV) is an important pathogen that infects 3% of the human population worldwide. Despite intense efforts to control this pandemic, 3 to 4 million people are infected, and about 350,000 individuals die of HCV-related diseases every year. New strategies to control and eradicate this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

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

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



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



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SELECTED PUBLICATIONS

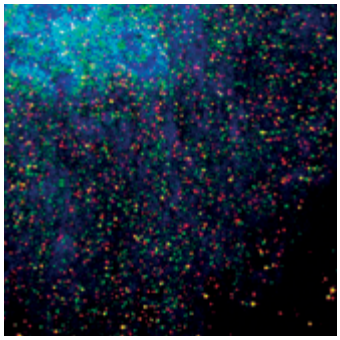
Johnston IG, Gaal B, Neves RP, Enver T, Iborra FJ, Jones NS. Mitochondrial variability as a source of extrinsic cellular noise. PLoS Comput Biol. 2012;8(3):e1002416

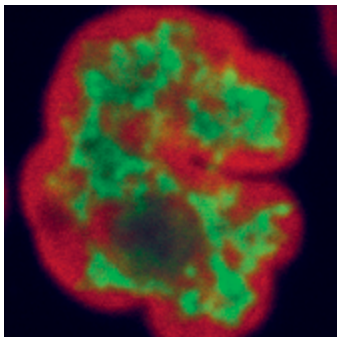
Biological noise

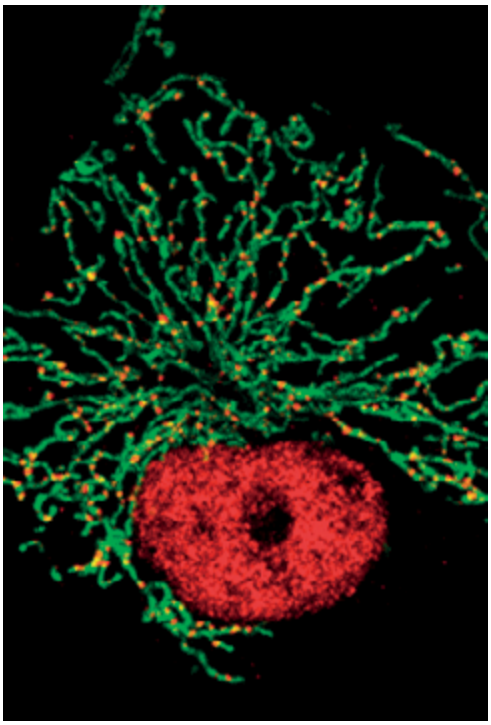
Our lab is interested in the origin of the phenotypic variability between genetically identical individuals. The reason we pursuit this endeavour is that non-genic variability is the basis of many pathophysiological processes such as cell differentiation, cellular responses to drugs, and even the execution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

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

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







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

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

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

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

Furlan-Magaril M, Rebollar E, Guerrero G, Fernández A, Moltó E, González-Buendía E, Cantero M, Montoliu L, Recillas-Targa F. An insulator embedded in the chicken α-globin locus regulates chromatin domain configuration and differential gene expression. Nucleic Acids Res. 2011 Jan;39(1):89-103

Román AC, González-Rico FJ, Moltó E, Hernando H, Neto A, Vicente-Garcia C, Ballestar E, Gómez-Skarmeta JL, Vavrova-Anderson J, White RJ, Montoliu L, Fernández-Salguero PM. Dioxin receptor and SLUG transcription factors regulate the insulator activity of B1 SINE retrotransposons via an RNA polymerase switch. Genome Res. 2011 Mar;21(3):422-32

Martin D, Pantoja C, Fernández Miñán A, Valdes-Quezada C, Moltó E, Matesanz F, Bogdanovi O, de la Calle-Mustienes E, Domínguez O, Taher L, Furlan-Magaril M, Alcina A, Cañón S, Fedetz M, Blasco MA, Pereira PS, Ovcharenko I, Recillas-Targa F, Montoliu L, Manzanares M, Guigó R, Serrano M, Casares F, Gómez-Skarmeta JL. Genome-wide CTCF distribution in vertebrates defines equivalent sites that aid the identification of disease-associated genes. Nat Struct Mol Biol. 2011 Jun;18(6):708-14

Tiana M, Villar D, Pérez-Guijarro E, Gómez-Maldonado L, Moltó E, Fernández-Miñán A, Gómez-Skarmeta JL, Montoliu L, del Peso L. A role for insulator elements in the regulation of gene expression response to hypoxia. Nucleic Acids Res. 2012 Mar;40(5):1916-27

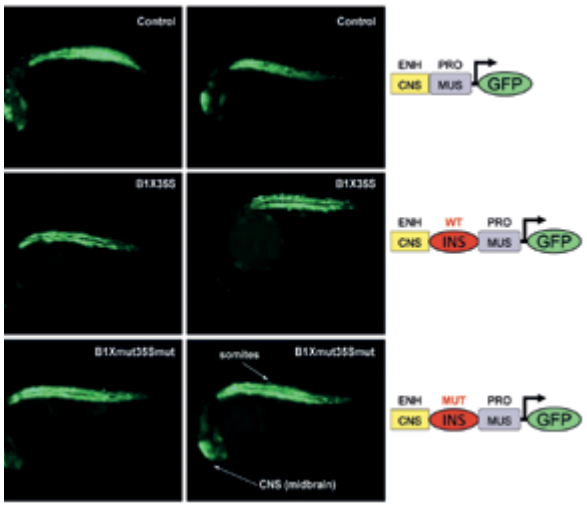
PATENT

P201231296 New animal model for acromatopsia.

Animal models by genetic manipulation

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

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



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

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Functional analysis of the transcriptional repressor DREAM

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

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

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

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

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

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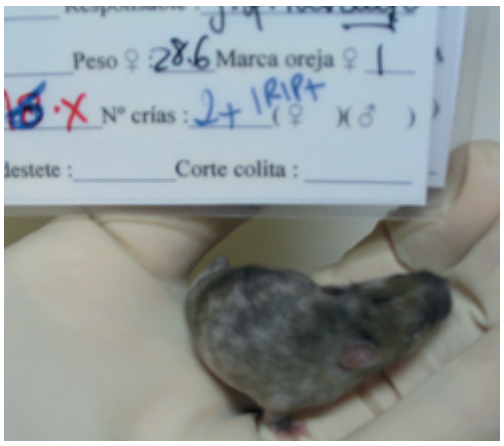
PATENT

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

❶



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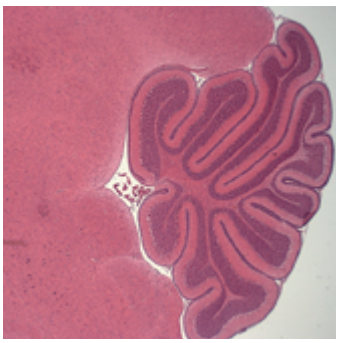

❶ *Double DREAM knock-in mouse carrying three mutations in the DREAM gene. This mouse chimaera was prepared in collaboration with the Transgenesis Service at CNB using consecutively two different zinc finger nucleases (ZFN) that targets exon II and exon VII of the DREAM gene.*

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

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SELECTED PUBLICATIONS

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

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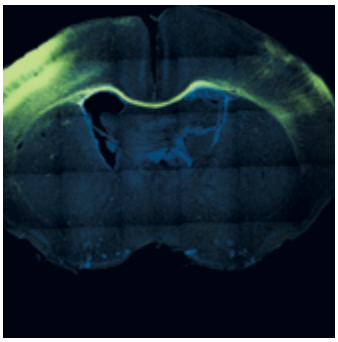
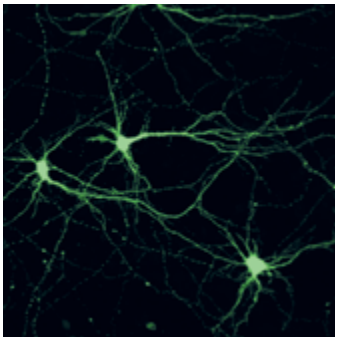
Cerebral cortical development

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

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





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

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

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

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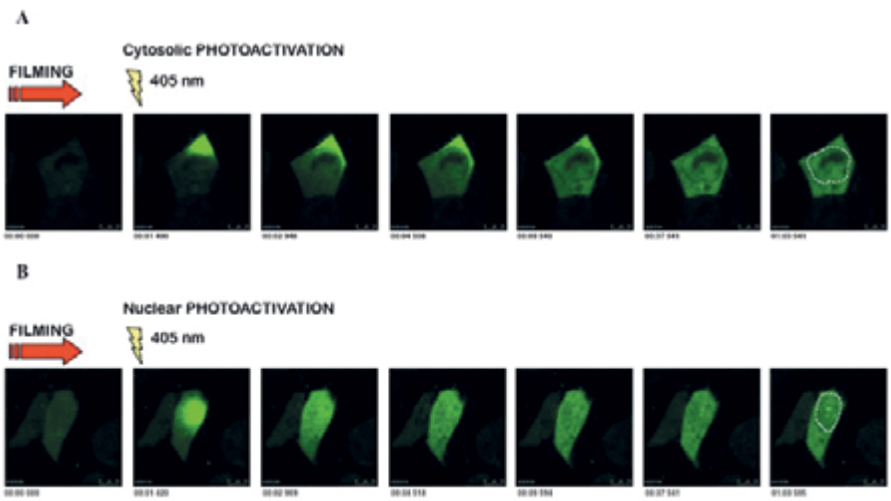
PATENT

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

Mechanisms of interaction between the influenza virus and the infected cell

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

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



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

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SELECTED PUBLICATIONS

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

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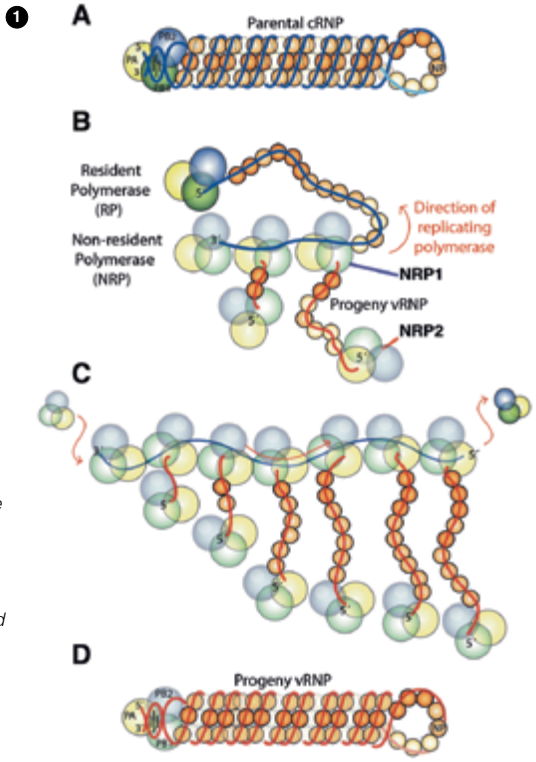
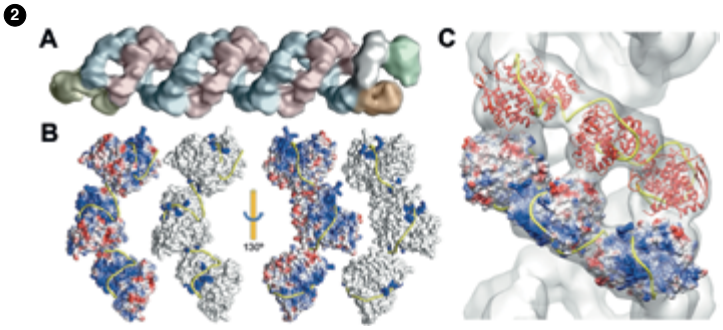
Transcription and replication of influenza virus RNA

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

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





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

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

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SELECTED PUBLICATIONS

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Virus and cancer

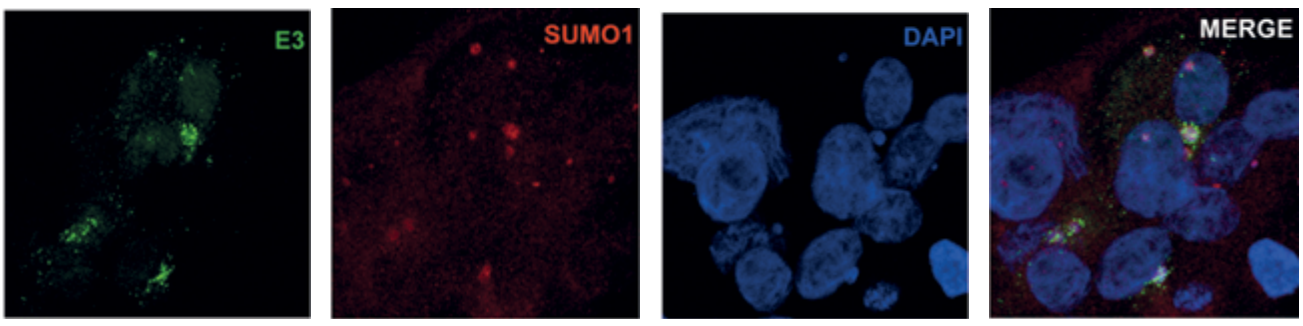
Our group is interested in the relationship between viruses and cancer. Tumour viruses induce oncogenesis by manipulating an array of cellular pathways, some of which are extensively regulated by the small ubiquitin-like protein SUMO. SUMOylation is a reversible post-translational modification by which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

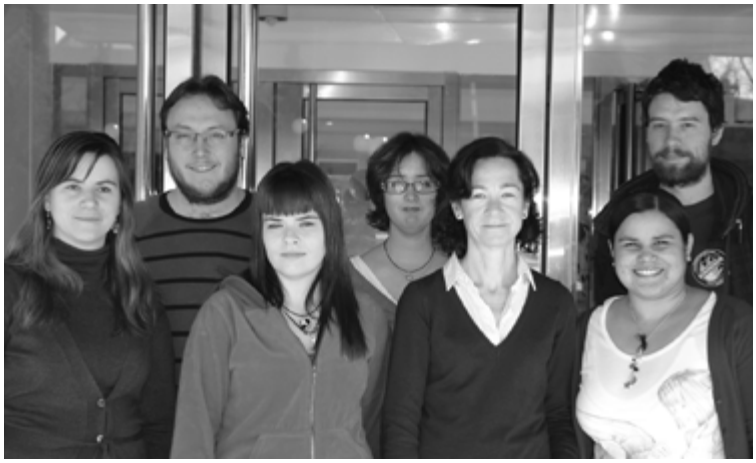
1. identifying how viruses and their regulatory proteins exploit the host cell SUMO modification system
2. evaluating the role of SUMO modification in virus replication, and
3. determining how alteration of the SUMOylation pathways by tumour viruses might affect cell transformation

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

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

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





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

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

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

Alonso-Padilla J, Pignatelli J, Simon-Grifé M, Plazuelo S, Casal J, Rodríguez D. Seroprevalence of porcine torovirus (PToV) in Spanish farms. BMC Res Notes. 2012 Dec 5;5:67.

Molecular characterisation and epidemiology of torovirus

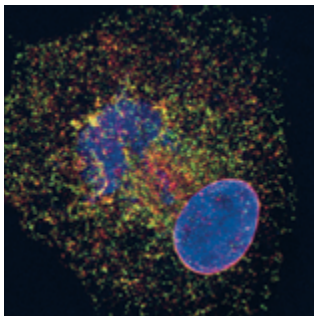
Toroviruses (family *Coronaviridae*, order *Nidovirales*) are emergent viruses with a potential of zoonotic transmission that can cause enteric disease and diarrhoea in various animal species and in humans. Four torovirus species or genotypes have been recognised thus far, on the basis of their hosts: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

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

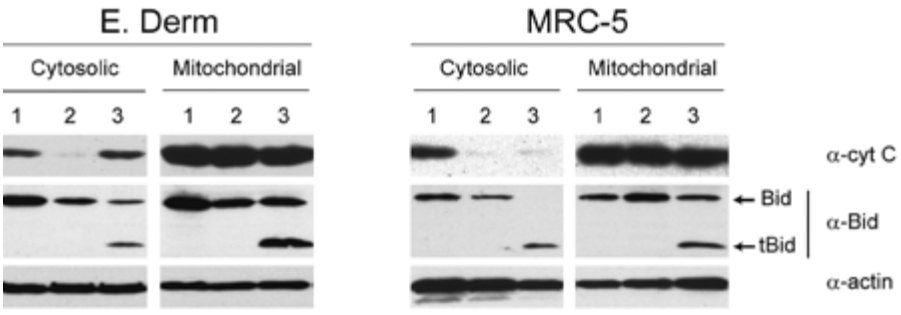
Examination of BEV-infected cells by electron and confocal microscopy suggests that, as for other RNA viruses, torovirus replication/transcription complexes are built in association with cell membranes. To characterise these complexes morphologically and functionally, we generated a panel of antibodies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

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

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



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



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

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

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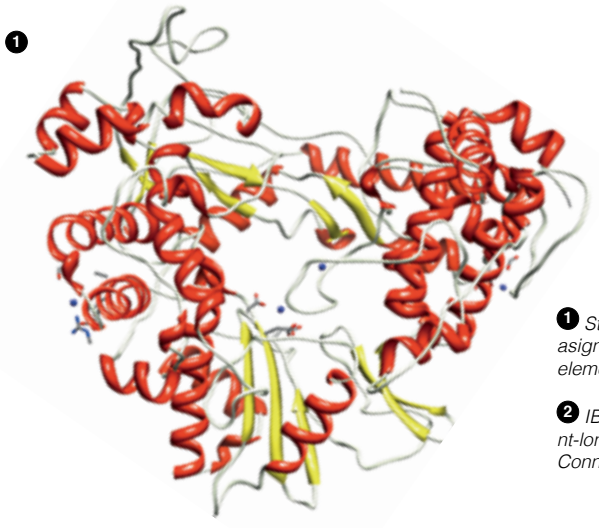
Molecular biology of birnavirus

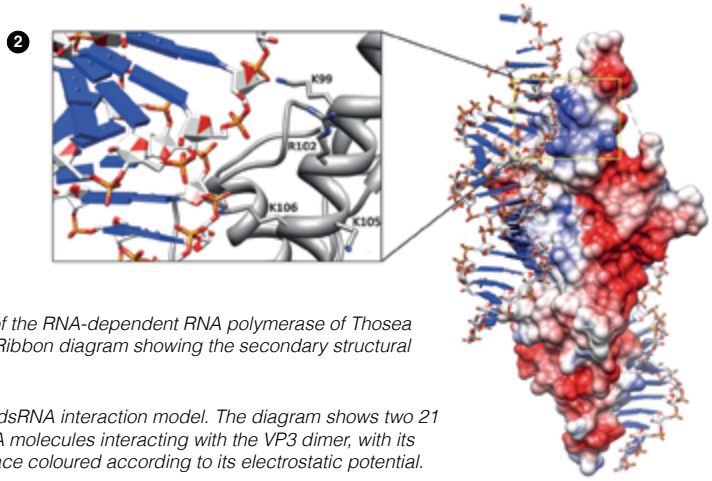
The *Birnaviridae* family groups icosahedral naked viruses with bipartite dsRNA genomes. Members of this family infect a wide variety of animal species including insects, aquatic fauna and birds. Our main virus model is the infectious bursal disease virus (IBDV), the aetiological agent of an acute immunosuppressive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

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

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

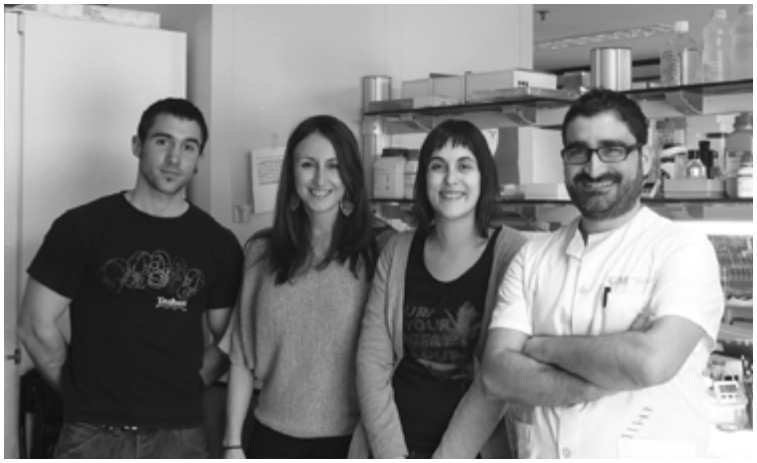
Birnaviruses exhibit a number of features that greatly resemble those of ssRNA viruses of the *Noda-* and *Tetraviridae* families, suggesting that birnaviruses might represent an evolutionary link that connects dsRNA and ssRNA viruses. To analyse this hypothesis, we are comparing the structure and function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





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

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



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SELECTED PUBLICATIONS

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

Visvikis O, Boyer L, Torrino S, Doye A, Lemonnier M, Lorès P, Rolando M, Flatau G, Mettouchi A, Bouvard D, Veiga E, Gacon G, Cossart P, Lemichez E. Traffic. 12(5):579-90. 2011

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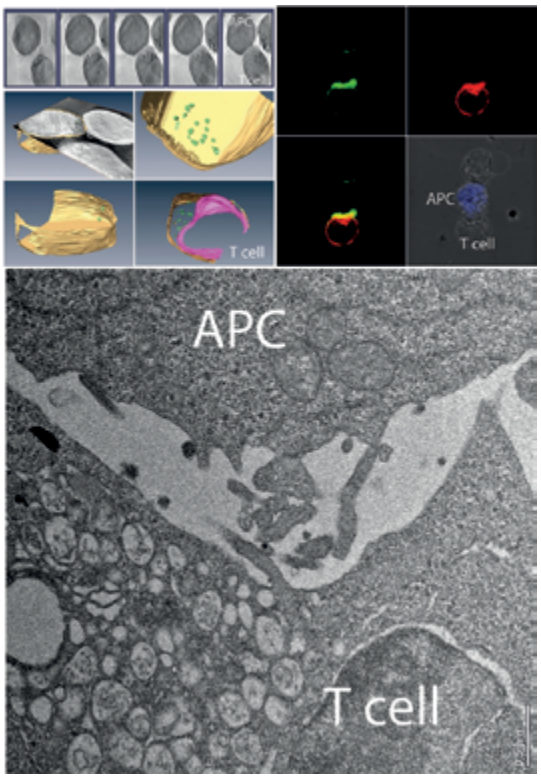
Cellular immunobiology and microbiology

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



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