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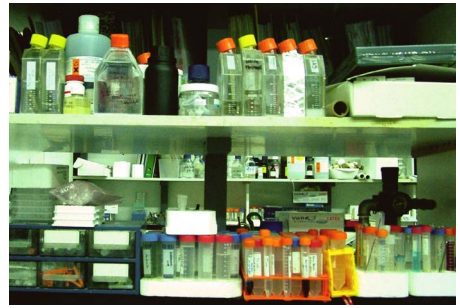
Letter from the Director

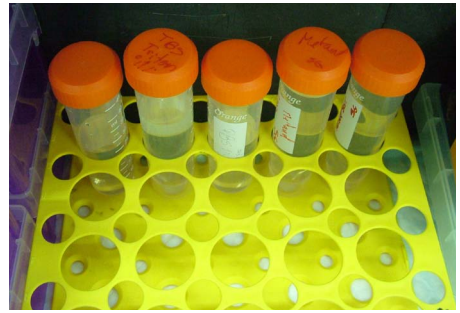
IBMC was created 15 years ago with the participation of researchers from various faculties, hospitals and institutions that were through some means associated with the University of Porto. IBMC has developed strong basic research in the field of Life Sciences, which has allowed a successful interface with applied and clinical research at the highest international level. This has resulted in a very productive multidisciplinary environment in which Research Groups can collaborate to find innovative answers to biologically relevant medical questions. In more recent years, we have continued to progress towards understanding basic biological questions, but also translated the results of research carried out within IBMC into clinical applications for an effective value creation in close contact with industry. IBMC includes excellent Research Groups with a strong basis on Molecular, Cellular and Organismic Biology, providing a solid foundation on which to address all other current questions in Life and Health Sciences. These questions currently include the crucial problem of phenotype versus genotype in Human Genetics, the organization and function of the Nervous System and the area of Host-Pathogen Interaction. We also strive to look to the future and keep new developments in sight, such as Tissue Regeneration and Repair, which have recently become of major medical relevance and will certainly deliver innovative new medical treatments in the future. IBMC has achieved national and international recognition in many areas but must continue to aspire to higher levels of excellence, which will in turn require transdisciplinary research initiatives. I invite you to read this document and become immersed in the research carried out at IBMC; a highly dynamic and exciting place to do science in Portugal.

Claudio Sunkel

Mission Statement

IBMC- Instituto de Biologia Molecular e Celular is a non-profit association of public utility. Our main mission is to foster research in the Life Sciences and Biomedicine at highest international level, to promote postgraduate training of young researchers in these areas, to encourage technology transfer and the public engagement with Science. Our vision is to become an international leader in multidisciplinary research into fundamental biological problems, while pursuing scientific innovation and social progress. Since it was founded in June 1991, IBMC contributes to the cutting-edge science within Universidade do Porto.





Methods

In our effort to achieve these goals we have invested heavily in attracting talented young group leaders and in providing broad-based interdisciplinary training to our graduate students. We have aggressively pursued technology transfer and have been addressing a wide range of science-in-society issues.

The IBMC was formed with the aim of developing research in the Biological Sciences with distinct applications in medical research; and as expected, a large component of the fundamental and applied work that is undertaken is related to health and biomedicine. Most of this research is underpinned by a strong molecular and structural foundation. Currently, the different Research Groups of the IBMC are organized into three Thematic Units:

- Infection and Immunity
- Molecular and Cellular Biology
- Neuroscience

there are also some:

- Associated Groups

the research supported by:

- Technological Platforms

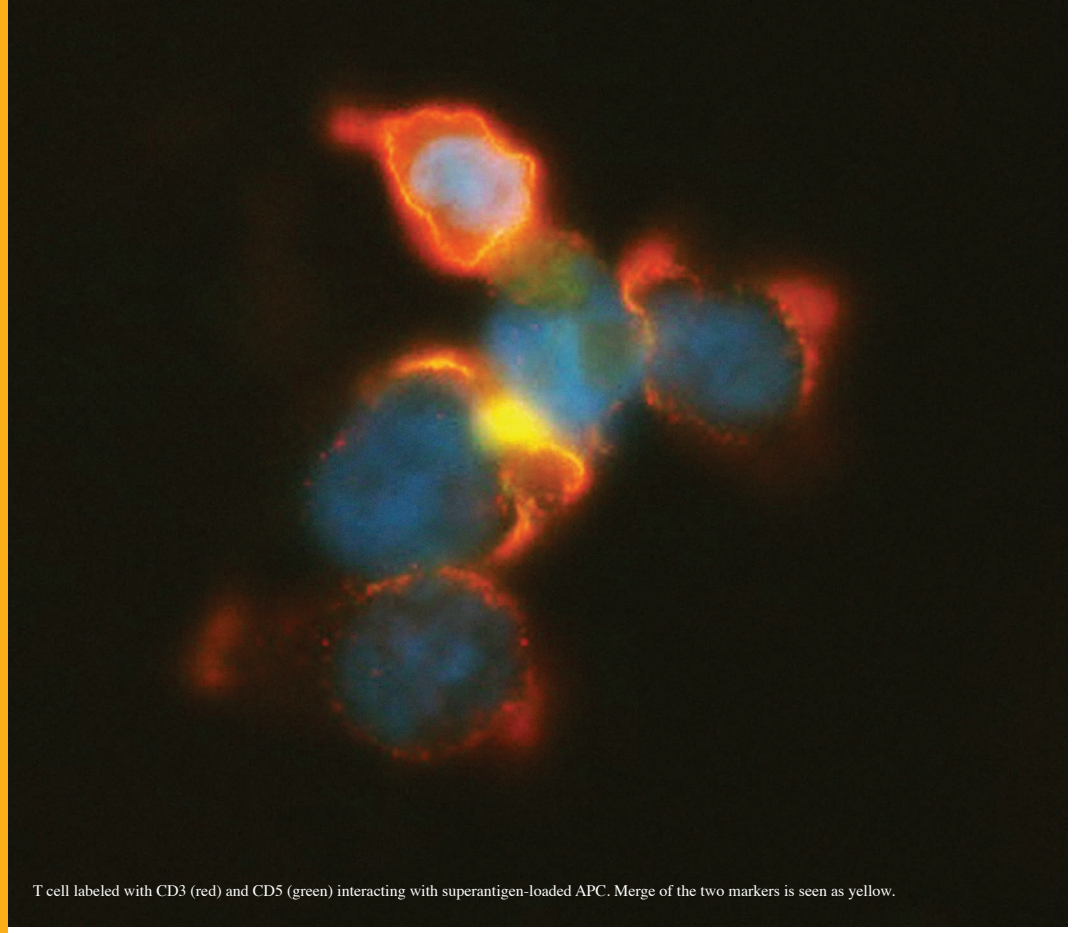
and translated into:

- Translational Iniciatives

Infection and Immunity

The interaction between pathogens and their hosts is a complex and dynamic process, with each player having to recognize, respond and adapt to the other. Pathogens have evolved strategies to manipulate and evade host defenses to optimize their survival and/or transmission. Meanwhile, the host defense system must balance the requirement to control the pathogen with the potential for damaging its own tissues. An infection is thus accompanied by responses from both the pathogen and the host and it is the balance between these responses that defines the infection outcome.

The principal aims of the work in this Unit are to identify and study the molecules and pathways that play critical roles during the interaction of pathogens with their hosts. The research groups examine host-pathogen interactions from both angles: the virulence mechanisms employed by pathogens to infect, colonize and persist in their hosts, and the mechanisms engaged by the host to resist infection at the cellular and organism levels. In addition, they analyze the mechanisms of pathology induction and investigate potential therapeutic and preventive strategies. The research uses in vivo and in vitro experimental models of host-pathogen interactions: from bacteria, fungi and pathogens to parasites, from extracellular to obligate intracellular pathogens, and from culture human cells to mouse and fish animal models.



T cell labeled with CD3 (red) and CD5 (green) interacting with superantigen-loaded APC. Merge of the two markers is seen as yellow.

Cell Activation and Gene Expression

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Previous research results

The group aims to study the molecular mechanisms that regulate transcription and protein expression in complex systems including tissues, cellular differentiation and immune cell activation. T cell receptor recognition of peptide-MHC complexes largely dictates the outcome of adaptive immune responses, however a varied number of other molecules regulate these responses. CD5 and CD6 are receptors expressed at the surface of T lymphocytes that localize at the immune synapse during T cell:APC interactions and modulate T cell signaling. CD5 has long been known to functionally work as an inhibitor, recruiting to the sites of antigen recognition the tyrosine phosphatase SHP-1, which dampens ongoing phosphorylation reactions. We have recently proposed an alternative mode of CD5 inhibitory signaling: stimulation of CD5 results in the tyrosine phosphorylation of the kinase Fyn at its C-terminal inhibitory tyrosine residue, thus inactivating the kinase. These inhibitory actions of CD5 are further amplified by an increased expression of CD5 upon T cell receptor triggering. This rise in protein expression is partly due to the alternative polyadenylation-mediated regulation of mRNA CD5 expression.

Future research goals

We aim to characterize the mechanisms of alternative polyadenylation of the CD5 transcript upon T cell activation and identify putative microRNAs controlling CD5 mRNA expression. Regarding the CD5-related receptor CD6, will further characterize the range of CD6 mRNA alternatively-spliced isoforms including their differential expression during thymocyte development. Finally, we will address the molecular mechanisms involved in the termination of transcription using global analysis of distant and closely-spaced genes.

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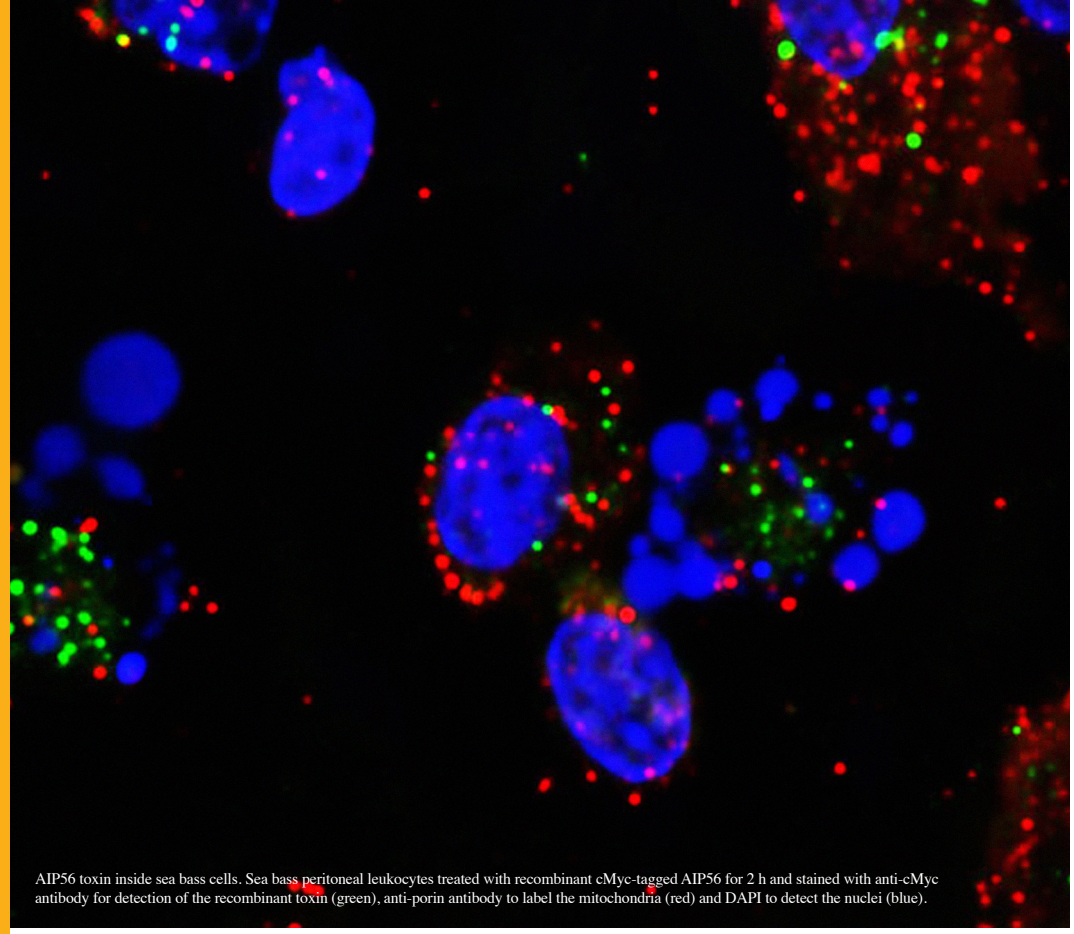
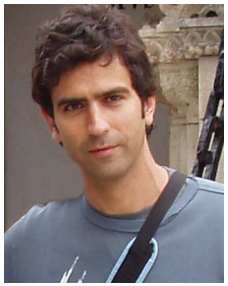


AIP56 toxin inside sea bass cells. Sea bass peritoneal leukocytes treated with recombinant cMyc-tagged AIP56 for 2 h and stained with anti-cMyc antibody for detection of the recombinant toxin (green), anti-porin antibody to label the mitochondria (red) and DAPI to detect the nuclei (blue).

Fish Immunology and Vaccinology



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Previous research results

At our laboratory we work on host-pathogen interaction using fish as host model.

Fish are the first phylogenetic group exhibiting a fully developed adaptive immune system, similar to that of mammals, and therefore, representing a unique link in the study of the immune system. We have been contributing to advances in this topic by sequencing and characterizing important molecules involved in the immune response in sea bass (*Dicentrarchus labrax*). In addition to their phylogenetic relevance, these tools have allowed us to dissect the immune response to infection at both transcriptional and translational levels.

We have also been studying a pathogenic mechanism triggered by an exotoxin (AIP56) that induces apoptosis of macrophages and neutrophils, resulting in lysis of these phagocytes by secondary necrosis. AIP56 is a major virulence factor secreted by a Gram-negative bacterium (*Photobacterium damselae piscicida*) that causes massive mortality in several important marine fish species, including sea bass. We found that AIP56 is an AB toxin: the A domain (N-terminal) is a zinc-metalloprotease that cleaves NF-kB p65, similarly to NleC (a type III secreted effector, from enteric bacteria, homologue to AIP56 N-terminal), and the B domain (C-terminal), homologue to a protein of unknown function from the bacteriophage APSE2, is responsible for the binding/entry of the toxin into the cells.

Future research goals

Studying host-pathogen interactions will continue within the scope of our group.

As short-to-medium term goals we will focus on three main issues: (i) development of tools for monitoring fish immune responses to pathogens, including production of antibodies against important molecules involved in immune responses, development of the tetramer technology for exact enumeration of antigen-specific T cells, and the development of immortalized lymphoid cell lines; (ii) detailing the mechanisms involved in the apoptotic and inflammatory responses as well as antigen presentation in fish; (iii) studying the mechanism of action of AIP56 and its structure-function relationship. This includes defining its internalization and trafficking pathways, disclosing its 3D structure, and developing an AIP56-based vaccine as well as potential use as pharmacological and biological tool.

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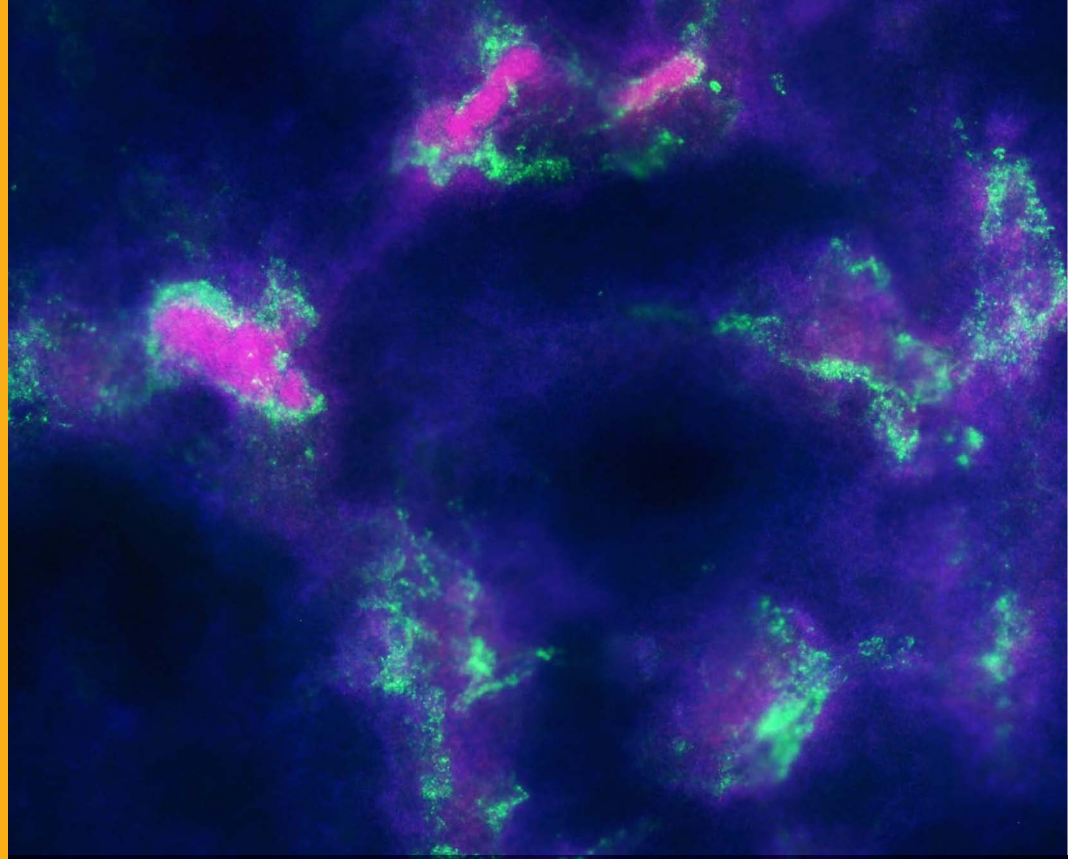

Co-localization of poly-N-acetylglucosamine (green) and dead cells (red) within *S. epidermidis* biofilms. Biofilm cells were stained with DAPI (blue).

Immunobiology

Manuel Vilanova

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Previous research results

We are interested in the broad field of immunology of infection and our main objective is to develop novel strategies to prevent and treat infectious diseases. Group B streptococcus (GBS) or *Streptococcus agalactiae* is a causative agent of severe infections in human neonates. We have previously identified GBS GAPDH, as an important virulence factor for this bacterium through a mechanism encompassing host IL-10 production. Moreover, recombinant GBS GAPDH was successfully used to vaccinate neonate mice against lethal GBS infection. This vaccination strategy is currently patented. In another research line, innate and acquired immune mechanisms, elicited in the murine host by *Neospora caninum*, were characterized. Namely, conventional and plasmacytoid dendritic cells were identified as the main producers of host-protective IL-12 upon infection. This protozoan is the main infective agent causative of abortions and stillbirths in cattle. More recently, we have initiated the study of *Staphylococcus epidermidis* biofilms, a main cause of medical-device associated infections. Flow cytometry-based tools were developed to analyse the physiological state of *S. epidermidis* biofilm cells and how biofilms with different proportions of dormant cells interact with the host immune system.

Future research goals

Our main objective in the long-term concerns the clinical application of the vaccines developed and studied in animal models. To reach this objective, a more detailed characterization of the interaction of *S. agalactiae* GAPDH with host immune cells will be carried out. This includes the identification of host cell receptors for this immuno-modulatory protein and signal transduction pathways. Mucosal immunization with *N. caninum* antigens will be attempted in mice and bovine hosts as a novel approach to prevent neosporosis. Using the *N. caninum* model, immunotherapy of immune defective hosts using specific monoclonal antibodies will be also studied. The interaction of *S. epidermidis* biofilms with the host immune system will be also characterized in further detail in order to conceive an immune-based approach able to prevent nosocomial infections caused by this pathogenic agent.

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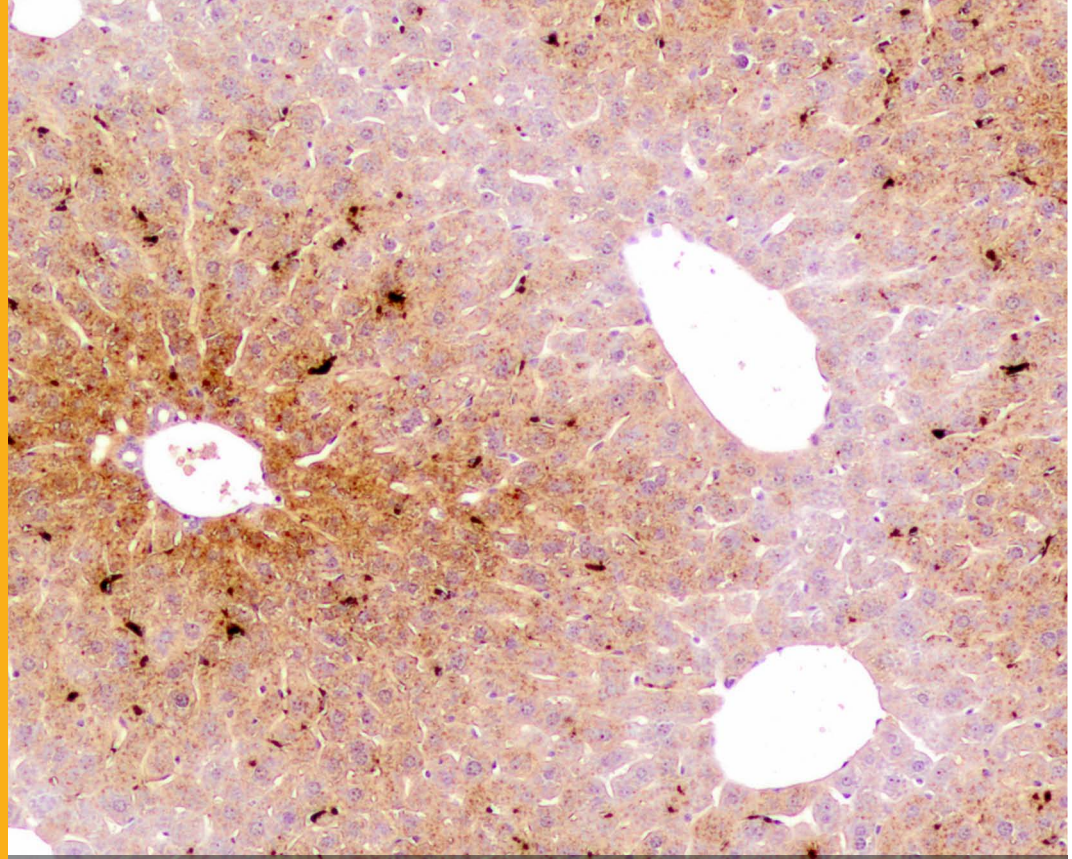

Iron staining (DAB-enhanced Perls reaction) of a C57BL6 mouse liver section after Fe-dextran injection showing iron deposition in periportal hepatocytes and Kupfer cells

Iron and Innate Immunity

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MSc in Immunology, University of Porto/Free University of Amsterdam, Holland, 1992
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Previous research results

We have previously shown that iron overload favours bacterial growth in mouse[1] and fish[2,3], whereas iron chelation has the opposite effect[4]. We contributed to the characterization of two mouse models of hereditary haemochromatosis, the B2m-knock-out and the Hfe-ko, both of which show parenchymal iron accumulation with sparing of tissue macrophages[5]. We reported that iron distribution is altered during infection with *M. avium*, accumulating inside infected macrophages[6].

Hepcidin decreases iron release from macrophages and enterocytes, resulting in decreased serum iron levels and, in the long term, in anaemia. However, when we infected mice with *M. avium*, the animals developed mild anaemia with no significant induction of hepcidin[6]. Mice infected with *M. avium* showed altered expression levels of other iron-related genes such as ferritin, lipocalin-2 and genes involved in heme metabolism[6]. On the other hand, we showed that the dual activation of hepcidin by iron and by infection has been conserved throughout evolution since it is also observed in fish[2].

Recently, we hypothesized that iron exacerbates infection through oxidative stress and inflammatory mechanisms. Iron induces Nrf2 signalling *in vivo* and primary cells derived from Nrf2-/- mice are more susceptible to iron-mediated oxidative stress (Duarte et al, in preparation). Likewise, mice genetically deficient in heme-oxygenase-1, a transcriptional target of Nrf2, are more susceptible to *M. avium* (Gomes et al, in preparation).

Interestingly, infection by the protozoan parasite *Leishmania infantum* is more easily circumvented in iron overloaded mice and we have evidence that iron-induced parasite killing is mediated by host-derived oxygen and nitrogen species (Costa et al, in preparation).

Future research goals

Future studies on the links between iron metabolism, oxidative stress and inflammation in the context of the innate immune response to infection are thus granted. In particular, we will aim at understanding the relevance of Nrf2 activation by iron in vivo and at identifying the mechanisms by which heme-oxygenase-1 protects against *M. avium* infection. We are also determined to investigate the molecular and cellular mechanisms of the anemia induced by *M. avium* in mice. We previously showed that *M. avium* infection has an impact on the iron distribution of the host and causes mild anemia after one month of infection. Lipocalin-2 was one of the most highly induced iron-related genes in the liver. Since it has been suggested in the literature that lipocalin-2 inhibits erythropoiesis, we will analyse the impact of *M. avium* infection on the iron metabolism of lipocalin-2-deficient mice, particularly in what concerns the red blood cell compartment. Finally, we will collaborate with the Chemistry Department (FCUP) in the development of new therapies against Mycobacteria and Leishmania infection, based on iron chelators that we have previously shown to be able to inhibit the growth of *M. avium in vitro*.

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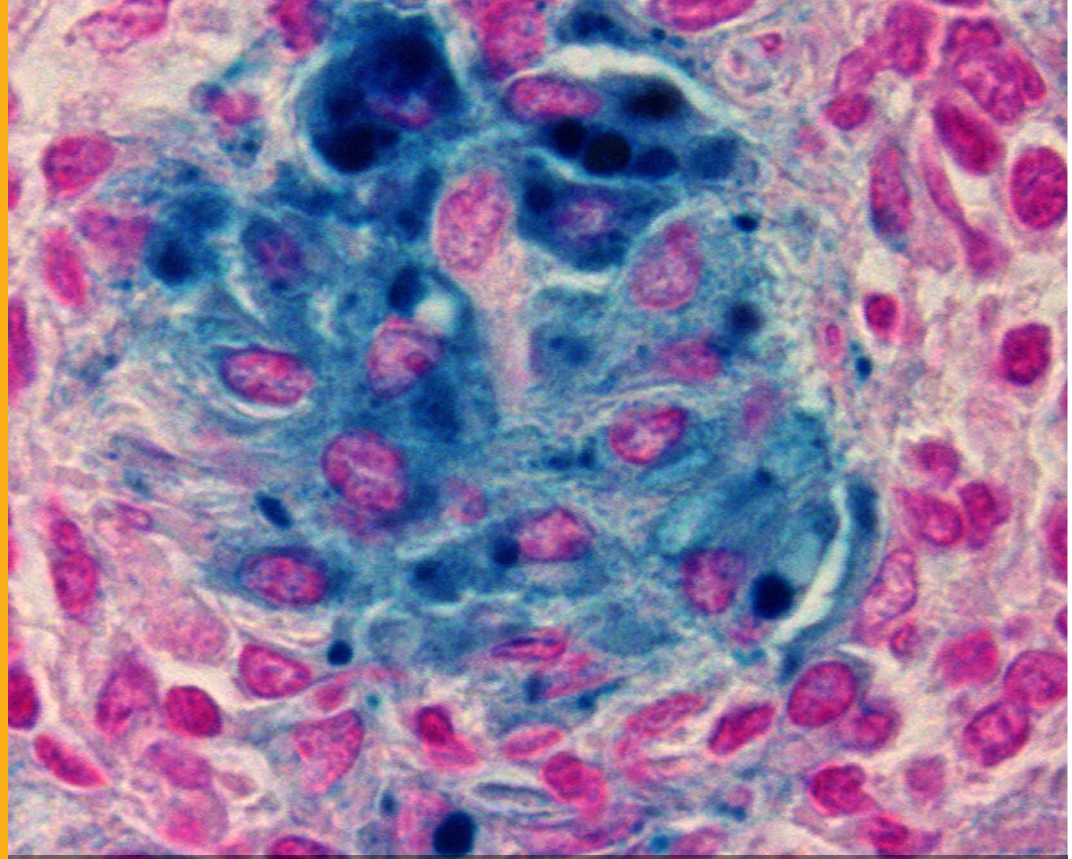

Perls staining of a *mycobacterial granuloma*

Microbiology and Immunology of Infection

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Previous research results

We have used a mouse model of *Mycobacterium avium* infection to dissect the immune response to mycobacterial infections looking at protective immunity mechanisms (both innate and adaptive) and immunopathology (granuloma formation, fibrosis, and necrosis, peripheral lymphopenia, and thymic atrophy). Thus we now know that protective immunity requires gamma interferon (IFNg), the interleukins 6 and 12, tumor necrosis factor (TNF), the CD30 and CD40 molecules and the TLR2 receptor. We pinpointed the pivotal role of IFNg in the mechanisms leading to granuloma formation and necrosis as well as the cell loss of central and peripheral lymphoid organs while excluding major players as the mediators of such pathology. For example, granuloma necrosis does not require the participation of apoptosis-inducing mediators such as NO, oxygen reactive species, TNF, Fas or TRAIL nor can it be prevented by Bcl-2. The deficiency in the same molecules does not affect the development of peripheral lymphopenia.

Amélia Sarmento has looked at immune alterations in the physiology of monocytes from Inflammatory Bowel Disease (IBD) patients and identified differences in the production of TNF in the cells of Crohn's disease patients.

Future research goals

In addition to pursuing the research described above, recent collaborations with extramural groups have led to the analysis of the immunomodulatory role of mycobacterial lipoglycans (with Ben Appelmelk, Germain Puzo and Jérôme Nigou) and the role of apoptosis in *M. tuberculosis* control (with Otília Vieira and researchers from Harvard Medical School).

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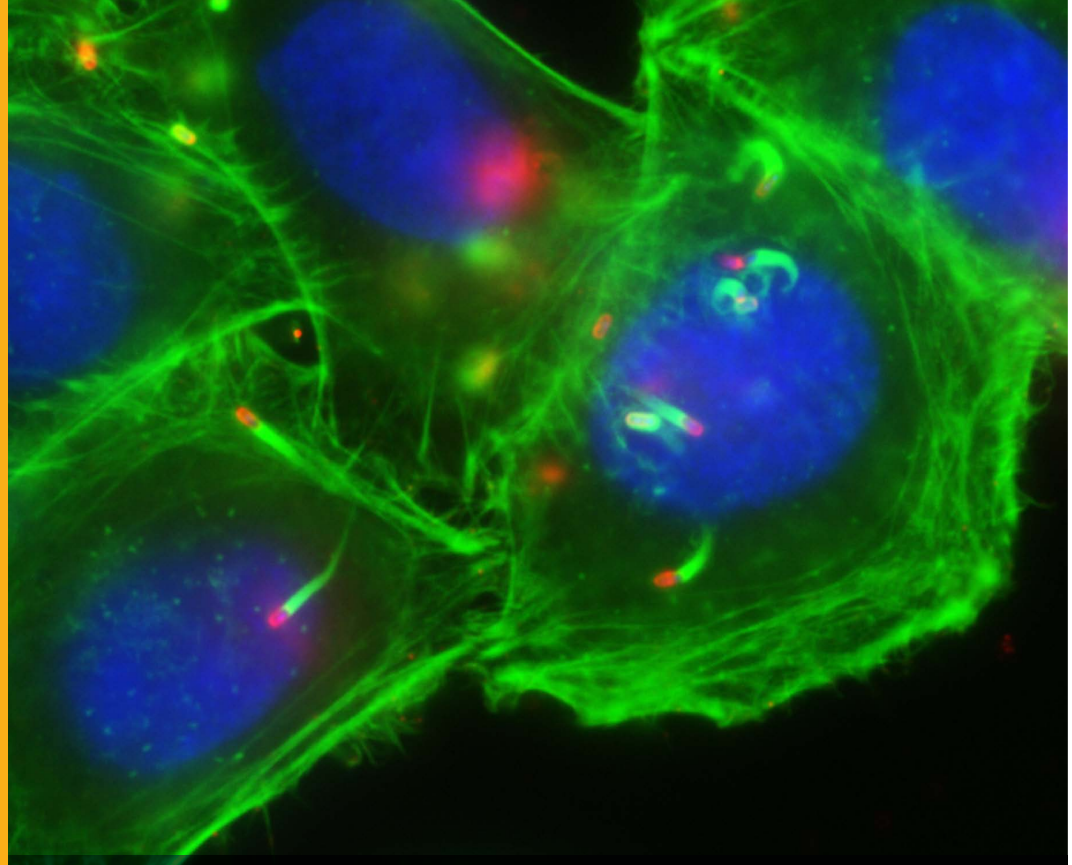

Listeria monocytogenes infecting human cells. Bacteria are visualized in red, cell nuclei in blue and cellular actin in green.

Molecular Microbiology

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Previous research results

Listeria monocytogenes is an intracellular human food-borne pathogen that causes listeriosis, an infection characterized by gastroenteritis, meningitis, encephalitis and maternofetal infections. *L. monocytogenes* enters the host via the ingestion of contaminated foods, invades the intestine, translocates to mesenteric lymph nodes and spreads to the liver, spleen, brain and to the placenta. During infection, *Listeria* has the ability to cross the intestinal, the blood-brain and the placental barriers, entering, surviving and multiplying inside phagocytic and non-phagocytic cells. *L. monocytogenes* thus emerged as an exceptional model to address the different facets of host-pathogen interactions. Our research is focused on the identification and analysis of virulence mechanisms used by *L. monocytogenes* to enter, survive and proliferate into its host.

We have identified and characterized several *Listeria* factors crucial for virulence and involved in cell adhesion, invasion or resistance to host defences. We performed the first genome-wide expression analysis of a bacterial pathogen in deep infected mouse organs, revealing how *Listeria* adapts to host conditions, activates virulence mechanisms and subverts host defence functions. We also identified new host factors hijacked by *Listeria* to promote infection, performed the molecular characterization of the interaction between *Listeria* virulence factors and host receptors, and deciphered signalling cascades downstream these interactions.

Future research goals

Our current and future objectives include not only the description of new aspects of the *Listeria*-host interaction, but also the involvement of newly identified proteins and pathways in the infectious process of other pathogens.

We are characterizing new *Listeria* virulence factors that we identified by *in vivo* transcriptomics. In particular, we try to assess the role of wall teichoic acids glycosylation and cadmium efflux system in *Listeria* virulence.

Host phosphorylation cascades are preferential targets of infecting bacteria. We recently identified two new cytoskeletal proteins differentially phosphorylated in response to *Listeria* uptake. Our goal is to address the role of these phosphorylations in the infectious process and also in general cellular processes.

We will also investigate the possible interplay between *Listeria* and the host cell cycle, and address the role of this crosstalk in the establishment and progression of cellular infection.

As different pathogens often hijack same signalling pathways, we will investigate the involvement of the newly identified proteins/pathways in the infectious processes of other human pathogens, as pathogenic *E. coli* and *Yersinia*.

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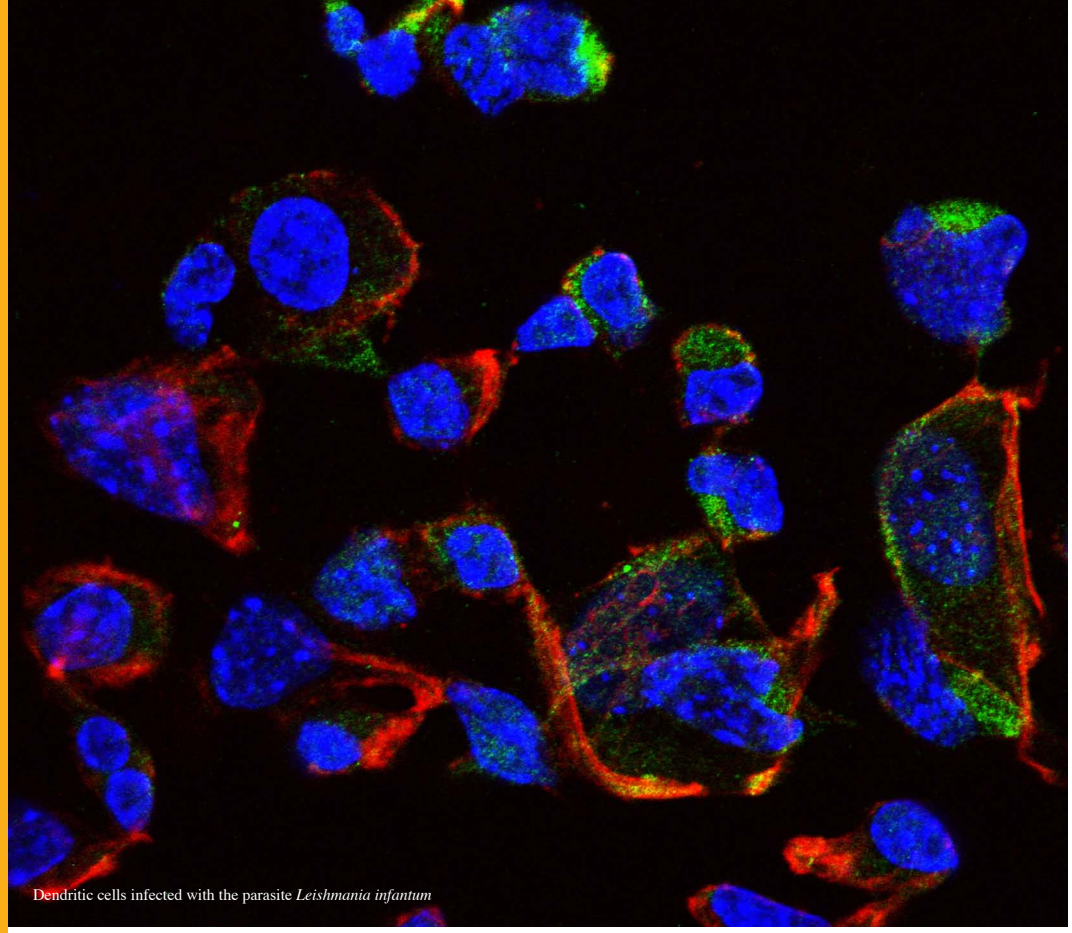

Dendritic cells infected with the parasite *Leishmania infantum*

Parasite Disease

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Previous research results

The research focus in our laboratory is the kinetoplastid protozoa, organisms responsible for major human and veterinary diseases such as leishmaniasis and the African and South American trypanosomiasis. Disease control is dependent on limited chemotherapy since no human vaccine is available. One of the main interests of the group is the understanding of the host immune mechanisms involved in the control/susceptibility to infection, in particular the signaling profile induced by *Leishmania* parasites in host immune cells. Our recent achievements demonstrated that a visceral *Leishmania* species can differentially target PI3K/Akt, MAPKs, and NF-κB to modulate the maturation, activation, and immunostimulatory abilities of dendritic cells (DCs). Moreover, the control of host cells in early host-pathogen interaction derives also from surface and secreted protozoan proteins. For that we have been developing and characterizing a new approach to study, in particular, exosome-based secretion pathways. Another area of interest is the identification and validation of new therapeutic targets and drug delivery systems based on nanoformulations. In particular, we have been developing PLGA nanoparticle encapsulation of anti-*Leishmania* drugs to solve several limitations of conventional drug delivery systems. The team is also interested in the improvement of diagnostic tools for leishmaniasis. We reported a strategy of combining two well-defined *Leishmania* antigens, *Lic*TXNPx and rK39, which proved to be a sensitive and specific improvement to current serological diagnosis of canine leishmaniasis.

Future research goals

In the future we will continue to focus on the understanding of the immune response developed during these pathologies that may lead to improved chemotherapies, vaccines and current diagnostic methods.

The long-term objectives are: 1- Identification of new virulence factors; 2- Dissection of cellular and molecular mechanisms determining the susceptibility of the host to the infection; 3- Understanding the impact of *Leishmania* infection on mitochondrial homeostasis; in particular the role of the sirtuins family proteins in the modulation of host mitochondria during infection; 4- Application of nanotechnology in trypanosomatids therapy – from drug screening to nanoformulation development 5- Development of a new immunological screening method for canine leishmaniasis using colloidal gold nanoparticles.

The group achievements are being supported by interdisciplinary international collaborations that we intend to reinforce and expand, in particular with industrial partners.

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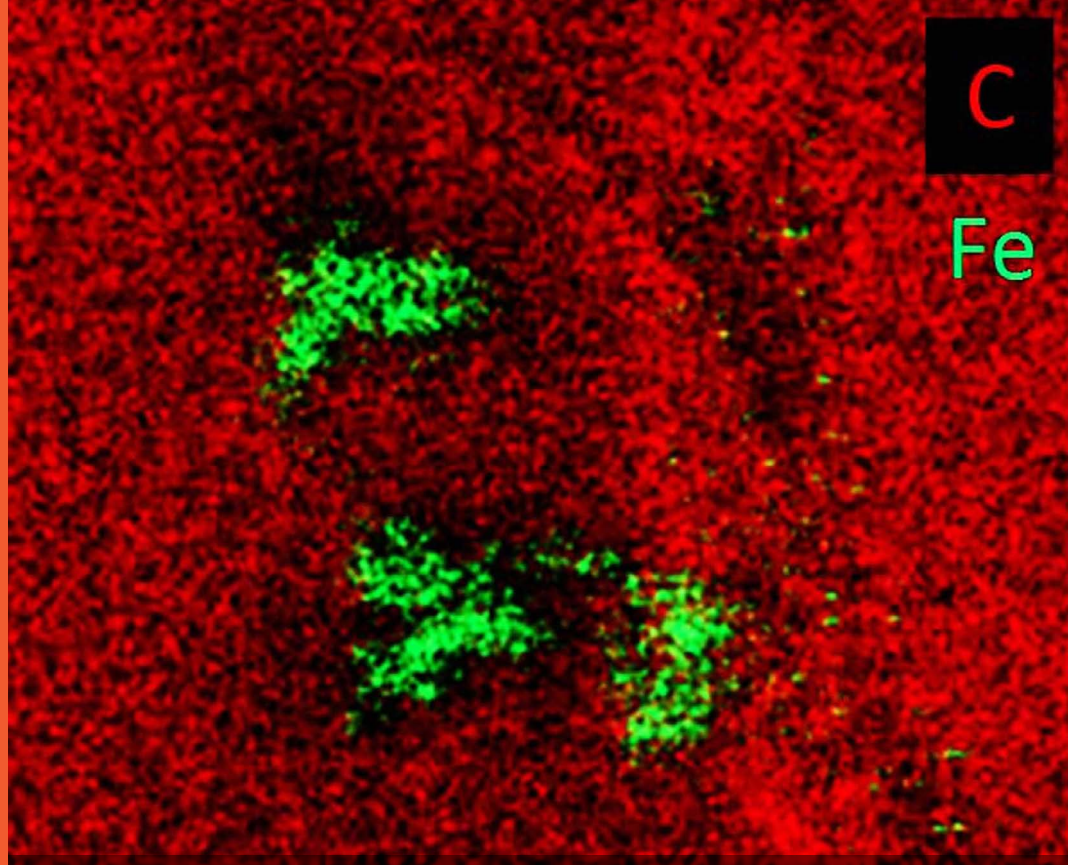
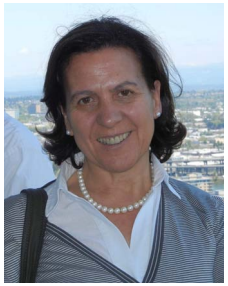
Molecular and Cellular Biology

The main aim of this Unit is to study the basic underlying mechanisms of biological organization at different levels including protein structure, cellular homeostasis, tissue growth and organization, and evolution. To gain insight into these questions, it is critical to develop new and more comprehensive explanatory and unifying models of how complex biological systems work, so that ultimately this understanding can be used to design better therapeutic strategies for a wide range of human diseases. The work undertaken in this Unit is based on a broad transdisciplinary approach, which reflects the variety of research interests of the participating groups. A number of different problems are studied including the structure and function of membrane proteins, the biogenesis of organelles, the mechanisms involved in cell division and its relation to cancer, cellular processes such as protein targeting, secretion and degradation, cellular physiology and homeostasis, gene regulation during cellular differentiation and embryogenesis, ageing and evolution. These issues are addressed in systems as diverse as yeast, fungi, plants, fruit flies, fish, mice and mammalian cells in culture, employing a range of techniques such as protein biochemistry, molecular biology, electron microscopy, advance light microscopy, digital imaging and mathematical modeling, amongst others.



Energy dispersive X-ray (EDX) elemental analysis showing Iron (Fe, green) accumulation in a carbon-rich (C, red) intracellular region of a human monocyte.

Basic and Clinical Research on Iron Biology



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Previous research results

The work of our group focuses on the study of the reciprocal interactions between iron metabolism and the immune system. Iron is essential for many fundamental cellular processes but in excess is toxic. Dysregulation, i.e., depletion, overload, or inappropriate distribution of iron can lead to cellular damage and disease. Iron homeostasis is therefore essential and is maintained through a tightly regulated process, involving an intricate network of proteins and cell types. Over the past years, using human and mouse models of iron overload and appropriate *ex vivo* and *in vitro* systems, we have investigated the reciprocal interactions between iron and the immune system. Our results identified $CD8^+$ T cell numbers and the protein calreticulin as markers of severity of iron overload in hereditary hemochromatosis patients and demonstrated the role of hepcidin, the main effector protein in iron homeostasis, in the T lymphocyte response to activation.

Future research goals

In the future we will strengthen our focus on the clarification of the mechanisms underlying the iron-immune system interaction. We will (1) characterize the contribution of iron and iron-related genes to lymphocyte differentiation and proliferation, using knock-out and knock-in mouse models, gene and protein expression arrays, siRNA technology and *in vitro* models of thymocyte differentiation; (2) identify the molecular mechanisms mediating the response of lymphocytes to iron challenge or deprivation, using state of the art proteomics and genomics approaches, siRNA technology and advanced optical and electron microscopy; (3) identify the molecular players involved in the genetic regulation of lymphocyte homeostasis in humans using massive parallel sequencing, genome wide transcriptome analysis and appropriate functional assays.

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The medicinal plant *Catharanthus roseus*

Bioactive Natural Products

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Previous research results

Plants display a unique metabolic plasticity which includes thousands of natural products. These features help them deal with a wide range of environmental threats, from which they cannot escape being sessile organisms. Many of these natural products were shaped by evolution to function as herbivorism deterrents, possessing strong physiological activities in animals, which may translate into important therapeutic actions in humans, under adequate dosages. We are mainly interested on the biosynthesis of the terpenoid indole alkaloids produced by the medicinal plant *Catharanthus roseus*, which include the anticancer drugs vinblastine and vincristine. Previous work involved the biochemical and molecular characterization of a key biosynthetic step leading to the production of the anticancer alkaloids and involving a class III peroxidase – CrPrx1. Recently, we have shown that CrPrx1 seems also to be involved in the homeostasis of hydrogen peroxide in leaves, with a protection effect especially important under conditions of excess light. We have also characterized the vacuolar sorting of CrPrx1 and developed important tools for the future research of the anticancer alkaloids pathway, namely a transient expression protocol in leaf protoplast cells and a regeneration methodology aiming at the production of transgenic plants. Finally, we have characterized the functions of the main peroxidases of leaves of the model plant *Arabidopsis thaliana*, namely through reverse and forward genetics.

Future research goals

We are now committed to unraveling important biosynthetic, transport and regulatory genes implicated in the anticancer alkaloid pathway, through omic approaches. We have devised a strategy involving the isolation of the blue fluorescent cells accumulating the alkaloids (see picture above) by fluorescence activated cell sorting, followed by the analysis of the differential transcriptome of those cells. This strategy has already enabled the detection of several candidate genes that may be involved in the regulation and the multiple transmembrane transport events of the alkaloid pathway. Those genes will now be investigated using molecular and biochemical approaches, with the aims of understanding the regulation and metabolite fluxes of plant secondary metabolism, and of applying the knowledge gained to the metabolic engineering of *C. roseus* to improve the current low yields of the anticancer alkakaloids, and produce new bioactive derivatives.

In parallel, we will continue the investigation of *A. thaliana* peroxidases, especially in what concerns their interaction with natural products, arabinogalactan proteins, and hydrogen peroxide.

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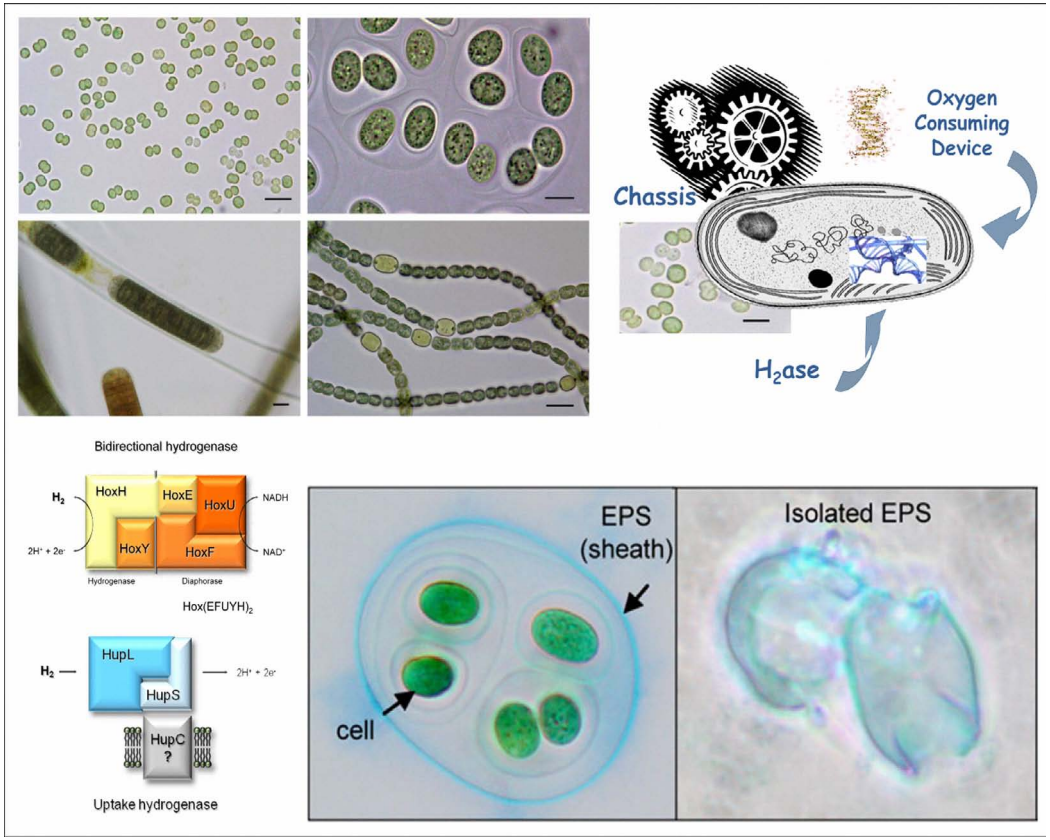

Cyanobacteria: diversity, hydrogenases, as a photoautotrophic chassis, extracellular polymeric substances (EPS)

Bioengineering and Synthetic Microbiology

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Previous research results

The main research topic of the group has been **cyanobacterial hydrogenases** and **hydrogen metabolism**. We investigated the transcription and expression patterns of genes related to hydrogenases, as well as the involvement of several transcriptional factors. In addition, a **synthetic biology approach** was introduced to solve fundamental issues related to hydrogenases function(s)/hydrogen production. *Synechocystis* PCC 6803 was chosen as a **photoautotrophic chassis** to accommodate the standardized parts and devices primarily designed for H_2 production. To prepare the chassis, and since a heterologous hydrogenase was going to be introduced, genes encoding the native bidirectional hydrogenase were deleted. Furthermore, an in silico analysis of the genome was performed to identify neutral sites for the integration of synthetic parts/devices. In parallel, to achieve the microaerobic intracellular environment required for optimal hydrogenase activity, several **synthetic oxygen consuming devices** (OCDs) were designed. The group is also exploring the use of exopolysaccharides(EPS)-producing cyanobacteria to remove metallic ions from polluted waters, and other forms of **bioremediation**. Previously, we tested the capability of several strains to remove cations from aqueous solutions, characterized the polymers produced, and identified the functional groups involved in the removal.
Together CIIMAR, we described the **diversity of cyanobacterial strains** isolated from the intertidal zones along the Portuguese coast. This study revealed novel diversity and that one-third of the isolates are potential nitrogen fixers, while no conventional toxin producers were detected.

Future research goals

In the future, the group will continue to investigate the **biosynthesis/maturation of cyanobacterial hydrogenases**, notably the involvement of Hyp proteins in the maturation of the uptake hydrogenase and the specificity of the endopeptidases (HoxW and HupW) in the cleavage of the C-terminal polypeptide of each large subunit precursors, the validation of the **neutral sites** in the genome of *Synechocystis* PCC 6803, and the characterization of the **synthetic oxygen consuming devices** in *E. coli* and in our photoautotrophic cyanobacterial chassis. Moreover, within the bioremediation project the genes encoding proteins involved in the last steps of EPS production and the physiological/environmental conditions promoting their production and export will be characterized, and small scale metal bioremoval assays will be performed. In the isolated marine cyanobacterial strains, no genes involved in the production of conventional freshwater toxins were found. However, cell extracts were toxic to invertebrates suggesting the presence of other compounds, which are currently being investigated.

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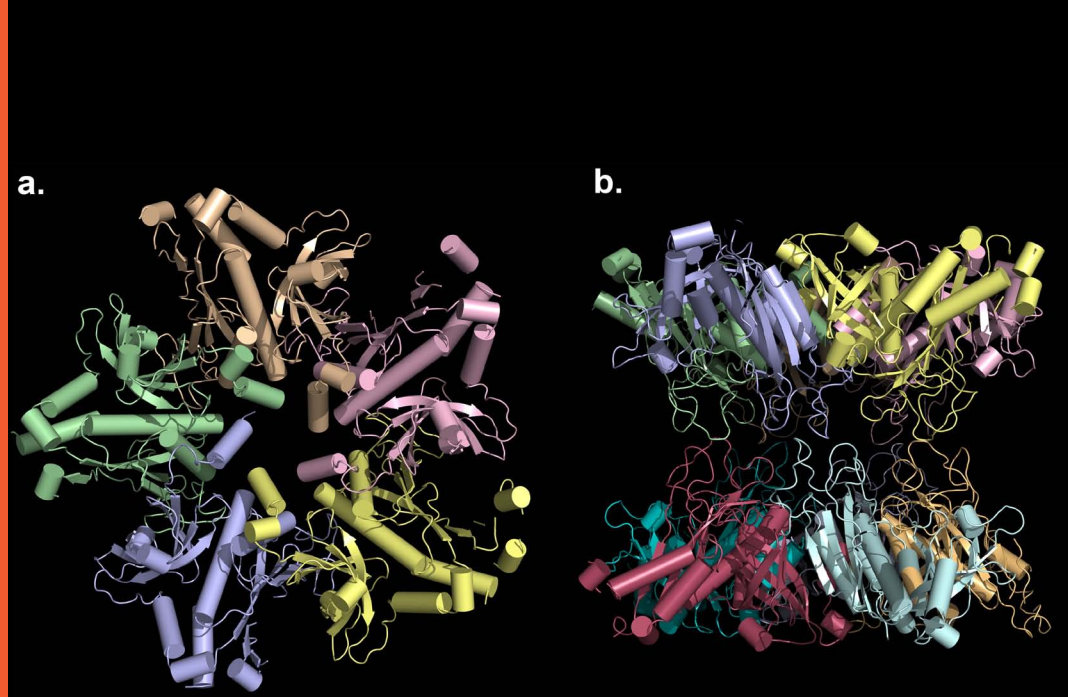

Top (a) and side (b) view of the experimental three-dimensional model of the decameric cytosolic glutamine synthetase from the model legume *Medicago truncatula*.

Biomolecular Structure

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Previous research results

We focus our research on protein structure and function, with a particular emphasis on enzymes with potential biomedical implications. Using X-ray crystallography as the main technical approach, complemented by a plethora of other biochemical, biophysical, and computational techniques, we try to understand the function of macromolecules and macromolecular complexes from their high-resolution structures. We are currently interested in the characterization and validation of potential drug targets from bacterial human pathogens and in elucidating the molecular details of specific coagulation factor IIa recognition and inhibition by natural macromolecular anticoagulants from haematophagous parasites.

In this context, we have recently determined the three-dimensional structure of a novel glucosyl-3-phosphoglycerate synthase from *Mycobacterium tuberculosis,* which integrates an essential and unique pathway of this human pathogen. Together with the structure of the closely related mannosyl-3-phosphoglycerate synthase from the hyperthermophile *Rubrobacter xylanophilus*, this structure revealed the molecular determinants of substrate specificity and established a knowledge base for the rational design of specific inhibitors. In a similar line, we have determined the three-dimensional structure of human thrombin in complex with three small synthetic inhibitors, unveiling important details of their specific mode of action.

Future research goals

The recent uprising of life-threatening infectious diseases of bacterial origin, brought about by the increasing resistance of many pathogens to the available antibiotics, prompts for the functional and molecular characterization of novel pathways that are amenable to therapeutic intervention. Our group will therefore continue the molecular and structural dissection of the unique *M. tuberculosis* pathways that lead to cell wall synthesis, in order to identify and validate suitable drug targets.

Coagulation disorders are other life-threatening and highly incapacitating pathologies for which more efficient and safer therapies are much needed. By pursuing the characterization of the structural determinants of thrombin recognition by natural macromolecular anticoagulants, we will contribute towards the development of better antithrombotic drugs.

In summary, our group will carry on with the structural and biochemical characterization of medically relevant enzymes and drug targets from human pathogens and of unique natural anticoagulants from haematophagous animals, ultimately envisaging the development of novel therapeutic strategies.

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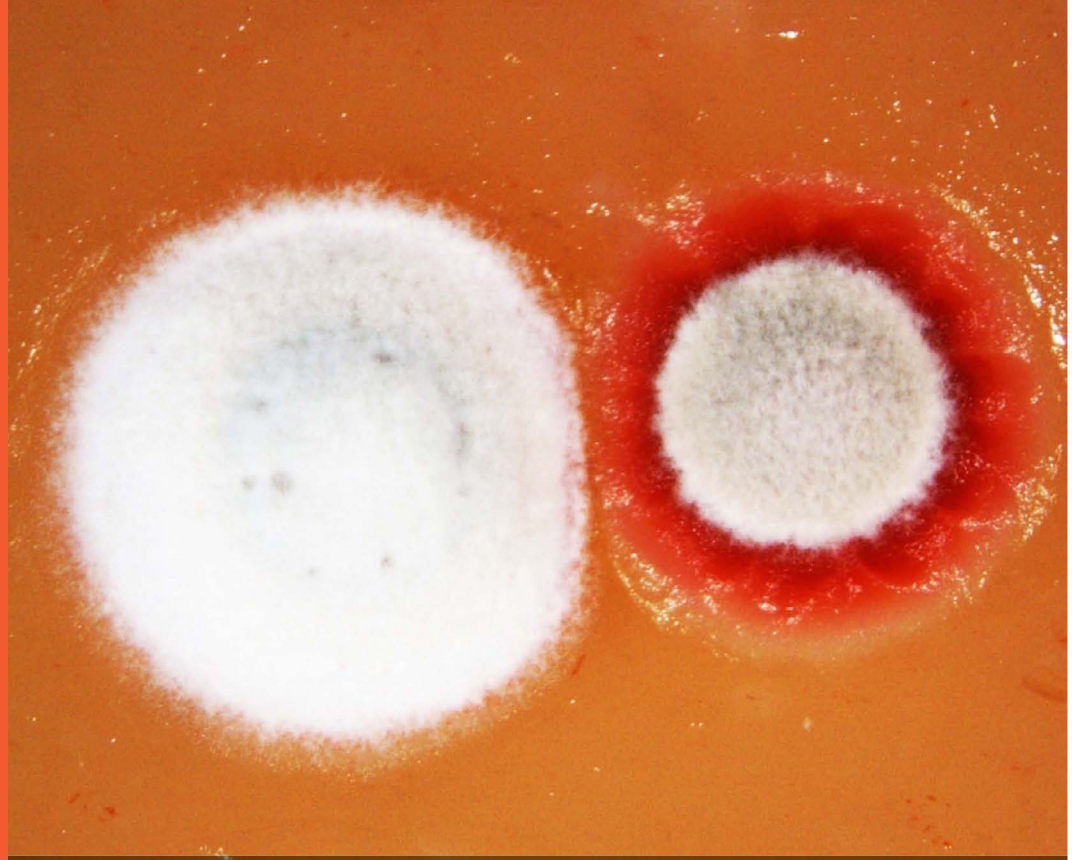
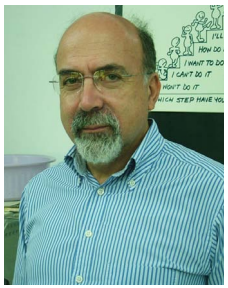


Immunofluorescence of dendritic cells infected with the parasite *Leishmania infantum*.

Cellular and Applied Microbiology



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Previous research results

The Cellular & Applied Microbiology group has been addressing the molecular mechanisms underlying the stress response, namely oxidative stress in yeast and bacteria. At present the work focuses on the effect of reactive oxygen species (ROS), such as hydrogen peroxide, in the production of secondary metabolites in *Streptomyces*.

Streptomyces are Gram-positive, filamentous, soil-dwelling bacteria well known for their ability to produce a wide variety of secondary metabolites such as antibiotics and immuno-suppressant agents, among others. *Streptomyces* secondary metabolism is regulated by a complex network involving multiple factors and taking place at different levels: from pathway-specific regulatory genes to pleiotropic regulators which control both secondary metabolism and morphological differentiation

The work aims at identifying the molecular mechanism(s) induced by hydrogen peroxide that can play a role in the regulatory network governing the synthesis of secondary metabolites. Recently we were able to construct *Streptomyces* knock-out mutants on H_2O_2-related anti-oxidative enzymes that allowed us to modulate the intracellular redox status. In doing so, we have highlighted the cross-talk between intracellular ROS homeostasis and secondary metabolism in *Streptomyces*.

Future research goals

We will continue studying the bacterial signaling networks and the molecular interactions between them. We will focus on the molecular basis of the redox-dependent regulation of secondary metabolism in *Streptomyces*.

In the short-term two main questions will be addressed: (i) what are the molecular mechanisms behind the crosstalk between ROS homeostasis signalling pathways and secondary metabolism in *Streptomyces* spp. and (ii) can ROS homeostasis modulation be used to obtain improved fermentation yields.

In the long term we will seek to expand the research to other physiological processes and how they integrate into the binomial redox signaling / secondary metabolism. The first process that we intend to focus is quorum-sensing, a phenomenon directly related to secondary metabolism in *Streptomyces*. For that purpose a new project that integrates phylogenomics and physiological approaches on the study of quorum-sensing in the actinobacteria phylum is already in place.

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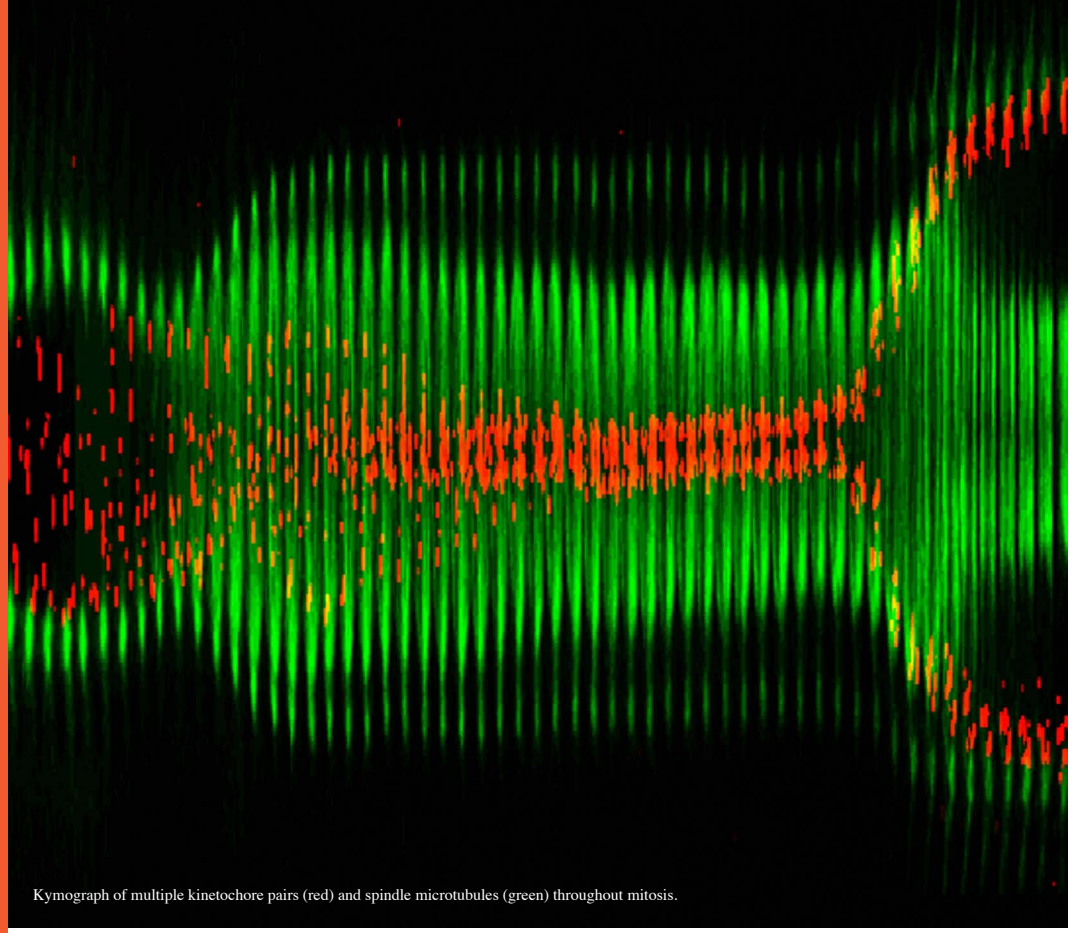

Kymograph of multiple kinetochore pairs (red) and spindle microtubules (green) throughout mitosis.

Chromosome Instability and Dynamics

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Previous research results

Our laboratory aims to understand how chromosome segregation is coordinated in space and time, focusing on the molecular and structural perspective behind concurrent pathways involved in mitotic spindle assembly and function. We are also very interested in how failure of this process contributes to aneuploidy and chromosomal instability in animals. We have established the roles of key molecular players at the kinetochore-microtubule interface, such as the microtubule plus-end tracking proteins CLASPs. CLASPs are part of a molecular switch at kinetochores that is critical to ensure error correction, while stabilizing correct kinetochore-microtubule attachments, prior to their synchronous segregation during anaphase. Being part of the microtubule flux machinery, the study of CLASPs revealed that spindle microtubule flux plays an important role in in force distribution and anaphase synchrony. We have additionally identified acentriolar MTOCs in living animal somatic cells, which are required for cytoskeleton remodeling at the entry and exit from mitosis. More recently, we provided a new conceptual view of a spindle-matrix, not as a rigid structural scaffold as classically envisioned, but as a dynamic spatial determinant of the mitotic checkpoint. These and other studies pioneered the combined use of RNAi, laser microsurgery and fluorescent-speckle microscopy to study kinetochore function in living animal cells. We aim to promote the interaction between a multidisciplinary team to answer key questions at the forefront of cell division research that were hitherto unapproachable, elucidating how interference with critical players impairs mitotic fidelity and the respective implications for human health.

Future research goals

Our present research interests are focused in understanding how mitotic fidelity is regulated in space and time, paying particular attention to how the spindle matrix confines SAC signaling and how error-correction mechanisms at the KT-MT interface are regulated throughout mitosis. For this purpose we have adopted a multi and interdisciplinary approach to address key biological challenges with the following goals: 1) Molecular and functional dissection of mitotic spindle assembly pathways; 2) Spatiotemporal regulation of spindle-chromosome interactions; and 3) Implementation and functional analysis of in vivo mammalian models with compromised mitotic fidelity.

Specifically, we have completed a genome-wide screen in Drosophila for genes required for acentrosomal spindle assembly and are currently investigating the function of potential novel genes required for this process in animal somatic cells. We intend to continue to apply and further develop state-of-the-art laser microsurgery and other optical tools to investigate fundamental questions behind animal cell division, and combine them with modern molecular biology techniques. Finally, we aim at extending the investigation of the role of CLASPs during mitosis in mammals, focusing on the identification of new molecular partners, as well as in the generation and characterization of mammalian models in mice.

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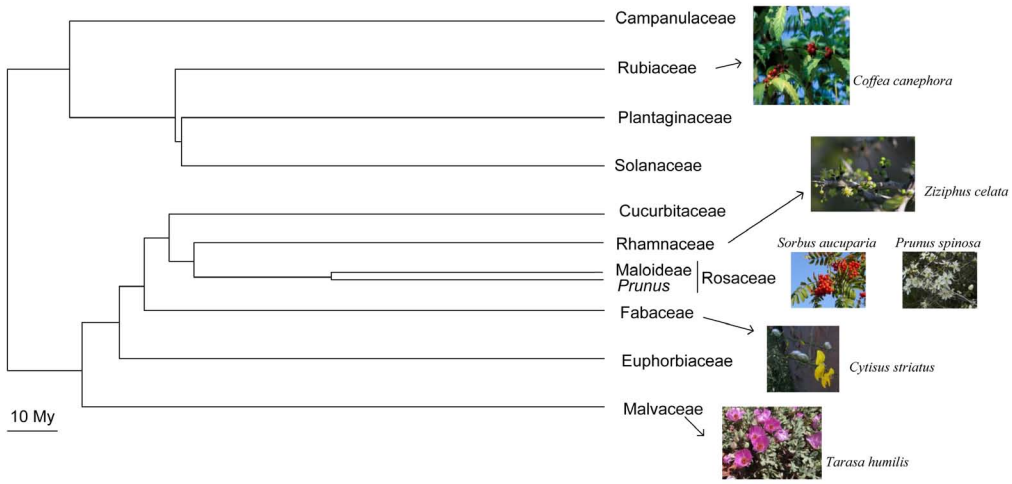

Plant families presenting *S-RNase* based GSI. Species under study are shown.

Evolutionary Systems Biology

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Previous research results

Lessons on the evolutionary forces and molecular mechanisms that drive the divergence of populations and species, and ultimately speciation, can be taken from the analyses of genes that contribute to the cessation of gene flow between populations. Barriers to gene flow can arise at multiple prezygotic and postzygotic life-history stages. In flowering plants, one of the most common post-pollination prezygotic barriers, is gametophytic self-incompatibility (GSI). In this widespread system, when the S-pollen specificity matches that of the S-pistil, the pollen is recognized as "self" and is rejected by the pistil. This system is ideal to address general principals on how life can innovate, since it has reappeared independently several times during plant evolution.

Although in eudicots there is evidence for a single *S-RNase* based GSI origin, in Rosaceae species, at the *S-RNase* gene, different amino acid positions are involved in specificity determination. Moreover, levels of recombination, the rate at which new specificities arise, the number of ancestral lineages and the degree of specificity sharing between closely related Rosaceae species are also different. Furthermore, in *Prunus* the *S*-pollen gene is a single gene. In contrast, in Pyrinae (former Maloideae) multiple genes determine *S*-pollen specificity. Different mechanisms may thus, be used to achieve the rejection of incompatible pollen in different plant families.

Future research goals

To highlight the features of the ancestral GSI system we are characterizing the S-RNase and S-duplicated genes in Fabaceae, Rhamnaceae, Malvaceae, and Rubiaceae species. The current hypothesis is that the common ancestor of these plant families possessed an *S-RNase*-based GSI system. Nevertheless, remarkable differences are observed in Rosaceae species such *S-RNase* gene intron number, number of S-pollen genes, or competitive interaction (the breakdown of SI in heteroallelic pollen) documented in *Malus* but, that does not exist in *Prunus*. The alternative hypothesis, that different gene family members have being recruited for GSI in these familes is thus, being tested.

In *Prunus*, the *S*-pollen protein is assumed to protect self *S-RNases* from being inhibited by a general *S-RNase* inhibitor. We are addressing the predictions of the general inhibition model such as: testing the interaction of the two proteins, expression and purification of recombinant *Prunus* SFB and S-RNase, determining the three-dimensional structure of the S-RNase, SFB and their complex by X-ray crystallography, and identification of SFB and S-RNase binding partners.

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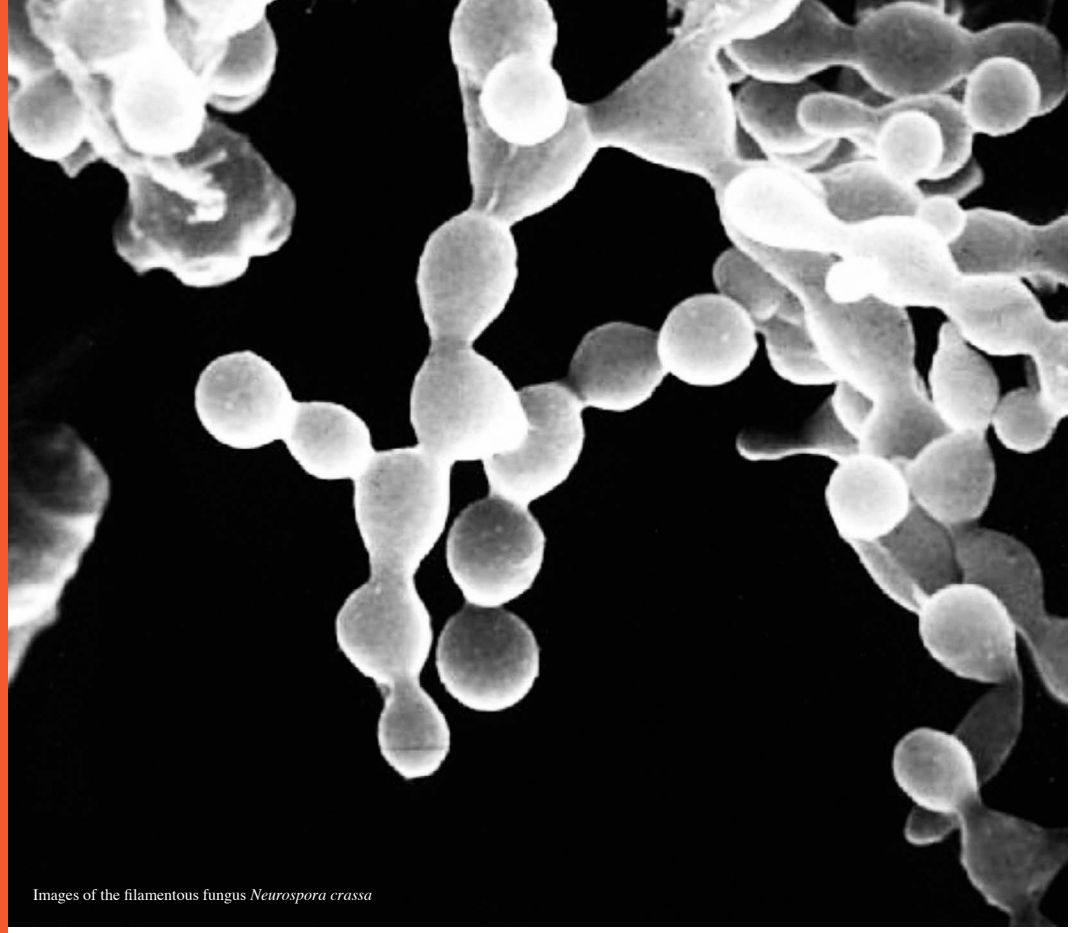

Images of the filamentous fungus *Neurospora crassa*

Mitochondria

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Previous research results

Our laboratory has been interested in the molecular biology of mitochondria, the organelle responsible for the generation of most cellular energy. In particular, we have been studying the bioenergetics processes using the filamentous fungus *Neurospora crassa*. We have identified and characterized all respiratory chain NADH dehydrogenases from Neurospora, namely the proton-pumping complex I and the four alternative NAD(P)H dehydrogenases (one internal and three external enzymes). We found that complex I is essential for sexual development and the alternative enzymes are important for the germination of both sexual and asexual fungal spores. We also described the composition of the fungal complex I (about 40 proteins). We have defined the role of many of theses polypeptides in the assembly, structure and function of complex I. We also generated fungal models of human mitochondrial disease associated with complex I, finding that some mutations result in diminished levels of the enzyme rather than affecting its activity.

We have also identified and determined the cellular location and characterized of all FKBP proteins (ligands of immunossupressant FK-506) from *Neurospora crassa*, unraveling some of their roles within the cell. We cloned two proteins (enolase and NAD+ synthetase) from *Streptococcus sobrinus*, an agent of dental caries, which affect the mice immunological system. We identified and collected Neurospora species from nature for the first time in Europe, participating in a study of fungal diversity at continental scale. We also showed that *N. crassa* can be used as a model organism to investigate programmed cell death.

Future research goals

Mitochondria are crucial for the life of organisms, through its capacity for energy production and regulation of other cellular processes, but also have a central role in programmed cell death (PCD). The death program is essential for the development of metazoan organisms and its dysfunction may result in human disease, like cancer. Our main objective for the future is the characterization of genes/proteins and mechanisms involved in programmed cell death, with particular emphasis in the mitochondrial involvement. We anticipate that knowledge about novel processes associated with programmed cell death will be useful to develop drug combinations that can be used as anti-fungal and/or anti-tumor agents. For this, we plan to establish and use the filamentous fungus Neurospora as a model organism to investigate PCD. We also plan to continue the characterization of mitochondrial proteins/complexes, with particular emphasis in respiratory chain components, and their role in mitochondrial biogenesis and function.

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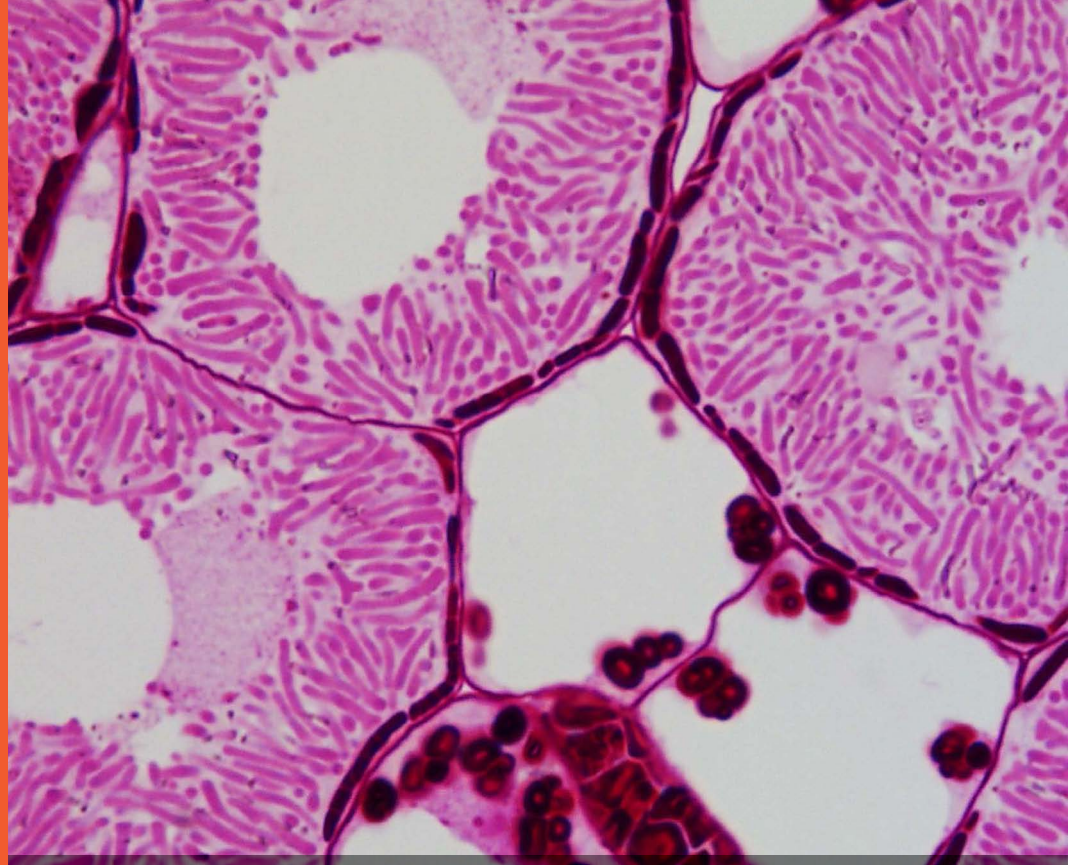

Cross section of a *Medicago truncatula* root nodule formed by *Sinorhizobium meliloti*. The figure shows infected plant cells containing numerous bacteroids.

Molecular Biology of Nitrogen Assimilation

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Previous research results

Glutamine Synthetase (GS) is a crucial enzyme in nitrogen metabolism, as it catalyses the first step at which nitrogen is brought into cellular metabolism. The complete understanding of the mechanisms controlling GS activity is of uppermost importance, not only for plants but for all forms of life. We use the model legume *Medicago truncatula* to investigate the regulatory mechanisms that control this key enzyme and evaluate its in¬volvement in the regulation of nitrogen use efficiency (NUE). Our previous studies provided important insights towards the understanding of the genetic and molecular basis of GS expression in *Medicago truncatula*. We have identified important regulatory controls operating both at the gene and protein levels which modify GS activity according to the context in which the metabolism is taking place and we have determined the tridimensional structure of the proteins. Using reverse genetics we succeeded in obtaining transgenic plants altered for the levels of GS specifically in root nodules. Analysis of these plants indicates that nodule GS activity is positively correlated with symbiotic nitrogen fixation and plant nitrogen utilization efficiency. Using the tools of trancriptomics and metabolomics we identified the major transcript and metabolite changes associated with the altered nodule metabolism. Taken together, these studies provide important knowledge for the manipulation of nitrogen assimilation, towards the goal of obtaining plants more efficient in terms of nitrogen utilization, better protein content and increased productivity.

Future research goals

For the coming years we aim to continue our research on the biochemical and genetic regulation of the metabolic processes that are required for nitrogen assimilation in the model legume *Medicago truncatula*. Whole genome approaches using plants in which the activity of key metabolic enzymes has been modified either by reverse genetics or by the use of specific inhibitors, will be used to identify novel regulatory and signal transduction genes. The research involves the use of functional genomics approaches together with traditional tools of genetics, biochemistry, and physiology to dissect the molecular mechanisms regulating gene expression and enzyme activities in response to alterations in nitrogen metabolism. In the continuation of the dissection of the post-translational mechanisms that regulate glutamine synthetase activity and its physiological implications for nitrogen metabolism, three major processes will be studied: protein-protein interactions, phosphorylation and nitration. We also aim to investigate the contribution of GS for seed metabolism, with a special focus on a novel GS isoenzyme that we identified recently and is specifically expressed in seeds of *M. truncatula*.

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As old as it gets (at 25ºC): a ten month old *D. americana* male fly

Molecular Evolution

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Previous research results

Due to the recent advances in genome sequencing techniques, it is now feasible to have data on the genome of many individuals, yet the meaning of the observed variation is still largely unclear. Understanding the genetic basis of phenotypic variation is thus still one of the main goals of research in Biology. Indeed, even in model systems, such as *Drosophila*, where this problem is more tractable than in humans, progress on the identification of the variants responsible for the within and between species phenotypic differences in important life-history traits such as size, cold resistance, developmental time or lifespan to name a few, is still very limited. Not surprisingly, within the genus *Drosophila*, most studies have been performed in the model species *D. melanogaster*. Therefore, for most phenotypic traits it is unclear whether the results obtained for *D. melanogaster* can be generalized to other distantly related species.

When using a candidate gene approach, we have found important differences regarding the genetic basis of immune response traits, cold resistance and lifespan. Remarkably, several genes identified in *D. melanogaster* as responsible for variation in important phenotypic traits are not present in distantly related *Drosophila* species. Variation in gene content is observed even when studying highly conserved gene networks (such as genes involved in meiosis). Since our main aim is to identify the causative polymorphisms responsible for variation in ecologically important traits, we have been performing as well classical association studies in species of the *virilis* group.

Future research goals

Variation in phenotypic traits such as size, developmental time, cold resistance, propensity to enter diapause, and lifespan, are likely responsible for present day *Drosophila* species distributions, and for the degree of adaptation to the ongoing climate change. Very likely, these traits were also important in determining the fate of different *Drosophila* species in the past (for instance, during the last glaciation). The identification of the climatic features that limit species distributions can be inferred using GIS and ancestral state reconstruction techniques. Such information can help in the identification, through the association with a given amino acid variant or expression pattern at candidate genes, of the genetic basis of within and between species phenotypic variation. Our recent work led to the identification of a likely causative variant at two genes involved in the setting of lifespan and one involved in cold resistance. Further work is now being conducted to understand why such variants affect the phenotype the way they do.

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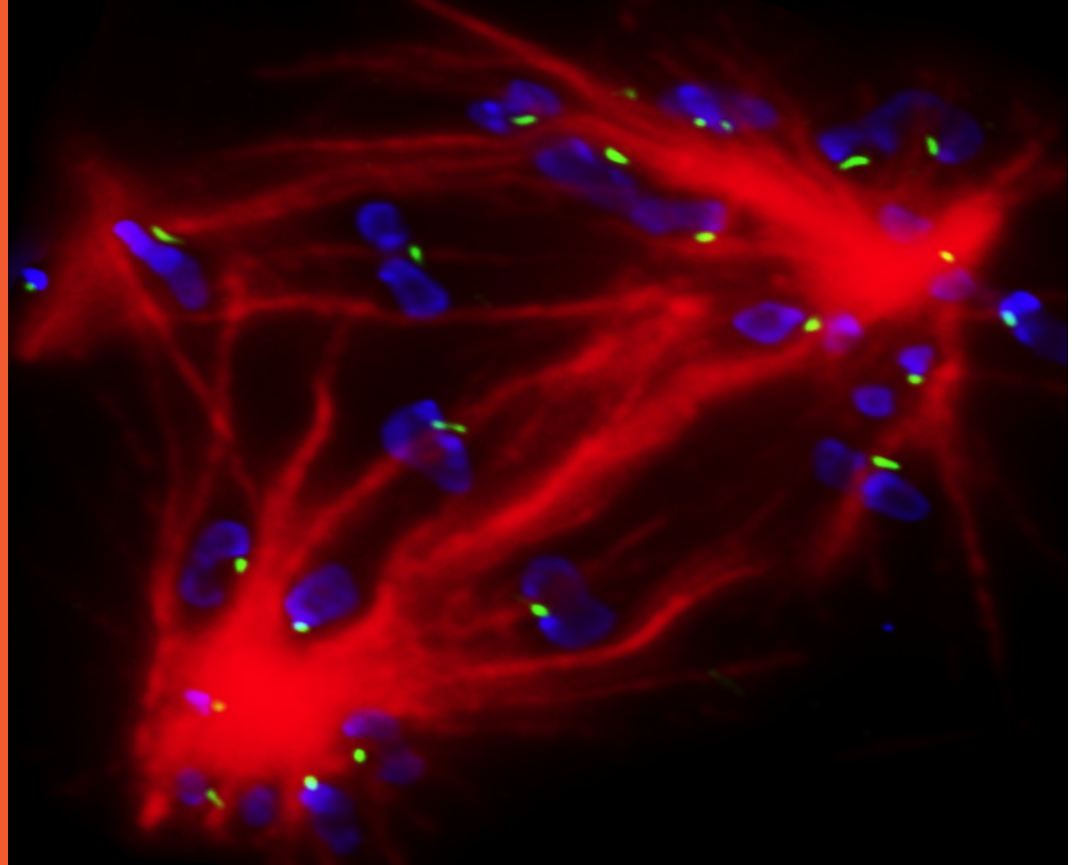

A Drosophila S2 tissue culture cell during early anaphase in which the kinetochore protein involved in microtubule binding Ndc80 was depleted by RNAi. The image shows kinetochores (green), spindle microtubules (red) and DNA (blue).

Molecular Genetics

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Previous research results

Our research group has been for a number of years involved in studying the molecular mechanisms required for maintenance of genomic stability during cell division. Towards this aim we have used Drosophila melanogaster as our model organism resorting to classical genetics, biochemistry and cell biobogy to identify and characterize genes that are essential for this process. During these studies we identified, characterized and performed functional studies of proteins involved in a number of events that take place during cell division. We were involved in the identification of the founding member of the Polo-like kinase family and demonstrated its role in mitotic progression and centrosome maturation. Subsequently we identified mutations for γ-tubulin and showed that although it was involved in microtubule nucleation from centrosomes, partly functional spindles could be made in its absence. During subsequent years we concentrated our work on the proteins required for mitotic chromosome organization and demonstrated that the condensin complex is not required for overall chromosome architecture but is essential for sister chromatid individualization. During the last few years our attention has turned to the functional analysis of proteins involved in the Spindle Assembly Checkpoint that monitors proper attachment of chromosomes to the mitotic spindle. We have shown that some of these proteins like Bub3 and BubR1 have essential roles in mitosis that are additional to their roles in the checkpoint. Also we demonstrated that the kinase activity of BubR1 plays a major role during regulating meiotic recombination.

Future research goals

The work in progress to uncover the molecular mechanisms that integrate the signals that result after microtubules bind the kinetochore with those involved in checkpoint signalling. For this we are studying at the biochemical level the role BubR1 and the kinase Mps1 play in signal transduction. In parallel we are continuing to study the role of BubR1 in meiotic progression and have identified Polo as an important genetic interactor to supress nondisjunction. Moreover, we have started two new lines of research designed to study 1) the molecular requirements for SAC deficient cells to become tumorigenic and 2) the role os SAC proteins and other mitotic regulators in cell division within well-defined epithelia and its relation to Apico-Basal polarity. We continue to use tissue culture cells, dsRNAi, in vivo fluorescence imaging and biochemical approaches to develop models for microtubule attachment and checkpoint function. In parallel we are designing experiments that will test the models derived from these studies in different cell types within the whole organism including mitotic. With these approaches we aim to determine the possible contribution of chromosome missegregation in tumour development.

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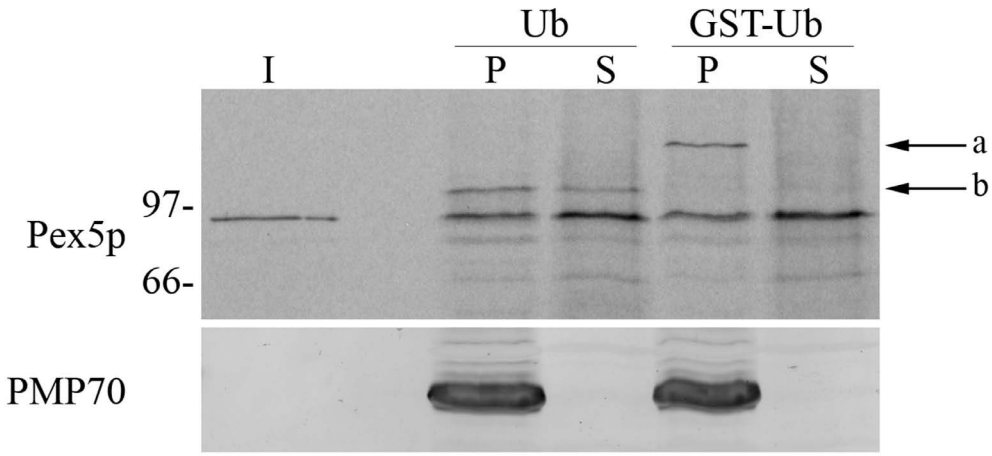

In vitro systems are extremely powerful tools to study the mechanism of peroxisomal protein import. Here, 35S-labeled PEX5 was incubated with purified peroxisomes and components of the ubiquitin-conjugating cascade. The experiment shows UbcH5-mediated mono-ubiquitination of PEX5 and its partial export back into the cytosol. Conjugation of a GST-Ubiquitin fusion protein to PEX5 is also possible in this system but the corresponding conjugate is no longer a substrate for the export machinery.

Organelle Biogenesis and Function

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Previous research results

Peroxisomal proteins are synthesized on cytosolic ribosomes and post-translationally targeted to the peroxisome by cycling receptors. There are two main receptors: PEX19 is the receptor/chaperone for intrinsic membrane proteins; PEX5, alone or with the help of the adaptor protein PEX7, is the receptor/translocator for proteins destined to the matrix of the organelle. Our main aim has been to understand the mechanisms of these two protein sorting pathways. Using *in vitro* import systems developed in our laboratory, we have shown that PEX5 becomes transiently inserted into the peroxisomal membrane docking/translocation machinery during the transport cycle. Our data further suggest that protein-protein interactions involving PEX5 on one side, and the membrane machinery on the other, provide the energy for the cargo translocation process. Following this step, PEX5 is extracted from the peroxisomal membrane machinery in an ATP-requiring step. Remarkably, this requires monoubiquitination of PEX5 at a conserved cysteine residue. Finally, the ubiquitin moiety is removed from the cytosolic PEX5 conjugate probably by a combination of enzymatic and non-enzymatic mechanisms. Our goal at present is to understand why this unconventional ubiquitination is used in this pathway.

Future research goals

We will continue to study the mechanism of protein sorting into peroxisomes. Efforts will be focused on PEX7, an adaptor protein that increases the range of cargo proteins recognized and transported by PEX5. In parallel, we will try to understand how PEX5 interacts directly with most cargo proteins and, after insertion into the peroxisomal docking/translocation machinery, releases them into the organelle matrix in a process that apparently does not require ATP-hydrolysis.

Besides the work on peroxisomal biogenesis we are also developing a new research line on protein regulation by ubiquitin and ubiquitin-like molecules.

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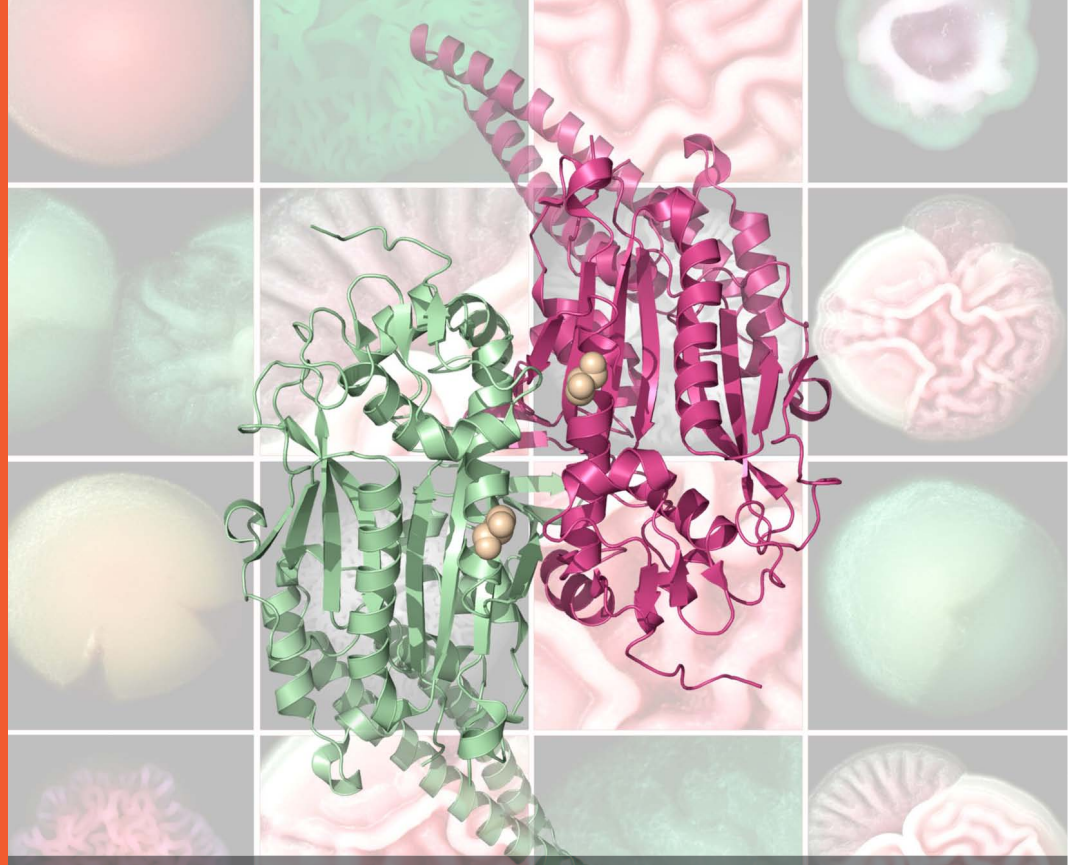

Crystal structure of *C. albicans* seryl-tRNA synthetase dimer.

Protein Crystallography

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Previous research results

Our group aims at characterizing protein function at an atomic level, focusing on the structural and functional study of biomedically relevant enzymes. The core of the research methodology is X-ray crystallography, complemented by other biophysical and biochemical techniques aiming at elucidating the biological function and biomolecular interactions of target proteins. Current research includes the following topics:
(1) Structure-function relationships in enzymes implicated in neurodegenerative disorders, especially ataxin-3, the protein affected by polyglutamine expansion in Machado-Joseph's disease;
(2) Structural characterization of novel therapeutic targets from human pathogens (e.g. *Candida albicans*).
Major achievements include contributions towards the understanding of the fibrillization pathway and of the nucleocytoplasmic shuttling activity of human ataxin-3, as well as the identification of novel post-translational modifications regulating the stability and proteolytic activity of this ubiquitin hydrolase. Moreover, we have determined the three-dimensional structure of seryl-tRNA synthetase from *C. albicans*, a central enzyme in the peculiar mechanism of ambiguous decoding of the universal leucine CUG codon both as serine and as leucine. This structural analysis unveiled novel structural features and showed that serine or leucine insertion within the CUG-encoded position induced only subtle structural changes, which were associated with small changes in aminoacylation activity.

Future research goals

Serious pathologies often arise from the impairment of the regulatory mechanisms controlling cellular processes, which can be circumvented by understanding the atomic details of the enzymes involved in specific pathways. Specific inhibition, spatial and temporal compartmentalization, and post-translational modifications of the intervening enzymes allow a tight regulation of those processes. A major goal for the following years will be the molecular and biochemical characterization of the role of ataxin-3 post-translational modifications on its biomolecular interactions, proteolytic activity, structure, and aggregation behavior using *in vitro* and *in situ* models. The recent characterization of the *C. albicans* proteome potentially affected by CUG ambiguous decoding prompted the structural and functional characterization of specific pathways associated with virulence and pathogenesis. We will therefore concentrate on the structural and biochemical characterization of medically relevant enzymes and drug targets from human pathogens, aiming at contributing to the development of novel therapeutic strategies.

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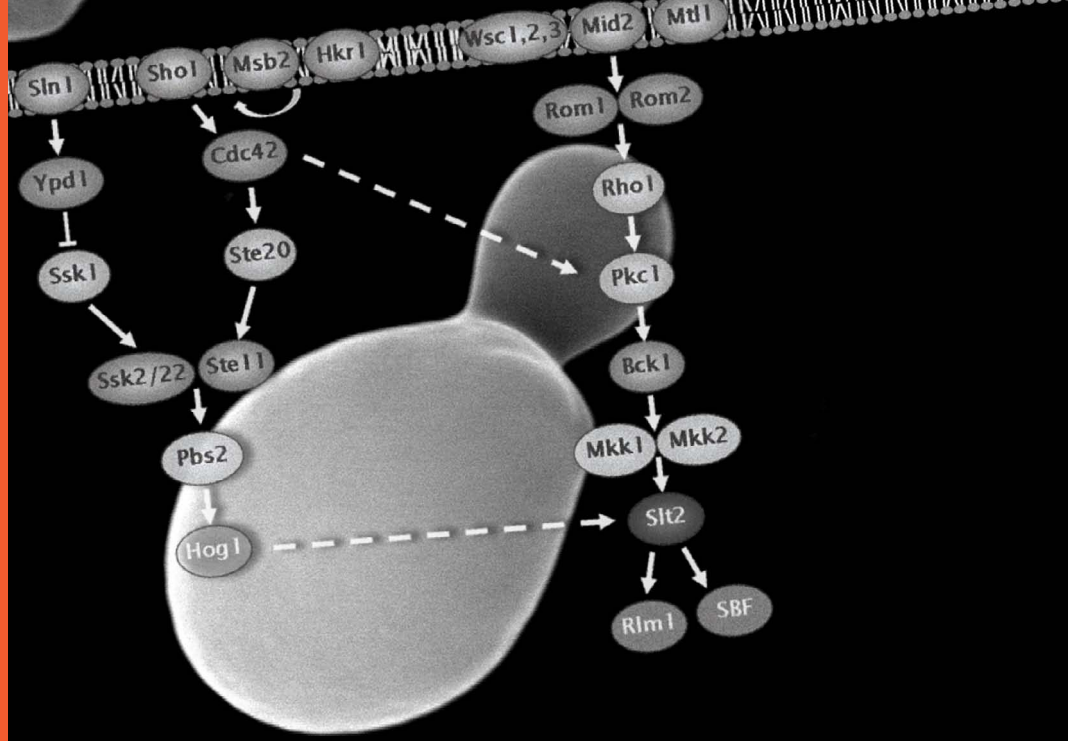

Two signalling cascades involved in stress responses in yeast.

Redox Cell Signalling

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Previous research results

Changes in redox homeostasis and the accumulation of oxidative damages is a hallmark of ageing and a number of diseases associated with ageing. The yeast *Saccharomyces cerevisiae* is an excellent eukaryotic model organism and yeast molecular genetics has been extensively exploited to provide powerful insights into the redox signalling pathways mediating oxidative stress protection and cell longevity. Our group has identified major protein targets oxidised in yeast under oxidative stress conditions and shown that the turnover of oxidised proteins (by the vacuolar proteinase Pep4p) as well as scavenging of reactive oxygen species (by endogenous antioxidant defences or quercetin, a polyphenolic compound present in the diet) play a key role in cell homeostasis during chronological ageing.

We have also shown that Isc1p, the yeast orthologue of mammalian neutral sphingomyelinase-2, modulates redox homeostasis, iron levels and apoptosis and its deficiency leads to a shortened chronological lifespan. Moreover, Isc1p is an upstream regulator of Sit4p, a ceramide-activated protein phosphatase, and our results implicate Sit4p activation in mitochondrial dysfunction and premature ageing.

Our current goal is to identify and characterize downstream targets of Sit4p. We are also investigating whether polyphenolic compounds exert their protective effects through modulation of cell signalling.

Future research goals

We aim to further characterize the molecular mechanisms by which bioactive sphingolipids, such as ceramide, and enzymes of sphingolipid metabolism modulate redox homeostasis and chronological lifespan. For that, we are developing two major lines of research:

1. Role of neutral sphingomyelinase: we will use biochemical assays and molecular and cell biology methods to identify and characterize downstream targets of Isc1p and Sit4p. We will focus on the characterization of signalling pathways that modulate mitochondria and vacuoles, two organelles with critical roles in redox homeostasis.

2. Molecular mechanisms of Niemann Pick type C disease: We will use yeast as a model organism to investigate the role of Ncr1p, the yeast orthologue of hNPC1, in redox homeostasis and lifespan. Loss of function mutations in hNPC1 are associated with the Niemann Pick type C (NPC) disease, characterized by changes in cholesterol and sphingolipid metabolism. Insights from yeast studies will be validated in cultured fibroblasts isolated from patients with different NP-C1 mutations (collaboration with the Lysosome and Peroxisome Biology Unit, IBMC).

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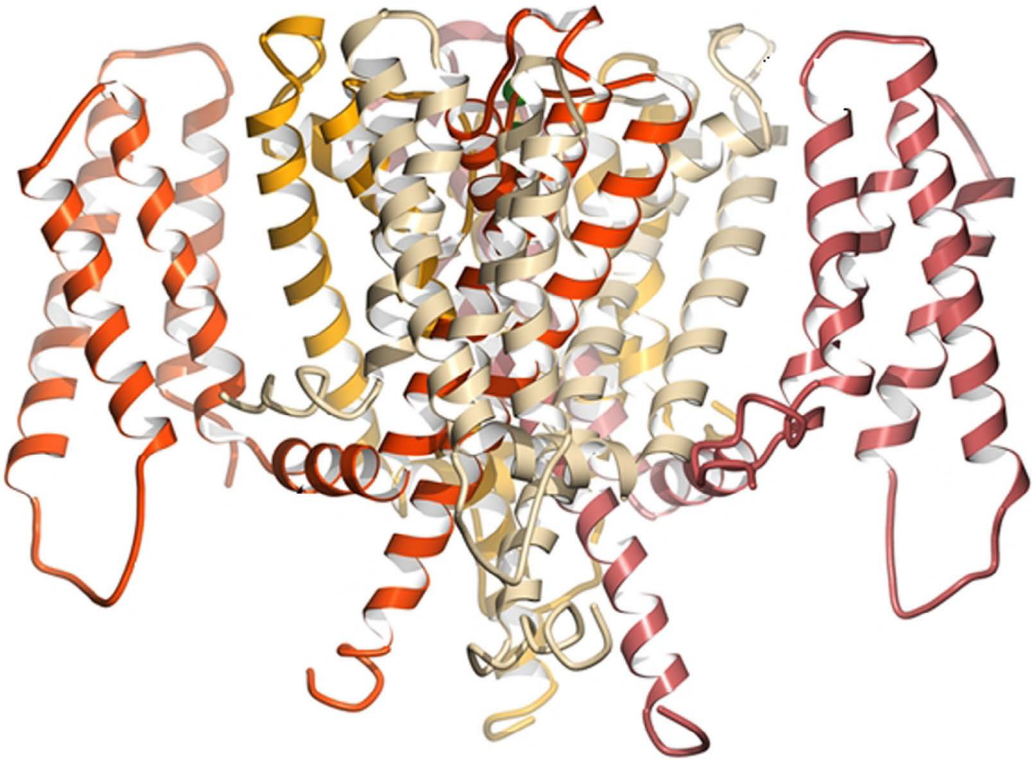

Side-view of the cyclic nucleotide regulated potassium channel.

Structural Biochemistry

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Previous research results

We are interested on the molecular mechanisms of transport and regulation in ion transport membrane proteins. Our studies are focused on a couple of potassium channels, both from eukaryotic and prokaryotic organisms, one ion symporter and an ion antiporter. In these studies we combine X-ray crystallography, for three-dimensional structure determination, with different biochemical and biophysical techniques.

The MlotiK1 cyclic nucleotide regulated potassium channel is one of the proteins which we have been studying. This channel is activated upon binding of cAMP just like the CNG and HCN channels in humans. In the past few years, together with the lab of Lise Thomas in the US, we have characterized the functional, structural and biochemical properties of this potassium channel. In particular, we have determined the structure of the full-length channel as well as several different structures of its regulatory domain. We now understand fairly well the molecular changes that occur at the level of the regulatory domain when it binds or unbinds cAMP. Currently, we are developing a molecular model for the molecular changes which occur at the level of the channel during gating. For this purpose, and together with Daniel Müller in Switzerland, we have recently characterized the conformational transition of the channel using Atomic Force Microscopy.

Future research goals

At present one of our aims is the expansion of the set of technical tools used in our studies. We have started using fluorescence spectroscopy to characterize the mechanism of the membrane proteins. In particular, we have been developing ion transport assays using fluorescence probes that are sensitive to K^+ concentration. We are now hoping to start using FRET techniques in the cell to characterize large multiprotein complexes that include the channels we have been studying.

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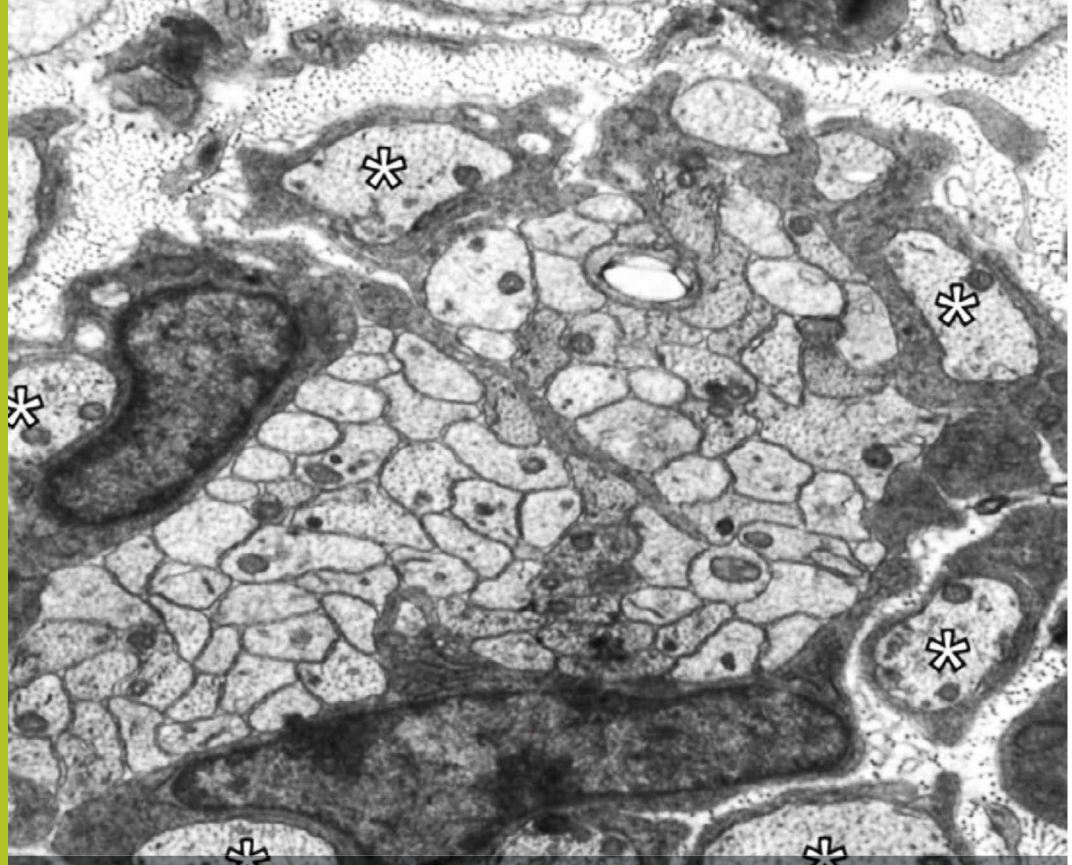
Neuroscience

The main objectives of this Unit are to consolidate ongoing studies in the areas of Neurodegenerative Diseases, neuronal repair and regeneration, neuronal protection and development, and pain. The interdisciplinary nature of the research takes advantage of the knowledge and expertise from different research groups. The areas explore human genetic studies in dominant ataxias like MJD, FAP and other lysosomal diseases, including their genetic and epigenetic associations, the development of in vitro and in vivo models for these diseases, structural and cell biology studies, development of animal models, clinical studies and the transition of this research into therapeutics. Work in the Unit also aims to search for neuroprotective molecules in a variety of neurodegenerative disease models and in particular to identify molecular strategies that promote regeneration and repair. The Unit also includes groups that work in the area of chronic pain using primarily model organisms, as well as direct clinical research. The Unit has made a strong effort to promote translational research for, the Center for Predictive and Preventive Genetics, which provides Genetic counseling for a large number of individuals for a variety of human conditions.



Inhibition of ROCK signaling facilitates axonal sorting in *ILK* mutant mice (Pereira et al., 2009).
In the figure asterisks mark axons that have been recently sorted from the axon bundle by Schwann cells.

Glial Cell Biology

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Previous research results

The work of our laboratory focuses mainly on molecular mechanisms regulating myelination and remyelination of the nervous system. Oligodendrocytes in the central nervous system (CNS) and Schwann cells in the peripheral nervous system (PNS) produce myelin, a lipid-rich biological membrane, which forms multilamellar, spirally wrapped sheets around axons. Myelination allows rapid saltatory conduction of action potentials, and contributes to the maintenance of axonal integrity. The devastating neurological effects caused by demyelinating diseases in both CNS and PNS illustrate the importance of the process. Despite being intrinsically different, both glial cell types proliferate and migrate over long distances before undergoing the remarkable morphological changes associated with the ensheathing and myelination of axons. Precise control of these processes derives, at least in part, from instructive cues originating within the extracellular environment, in which proteins of the extracellular matrix (ECM) are essential components. Cell-ECM contact is largely mediated by integrins, a major group of ubiquitous cell-adhesion receptors for the proteins of the ECM. Over the past five years, using conditional transgenic approaches in mice together with appropriate *in vitro* cell culture systems, we have investigated the role of integrin and integrin-associated molecules in the regulation of myelination. Our results showed essential roles for integrin β1, integrin-linked-kinase, particularly interesting Cys-His-rich protein (PINCH) and for the RhoGTPases cdc42 and rac1 in the regulation of different stages of Schwann cell and oligodendrocyte development and myelination. More recently, we have also shown roles for some of these molecules in nervous system remyelination and neural progenitor differentiation.

Future research goals

In the future we will continue focussing mainly on fundamental mechanisms of myelination and remyelination. We aim to: 1. Characterize further the functions of integrin-associated proteins and of the Rho-family of small guanosine triphosphatases (Rho GTPases) in the regulation of CNS and PNS developmental myelination and remyelination. To do that we will use a complementary set of experimental approaches including conditional tissue-specific gene ablation in mice, *in vitro* myelination systems, genomic and proteomic methods, siRNA technology, as well as optical and electron microscopy. 2. Identify novel regulatory molecules and pathways in the context of myelination and remyelination of the nervous system using state-of-the-art proteomic approaches followed by siRNA-based functional assays. We will also use proteomics to identify interacting partners for some of the proteins we have previously described to be involved in the regulation of myelination and remyelination, such as those belonging to the atypical RhoGTPases sub-family. 3. Expand our research in regenerative neuroscience. We will investigate whether regulation of RhoGTPase signaling can promote neuro-protection after injury or disease of the nervous system.

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Scientifically valid and ethically responsible animal research starts with breeding and rearing the animals. This photo of a litter of young mouse pups comes from one of our ongoing research projects into maternal behaviour and pup survival in laboratory mouse breeding. Photo: Robert Eriksson

Laboratory Animal Science

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Previous research results

We work towards improving animal-based research in a way that addresses both scientific validity and animal welfare. In studies of behaviour and welfare, we study how animals behave in different housing environments, and how providing resources animals value affects parameters measured in research in neurosciences and immunology. Our results indicate that – while improving animal welfare - furnishing mouse cages with nesting material and shelters does not compromise research results. We also use different experimental and epidemiological methods to understand the problems with early pup mortality in laboratory mouse breeding. Research into anaesthesia investigates how different concentrations affect learning, memory and brain morphophysiology in rats and mice and also develops refined anaesthesia protocols. In ethics, we ask how the harm-benefit balance of research can be improved. Analyses of different types of research have identified critical points in which animal welfare can be improved, and more recently we have also started to address the question of how to optimize benefit. We have also analyzed biotechnology applications from an animal-ethics perspective.

Future research goals

The overall goal remains to develop animal-based research in a way that takes both research quality and animal welfare into account.
During the coming years, focus will be on an integration of our studiesof behaviour and welfare with the biomedical research where the animals are used. Similarly, we will take an integrative approach to the ethical harm-benefit analysis of animal-based research, further developing methods for critical evaluation of harm reduction and benefit optimization. In anaesthesia, the work on assessing different protocols and their effect on different levels of the nervous system continue. To achieve these goals, we are expanding our interdisciplinary and international collaborations, which now include sociologists and engineers as well as philosophers and life scientists. We welcome collaboration with industry.

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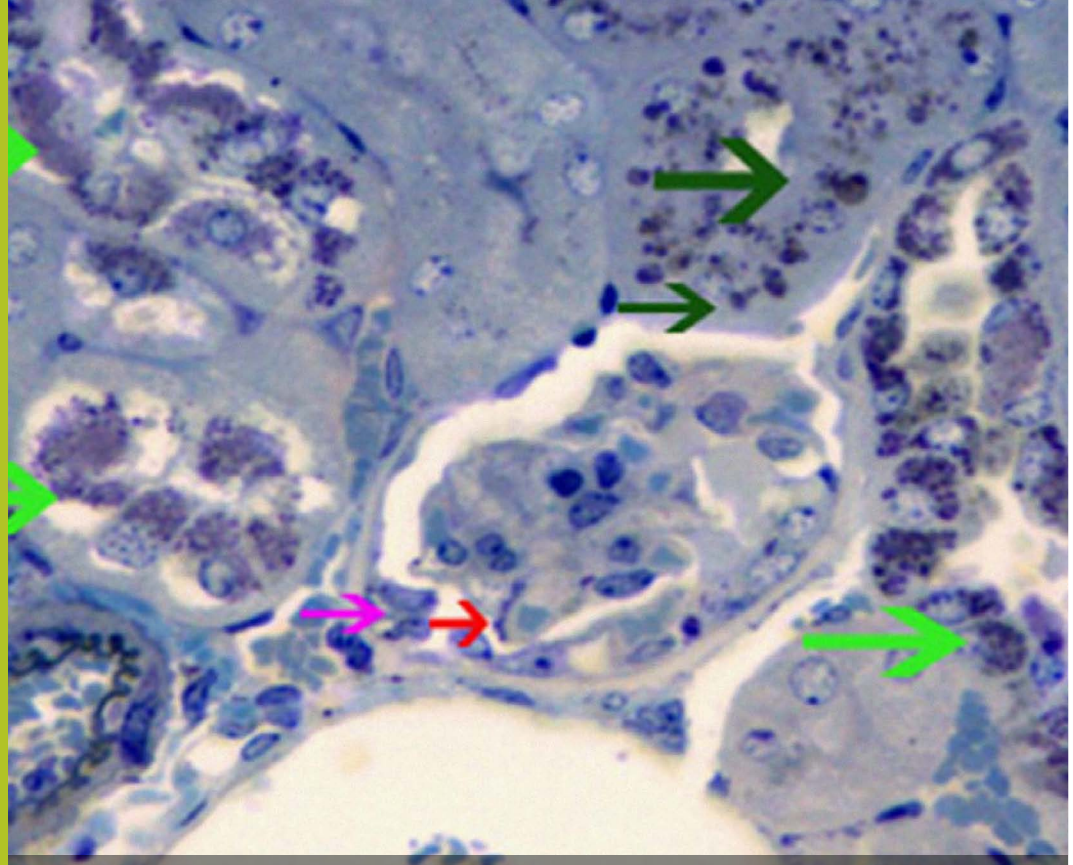

Glycosphingolipid deposits in the kidney of the Fabry mice.

Lysosome & Peroxisome Biology

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Previous research results

UniLiPe works in the field of lysosomes and lysosomal storage diseases (LSDs). During these years our work contributed to the knowledge of the genetic epidemiology of LSDs and the pathogenic mechanisms of Gaucher disease (GD), Fabry disease (FD) and a new LSD due to the deficiency in LIMP-2. The birth prevalence of 27 different LSDs in the Portuguese population was determined. Overall, LSDs have a prevalence of 1:4.000 live births, a value significantly higher than the ones previously reported (Pinto R. et al 2004). The impact of sphingolipids storage in the immune and neurological systems function was investigated. GD patients presented an upregulation of CD1d and MHC-class II and imbalances in T and iNKT cell subsets (CD4+, CD8+, and Vα24+) T cells (Balreira A et al., 2005). FD "knockout mice" showed alterations in T and in iNKT cells, however these anomalies were not observed in FD patients (Balreira A et al., 2008). We demonstrated for the first time that Fabry mice have Gb3 accumulation in the peripheral nervous system, alterations in sensorimotor function, hypoalgesia and no impairment of motor nerve conduction (Rodrigues L et al., 2009). Recently, we described a deficiency in LIMP-2, in two sisters with Action Myoclonus-Renal Failure syndrome. Accordingly with our findings, in the absence of LIMP-2, the sorting receptor for β-glucocerebrosidase, a cell type specific glucocerebrosidase deficiency is observed (Balreira A et al., 2008).

Future research goals

In UniLiPe, our main goal is to study the biology of lysosome and LSD, particularly, sphingolipidosis. Our main objective is to investigate the impact of lysosomes dysfunction in cell, tissue and organism homeostasis, exploring the effect of abnormal sphingolipids metabolism in the development of symptoms common to sphingolipidosis and to complex disorders such as obesity, hypertension, stroke and left ventricular cardiac hypertrophy. Our hypothesis is that the storage of sphingolipds in the endolysosomal system interferes with the recycling mechanisms altering the lipids distribution and the lipid rafts membranes composition, what may result in abnormal function of cells, namely of the nervous and immune systems. In order to investigate this, the lipid profile of plasma and tissues from Gaucher disease (GD) and Fabry disease (FD) patients and animal models will be determined; the impact of the metabolic abnormalities, in the immune and the nervous system of GD and FD, will be evaluated. The role of LIMP-2 in the biogenesis of lysosomes and the mechanisms of sorting of glucocerebrosidase in different types of cells will be also investigated.

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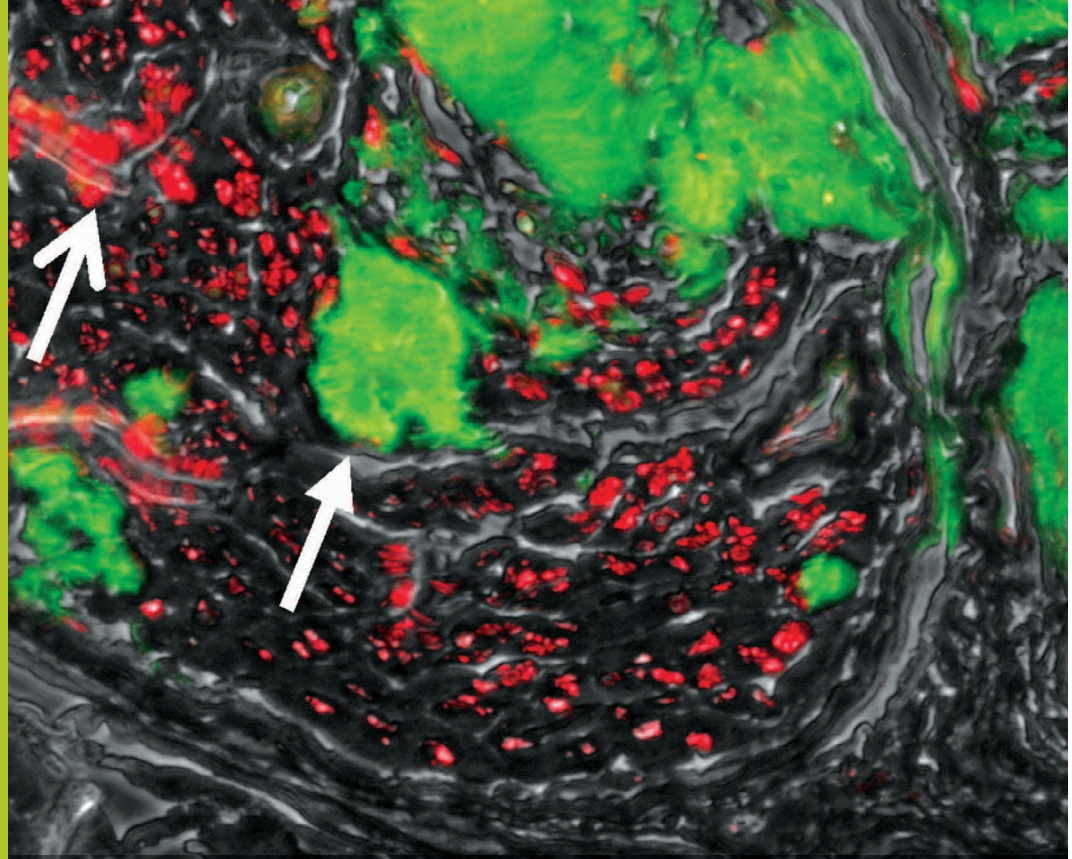

TTR amyloid plaque in the nerve of a FAP patient (green) and up-reagulation of head shock protein HSP 27 (red)

Molecular Neurobiology

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Previous research results

Studies transthyretin (TTR)-related *degenerative diseases* and the role of amyloidogenesis inhibitory and enhancing factors in disease development. In particular, we focus on familial amyloidotic polyneuropathy (FAP) an autosomal dominant disease related to TTR mutations, prevalent in Portugal. Other lines of research devote to signalling pathways involved in brain injury, particularly those related to Alzheimer disease, brain ischemia, and oxidative conditions using unique animal models and seek neuroprotective strategies to rescue phenotypes related to these conditions. Specific objectives: - To identify unknown ligands of TTR important in the biology of the protein in health and disease by determination of TTR- protein ligand interactions both "in vitro" and "in vivo" using a TTR-KO mouse model.

- To understand why TTR has propensity to aggregate as amyloid fibrils through studies of intermediate forms in TTR amyloidogenesis and effect of inhibitory and enhancing factors.

- To generate newer improved models for FAP for investigation on signalling cascades triggered by TTR aggregates, for biochemical markers of the disease, for elucidation of neurodegeneration, and to test newer drugs/protocols to inhibit cytotoxicity in tissues related to amyloid deposition.

- To understand neuroprotection of TTR in Alzheimer disease and in ischemia; to understand neuroprotection in models of oxidative stress.

Future research goals

We will foccus on Neurodegeneration and Neuroprotection: We will dissect molecular pathways triggered by TTR aggregates and forms to ameliorate cell death; these studies encompass indepth knowledge of unknown functions of TTR in the nervous system; will seek for the molecular basis of neuroprotective properties of TTR in the nervous system; we will follow on with the characterization of acyl-L-carnitine neuroprotective properties.

A large effort of the Group is on translational medicine on unique pre-clinical models for FAP, and participation on clinical trials on FAP patients. For that purpose we collaborate with international up-front companies in the development of new therapeutic and prophylatic drugs in the treatment of protein misfolding disorders.

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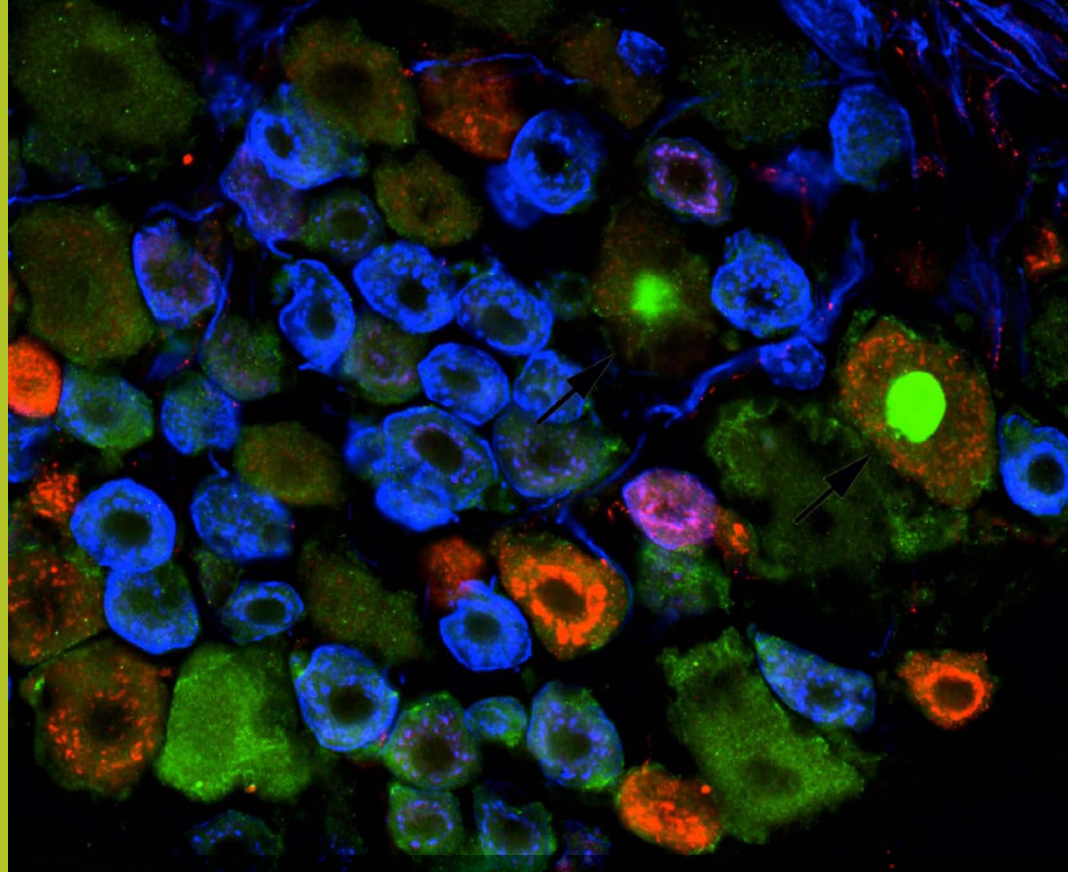

ATF-3 is mainly expressed in small and medium peptidergic nociceptors. Immunolabelling for the neuronal injury marker ATF-3 (green), CGRP (red) and IB4 (blue) in a dorsal root ganglion innervating the inflamed ankle joint of a monoarthritic rat (Nascimento et al., 2011)

Morphophysiology of the Somatosensory System

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Previous research results

The work of our research group aims at elucidating the mechanisms that underlie the physiological processing of pain at the molecular, cellular and network levels, and at characterizing the changes that take place during the establishment of chronic Pain. Pain is a phylogenetically old organism protective function that involves the recognition of internal and external threats and the organization of adequate protective and reacting responses. The nervous system is a major player in the entire process by detecting damaging or putatively damaging conditions, conveying the information to perception and response centers and modulating input flow according to a multitude of past and present events. In various pathological situations implying particularly intense or sustained noxious stimulation or lesion of the nervous system, pain becomes a threat in itself by determining structural and physiological changes in the pain circuitry, as well as in various cognitive and affective circuitries intimately implicated in pain processing. In spite of all the pain killers available, there are various types of pain that still escape pain treatment, chronic pain being a major public health problem with enormous impact in the world economy. By the use of multiple approaches, from molecular biology and network design to *in vivo* multi-electrode electrophysiology coupled to behavior analyses, our group has made major contributions to our present knowledge of pain. These include the characterization of the spinal system conveying nociceptive information to brain centers, the uncovering of facilitatory supraspinal pain modulation, the neurochemical spinal and supraspinal changes occurring upon the establishment of chronic pain and the cognitive impairment resulting from chronic pain. In addition, epidemiological studies have revealed the high impact of chronic pain in Portugal.

Future research goals

The group will pursue his main objective of further understand the physiopathology of pain by investigating along four different lines:

1. We will continue the study of the molecular players in the embryonic development of the nociceptive system as a way of identifying molecular markers of its various components. This should enable us to investigate the differential role of each part in pain processing and identify molecules of putative therapeutic interest directed specifically to each one. This study will focuse on the primary-afferents/ second order circuit, where past studies by the group revealed the transcription factor Prrxl1 to relate specifically to the development of the excitatory component and to be over expressed during inflammatory chronic pain.
2. Molecular changes occurring during chronic pain will continue to be studied at the peripheral, spinal and supraspinal level in the monoarthritis, osteoarthritis, spared nerve injury and diabetic neuropathy models. Gene manipulation of multiple neuronal systems together with microdyalisis will be used to better reveal the plasticity of neurotransmission in such chronic pain conditions.
3. Chronic preparations using multielectrode electrophysiological recording coupled to behavior recording will be used to study chronic pain-induced cognitive abnormalities related to misfunctioning of prefrontal and striatal regions.
4. Epidemiological studies will continue in order to establish the prevalence of acute and chronic postoperative pain.

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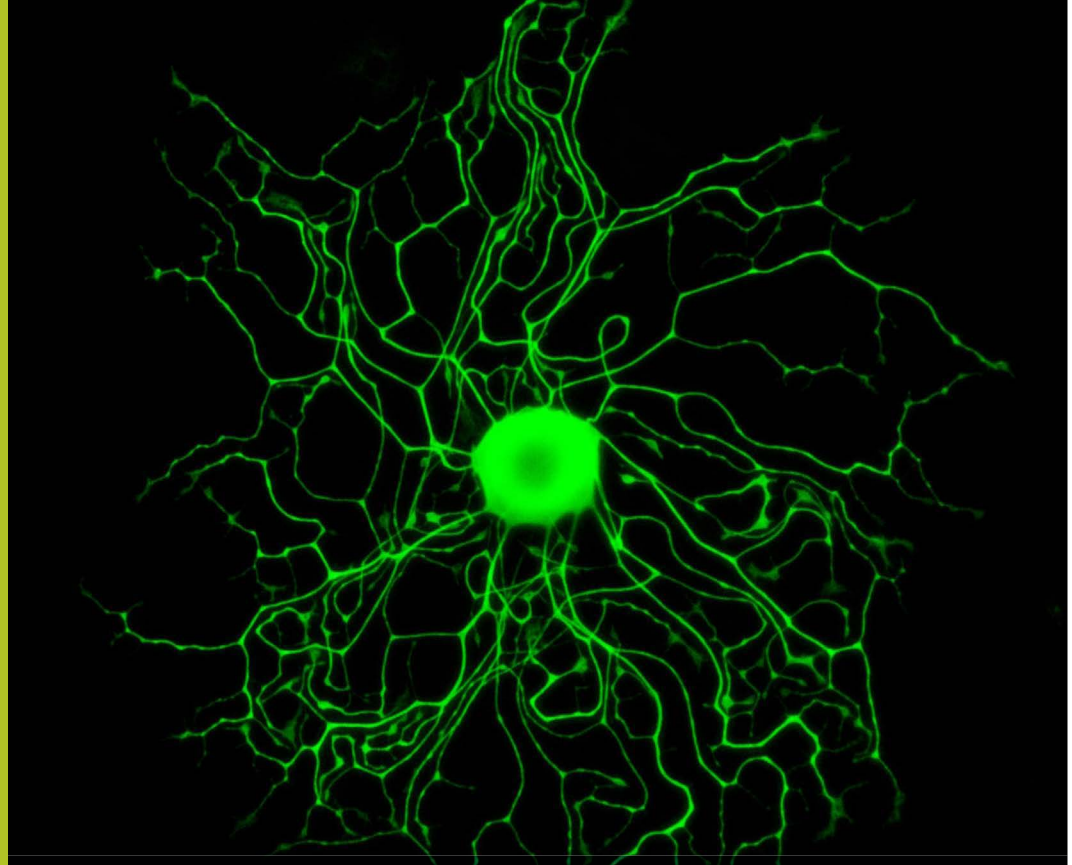

Neurite outgrowth in dorsal root ganglia neuron cultures (β-tubulinIII labeling).

Nerve Regeneration

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Previous research results

The inability of axons to regenerate in the mature central nervous system (CNS) is a major obstacle in the treatment of neurological disorders and CNS injury. A key principle guiding research in axon regeneration is that extrinsic cues in the environment of neurons, as well as cell-intrinsic mechanisms, contribute to the limited capacity of neurons to regenerate. While progress has been made in characterizing the extrinsic cues that inhibit axon growth, the cell-intrinsic mechanisms that govern axon regeneration remain poorly understood.

Through the use of in vivo models of injury, in vitro neurite outgrowth assays, as well as proteomics and cell biology approaches, our group has identified modulators of nerve regeneration:

- In the peripheral nervous system (PNS), we identified transthyretin (TTR) as a nerve regeneration enhancer.
- In the CNS, we identified candidates that enable axonal regeneration following a lesion to the PNS (conditioning lesion).
- We determined that plasmalogens, a membrane phospholipid, affect neurite outgrowth and neuronal membrane fluidity.

The ongoing characterization of basic mechanisms enabling axonal regeneration will support future therapeutic applications aiming to promote axonal growth.

Future research goals

We are interested in understanding cell-intrinsic pathways enhancing axonal growth. For that, we are dissecting the following mechanisms:

- Axonal transport of injury signals and regeneration enhancers: The activation of a regeneration program comprises the transport of injury signals from site of lesion to the cell body. These signals will then induce the expression of regeneration enhancers. We are characterizing injury signals and putative novel axonal regeneration enhancers identified by our group. Moreover, the relevance of unknown genes highly expressed by sensory and motor neurons is being studied.
- Cytoskeleton remodeling: Cytoskeleton remodelling is crucial to support axon extension. In this context, we identified GSK3beta and adducin, which are involved in microtubule and actin dynamics respectively, as differentially regulated in conditions where increased axonal regeneration is observed. Now, we will evaluate the potential of modulating GSK3beta and adducin in vivo, using the spinal cord injury model, to promote CNS regeneration.
- Membrane fluidity: Besides cytoskeleton remodeling, membrane refashioning is essential for growth. Given the involvement of plasmalogens in membrane fluidity and neurite outgrowth in vitro, we will evaluate in vivo their role in neurons and in myelin-forming glia, physiologically and during degeneration/regeneration.

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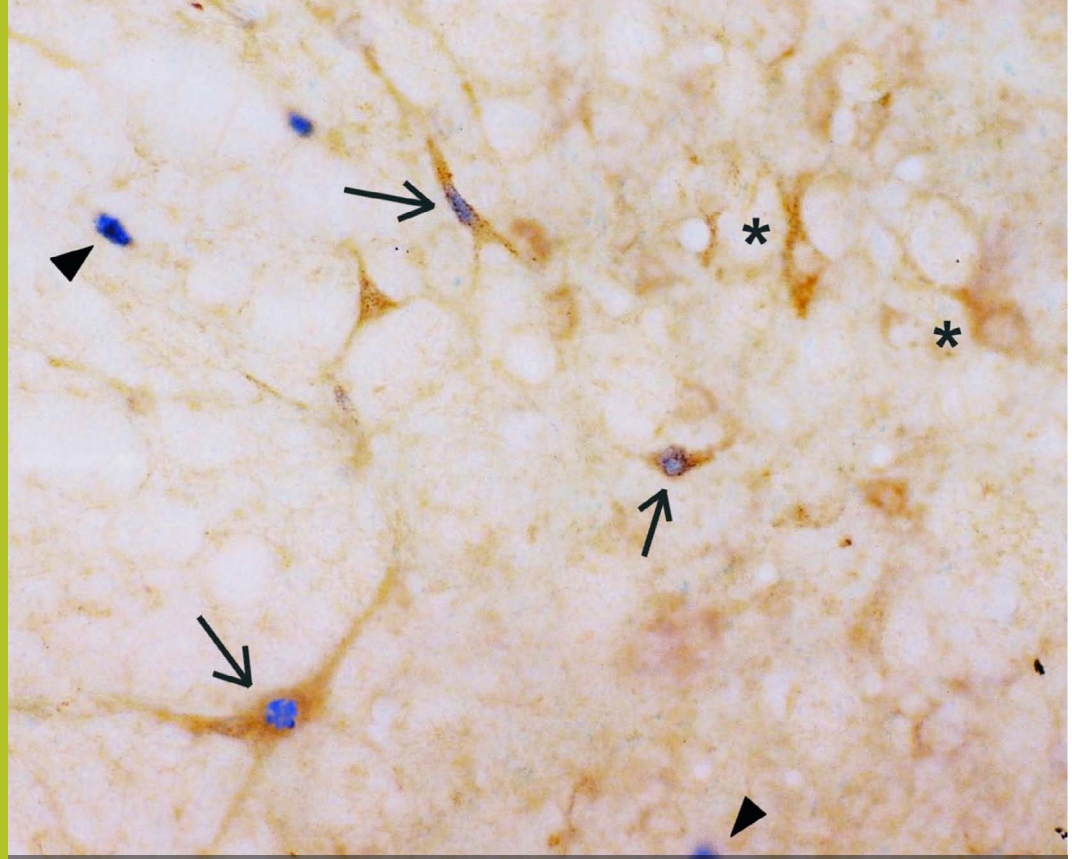

Dorsal horn spinal sections immunoreacted for the Fos protein and GABAB receptors in noxiously stimulated rats. Fos: bluish nuclei (arrowheads), GABAB receptors: brown perikarya and dendrites (asterisks) and double-labeled neurones (arrows).

Neuropharmacology

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Previous research results

Our group has long been studying the pathophysiology of arterial hypertension and its regulation by catecholamine, the rennin-angiotensin system (RAS) and reactive oxygen species (ROS). Hypertension has been associated with reduced sensitivity to acute pain both in animal models and in humans. This hypertension-induced hypoalgesia appears to be due to inhibition of nociceptive transmission. Our studies addressing the mechanisms of the physiological and pathological interaction between cardiovascular and pain regulatory systems showed that there is a decrease of nociceptive activation of spinal cord neurones, due to changes of GABAergic inhibitory system. In chronic pain there are also decreased responses to pain in Angiotensin II hypertensive animals.

The NTS and VLMlat are involved in cardiovascular and pain control. CVLM neurons expressing AT1 receptors, and involved in the CVLM-A5 pathway are mainly non-catecholaminergic. The decreased expression of NMDA receptors in the NTS elicits hypoalgesia and hypertension. The administration of NMDA reverses the nociceptive and cardiovascular effects.

NOX activation is involved in the pathophysiology of SNI peripheral neuropathy, although different pain sub modalities (mechanical and thermal) were differentially affected, suggesting differential modulation of C and Aδ fibers.

Selective alpha2C-adrenoceptor blockade produces an increase in brain tissue levels of dopamine; by enhancing the transport activity of the catecholamine precursor L-DOPA.

Future research goals

Our future work intends to further enlightening the dispute about the existence of separate neuronal populations for pain modulation and cardiovascular control and the role and mechanisms of angiotensin II involvement.

Evaluate the interaction between the hypertensive and chronic pain pathological states, involving not only inflammatory but also neuropathic chronic pain. Evaluate the oxidative status in spinal and supraspinal pain processing areas.

Characterize an alternative therapeutic target for the prevention/treatment of diabetic nephropathy, eventually associated with hypertension.

Unravel the mechanisms contributing to the cardiac and renal protection exerted by aspirin and RAS blockers.

Provide novel insights to the physiology of brain alpha2C-adrenoceptors but also evaluate these receptors as possible targets in diseases associated to dopamine imbalance such as Parkinson's disease.

Characterize the role of adrenaline on the maturation of the different β-adrenoceptor subtypes.

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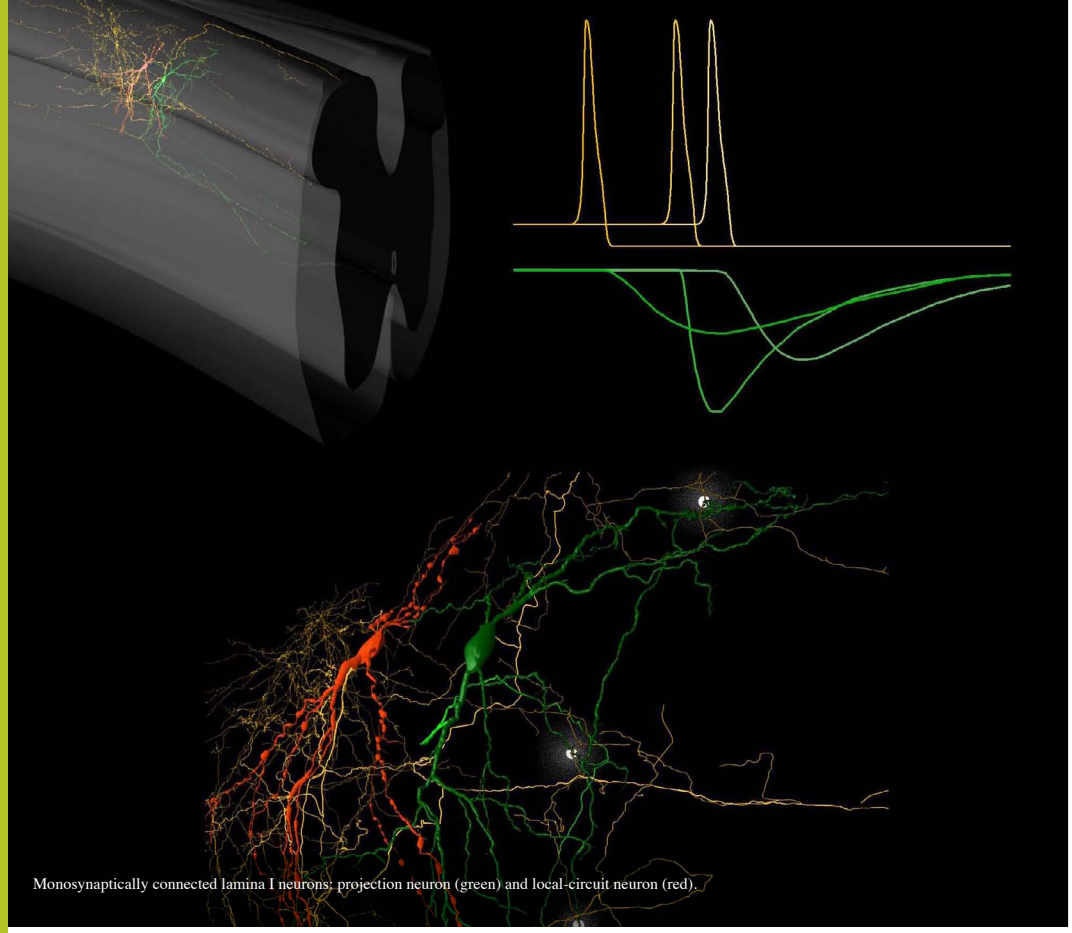

Monosynaptically connected lamina I neurons: projection neuron (green) and local-circuit neuron (red).

Spinal Neuronal Networks

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Previous research results

We are studying the basic principles of organization and physiological functioning of spinal nociceptive processing circuitries. A combination of imaging, labeling and recording techniques is used to describe the anatomical structure of spinal nociceptive neurons, their interconnectivity, efficacy of transmission in sensory synapses and the mechanisms of cell-specific firing pattern generation. We have recently described several new types of the local axon collaterals issued by the lamina I projection neurons, implying strong involvement of projection neurons in intra- and intersegmental spinal integration. We have also found that sensory processing in the superficial dorsal horn is dominated by excitatory interneurons. The synapses formed by the excitatory interneurons activate Ca^{2+}-permeable AMPA receptors, show several forms of functional plasticity, and a release from synapses of one neuron can be sufficient to excite another neuron. Our former studies of the spatial distribution of Na^{+} and K^{+} channels in dorsal horn neurons elucidated the roles of the soma, axon initial segment and dendrites in spike generation. We have also shown that Aδ- and C-afferents from several segmental dorsal roots converge monosynaptically on individual neurons in laminae I and II, what can form the basis for the somatovisceral integration underlying phenomenon of referred pain.

Future research goals

Our future research will deal with the neurons in the most superficial dorsal horn layer, lamina I, which is a key element of the spinal nociceptive processing system. Based on technological progress achieved in our laboratory during the last five years, we shall study several unknown properties of the neuronal circuitry organization in lamina I: functional connectivity between different morphological types of lamina I neurons, the monosynaptic transmission between lamina I neurons, as well as the activity-dependent plasticity and types of transmitter-activated receptors involved. We shall study the axonal architecture of lamina I local-circuit neurons and monosynaptic convergence of somatic and visceral primary afferent fibers on individual lamina I neurons. We are also planning to study the descending input to lamina I neurons from the brainstem and other supraspinal areas and to create realistic computer models of major classes of spinal lamina I neurons based on detailed 3-D reconstructions of completely filled cells.

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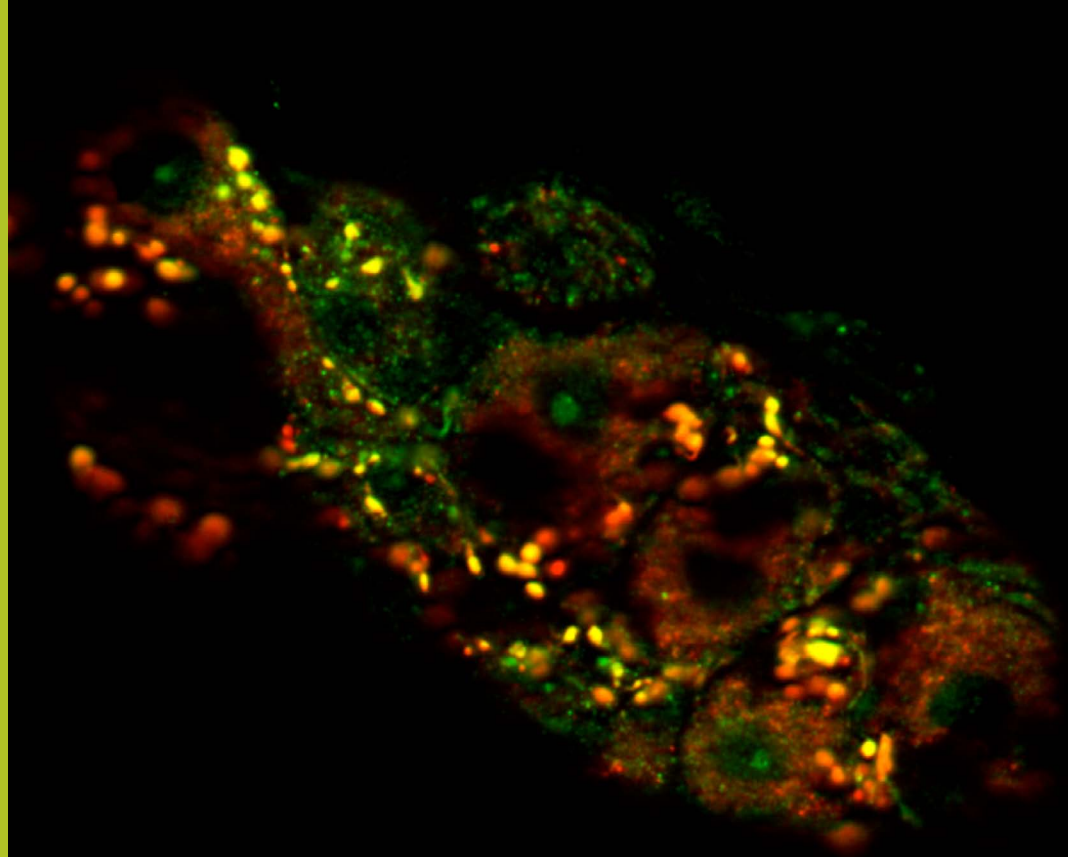
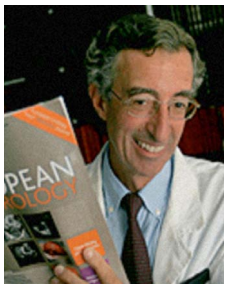


Co-expression of cleaved SNAP-25 (red) with VAChT (green) in a pasympathetic ganglion of the guinea pig bladder following Botulinum toxin injection. The end product of the neurotoxin is localized in cholinergic terminals (yellow). Coelho A, Cruz CD, Cruz F, Avelino A. Journal of Urology, in press

Translational Neuro-Urology



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Previous research results

Our group aims at uncovering the fine mechanism of micturition control in health and in several disease states and at offering better and safer treatments to patients with lower urinary tracts symptoms, like urinary incontinence or bladder pain.

At an experimental level we make use of animal models that mimic diseases as spinal cord lesions, overactive bladder or bladder obstruction due to prostate enlargement. We have studied the importance of neurotrophins and TRP receptors and its endogenous agonists in these processes and in terms of therapeutic possibilities we are making progresses in finding effective TRPV1 and TRPV4 antagonists and clarifying how Botulinum toxin works once injected in the bladder or in the prostate gland.

At a clinical level we were involved in a large multicentre clinical trial that demonstrated for the first time the efficacy and safety of Botulinum Toxin type A in the treatment of urinary incontinence due to neurological disorders and defined the ideal dose of this toxin. We also contributed decisively to introduce this toxin in the treatment of chronic bladder pain states. We made substantial progresses in the definition of urinary biomarkers in overactive bladder. We are currently investigating the possibilities of the pharmacological manipulation of the bladder adrenoreceptors for the treatment of this syndrome. Finally we investigated new surgical options for treatment of urinary incontinence in women.

Future research goals

The future research of the translational neuro-urology group will be directed into two main new areas. One is the prevention of bladder overactivity and the other is the identification of urine and blood biomarkers to aid the diagnosis and treatment of bladder overactivity.

Prevention of bladder overactivity will start to be investigated in animals model of spinal cord injury, which most closely reproduce the equivalent human condition (spinal cord lesion). Fast administration of TRP antagonist and sequestration of neurotrophins appear as ideal pharmacological intervention to prevent the appearance of bladder overactivity. In addition we hope to investigate the effect of electrosacral neuromodulation in this process, in collaboration with the Tubingen Group of Neurourology (Germeny). As collateral to this research we expect to gain relevant information about the role of neurotrophins and TRP receptors in micturition control.

Biomarkers are measurable characteristics that reflect physiological, pharmacological, or disease processes. Bladder overactivity lacks objective diagnostic tests. Therefore, identification of objective parameters will be extremely relevant to aid diagnosis, in addition to clinical examination, and monitor treatment. Although our recent studies indicate that neurotrophins are the most obvious candidates, we intent to perform proteomic analysis of urine and blood of these patients in order to detect additional biomarkes candidates.

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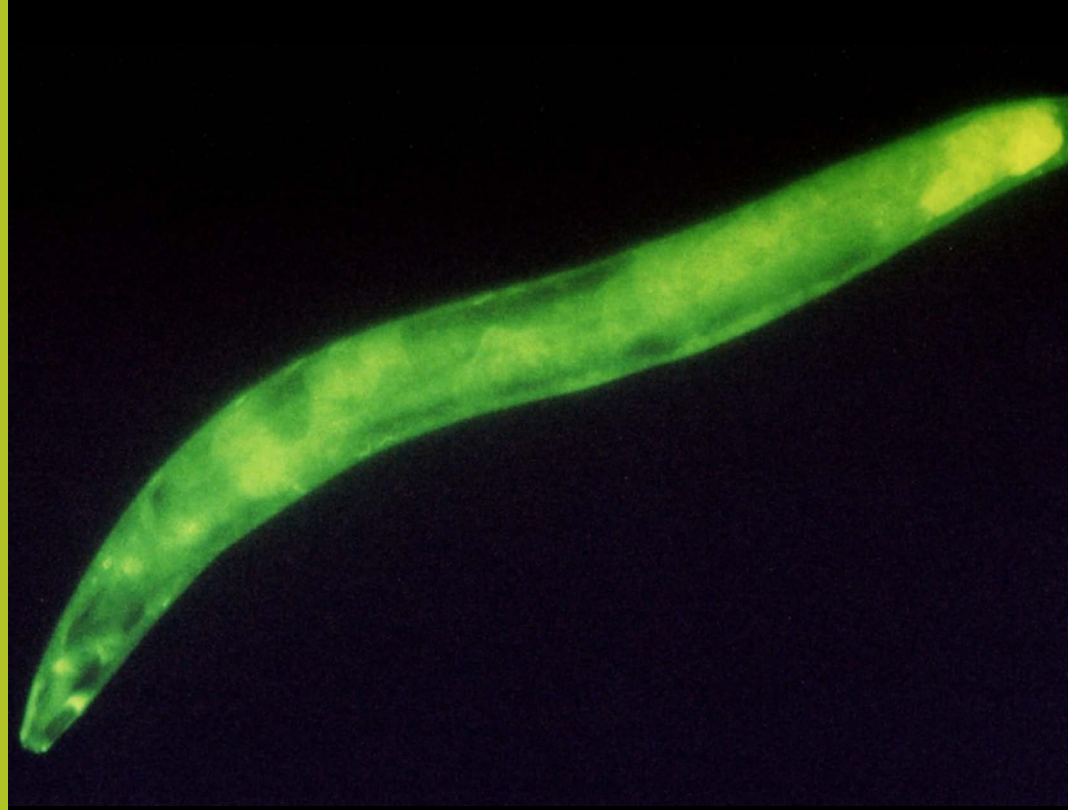

"This image shows a *C. elegans* worm, an excellent model to study neurodegenerative diseases. Our interests start with the clinical characterization of patients and families, using genetic epidemiological tools and the identification of mutations and mechanisms responsible for disease, and continue up to the functional characterization of normal and mutant proteins, but also population studies and evolution. Our group has large experience with the use of human patients, and animal and cell-based models."

UnIGENe

Unit for Genetic and Epidemiological Research in Neurological Disorders

Jorge Sequeiros

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Previous research results

Our group focuses on genetics of neurological diseases, mainly the spinocerebellar ataxias (SCAs), Huntington disease and other movement disorders.

We have been involved in the identification and characterization of genes and mutations for dominant (SCA) and recessive hereditary ataxias (AOA), Huntington disease (HD) and HD-like disorders and hereditary spastic paraplegia (HSP/SPG).

We have also been interested in complex disorders, particularly in the study of migraine (familial and association studies), to assess familial aggregation and ascertain susceptibility genes. We have also studied paediatric stroke, multiple sclerosis and hypodontia.

Our animal-based research (mice and C. elegans) has been centred on Friedreich ataxia, Machado-Joseph disease (MJD/SCA3) and SCA6, to evaluate disease mechanisms as well as the function of proteins involved. Cell models have been used to characterize ataxin-3 interactors and assess the role of protein processing and protein degradation systems in neurodegenerative disorders.

We have been also involved in the identification of disease modifiers and epigenetic modification in HD, MJD and familial amyloid polyneuropathy (FAP) ATTRV30M.

Another interest has been the study of founder effects, ancestral haplotypes and mutational origins mainly in MJD, but also SCA2 and SCA10.

Our unit is served by a large multidisciplinary team, including clinical neurology and epidemiological studies, psychosocial genetics and ethics; population genetics, genetic epidemiology and historical genetics; gene mapping and mutational analysis, functional genomics and animal models.

Future research goals

Identification of new genes and mutations in neurodegenerative disorders are still one of our major goals, as these can be translated into regular clinical practice and have a direct impact in patient management and genetic counselling in their families.

We will continue the search for cell pathways and pathogenic mechanisms in neurodegeneration, through the study of cell and animal models. We are particularly interested on the impact of mutations in protein folding and aggregation, as well as on toxic intermediate species and the role of protein degradation systems in aggregate formation and clearance.

We will further invest on the search for disease modifiers (genetic, epigenetic and environmental), through human, cell and animal-based approaches that might help clarifying the disease mechanisms contributing to Huntington, SCAs, FAP ATTRV30M or Parkinson disease.

Patient studies and genetic epidemiological tools will be used also for identification of modifiers. In addition, understanding the genetic contribution to the aetiology of complex diseases will remain one of our aims.

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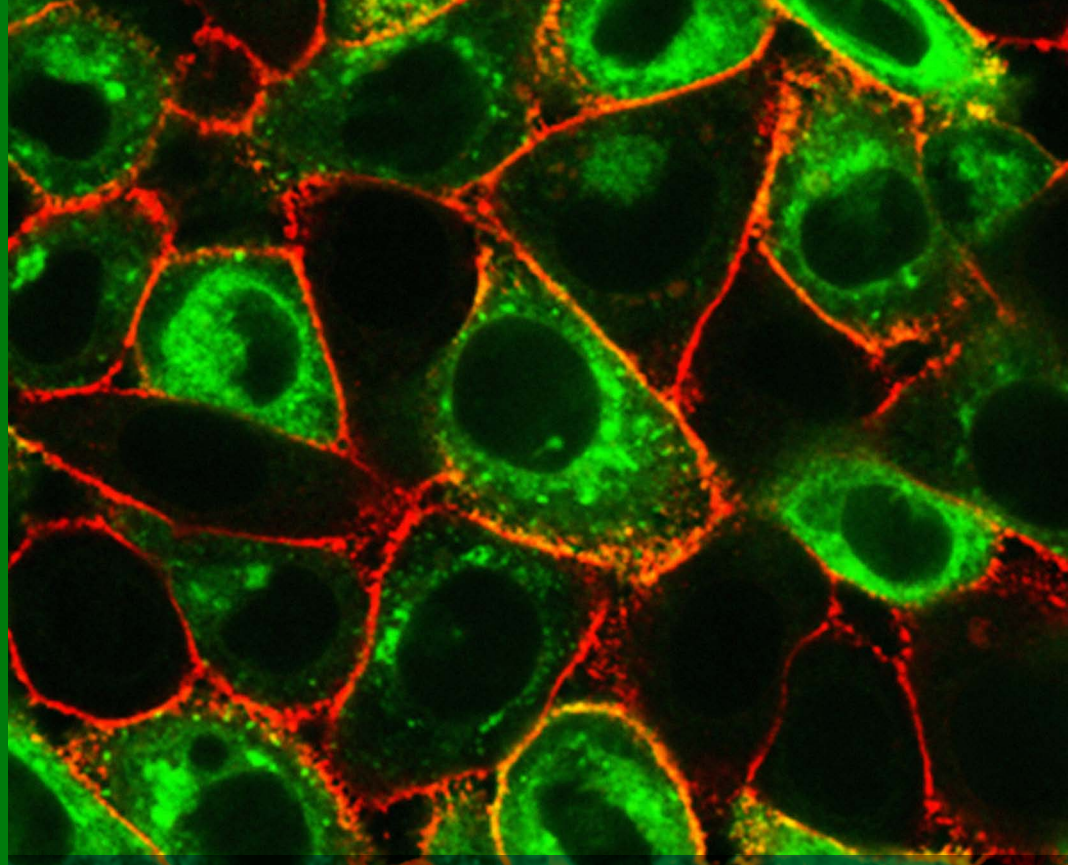
Associated Groups



c-myc tagged melanocortin 5 receptor (green) and Con-A (red)in HeLa cells. Microsc. Microanal., 2008

Ageing and Stress

Henrique de Almeida

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Current Associate Editor of Microscopy and Microanalysis
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Previous research results

The interest on ageing & stress has led us to focus on patterns of expression in human cells and tissues.

Stress-induced cell senescence mimicks replicative senescence of cells and is known to result from the action of a very limited number of physical or chemical agents. Recently, we added copper as one new such agent, and are now searching specific mechanisms involved, which may underlie copper related human disorders. Reproductive ageing presents a very peculiar aspect of human ageing. In fact, whereas middle aged males evidence age-related deterioration of the penile structure and vascular remodelling (as reduced smooth muscle cells and enhanced lipid cells deposition in the corpus cavernosum and VEGF receptor variation), the reduced antioxidant protection offered to oocytes by the surrounding cumulus cells is already noticed in younger females and associates to reduced number of successful pregnancies. Ageing thus imparts an oxidative stress upon cells which may be due to enhanced protein carbonylation, a point under investigation. Specific conditions impinging on stress receptors, as melanocortin-5 receptor, start a transductive process which activates cAMP/PKA and MAPK pathways. An unexpected lypolitic effect recently observed, became a point of interest.

Future research goals

While exploring mechanisms that make copper induce cell senescence, we are now extending the range of oxidative stress regulation of human oocytes along reproductive ageing, through a major grant from an international pharmaceutical company. In addition, we are including the study to oocyte derived growth factors which work as cumulus oophorus cells modulators. To explore further the atherosclerosis-prone high fat diet effect upon the rat corpus cavernosum, we are testing the use of antioxidants and their putative targets or modulators. To examine the role of carbonylated proteins as intermediates in the modulation of metal induced oxidative stress on human fibroblasts and enlarge the prospects in ageing cell research, we are using now a human T cell line. The lypolitic effect under stressful conditions that was found is now being explored, employing a human adipose cell line (SGBS).

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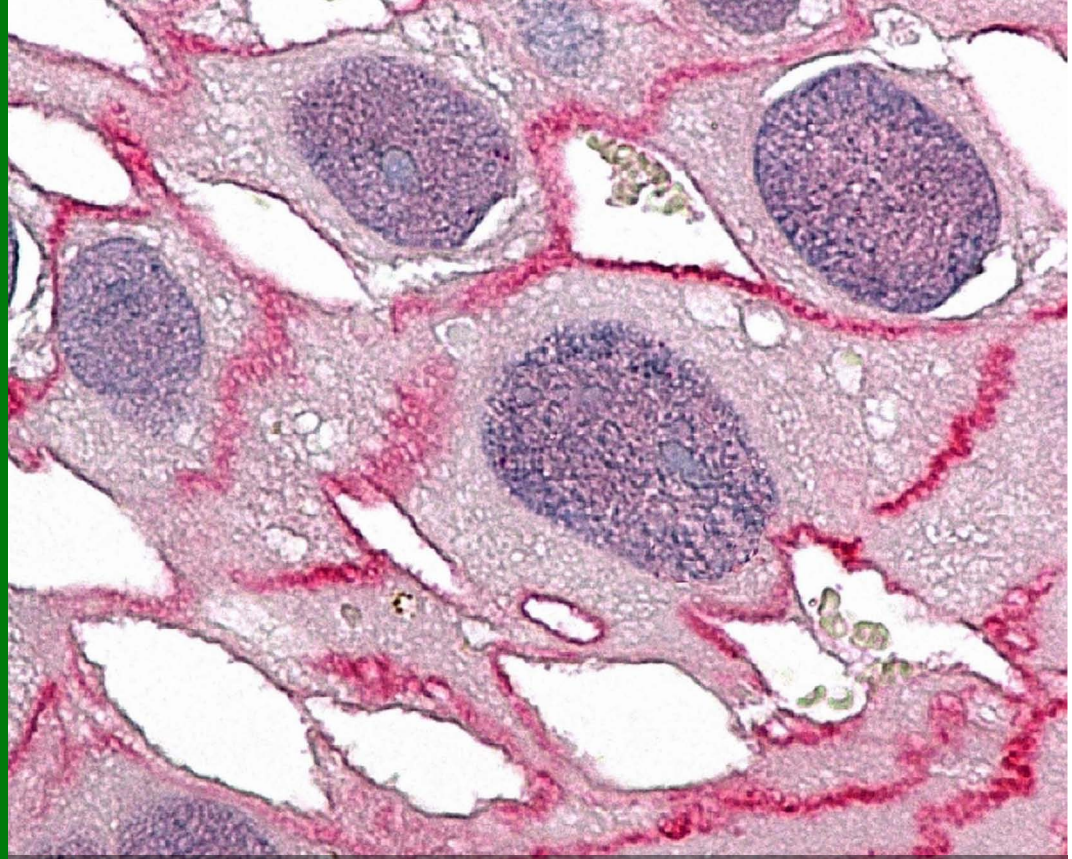

Immunoreactive N-acylphosphatidylethanolamine-hydrolyzing Phospholipase D (NAPE-PLD), which generates the main endocannabinoid - anandamide, in giant trophoblast cells on day 14 of pregnancy

Biology of Inflammation and Reproduction

Natércia Teixeira

Is the group leader, since 1996, and is also a professor of Cell Biology at the Faculty of Pharmacy. She obtained her degree in Pharmacy at the University of Porto and has a PhD in Biochemistry from the University of Strathclyde (Glasgow).
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Previous research results

The principal aim of our research has been the understanding of the cellular and molecular mechanisms underlying different inflammatory conditions, associated with physiological and pathological situations that can trigger serious or fatal events. Our group has been focusing on three main areas: i) study of several blood markers, to estimate in a quantitative way risk factors associated with serious or fatal events occurring alongside with inflammatory conditions; ii) understanding of the mechanisms that underpin the dynamic uterine-embryo interactions during successful and complicated pregnancy, as well as the role of endocannabinoids in fetoplacental development; iii) biological evaluation of new compounds and drugs for the treatment of hormone-dependent tumors and inflammatory diseases.

Future research goals

We plan to further study the biology of inflammatory diseases (psoriasis, chronic renal disease, obesity) and the role of inflammation in i) worsening of psoriasis; ii) erythropoietin resistance and the associated cardiovascular risk, in patients with chronic kidney disease, and in animal models with induced renal failure; iii) cardiovascular risk in obese children and adolescents. We studied about 100 Portuguese families with Hereditary Spherocytosis; we intend to further study the biology of HS and to develop flow cytometry assays to diagnosis of RBC membrane diseases. In addition, the pharmacological and toxicological effects of natural antioxidants and of therapies used in inflammatory conditions are also future goals.

Other main objective will focus on the biological evaluation of new potent anti-tumor compounds for hormone-dependent breast and prostate cancers, as well as the investigation of the type and signaling pathways of cell death induced by these compounds

The biological actions of endocannabinoids are now emerging in various physiological processes. Our group showed a pro-apoptotic activity for anandamide in uterine decidual cells. We intend to extend our study to the mechanisms underlying endocannabinoid effects in the fetoplacental bed in order to understand the involvement of these lipid modulators in tissue remodelling during gestatio.

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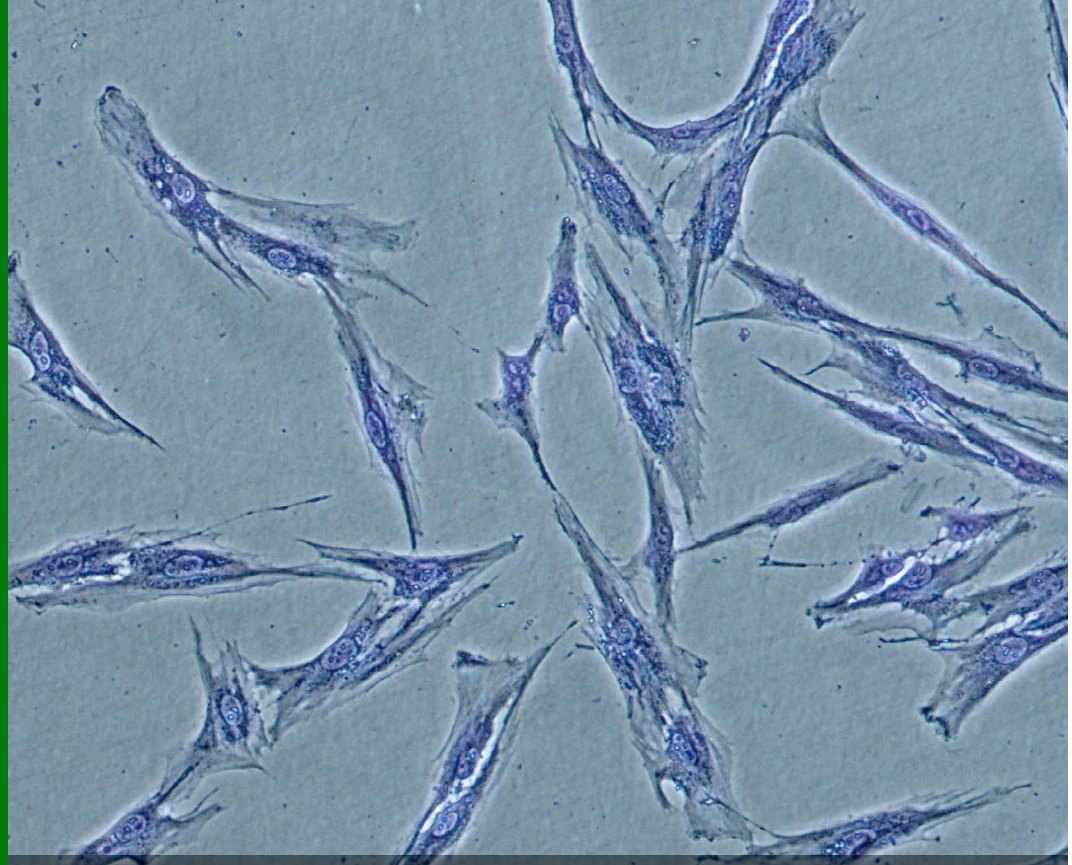

Bone Marrow Mesenchymal Stem Cells from patient with DISH. Staining with 1% of crystal violet. Amplification:10X.

Genetics and Arthritis Research

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Previous research results

We are currently devoted to two different areas: 1) the study of detrimental calcifications characterizing and complicating a number of skeletal disorders, and 2) the study of additional genetic factors associated with the neurological disorder Machado Joseph Disease (MJD).

Rheumatic disorders: The etiology and clinical characterization of rheumatic disorders with ectopic calcification is still our main research interest. The investigated disorders are Diffuse Idiopathic Skeletal Hyperostosis (DISH), chondrocalcinosis (CC) and ankylosing spondylitis (AS). Whole genome expression studies in cartilage and synovial tissue biopsies from AS patients are currently being analyzed. The possible AS genetic association of three genes (*TNFSF15, ERAP* and *IL23*) is currently being scrutinized in a cohort of Portuguese AS patients and controls. The non classical HLA loci E, G, F, DPA and DPB are also being investigated in AS patients and controls to investigate its possible involvement in the etiology of this disorder.

Neurological Disorders: Machado-Joseph disease (MJD/SCA3) is the most common autosomal dominant spinocerebellar ataxia, being caused by a gain of function of ataxin-3, which occurs when an abnormally expanded CAG motif is present in the coding region of *ATXN3* gene. In MJD, the causative mutation itself is not sufficient to determine the disease expression, supporting the contribution of additional genetic factors. We have recently focused our efforts on the regulation and transcriptional variation of the *ATXN3*, and its potential as a modulator of the clinical variability of MJD. Investigation aiming to understand the clinical spectrum of MJD has also resulted in the identification of the Apolipoprotein E gene as a modulator of MJD's phenotype.

Future research goals

Rheumatic Disorders: Future work will involve the collection and organization of biological samples, from patients with the rheumatic disorders under study – DISH, CC and AS - in a BioBank. This project will enable the assemblage of important information that can be used for studies of etiology, therapy and clinical outcome. Cell culture of human bone marrow mesenchymal stem cells and human chondrocytes were also recently started; expression studies involving the cultured cells will surely be of major interest. Whole exome sequencing of patients with the phenotype DISH/CC will be performed/analyzed with the objective of identifying mutated genes that can be the underlying cause of the phenotype DISH/CC. Methylation and proteomic studies, with the AS biospecimens collected so far, will hopefully establish new investigation lines and identify new genes of interest.

Neurological Disorders: in addition to ApoE, other genetic modifiers remain to be identified. Microarray expression data is being generated for patients, aiming to pinpoint potential new candidates. Presently, accurate measurement tools, to detect the first signs of the disease and subtle therapeutic benefits, are needed for MJD. We are using a non-biased whole-genome approach to detect such changes in peripheral blood of patients.

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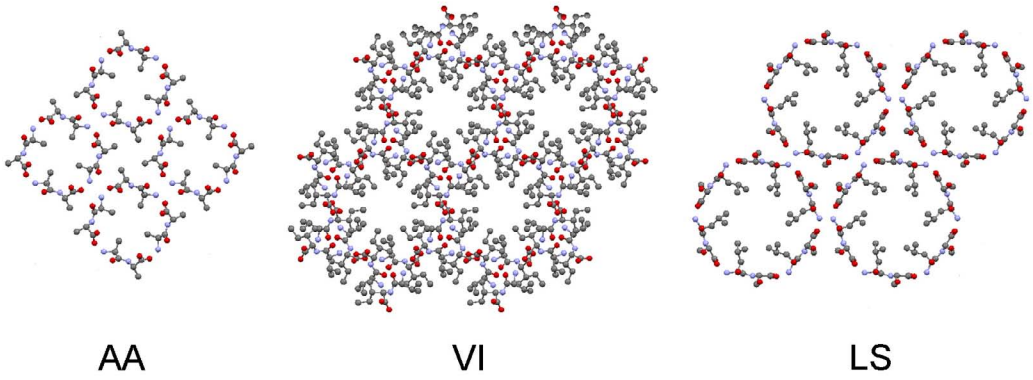

Immunoreactive N-acylphosphatidylethanolamine-hydrolyzing Phospholipase D (NAPE-PLD), which generates the main endocannabinoid - anandamide, in giant trophoblast cells on day 14 of pregnancy

Molecular Biophysics

Luís Gales

Luís Gales (born 1971) received his PhD degree in Chemical Engineering in 2000 at Universityof Porto. Currently he is Associate Professor of Biophysics at Instituto de Ciências Biomédicas Abel Salazar of the University of Porto. He authored or co-authored more than 40 papers and his research focuses on self-assembling processes involving peptides and proteins, especially for applications in biomedicine.

Group Leader at IBMC, since 2011

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Previous research results

We are interested in combining approaches of physics, chemistry and biology to understand, characterize and manipulate biological systems with molecular precision. The main lines of research are:

a) supramolecules - structural characterization and manipulation of peptide and protein assemblies. Projects: peptide-based assemblies with functional properties for molecular recognition and delivery of guest molecules (1,2); protein self-assembly processes involved in amyloid diseases (3,4).

b) molecules - protein structure elucidation using X-ray crystallography complemented with other techniques. Projects: structural and functional characterization of the enzyme responsible for the degradation of the pesticide molinate (5) and structure-based design of transthyretin amyloid inhibitors(6).

Future research goals

In the future we will focus on the study of the self-assembly of biomolecules (peptides or proteins) oriented for two complementary goals: i) development of supramolecular complexes for molecular recognition and delivery of bioactive compounds and ii) mechanistic studies of amyloid fibril formation. In addition, we are also interested in using X-ray crystallography for structure-oriented research of amyloid inhibitors.

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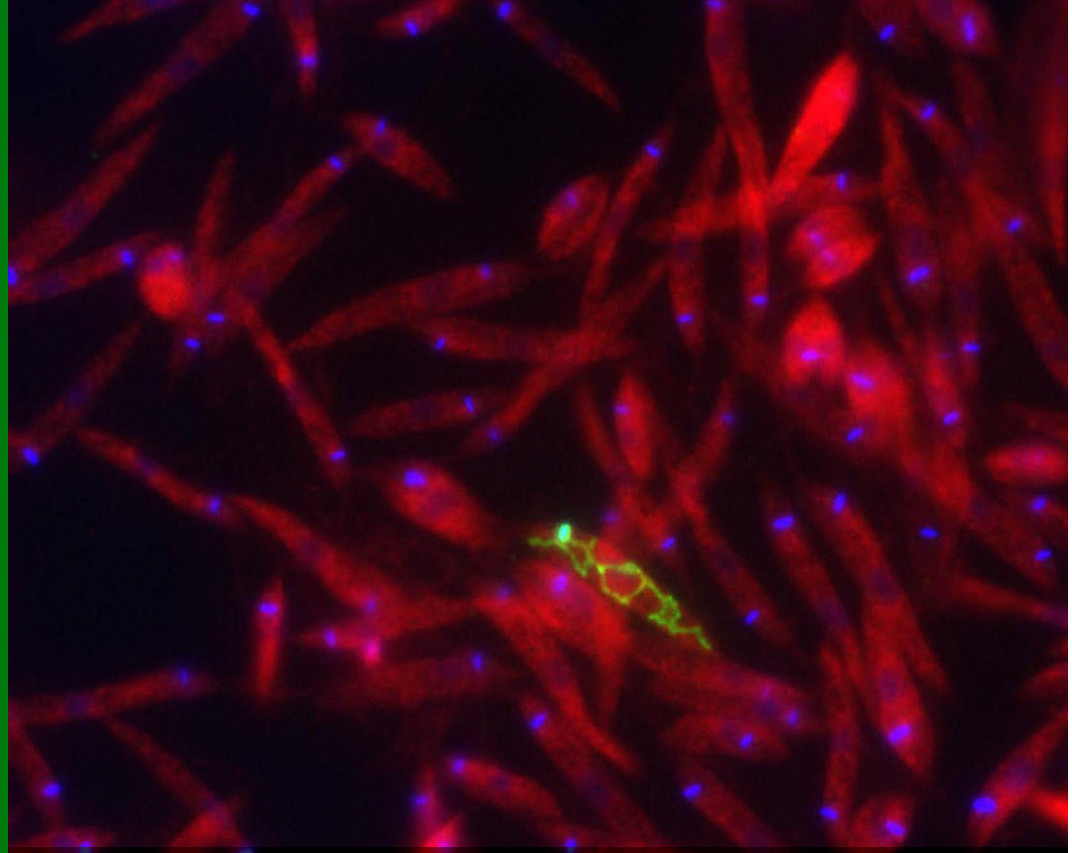

Immunofluorescence micrograph of one wild type *Leishmania infantum* contaminating a culture of parasites knockout for a mitochondrial peroxiredoxin. Analysis of these mutants revealed this protein to be crucial for parasite pathogenicity (Castro *et al*. 2011, *PLoS Pathogens*, 7:e1002325, 2011)

Molecular Parasitology

Ana Tomás

Ana Tomás is a Molecular Parasitologist and an Associate Professor in Parasitology. Her scientific interests are the study of fundamental aspects of the biology of protozoan parasites such as Leishmania and of the interaction of these with their mammalian hosts.

Group Leader at IBMC, since 2010

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Previous research results

Our laboratory focuses on *Leishmania*, the agents of human and canine Leishmaniasis in many regions of the world, Portugal included. Leishmaniasis is a serious, often fatal, condition for which no satisfactory therapy exists. We have been studying aspects of the thiol metabolism of *Leishmania*, namely the processes used by these parasites to eliminate peroxides. Initiated with the identification and the characterization of several *Leishmania* antioxidant enzymes, our work has recently led to two important findings. One of these refers to the discovery that redox metabolism in the mitochondrion of *Leishmania* and other trypanosomatids is not dependent on the activity of a class of trypanosomatid-specific oxidoreductases named as tryparedoxins. Our most recent significant contribution relates to another *Leishmania* mitochondrial enzyme, the peroxiredoxin LimTXNPx which we identified as a factor essential for the parasites to thrive in their mammalian hosts. Noticeably, the *in vivo* crucial function of LimTXNPX could not be explained by its well characterized peroxidase activity. Rather, our observations suggested this mitochondrial peroxiredoxin to function as a chaperone, an activity that may allow the parasite to sustain the change of temperature as it passes from the insect to the mammalian host.

Future research goals

Our research on thiol metabolism has 3 immediate aims:

i) To characterize the chaperone activity of LimTXNPx at the biochemical level and confirm its *in vivo* significance.

ii) LimTXNPx is essential for *Leishmania* survival in the mammalian hosts but is redundant in the insect stage, the promastigote. Our objective is to investigate if *Leishmania* promastigotes devoid of LimTXNPx can be used as a basis for a life attenuated vaccine.

iii) *Leishmania* thiol metabolism is interesting for drug development because it depends on trypanothione and not, as their hosts, on glutathione. This suggests that it can be used as a target for new anti-*Leishmania* therapeutics. Presently, we are genetically and chemically validating the enzymes involved in the synthesis of trypanothione in *Leishmania infantum*.

We have now initiated a second area of research, metal acquisition in intracellular *Leishmania*. A detailed knowledge of the parasite metal transport machinery and of the modifications in metal metabolism occurring in infected cells will not only increase our understanding of the infective process itself, but may provide also an opportunity for therapeutic intervention. Our aims are:

i) To identify and characterize components of metal uptake systems in *Leishmania infantum*.

ii) To investigate the changes taking place in iron metabolism proteins and in iron traffic in *L. infantum*-infected macrophages.

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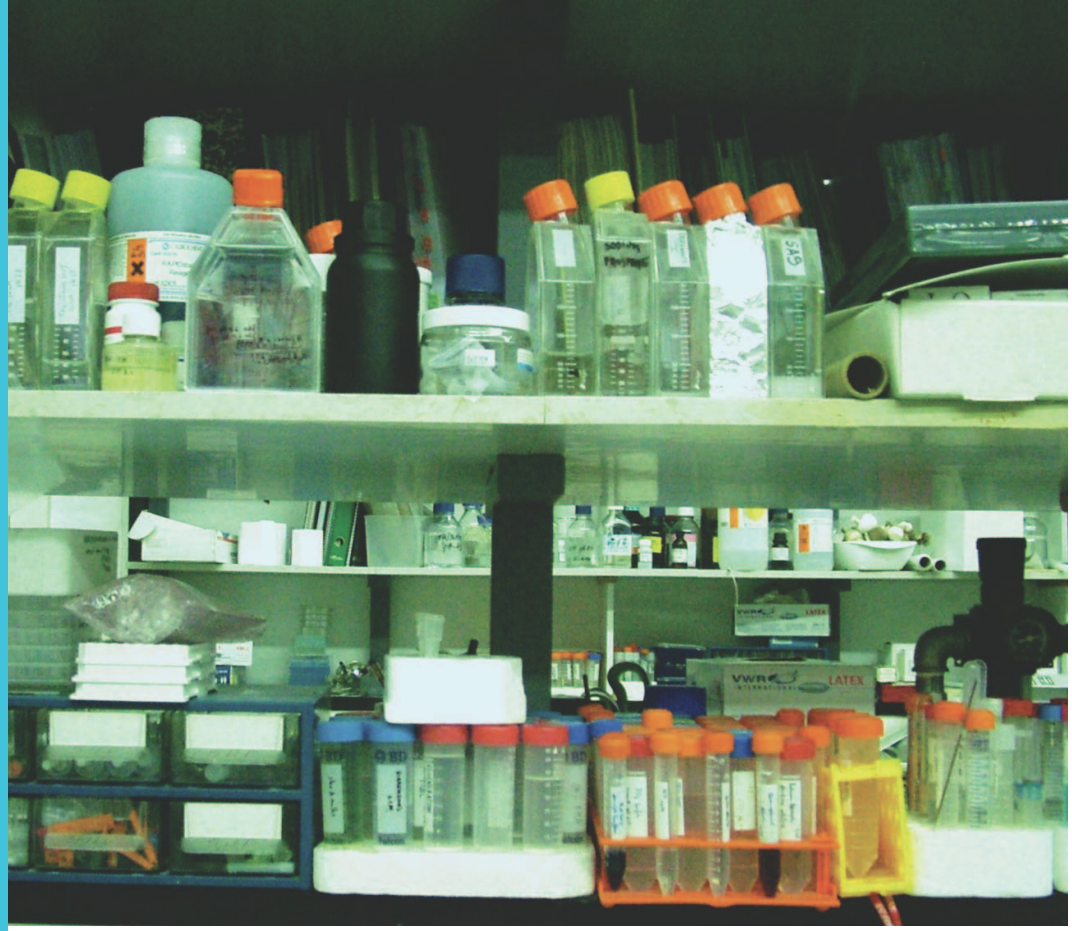
Technological Platforms



Technological Platforms

The IBMC has several scientific, administrative and general services that are shared by all Research Groups and also provide external services for the community. These scientific services are run by dedicated Technical Staff; some of them already hold PhDs. These technicians are encouraged not only to provide services but also to undertake technical development as well as training.

Advanced Flow Cytometry
Advanced Light Microscopy
Animal House
Cell Culture and Genotyping
Histology and Electron Microscopy
Programs' Office
Protein Production and Purification
Technology Transfer Office

Advanced Flow Cytometry

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The mission of the Advanced Flow Cytometry Unit (AFCU) is to offer researchers efficient and reliable flow cytometric services with the highest standards of quality control and productivity.
The AFCU provides investigators with equipment for acquisition and analysis of flow cytometric data and for cell sorting from single cell suspension using fluorescence. Furthermore, the AFCU gives training and consulting for researchers that intend to use the flow cytometry in their projects; and helps analyzing and interpreting data. A number of applications, including the multicolor analysis of cell phenotype, gene expression, membrane potential, Ca2+ and Mg2+ influx and cell cycle can be performed.
For analyzing the flow cytometric data, the AFCU is equipped with a Flowjo workstation (a computer with a licence for the flowjo software). The available equipment can be booked on line.

Advanced Light Microscopy

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The Advance Light Microscopy (ALM) is the scientific core facility of IBMC dedicated to state-of the-art optical microscopy applications for biosciences. Multidimensional (6D) imaging of cells and tissues, high speed live cell microscopy, molecular analysis techniques and in vivo microscopy are some of the applications available. The ALM provides access to advanced light microscopy systems as fluorescence widefield and laser scanning confocal microscopes, training in equipment use, scientific advisement in experiment planning, consulting and collaboration in research projects, and develops educational activities. The ALM deals with a broad range of biological problems and acts to establish connections between different investigators and areas of research. Furthermore, we have high motivation to implement new experiments and techniques. The ALM works as an open-access facility to all members of the IBMC•INEB Associated Laboratory and outside researchers from scientific and technological communities.

Animal House

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The Animal House (AH) is a licensed breeding and experimental facility holding 2000 cages of rodents (mice and rat) and 2-4 rabbits. There are several strains of genetically modified mice, most of which are immunocompromised. The AH provides different services that support animal based research:
a) Care and management of genetically modified animals (breeder selection, mouse breeding, weaning, animal identification, sample collection for genotyping, weekly animal records and care of sublethal KOs;
b) Supply of common rodents from different strains (C57BL/6J, BALB/c, 129, NMRI and Wistar Han rats), timed pregnant females, embryos and neonates;
c) Specialized services: Polyclonal antibody production, Rederivation techniques; PCR diagnostic for common rodent pathogenic agents;Veterinary care and surgical interventions: administration of substances, blood collection, and post surgical assistance; Training of researchers and animal caretakers (FELASA category A and B).

Cell Culture and Genotyping

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The Cell Culture and Genotyping Service (CCGen) cooperates with researchers through: a) Cell culture service that offers individual, fully equipped and monitored rooms for cell culture, mycoplasma tests and N2 reservoir for cell storage; b) genotyping and gene expression service that facilitates the implementation of these technologies, including expert consultation and training and providing equipment for gene expression analysis (*iQ5 Real-Time PCR Detection System*); automated DNA and RNA extraction (*Maxwell 16 System*); quantification of DNA, RNA and protein (*NanoDrop*); and automated electrophoresis system for nucleic acids and protein analysis (*Experion*); c) Mouse genotyping service for that implements and optimizes genotyping protocols and performs routine analysis of 46 different genes. All services offer technical assistance to all researchers.

Histology and Electron Microscopy

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This Core Research Facility is centred on Transmission Electronic Microscopy (TEM) namely conventional Ultrastructure; immunoelectromicroscopy; elemental analysis – EDX; STEM; in situ hybridization; Autoradiography; training in tissue preparation, and Optical Microscopy namely criomicroscopy and paraffin embedding with the ancillary equipment.
The equipment available to perform these techniques includes an electron microscopes Jeol JEM 1400, Zeiss model EM 10C and model EM 902A with a SC1000 OriusTM CCD camera gatan, ultramicrotomes, and for optical microscopy: vibratome, freeze and paraffin microtomes, paraffin tissue processor and a modular embedding system.
The Facility provides both the equipment and technical support to researchers needing high level optical and TEM to tackle studies either of cell or material sciences. Besides the instruction of researchers the Facility also offers training courses as well as guided visits to high school students. Currently the Facility is also engaged on workshops or exhibitions for the general public organized by the Office of Science Communication of the IBMC.

Programs' Office

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The Programs' Office is a joint department of IBMC, INEB and IPATIMUP and its main purpose is to support the researchers with project applications, by searching and announcing funding opportunities, studying the specific call requirements and helping to prepare and submit the project proposals.
The office is also involved in other research related activities, such as circulating information about scientific events, fellowships and job positions, managing the requests for licensing projects with animal experimentation and supporting ongoing projects and programs.

Protein Production and Purification

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The Protein Production and Purification Unit (UP3) provides access to state-of-the-art equipment for the expression, purification and biochemical/biophysical analysis of recombinant proteins. This facility provides both scientific and technical expertise, actively participating in the establishment of interdisciplinary research and networking activities involving internal and external research groups.
Users have access to facilities for the heterologous expression of proteins in prokaryotic and eukaryotic systems, as well as to chromatographic techniques, and to a wide range of analytical methods such as absorbance spectroscopy, spectrofluorimetry, circular dichroism spectrometry, microcalorimetry and surface plasmon resonance. Proteins, as well as a wide variety of other biomolecules (such as nucleic acids and small molecules), can be studied in terms of their specific activity, stability, and molecular interactions.

Technology Transfer Office

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The mission of the TTO is to promote the research activities of the Associate Laboratory IBMC to public and private investors, to protect the intellectual property capital and to promote social development. It has one FTE with a degree in Biology and a MBA.

Its main tasks are:
1) Management of Material transfer agreements.
2) Scouting of licensing opportunities
3) Spinning out and starting new ventures
4) Attraction of foreign direct investment
The objective is to maintain a profitable business unit that contributes to the financing of IBMC.

Translational Iniciatives

CGPP

CENTRO DE GENÉTICA
PREDITIVA E PREVENTIVA

Testes genéticos | Aconselhamento genético | Formação

The Center for Predictive and Preventive Genetics (CGPP)

Jorge Sequeiros

Full professor, ICBAS; PI, UnIGENe; Director, CGPP; President, National Medical Genetics Commission; Board, Port. College of Medical Genetics; Member, National Council for Ethics in the Life Sciences; Board, ESHG; Member, PPPC, ESHG; Organizer, SCAs EQA, EMQN

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Previous research results

The CGPP develops its activities in three main areas: (1) medical genetics clinic and genetic counselling, (2) genetic testing for hereditary diseases and (3) training in human genetics for health professionals.

At the outpatient clinic, patients and families with genetic diseases are observed and counselled by a multidisciplinary team of clinical geneticists, neurologists, haematologists, psychologists & social service. A specific protocol for presymptomatic testing and counselling in late-onset neurological diseases is in place for relatives at-risk.

At our molecular genetics lab, diagnostic, carrier, pre-symptomatic and prenatal tests are available for a large number of diseases, namely dominant and recessive ataxias, Huntington (HD) and HD-like diseases, spastic paraplegias, Wilson, Parkinson, familial Alzheimer and frontotemporal dementia, familial hemiplegic migraine, Charcot-Marie-Tooth, familial amyloidosis, epilepsies, neurofibromatosis and other. A quality management system is in place. The DNA/cell-line bank storages thousands of samples, coupled with clinical and pedigree data, available for research.

The European EQA for SCAs is administered by us, since 2004, for the European Molecular Quality Network. EMQN Best Practice guidelines for genetic testing of the SCAs were developed and consensus and controversies identified and discussed. SCAbase (scabase.eu) is an evidence-based online diagnostic resource for the dominant ataxias (SCAs) and publicly available.

CGPP is the hosting institution for Orphanet-PT (www.orpha.net - the portal for rare diseases and orphan drugs), collecting the national data for the database and translating its contents into PT.

15 EuroGentest leaflets (www.eurogentest.org) for patients and families were developed, revised and/or translated to PT. CGPP participates in other European networks, as EHDN, Euro-Wilson and SPATAX, and collaborates closely with the national patient organizations for ataxias, HD and amyloidosis. CGPP has also been offering consultancy in public policies and ethics, to national health authorities and international organizations, participating in several international guidelines for the OECD, EC, CoE, ESHG, ESHRE and EASAC and FEAM.

Future research goals

CGPP activity focus on the following services to the community:

Genetic testing mostly for neurological diseases and haemocromatosis. Full list of genetic tests offered is available at www.cgpp.eu;

Genetic counseling, Medical Genetics and Neurology consultations to patients and families, as well as psychological evaluation and follow-up;

Education and training in human and medical genetics for physicians and other health professionals; clinical and lab rotations for medical residents; education for biologists, biochemists and others, in a diagnostic laboratory context; rotations for psychologists, nurses, social service and the professional master course in genetic counselling;

Biobank of DNA, cell lines and other biological materials for potential future studies.

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