

Cellular & Molecular Biology

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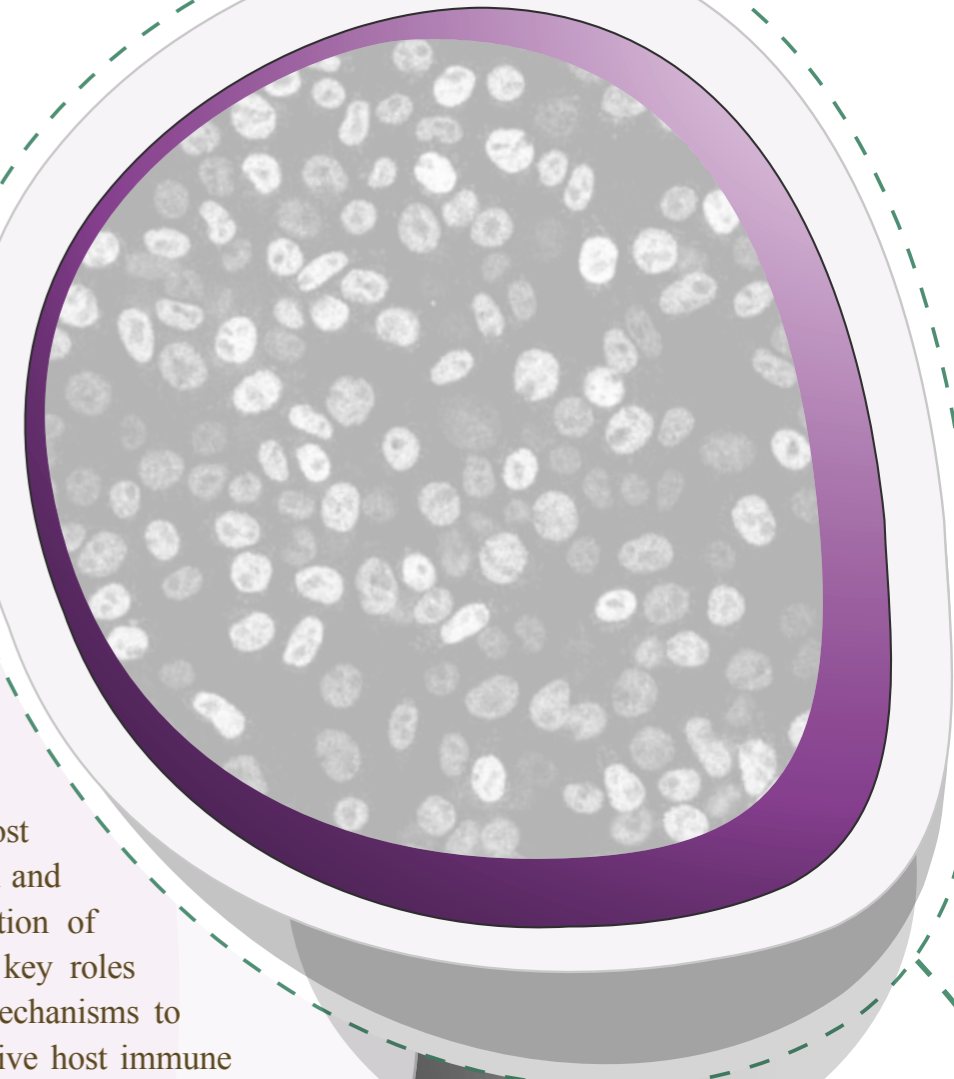
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The Department hosts fifteen independent research groups working on two broad and highly intertwined research areas with the ultimate scientific goal of identifying specific therapeutic targets potentially useful for disease prevention and control. The first research area is focussed to the dissection of virus-host interactions of highly relevant human and veterinary pathogens. The identification of virus and cellular elements playing key roles in virus replication as well as on mechanisms to evade or counteract innate and adaptive host immune responses at cellular and organism level is essential for the rational design and implementation of new strategies for disease control, including the generation of innovative vaccination strategies and virus-based vaccine vectors. The second area is centred on the understanding of networks controlling mammalian gene expression, and on the characterization specific genes playing critical roles on both normal and pathological processes. The focal point of this research program is the identification and exploitation of relevant diagnostic and therapeutic molecular targets. In addition to the generation of frontier knowledge, studies carried out at the Department are intended to provide the essential scientific background for the development of novel biotechnological tools of biomedical importance.



Cellular & Molecular Biology

LEAD INVESTIGATOR

Inés M. Antón Gutiérrez

PREDOCTORAL SCIENTISTS

Inmaculada Bañón Rodríguez
Alejandra Bernardini
Estefanía Fernández López
Ana Franco Villanueva
Esther García González



UNDERGRADUATE STUDENTS

Inmaculada Pereda Pérez
Sonia Pérez Carpintero

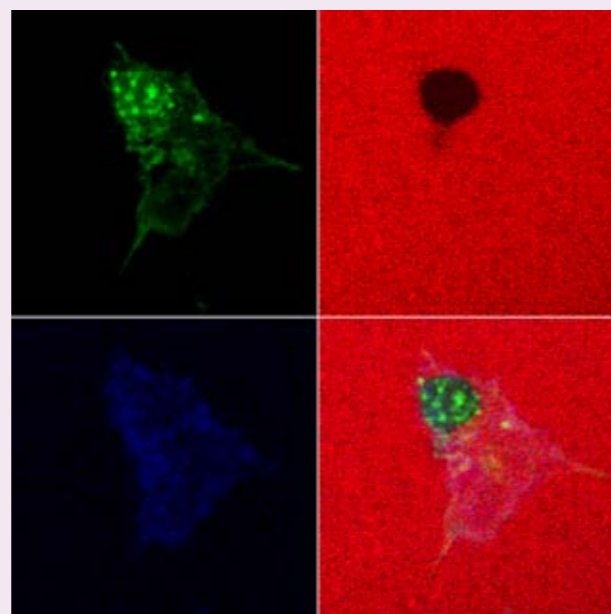
TECHNICIANS

María Lozano
Chiara Ragazzini

Molecular Bases of Cytoskeletal Reorganisation: Role of Actin Polymerization in Neuritogenesis, Inflammation and Metastasis

Essential physiological processes such as neuronal morphogenesis, cell motility and tissue invasion rely on the spatial and temporal regulation of actin dynamics and therefore, their deregulation is at the root of severe pathologies.

Actin reorganization is controlled by nucleation-promoting factors like neural Wiskott-Aldrich syndrome protein (N-WASP) and cortactin, and associated proteins that regulate their activity such as WIP (WASP-interacting protein), a ubiquitously distributed protein that stabilizes actin filaments. Our goal is to define the role of WIP- and WASP-family proteins in actin dynamics within a variety of cellular processes in an array of cell models (fibroblasts, dendritic cells (DC), neurons and astrocytes).



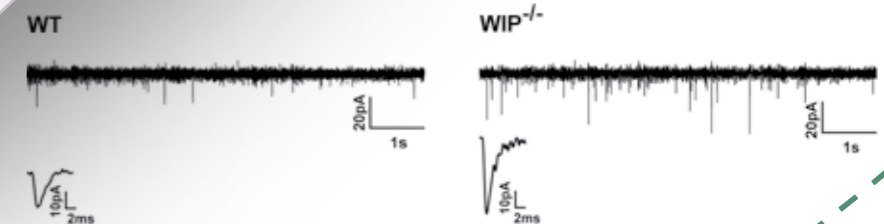
WIP expression restores podosome formation and ECM degradation. WIP KO DC infected with a lentivirus coding for WIP-eGFP (green) were plated onto gelatin-rhodamine (red) and labelled with anti-cortactin (blue).

In the past two years, we have contributed toward defining the molecular mechanisms that underlie invasion and extracellular matrix (ECM) degradation by matrix metalloproteinases (MMP). We described that WIP is essential for MMP secretion, but not for MMP synthesis, in murine DC. Moreover, absence of WIP prevents the formation of podosomes, actin-rich migratory structures in which MMP activity localizes. Cortactin binding to WIP is required for correct podosome formation, MMP activity and ECM degradation. Our future work aims at unravelling the WIP contribution to invasion and metastasis by human tumour cell lines.

Since the ability to reorganize actin filaments is at the basis of neuronal plasticity, we studied the role of WIP on neuron differentiation both in early and in late developmental stages, *in vitro* as well as *in vivo*. Using embryonic hippocampal primary WIP^{-/-} neurons, we characterized, at the biochemical and morphological levels, the effect of WIP as a negative regulator of sprouting and neuritic/dendritic branching without affecting axon generation. Similar to our observations in dendritic cells, we found that WIP expression in neurons is required for the correct cortactin localization. Our results point to WIP as a novel regulator that prevents premature dendritic and synaptic maturation. We aim to deepen our understanding of the contribution of WIP to neuron and astrocyte migration, to brain lamination and development, and the functional effects of WIP deficiency on the murine nervous system.

The results of our work should yield fundamental information on the function of these cytoskeletal molecules and offer new insights into the molecular mechanisms that underlie actin dynamics and related functions, providing new diagnostic and/or therapeutic tools for neurological diseases, inflammation-mediated affections and tumour invasion.

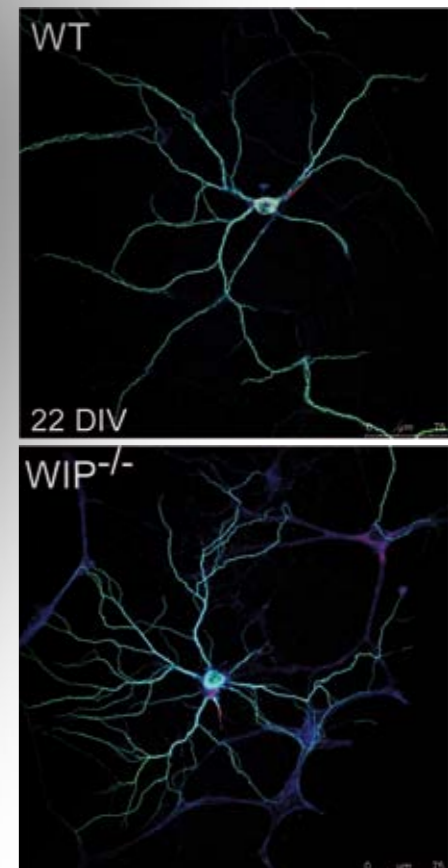
Enhanced dendritic and synaptic maturation in WIP^{-/-} neurons. Top, Hippocampal primary neurons from control (WT) or WIP^{-/-} embryos were grown for 22 days (DIV) onto an astrocyte monolayer and stained for F-actin (blue), MAP2 (green) and ankyrin G (red). Bottom, Representative traces of miniature excitatory postsynaptic currents recorded from a WT and a WIP^{-/-} neuron. Amplitude (pA) and frequency (s) are indicated.



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LEAD INVESTIGATOR

Luis Enjuanes

SENIOR SCIENTISTS

Isabel Sola
Fernando Almazán

PREDOCTORAL SCIENTISTS

Aitor Nogales
Jazmina L. González
José Luis Nieto
Pedro A. Mateos
Lorena del Palacio

TECHNICIANS

Carlos M. Sánchez
Sarhay Ros

VISITORS

José Gallego
Tatiana Ostrova
George Lomonosoff
Marcel Muller
Paul B McCray
Shinji Makino

POSTDOCTORAL SCIENTISTS

Sonia Zúñiga
Marta L. De Diego

Silvia Márquez
José Manuel Jiménez
Martina Bécares
José Ángel Regla

Margarita González

Olga Zaberezhnaya
Jaume Torres
Christian Drosten
Valerian V. Dolja
Shahid Jameel



Replication, Virus-Host Interactions, and Protection in Coronavirus

The main focus of our research is the study of the molecular basis of coronavirus (CoV) replication and virulence, and the identification of signalling pathways modified by the virus, to control disease.

The information from these basic projects will be used to design protection strategies against CoV-induced diseases, particularly human severe pneumonia that can end in acute respiratory distress syndrome (ARDS).

The CoVs are single-stranded positive-sense RNA viruses with genomes of around 30 kb, responsible for respiratory and enteric diseases with high impact on animal and human health. Our group is interested in the molecular basis of replication and transcription, assembly, and virus-host interaction using transmissible gastroenteritis coronavirus (TGEV) and the severe and acute respiratory syndrome virus (SARS-CoV) as models.

Virus replication and transcription, and virus-host interactions are mediated by binding of virus RNA motifs to viral and host cell proteins, and by protein-to-protein recognition. These interactions are analysed in all the processes that we study, including the replication complex. We postulated that coronavirus transcription and replication involve 5' and 3' genome end interactions, mediated by proteins that are being identified using functional genomics and proteomics. The relevance of host cell factors involved in these processes is being evaluated by inhibiting their expression using siRNAs.

Coronavirus transcription requires discontinuous RNA synthesis to link the leader to coding sequences in the subgenomic RNA, a process similar to high frequency copy-choice, similarity-assisted RNA recombination. Based on a large amount of data generated by reverse genetics, we described two mechanisms that regulate transcription in coronaviruses at different levels. We showed that basepairing between the nascent minus RNA chain and the genomic RNA leader regulates the amount of all subgenomic RNAs produced. A transcription enhancer that regulates the expression of an specific mRNA was also recently identified. There are ad-

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PATENT

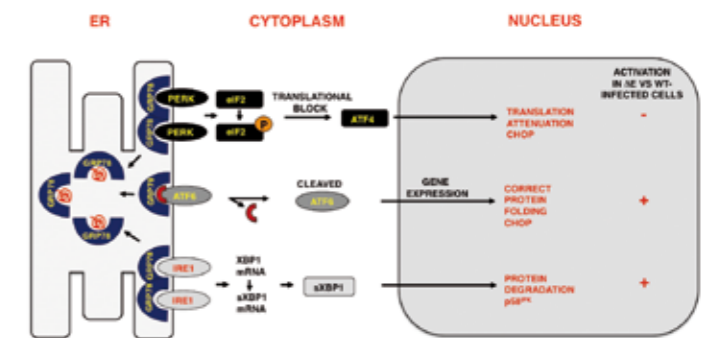
L. Enjuanes, S. Zúñiga, M. Bécares, J. Plana Duran. Nucleic acids encoding PRRSV GP5-ecto-domain and M protein. Nº Registro: **10192693.9**. CSIC and Fort-Dodge Veterinaria. 2010. Internacional, PCT.

ditional regulatory mechanisms that influence the amount of subgenomic RNA, such as RNA-protein interactions, one area in which our laboratory is highly active at present. The role of viral and cell proteins, such as RNA chaperones, that might be involved in CoV replication, transcription and packaging is being addressed; we showed that coronavirus N protein is essential for coronavirus RNA synthesis and acts as an RNA chaperone.

A main area of interest in our laboratory is the study of the molecular basis of virus virulence, virus-host interaction, and signalling pathways affecting virus replication or, alternatively, cell pathways altered by coronavirus infection leading to diseases such as those associated with inflammation of respiratory tissues. Comparative genomics and proteomics information is essential in these studies. We described that specific virus structural proteins, such as the TGEV and SARS-CoV envelope (E) proteins, influence virus virulence and modulate signalling pathways. The deletion of E protein led to the generation of propagation-deficient TGEV, 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 significantly affects viral and cell translation and apoptosis. Viral and host cell factors involved in these signalling pathways are being studied.

The information derived from academic studies is being applied to the engineering of coronavirus vectors. Using reverse genetic approaches based on two infectious cDNA clones produced in our laboratory for TGEV and SARS-CoV, viral vectors have been engineered using virus-attenuated phenotypes. These vectors have led to promising recombinant vaccine candidates for animal and human health, including SARS.

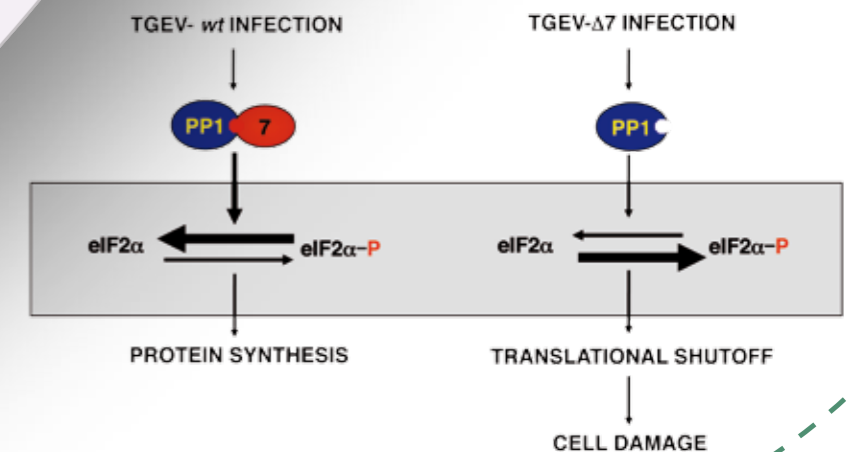
UNFOLDED PROTEIN RESPONSE



SARS-CoV gene E and unfolded protein response. A scheme of the three pathways (PERK, ATF-6 and IRE1) comprising the unfolded protein response (UPR). Infection with a SARS-CoV deletion mutant lacking gene E expression (ΔE) enhanced UPR compared with wt virus infection. This effect was due mostly to the activation of IRE1 pathway, leading to increased XBP1 cleavage.

Role of coronavirus alpha gene 7 on translation. After TGEV infection, viral protein 7 interacts with protein phosphatase 1 (PP1). This interaction promotes eIF2 α dephosphorylation, leading to protein translation. Infection with a TGEV mutant lacking gene 7 expression ($\Delta 7$) increased eIF2 α phosphorylation, leading to translational shutoff and enhanced apoptosis.

WORKING HYPOTHESIS



Cellular & Molecular Biology

LEAD INVESTIGATOR
Mariano Esteban

POSTDOCTORAL SCIENTISTS
Susana Guerra
Carmen E. Gómez
José Manuel González
Alan Goodman
Mariang García
Beatriz Perdiguero
Juan F. García
José Luis Nájera

PREDOCTORAL SCIENTISTS
Magdalena Krupa
Aneesh Vijayan
Jacobo Nieto
Ana Caceres
Lucas Sánchez
Lidia Mingorance
Pilar Arnáez



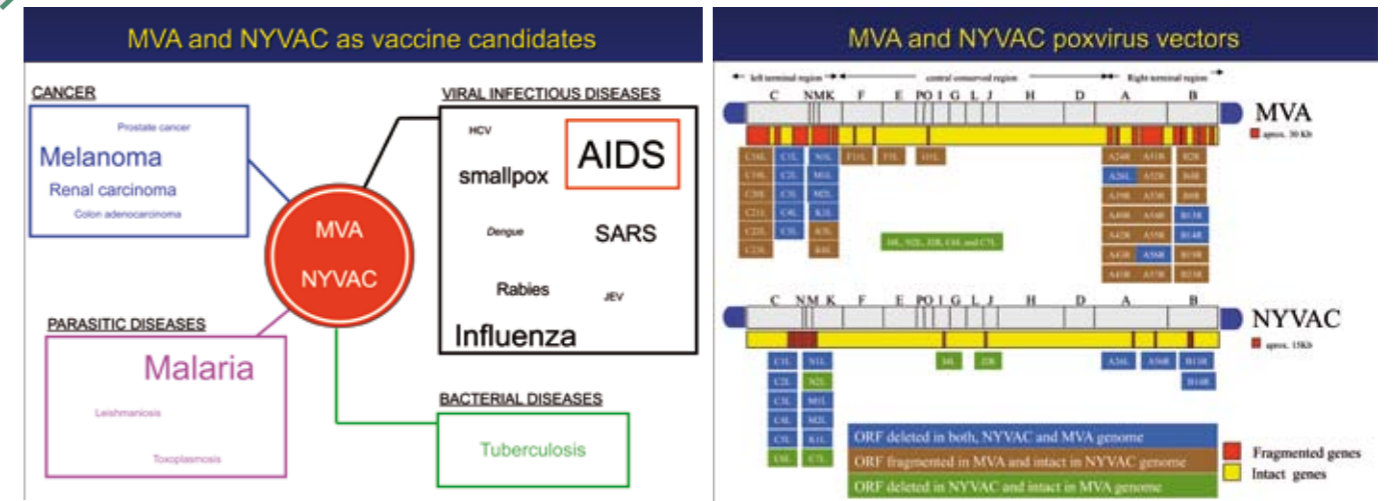
TECHNICIANS
María Victoria Jiménez
Ruben Sanz

Poxvirus and Vaccines Lab

The main objectives of our laboratory are geared toward understanding the molecular basis of 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 and leishmaniasis.

As a model system of an infectious agent and as a delivery vector for expression of genes of interest, we used vaccinia virus (VV), a member of the poxvirus family.

Our group at the CNB has developed prototype poxvirus (NYVAC and MVA strains) vaccines against HIV/AIDS based on subtypes B and C, which account for nearly 80% of all HIV infections worldwide. In preclinical studies in mice and in macaques, the prototype vaccines expressing four HIV antigens (Env/Gag-Pol-Nef) have fulfilled the expected characteristics of a potentially effective vaccine, i.e., high immunogenicity; in monkeys, they elicit protection after challenge with pathogenic simian immunodeficiency virus; the vaccines can be delivered safely by aerosol, which facilitates their administration, especially in poor countries; and when given to human healthy volunteers in DNA/poxvirus combination, triggered HIV-specific T cell immune responses in over 90% of volunteers, with a polyfunctional and durable immune response. With the vaccine prototype MVA-B, in 2009 we initiated a phase I clinical study in Spain, with the participation of the Hospital Clinic in Barcelona and the Hospital Gregorio Marañón in Madrid, which has received wide attention from the media. Follow-up phase I clinical trials in Spain will be started in 2011, with only MVA-B administered in HIV-positive individuals on HAART therapy, to assess safety and immunogenicity of the vaccine protocol. Phase I clinical trials with the MVA-C and NYVAC-C prototypes will be started in Africa in 2011. To determine the impact of these vaccines on human cells, we have identified gene signatures triggered by the HIV/AIDS vaccine MVA-B that might play important roles in the innate immune response. We also identified the TLR trig-



Attenuated poxvirus vectors as vaccine candidates against prevalent human diseases and, diagram showing genome deletions in the two viral vaccine candidate vectors MVA and NYVAC.

gering signals induced by the poxvirus vectors. To potentiate the immune responses to the poxvirus vectors, we followed two strategies: use of adjuvants such as Mega CD40L and selected deletions in viral immunomodulatory genes. In collaboration with other groups, we have provided important insights into the structural organization of the virus core and the role of some oncogenes in antiviral action.

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Modified Immunization Vectors. US Provisional Patent Application, April 2009. Nº **61174024, PCT/US2010/032966**. Mariano Esteban, Bertram Jacobs, Giuseppe Pantaleo, Cornelius Melief, Rafick Sekaly, James Tartaglia.

Cellular & Molecular Biology

LEAD INVESTIGATOR

Alberto Fraile

PREDOCTORAL SCIENTISTS

María Victoria Cepeda



Pathogenesis and Cell Biology of Human Cytomegalovirus

Human cytomegalovirus (HCMV), a member of the herpesvirus family, is responsible for a number of diseases in immunocompromised patients.

Furthermore, HCMV infection has been associated with colon cancer and gliomas, but there is no evidence supporting a role for HCMV-encoded genes in cancer pathogenesis. As HCMV is an enveloped virus, replication is dependent on interactions with cell membrane systems. The mechanisms of HCMV envelopment and secretion nonetheless remain to be defined. In our group, we study molecular mechanisms that underlie HCMV pathogenesis and morphogenesis. To investigate the molecular basis of pathogenesis, we study the role of the HCMV-encoded chemokine receptor US28 in cancer. To provide new insights into viral morphogenesis, we explored the function of cell components involved in vesicle-mediated transport, including small GTPases of the Rab family and SNARE proteins, in this process.

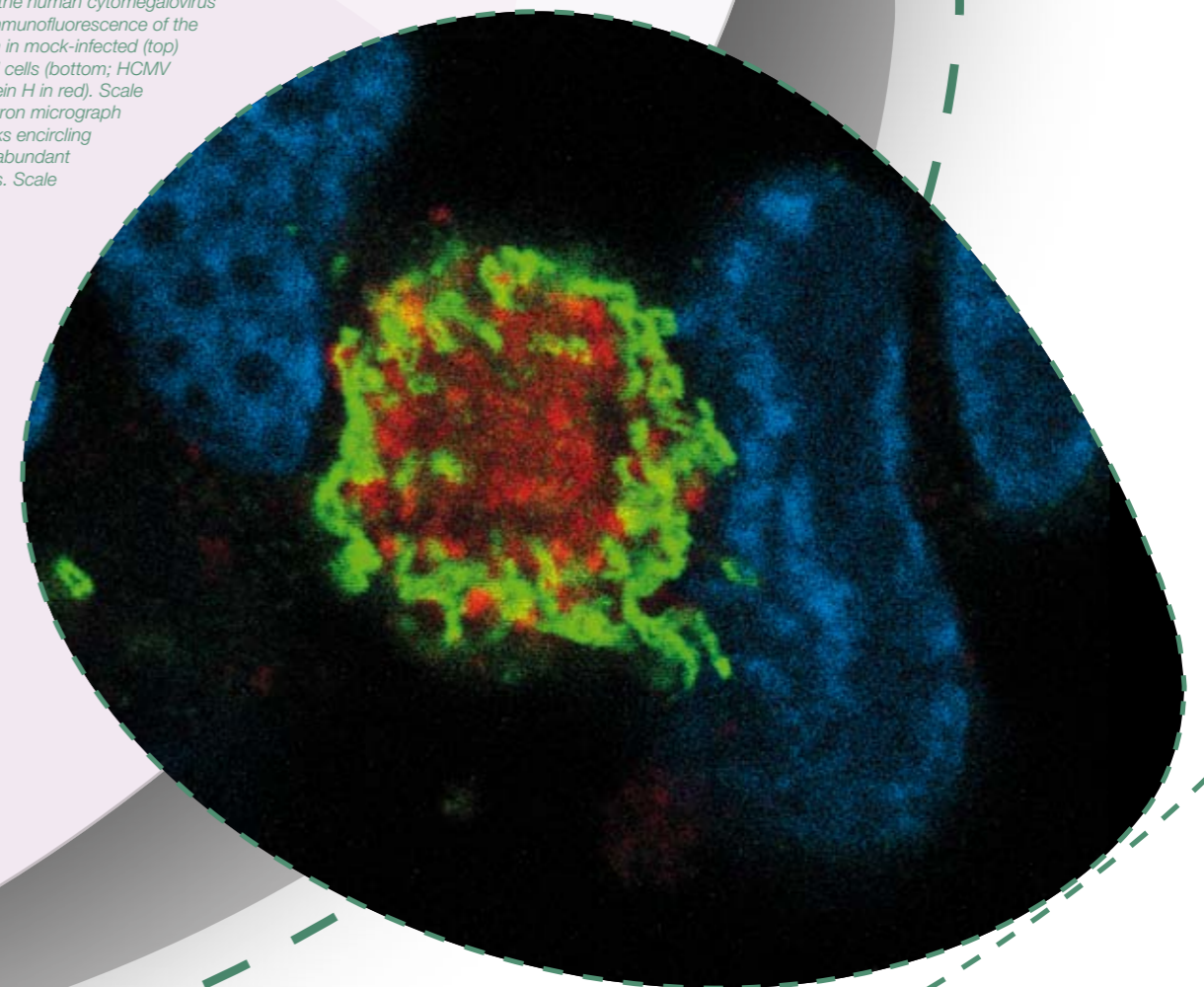
We found that US28 expression in transgenic mice promotes development of dysplasia. We also showed that US28 proliferative signalling occurs through the interleukin-6/signal transducer and activator of transcription 3 (STAT3) axis. Moreover, analyses of tumour specimens from glioblastoma patients demonstrated colocalisation of US28 and phosphorylated STAT3 in the tumours.

We observed that HCMV induced the expression of Rab27a, a small GTPase involved in lysosome-related organelle transport, as well as its recruitment to the assembly site where Rab27a associated with viral envelopes. CMV production was reduced after silencing of Rab27a and in Rab27a-deficient ashen melanocytes. This study indicates a role for Rab27a in the CMV life cycle, and that CMV and LRO biogenesis share common molecular mechanisms. In addition, we found that HCMV induces expression of the SNARE protein syntaxin 3 (STX3). STX3 was located at the plasma membrane and at the assembly site, where it is associated with virus wrapping membranes. STX3 silencing

reduced HCMV production; in contrast, STX3 depletion did not affect herpes simplex virus type-1 production. The mechanism through which STX3 affected HCMV morphogenesis probably involves late endosomes/lysosomes, since STX3 depletion reduced expression of lysosomal membrane glycoproteins. These results demonstrate a function for STX3 in HCMV morphogenesis, and help unravel the role of this SNARE protein in late endosome/lysosome compartments.

In summary, our work provides new insights into the basis of HCMV pathogenesis and replication and also shed light on basic aspects of cell membrane biology. The results of our work might suggest novel targets for antiviral strategies against these important pathogens.

Characterisation of the human cytomegalovirus assembly site. A) Immunofluorescence of the Golgi marker giantin in mock-infected (top) and HCMV-infected cells (bottom; HCMV envelope glycoprotein H in red). Scale bar, 20 µm. B) Electron micrograph showing Golgi stacks encircling virus factories with abundant vesicles and tubules. Scale bar, 250 nm.



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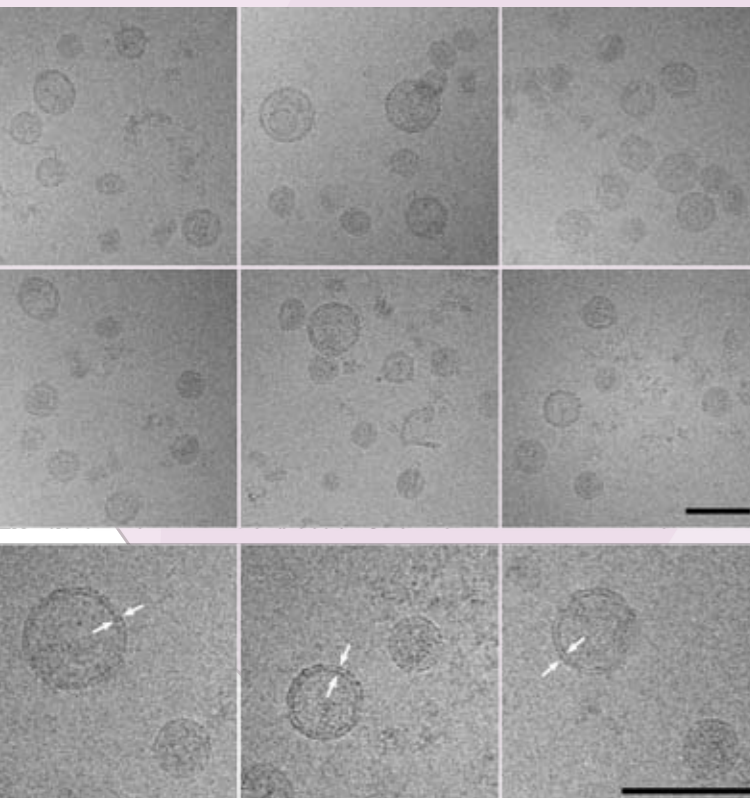
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Cellular & Molecular Biology

LEAD INVESTIGATOR

Pablo Gastaminza Landart



Cellular Factors Involved in Hepatitis C Virus Infection and Pathogenesis

Hepatitis C virus (HCV) is a pathogen that infects 3% of the human population worldwide.

Our laboratory is interested in the cellular and molecular processes underlying aspects of HCV biology and pathogenesis. After contributing to the development of a cell culture model for HCV infection, we focused on the process of infectious virus assembly and virion morphology. Previous studies at the Scripps Research Institute (La Jolla, CA) showed that infectious HCV assembly relies on cellular lipoprotein biosynthesis, and that lipoprotein components are incorporated into HCV particles. We showed that apolipoprotein B (apoB) expression levels are rate-limiting for HCV assembly and that apolipoprotein E (apoE) is incorporated into HCV particles. To determine how viral and cell components are organised in the infectious virions, we optimised virus production and purification, and obtained virus preparations that enable the study of virion morphology by cryoelectron microscopy. This analysis revealed that HCV particles are pleomorphic and heterogeneous in size, some of which (those associated with greatest infectivity) show a visible envelope surrounding an apparently disordered capsid. At the CNB, we are conducting biochemical and functional studies to determine the molecular mechanisms that underlie the dependence of HCV assembly on apoB, apoE and other cellular genes involved in lipid metabolism, and how this relationship might alter normal cell lipid homeostasis.

In addition to studying cellular factors that mediate HCV infection, we explored new methods to identify molecules with antiviral potential against HCV. We developed a novel unbiased cell-based screening system that enables interrogation of chemical libraries for compounds that target

CryoEM micrographs of highly purified HCV particles showing heterogeneous size and morphology of the viral population. Some particles display a visible bilayer that probably represents the viral envelope (white arrows).

known and unknown aspects of the viral life cycle, be they cellular or viral. We established the efficacy of this system using a library of clinically approved compounds and demonstrating the capacity of the system to identify compounds that inhibit entry, replication and assembly processes, as well as compounds that target undefined aspects of HCV infection. Not surprisingly, some of the antiviral compounds target cellular factors involved in lipid metabolism regulation. After this proof of principle, we interrogated a chemical library of compounds susceptible to chemical derivatisation using click chemistry, a highly modular and predictive synthetic method, optimal for structure-activity relationship studies. The screening process led to identification of a new family of anti-HCV compounds, and their derivatisation permitted us to optimise the antiviral molecules to obtain compounds with antiviral activity at nanomolar concentrations. Study of the mechanism of action showed an unprecedented mode of action at the outset of infection, in which the non-structural protein NS5A is involved after primary translation and leads to the establishment of replication complexes.

Micrograph of a HCV infection focus, determined by immunofluorescence microscopy using antibodies against viral antigens (red). Cells are counterstained with DAPI. Counting infection foci permits calculation of infectivity titres (focus-forming units per ml; FFU/ml) in samples from infected cells.

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Cellular & Molecular Biology

LEAD INVESTIGATOR

Francisco J. Iborra

Control of Cell Differentiation

We would like to understand the fundamental question of the control of cell differentiation, how a cell moves from one genetic program to another.

The difference between the cells of our body is the set of genes they express. Pathological situations tend to be associated with aberrant patterns of gene expression. Gene expression starts at the DNA, which can be modified to be silent. The second control level is the kind of modification of the proteins wrapping the DNA, again rendering the gene silent or active. Third, the right protein factors must direct the transcription machinery to the site at which to start transcribing the gene. These proteins work in teams, and the relative abundance of each will drive the cell to take one path or another. Our group plans a detailed analysis of these factors at the single cell level, to understand the regulation of cell differentiation and pathology.

All cells in the organism are derived from the same progenitors through differentiation, which can be seen as a progressive change in phenotype. The phenotype of any cell is ultimately determined by the set of genes transcribed, which are determined by specific transcription factors and epigenetic modifications of chromatin in the cell nucleus.

This idea was recently challenged by the observation that genetically identical populations of cells can exhibit cell-to-cell variations in the amount of protein a gene produces, resulting in phenotypic diversity. It is thought that the variations arise from the typically small number of molecules

involved in gene expression. Protein numbers are often in the order of hundreds of molecules, mRNA in the order of tens of molecules, and the genes themselves are often present in just one or two copies per cell.

We attempt to understand how the organisation of transcription in the nucleus affects haematopoietic cells during differentiation, how transcription factors and extrinsic factors modulate gene expression. These analyses will provide some essential rules to understand how gene expression is integrated in the nuclear context and how nuclear structure and cellular processes influence gene activity.

The factors leading to cell-to-cell variation can be classified as originating from two sources: (a) variations in global, or extrinsic, factors, such as varying amounts of transcriptional activators, or (b) essentially random, or intrinsic, molecular events, such as the transcription of a particular gene that is regulated by a specific set of activators.

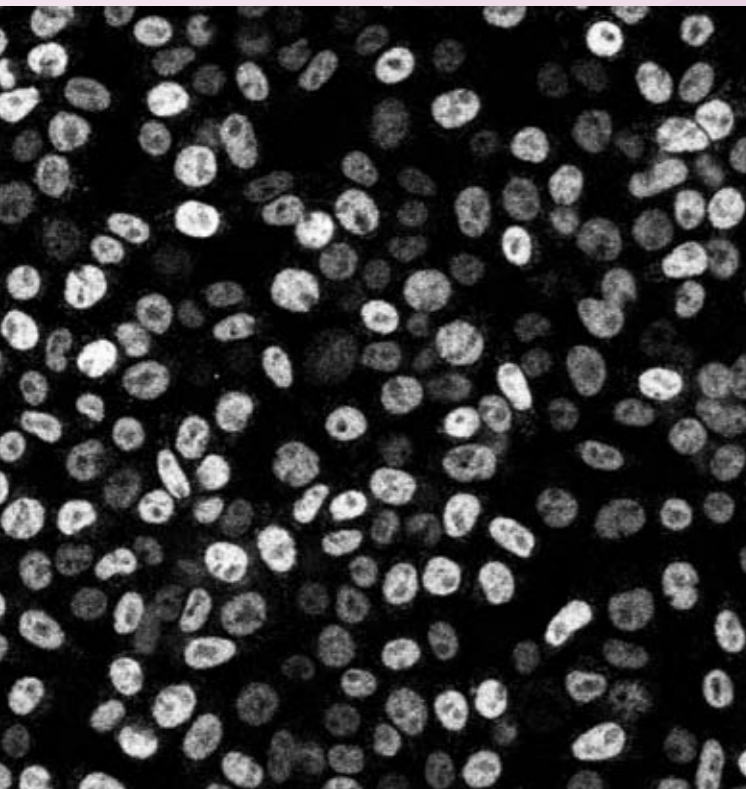
My group studies the contribution of the different factors to cell-to-cell variation, using single-cell and genomic approaches. Our long-term goal is to build a detailed map of processes such as cell differentiation and transformation.

SELECTED PUBLICATIONS

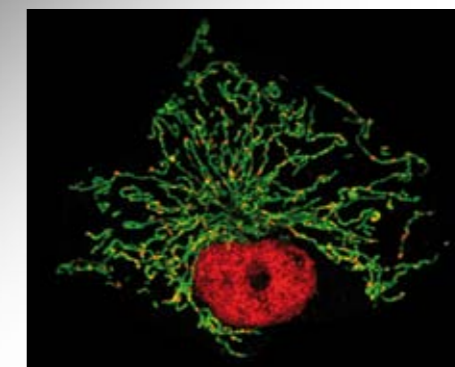
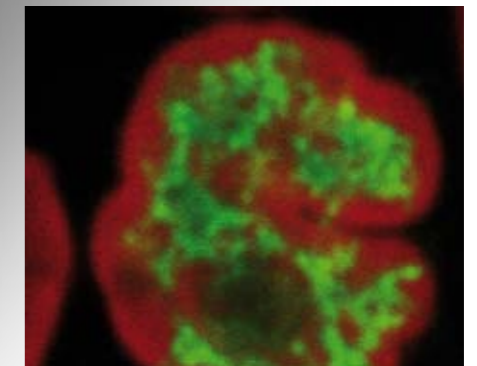
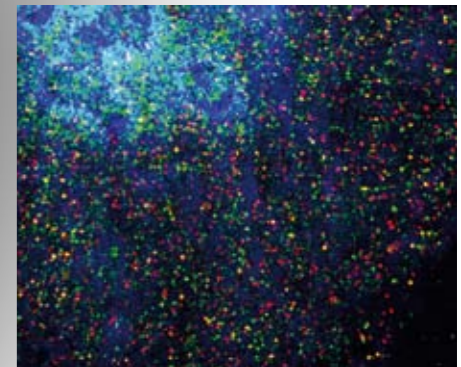
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Noise in transcription.



HeLa cells grown in presence of BrU, as a precursor of transcription, show a high degree of variability in Br-RNA production.

Cellular & Molecular Biology

LEAD INVESTIGATOR

Lluís Montoliu José

POSTDOCTORAL SCIENTISTS

Eduardo Moltó
Almudena Fernández

PREDOCTORAL SCIENTISTS

Julia Fernández
Esther Zurita
Diego Muñoz
Cristina Vicente
Davide Seruggia



TECHNICIAN

Marta Cantero

VISITORS

Clara Isabel Gómez
Almudena Tello
María Tiana
Zahira Corrales

Animal Models by Genetic Manipulation

In our laboratory, we are interested in understanding how mammalian expression domains work and how they are organised within genomes.

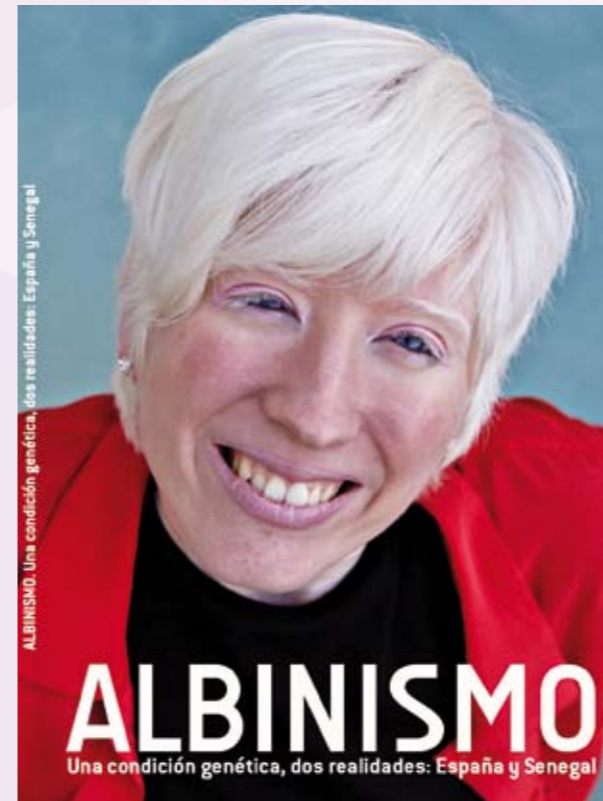
In particular, we focus on the identification and characterisation of genomic boundaries or insulators. By studying insulator elements, we aim to contribute to understanding of the functional and structural organization of vertebrate genomes. Insulators can be used effectively in biotechnological applications, as spacers, as boundaries, in any gene expression construct to be used in gene transfer experiments. They prevent inappropriate expression transgenes or gene therapy constructs and insulate them from neighbouring sequences at the insertion site in the host genomes. We are searching for new insulator sequences in vertebrate genomes, through initial bioinformatic analyses. Insulator candidates are functionally validated *in vitro* using cells and the enhancer blocking assay, and possibly *in vivo*, using transgenic animals bearing appropriate constructs (zebrafish in collaboration with JL Gómez-Skarmeta at the CABD, and mice at the CNB).

In addition, our laboratory generates and analyses new animal models to study neural alterations in vision, as well as in hearing, associated with albinism, a rare disease whose research effort is the focus of work within the CIBERER (www.ciberer.es). Using transgenic pigmented and albino mice in collaboration with the laboratory of I Varela-Nieto (IIB-CSIC/UAM), we showed that albino mice display premature severe hearing loss and do not recover after a noise-induced hearing loss, compared to their pigmented counterparts.

We collaborate with ALBA (www.albinismo.es), the Spanish association in support of people with albinism, and have published a book to inform society about the different types of albinism and their phenotypic consequences.

The expertise of our laboratory and its leadership in the Mouse Embryo Cryopreservation and Histology Facilities at the CNB have also been fundamental for our participation in the FP7 European Projects within the field of mouse functional genomics (INFRAFRONTIER) and in EMMA,

the European Mouse Mutant Archive, whose Spanish node at the CNB began operation in 2009 under our coordination. This work has produced additional publications within the field of animal transgenesis.



Cover of the book on albinism published by ALBA (2009) with the collaboration of L. Montoliu (photo by A Yturralde).

Finally, through collaborations, we have generated and are analysing a number of additional animal models (transgenic mice) to study human diseases, including Alzheimer, exploiting our technology of yeast artificial chromosome (YAC)-type of transgenes, which have been instrumental in the execution of scientific contracts with biotechnology and pharmaceutical companies.

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Transgenic zebrafish expressing GFP, used to validate insulator function of selected vertebrate genome elements. See Bessa et al. (2009) for additional information (photo by JL Gómez Skarmeta (CABD)).

Cellular & Molecular Biology

LEAD INVESTIGATORS

José R. Naranjo & Britt Mellström

POSTDOCTORAL SCIENTISTS

Diego Villar
Marcos Rivas
Malorzata Palczewska
Begoña Torres



PREDOCTORAL SCIENTISTS

Tomaso Benedet
Sofía Domingo
Kedar Ghimire

STUDENT

Paula Martínez

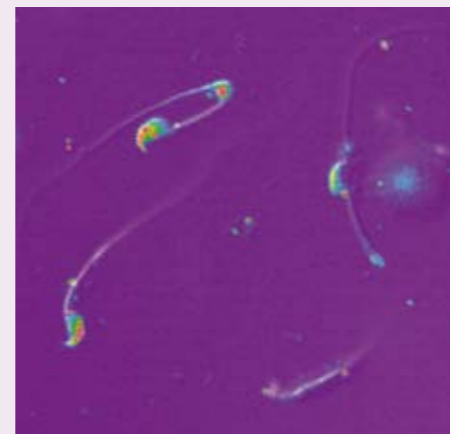
TECHNICIANS

M. Paz Gonzalez
Veronica Sanz
Xose Manuel Dopazo

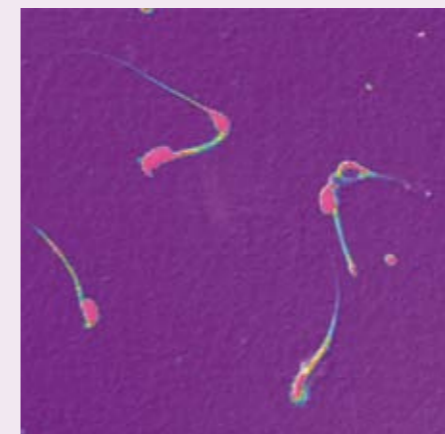
Functional Analysis of Transcriptional Repressor DREAM

We study the nuclear components of activity- and Ca²⁺-dependent transcriptional responses in neurons and immune cells to

i) understand the molecular determinants of downstream events responsible for plastic changes in synaptic function, ii) to regulate the output of physiological functions including learning and memory, pain sensitization and immune response, and iii) to develop tools to intervene in pathological processes during neurodegeneration. We foresee the Ca²⁺-dependent transcriptional repressor DREAM as an active/central component of several nucleoprotein complexes that mediate specifically the various transcriptional cascades triggered



DREAM regulates Ca²⁺ mobilization in sperm cells. The calcium signal (red) in response to progesterone (top) is reduced in transgenic spermatozoa overexpressing DREAM (down).



DREAM regulates thyroid gland development. High DREAM levels result in abnormal growth of the thyroid.

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PATENT

Mellström, Villar, Rábano, Dierssen y Naranjo. Compuestos para el tratamiento de enfermedades neurodegenerativas. CIBERNED, CSIC, CRG y Fundación CIEN. Patente referencia **P6529ES00**.



Cellular & Molecular Biology

LEAD INVESTIGATOR

Amelia Nieto

POSTDOCTORAL SCIENTISTS

Alicia Pérez
Ariel Rodríguez
Emilio Yángüez
Catalina Llompart



PREDOCTORAL SCIENTISTS

Roberto Alfonso
Paloma Rodríguez
Juan Pablo Chávez

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 RNA polymerase II (RNAP II) transcripts; this fact entirely decides its life cycle.

It first requires functional coupling between viral and cellular transcription machineries. Second, viral and cell mRNAs contain a 5' cap structure and a 3' polyA tail and are thus structurally equivalent; influenza virus must therefore have developed sophisticated strategies to discriminate and favour translation of its own mRNA. As a consequence of its transcription mechanism and the nature of the viral mRNA, influenza virus thus requires a complex system of virus-host factor interactions to complete a successful viral replication cycle. The viral polymerase plays a key role establishing productive interactions with host-cell factors involved in both cell transcription and translation.

Of the cell factors that interact with viral polymerase, we have been involved in the characterization of two transcription related factors: hCLE, a positive modulator of the RNAP II and CHD6, a chromatin remodeller. We observed that whereas hCLE also positively modulates influenza virus replication, CHD6 is a negative modulator.

Although viral and cell transcription are functionally coupled, degradation of cell RNAP II occurs once synthesis of viral mRNAs is complete, probably avoiding competition; this degradation appears to be a virulence marker. Using recom-

binant viruses containing polymerase segments from strains that do or do not induce RNAP II degradation, we observed that PA and PB2 polymerase subunits contribute individually to the degradation process.

Among the mechanisms that influenza virus uses to ensure selective translation of its mRNA, the eIF4F translation initiation complex has a key role. We characterized the influenza virus mRNA translation requirements for the components in this complex. Whereas eIF4A and eIF4G components are absolutely necessary, the cap-binding factor eIF4E is dispensable. Despite the fact that influenza virus mRNAs are capped, their translation is therefore independent of the cell cap-binding factor used by cellular mRNAs, and the viral polymerase might play the role of specific viral cap-binding complex for viral mRNA translation.

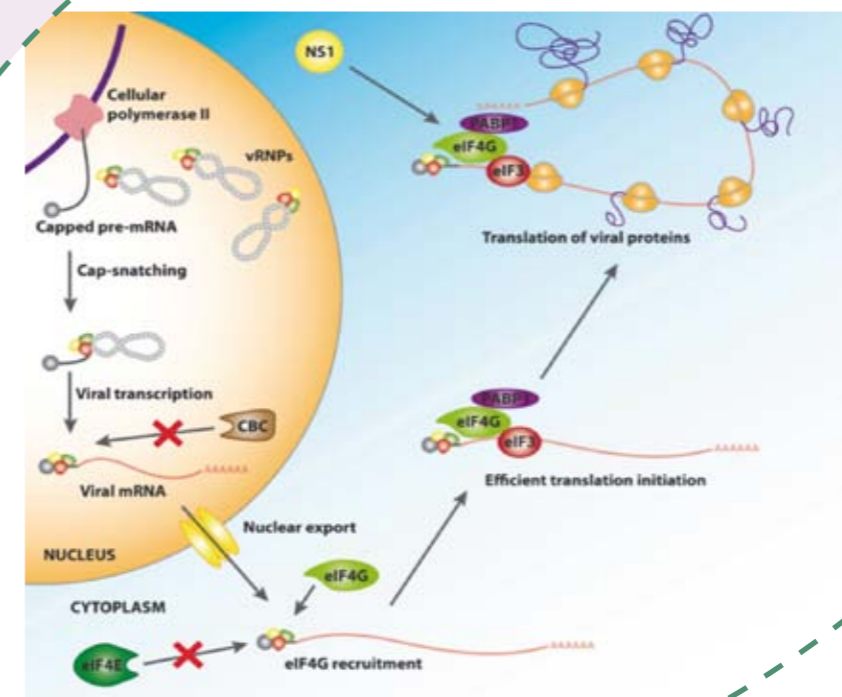
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Model for viral mRNA translation. Viral transcripts generated in the nucleus are transported to the cytoplasm with the polymerase bound. This situation avoids the replacement by eIF4E, permitting the recruitment of translation initiation complexes through the eIF4G binding capacity of the polymerase. In addition, the interaction of NS1 with eIF4G and PABP1 could help to the formation of a "closed loop" between the 5' and 3' ends of the viral mRNA activity co-operating to recruit translation complexes.



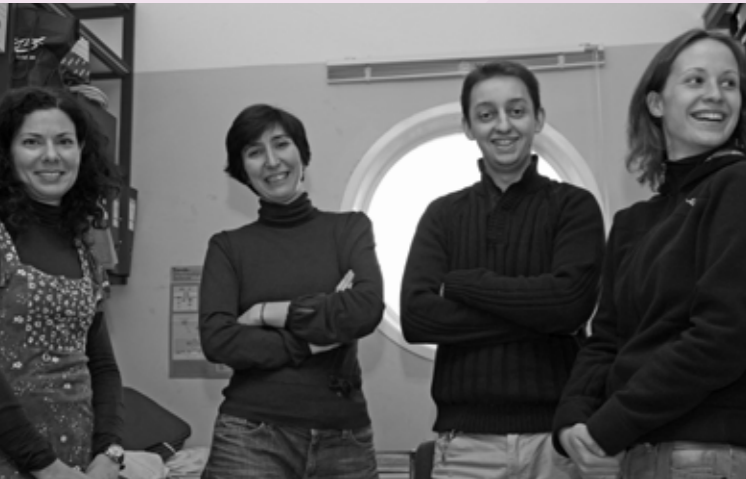
Cellular & Molecular Biology

LEAD INVESTIGATOR

Marta Nieto López

PREDOCTORAL SCIENTISTS

Alvaro Sebastian Serrano
María Fernanda Rodríguez



Cerebral Cortical Development

Human cognition is rooted in the exact formation of stereotyped complex patterns of connectivity among an enormous diversity of neurons during cerebral cortical development.

A fundamental question is to understand how the neurons of the cerebral cortex establish these precise patterns of connectivity. The cortex is organized in several functional and anatomical areas that are interconnected through stereotyped networks. Radially, the vertebrate cortex is organized into several neuronal layers (layers I-VI), and each layer contains neurons with similar molecular identities and connectivity patterns. In general, the selective expression of transcription factors (TF) in each layer and area couples the differentiation of neuronal subtypes to the establishment of their connectivity during embryonic and early postnatal stages.

Our most recent work, using knockout and knockdown studies combined with morphological, molecular and electrophysiological analysis, demonstrates that *Cux1* and *Cux2* are intrinsic and complementary regulators of dendrite branching, spine development, and the functional synapse of neurons of layer II-III of the cerebral cortex. *Cux* genes control the number and maturation of dendritic spines, partly through direct regulation of the expression of *Xlr3b* and *Xlr4b*, chromatin remodelling genes previously implicated in cognitive defects. We identified *FAM9A, B* and *C* (Martinez-Garay et al., 2002) as the closest orthologues of *Xlr* genes in humans, and found that *Cux1* and *2* proteins bind to sites in *FAM9A, B* and *C* loci that are conserved between primates and humans. This indicates that it is possible that similar *Cux*-mediated synaptic mechanisms act in humans. Downstream of *Cux*, we also found mechanisms of synaptogenesis key to cognition, including the downregulation of protein levels of NMDA receptor 2B (NMDAR2B), PSD95 and β -actin. Accordingly, abnormal dendrites and synapses in *Cux2*^{-/-} mice correlate with reduced synaptic function

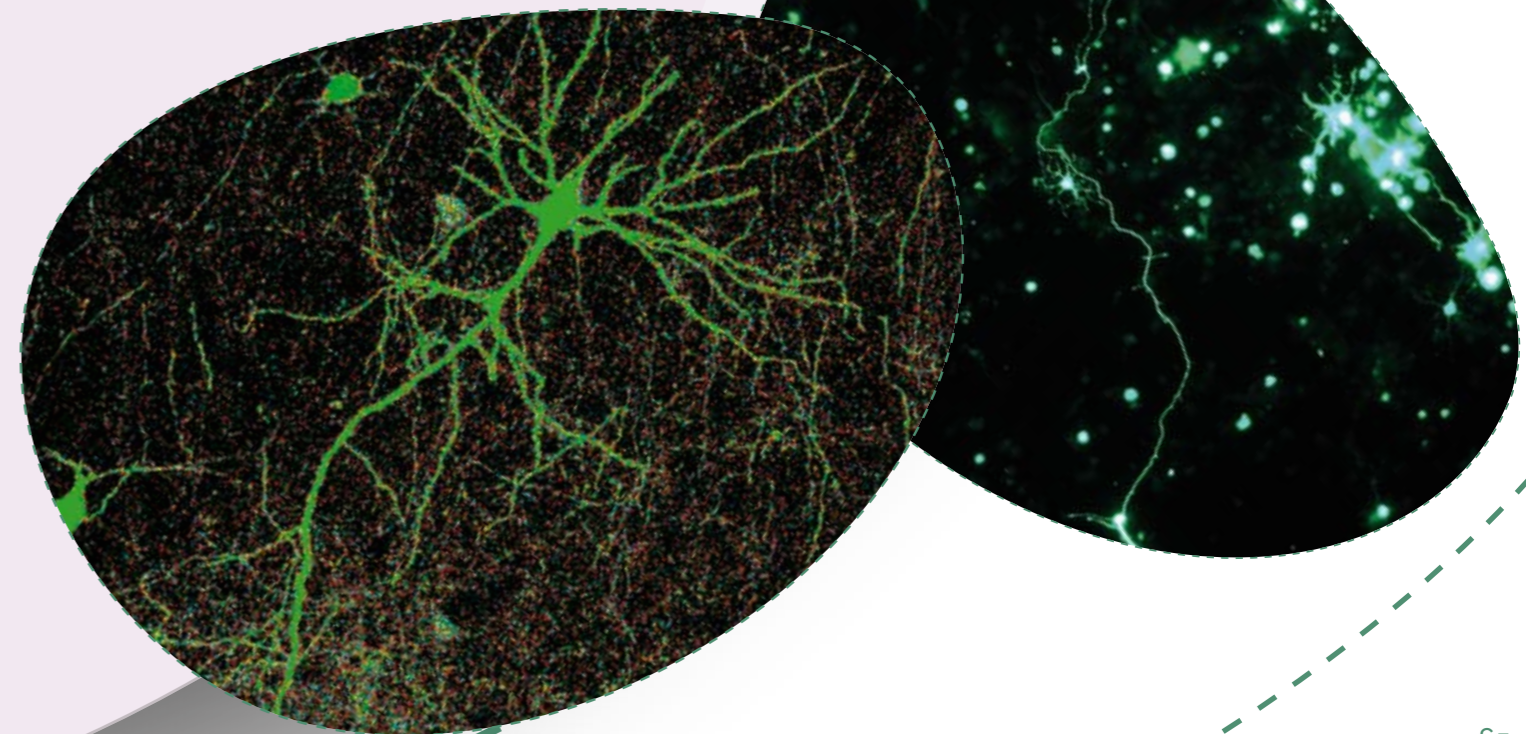
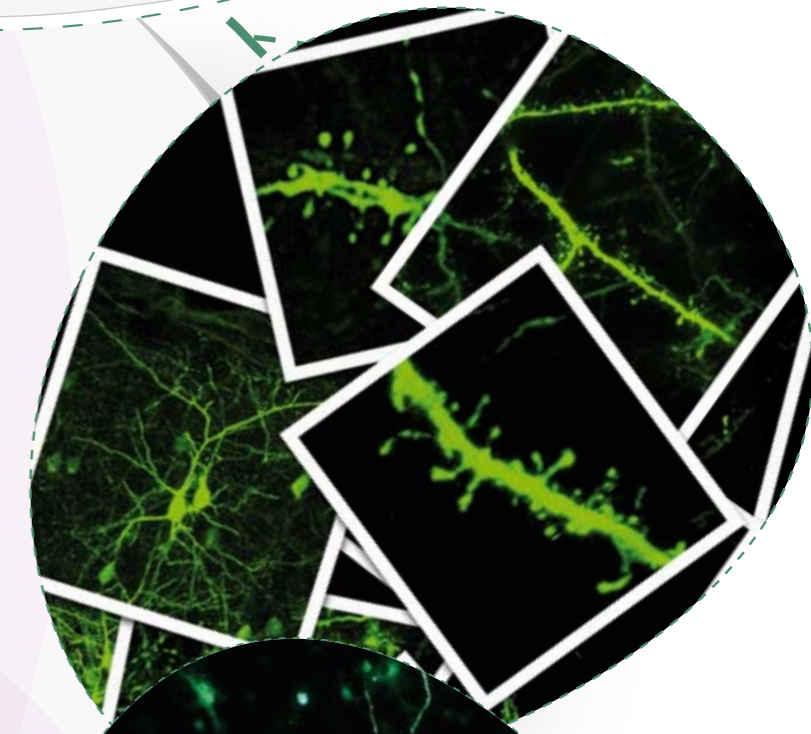
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and defects in working memory. Our work demonstrates critical roles for *Cux* in dendritogenesis and highlights novel subclass-specific mechanisms of synapse regulation that contribute to the establishment of cognitive circuits. Understanding brain wiring is an enormous task, fundamental for the dissection of normal cognitive processing and the molecular basis of disease. In particular, abnormalities in the development of layer II-III neurons are pathological, and associate to mental retardation and autism.

Dendritic structures. Green fluorescent protein (GFP)-labelled neurons and dendritic extensions generated after in utero electroporation of embryonic neural precursors.



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LEAD INVESTIGATOR

Juan Ortín Montón

POSTDOCTORAL SCIENTISTS

Susana de Lucas
 Lorena Ver
 Rocío Coloma
 Patricia Resa-Infante



PREDOCTORAL SCIENTISTS

Maite Pérez-Cidoncha
 Sara Landeras-Bueno
 Joan Peredo

TECHNICIANS

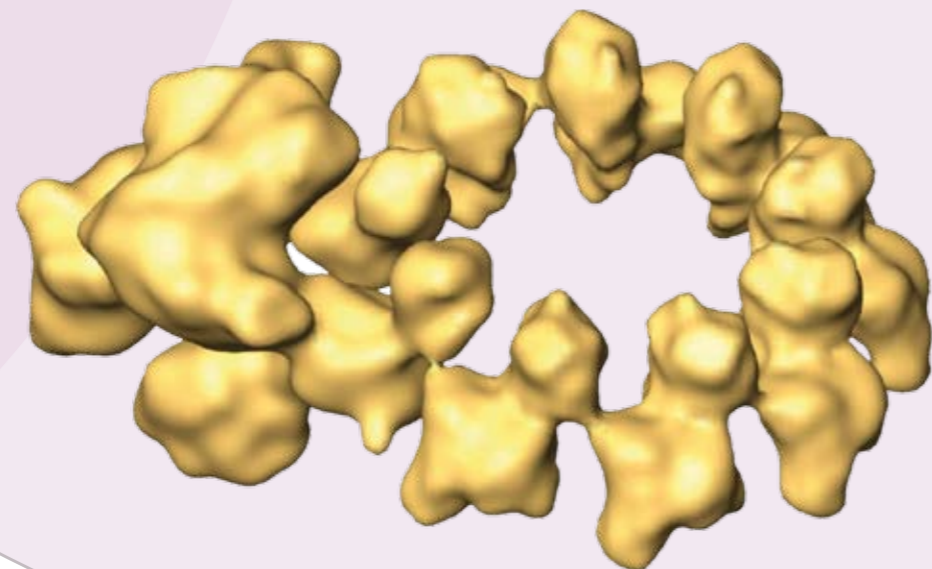
Yolanda Fernández
 Noelia Zamarreño
 Marcela Benavides

Transcription and Replication of Influenza Virus RNA

In the last two years, our group has continued the study of the structure and function of the influenza virus ribonucleoproteins (RNP) and the included polymerase complex. We have purified recombinant RNP as well as viral polymerase complex expressed in human cells.

The structure of these viral complexes was studied by electron microscopy of negative-stained or frozen samples and three-dimensional reconstruction, in collaboration with Jaime Martín-Benito, José María Valpuesta (CNB) and Óscar Llorca (CIB).

On the one hand, we established the first structure of an influenza RNP by cryo-EM at 12-18 Å resolutions that describes the interactions among NP monomers as well as the interactions of NP with the polymerase complex. The structure of the polymerase contained in the RNP is the best information available so far on any negative-strand RNA virus polymerase, and has allowed the localisation of specific domains of the included subunits. In addition, we described the structure of the polymerase in association to the template RNA but devoid of NP. These complexes were generated by replication *in vivo* of a micro-RNA template and show similarities, but also differences, with the polymerase integrated in



a functional RNP. The mechanism of viral RNA replication and transcription was analysed by genetic *trans*-complementation tests. We used highly efficient procedures for the generation and purification of recombinant RNP, and also characterised polymerase mutants defective in either RNA replication or transcription, to determine whether the polymerase associated to the parental RNP is responsible for the synthesis of the progeny RNA and/or its encapsidation into progeny RNP. The results are compatible with a new model for virus replication whereby a polymerase complex distinct from that present in the parental RNP carry out RNA synthesis *in trans* and yet another polymerase complex is in charge of encapsidating the progeny RNA. In contrast, the polymerase complex associated to the parental RNP carry out RNA transcription *in cis*.

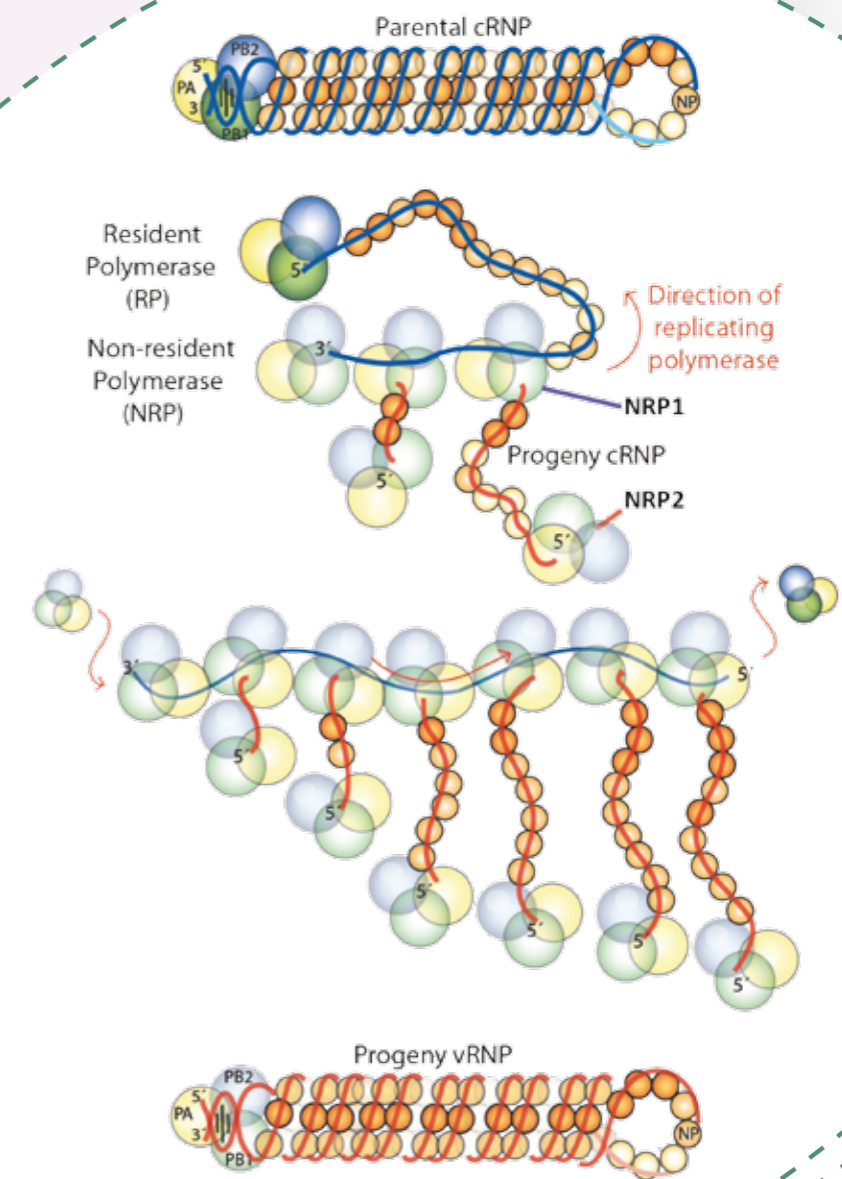
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Cellular & Molecular Biology

LEAD INVESTIGATOR

María del Carmen Rivas Vázquez

POSTDOCTORAL SCIENTISTS

Dolores González de Llano
Michela Campagna
Laura Marcos Villar



PREDOCTORAL SCIENTISTS

José del Carmen González Santamaría
Pedro Gallego Jiménez

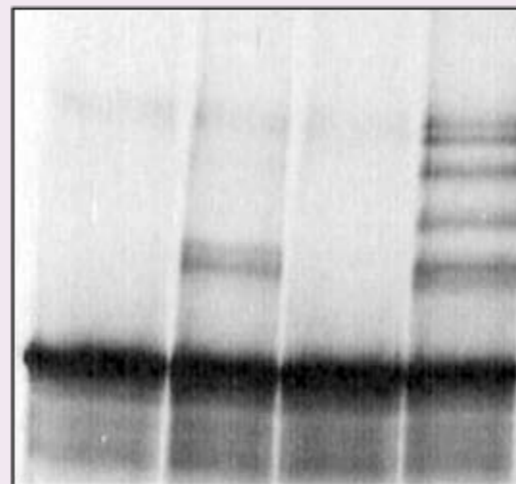
Virus and Cancer

Our group is interested in the relationship between virus and cancer.

One of our lines of investigation is “virus as the driving force of cancer”. Viral infection has long been associated with human cancer; one of the most recent links is with Kaposi’s sarcoma-associated herpesvirus (KSHV), the etiologic agent of Kaposi’s sarcoma, primary effusion lymphoma and multicentric Castlemann’s disease. The mechanisms by which KSHV infection might cause cancer are still being clarified. Most of the tumour cells in these neoplasms are latently infected with KSHV, suggesting that the latent KSHV genes are responsible for the viral pathogenesis. LANA2 is one of the latent KSHV proteins, and was shown to be absolutely necessary for survival of KSHV-infected primary effusion lymphoma cells. In recent years, our group has identified some of the functions of this viral protein, which suggest that LANA2 has an important role in the transforming activity of the virus. Continuing with these studies, in the last two years we have:

- Identified a new function that might contribute to the pathogenesis of the virus, the disruption of cell PML-NB by LANA2.

SAE1/2	+	+	+	+
Ubc ^o	+	+	+	+
SUMO-1	-	+	-	-
SUMO-2	-	-	-	+

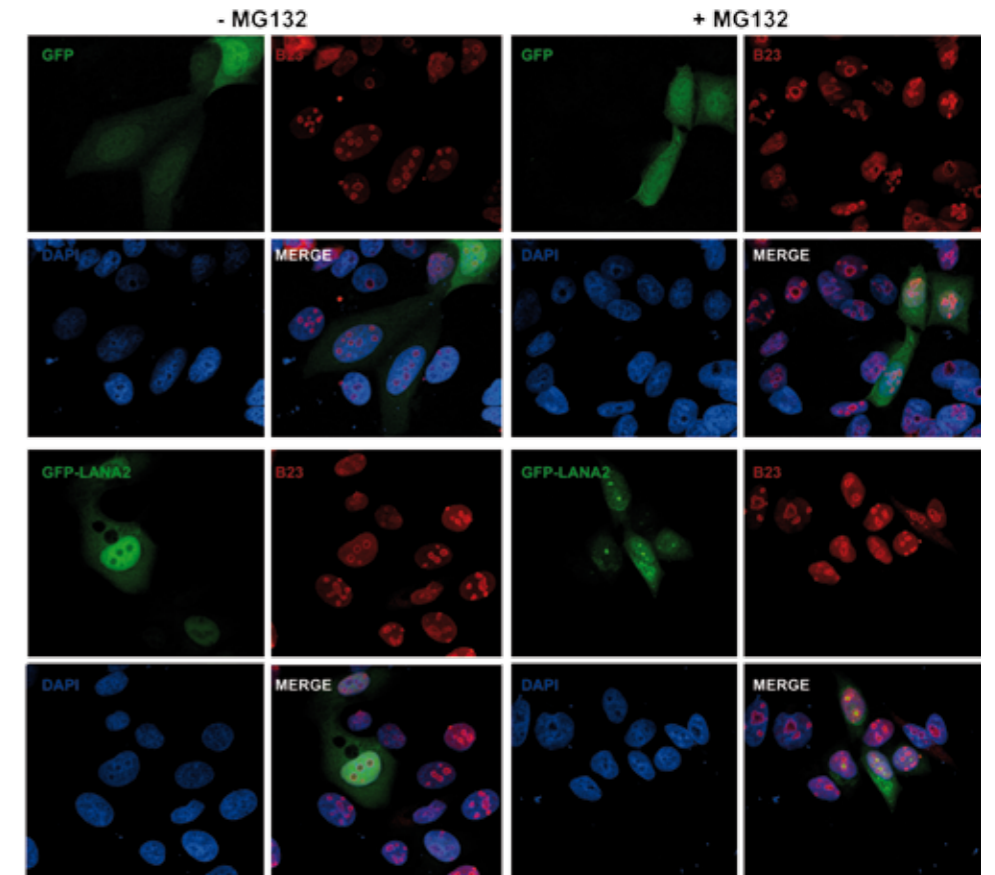


Sumoylation of LANA2 in vitro.

- Demonstrated that LANA2 activity is regulated by SUMO modification.
- Demonstrated the induction of chromosome instability by LANA2.

In addition, we are interested in evaluating the importance of different tumour suppressors in the complex innate antiviral host defence. DNA tumour viruses have developed mechanisms to inhibit tumour suppressors, and activation of some tumour suppressors after interferon treatment has been described. Together, these results suggest that tumour suppressors could be important for the antiviral response of the cell, providing new links between tumour suppression and the antiviral host defence. As a result of our studies on the regulation of virus infection by major components of the cell tumour suppression mechanisms carried out in the last two years, our group has:

- Identified the tumour suppressor pRb as necessary for correct activation of the NFκB pathway in response to virus infection.
- Described a new function of the deacetylase SIRT1: regulation of correct PML-NB formation.



Nucleolar translocation of LANA2 upon inhibition of proteasome-dependent protein degradation.

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Cellular & Molecular Biology

LEAD INVESTIGATOR

Dolores Rodríguez Aguirre

POSTDOCTORAL SCIENTISTS

**Jaime Pignatelli Garrigós
Julio Alonso Padilla**



PREDOCTORAL SCIENTISTS

**Ginés Ávila Pérez
Laura Santamaría Rubio
Gliselle Nieves Molina**

TECHNICIANS

**Miriam Jiménez de Olmo
Henar Petit Balmaseda
Susana Plazuelo Calvo**

Molecular Characterization and Epidemiology of Torovirus

Our general objective is to study different biological aspects of the toroviruses, emergent viruses that are practically ignored although they have the potential to infect and cause enteric diseases in various animal species and in man.



Double-membrane vesicles (DMV) in cryosections of BEV-infected cells.

Toroviruses are enveloped viruses with a single-stranded RNA genome, which belong to the *Coronaviridae* family within the order *Nidovirales*. Four genotypes or species have been identified so far: equine torovirus (EqToV or BEV), bovine torovirus (BToV), porcine torovirus (PToV) and human torovirus (HToV). The impossibility of growing these viruses in cultured cells, with the single exception of the equine torovirus BEV, has probably contributed to their insufficient study.

Our group has developed two specific assays for diagnosis of porcine torovirus (PToV): an ELISA to determine antibodies to torovirus in serum samples, and a real time RT-PCR for detection and quantitation of PToV in clinical samples.

Results using these assays show that porcine torovirus is highly prevalent in Spanish porcine livestock; this was the first study to show the presence of torovirus in Spain. We later performed a longitudinal study analysing samples taken from animals at different times during the piglet's life, which showed that they are infected shortly after weaning, when maternal protection transferred through colostrum declines. We are currently performing an extensive epidemiological study and genetic characterization of the viral strains identified.

Another research line focuses on the study of virus-host interactions. We observed that BEV causes apoptosis at late times post-infection, once the new viral progeny have been produced. In addition, we showed that the structural proteins S and M are able to trigger this process when expressed individually. Thus, the induction of apoptosis in the infected cells, specifically in the enterocytes and the crypts of the enteric tract, could be the cause of the diarrhoea exhibited by infected pigs.

A third area of interest in our group is the characterisation of the torovirus replication/transcription complexes. Positive-strand RNA viruses use virus-modified cell membranes to build their replication machineries. By electron microscopy, we observed clusters of double membrane vesicles (DMV) in the cytoplasm of cells infected with BEV, the prototype torovirus. They were observed both in thin sections of infected cells embedded in conventional epoxy resin and in cryosections that were immunogold-labelled with antibodies to double-stranded RNA. We are now determining the composition of these structures and attempting to identify the origin of the associated membranes.

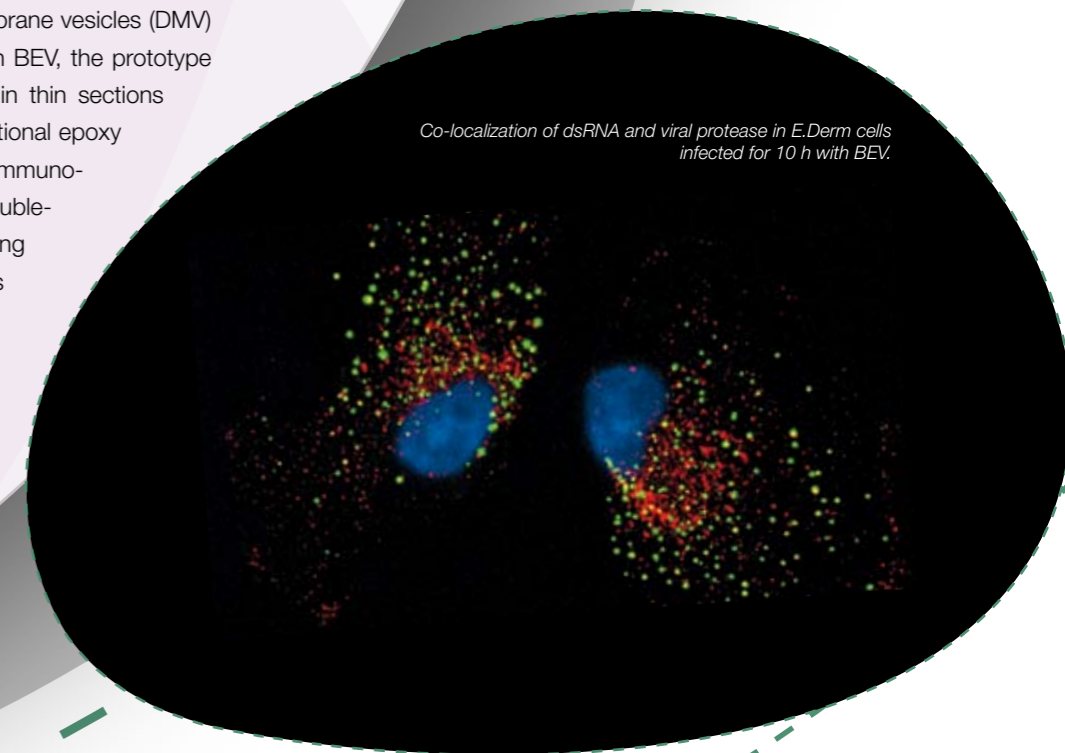
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Co-localization of dsRNA and viral protease in *E.Derm* cells infected for 10 h with BEV.



Cellular & Molecular Biology

LEAD INVESTIGATOR

José Francisco Rodríguez Aguirre

POSTDOCTORAL SCIENTISTS

Rosa M^a Moreno Dalton
Damià Garriga Rigau



PREDOCTORAL SCIENTISTS

Nerea Irigoyen Vergara
Idoia Busnadiego Rezola
Diego Ferrero

TECHNICIAN

Antonio Varas Rios

VISITORS

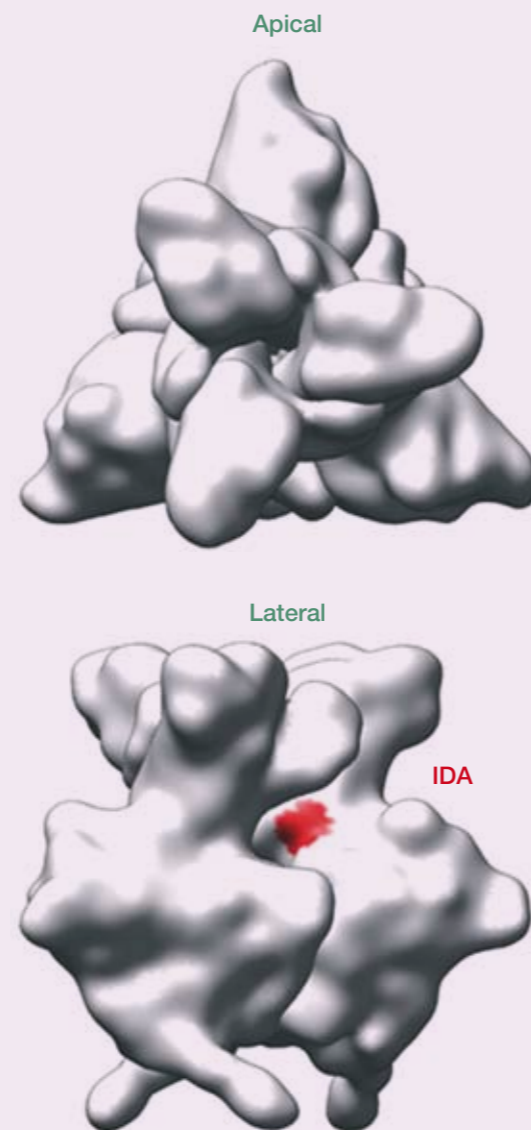
Flavia Zanetti
Ariel Talavera Pérez
María del Carmen Lago
Ana M^a Oña
Ignacio Jiménez
Fernando Méndez

Confocal laser scanning micrograph of QM7 cells infected with a recombinant vaccinia virus expressing a mutant version of the IBDV polyprotein encoding the capsid precursor (pVP2), the virus protease (VP4) and the scaffolding (VP3) polypeptides. Green corresponds to pVP2, Red to VP3, and Blue to nuclear staining.

Biología Molecular de 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. Some of these viruses cause diseases of great socio-economic significance. Our main virus model is the infectious bursal disease virus (IBDV), the etiological agent of an acute immunosuppressive disease affecting domestic chickens. Our group is mainly interested in two broad research areas: (i) Birnavirus structure and morphogenesis; and (ii) the molecular basis for IBDV virulence.



We have continued our molecular analysis of the IBDV assembly centered on the role of the multi-functional VP3 polypeptide and the precursor of the capsid polypeptide (VP2). The characterization of the particle structure led us to demonstrate that the largest fraction of infectious IBDV particles contain four genome segments, thus providing the first evidence about the existence of icosahedral viruses with polyploid genomes. These studies led to the observation that the inner capsid space of the IBDV particles is occupied by a ribonucleoprotein (RNP) complex formed by the dsRNA genome associated to the VP3 polypeptide and the RNA dependent RNA polymerase (RdRp). The presence of birnavirus RNPs represents a sharp divergence with respect to prototypical icosahedral dsRNA viruses whose genomes remain enclosed within an inner capsid (T=2) known as transcriptional core. The unique birnavirus structural and functional configuration pose major questions about their replicative strategy, i.e. the mechanisms for RNA transcription and replication mechanisms, and those used to counteract cellular dsRNA sensors and control host-cell innate immune responses. We are currently focusing a great deal of effort to functional characterization of the VP3 polypeptide. This multifunctional and highly conserved protein that plays different roles during virus replication and morphogenesis is a key element responsible for the control of the host's innate antiviral responses.

As part of our work on virus-host interactions, we have identified the $\alpha 4\beta 1$ integrin as the major IBDV binding receptor. IBDV cell binding entails the recognition of a strictly conserved sequence motif located in the projection domain of the capsid polypeptide by the $\alpha 4\beta 1$ heterodimer. Additionally, we have shown that under certain conditions IBDV is capable of establishing persistent infections in chicken lymphocytes. These studies have opened new venues to characterize the mechanism of IBDV entry, and the molecular determinants for virus tropism, and for the development of new strategies to control IBDV infection.

SELECTED PUBLICATIONS

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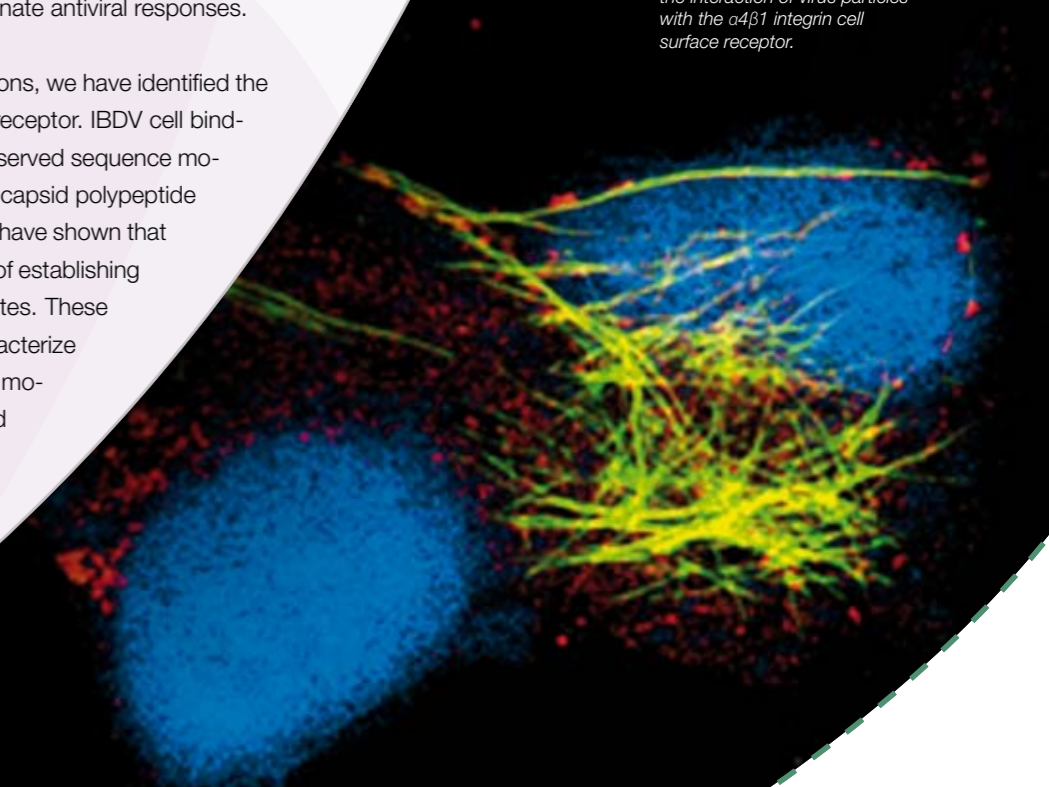
PATENTS

Bionostra SL. Empty Capsids (VLPS(-VP4)) of the infectious bursal disease virus (IBDV), obtainment process and applications. Aug 20, 2009. **US 2009/0208528 A1.**

Bionostra SL. chimeric empty capsids of the infectious bursal disease virus (IBDV), obtainment process and applications. Mar 4, 2009. **EP 1706486 B1.**

Chimera Pharma SL. chimeric empty viral-like particles derived from the infectious bursal disease virus (IBDV), process for their production and applications. Jan 13, 2009. **US 7476387.**

Apical and lateral views of a three-dimensional model of the VP2 trimer, the building block of the IBDV capsid. The red path indicates the position of the "IDA" tripeptide responsible for the interaction of virus particles with the $\alpha 4\beta 1$ integrin cell surface receptor.



Cellular & Molecular Biology

LEAD INVESTIGATOR

Esteban Veiga Chacón

POSTDOCTORAL SCIENTIST

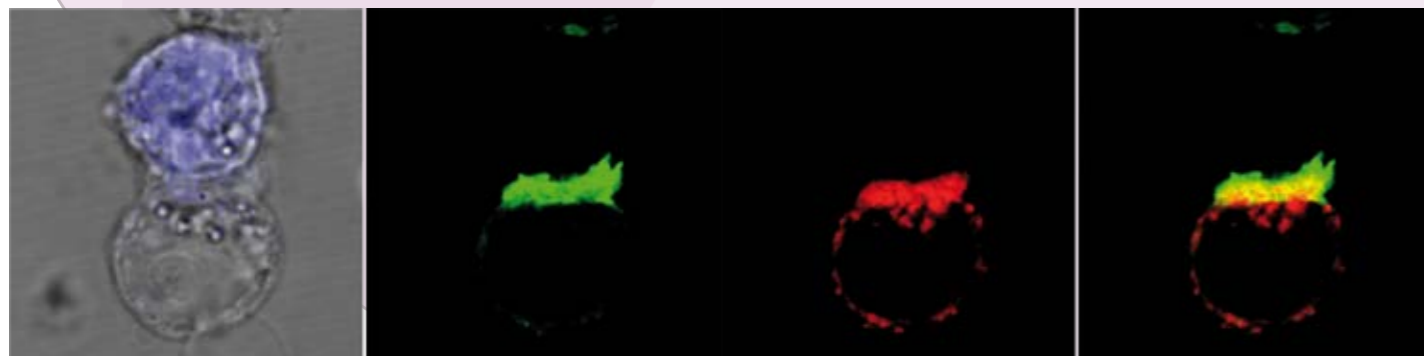
Aranzazu Cruz Adalia

PREDOCTORAL SCIENTISTS

Carmen Calabía-Linares
Mónica Torres Torresano



Clathrin accumulates at the IS. Combination fluorescence and phase contrast image showing an antigen (SEE)-loaded APC (blue) conjugated with a T cell expressing td-Tomato-LCa (clathrin; red), actin-GFP (green).



Cellular Immunobiology and Microbiology

In the last two years (2009-2010), our group, “Cellular Immunobiology and Microbiology (ICM)” has been studying the molecular and cellular mechanisms that allow the formation of immunological synapses.

To become activated, T cells must establish cell-cell contact with antigen-presenting cells (APC). This contact, known as the immune synapse (IS), drives major morphological and functional changes in T cells, including massive actin rearrangements necessary for productive IS formation. The IS serves as a platform for large-scale molecular exchange between the IS-forming cells. Multiple cytokines and vesicles, which drive intercellular communication, are released to the synaptic cleft. We were the first to show that clathrin is essential for the massive actin polymerization observed at the IS. This finding, observed in cell lines and primary cells, is of outstanding relevance not only to immunologists, but also to cell biologists and scientists in the field of cellular microbiology. We are currently carrying out studies on the molecular mechanisms that drive pathogen infections, and extending these studies to the intimate relationship between bacterial and fungal pathogens with the cells of the immune system. We are also exploring the possible therapeutic use of bacterial products able to modify the immunological system.

We are currently financed by a project from the Spanish Ministry of Science and Innovation, BFU2008-04342/BMC.

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Electron micrograph of the IS. The MVB are clearly visible near the cell-cell contact area.

