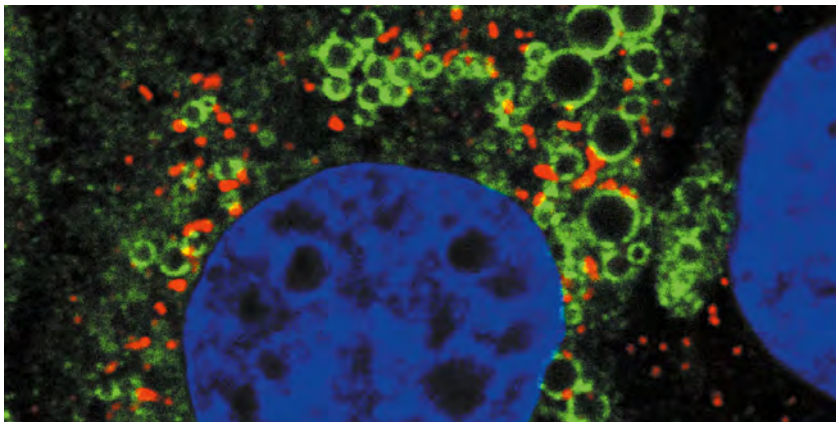

Molecular and Cellular Biology

The Department of Molecular and Cellular Biology hosts 19 independent research groups working in two broad, closely interwoven research areas, with the goal of identifying specific therapeutic targets for use in disease prevention and control. The first area focuses on Virology, dissecting viral replication mechanisms and on structural studies of key viral proteins, as well as virus-host interactions for important human and veterinary pathogens. The identification of virus and cell elements with key roles in virus replication is essential for the rational design and implementation of new strategies for disease control. Understanding the mechanisms that allow a virus to evade or counteract innate and adaptive host immune responses will allow generation of innovative vaccination strategies and virus-based vaccine vectors. The second area regards different aspects of Biomedicine, from gene expression, to cellular biology of tumors and the development of novel antitumor therapies to understand the molecular networks allowing the generation of neurons and circuits of the mammalian cerebral cortex. The main aim of this research program, is to improve current diagnostics and therapies for different human diseases.

Our department developed the virus biotechnology platform (VBP), created with the aim of providing integral biotechnological solutions to health challenges caused by viruses. In the context of the COVID-19 pandemic several groups have devoted their efforts to fight against SARS-CoV-2 by: i) developing vaccines based on non-replicative SARS-CoV-2 replicons and on poxvirus recombinants; ii) developing a high throughput screening platform to test compound libraries for their antiviral potential against SARS-CoV-2; iii) developing ultra-deep sequencing techniques to study intra-host variability of SARS-CoV-2; iv) producing recombinant SARS-CoV-2 proteins as antigens for the development of serological test and potential vaccines; v) producing monoclonal antibodies for anti-viral therapy; vi) controlling viral infection through the modulation of cellular energy metabolism; and vii) using the CRISPR/cas13d technology as a therapeutic tool to target coronavirus RNA genome.

HEAD

Esteban Veiga

Figure legend: Confocal microscopy image of hepatitis C virus-infected Huh7 cells. HCV core protein (green) localises at the surface of lipid droplets, which are prominent in this hepatoma cell line. A host factor required for efficient initiation for viral RNA replication, LPIN1, was labeled in red. (63X magnificaton with a 7X digital zoom) (Pablo Gastaminza´s lab).

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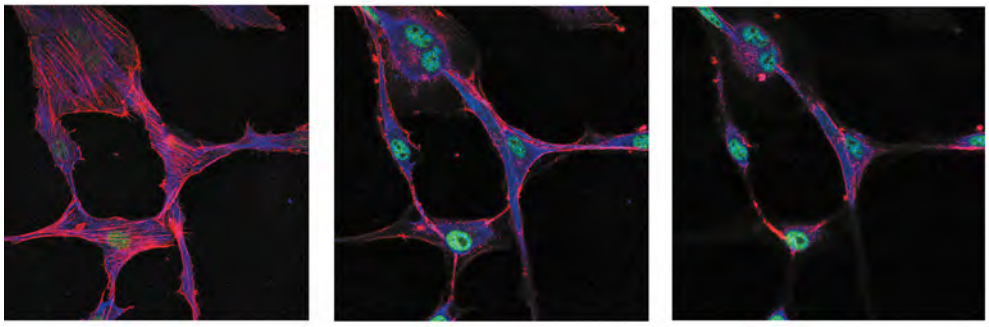
Molecular bases of actin cytoskeleton reorganisation in cell motility, tumour generation and invasiveness

Cancer can generate from oncogene-mediated transformation of stem cells. Aged stem cells are more vulnerable to malignant transformation, making aging a risk factor for developing cancer. Altered integrity of the actin cytoskeleton perturbs cell metabolism and tissue homeostasis contributing to functional decline in older individuals. Thus, modifiers of the actin cytoskeleton dynamics can be additional inducers of age-associated diseases like cancer. WIP (Wiskott-Aldrich Syndrome Protein (WASP) Interacting Protein) serves as an appropriate model to study cancer development and aging, as it regulates the organization of the actin cytoskeleton and participates in cell proliferation, migration, invasion, differentiation and tumour progression. Our group aims to define the molecular and physiological bases of WIP function in relation to stem cell activity during cell transformation and organism aging.

A combination of biochemical, proteomic and transcriptomic approaches, advanced imaging and 2/3D cell cultures, have led us to describe the pro-oncogenic activity of WIP in solid tumours like GBM (glioblastoma), colorectal and breast cancer mediated by transcription regulators YAP/TAZ. Database analysis confirmed that low WIP levels correlate with a higher overall survival of cancer patients (GBM, head and neck, gastric and breast). Interestingly, WIP acts as a tumour suppressor in ALK+ (anaplastic lymphoma kinase) haematological cancers. Our proteomic analyses of the WIP interactome have identified potential candidates that could explain WIP specific activity in solid tumours, focusing on GBM. Complementary, we have observed that WIP-deficient mice present shorter lifespan and phenotypic characteristics compatible with premature aging, such as immunological disorders or homeostatic alterations of tissues relying on proper stem cell activity. Our results reinforce the importance of WIP as a promising therapeutic target both for cancer and aging. They also open new venues to study the contribution of actin cytoskeleton regulatory proteins in (cancer) stem cell development and functionality, and their contribution to associated human diseases.



Confocal microscopy image of WIP and YAP/TAZ distribution in glioblastoma cells. Low, medium and upper stacks (from left to right) of U-87 human glioblastoma cells fixed and stained for actin (red), YAP/TAZ (green) and WIP (blue).

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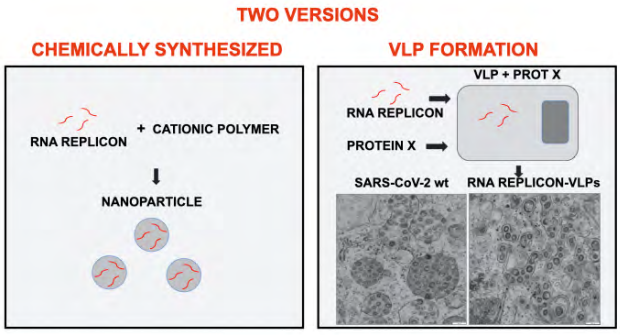
Coronavirus: replication and transcription, virus-host interactions, and protection

Coronaviruses are emerging viruses with pandemic potential. To date, seven coronaviruses (CoV) that infect humans are known. HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1 cause up to 15% of mild respiratory infections. In contrast, SARS-CoV, MERS-CoV, and SARS-CoV-2 cause severe pneumonia and acute respiratory distress syndrome (ARDS), which are potentially deadly conditions. The problem is even greater in the elderly population, which responds with lower efficacy to vaccination. SARS-CoV, MERS-CoV, and SARS-CoV-2 emerged from animal reservoirs in the 21st century, being SARS-CoV-2 the causative agent of the COVID-19 pandemic.

Our laboratory focuses on the study of virus-host interactions, the design of vaccines and the selection of antivirals to protect against severe respiratory CoV infections by modulating the innate immune response in young and elderly populations.

The main aims of our research are:

- **Development of next generation of SARS-CoV-2 vaccines consisting in replication-competent propagation-deficient RNA replicons and to determine their efficacy in animal model systems.** Vaccine development includes: (i) Engineering the SARS-CoV-2 RNA-replicons by deleting or modifying viral genes responsible for propagation and virulence, using reverse genetics; (ii) Development of packaging cell lines that efficiently complement the generation of virus-like particles (VLPs); (iii) Identification of RNA-replicon delivery systems; (iv) Engineering simplified and safer versions by reducing the replicase size.
- **To identify cell-signaling pathways involved in CoV replication and pathology** in order to select antiviral drugs that inhibit these pathways. We study PBM-PDZ protein-protein interactions and viral accessory genes involved in the innate immune and inflammatory responses, since activation of these pathways is responsible for virulence.
- **To determine the relevance of post-transcriptional regulation of gene expression to the inflammatory pathology.** We study the contribution to dysregulated inflammation of small non-coding RNAs (host miRNAs and virus-derived RNAs) and RNA-protein complexes. RNAs and proteins involved in these regulatory networks represent potential antiviral targets.



RNA replicon vaccines to protect against highly pathogenic human coronaviruses. Two types of replicon delivery systems have been designed: (i) a chemically synthesised one that includes two components, the RNA replicon and a cationic polymer to form nanoparticles. (ii) formation of virus like particles (VLPs) complemented in packaging cells lines with the proteins required for propagation from cell to cell.

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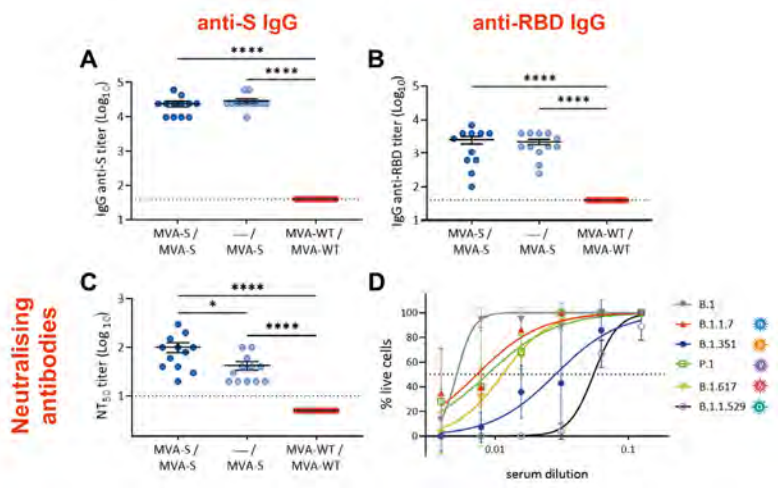
Poxvirus and vaccines

The main objectives of our laboratory are directed to understand the molecular basis of the biology 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 emerging and reemerging viruses**, like HIV, chikungunya, Ebola, zika, hepatitis C, coronavirus SARS-CoV-2, as well as against cancer. As a model system of an infectious agent and as a delivery vector for expression of genes of interest, we used vaccinia virus (VACV) and the attenuated vaccine strains MVA and NYVAC, members of the poxvirus family. Our goal is to develop the best-in-class immunogens and vaccination protocols to be applied as vaccines against **prevalent human diseases.**

Due to the SARS-CoV-2/COVID-19 pandemic, during the period of 2021-2022 we focused our research in the development of a vaccine against this coronavirus. We generated modified vaccinia virus MVA vectors expressing the S (spike) protein of SARS-CoV-2, as well as conserved domains of the main structural proteins of the virus. In three animal models (mouse, hamsters and macaques), we demonstrated that the vaccine MVA-CoV2-S triggered potent immune responses and complete protection against SARS-CoV-2 infection. Moreover, the vaccine induced long-term high titers of binding antibodies to S and of virus neutralising antibodies, together with activation of specific CD4+ and CD8+ T cells, markers that correlated with protection against infection. In addition, the vaccine produced sterilising immunity in the brain and broad spectrum of neutralisation against SARS-CoV-2 variants of concern (VoCs). GMP lots of the vaccine were produced by the company Biofabri in Spain and a phase I clinical trial is under consideration. The vaccine was patented and received wide coverage in the media (radio, press and TV). Financial support was obtained from various private and public institutions.



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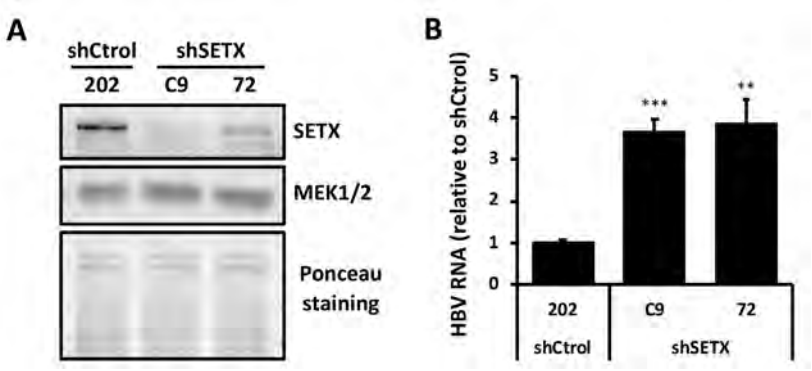
Virus-host interactions in hepatitis B virus infection

Our laboratory is interested in understanding the virus host interactions that regulate the outcome and pathogenesis of hepatitis B virus (HBV) infection in order to identify vulnerabilities that could be exploited to develop new antiviral therapies against this important human pathogen. Indeed, HBV is responsible of millions of cases of acute and chronic hepatitis and represent the major etiological agent of liver cancer worldwide.

During the 2021-2022 period, we focused on understanding the role of cellular proteins in the replicative cycle of HBV. We have confirmed our initial observations that DNA damage response related proteins, such as Senataxin and Ku70/Ku80, are key restriction factors for HBV infection and described their role as negative regulators of HBV gene expression. Moreover, we have expanded these observations to other viral and non-viral systems which gene expression relies on episomal DNA as template for transcription. These results suggest that the identified factors are master regulators of episomal gene expression.

Complementary to those studies, we are also working on the identification and characterisation of cellular genes and pathways that are required for HBV DNA integration in the host cell, an aspect that is important for HBV-related hepatocellular carcinoma development.



Effect of Senataxin silencing on HBV gene expression. *(A) Senataxin downregulation efficiency measured by WB analysis by two different shRNA sequences (C9 and 72). (B) Effect of Senataxin silencing on the accumulation of intracellular HBV RNA during HBV infection.*

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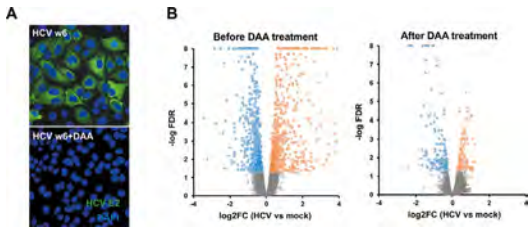
Hepatitis C and related virus infection

The main objective of the laboratory is to understand the molecular mechanisms underlying efficient infection by pathogenic human viruses, with the ultimate goal of identifying novel targets for antiviral therapy. The lab uses multidisciplinary approaches studying functional and structural aspects infection. The approaches and methodologies implemented in our laboratory for hepatitis C virus (HCV) infection are already being applied to study other pathogens, mainly Flaviviruses and recently SARS-CoV-2.

We used models of persistent HCV infection to determine the ability of direct-acting antiviral (DAAs) drugs of restoring normal cell homeostasis and ultrastructure in formerly infected cells. We have focused on the differential transcriptomic profiles of human hepatoma cells persistently infected with HCV, before and after infection elimination by treatment with DAAs. Our studies suggest that persistent HCV infection causes irreversible transcriptional alterations in the host. Studies in differentiated, growth arrested cell cultures enabled identifying a de-differentiation profile of the formerly infected cells, providing insight on the contribution of HCV replication on hepatocarcinogenesis, a biomedical problem that remains elusive for patients that have cleared the infection. Cell culture HCV models enabled monitoring reversion of the ultrastructural alterations caused by persistent HCV replication using cryo soft X-ray tomography, a technique producing 3D maps of whole cells in a quasi-native state that facilitate investigating the host mechanisms of viral replication organelle elimination after treatment with DAAs.

1



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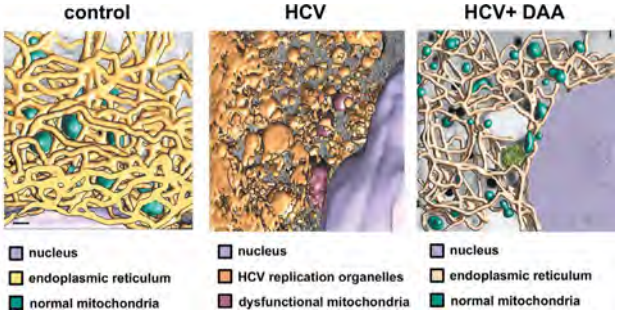

1 ***Irreversible transcriptional alterations in persistently HCV infected cells.*** *A-Immunofluorescence microscopy showing the loss of viral antigen staining (green) in cells counterstained using the nuclear stain DAPI (blue). B-Vulcano plot (log2 fold change in expression versus -log of false discovery rate (FDR) for individual transcripts illustrating the impact of HCV infection on the host cell transcriptome before and after virus elimination using direct acting antivirals. Data from 4 biological replicates show how virus elimination does not entail complete normalization of the cell transcriptome despite elimination of all viral proteins and RNA. Significantly (FDR<0.05) upregulated transcripts are shown in orange and downregulated transcripts are shown in blue.*

2 ***Tridimensional model of cells replicating HCV before and after antiviral treatment.*** *Cryo-SXT of control, HCV-replicating cells untreated or treated with DAAs. Manual segmentation of the surface boundaries identifying the organelles: normal mitochondria in dark green, abnormal mitochondria in dark purple, ER in yellow-brown, nuclear envelope in light purple and HCV-modified ER in orange. The scale bar represents 1 µm.*

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Animal models by genetic manipulation

Our laboratory is interested in understanding the underlying pathological mechanisms of a group of human rare diseases known as albinism, a heterogeneous genetic condition associated with mutations in at least 22 genes, characterised by visual impairment, present in all types, and pigmentation alterations, not obvious for some cases. This work on human rare diseases is associated with our participation in the CIBERER-ISCIII.

Our laboratory has generated and analysed new animal models to study visual abnormalities and different anomalies affecting retina development that are associated with albinism. In collaboration with Angel Carracedo (USC) and Carmen Ayuso (FJD), we have devised, within the CIBERER-ISCIII, a project for the universal genetic diagnostic of all known mutations in albinism. We apply this knowledge in cooperation with ALBA, the Spanish association in support of people with albinism and have been able to genetically diagnose numerous Spanish families.

We are also interested in understanding the function of regulatory elements that are required to define gene expression domains in mammalian genomes. We have used the mouse tyrosinase locus (Tyr) as experimental model. This approach has allowed us to identify several key regulatory elements, such as genome boundaries or insulators, which protect the locus from surrounding genes and ensure the faithful gene expression pattern.

As a general strategy, we regularly use transgenic and genome-edited mice to introduce different type of gene constructs in order to investigate the relevance of specific DNA regulatory sequences. The functional analysis of regulatory elements found within the intergenic non-coding genomic sequences can now be addressed more efficiently thanks to the efficient genome editing CRISPR-Cas9 tools. In Spain, where we pioneered the application of CRISPR technology in mice, we have generated a series of genome-edited mice carrying patient-specific mutations that we are currently phenotyping and using to explore new therapies for albinism.

CRISPR genome edited mice as animal models of (from left to right): OCA2, wild type, OCA1A and OCA1B oculocutaneous albinism types. (Fernandez et al 2021 Pigment Cell Melanoma)

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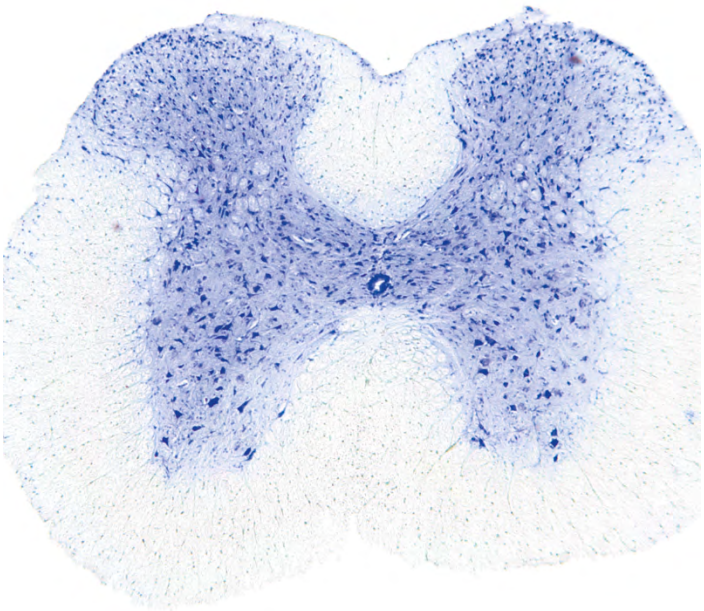
Functional analysis of transcriptional repressor DREAM

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

DREAM (downstream regulatory element antagonist modulator), also known as calsenilin or KChIP3, is a Ca2+ binding protein of the neuronal calcium sensors (NCS) superfamily that interacts with specific sites in the DNA to repress transcription of target genes in a Ca2+-dependent manner. In addition, DREAM interacts with specific proteins to exert various specialised functions in different subcellular compartments. Thus, through the control of activity-dependent gene expression and through specific protein-protein interactions, DREAM participates in many physiological processes in and outside the central nervous system. Work reported by us and other groups has shown important regulatory roles for DREAM in learning and memory in the hippocampus, in pain control in the spinal cord as well as in the immune response, in inflammation, in the thyroid gland and in the placenta. Moreover, recent studies have shown the involvement of DREAM in neurodegenerative disorders including Huntington disease (HD), Alzheimer disease (AD) and Amyotrophic Lateral Sclerosis (ALS).

DREAM was originally associated with AD because of its interaction with presenilins, however, altered neuronal calcium and protein homeostasis and early compensatory changes in transcriptional programs are common features of many neurodegenerative disorders which open the opportunity to explore a role for DREAM in these pathologies. In physiological conditions, binding of calcium or membrane lipids (e.i. arachidonic acid) regulate the interaction with DNA or with other proteins. Newly identified molecules, including glinides, modify DREAM conformation and activity upon binding. In this respect, our interest is to contribute to the definition of more specific DREAM binding molecules, to reveal the molecular mechanisms underlying their effect upon binding to DREAM and to assess their potential therapeutic actions on appropriate cellular and/or mouse models of target



Nissl staining of lumbar spinal cord (coronal) in transgenic mice overexpressing human TDP-43 with the A315T mutation, a mouse model of familial ALS (Wegorzewska et al. PNAS 106:18809, 2009). Project funded by Asahi Kasei Pharma (Japan). Tissue preparation and staining was performed in the Histology service at the CNB.

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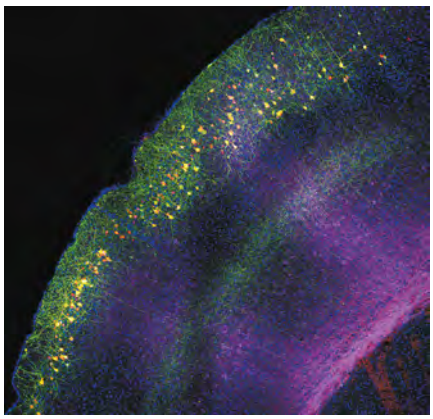
Cerebral cortical development

The cerebral cortex mediates the high functions of the human brain. It provides optimal responses to the external world, intellectual processing, and social behaviours. It is one of the most complex functional networks in biological systems and comprises an extraordinary number and diversity of neurons. Despite their complexity, cortical circuits, which ensemble during a protracted period of embryonic and postnatal development, wire stereotypically and reproducibly in all individuals of the same species. We think, see, feel, or interpret social behaviours similarly because our circuits are similar, allowing us to interact and evolve as a population.

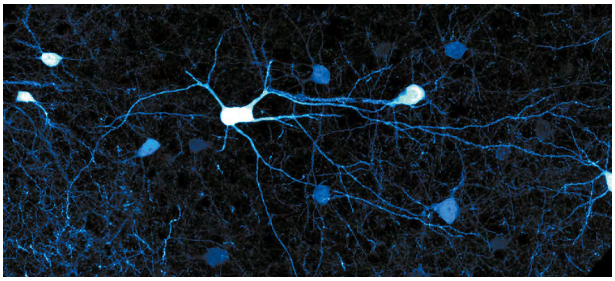
We study the developmental mechanisms that build cortical circuits. We aim to improve the understanding, diagnosis and treatment of neurodevelopmental disorders that appear when these mechanisms fail, such as epilepsy, dyslexia, autism, schizophrenia, mental retardation, and many syndromic and nonsyndromic disorders. During these two years, our principal focus has been investigating the development of corpus callosum (CC) connections. The CC connects the cerebral hemispheres and creates a more complex information processing. It is an evolutive addition to the mammalian brain, and focusing on the CC facilitates us to delve into the specific properties of the mammalian neurons. We have found that one of the hallmarks of mammalian neurons is extraordinary molecular and axonal plasticity under the control of activity. Bragg *et al*., discuss these ideas in a beautiful review. We also investigated the axonal cues that guide specific connectivity. Neuropilin-1 (Nrp1) is a receptor that binds to various ligands and signals differentially upon its association with distinct coreceptors. We demonstrated that dynamic expression of Neuropilin-1 during postnatal development is key to establishing a topographic organization of axonal projections in the contralateral hemisphere. Together our data support a model of cortical assembly due to the temporal evolution of molecular and wiring trajectories.

1



1 *Neurons of the cerebral cortex were labeled genetically with fluorescence proteins Dsred Express (red) and GFP (green). Fluorescence-CTB (magenta) illuminates callosal neurons and their axons. Cell nuclei (blue).*

2



2 *Genetic labeling of Parvalbumin GABAergic inhibitory neuron from the mouse cerebral cortex.*

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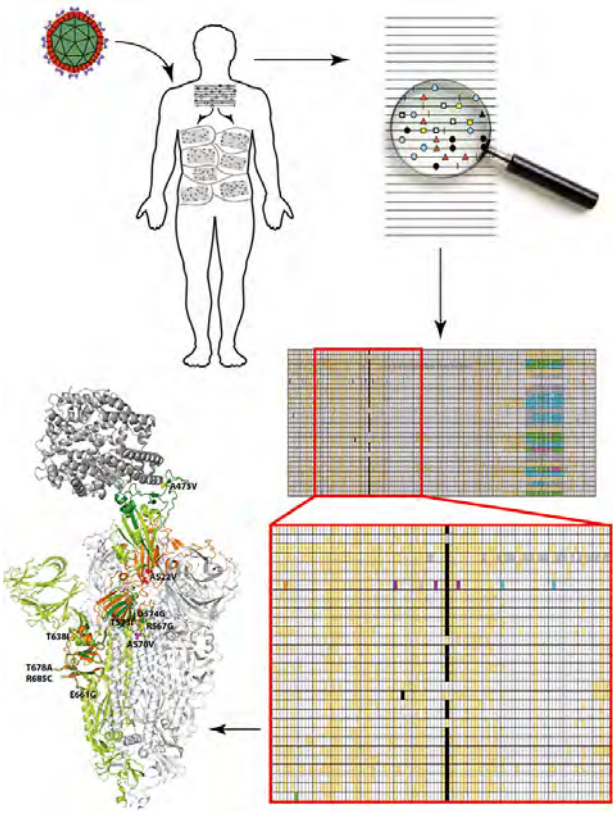
Dynamics of RNA viruses in infected patients. New antiviral designs

Virus variability is one of the main obstacles for the effective prevention and treatment of viral diseases. The main objective of our laboratory is to understand dynamics of RNA viruses, based on deep sequencing data of *in vivo* and ex vivo viral populations. Genomes present at low frequency in a mutant spectrum fuel virus adaptability, and may also influence the behaviour of the population ensemble. Selective pressures (such as antiviral agents or vaccination) may favour replication of some components of a mutant spectrum over others. Quasispecies dynamics demands that new approaches be investigated for the prevention and treatment of diseases associated with RNA viruses, to counteract the adaptive capacity conferred by the mutant clouds.

In our laboratory, we are extending previous studies on population dynamics with other RNA viruses to SARS-CoV-2 with the aim of increasing our understanding of quasispecies implications in a comparative manner in cell culture and *in vivo*. To this aim, we have available more than 7,000 SARS-CoV-2 positive nasopharyngeal swabs from the Fundación Jiménez Díaz in Madrid that cover all pandemic waves. We are currently analysing the intra-host mutant composition of SARS-CoV-2 populations from infected patients by ultra-deep sequencing, and exploring synergistic combinations between inhibitors to achieve viral extinction. Most of these compounds are nucleotide analogues and some of them act as lethal mutagens, driving virus extinction by an excess of mutations. These projects are performed in collaboration with other teams, as reflected in recent publications.



When a viral particle enters into a host, rapidly replicates and becomes a distribution of different mutants called a viral quasispecies. Ultra-deep sequencing analyses are revealing a huge complexity of viral populations represented here through heat maps. Possible structural and functional effects of amino acid substitutions are routinely analysed in the three-dimensional structure of the corresponding proteins.

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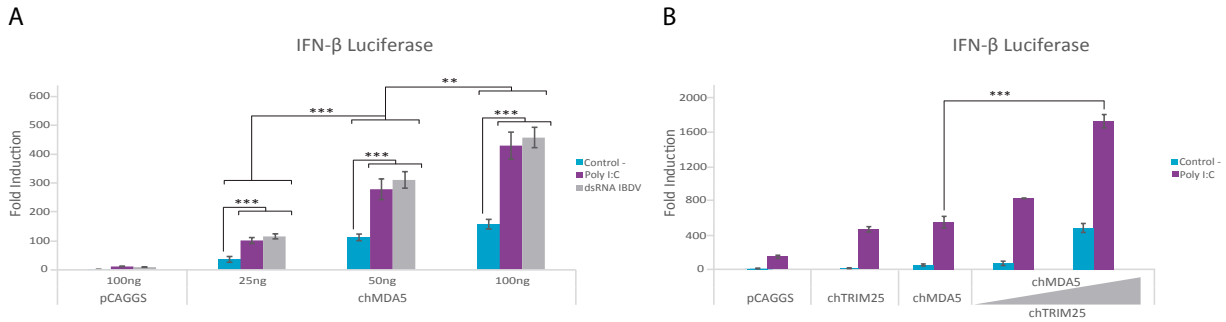
Molecular characterisation and epidemiology of torovirus

One of the main focuses of our research is the study of the virus-host interaction that would determine the outcome of the disease. For many years our efforts have been concentrated on toroviruses, emergent viruses (*Nidovirales* Order) that cause enteric diseases in different species of domestic animals and could represent a zoonotic threat. Related with them, and in the context of the COVID-19 pandemic, during this period we initiated new research projects on SARS-CoV-2 for the development of new strategies of control. We found that SARS-CoV-2 downregulates several enzymes of the Krebs cycle affecting the mitochondrial function and that the use of therapies directed to maintain mitochondrial function could be effective in combating SARS-CoV-2 infection (manuscript submitted). In addition, we have also collaborated with Drs. Montoliu and Fernández (CNB), and Dr. Moreno (CABD-CSIC)/UPO) in a project aimed at using the CRISPR-Cas technology to target coronavirus RNA genome.

In addition, we maintain a longstanding collaboration with the group of Dr. J.F. Rodríguez (CNB) to study the molecular bases of infectious bursal disease virus (IBDV) pathogenesis. IBDV infection is responsible for the immunosuppression and/or death of infected birds, causing heavy losses to the poultry industry worldwide. IBDV infection causes an exacerbated expression of proinflammatory cytokines, including IFN. Our initial results revealed that IFN contributes to exacerbate apoptosis of infected cells and therefore may collaborate to aggravate the disease caused by this virus in chickens. Additionally, we have determined that IBDV can establish long-term persistent infections in cultured cells. Significantly, the characterization of persistently infected cell clones revealed that they lack the capacity to respond to type I IFN. All the above highlights the importance of IFN in the outcome of IBDV infection. Our knowledge of the chicken IFN system is still very limited. Therefore, during this period we have approached the regulation of the signalling pathway initiated in chicken cells upon recognition of the dsRNA IBDV genome. We uncovered the regulatory role of TRIM25 on the MDA5 signalling pathway in chicken cells, and its contribution to control IBDV infection.



Activation of IFN-β promoter by the chicken pathogen recognition receptor MDA5, and its regulation by chicken TRIM25. *(A) DF-1 cells were co-transfected with different amounts of chMDA5 expression vector together with plasmids, pLucter, carrying the firefly* luciferase *gene under the IFN-β promoter, and pR-null, harboring the* Renilla luciferase *gene. At 8 h pt, cultures were either mock transfected (control) or transfected with IBDV dsRNA or synthetic dsRNA (Poly I:C) and harvested 24 h after plasmid transfection. (B) DF-1 cells were transfected with pLucter and pR-null in combination with the plasmids expressing either chTRIM25 or chMDA5, or chMDA5 with two amounts of the chTRIM25 expression plasmid. At 8 h pt the cells were transfected with Poly I:C. 24 h after plasmid transfection the firefly luciferase expression level of each sample was determined and normalized using* Renilla *values. ** and *** indicate P values of <0.01 and <0.001, respectively, as determined by unpaired Student´s test.*

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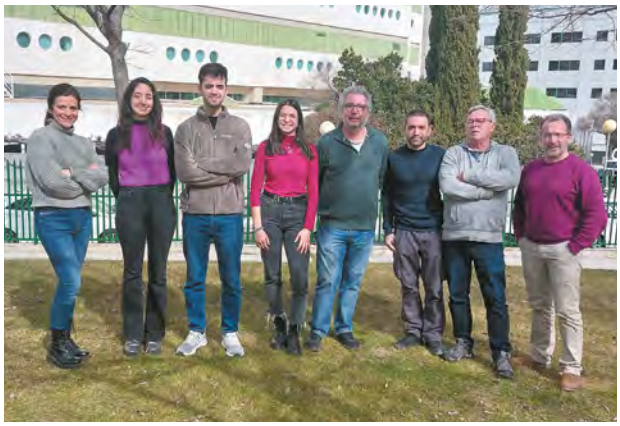

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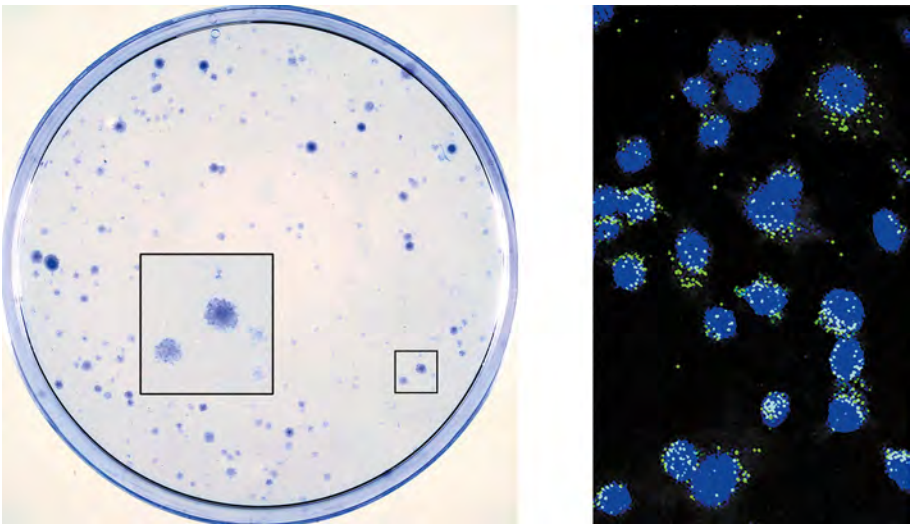
Molecular biology of birnaviruses

The *Birnaviridae* family groups a small number of species of non-enveloped, single shelled, icosahedral viruses harboring bipartite dsRNA genomes. Members of this unconventional dsRNA virus family include pathogens affecting a wide variety of animal species. The infectious bursal disease virus (IBDV), our main study model, is the etiological agent of an extremely contagious, immunosuppressive disease affecting domestic chickens with a major socio-economic impact to the Poultry Industry world-wide. During the past few years, our work has been mainly focused to deciphering the molecular basis underlying IBDV pathogenesis and the establishment of persistent infections. In addition to this, our team has devoted a great deal of effort to SARS-CoV-2 research, developing new diagnostic tools as well as a subunit vaccine candidate using dimers of the receptor binding domain (RBD) of the SARS-CoV-2 spike polypeptide.



Generation of DF-1 cell cultures persistently infected with IBDV. DF-1 cell monolayers were infected with IBDV using a multiplicity of infection of 3 plaque forming units per cell. Cultures were maintained for three weeks and then either stained with crystal violet, to visualise surviving cell clones (left panel), or processed for immunofluorescence microscopy (right panel) to detect the virus-encoded VP3 polypeptide (green). Cell nuclei (blue) were stained with DAPI. The inset at the left panel show a x2.5 magnification of the boxed area.

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Development, differentiation and regeneration in vertebrates

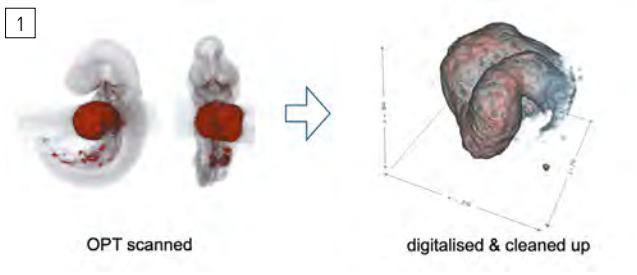
Our group is interested in understanding the molecular and cellular basis of organ formation during embryonic development. Signalling pathways involved in embryogenesis are also required for adult tissue homeostasis and repair. Moreover, malfunction of these pathways can lead to disease. Therefore, studying developmental genes and signals can offer new avenues for treatment of prevalent diseases such as inflammatory diseases and cancer and also to improve the regenerative ability of tissues.

The focus of our research in embryology is the development of limb and heart, studying the mechanisms of their morphogenesis. In a long-standing collaboration with James Sharpe (EMBL Barcelona) on this topic, we have participated in the generation of a computer model of organ formation in the mouse embryo. The result allows for a data-driven quantitative 4D description of limb and heart morphogenesis (Figure 1).

We are also interested in analysing the relationship between inflammation, regeneration and disease. We want to understand how uncontrolled or chronic inflammation can lead to disease, particularly in the context of inflammatory bowel disease (IBD) and colon cancer. In collaboration with the group of Ana Cuenda (Department of Immunology and Oncology, CNB) we are studying the functions of p38MAPKs in those pathologies. Using mice models and also samples from patients, we have shown that an increase in p38*γ* and a decrease in p38*δ* protein expression correlates with more inflammatory bowel disease and tumour development, making p38*γ*/*δ* useful biomarkers for colitis and early colon cancer. We are also investigating the role of gut microbiota in inflammation and cancer.

Finally, we have studied the evolution of p38MAPK along the tree of life in collaboration with Iñaki Ruiz-Trillo (Institute of Evolutionary Biology, Barcelona), showing that a p38MAPK homolog was already present in the closest unicellular relatives of animals, where it can respond to osmotic stress (Figure 2).

1



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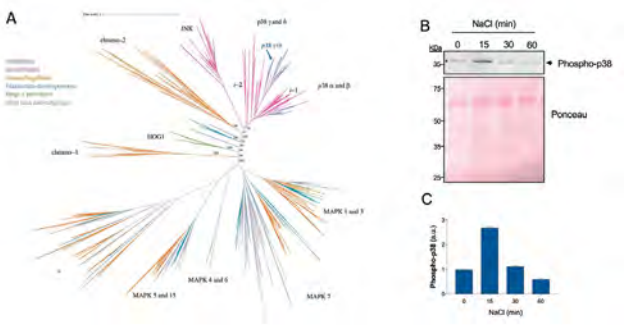

1 *Mouse embryo showing heart stained with myosin heavy chain, imaged with Optical Projection Tomography and digitised for computer reconstruction.*

2 *(A) Maximum-likelihood phylogenetic tree of the p38MAPK subfamily, including the closest unicellular relatives of animals. (B, C) Osmotic stress induces the phosphorylation of Capsaspora owczarzaki p38MAPK. B, Western blot and C, quantification of band intensity*

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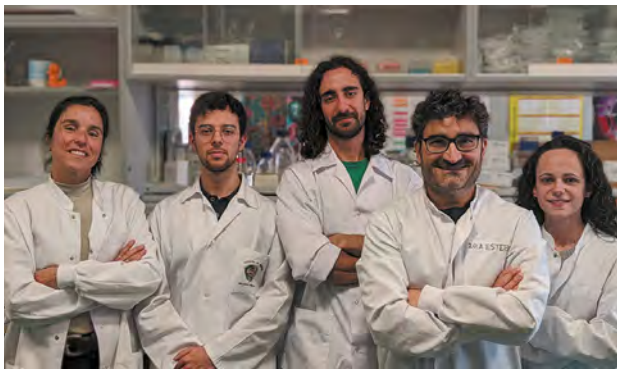

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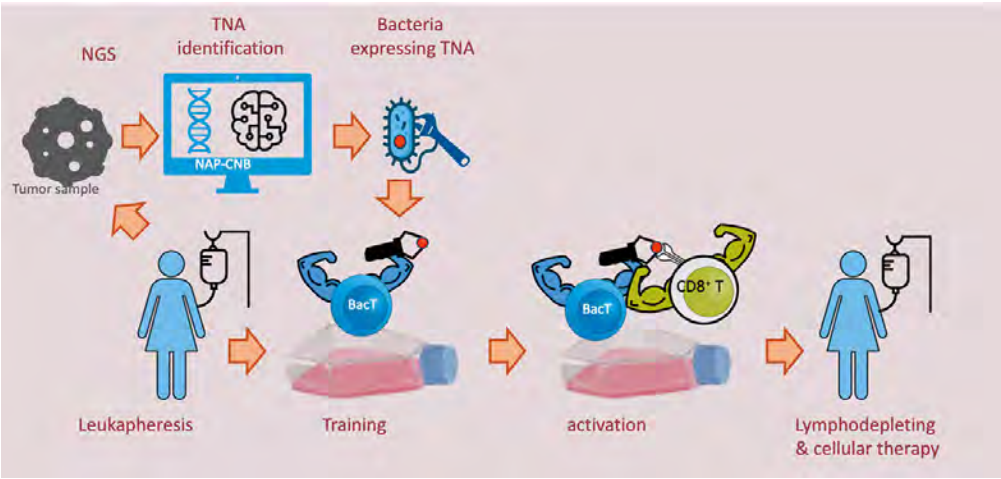
Bacteria-based immunotherapies against cancer

Our group is focused on generating novel cellular immunotherapies using the ability of bacteria to modify immune responses. We discovered that CD4+ T cells could be *"trained"* with some bacteria expressing tumor antigens to generate potent antitumor responses. This discovery that challenged the dogma of adaptive/innate immunity role separation are at the leading edge of the immunology and oncology fields as recognised by different prizes, for example, the XIX FERO foundation award for the best project against breast cancer.

CD4+ T cells can capture and destroy bacteria by transphagocytosis. Moreover, bacteria exposure "trains" conventional CD4+ T cells. Trained T cells (bacT), cross-present antigens from captured bacteria, activating naïve CD8+ T cells that became effective cytotoxic cells and differentiated into central memory cells expressing very low amounts of PD1 or CTLA-4; desired features for antitumor cells. The antitumor effects of bacT cell therapies are being tested in proof-of-concept experiments against different mouse model of cancer.

In addition, in order to fight one of the major bottlenecks in cancer immunotherapies, we are generating an easy-to-use platform supported in machine-learning based algorithms that would allow to rapidly identify tumor neoantigens (TNAs). Our predictions were more accurate than any competing algorithms. The (NeoAntigen Prediction; NAP-CNB) platform is open and only requires RNAseq data from malignant and healthy tissues. NAP-CNB is being generated under the direction of Drs. Carlos Oscar Sánchez Sorzano (CNB) and Arrate Muñoz Barrutio (Universidad Carlos III de Madrid).



Summary of bacT therapy. Tumour neoantigens (TNA) will be identify by using NAP-CNB platform developed by us from NGS sequencing. TNA will be cloned in engineered bacteria generated to optimise cellular training. Lymphocytes from the patients will be trained with bacteria expressing TNA and bacT cell-activated CD8+ T cells will be re-infused as therapy.

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