

PhD 2007 Welcoming Meeting

7th April 2008



FUNDAÇÃO CALOUSTE GULBENKIAN

Instituto Gulbenkian de Ciência

Schedule and abstracts

Welcome!

This meeting aims at introducing the 1st year PhD students to the IGC research community, especially other PhD students. Each student will have the opportunity to present his or her project in 10 minutes followed by 5 minutes for questions/discussion. At the end of the meeting a beer hour with buffet will be organized so that the students have the opportunity to meet in a relaxed environment.

Schedule

14:00 – 14:30	Welcoming word by Prof.Coutinho and Henrique Teotónio
14:30 – 14:45	Ivo Marguti (Page 3)
14:45 – 15:00	Ricardo Paiva (Page 3)
15:00 – 15:15	Clara Pereira (Page 4)
15:15 – 15:30	Patricia Inácio (Page 4)
15:30 – 15:45	Mariana Silva (Page 5)
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16:15– 16:30	Ana Teresa Avelar (Page 5)
16:30 – 16:45	Ana Inês Ferreira (Page 6)
16:45 – 17:00	Catarina Samora (Page 6)
17:00 – 17:15	Tânia Ferreira (Page 7)
17:15 – 17:30	Barbara Jezowska (Page 7)
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18:15 – 18:30	Rita Isabel Cardoso (Page 8)
18:30 – 18:45	Migla Miskinyte (Page 9)
18:45 – 19:00	Sandra Trindade (Page 9)

Beer hour and Buffet

Ivo Marguti

Inflammation (Miguel Soares)
PGD 2007

A role for the heme/HO-1 system in dendritic cell biology

Heme oxygenase-1 (HO-1) is the rate-limiting enzyme in the catabolism of free heme, a process leading to the generation of biliverdin, carbon monoxide and free iron. Dendritic cells (DC) are professional antigen presenting cells that bridge innate and adaptive immunity. DC can regulate the quality of adaptive immune responses by inducing TH cell differentiation into distinct effector phenotypes. Moreover, DC can also induce TH cell differentiation into tolerogenic phenotypes. The molecular mechanism(s) regulating the immunogenic versus tolerogenic status of DC are fundamental in determining the outcome of adaptive immune responses and thus must be tightly regulated.

HO-1 expression controls the outcome of a variety of pathologies in which adaptive immunity is involved. As DC play a critical role in regulating adaptive immune responses, we hypothesized that HO-1 expression in DC might modulate TH cell activation in a manner that suppresses these pathologic conditions. This hypothesis is supported by the following observations: i) pharmacologic induction of HO-1 in DC suppresses the re-activation of TH cells, ii) some of the end products of heme catabolism can promote T cell tolerance, iii) induction of immune tolerance to allografts requires the expression of HO-1. The main hypothesis to be tested in this project is that the heme/HO-1 system might regulate DC immunogenicity.

Ricardo Paiva

Lymphocyte physiology (Jocelyne Demengeot)
PGD 2007

Regulatory T-cell dynamics

Regulatory T-cells (Tregs) are a T-cell subset involved in maintenance of self tolerance and control of immune responses. Its identification is nowadays achieved through the detection of a transcription factor shown to be essential for their activity – the forkhead box P3 (Foxp3). Until very recently it was widely accepted that Tregs originated exclusively in the thymus as a functionally mature cell population. However, recent research has produced considerable evidence showing that circulating naïve conventional T-cells can also be induced to express Foxp3. This poses diverse conceptual challenges: can any T-cell become regulatory and inhibit responses to its cognate antigen? If so, what determines if a cell should become a helper T-cell or be diverted to the Treg subset when it encounters its cognate antigen? In which conditions, with which role, and to what extent does this happen? Under this framework a project was set up in order to identify and study the conversion of Foxp3⁻ to Foxp3⁺ T-cells in mice which immune system is unchallenged, challenged with self peptides or challenged with foreign peptides. Moreover, since the expression of the Foxp3 transcription factor is determinant for T-cell regulatory activity, the regulation of its transcription will also be assessed. This project aims to study the Treg pool origin and in particular clarify the role of Tregs which differentiate in the periphery.

Clara Pereira

Epigenetics and Soma (Vasco Barreto)
PGD 2007

Random monoallelic expression of autosomal genes

I am interested in studying random monoallelic gene expression of autosomal genes as a general epigenetic phenomenon beyond genomic imprinting and X-chromosome inactivation. Random monoallelic gene expression is a poorly understood mechanism that may represent an additional way organisms have found of generating diversity further than polymorphisms, with relevance for understanding haploinsufficiency-related diseases, and for lineage commitment in stochastic processes of cell differentiation. By using one or several simple model organisms, with a focus on zebrafish, I will search for random monoallelically expressed genes, and study how frequent they occur in the genome, their tissue specificity and distribution, and the relevance of this phenomenon during development. I will further study genetic systems that are known to undergo allelic exclusion. I will start by analysing the epigenetic nature of the immunoglobulin (Ig) genes allelic exclusion. My goal is to understand how the allelic exclusion of Ig genes is first established. Specifically, I will see if this process is dependent on a pre-determined (epigenetic) monoallelic expression or is simply a stochastic event, as has been suggested many times before. This will support the idea that random monoallelic expression of autosomal genes is a relevant broad mechanism of gene transcriptional regulation.

Patrícia Inácio

Cellular Pathogenesis of Malaria (Miguel Seabra)

In collaboration with:

Maria Mota (Instituto de Medicina Molecular)

PGD2007

Host-plasmodium interactions: understanding intrahepatic sporozoite development

Malaria, caused by the Plasmodium parasite, remains one of the most prevalent infectious diseases worldwide. Still, the requirements and strategies developed by Plasmodium sporozoites in order to survive and to be successful inside its host cell during the liver stage of infection remain poorly understood. As the parasite matures and replicates its size increases dramatically leading to the expansion of the parasitophorous vacuole membrane (PVM). However, the biogenesis and composition of the PVM is still unknown.

The overall goal of my project is to understand the biology and the interactions occurring during the development of Plasmodium sporozoites inside the hepatocytes. To this end, we will try to isolate the parasitophorous vacuole. This will allow studying the PVM in detail. In parallel, we will apply a quantitative proteomic technique, SILAC, at different time points of liver infection in Huh7 cell line to identify protein modifications in cells as a result of parasite invasion and development. The data generated by SILAC will allow not only a selection of host genes crucial for the hepatic stage of the infection, that will be used for further functional assays both in vitro and in vivo, but

also to identify potential new parasite proteins crucial for liver infection. Thus, the project is designed to study host-Plasmodium interactions from both the parasite and host sides.

Mariana Coelho Correia da Silva

Laboratory for Epigenetic Mechanisms (Lars Jansen)

PGD 2007

Epigenetic control of centromere function: CENP-A as a key epigenetic mark for centromere function and propagation.

The centromere is a specialized chromosomal locus that ensures proper chromosome segregation thereby preventing aneuploidy, a major feature of human cancers. Although directly associated with chromosomal DNA, the specific DNA sequences are neither necessary nor sufficient for centromere identity and centromere inheritance is therefore controlled by epigenetic factors. CENP-A, a histone H3-like protein only present at centromeres, is the primary candidate for centromere identification and function. Although it is known that CENP-A is critical for centromere propagation, how CENP-A loading is initiated and restricted to a defined region and how CENP-A nucleosomes are stably maintained across generations remains to be clarified. During my PhD thesis I am interested in clarifying the assembly mechanism of CENP-A and I will use a gene down regulation approach combined with a novel developed labelling technology (SNAP technology) to address that. I am also interested in determining how CENP-A nucleosome stability are stably maintained during several cell divisions, and how centromeric chromatin is rearranged during S phase, as CENP-A synthesis only occurs during late G2. Defining the epigenetic mechanism responsible for centromere function and propagation will be instrumental in elucidating general principles of epigenetic inheritance that are crucial to transcription regulation, developmental programs, genome organization and, when defective, pathology.

Ana Teresa dos Santos Avelar

Telomere and Genome Stability (Miguel Godinho Ferreira)

In collaboration with:

Isabel Gordo, Evolutionary Biology (IGC)

PGD 2007

Genome dynamics and variability: the influence of genomic instability

The main question of my project is to understand how large modifications in the genome of cells can contribute to the generation of sustainable diversity, and allow rapid cellular and organismal adaptations. My project consists of studying the outcomes of telomere-driven genomic instability as a model for generation of chromosomal rearrangements, using the fission yeast *Schizosaccharomyces pombe* as biological model.

Ana Inês da Cunha Ferreira

Cell Cycle Regulation (Mónica Bettencourt Dias)

In collaboration with:

José Pereira Leal, Computational Genomics (IGC) and David Glover,
Regulation of Mitosis and Meiosis (University of Cambridge)
PGD 2007

Unraveling the control of centriole number and fate

My interest in cell cycle regulation led me to apply for a PhD position in the group of Cell Cycle Regulation headed by Mónica Bettencourt-Dias at the IGC. My project is focused on understanding the factors that are involved in the control of centriole biogenesis. The centriole is a structural component of the centrosome, the primary microtubule-organizing centre (MTOC) in animal cells regulating cell motility, adhesion and polarity in interphase and facilitating the organization of the spindle poles during mitosis. In addition the centriole has another quite distinct function as a basal body, seeding the growth of cilia and flagella known to have important roles in cell movement or as cellular antenna sensing substances. Little is known about the processes that are involved in centriole biogenesis, namely the regulation of centriole number and centriole fate. This PhD project aims at a better understanding of the molecules and mechanisms that regulate centriole biogenesis. This project will use a combination of computational, genetic, biochemical and cell biological approaches. The computational side of this work will be co-supervised by José Pereira-Leal (Computational Genomics Group at IGC).

Catarina Samora

Mitosis (Álvaro Tavares)

In collaboration with:

David Glover, Regulation of Mitosis and Meiosis (University of Cambridge)

Regulation of Mitotic exit in higher eucaryotes - The role of Cdc14 and Mob genes

Cytokinesis is a key point in cell proliferation and fundamental for the growth and development of all eukaryotic organisms. In order to ensure that each daughter cell receives only one copy of each chromosome, exit of mitosis and cytokinesis must not occur before chromosome segregation has been completed. Exit from mitosis is the transition from the mitotic state, characterized by high mitotic cyclin-dependent kinase (CDK) activity, to the interphase, when mitotic CDK activity is low.

A family of structurally related proteins (Mob proteins) has been demonstrated to be important for both mitosis completion and cytokinesis. Its founding member, Mob1, was identified in *Saccharomyces cerevisiae*, where it is an essential component of the MEN, a complex signal transduction cascade that co-ordinates mitotic exit with cytokinesis. This pathway promotes the inactivation of the mitotic Cdk1-cyclin B complexes and drives mitotic exit by leading to the release from the nucleolus and subsequent activation of the Cdc14p phosphatase.

We wish to establish whether higher eukaryotes have a defined mitotic exit signaling pathway. For such we will determine the function of the *Drosophila* Cdc14 and Mob-like proteins. We wish to determine if their malfunction lead to genetic instability and the formation of tumors in *Drosophila*. Following previous work in our groups, we wish to characterize in detail the mitotic defects of Cdc14 and Mob mutants. Finally, we want to determine if Cdc14 reverts the activity of CDK and induces an entry into interphase.

A major breakthrough we expect is the finding of possible new components of the mitotic exit signalling cascade. We will also determine if the subcellular localization of these proteins follows a dynamic distribution to the centrosomes and kinetochores.

Tânia Ferreira

Early Fly Development (Rui Martinho)

Defining novel mechanisms required for the formation and morphogenesis of a polarized epithelium.

Epithelial cells are essential for organism homeostasis and they play an important role during the morphogenetic development of the embryo. In *Drosophila*, early embryonic development is syncytial and a polarized epithelium forms *de novo* through a process known as blastoderm cellularization. Once completed, the embryo starts a complex set of morphogenetic movements that leads to the formation of an embryo with three germ layers.

One of the main goals of this work is to better define the molecular mechanisms responsible for the formation and morphogenesis of a polarized epithelium. We are going to follow two distinct experimental approaches: 1) Characterization of previously isolated *Drosophila* mutants defective for epithelial morphogenesis. 2) Functional characterization of a novel allele of *atypical protein kinase C (aPKC)* that behaves like a loss-of-function allele of *aPKC* during gastrulation, but fully rescues *aPKC* function during later stages of development.

Barbara Jezowska

Actin Dynamics (Florence Janody)
PGD 2007

Roles of capping protein during *Drosophila* wing morphogenesis

Capping protein (CP) is a highly conserved protein heterodimer, composed of α (Cpa) and β (Cpb) subunits. This heterodimer is known to belong to the group of proteins assigned as

actin binding proteins since it possesses a high affinity to actin, and was shown by in vitro experiments to bind the barbed end of the actin filaments thereby preventing the addition of new actin monomers and restricting actin polymerization.

Until very recently, it was considered that CP had a general actin capping activity in all cell types. However, in vivo experiments suggest that CP can act on specific subpopulations of actin filaments in a tissue and time specific manner during *Drosophila* development which result in different cellular and tissue responses.

For my PhD project I would like to elucidate the different molecular functions of CP using the morphogenesis of the *Drosophila* wing as a model system. This will allow me to get some insights on the molecular mechanisms that translate cell shape changes into the formation of the proper adult organ.

Barbara Vreede

Evolution & Development (Élio Sucena)
PGD 2007

Studying the genetic basis of evolutionary novelties: dorsal appendages in *Drosophila* eggs as a unique model system

One of the most intriguing concepts in current evolutionary biology is the origin of a new structure (or other property), a so-called evolutionary novelty. This provides a lineage with new opportunities for diversification, and hence, new adaptive possibilities. We will use dorsal appendages on *Drosophilid* eggs as a model system for evolutionary novelty. These appendages are unique structures in most *Drosophilid* species, and are not seen outside this clade. Additionally, within the *Drosophilidae* a wide variety exists, ranging from secondary loss to up to 12 appendages per egg. The patterning pathways that underlie these structures are well known and involved in forming the main axes of the egg. We will use this knowledge to compare the formation of dorsal appendages in different species, and to determine the points in the developmental network that have played a role in the evolution leading up to both the formation and divergence of this evolutionary novelty. Using recently found mutants of *Drosophila melanogaster* that do not form dorsal appendages, we will determine (additional) candidate genes. The final aim of the project will be to perform a functional analysis, which is unique in the study of evolutionary novelty, to functionally link candidate developmental points to a novel phenotype. For this, we will test the candidate genes determined in the early phase of the project, taking advantage of the many genetic tools available in *Drosophila melanogaster*.

Rita Isabel de Amorim Cardoso

Stress and Cytoskeleton (Helena Soares)
In collaboration with:

Dr. José Alexandre da Costa Perdigão e Cameira Leitão
(Instituto de Investigação Científica Tropical) and Professor Helder Carola
Espiguinha Cortes (University of Évora)

The role of components of the tubulin folding pathway in *Besnoitia besnoiti* host cell invasion

Besnoitia besnoiti is an *Apicomplexa* parasite, related to *Toxoplasma gondii* that affects bovine cattle, leading to significant economic losses. During the last two decades, an increasing number of cases of bovine besnoitiosis has been reported in Portugal, Spain, and France. The striking features of the disease are fever and abortion in the initial phase, and, in the subsequent chronic stage, thickening of the skin, difficult locomotion, wounds and secondary infections in areas of higher elasticity demand, accompanied by anorexia and severe weight loss.

Our results show that host cell invasion requires a cross-talk between the two cytoskeletons, which leads to *Besnoitia besnoiti* microtubule cytoskeleton remodel. We postulate that tubulin folding machinery controlling synthesis, flux, and transport of tubulin, will play a crucial role in microtubule arrays rearrangement and dynamics, and will thus be essential for infection. Our aim is to characterize central components of this by cloning the genes, and studying their pattern of expression and their role during host cell invasion. Based on literature we have chosen the CCTa-subunit and the Cofactors B and E. We will establish a transfection system for *Besnoitia besnoiti* that will permit to study intracellular localization and microtubule rearrangements during invasion. Studying the mechanisms underlying cytoskeletal functions in this apicomplexan parasite, may open perspectives for developing new strategies for controlling infection.

Migla Miskinyte

Evolutionary Biology (Isabel Gordo)
PGD 2007

The role of biotic interactions in generating and maintaining biodiversity

In my current project I want to test a long-standing hypothesis that biotic environments exert higher selection pressures on other organisms than abiotic environments, leading to higher rates of adaptation and hence generating more diversity. It remains an open question whether theoretical predictions and short-term observations will stand the critical test of long-term evolution. On the other hand, experimental evolution studies have mostly focused on the adaptation of a single species to an abiotic environment, despite the fact that biotic interactions are overwhelming in the real world. Investigating the long-term consequences of biotic interactions is clearly an emerging field of research to which I hope to contribute substantially. I will do that with this project, employing the most overwhelming key biotic interactions in the real world – competition and predation. To test this hypothesis I will use two genetically related microorganisms, *Escherichia coli* and *Salmonella enteric* serovar Typhimurium undergoing long-term experimental evolution. Apart from contributing to the understanding of biodiversity patterns in nature, this project has also bearings on applied biomedicine, as I will be able to generate predictions concerning the nature of adaptations of micro-organisms to multiple infections and to the immune system.

Sandra Trindade

Evolutionary Biology (Isabel Gordo)

The impact of genetic interactions in antibiotic resistance

Epistasis is the interaction between genes in different loci. Understanding the nature of genetic interactions, known as epistasis, is crucial in several aspects of biology. Epistasis may also play an important role in medical sciences when the traits under study are of clinical interest, such as antibiotic resistance in bacteria. Here we measured the degree of epistasis between alleles that confer resistance to commonly used antibiotics in an environment that is antibiotic free and, in particular, ask how costly it is to eradicate multi-resistant bacteria in an antibiotics-free environment. The resistance is conferred by single point mutations and the mutational effect caused by the different combinations of alleles were estimated for each clone and compared with the predicted, under the null hypothesis of nonepistatic interactions.

NOTES