



INSTITUTO GULBENKIAN DE CIÊNCIA

ANNUAL REPORT 2003

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FUNDAÇÃO CALOUSTE GULBENKIAN

BOARD OF ADMINISTRATION

The Fundação Calouste Gulbenkian, established by Calouste Sarkis Gulbenkian by his Will dated June 18, 1953, is a private Institution of general public utility, endowed with legal personality. The aims of the Foundation are charity, art, education and science. The members of the Board of Administration in 2003 were:

President

Emílio Rui Vilar

Honorary President

Mikhael Essayan

Executive Trustees

José Blanco

Diogo de Lucena

Isabel Mota

Eduardo Marçal Grilo

Non-Executive Trustees

André Gonçalves Pereira

Eduardo Lourenço

Artur Santos Silva

INSTITUTO GULBENKIAN DE CIÊNCIA

BOARD OF DIRECTORS

The Board of Directors for the Instituto Gulbenkian de Ciência (IGC) ensures that the activities at the Institute follow the guidelines and objectives defined by the Board of Administration of the Fundação Calouste Gulbenkian. The members of the Board of Directors for 2003 were:

Board of Directors

Diogo de Lucena (Chairman)

João Caraça

Manuel Rodrigues Gomes

Manuel Carmelo Rosa

Horácio Menano

António Coutinho

SCIENTIFIC ADVISORY BOARD

The Scientific Advisory Board of the IGC scrutinises the scientific progress and teaching programmes, as well as the recruitment and activity of personnel and research groups. The Scientific Advisory Board also advises the Board of Administration of the Fundação Calouste Gulbenkian on all matters relevant to the mission of the Institute. The members of the Scientific Advisory Board for 2003 were:

Prof. Sydney Brenner (Chairman)
Prof. Philippe Kourilsky
Prof. Nicole Le Douarin
Prof. Martin Raff
Prof. Kai Simons
Prof. Susumu Tonegawa
Prof. Hans Wigzell
Prof. Lewis Wolpert

The Scientific Advisory Board met at the IGC on 1-2 May 2003.

In 2004, the renewal process of the Scientific Advisory Board was initiated. After serving for 5 years, Prof. Hans Wigzell has now left the Board. We are all very grateful for his support and advice. We welcome Prof. Jonathan Howard as the new member of the Scientific Advisory Board.

STAFF

DIRECTOR

António Coutinho

DEPUTY-DIRECTORS

Sérgio Gulbenkian
José Mário Leite

CHIEF TECHNOLOGICAL OFFICER

Matthias Haury

RESEARCH MEMBERS

The IGC is not divided into departments, and its scientific activities are organised in relatively small groups. Research is autonomously conducted by individual scientists and small groups who are free to associate in projects.

It should be noted that nearly all the scientists at the IGC are affiliated at other institutions or supported by national or international organisations; these are indicated in parenthesis. Some of those listed below were present at the IGC for only part of the year.

Jan Andersson (Univ. Stockholm/FCG)
 Jörg Becker (FCT)
 Juan Carlos Belmonte (Salk Institute)
 José António Belo (UALG)
 Mostafa Bendahmane (FCT)
 Marie-Louise Bergman (EMBO/IGC)
 Jorge Carneiro (Lab. Associado)
 Pierre-André Cazenave (Univ. Paris VI/Institut Pasteur/CNRS/FCT)
 Sukalyan Chatterjee (FCG)
 Melvin Cohn (Salk Institute/FCT)
 Ana Paula Coutinho (FCT)
 António Coutinho (CNRS/FCG)
 Pedro Coutinho (FCT)
 Ana Crespo (UE)
 Jocelyne Demengeot (FCG)
 Sérgio Dias (IPOFG/FCT)
 Francisco Dionísio (FCT)
 Sabrina Epiphany (FCT)
 José Faro (Univ. Salamanca)
 José Feijó (FCUL)
 Lisete Fernandes (ESTSL)
 Carlos Alberto Ferreira (HUSM)
 Constantin Fesel (FCT)
 Carlos Penha Gonçalves (Lab. Associado)
 Alexis Gonzalez (FCT)
 Simone Gines (FCT)
 Gabriela Gomes (FCT)
 Isabel Gordo (FCT)
 Christophe Gregoire (FCT)
 Isabel Pombo Gregoire (FCT)
 Sérgio Gulbenkian (FCG)
 Werner Haas (FCG)
 Matthias Haury (FCG)
 Domingos Henrique (FMUL)
 Dan Holmberg (Univ. Umea)
 António Jacinto (FCT)
 Gregory King (Univ. Warwick)
 Abdelkader Lakmeche (FCT)
 Joaquin Rodriguez León (FCT)
 Moises Mallo (Lab. Associado)
 Moises Marinho (UE)
 Kalet Leon Monzon (Cent. Immunol. Mol. Cuba/IGC)
 Maria Mota (FCT)
 Maria Teresa Faria Pais (FCT)
 Isabel Palmeirim (ECSUM)
 Ana Maria Pamplona (FCT)
 Michael Parkhouse (FCG)
 Paula Parra (FCT)
 Leonor Parreira (FMUL/CEBIP)
 Sylviane Pied (INSERM/Inst. Pasteur)
 Ricardo Pimenta-Araújo (FMUL/IGC)

Ana Rita Ponce (IGC)
Gabriela Rodrigues (FCUL)
Ana Paula Santos (FCT)
Leonor Tavares Saúde (FCT)
Elsa Seixas (FCT)
Gabriela Silva (FCT)
Helena Soares (ESTSL)
Miguel Che Parreira Soares (Lab. Associado)
João Pedro Simas (FMUL)
John Stewart (Univ. Tech. Compiègne/CNRS)
Élio Sucena (FCT)
Ana Teresa Tavares (FCT)
Álvaro Augusto Tavares (ISTUL)
Vera Lucas Teixeira (FCT)
Henrique Teotónio (FCT)
Solveig Thorsteinsdottir (FCUL)
Maria de Jesus Trovoadá (FCG)
Johann Truccolo (FCT)
Filipa Vala (FCT)
Tatiana Vassilevskaia (Astrazeneca/FCT)
Astrid Vicente (FCG)
Luisa Mota Vieira (HDES)
Ana Margarida Vigário (FCT)
Andrew Waters (Univ. Leiden/FCG)
William Wood (FCT)

STUDENTS

Ph.D. Students

Sónia Albuquerque (ICBAS/FCT)
Isabel Alcobia (FMUL/FCT)
Emília Almeida (ITQB/UNL/FCT)
Sílvia Almeida (ITQB/UNL/FCT)
Paulo Alves (FMUL/FCT)
Fernanda Bajanca (FCUL/FCT)
Marta Barreto (FCUL/FCT)
Leonor Boavida (FCUL/FCT)
Ana Cristina Borges (UALG/FCT)
Ana Sofia Cachão (FCUL/FCT)
Dinis Calado (FMUL/FCT)
Susana Campino (FCUL/Univ. Umea/FCT)
Marta Campos (FMUL/FCT)
Iris Caramalho (ICBAS/FCT)
Daniel Carapau (FCUL/FCT)
Marta Carapuço (ITQBUNL/FCT)
Thiago Lopes Carvalho (ICBAS/FCT) left June 2003
Ana Catarina Certal (FCUL/FCT)
Ângelo António Chora (FMUL/FCT)
Jaime Combadão (ITQB/UNL/FCT)
Sofia Cordeiro (FCUL/FCT)

Sívia Correia (ITQB/UNL/FCT)
Vasco Vieira Correia (ICBAS/FCT)
Sofia Côrte-Real (FMUL/FCT)
Sílvia Costa (FCUL/FCT)
Ana Margarida Coutinho (FCUL/FCT)
Margarida Cunha (ICBAS/FCT)
Célia Domingues (FCUL/FCT)
Mariana Faria (FCUL/FCT)
Elisabete Fernandes (FCT)
Beatriz Fernandez (ITQB/UNL/FCT)
Catarina Figueiredo (ITQB/UNL/FCT)
Mário Rui Filipe (FCT/UNL/FCT)
Cláudia Florindo (FCT/UNL/FCT)
Francisca Fontes (Hospital Egas Moniz/ Ministério da Saúde)
Ana Rita França (FMUL/FCT)
Catarina Freitas (FCUL/FCT)
Rui Freitas (ITQB/UNL/FCT)
Rui Gardner (ICBAS/FCT)
Susana Godinho (IST/FCT)
Mário Grãos (FCUL/FCT)
Vincent Guiyedi (Inst. Pasteur)
Anja Hagemann (FCUL/FCT)
Pedro Laires (FCUL/FCT)
Patrícia Leirião (IHMTUNL/FCT)
Patrícia Madureira (FMUL/FCT)
Sofia Marques (FMUL/FCT)
Maria Hortense Matos (ITQB/UNL/FCT)
Joana Monteiro (FCUL/FCT)
Joana Moreira (FCUL/FCT)
Inês Mota (Univ. Évora)
Filipa Moraes (ITQB/UNL/FCT)
Rute Nascimento (FMUL/FCT)
Hélia Neves (FMUL/CEBIP/FCT)
Sofia Nolasco (FCUL/FCT)
Helena Nunes (ITQB/UNL/FCT)
Vivian Oliveira (ITQB/UNL/FCT)
Tiago Paixão (ICBAS/FCT)
Diogo Pimentel (PGDB/FCT)
Ana Margarida Prado (FCUL/FCT)
Ana Sofia Quina (FMUL/FCT)
Alessandro Ramos (FCUL/PDEE)
Manuel Rebelo (FCUL/FCT)
Ana Luisa Reis (FMVUTL/FCT)
Cristina Rodrigues (FCUL/FCT)
Lénia Rodrigues (FMUL/FCT)
Sofia de Albuquerque Rodrigues (ECSUM/FCT)
Ana Cristina Santos (FMUL/IEFP)
Margarida Santos (FMUL/FCT)
Ana Cecília Seixas (FCUL/FCT)
Mark Seldon (ICBAS/FCT)
Ana Cristina Silva (FCUL/FCT)

Susana Silva (FCUL/FCT)
Sérgio Simões (FCUL/FCT)
Laszlo Tokaji (PGDB/ ITQB/UNL/FCT)
Ana Sofia Veloso (ITQB/UNL/FCT)
Ana Maria Vieira (FCUL/FCG)
Santiago Zelenay (FCUL/IGC/FCT)

B.Sc. Students

Ricardo Águas (Univ. Évora)
Ricardo Ataíde (Univ. Lusófona)
Cláudia Bicho (FCUL)
Inês Conceição (FCUL) left February 2003
Catarina Correia (FCUL)
Vanessa Cristão (FCUL)
Tânia Cruz (Univ. Lusófona)
João Duarte (Univ. Coimbra)
Lurdes Duarte (Univ. Évora)
Diogo Fonseca (Univ. Algarve)
Joana Duarte (FCUL)
Andreia Cunha (FCUL)
Dinis Gokaydin (FCUL)
João Gonçalves (FCUL)
Raquel Lourenço (Univ. Lusófona)
Marta Luz (FCUL) left July 2003
Susana Matias (FCT/UNL)
Inês Matos (Univ. Évora)
Ester Morgado (École Sup. Techniques de Biol. Apl., Paris, France)
Ana Margarida Nunes (FCUL)
Nuno Pedroso (FCUL) left October 2003
Lília Perfeito (FCUL)
Carlos Pereira (FCUL) left July 2003
Joana Ribeiro (FCUL) left September 2003
Pedro Rifés (FCUL)
Yara Reis (Univ. Beira Interior)
Joana Rodo (Univ. Évora)
Luís Saraiva (Univ. Évora)
Ana Filipa Simões (FCUL)
Pedro Vale (Univ. Évora)

Laboratory Technical Support

Ana Água-Doce (BIC/FCT)
Ana Alexandra Almeida (BI/FCT)
Paulo Almeida (Lab. Associado)
Dolores Bonaparte (BTI/FCG)
Daniela Brites (BI/FCT)
Marisa Cabrita (BTI/FCG)
Lara Carvalho (BTI/FCG)
Carl Collins (BIC/FCT) left March 2003
Maria do Céu Conceição (BTI/FCG)
Ana Neves Costa (BI/FCT)

Sofia Couceiro (IEFP)
Joana Costa Dávila (BIC/FCT) left August 2003
Ligia Deus (IEFP)
Carla Fernandes (BTI/FCG)
Ricardo Ferreira (IEFP)
Lidia Fonseca (BTI/UE)
Ana Cristina Gaspar (BTI/FCG)
Alexandre Gonçalves (BIC/FCT)
Susana Magrito (BTI/FCG)
Sara Marques (BI/FCT)
Sara Rute Marques (BIC/FCT) left July 2003
Bruno Mateus (IEFP/FCT)
Ana Nóvoa (Lab. Associado)
Dominique Ostler (BTI/FCG)
Marcos Pinho (IGC)
Rui Rodrigues (BIC/FCT)
Nuno Sepúlveda (BIC/FCT)
Catarina Silva (BTI/FCT)
Susana Silva (IEFP)
Sofia Simões (BTI/UE)
Sónia Ventura (BI/FEDER)

ADMINISTRATIVE, SECRETARIAL AND TECHNICAL STAFF

The administrative, secretarial, and technical staff of the IGC provide support to the research and teaching activities. Everyone here worked at the IGC for all or part of 2003.

Administrative and Secretarial Staff

Sofia Andrade (IEFP)
Manuel Carvalho
Manuela Cordeiro
Jorge Costa
Greta Martins
Fátima Mateus
Maria Matoso
Ana Carolina Maya
Margarida Meira
João Nunes
Ana Lícia Pires
Ana Maria Santos
Vitor Santos
Abílio Simões
Teresa Maria Sousa
Lurdes Torres

Laboratory Technical Staff

Ana Cristina Leitão Homem
Júlia Lobato
Isabel Marques

Nuno Moreno
Rosa Maria Santos
João Sousa

Technical Support Staff

António C. Ligeiro
João Carlos Lopes
Severino Matias
Carlos Nunes
António Sousa
Vitor Varão

UNITS AND SERVICES

The IGC has set up and runs a series of differentiated Services and research-supporting Units that are manned, operated and financed under institutional responsibility. These Services and Units provide regular scientific and technological expertise and advice, as well as personnel support, to the researchers at the IGC and elsewhere in the campus, while open to others in Portugal and abroad.

Animal Facility: Jocelyne Demengeot

Bioinformatics: Pedro Fernandes

Cell Imaging: Sérgio Gulbenkian/Matthias Haury

Histology and Histopathology: Miguel Soares/Sérgio Gulbenkian

Informatics: Matthias Haury

Library and Scientific Information: Sérgio Gulbenkian

Science and Society: Ana Paula Godinho Coutinho

Sequencing and Genotyping: Carlos Penha-Gonçalves

Theoretical and Computational Biology: Jorge Carneiro

Transgenic Unit: Moises Mallo

The activity in all Units and Services is accompanied and adjusted to current needs by Users Committees that include a good fraction of all scientists of the Institute.

Animal Facility

This year the facility doubled its capacity both in the production and the experimental areas. Major efforts have been put in setting all animals of the production and the main experimental area in Specific Pathogen Free conditions according to the novel regulation that strictly excludes *Helicobacter* colonization. This implementation required efficient embryo transfer technology, a procedure we now perform twice a week. Moreover, the production of conventional mouse strains has been scaled up and now satisfies the requests of both internal and external users. Expansion of the most used experimental colonies is being implemented to reach the same satisfaction. At the closing of the year, the animal house facility is hosting about 20 000 mice representing 80 different strains. It has accommodated the experiments of 18 IGC and 2 external research groups, and delivered animals to 15 Portuguese Institutions.

Bioinformatics

The activity of the Bioinformatics Unit was spread in four main directions: organization of training courses under the GTPB; organization of the first year of the PGBIOINF, the new Post-Graduation course; provision of counselling services for IGC members, mainly from the research groups on Mitosis, Inflammation, Lymphocyte Physiology, Malaria Cell-Biology, Organogenesis, Segmentation, Stress & Cytoskeleton; and finally, provision of counselling services for external users, mainly from Faculdade de Medicina Veterinária (Lisboa), Laboratório Nacional de Investigação Veterinária (Lisboa), Faculdade de Ciências da Universidade de Lisboa, Faculdade de Ciências da Universidade de Coimbra.

Cell Imaging Unit

The year 2003 was again a year with a further increase in the already extensive usage of the UIC Services by most IGC scientists and many external users. In order to suit better the increased demand in equipment availability and to complete the spectrum of microscopes available we purchased one more Leica Inverted DM IRE2, one Leica DMLB2 and one DMLS2. We additionally purchased 5 new computers and 2 CCD cameras in order to improve the image analysis capacities.

The most used equipment were again the Leica DMR2 Microscope with over 2800 hours of usage, followed by the Leica Confocal, the FACSCalibur and the Moflo Highspeed Cellsorter (all between 1200-1800 hours).

The histology section produced again more than 2500 slides for IGC users, and over 40 monoclonal antibodies are available purified and labeled with at least 3-5 different fluorochromes. Some of these purified monoclonal antibodies are used in large quantities (especially for in vivo injections and cell-sorting), and together with our custom enzyme production the UIC unit is contributing considerably to reduce the costs for commonly used reagents.

The media preparation service centralises media and buffer preparation for all of the IGC, and is also managing the central cleaning and sterilisation facility.

UIC personnel is also involved in the maintenance and programming of the IGC website, the UIC website, the IGC Online equipment reservation system, the inventory and ordering system, as well as laboratory security.

Informatics

In 2003 was the first year of the complete restructuring of the IGC Informatic Services under a new direction. This restructuring was absolutely necessary in order to keep the IGC on level with new technologies and allow the implementation of better security measures in order to fight an increasing number intrusion attempts and virus attacks.

A new email system was setup on a specially secured and daily backed up server, with integrated antivirus control and SPAM filters. Various mailing lists are now standard for internal and outside communication in the IGC.

New load-balanced and clustered DNS (domain name servers) Servers were setup for both the new internal and the from the outside accessible DMZ (demilitarized zone) network zones. New central switching equipment was purchased and 2-4 Gbit/s

Network Backbone was setup, with desktop gigabit connectivity established in most areas of the IGC (still ongoing in 2004).

The internet service provider was changed to the FCCN, which allowed us to increase the internet bandwidth over 6 times while reducing costs by over 30 %. A new network topology was implemented allowing us to use an internal IP address space to end the limitation of 256 IP addresses in the previous system. A new router and a special firewall was installed in the network in order to secure the internal network from outside attacks. VLAN (Virtual Local Area Networks) are implemented in order to limit local traffic inside the various zones of the IGC (wings) and VPN (virtual private networks) technology is being made available for special high security access for scientists requiring special access from their homes.

Several new SQL databases were setup and web-interfaces programmed in order to implement central management of human resources, equipment inventory, computer network connections, access card systems and a fully dynamic database driven website was build.

The electronic door access surveillance system was re-configured and interfaced with our user databases for minimal administrative overhead.

Several new wings were equipped with networking infrastructure (cables, switches etc).

Computer software licensing has also been organised in order to maximize benefits through high volume educational discount prices.

All together, this first part of the restructuring yielded an significant number of improvements for all IGC users and we are confident to be able to solve most of the outstanding issues in 2004.

Library and Scientific Information

In 2003, the IGC library received 125 subscriptions and provided local online access to 122 titles.

The IGC library was visited by some 20.000 readers in 2003.

Sequencing and Genotyping

In the year 2003 the Sequencing and Genotyping Unit has sequenced some 4780 samples for IGC users and for the following institutions: Centro de Genética e Biologia Molecular da Universidade de Lisboa; Faculdade de Farmácia da Universidade de Lisboa; Faculdade de Medicina da Universidade de Lisboa, Instituto de Medicina Molecular da Faculdade de Medicina de Lisboa; Laboratório de Biologia Molecular do Instituto Português de Oncologia; Instituto Superior de Ciências de Saúde.

One of the sequencing machines was also used for genotyping in a "self-service" mode by IGC users.

Transgenic Unit

During 2003 the Transgenic Unit at the IGC consolidated its activities and increased its capacity to produce transgenic lines and embryos by pronuclear microinjection. During this period, a total of 14 different constructs were injected. 4 of these constructs produced 12 different transgenic lines that are currently analyzed for transgene expression and phenotypic consequences. In addition, 10 constructs were injected to perform transient transgene analysis. In these experiments, a total of 60 transgenic embryos were obtained by cesarean section at embryonic stages E9.5, E10.5 and E14.5.

In 2003 the Transgenic Unit moved its physical location within the IGC's Animal House, from a non-SPF room into the SPF area. This move has allowed for the production of transgenic founders with a SPF status, thus avoiding the need of rederivation of specific transgenic lines (particularly, those produced for immunological studies) into the SPF area.

INTRODUCTION

The Instituto Gulbenkian de Ciência (IGC) was founded and is supported by the Fundação Calouste Gulbenkian, representing the Foundation's direct intervention in Science, one of its statutory goals.

The Institute harbors two Research Centers (Unidades Plurianuais) of the National Research Council (Fundação para a Ciência e a Tecnologia), and it integrates, together with the Instituto de Tecnologia Química e Biológica and the Instituto de Biologia Experimental e Tecnológica, one Associated Laboratory at the Research Council. The IGC is a member-institution of the European Mouse Mutant Archive, and it hosts a Laboratoire Européen Associé au Centre National de la Recherche Scientifique (France), as well as the Portuguese node of the EMBnet.

The Institute is responsible for the Gulbenkian PhD Program in Biomedicine - supported by the Fundação para a Ciência e a Tecnologia, the Secretaria de Estado da Ciência e do Ensino Superior, and the Fundação Luso-Americana para o Desenvolvimento – and it runs its own PhD Program (PDIGC), for all doctoral students working in our laboratories.

Support for projects or individual students and scientists has also been obtained in 2003 from the Fundação para a Ciência e a Tecnologia, the European Union, the Fundação Calouste Gulbenkian, the European Molecular Biology Organization, the Juan March Foundation, and the National Institutes of Health (USA). Research contracts have been passed with Astra-Zeneca (UK) and Alfama (P).

Strategy and Results

Six years have passed on the decision of the Calouste Gulbenkian Foundation's Board of Administration to reform the Instituto Gulbenkian de Ciência, opening a new period in its history. A history that belongs now to many more, a history of enthusiasm and much work, of rigor and creativity, of openness, exchange and community, of many a difficulty, but of many joys as well. The hand-full of starting groups working in the cellar while waiting for the renewal of laboratory spaces, have now been joined by many others, one after the other, at the pace of reconstruction. We are now over 200 people at the IGC, scientists, students, technicians and administratives; everyone convinced – I would think - that it is enriching and fun to work here, and share the responsibility for the Institute's performance, reputation and attractiveness. Over 200 new individual plans were thus cherished, and many common projects were launched, some with success, all with the same engagement and conviction that doing science is a most relevant and rewarding activity in the pursuit of a better world. For what this represents in personal coherence, and regardless of the individual achievements or disappointments, we are all very grateful to the Gulbenkian Foundation for the support we have received. The Institute is a space of liberty and rationality, yet of excitement and passion. We are aware that our strength can only be derived from the uniqueness of each one and the cooperativity it engenders, rooting our hopes on both the individual diversity and the community of ideals. As in biology, cooperativity is the key to higher levels of organization that bring about further complexity, a fountain of novelty, a source of increasingly interesting properties that emerge, as by miracle, from the limited abilities of each component.

The IGC has adopted a model of “host institution” with a high turnover, which others have declared “suicidal” in terms of institutional building. Most of the groups now at

the IGC have been “in house” for less than 5 years, but several of the initial groups have already left, and others are preparing to do so ! Such a high turnover strategy is a continuous re-start, representing the never-ending repetition of much work, worries and uncertainty, but also of excitement, joys and gains: for every group that leaves, the Institute loses an important asset but gains another... and a new root elsewhere. The strategy seems to be working after all, precisely in terms of the institution. Thus, we all know that the time we spend here - good as it might be - is limited. None of us, therefore, aim at establishing “private” domains, power or influences that might be promises of individual advantages, for these would soon be useless. Moreover, we do not really have the time to accumulate bad feelings or a history of disaffections. Rather, we know that we should better try and do the best out of this short time, which we hope to keep as a good life memory. We all know that the better the institution fares, the greater the advantages that will emerge and the more abundantly they will be “distributed” by all those who are, have been, and will be members of the IGC. Paradoxically, in such a “transient” set-up where “nobody has a future”, the Institute seems to have one, as it continues to produce good science and a lot of it. Indeed, looking at the set of publications in the most competitive journals in the world¹ over the last three years, the IGC has produced a third of all science of excellence in Portugal ! This is perhaps the first thing to underline in the present report, for the trend over the last five years cannot be due to chance, to the coincidences of small numbers, or to some sort of beginners’ luck².

This result is most surprising, considering the very small size of the Institute, relative to the research community in the country, and the fact that many of the groups at the IGC have been operating for a few years only. In turn, this performance validates the institutional strategy and demonstrates the operability of the model, providing an exceptional return for an investment that represents only 0.5-1% of the total spending in Science & Technology in Portugal³. This is reassuring, as it seems difficult to find better ways to spend money in science. All the more so as the same investment also pays for the Gulbenkian PhD Programs, which have now gained ample international recognition as an example of “best practice”, innovation and quality. It is thus possible to produce internationally competitive science and science education in Portugal, as other groups and institutions had previously given proof and continue to do so. Clearly, however, in spite of recent progress, the Portuguese Science & Technology system remains fragile in terms of producing science at the highest level. Science is universal, just as “the market” is global, and there is no valid reason to invoke a putative “national interest” in the defense of scientific production that is only “locally relevant”. Supporting research by criteria that place local interests above international competition is inexorably doomed to failure, as it promotes institutions and individuals whose local reputation far exceeds their real performance in the

¹ Scientific performance is currently measured by several parameters, the first of which is the quality and the number of scientific contributions to the world scientific literature. While it is recognized that the very high impact journals often make decisions on publications that are not restricted to the quality and interest of the work, while very low impact journals continue to harbor very interesting papers, it is clear that publication in the top journals continues to be the aim of all scientists.

² For journals with Impact Factors equal or above 15.0, the IGC’s share in all papers that were published with an address in Portugal is 25.0%, 28.4% and 34.0%, respectively, for the last 5, 4 and 3 years. I have hesitated a lot in dealing so bluntly with these matters, which can readily be taken for immature and valueless boasting. As I have contributed very little to this performance, however, I feel free to promote the excellent work of my colleagues who give so much of their life, competence and enthusiasm to this project.

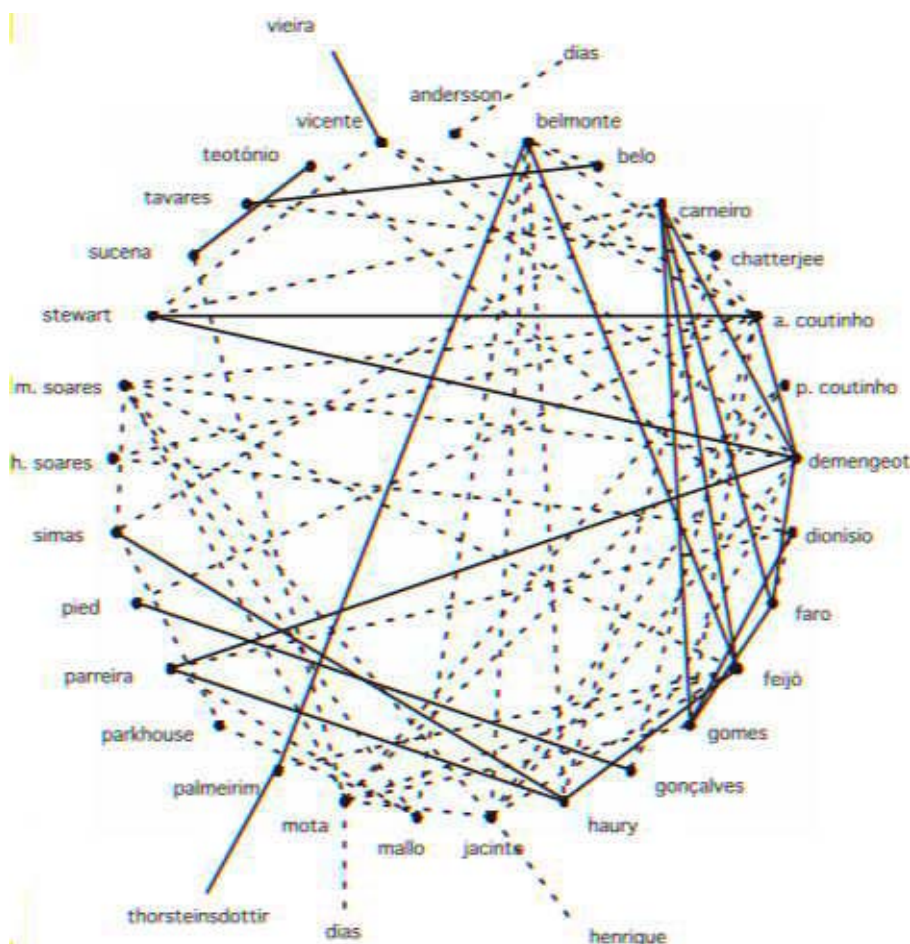
³ Estimated from the data available at Observatório da Ciência e do Ensino Superior (MCES) for the total I&D spending in Portugal.

world. Development and innovation urgently require discontinuing such policies of investment in the “unproductive mediocrity”, which have long contaminated national (and European) public investments. Most importantly, these results relate to institutions, rather than to groups or individuals, making it likely, therefore, that performance depends on institutional strategies, rather than on isolated individuals, whoever they are. The great advantage of the IGC is that it can diverge from institutional models based on hierarchy and departmentalization, on a civil servant mentality emerging from the safety and protection of tenured positions, on the majority rule of individual interests. Critical institutional decisions on the choice of research themes and of individuals cannot be left to systems that, permeated by “influences” and diluting responsibilities, can only be “democratic” in the rule of vote.

While the publication record is currently the only indicator that allows for a comparative analysis in Portugal, there are great limitations in reducing scientific production to articles in high impact journals. The IGC should also be evaluated by other parameters: education of new scientists, impacts in the local scientific community, the number and performance of research groups it attracts, installs and exports, patents and gains from intellectual property, contribution to entrepreneurial initiatives, the promotion of science in society, its international reputation. We are well aware that the IGC has a long way to become a reference institution in the world, and these are our standards. Yet, by its outputs in scientific research and education, in technology and innovation, the Institute contributes, if modestly, to the ultimate goals of the Gulbenkian Foundation - a better society, more productive and cultured, more responsible and just. Additionally, the IGC serves as a “field experiment” in institutional models, such that the Gulbenkian Foundation provides public authorities with timely “hard data” on alternative modes of research organization and science support. With the validation of the IGC model, the Foundation has now good reasons to support other institutions adopting comparable strategies and reaching similar outputs. Furthermore, with first-hand information on a number of young scientists who have given proof of autonomous competence and determination, the Foundation may wish to take further risks in innovation and engage in the next strategic step: a network of research groups throughout the country, operating in a similar model, sharing a set of principles in the pursuit of common goals. In other words, for a strategic model of this kind, an institute goes well beyond its walls and it actually needs not them at all.

It may be interesting to analyse the reasons that brought about the IGC’s performance. Obviously, good science can only be produced by good scientists, and the very first explanation for those results is, certainly, the quality of the scientists at the Institute. Yet, I would think that none of my colleagues at the IGC would object to my contention that, individually, we are no better nor worse than scientists in other Portuguese institutions. It follows that the difference must be in the institutional structure and mode of operation. At the IGC, quality and competence, generosity and individual responsibility take the place of hierarchy. The organizational structure is characteristically “horizontal” and, thus, cooperative, based on the complementarity of scientific interests and on sharing of resources, equipments, and services. In short, the fundamental thing here is perhaps not the scientists, but the relationships amongst them that, nevertheless, require the scientists to be what they are, and promotes that students and beginners learn to be that way. This apparent paradox is solved in a discussion between the Great Kahn and Marco Polo that was invented by Calvino in one of his beautiful books. Polo is describing to the Kahn what is a bridge and how it is made, stones laid on each other in the form of an arch. The Kahn interrupts him to

ask “of all those stones, which one supports the weight of the bridge ?” Surprised, Polo says that “the weight is not supported by this or that stone; it is the arch they form that takes the weight”, only to hear from the Kahn “Why, then, do you tell me about the stones ? Only the arch is interesting !” Polo reflects a moment and closes “Without stones there is no arch”. Institutional building has a lot to learn from this piece of Calvino. Figure 1 shows the structure of the network defined by the research interactions amongst the groups at the Institute⁴.



As compared to most Small World Networks (SWN), this is an extremely small SWN. Yet, it shares many of the SWN properties, notably, a degree of randomness in the establishment of its connections, short average “path lengths” between any two individuals, and predominantly “weak” connections. Currently, laypersons know of SWNs by one of their characteristics: once you enter the network, you can progress to any other node with very few steps (or “degrees of separation”). In other words, you may not know Madona personally, but if you know some one in the “show-business world” – which is a small one – this acquaintance of yours will know somebody, who will know somebody who knows Madona, perhaps with another few steps in between. As the graph shows, there are very few “degrees of separation” between any of the actual research projects developed by IGC groups.

This was a central goal in the institutional strategy, which determined the choice of operational model: sharing of resources (space, equipments, and service competences)

⁴ The graph maps collaborative research projects, obviously not any other type of interactions or exchanges between individuals in different groups.

that fostered sharing of ideas and projects amongst the various groups. This seemed absolutely essential for several reasons. First, because the limited size of the groups at the IGC – imposed, in turn, by the “export” rates that were necessary for ensuring a significant overall impact - precluded that each group had the ability to set-up various techniques and competences. Hence, these would have to be centralized and shared. Second, limited financial capacity imposed the need to operate under conditions for maximal returns (e.g., machine occupation, personnel multivalence). Last, but not least, because scientific competitiveness would certainly increase if the IGC’s scientific contributions would center in areas of overlap in specific interests, at the edges of competences that are available in the various groups. In short, the IGC is too poor, and its research groups are too small and internationally too insignificant to excel in conditions other than finding strength in cooperation and community of achievement. In turn, this strategy required thematic diversity (dispersion, as others would call it), laying the foundation for the IGC’s diverse scientific production in top journals, rare amongst Portuguese research institutions. Thematic diversity, on the other hand, while limiting investments in each area, brought additional advantages in graduate education, and it did contribute to higher cooperative levels. Accordingly, the graph shows that, as expected, connections are usually more frequent among the groups with the same specific interests. Yet, there are now many productive interactions between apparently unrelated groups (e.g., immunologists and developmental biologists, parasitologists and geneticists), with a solid basis of mutual interest between theoreticians and empiricists. I shall add that such collaborations are not based on technology only: it is in the essence of the technology support Units at the Institute to “collaborate” with any group who needs them, but such “services” are not counted in this graph.

How did this network come about and which are the perspectives for its evolution ? A structural organization that encourages interactions, and an enhanced performance that derives from cooperation, were obviously not enough. As Calvino’s Marco Polo argued, any design or strategy would fail miserably if it were not based on the appropriate choice of people. Hence, in addition to intellectual excellence and the scientific track record of individuals, two other major selective criteria have been essential in choosing them: population diversity, as well as personal generosity and openness. If most of the IGC’s problems arise from my poor performance in “organizing” the network, my truly serious mistakes have been in the choice of individuals, some of which may have been most concerned with their own short-term advantages, or else, too insecure to engage in open exchange and freely contribute their own competences to a better performance of others. Now, the evolution of networks in time goes well beyond their internal dynamics, representing what Francisco Varela and I designated, years ago, by “meta-dynamics”. One of the many interesting properties of networks is their dynamic robustness and resilience, which depend on network structure – defined, as in Figure 1, by numbers of “nodes” and “connections” and by the overall architecture. The architecture of the IGC’s very small SWN is not of the so-called “aristocratic” type, that is, with most connections being single and organized around a few highly connected “nodes”. Thus, while some “nodes” are more connected than others, the respective values are not much higher than average. This is interesting, as “aristocratic SWN” have an “Achilles tendon”: they often fall apart upon removal of one or a few “hubs”. In contrast, “distributed” SWN are more robust, such that they may be amputated of one or more “nodes” and respective connections, while maintaining, nevertheless, their basic dynamic properties. In other words, networks of individuals may dispense of each of the individuals themselves. This is the goal of the IGC: to practice thoughtful and

interactive science on principles that provide for an institutional stability which becomes independent of the individuals and goes well beyond the current actors and set-up. As with any biological network, its operation depends upon a “domain of interactions” with the environment (e.g., food), but the nature of its product is not determined by the environment (e.g., the kind of fertilizer does not determine the kind of grass). Viability, on the other hand, requires that the network is able to adapt its dynamics to changes in the environment, but also that environmental conditions are kept within the boundaries of the interaction domain. Needless to say, the kind of dynamics and the stability of a SWN does not depend either on “external” factors, such as directors or organizers, the function of which is limited to ensuring those environmental boundaries of viability. The next issue is how to help this network growing within and without the walls of the Institute. Obviously, new groups arriving at the IGC must be provided with conditions to rapidly establish connections, while those leaving Oeiras should not necessarily have to abandon the Institute’s network. It follows that the addition of new research areas to those currently established can only be done if several groups are simultaneously installed. In turn, it will suffice that a few of these establish “bridges” to other areas, for a renewed dynamic vigor to emerge, perhaps with novel scientific contributions at these newly created “edges”, as one would hope. This has been the recent experience with evolutionary biology at the IGC, where the first four groups should soon be joined by a few more, but already share many a project with others. Conversely, for those leaving Oeiras there is no reason to break current links within the Institute. Rather, these might become even more important for survival, in the face of new environments. Interestingly, the maintenance of current “connections” automatically provides for a natural growth of the IGC’s SWN. As long as we share principles and goals, we all belong together, and as long as we maintain operative “connections”, we share a common dynamics wherever we are. Again, it is up to the “organizers” to ensure conditions for network viability, now in a larger and diversified environment.

As I come to the end of this introduction, I must add two more notices. First, it would be unfair and grossly inaccurate if the reader is left with an idyllic image of harmony in a problem-free community of systematically creative, generous and tolerant individuals. This is definitely not the case. The people at the IGC are not more inclined to an Assisian behavior or life-style than colleagues elsewhere. There are many kinds and lots of problems at the Institute, such that, everyday, we all have to cope with disappointments, and fight our ways through individual hang-ups and ill-adapted rules to more openness and reliability. Yet, if the above description is edulcorated, it certainly catches the essentials of the institutional condition. Second, again as in real biology, evolution goes without a blue-print, life today is what it happened to be in the domain of what it could be. Or, as the poet put it, “caminante no hay camino, se hace el camino al andar”. Hence, the IGC is today what we all made it to be in the conditions we had, largely the product of “chance and necessity”, the work of a “blind watch-maker”. If a glimpse of a strategy there was in the institutional design, this was a mere adaptation of older experiments to a new environment. Yet, some steps in the path were already walked and whatever the new way will be, it can only go from here.

Science and Society at the IGC

Necessary as it is for the Institute’s survival, heuristic as its practice might be for a set of principles and for education, scientific production is but one aspect of the strategic goal. The “IGC project” goes well beyond the current scientific activity at the Institute, and should be judged accordingly, if in some years only. One of these “other

missions” concerns the promotion of science outside the scientific community. In modern societies, science is the ultimate motor of all innovation and socio-economic progress, as it constitutes the unique origin of novel technologies. Hence, the designation of “knowledge-based” to which developed societies aim. Science is also a strong pillar of modern democratic socio-political systems. Thus, science produces explanations of the world and of ourselves that are based on the rational derivation of natural laws, using approaches which foster the fundamental values of doubt (or “objective skepticism”, as put by others) and of the contradictory debate of ideas and evidence. Clearly, democracy cannot be based on superstition, ignorance and intolerance. It is all the more surprising, therefore, that governments are generally little inclined to invest in education and science, systematically preferring the short-sighted view of the immediate returns promised by investments in the “productive sectors” (often using outdated technologies and processes). Accordingly, public economic measures, rather than re-inforcing education and R&D by enlarging the respective recruitment bases, often aim at ensuring the maintenance of employment in those “productive sectors” only. Many of us, however, feel that a better and richer society requires higher investments in science and science education, and are ready to contribute the appropriate information and arguments to the public. We hope that, in turn, public opinion will force politicians to follow.

For scientists and scientific institutions, therefore, aware as we are that the robustness of scientific activity varies with the strength of its roots in society, the defense of science by the promotion of its values and contributions becomes a principal concern. There are basically three alternative modes of promoting science in society. The first is, of course, in the schools, where dedicated professionals are in the best place, and have the best audience, to do it in the most productive way. Accordingly, the IGC has promoted regular activities either with secondary school biology teachers, or directly with very young students. Along the year, the Institute is visited by a considerable number of biology classes. Accompanied by the respective teachers, the youngsters are informed on the major questions in modern biology that are tackled here, visit laboratories and discuss with the scientists. The Institute also continues to organize regular sessions with secondary school teachers, who spend an afternoon at the Institute discussing with scientists, often PhD students, “hot” topics in modern biology: “life-span” and ageing, immunity and emergent infections, stem cells and developmental biology, biotechnology and transgenic food, genetics of disease and predictive medicine, among others. The second mode of communication between scientists and the public is indirect and “mediated” by the press, radio and television. The IGC hosted last year a number of radio and television programs, on both specific research topics and on general questions of scientific development in Portugal. Effective as it is, however, “media”ed science is not ideal for at least two reasons: it is unidirectional, and it requires interactions between scientists and journalists that are not always optimal. In order to promote mutual awareness of each side’s objectives and methods, to overcome natural suspicions, and to provide information in a most informal setting, the IGC has organized regular monthly encounters of journalists with scientists with a very loose program. From both sides of the fence, we have collected very positive reactions. Finally, the third way to contact the general public is simply to arrange such that scientists and science students meet with citizens to exchange concerns and expectations, objectives and dreams. This has also been done at the IGC, giving raise to a most productive interaction with Oeiras’ citizens.

These questions were debated at a workshop on Science Communication, organized at the IGC by three alumni of the Gulbenkian PhD Program with interests in this area. Scientists, communicators and authorities discussed for several days strategies for

improving the engrafting of science in modern society. While there were no “official” conclusions, my reading from those discussions includes: (1) scientists must do more to avoid some degree of “sensationalism” in their general attitude, in their own public announcements, even in the scientific journals they publish; in short, scientists must stick to their principal rule of ethics, and always speak the truth; (2) “science communication” should be done by the best scientists; even if these might be poor “communicators”, they represent our most honest image, better than that of frustrated scientists who find in communication a refuge to their disaffection or poor science performance; (3) public communication must be primarily directed at the “values” of science – truth, rationality, doubt, open debate, validation, progress - rather than to its contents, for these change with time and fashion; (4) science communication must follow “local rules” and, rather than organized in transnational campaigns, should be anthropologically and socially adapted to each community; (5) last, but not least, no Science & Society initiative, excellent as it may be, can make us forget that good schools ensure the best way to promote a culture of science.

The motor of all these activities has been Ana Paula Godinho Coutinho, a developmental biologist at the IGC, whose engagement in science communication brought us a whole new approach and competence, which I very much want like to acknowledge.

The Thinking of Art/Representations of the World

As their colleagues elsewhere, most students and young scientists spend many hours a day (and a night) at the Institute, and their free time is already too short for the kinds of leisure that are characteristic of their ages. Yet, scientific creativity, while requiring “to think about it all the time”, has everything to gain from opening to other sorts of human brain activities, provided these are serious and solid. It is also likely that exposure to alternative “representations of the world” will provide young scientists-to-be with the diversity of views and the zest of humbleness they need for a richer and happier living – whatever this might be. It appeared appropriate, therefore, to offer them a series of lectures and discussions on various forms of “art”: architecture and painting, music and dance, cinema and photography.

Leonor Parreira had the great insight to compose this series, and we were privileged to host some of the most representative experts in Portugal, giving us a glimpse of their own “representations of the world”. Many of the scientists manifested their disagreement with some of these views, and with current opinions on art evaluation, generating most interesting exchanges. Yet, we have all gained by understanding that people may use intelligence in ways other than our own. These series are now to be continued with yet other “explanations of the world”, discussing economics, history, philosophy and religion, but also basic physics and chemistry.

RESEARCH

The IGC's scientific interests are centered on the genetic basis of development and evolution of complex systems, privileging organism-centered approaches and using experimental models that include plants, yeast, flies and mice, while working on the genetics of complex human diseases as well. A strong theoretical sector is also one of the IGC's specificities.

Following a suggestion of our Scientific Advisory Board, this Annual Report is presented in a slightly different way, as compared to previous years. Individual projects of scientists continue to be listed here and their full description available in the IGC's web page. Some organization was now introduced, with short introductive summaries to the various areas of research. I thank all the colleagues who helped in the preparation of these summaries.

Experimental evolution

The four groups concerned with Evolutionary Biology aim at studying the processes of natural selection, genetic drift and mutation, in general, and of adaptation to novel environments, in particular. All groups follow an "experimental evolution" approach, where the experimenter seeks to control the conditions under which evolution occurs, in a reproducible manner, in order to observe its course of action. This approach has proven highly successful to test basic theory on the evolution of aging and life-history, on antibiotic and parasite resistance, on co-evolution and eusociality, on frequency and density-dependent natural selection, on the role of mutators in evolution, among many other topics. When coupled with the analysis of genes implicated during evolution, a description of adaptive landscapes can be integrated with the physiological and developmental mechanisms generating them.

The model organisms presently used in the Institute include *Escherichia coli*, *Drosophila spp.* and *Caenorhabditis elegans*. Our common research interests are centered around the genetics of adaptation, with specific projects in: 1) genetic mapping of life-history traits during reverse evolution and laboratory adaptation, using linkage disequilibrium association mapping in *Drosophila*; 2) mating system evolution in *C.elegans*; 3) genetic networks and *cis*-regulatory gene evolution generating interspecific morphological variation in *Drosophila*; 4) genetic mapping of adaptation to an environmental toxin in *D.melanogaster*; 5) evolutionary dynamics of mutator *E. coli*; 6) estimation of the distribution of effects of novel beneficial mutations in *E. coli* when adapting to novel environments; 7) co-adaptation between bacteria and plasmids; 8) population genetics models to access how adaptation shapes patterns of genetic variation in natural populations; 9) theoretical models for the evolution of cooperation.

In the next few years, we expect to install new groups in this area, to re-inforce evolutionary thinking in other programs at the Institute, and contribute to promoting the study and public knowledge of evolution in Portugal.

Evolution of virulence: the case of plasmids

Member: Francisco Dionisio

Students: Inês C. Conceição, Ana C. Marques, Luís Saraiva, Pedro Vale

The aim of this project is to study the reason(s) why most plasmids are non-virulent to their hosts. Here, the word “virulence” is used as a synonymous of fitness cost, or of decrease in growth rate of the host due to the presence of the plasmid. One of the main hypotheses explaining the non-virulence of plasmids is that, once a conjugative plasmid enters into a new host, co-evolution may occur. That is, either or both the conjugative plasmid and the bacterial cell may mutate such that the growth rate of plasmid-bearing cells becomes as high as ancestral plasmid-free cells. In fact, this may occur. This project will contribute for the understanding of the evolution and epidemiology of plasmids.

Mutator dynamics in *Escherichia coli*

Members: Francisco Dionísio, Isabel Gordo and Lisete Fernandes

Students and Technicians: Ana C. Marques, Lília Perfeito

Collaborators: Ivan Matic, Universite Rene Descartes-Paris V, France

The proportion of mutators observed in surveys of natural populations is some orders of magnitude higher than expected under a simple mutation/selection equilibrium model. For example, certain viruses, bacteria and cancer cells have abnormal high mutation rates. This mutator phenotype is a two-edged sword: it confers the potential for faster adaptation but also the short time cost of deleterious mutations. However, understanding how mutators can rise in frequency is still an unsolved problem. The aim of this project is to contribute for the understanding of this.

Models of natural selection in populations and chromosomes with restricted recombination

Member: Isabel Gordo

Collaborators: Doris Bachtrog, Cornell University, USA

We study the population genetics of adaptation in non-equilibrium haploid asexual populations. We find that the accumulation of deleterious mutations, due to the operation of Muller’s ratchet, can considerably reduce the rate of fixation of advantageous alleles. We show that, while the rate at which adaptive mutations fix is reduced, the rate of fixation of deleterious mutations due to the ratchet is not changed by the presence of beneficial mutations as long as the rate of their occurrence is low and the deleterious effects of mutations (s_d) are higher than the beneficial effects (s_a). When $s_a > s_d$, the advantage of a beneficial mutation can outweigh the deleterious effects of associated mutations and, under these conditions, a beneficial allele can drag to fixation deleterious mutations. We propose analytical approximations for the rates of accumulation of deleterious and beneficial mutations. Furthermore, when allowing for the possible occurrence of interference between beneficial alleles, we find that the presence of deleterious mutations of either very weak or very strong effect can marginally *increase* the rate of accumulation of beneficial mutations over that observed in the absence of such deleterious mutations.

Experimental evolution and the genetic basis of adaptation: analysis of candidate genes during reverse evolution

Members: Henrique Teotónio

Students: Daniela Brites

External Collaborators: Anthony D. Long (University California Irvine, USA)

The reversibility of evolution at the phenotypic level and its genetic mechanisms have been studied recently using an experimental evolution approach. This approach is characterized by imposing a specific laboratory environment in populations of known evolutionary history, with selection, drift and mutation being directly quantified. Until now however the reversibility of evolution at the molecular level has only been studied in viral populations. The extent to which the evolutionary dynamics of sexual populations is similar to those of asexuals is not known. Here we propose to address this question. Current approaches in sexual organisms devised to map the genetic factors of complex characters such as adaptation itself, suffer from several problems. One promising new venue is the use of a high density of molecular markers distributed throughout the genome and then associate differences in the phenotype of interest with differences in molecular marker frequencies. We propose to genetically map the genetic factors behind reverse evolution in laboratory adapted outbred populations of *Drosophila melanogaster*. We will determine the extent to which evolution is reversible at two candidate loci: CuZn-Superoxide dismutase (*Sod*), an enzyme involved in the catabolism of oxygen free radicals, and Phosphoglucosmutase (*Pgm*), an enzyme involved in the glycolytic pathway. The first phase of the project will give an extensive characterization of the polymorphisms and linkage disequilibrium present for two 10Kb regions, encompassing the two candidate loci. This information will be necessary for the second phase and it will also determine the density of markers necessary for future studies (not included here) where genome-wide mapping strategies will be undertaken. The second phase of the project will determine the extent of reverse evolution at the molecular level by tracking frequency changes in marker-alleles at the *Sod* and *Pgm* loci with the phenotypic evolutionary trajectories characterized in a previous study of experimental reverse evolution. To our knowledge it will be the first study which will use association mapping in populations of known evolutionary history.

Recombination activating genes 1 and 2 and vertebrate genome stability

Members: Jocelyne Demengeot, Carlos Penha Gonzalves, Antonio Jacinto, Moises Mallo

Students: Paulo Almeida, Thiago Carvalho

Collaborators: Vasco Barreto, Rockefeller Institute, NY, USA and Miguel Godinho, MRC, London, England.

We have previously reported the generation of transgenic mice expressing the Rag1 and 2 genes both continuously throughout lymphocyte development and constitutively in most non-lymphoid tissues. We showed that ectopic expression of the Rag genes is lethal, both to lymphocytes, and to the organism as a whole. These animals display growth retardation and early death reminiscent of mice deficient in double strand break repair molecules. We developed a novel transgenic system, where Rag1 and 2 are independently expressed. Analyses of these mice suggest that over-expression of

only one of the Rag genes is sufficient to induce severe pathological lymphopenia. We are also investigating the impact of Rag-1 and 2 on the vertebrate genome evolution, by introducing these genes in invertebrate organisms and by a bio-informatic approaches.

The Molecular Basis of Parallel Evolution

Member: Élio Sucena

Complex genetics

The genome sequencing projects resulted in a range of technologies and a volume of information that brought about unprecedented developments in genetic analysis, allowing biologists from all areas to address questions that had long been untractable. One of these relates to the genetics of “complex” phenotypes, which do not follow classical medelian inheritance, and are governed by many genetic and non-genetic factors. The approaches to complex phenotypes are differentiated but complementary: cell biology and molecular genetics, bioinformatics, and statistical genetics. Experimental systems, such as the fly and the mouse, aim at understanding the generation and the genetic architecture of such phenotypes. In humans, current work concerns common human diseases like diabetes, obesity, heart diseases, psychiatric disorders, but also behavioral traits. Beyond the importance of disease genetics to predictive medicine, it is hoped that detailed knowledge on genes and molecular mechanisms will contribute a better understanding of disease processes and novel possibilities of therapeutic intervention.

At the IGC, several groups are dedicated to the genetic dissection of complex traits, studying human disease, mouse models of disease, and the evolution of genetic traits at the population level. Research in human genetics, conducted in intimate collaboration with patients associations and MDs in several hospitals, is focused on family studies of autism, systemic lupus and Type I diabetes, while the mouse projects include the genetics of susceptibility to malaria and diabetes. Significant progress was achieved in the genetic epidemiology of autism, where a total of 235 nuclear families have now been studied, with several findings of particular interest, which are now under scrutiny: there is an unexpectedly high prevalence of mitochondrial disorders in autistic children; the prevalence of autism in the Azorian islands is 50% higher than in mainland Portugal; two genes have been found involved in autism, namely the *serotonin transporter* gene, and the *neuroligin 4* gene. In systemic lupus, the analysis of 76 multigenerational families showed the involvement of the CTLA-4 locus, and defined SLE-associated Regulatory T cell phenotypes segregating in the affected families. The chromosomal region containing the CTLA-4 gene had been previously associated with diabetes susceptibility in mice, allowing us to investigate the respective cellular and molecular mechanisms. The relevance of this locus for human Type I diabetes has now been proven. Statistical methods that incorporate multiple parameters in phenotype definition, as well as methods assessing the contribution of multiple genes to specific quantitative phenotypes, have also been developed. Research in bioinformatics and statistical population genetics has also been launched, and a new group in human disease genetics will be soon installed. Now that a gene expression unit is fully operational at the Institute, the success of this program

requires re-inforcing competences in bioinformatics, and the availability of a technology platform for medium-throughput DNA sequencing and genotyping.

Genetic epidemiology of autism

Members: Astrid Vicente, Constantin Fesel

Students and Technicians: Ana Coutinho, Marta Barreto, Ricardo Ferreira, Catarina Correia

External Collaborators: Guiomar Oliveira, Hospital Pediatrico de Coimbra; Luisa Mota Vieira, Hospital do Divino Espirito Santo; Patricia Maciel, Universidade de Braga; Steve Sommer, City of Hope National Medical Center

Genetic Epidemiology of Autism in Portugal: Our present database and sample collection now includes 235 nuclear families with one or more autistic patients and extensive clinical, behavioral, genealogical and biochemical information on patients and relatives. In this sample we have been analysing the association of candidate genes of the serotonergic system, and others, with autism and with associated quantitative endophenotypes, as well as exploring the hypothesis of the involvement of autoimmune mechanisms in the disease pathogenesis. Our work on the role of the serotonin transporter in hyperserotonemia in autism is being published in the February 2004 issue of *Molecular Psychiatry*, while reports on the work on *BDNF* gene in autism, depression and schizophrenia have been published or submitted. We have joined an international consortium for research on the genetics of autism, the *Autism Genetics Cooperative*, funded by National Alliance for Autism Research (NAAR), and progress in being done on organisation issues, namely re-consenting of samples for later public availability. This consortium proposes to conduct a genome wide association scan for autism in a sample population of unprecedented size. This collaborative effort will undoubtedly yield crucial information for the understanding of autism etiology, while opening opportunities for follow up research by the associate groups on the pathological mechanisms associated with autism symptoms and deficits.

Autism and Mitochondrial Disorder – microarray analysis of the expression profile of nuclear genes for mitochondrial enzymes in autism. We have found an unexpectedly high rate of mitochondrial disorder associated with autism in our sample (17% initially suspected cases, with 11/205 already confirmed and 24 under evaluation); no mtDNA mutations were detected; ongoing is the analysis of nuclear genes encoding mitochondrial-related proteins.

Clinical, epidemiological and genetic study of Rett Syndrome in Portugal. In the context of a collaborative project with Patricia Maciel at Universidade de Braga and Steve Sommer at the City of Hope National Medical Center, we screened our autism patients for mutations in the *MECP2* gene, which causes mental retardation and Rett syndrome. This disorder often presents with autistic symptoms, and our hypothesis is that milder mutations may cause autism in males. Several variants have been found in conserved regions both in the coding region and in the 3'UTR, and are being correlated with phenotype in autism.

Pharmacogenetics of risperidone therapy in autism spectrum disorders

Members: Astrid Vicente, Constantin Fesel

Students and Technicians: Ana Coutinho, Marta Barreto, Ricardo Ferreira, Catarina Correia

External Collaborators: Guiomar Oliveira, Hospital Pediatrico de Coimbra; Luisa Mota Vieira, Hospital do Divino Espirito

We are investigating the role of selected candidate genes in the variability of response, in efficacy/side effects, to specific medication for autism, aiming at the prediction of individual response based on specific genotypic and phenotypic information, with a major impact on therapeutic decisions in clinical settings. Clinical and psychological evaluation procedures are being validated, as well as genetic and biochemical characterization techniques.

Genetics of human Systemic Lupus Erythematosus (SLE)

Members: Astrid Vicente, Constantin Fesel

Students and Technicians: Marta Barreto, Ricardo Ferreira, Catarina Correia

External Collaborators: Associação de Doentes com Lupus; Carlos Ferreira, Hospital de Santa Maria; Carlos Vasconcelos, Hospital de S. João; Berta Martins, ICBAS; João Faro Viana, Hospital de Sta. Cruz; Luisa Mota Vieira, Hospital do Divino Espirito

SLE is a multifactorial disorder with heterogeneous presentation, in which genetic susceptibility plays a major role. The main objective of this study is the identification and characterization of genetic susceptibility factors for SLE. The strategy used is the identification of lupus-associated traits that are genetically less complex, and therefore more amenable to genetic mapping. The collection of SLE patients and family members has progressed throughout 2003, in collaboration with the Associação de Doentes com Lupus, Hospital de Santa Maria and ICBAS. Presently, 74 multigenerational families have already been collected, including 140 patients and 210 unaffected relatives. Identification and collection of familial cases is progressing in the Azorian islands. A database has been established, gathering clinical and serological information as well as disease-associated phenotype and genetic data. Given that antinuclear antibody (ANA) production is a main characteristic of SLE, we have been analysing autoantibody reactivities in patients and relatives, and determining heritability of these traits in multiplex families. The epitope specificities of the antinuclear antibodies in patients and unaffected relatives and controls are also being analysed, and preliminary results indicate that specific ANA are inherited among affected and unaffected family members. We have also been investigating the role of regulatory cells in SLE, analyzing regulatory cell numbers and its heritability in our families, as well as its genetic regulation. Specific genes involved in regulatory cell function are being tested as candidates for disease susceptibility.

Genetics of malaria in mouse models

Members: Carlos Penha Gonçalves

Students: Elizabete Fernandes, PhD student (FCUL/FCT), Nuno Sepúlveda, MSc Student (IST), Paulo Almeida, Bioinformatics technician (ITQB), Lúcia Deus, Laboratory technician (IEFP)

External Collaborators: Pierre-Andre Cazenave (Institut Pasteur, Paris, France), Dan Holmberg (Umea University, Sweden)

This project aims to identify genetic factors that confer resistance to malaria infection in mouse models. Unraveling the identification of such genetic factors that control resistance to infection will provide important contribution to the understanding of pathogenesis and will suggest therapeutic and vaccine strategies to improve resistance to disease.

We investigate phenotypes of malaria related to infection resistance in different stages of the disease, including the liver stage, the blood stage and the clinical complications like cerebral malaria.

The work-plan comprises (1) the genetic mapping of resistance loci using genetic crosses

of mouse strains that are resistant to malaria and (2) the isolation of the underlying genetic factors by an approach that combines candidate gene analysis and positional cloning.

To this point the project has been focused in the *P. berghei* infection model and has led to the identification of 2 loci controlling resistance to cerebral malaria, 2 loci controlling resistance to hyperparasitemia (submitted). Genetic mapping of loci controlling the hepatic phase is under way.

Genetic structure of the Azorean population – A surname study

Members: Claudia C Branco, Bernardo R Peixoto, Teresa Cymbron and Luisa Mota-Vieira.

Students: Rita Cabral, Sónia Costa and Paula R Pacheco

Collaborators: GianUmberto Caravello and Miro Tasso Dipartimento di Medicina Ambientale e Sanit Pubblica - sede di Igiene, Universit di Padova, Padova, Italy

To obtain a better understanding of the genetic structure of the Azorean population we conducted a survey based on surnames listed in the 2002 telephone book. First, we analyze the population living the 44 rural localities of São Miguel. In a total of 12,625 subscribers we found 812 different surnames. The highest value of surname diversity was found in Capelas (socio-economically more developed) and Rabo-de-Peixe (the most populated); and the lowest value was found in Lomba de S. Pedro (the least populated). Surname diversity was estimated by the coefficient of relationship between localities (R_{ij}) and Nei's genetic distance. The analysis of rural population relationships reveals that Povoação and Nordeste are the two localities with highest value of R_{ij} (0.217), whereas Capelas and Nordeste have the lowest value (0.0042). The rural locality displaying the greatest homogeneity is Salga ($F_{st}=0.0145$). The genetic similarity between localities was obtained by a dendrogram based on Nei's distance matrix. We identified two main clusters, one includes Nordeste and Achada, both located in the most eastern part of S. Miguel, and the other includes the remaining 42 localities grouped in three major subclusters. The subcluster that stands

out groups all the localities of the western part of the island. These data are consistent with the spatial distribution of surnames obtained by Principal Component (PC) analysis. In fact, the first two PC account for 59% of the total variance and shows a cluster at the upper left corner of the plot with 8 western localities.

Second, we outline the structure of the Azorean population. We identified 2,455 different surnames in a total of 55,530 subscribers. In order to characterize the influence of geographic discontinuity on surname diversity, we performed a surname specificity analysis. Our results reveal that each island has surnames 50% more frequent than in the rest of the archipelago (specific surnames). This is particularly evident in the islands of S. Miguel, Santa Maria, Terceira and Corvo. To evaluate the genetic similarity between the islands's populations, we then analyzed surname distribution by spatial autocorrelation (Moran's I). In a total of 161 surnames, we identified 24 (15%) surnames with statistically significant patterns for 4 distance classes. These patterns were classified as: isolation by distance and depression (37.5%), cline (12.5%), intrusion (12.5%) and long-distance differentiation (8.3%). The remaining 29.2% had no defined pattern. The overall spatial correlogram plot of the 24 surname frequencies shows that the highest Moran's I coefficient (0.69) was present in the first distance class (105 Km). Moreover, autocorrelation change from positive to negative for distances greater than 105-250 Km, indicating that mobility is higher between islands of the same geographic group. In population genetic terms, the data show that geographic distance shapes surname diversity among Azores Islands.

Y-chromosomal STR haplotypes in Sao Miguel's population (Azores)

Members: Paula R Pacheco, Teresa Cymbron and Luisa Mota-Vieira.

Students: Rita Cabral, Sónia Costa and Claudia C Branco

This project aims to improve our understanding of the genetic structure of São Miguel's population, through the analyze of seven Y-chromosome STR loci (DYS385, DYS389I, DYS389II, DYS390, DYS391, DYS392 and DYS393) in 100 unrelated individuals. Father's birthplace was used to select the samples: Nordeste (n=4), Povoação (n=5), Vila Franca do Campo (n=8), Lagoa (n=11), Ribeira Grande (n=22) and Ponta Delgada (n=50), representing all the municipalities in Sao Miguel. The samples were typed by PCR with fluorescently labeled primers and products run in a CEQ8000 DNA Sequencer (Beckman Coulter). In a total of 100 complete seven-locus haplotypes, we observed 71 different haplotypes of which 54 appear only once. The mean heterozygosity per locus in the overall sample was 0.59, with the highest value in Nordeste (0.71) and the lowest in Ponta Delgada (0.56). On average the mean pairwise difference between two random haplotypes ranged from 4.49 in Ponta Delgada to 5.66 in Nordeste. The mean F_{st} value over all 7 loci was very low (-0.00758), denoting the absence of heterogeneity among the municipalities of the island. Pairwise genetic distances were computed for all six municipalities and no genetic heterogeneity was detected. AMOVA analysis shows that the percentage of variation attributable to differences within municipalities is extremely high compared to the differences between municipalities. These results provide evidence for reduced levels of Y-chromosome variability in the male lineages of Sao Miguel's population. We are currently analyzing 8 SNPs and 1 indel polymorphism, in order to characterize the frequency and distribution of Y-chromosome haplogroups in the population of São Miguel.

Establishment of a human DNA banking in São Miguel island (Azores)

Members: Luisa Mota-Vieira and Bernardo R. Peixoto

Students: Paula R. Pacheco, Rita Cabral, Sónia Costa and Claudia C Branco

Collaborators: Pedro Mendonça, Maria L. Almeida, Júlio Carvalho, Catarina Matos, Marta Loura and Ana Luisa Araújo, Serviço de Hematologia do HDES, Ponta Delgada.

This project aims to build a DNA bank of 1,000 healthy unrelated individuals (about 0.8% of the current population) living in the Azorean island of São Miguel (131,609 inhabitants). Resulting of a collaboration with the Hematology Department of the Hospital of Divino Espírito Santo, the only hospital located in S. Miguel island, the DNA bank follows the international ethical guidelines and has been approved by the Hospital's ethical committee. Blood samples (7.5 mL) were collected with appropriate Informed Consent, which includes (1) the distribution of free informative leaflet explaining the goals of proposed studies, the confidentiality of the personal data, and the methods of identification and storage of DNA samples; and (2) an interview with a health professional for additional information. All samples in the repository are anonymous and have self-reported data concerning sex, age, birth and current living places (locality and municipality in the island), and parental birthplaces. To date (Dec. 2002 through Oct. 2003), we collected 500 blood samples, of which 435 (87%) are male. The mean age of all participants is 36.3y (18-63y). All the six municipalities of the island are represented. The analysis of birthplaces shows that 427 (86%) of all participants have both parents born in S. Miguel island. Moreover, 256 (60%) of these participants have both parents born in the same locality. This data corroborates the high rate of marriages within the rural localities. Thus, the DNA bank of S. Miguel island represents a important resource for population and biomedical genetic research and provides opportunities for us to participate in international collaborative projects.

Genetic and consanguinity of congenital heart disease in Azores

Members: Teresa Cymbron and Luisa Mota-Vieira

Students and Technicians: Rita Cabral, Claudia C Branco and Paula R Pacheco

External Collaborators: Rui Anjos (Serviço de Cardiologia Pediátrica – Hospital de Santa Cruz, Lisboa), and Carlos P Duarte and Clara Macedo (Serviço de Pediatria – HDES, Ponta Delgada).

Congenital heart defects (CHD) are the most common form of clinically significant birth defects. Based in the Azorean Registry of CHD, this project aims to study cardiac alterations in Azores, an archipelago with a population of 241,763 inhabitants, settled by Portuguese in the 15th century. Recently, we showed that this population is relatively homogeneous and has the highest value of consanguinity in Portugal. To date, 383 patients have been clinically evaluated and classified according to the predominant cardiac lesion. To investigate the epidemiology of CHD in São Miguel island, a retrospective 10-year (1993-2002) study was carried out. CHD shows a non-random geographic distribution in Azores, with the exception of Corvo, all islands present CHD cases. The highest frequency was found in São Miguel island where nine CHD clusters were detected. These clusters correspond to small localities with a number of inhabitants varying between 798 and 3,501. Now, we are interviewing

CHD families in order to reconstruct extended multigenerational pedigrees. Special attention is been taken to detect consanguinity in families with CHD. Because of the genetic structure of Azorean population, the possibility of CHD families sharing a common ancestor is being investigated.

Virology & Immunity

The pathogenesis of infections is not a one-sided issue, as it reflects evolving interactions between the host immune system and the pathogens, such that long-term survival of both the pathogen and the host can be achieved. Accordingly, emerging infections are often highly lethal, whereas adapted infectious agents tend to be less pathogenic, having evolved strategies to survive and replicate without severe pathological consequences. Viruses have been particularly efficient in evolving strategies, That impinge and modify the cell biology and immune responses of their hosts. It follows that viral genes constitute an exploitable library of ready-made tools for gene manipulation or therapy, and for the design of novel drugs and vaccines. In the past, the majority of such virus “host evasion” genes have been identified through their homologies, using bioinformatic approaches. It is clear, however, that some of these evasion molecules do not have structural homologues, but are functionally equivalents to components of the vertebrate immune system. These are identifiable through appropriate functional assays, and provide a source of novel modifiers of immunity and cell biology. This theme forms the basis of our research programme. The ability to genetically manipulate both the virus and the host, notably by producing transgenic mice for viral genes, offers the potential to dissect the molecular mechanisms involved in the virus/host interplay.

Using a gammaherpes virus model, several viral genes have been identified which are involved in the establishment of “latency” in B lymphocytes, and reveal alternative strategies for host evasion: neutralization of chemokines, increased ubiquitination and degradation of MHC molecules, interaction with signalling molecules or cascades in lymphocytes. Through structural (bioinformatic) and functional approaches, a number of genes in African Swine Fever Virus have been identified, which ensure evasion via inhibition of Toll-like receptor and Type I and Type II Interferon pathways, via induction of apoptosis, or via inhibiting transcription of key genes for both the innate (NFkB pathway) and acquired (NFAT) immune defense systems.

Another example of a virus that modulates host immunity for its survival is the human immunodeficiency virus (HIV). Progression of HIV infection into the acquired immunodeficiency syndrome (AIDS) is characterized by a relentless loss of CD4 cells, which is faster in HIV1 than HIV2 infection. Comparing several immunological and viral variables in HIV1 and HIV2 infections, and incorporating these in mathematical models led to the conclusion that immune activation, and not viremia as was proposed, is the causative agent responsible for the extent of CD4 depletion in both infections, while the rate of progression into AIDS depends critically on the interclonal infection rate, and scales monotonously with it.

Molecular interactions in murine herpesvirus 68 latent infection of B-Lymphocytes

Member: J Pedro Simas

Students: Patricia Madureira, Sofia Marques, Lenia Rodrigues and Claudia Istrate

Collaborators: Stacey Efstathiou, University of Cambridge, UK and Philip Stevenson, University of Cambridge, UK

Studies into the molecular basis of gammaherpesvirus latency have been hindered by the lack of amenable animal model systems and the lack of fully permissive cell lines, which are required for the genetic manipulation of these viruses.

Research on the utilisation of a gammaherpesvirus, designated murine herpesvirus 68 (MHV-68), whose pathogenesis can be readily investigated in the laboratory mouse (for recent reviews see Simas & Efstathiou, 1998; Doherty and Christensen, 2000). MHV-68 is genetically related to Epstein-Barr virus and Kaposi's sarcoma associated herpesvirus, which are important Human pathogens. Experimental infection of inbred strains of mice with MHV-68 results in acute productive infection of the lung followed by latent infection of B-lymphocytes. Comparison of the genomic organisation of MHV-68 with other gammaherpesviruses shows the presence of virus specific ORFs and a number of cellular homologues, which are predicted to determine the particular biological properties of these viruses, e.g. host range, immune evasion, latency and disease. MHV-68 has 14 unique such genes, designated M1 to M14, and several cellular homologues, including a complement control protein, a D-type cyclin and an IL8 receptor. In addition to these cellular homologues two of the CEM^1 genes, M1 and M11, show low level similarity to serpins and bcl2 cellular protein, respectively.

Our research interests are focused in trying to understand how co-ordinated expression of these cellular homologues and unique ORFs, in a developing B-lymphocyte, result in immune evasion and latent infection.

To this end, we have adopted the following strategies; (i) to determine the tropism and virus transcription pattern, during the establishment and maintenance of latent infection in B-cells and, (ii) to construct recombinant viruses with specific genes deleted and study their phenotype upon infection of mice and, (iii) to identify cellular molecular targets for virus latency-associated genes.

Antiviral peptides blocking herpes simplex virus type 1 entry into cells

Member: J Pedro Simas

Student: Lidia Fonseca

Collaborators: Massimiliano Galdiero, University of Napoli, Italy; Helena Browne, University of Cambridge, UK; William Gibbons, ARISTOS Scientific Ltd, UK and Paolo Sarmientos, PRIMM, Italy.

Human herpes simplex virus 1 (HSV-1) affects over 50% of the European Union adult population and is highly transmittable: No vaccines, despite repeated attempts, are yet available for prevention, but classical antivirals such as nucleosides are the choice chemotherapies. Nevertheless, HSV resistant strains and the failure to completely relieve symptoms (especially in immunocompromised individuals) make it imperative that antivirals that utilise new mechanisms of action are developed and marketed. Symptoms of HSV-1 infections include erythematous vesicles, at mucocutaneous

sites, which can ulcerate and be quite painful. The mouth, lips and genitalia are the dominant sites of these lesions. HSV-1 however causes herpes keratitis, a recurrent infection of the eye as well as the much feared manifestation of HSV-1 infection, herpes encephalitis, which has a 1/500,000 incidence and high mortality if not treated. Systemic infection of the newborn and the chronic lesions of immunocompromised patients (especially HIV and cancer patients) and patients undergoing organ transplantation (which necessitates use of immunosuppressive drugs) can often be complicated by HSV-1 infections.

All of the above, including the pain, loss of work and productivity, are potent reasons for possessing effective new and possibly multiple therapies against HSV-1 infections. New therapies and in particular those which act by novel antiviral mechanisms should be developed. Many arguments against the use of peptide therapeutic agents, due principally to their rapid proteolysis and short half-lives are now dissipated. An excellent example is the anti-HIV aspartyl protease which enters cells and inhibit viral assembly. A second development, the existence of virions technologies to stabilize and deliver therapeutic peptides, has permitted administration of peptide chemotherapeutics by oral, I.V., nasal and other routes.

The objective of this research program is to develop of a new class of anti-HSV-1 drugs – a peptide that acts at the cell surface blocking virus entry – when used alone or in combination with, for example, nucleoside antivirals should increase the quality of life of many in the EU who suffer pain, discomfort and sometimes morbidity. It is hoped that this work will form a basis for developing peptides moieties as anti-HSV (not part of this proposal) and provide an example of this class of antiviral that block virus entry into cells. When sold in the EU and globally it should prove economically beneficial to the EU and provide R&D training to young scientists. Because it operates via an extracellular novel mechanism these anti-HSV peptides will prove complementary not competitive with current antiviral chemotherapies; predictably combined therapy, as with anti-HIV therapies, may prove the most common use of antiviral peptides that block virus entry.

Transcriptome analysis of germinal centre B cells during gammaherpesvirus latent infection

Member: J Pedro Simas

Students: Sofia Marques, Lidia Fonseca

Collaborators: Stacey Efstathiou, Philip Stevenson, and Paul Lyons, University of Cambridge, UK

Analysis of genomes from gammaherpesviruses reveals the presence of large blocks of co-linearly arranged conserved genes interspersed with virus specific ORFs and cellular homologues. Hence, there are two classes of putative viral host control proteins, namely those encoded by genes with and without sequence similarity to cellular genes. The existence of viral homologues to cellular genes suggests that during co-evolution viruses have ‘hijacked’ host genes that were subsequently modified for the benefit of the virus. Virus specific ORFs may represent novel structures with functional activities homologous to cellular proteins or could simply be an example of proteins for which the host homologues have not yet been identified. Our objectives are focused in trying to reveal the molecular function that these ORFs and cellular homologues have in a context of infection, that result in evasion of the host immune response and life-long latency. To this end we use a gammaherpesvirus

designated murine herpesvirus 68, as its pathogenesis can be investigated in the laboratory mice.

This project investigates the effect that MHV68 latent infection has upon GC B cell physiology by analyzing their transcription profile. We propose to use a strategy involving transgenic mice with a floxed EGFP allele that only becomes functional upon Cre mediated excision. In this model, Cre will be provided by a recombinant MHV68 resulting in the fluorescent tagging of latently infected cells. This makes possible the purification of pure populations of latently infected GC B cells, a prerequisite for DNA microarray analysis.

It is hoped that this strategy will identify key cellular genes and biochemical pathways that are involved in cellular functions important for the control of gammaherpesvirus infection. Knowledge gained from this type of approach may not only help determine the molecular basis for gammaherpesvirus infection but also provide clues on what gene products (either cellular or viral) may have therapeutic uses themselves or may be targets for therapeutic intervention.

AIDS immunopathogenesis

Member: Jorge Carneiro

Student: Nuno Sepúlveda

External Collaborators: Rui Victorino (IMM) and Ana Sousa (IMM)

Together with Victorino's group (IMM, Lisbon), we study AIDS-associated immunopathology. Progression into AIDS is characterized by a relentless loss of blood CD4 cells, which is faster in HIV1 infection than in HIV2 infection. We compared several immunological and viral variables during the two infections, reporting identical immune activation in HIV1 and HIV2 patients matched for CD4 blood counts (Sousa et al, 2002 *J. Immunol.*). These results indicated that immune activation, and not viremia as proposed before, is closely linked to the extent of CD4 depletion in both infections. We made mathematically controlled comparisons of models describing alternative causal relationships between infected cell numbers, CD4 depletion and immune activation in an attempt to infer the mechanism of disease progression. Improving other models in the literature, our models incorporate in an analytically tractable way two subsets of cells undergoing cell cycle: one in which sporadic cell division follows homeostatic response to perturbations to CD4 cell counts, and one in which series of cell cycles take place during immune responses (the so called "bursts"). The analysis of these models indicates that while CD4 depletion may be caused by the HIV itself or by lymphocyte activation, the rate of progression into AIDS depends critically on the interclonal infection rate, and scales monotonously with it.

The potential and application of virus host evasion genes that modify apoptosis and cytokine responses

Member: R.M.E. Parkhouse and Ana Crespo

Students: Sílvia Almeida, Sílvia Correia, Rute Nascimento and Vívian Oliveira

The aim is to identify and exploit viral modifiers of apoptosis and cytokine responses (particularly TNFRs) as a potential source of novel health care pharmaceuticals for

manipulation of immune responses and treatment of certain diseases. Such virus genes will be identified by nucleotide sequence and functional analysis of cloned viral ORFs and cDNA libraries of three large DNA viruses (African swine fever (ASFV), Ectromelia virus (EV) and Mouse herpes virus (MHV 68)). As a direct approach towards identifying novel virus evasion genes which do not have homologies in the data base, the genes of these three viruses will be systematically screened in functional assays for their impact on apoptosis and cytokine responses.

To date, we have identified three novel viral genes inhibiting interferon responses, one gene inducing cell cycle arrest/apoptosis, and one gene inhibiting some, but not all, toll receptor-like signaling pathways.

The construction of mice transgenic for selected virus “evasion” genes is now well underway, and will provide a novel approach to explore the mechanism and exploitation of these genes. One particularly interesting transgenic mouse has a defect in T cell development and lymphoid homeostasis, and will provide a novel system to explore basic mechanisms operating in the development of the lymphoid system.

Control of human, bovine and porcine cysticercosis through vaccination and improved diagnosis

Member: R.M.E. Parkhouse

Collaborators: Dr. T. Garate (Instituto de Salud Carlos III, Centro Nacional de Microbiologia, Madrid, Spain), Dr. L. Harrison (University of Edinburgh, Department of Tropical Animal Health, Centre for Tropical Veterinary Medicine, Scotland), Dr. E. Sciutto (Universidad Nacional Autonoma de Mexico, Institute de Investigaciones Biomedicas, Mexico), Dr. M. Cortez (Universidad de Carabobo, Venezuela) and Dr. H. Garcia, Universidad Peruana Cayetano Heredia, Lima, Peru).

The zoonotic tapeworm *Taenia solium*, causal agent of life threatening human neurocysticercosis, constitutes an increasingly major health risk. The adult, or tapeworm stage, lives in the intestine of man, whilst the intermediate metacestode stage, responsible for cysticercosis, may occur both on pig and man. The related parasite, *Taenia saginata*, similarly infects man as an intestinal tapeworm but passes its metacestode stage only in cattle. Rural transmission is mediated by poor sanitation and uncontrolled pig and cow management practices, and so the prevalence of these parasites is an objective indicator of rural poverty. Recently, population movement linked to close human/pig and cow contact in the rural-urban interface has exacerbated the problem. Control through improved sanitation is a major, long-term and expensive goal. This project focuses on the shorter-term, more cost-effective strategies of improving pig and cow management, including village pig vaccination (transmission control) and the development of sensitive and specific diagnostic assays to detect parasites and anti-parasite antibodies; the latter based on synthetic peptides, recombinant reagents and PCR, not parasite material. New diagnostic assays will improve hospital patient monitoring/treatment and man/pig screening and hence epidemiological knowledge.

To date, we have succeeded in developing the following diagnostic tests: 1) PCR tests for the differential diagnosis of cestode parasites (*Taenia solium*, *Taenia saginata* and *E. chinococcus*); 2) Synthetic peptide based assays to detect antibodies to *Taenia* parasites and 3) An ELISA assay which detects secreted metacestode antigens and thus viable metacestode parasites in pigs, cattle and man. These are all now being

applied in endemic areas, principally Mexico, Peru, Bolívia and Venezuela, and, on occasions, clinical material in Spain.

Finally, we have developed a potential vaccine for bovine cysticercosis, based on a recombinant oncospherical surface and secreted molecule which, interesting is functionally, an adhesion molecule, possibly facilitating tissue invasion by the parasite in the intermediate host.

Control of African Swine Fever (ASF) through improved diagnosis

Member: R.M.E. Parkhouse

Student: Ana Luísa Reis

Collaborator: Alexandre Leitão (Laboratório de Doenças Infecciosas, Faculdade de Medicina Veterinária, Universidade Técnica de Lisboa)

African swine fever is one of the most important diseases of pigs. Its control relies solely on efficient diagnosis and application of strict sanitary measures. Vaccine has never been obtained. ASFV infects domestic pigs and *Ornithodoros sp.* ticks (shown as vectors in Iberian Peninsula before the disease was eradicated). Danger of ASF re-emergence and/or new introduction is a major concern for EU, due to lacking knowledge on mechanisms of viral persistence in the pig and in ticks and because several European countries have close contacts with African countries where the disease is nowadays devastating. The rationale of this project is to improve diagnostic methods to enable rapid implementation of prophylactic measures.

Our previous work has identified the 12 principle serological determinants of ASFV and the principal aim of this project is to express these viral proteins and assess their potential value as serological diagnostic probes using sera from infected pigs previously obtained. To date, the 12 viral genes have been subcloned into the expression vector and their purification is underway for testing as reliable diagnostic tools.

Improved strategies for a retroviral gene therapy vector development: an enveloped Virus

Member: Matthias Haury

Collaborators: Ana Sofia Valente Coroadinha (IBET/ITQB, UNL, Oeiras, Portugal), Manuel Carrondo (IBET/ITQB, UNL, Oeiras, Portugal).

Retroviral vectors are being used since the past decade in gene therapy for the treatment of diseases in hundreds of clinical trials. Although this was the first viral vector used still many hurdles arise in the production of high viral titers, which are mainly due to the inherent vector instability. The objective of this work is to develop new retroviral packaging cell lines analysing along this process some of cellular characteristics thought to be involved in the ability to produce high levels of stable virus, in order to obtain improved producer cell lines.

Inflammation & Immunity

Inflammation is a stress reaction causing '*rubor, calor, dolor, tumor*' (redness, heat, pain and swelling) but it represents the body's defense to a variety of injuries. Inflammatory reactions often occur as a result of microbial infections, involving both the immediate activation of the "innate immune system", as well as the adaptive response of lymphocytes, cooperating in the clearance of pathogens. Inflammatory reactions should thus be perceived as a beneficial response that allows the immune system to deal with invading microbes. If uncontrolled, however, "innate" responses might be lethal, as in septic shock, while chronic inflammation often leads to tissue damage, at the origin of degenerative diseases (e.g., atherosclerosis, rheumatism, multiple sclerosis), many of which are autoimmune and continue to represent a serious therapeutic challenge. To be effective and, yet, not provoke disease, inflammatory reactions must thus be regulated. The molecular basis of inflammation and respective controls are, therefore, of utmost importance in biomedicine. Several groups at the IGC are concerned, directly or indirectly, with these questions, analyzing cellular and molecular mechanisms regulating inflammation. The specificity of our research relates to the complementarity of approaches (disease genetics, cell and molecular biology, immunology, theoretical biology), and to common concerns with Regulatory T cells, tissue-protective genes and mechanisms, which the IGC groups have helped to establish.

Genetic analysis of inflammatory processes can provide relevant information on the molecular mechanisms involved. This approach has been undertaken in man and mouse, studying either patients and families, or various mouse strains and their crosses, in order to identify genes that are associated with susceptibility to inflammatory disease. In mouse, our work concerns the analysis of lymphocyte responses to bacterial activators. In humans, as seen above, we have studied systemic lupus and Type I diabetes, and identified several genetic regions, notably CTLA-4, that are associated with susceptibility. The protein encoded at this locus seems necessary for controls mediated by Regulatory T cells, which we demonstrated to be fundamental in mouse models of multiple sclerosis and tissue transplantation, giving further significance to the lower levels of this set of T lymphocytes in such patients. Another significant advancement in the past year has been the discovery that signaling via Toll-like receptors – a central pathway in "innate immunity" - modulates the activity of Regulatory T cells that control inflammatory reactions. This may explain why such T cells inhibit immune clearance of pathogens, as well as the "protective effects" of infections in the development of autoimmune diseases and allergy. In short, the ligands that promote inflammation also activate the respective regulation, suggesting a kind of "quality control" of responses that had previously been postulated.

Tissue "protective genes" also regulate and limit inflammation, acting as a "negative feed back loop" that interferes with inflammatory reactions via a dual mechanism: inhibiting expression of pro-inflammatory genes associated with the onset of inflammatory reactions, and protecting cells from undergoing apoptosis. One of such genes is heme oxygenase-1 (HO-1), a rate-limiting enzyme that cleaves heme, the expression of which in blood vessels was found to play a critical role in controlling the progression of atherosclerosis. This effect is mediated by the localized tissue production of the gas CO, which acts directly on smooth muscle cells of the vessel wall to stop their proliferation.

**Molecular mechanisms underlying the protective effect of HO-1 derived CO:
*Interaction with the NF- κ B signal transduction pathway***

Members: Miguel Soares (UL/FCT) and Gabriela Silva (Post Doctoral Fellow, IGC/FCT).

Students and Technicians: Mark Pena Seldon (ICBAS/FCT).

External Collaborators: Josef Anrather, University of Cornell, New York City, USA

Endothelial cells (EC) form the physical barrier between blood and tissues. Activation of EC, such as it occurs during microbial infection, is associated with the acquisition by EC of a pro-inflammatory phenotype. This is a critical step in assisting the sequestration and activation of leukocytes into sites of microbial infection. This in itself this is a critical step in the initiation of the immune responses that will ultimately lead to microbial clearance.

To avoid the development of chronic inflammation, the pro-inflammatory phenotype associated with EC activation must be tightly controlled. One of the mechanisms that controls the pro-inflammatory phenotype associated with EC activation relies on the expression by EC of a series of anti-inflammatory genes that we refer to as “protective genes”. These genes have a dual role in that they control the pro-inflammatory phenotype associated with EC activation and protect EC from undergoing apoptosis. We have tested if the stress responsive enzyme heme oxygenase-1 (HO-1) acts as a protective gene in EC. Under inflammatory conditions, HO-1 is the rate-limiting enzyme in the catabolism of heme into biliverdin, free Fe^{2+} and CO. Biliverdin is subsequently catabolyzed into bilirubin, through a process that is dependent on the expression of biliverdin reductase. Free Fe^{2+} up-regulates the expression of the iron sequestering protein ferritin as well as that of an ATPase iron pump. We asked whether HO-1 would interfere with the expression of E-selectin (CD62E), intracellular adhesion molecule-1 (ICAM-1/CD54) and vascular cell adhesion molecule-1 (VCAM-1/CD106), all of which are pro-inflammatory genes associated with EC activation. We found that HO-1 inhibited E-selectin and VCAM-1 but not ICAM-1 expression, as tested at the RNA and protein level (Soares et al., Journal of Immunology, in press). Exogenous bilirubin and/or Fe^{2+} chelation mimicked the effects of HO-1 while biliverdin or CO did not. Given the central role of the transcription factor nuclear factor kappa B (NF- κ B) in regulating the expression of these genes we asked whether HO-1 would modulate NF- κ B activity in EC. We found that HO-1 blocks NF- κ B activity in EC. This effect is mimicked by bilirubin and/or by a decrease of free intracellular Fe^{2+} but not by biliverdin or CO. We have focused thereafter on the mechanism by which a decrease in intracellular Fe^{2+} would regulated NF- κ B activity. We found decreased levels of free intracellular Fe^{2+} target directly the NF- κ B family member p65/RelA to suppress its transcriptional activity. We have recently identified that his effect is exerted at the level of the DNA binding domain of p65/RelA. The hypothesis, we are currently testing, is that the phosphorylation “status” of the p65/RelA N-terminal domain is modulated by HO-1 in a manner that decreases the overall activity of this transcription factor.

Molecular mechanisms underlining the anti-apoptotic effect of heme oxygenase-1 (HO-1) and of its catalytic product carbon monoxide (CO): Role of the p38 MAPK signal transduction pathway

Members: Miguel Soares (UL/FCT) and Gabriela Silva (Post Doctoral Fellow, IGC/FCT).

Students and Technicians: Mark Pena Seldon (ICBAS/FCT).

External Collaborators: Leo Otterbein, University of Pittsburgh, Boston, USA.

We have previously shown that the stress responsive gene heme oxygenase-1 (HO-1) protects endothelial cells (EC) from undergoing apoptosis. This accounts for the overall protective effect of HO-1 as EC apoptosis is a highly deleterious event during inflammation. We have also shown that this effect of HO-1 is dependent on the generation of the gas carbon monoxide (CO), via the degradation of heme. CO protects EC from undergoing apoptosis via a mechanism that requires the activation of the p38 mitogen activated protein kinases (MAPK). The molecular mechanism(s) by which CO interact with this signaling transduction pathway and how this contributes to the anti-apoptotic effect of HO-1/CO remains to be established and is the focus of this project. We have found that over-expression of HO-1 in cultured EC results in the specific decrease of the levels of expression of the p38 α MAPK isoform. HO-1 does not regulate the level of expression of the mRNA encoding this p38 MAPK isoform. Instead it targets the p38 α , but not the p38 β MAPK isoform for degradation by the 26S proteasome pathway. This effect is seen only when the p38 α MAPK isoform becomes activated. We believe that this mechanism of action is critical in mediating the anti-apoptotic effect of HO-1, based on the finding by others that the p38 α MAPK acts in a pro-apoptotic manner while p38 β is anti-apoptotic. Our working hypothesis is that by the specific reduction of the level of p38 α expression, HO-1 can signal in a specific manner via the p38 β to protect EC from undergoing apoptosis. To address whether or not the ability of HO-1 to suppress the expression of p38 α is linked to its anti-apoptotic action, we have generated siRNAs directed against the p38 α or the p38 β MAPK isoforms. These have been tested in EC and shown to be specific for their respective targets. Inhibition of the p38 β MAPK expression by an anti-p38 β -siRNA decreases the viability of human umbilical vein EC by around 80% while inhibition of the p38 α MAPK anti-p38 α -siRNA does not decrease EC viability. This is consistent with the notion that p38 β is required to maintain EC viability while p38 α is not. As to whether p38 α MAPK acts in a pro-apoptotic manner in EC remains to be established.

***In vivo* delivery of HO-1 to Inhibit the pathogenesis of sepsis**

Member: Miguel Soares (UL/FCT), Moises Calvacante (IGC/FCT) & Tatiana Vassilevskaia (IGC/FCT).

External Collaborators: AstraZeneca, Manchester, UK

Inflammation, such as it occurs during microbial infections, is critical to the immune response that will ultimately lead to microbial clearance. However, inflammatory reactions must be terminated as soon as microbial infections have been cleared. When this does not occur, acute or chronic inflammation develops leading to tissue injury, organ dysfunction and disease. An example of this phenomenon is sepsis, an inflammatory driven syndrome that remains one of the main causes of death in our

countries. Expression of the stress responsive gene heme oxygenase-1 (HO-1) plays a critical role in suppressing inflammatory reactions. This effect is mediated primarily via the ability of HO-1 to degrade heme into the gas carbon monoxide (CO). We thought of taking advantage of this protective effect to prevent the development of sepsis. We have used a technology allowing the delivery of proteins, such as HO-1, into cells *in vitro* and *in vivo*. The approach employs small peptides, referred to as protein transduction domains that have been shown to enter cells “spontaneously” with very high efficiency. When linked/fused to other molecules, e.g. HO-1, these are transported into cells as well. We have generated a fusion protein containing a protein transduction domains derived from the human immunodeficiency virus Tat protein, i.e. YGRKKRRQRRPPQ and a chimeric rat HO-1 protein that contains a 6 His tag in the N-terminal region allowing its specific detection and purification. The resulting protein (6HisTat-HO-1) has been produced, purified and shown to keep its enzymatic function. Expression of Tat-HO-1 in primary mouse monocyte/macrophages blocked bacterial lipopolysaccharide driven TNF- α production, an effect similar to the one observed in monocyte/macrophages that over-expressed HO-1. We have expressed 6HisTat-HO-1 *in vivo* and shown that it prevents the development of bacterial lipopolysaccharide mediated endotoxic shock. We are in the process of assessing whether it can revert sepsis as well. In addition we will use this approach to assess the molecular mechanism by which HO-1 affords such a potent protective effect. We plan to expand the use of this approach to assess the protective effects of other molecules involved in the degradation of heme by HO-1, i.e. heavy chain ferritin.

Regulation of T-cell mediated immune responses by the stress responsive gene heme oxygenase-1

Member: Miguel Soares (UL/FCT)

Student: Angelo Chora (FML/FCT).

Collaborators: Jocelyne Demengeot, Lymphocyte Physiology Laboratory, Instituto Gulbenkian de Ciência, Oeiras, Portugal & Abelhadi Saoudi PhD HDR Autoimmunité et Immunorégulation Inserm - Unité de Recherche U563 Centre de Physiopathologie Toulouse-Purpan Pavillion Lefèvre, CHU Purpan.

Experimental autoimmune encephalomyelitis (EAE) in rodents is well-established prototypic CD4 T helper cell-mediated autoimmune disorder that recapitulates the human demyelinating disease multiple sclerosis. In both cases the pathogenesis of the disease is characterized by an initial phase associated with visual, motor and sensory disturbances. These symptoms are all directly attributed to injuries to the central nervous system. They manifest over relatively short periods (relapse episodes) followed by longer ones during which the intensity of the clinical signs decreases significantly (remission episodes). In about half of patients that suffer from multiple sclerosis, relapse episodes evolve more frequently and with higher intensity over time. We have hypothesized that the remission and resistance phases of this disease are regulated through the expression of specific set of genes that protect target cells in the brain (i.e. oligodendrocytes) from T cell and monocyte/macrophage (M ϕ) mediated injury. In addition these genes may also down-modulate the immune response that is directed against the target cells. We have obtained data that suggests that the stress responsive gene heme oxygenase-1 (HO-1) acts in such a manner. Based on the well-established protective effect of IL-10 in EAE and the recent demonstration that the anti-inflammatory effect of HO-1 is mediated via IL-10 we have hypothesized that IL-

10 may prevent the development of EAE via a mechanism that is dependent on the expression of HO-1. This would imply that HO-1, in itself, would suppress the development of EAE. We tested whether induction of HO-1 expression would modulate the activation/proliferation of T cells that recognize the myelin oligodendrocyte glycoprotein (MOG 35-55) antigen and that trigger EAE in C57BL/6 mice. We found that this is the case. Induction of HO-1 expression suppresses the activation/proliferation of this self-reactive T cell population. This effect is dependent on the generation of IL-10 as the immunosuppressive effect of HO-1 is overcome in cells from IL-10 deficient mice. We also found that the ability of HO-1 to suppress the activation/proliferation of anti-MOG CD4 T cells is dependent on the presence of antigen presenting cells. We are now testing whether these are dendritic cells. In keeping with this set of observations we also found that induction of HO-1 expression in vivo suppresses the development of EAE in C57BL/6 mice. We are in the process of assessing whether this effect is also dependent on the expression of IL-10 and whether this can be used in a therapeutic manner to suppress the development of ongoing EAE in mice. If we prove this to be the case then this would suggest that such an approach might be useful to overcome the pathogenesis of multiple sclerosis in humans.

Inhaled carbon monoxide suppresses the development of atherosclerotic lesions: assessment of mechanism of action and possible therapeutic applications

Member: Miguel Soares (UL/FCT) & Isabel Pombo Gregoire (FCT)

Student: László Tokaji (UL/FCT).

Collaborators: Moises Mallo, Neural Crest Laboratory, Instituto Gulbenkian de Ciência, Oeiras, Portugal

Heme oxygenase-1 (HO-1) is a stress responsive enzyme that catabolyzes heme into three products: the gas carbon monoxide (CO), biliverdin and iron. The hypothesis tested in this proposal is that inhaled CO prevents the pathogenesis of atherosclerosis via a mechanism that involves the modulation of monocyte/macrophage activation as well as the inhibition of smooth muscle cell (SMC) proliferation. We have previously shown that inhaled CO prevents the development of intimal hyperplasia that originates from acute vascular injury, associated with organ transplantation and/or balloon injury. We have also shown that this effect is associated with the ability of CO to suppress the pro-inflammatory phenotype of monocyte/macrophage activation and to block SMC proliferation via a sequence of events that requires the activation of the p38 mitogen-activated protein kinases (MAPK). In this proposal we aim to investigate whether inhaled CO can be used therapeutically to suppress the development of atherosclerosis. Further, we aim to understand the molecular mechanisms underlying this effect. We are in the process of generating a mice in which HO-1 expression can be suppressed in a regulated and reversible manner. We will backcross this mouse strain in the ApoE^{-/-} background and will assess the effect of endogenous HO-1 expression on the development of atherosclerosis in these mice. We will then assess whether inhaled CO can be used therapeutically to suppress the development of atherosclerosis in this mice. We will cross these mice with mice that are deficient for genes involved in the signal transduction pathway triggered by CO in smooth muscle cells so that we can assess whether these genes are involved in the anti-atherogenic effect of CO.

Regulatory T cells origin and specificity

Members : Antonio Coutinho and Jocelyne Demengeot
Students: Alexis Perez, Manuel Rebelo, Santiago Zelenay

In the previous years we assessed TCR specificity requirement for Treg development and effector function. The bulk of our work as those of others strengthened the original hypothesis that Treg are selected in the thymus for high affinity/avidity for Ag presented by epithelial cells. However most studies concerning Treg functions are conducted in lymphopenic animals, either by creating a severe bias in T cell repertoire or by adoptive transfer of small number of T cells in alymphoid mice. In these models, Treg prevent uncontrolled inflammatory reactions and autoimmune disease associated with the expansion of pathological cells. This year we developed approaches aiming at assessing Treg biology in more physiological conditions. We revisited two model of spontaneous lymphopenia not associated with pathology, namely neonates and IL-7 deficient animals, to assess the molecular requirement for Treg differentiation. Our analyses suggest that in addition to TCR specificities, Treg differentiation follows a developmental program distinct from the bulk of other conventional T cells. In parallel, we explored the physio-pathologic state of animals transiently depleted of Treg at adult age. Although these animals remain apparently healthy, they show severe alteration in serum Ig reactivities, that persist for the remaining lifetime. This alteration is not corrected by de novo thymic production of Treg, supporting the idea that Treg function is limited to the maintenance of physiological equilibrium. This experimental system should therefore allow for the characterization of the auto-antigen repertoire for which tolerance is solely ensured by Treg function.

Effect of immuno-suppressors on the onset and severity of autoimmune disease

Members: Antonio Coutinho, Jocelyne Demengeot, Werner Haas
Students: Francisca Fontes, Alexis Perez, Manuel Rebelo

Immune-suppressants such as steroids (e.g. hydrocortisone, HC) are widely used to treat autoimmune disease. Among other effects, steroids preferentially affect cycling cells. Elsewhere we have produced evidences that peripheral regulatory T cells are enriched for proliferating cells. We used the anti-myelin basic protein (MBP) TCR transgenic mice, a murine model for human Multiple Sclerosis developed by J.Lafaille (Cell 1994, 78: 399) to investigate the effect of HC treatments on regulatory T cells. Hydrocortisone, administrated in equivalent doses to what is currently prescribed to patients, induces EAE in otherwise healthy Tg mice provided the treatment is combined with the inflammatory compound pertussis. Similar results are obtained upon treatment with depleting anti-CD25 antibodies. Treg purified from HC treated donors loose their regulatory function as evidenced in adoptive transfer experiments. Ongoing work aims at the identification of the molecular defect in these affected Treg. While steroids are effective therapy for relapses in Multiple Sclerosis they do not improve the progression of the disease, and our results may contribute to the understanding of this failure. This study encourages us to apply this approach to test other therapeutic drugs potentially enhancing Treg functions.

A novel assay to quantitate regulatory T cell activity

Member: Jocelyne Demengeot and António Coutinho

Student: Santiago Zelenay

Development of *in vitro* model systems has facilitated the analysis of functional properties and mechanisms of action of regulatory T cells. The available assays, however, require relatively high numbers of cells, thus limiting the evaluation of regulatory activities to T cell sub-populations that can be purified in large numbers.

We have now developed an *in vitro* assay that allows monitoring regulatory activities of very small numbers of T cells. CD4⁺ CD25⁺ regulatory T cells do not produce IL-2 and suppress IL-2 production by naïve CD4⁺ T cells. In conventional assays, regulatory activity is read out as inhibition of (IL-2-dependent) proliferation of “target” cells. The novel assay instead measures IL-2 in (co-)culture supernatants, using a very sensitive biological indicator system. In short, sorted CD4⁺ CD25⁺ T cells are titrated on fixed numbers of CD4⁺ CD25⁻ “target” cells and irradiated spleen cells (as APCs), in the presence of anti-CD3 mAb for 48 hours. The IL-2 concentration in the culture supernatants is thereafter quantitated by monitoring the proliferation of an IL-2-dependent cell line (CTLL-2). This protocol permits detection of regulatory activity with at least ten fold less cells than conventional suppression assays. Typically, we could demonstrate a significant reduction of IL-2 production by as few as 100 CD4⁺ CD25⁺ T cells in cultures containing 1000 CD4⁺ CD25⁻ target T cells.

CD4⁺CD25⁺ T cells facilitate murine infection by *P. berghei*

Member: António Coutinho and Elsa Seixas

Student: Dominique Ostler

It has been recently demonstrated at the IGC that regulatory T cells, in addition to controlling inflammation, autoimmune diseases and allergy, reduce protective responses and the elimination of microorganisms in naturally infected animals (*see 2001 Annual Report*). We have now investigated the influence of regulatory T cells in the course of *P. berghei* infection in mice, and found that such cells facilitate infection. Thus, preferential elimination of regulatory T cells in BALB/c mice, by treatment with cyclophosphamide, results in significantly lower parasitemias that are reconstituted by the transfer of CD4⁺CD25⁺ (but not of CD4⁺CD25⁻) cells. Similar results were obtained in C57BL/6 mice. Furthermore, alymphoid (Rag-1 ^{-/-}) mice show significantly higher parasitemias if reconstituted with CD4⁺CD25⁺ cells, as compared to animals receiving CD4⁺CD25⁻ T cells.

The molecular and cellular mechanisms underlying such facilitation of *P. berghei* infection by CD4⁺CD25⁺ T cells are currently under investigation. We hypothesise that they might owe to the production by the parasite of “nonspecific” mediators on “innate immunity”.

Wound repair using *Drosophila* as a model system

Members: Antonio Jacinto, William Wood
Collaborators: Paul Martin (University College London, UK)

The capacity to repair an epithelial wound is a fundamental survival mechanism that can be activated at any site of damage throughout embryonic and adult life. During embryogenesis, several morphogenetic movements, such as dorsal closure, closely resemble the artificially activated tissue movements of wound healing. Understanding how epithelia move and fuse together in these embryonic situations may reveal clues as to how these same processes are accomplished during tissue repair and provide a way in which we might modulate wound healing. We are investigating how far the similarities between dorsal closure and wound healing extend by using what we know about gene cascades regulating morphogenetic movements in *Drosophila*, to dissect out which genes may also be necessary for the cell and tissue movements that occur in wound repair. We have established an embryonic wounding assay using a laser ablation system that has allowed the initial characterisation of epithelial wound healing in wild-type *Drosophila* embryos using confocal microscopy. It is now possible to test which of the factors that are involved in dorsal closure are also functionally required to initiate or drive the wound closure. We are using our wounding system to test mutants defective for morphogenetic genes that may be required during wound healing.

***Drosophila* hemocyte recruitment to wound sites**

Members: Antonio Jacinto, William Wood
Collaborators: Paul Martin (University College London, UK)

During wound healing in vertebrates the inflammatory leukocytes, such as neutrophils and macrophages, act to clear contaminating microorganisms and debris, and to amplify the earlier wound signals by the release of further pro-inflammatory factors, which instruct neighbouring cells, mainly fibroblasts and keratinocytes, to contribute to the repair process. Several of the chemoattractants that can recruit blood cells to the wound site have been identified. However, the exact regulation of this process is not understood and the mechanisms that act *in vivo* are difficult to unravel due to the number of factors involved and complexity of interactions between the different cell types. Studies in simpler models like *Drosophila* are expected to reveal some of the fundamental mechanisms of cell recruitment to wounds. Our laser ablation wounding system is used to test the function of factors potentially involved in hemocyte chemoattraction, that have homologues in flies.

Control of inflammatory responses by regulatory T cells

Members: Jorge Carneiro, Jocelyne Demengeot, Matthias Haury
Students: Iris Caramalho, Thiago Carvalho, Dominique Ostler, Gustavo Rosa, Santiago Zelenay

Acute inflammatory immune responses to normally innocuous microbes can be lethal and are a frequent cause of death in immuno-suppressed patients. In several

experimental systems of infection or colonization by commensals, CD4⁺ CD25⁺ regulatory T cells (Treg) prevents these deleterious inflammatory responses. We have shown that Treg prevent lethal inflammatory pneumonia induced by the hyper-responsiveness of naïve CD4⁺ cells triggered by *Pneumocystis Carinii* infection in mice. This study revealed that Tregs limit also protective immune responses. Our latest efforts concentrated on understanding the nature of the triggering signal necessary for Treg function during inflammatory responses. We evidenced that regulatory T cells are activated, expanded, and acquire higher effector efficiency upon inflammatory stimuli. This activation is in part mediated by Toll Like Receptors selectively expressed by this subpopulation of T cells. In addition other pro-inflammatory signals such as IL-15 participate in this dynamic. To assess the contribution of foreign antigen to the regulation of inflammatory reactions by Treg, we developed and explored an *in vivo* short-term assay of induced sterile inflammation. This approach revealed that Treg control the innate and adaptive responses to inflammatory stimuli by a mechanism independent of foreign protein. Finally we evidenced that interaction with naïve T cells is necessary for the stability of the CD25 molecule expression on regulatory T cells. Together these findings may offer a cellular and molecular basis for several unexplained phenomenology, such as the inverse correlation between infection and autoimmune disease incidence, inefficient immuno-therapy of cancer and chronic infections. Ongoing work aims at establishing the significance of this finding for pathological autoimmunity.

IL-10 and its role in regulation of immunological tolerance

Member: Matthias Haury

Collaborators: Dan Holmberg, (Umea University, Sweden) Antonio Bandeira (Institut Pasteur, Paris, France); Paulo Vieira (Institut Pasteur, Paris, France)

We have generated a new mouse transgenic mouse strain to study the expression of IL-10 *in vivo*, and we are currently characterizing this strain to study expression patterns of IL-10 in various cell types. We are also characterising in more detail the immuno-subphenotype of regulatory T-cells and their localisation using multicolor (5-6 colors) flowcytometry and multiphoton confocal microscopy. These studies are carried out in collaboration with the laboratory of Dr. Dan Holmberg, Umea University, Sweden and Antonio Bandeira and Paulo Vieira, Pasteur Institute Paris.

Role of microglia activation in inflammation models and associated neuropathogenesis

Member: Sukalyan Chatterjee, Teresa Faria Pais

Students: Catarina Figueiredo, M^a Hortense Matos, Rui Peixoto

Collaborators: Laura Santambrogio, Harvard Medical School Boston, USA.

Microglia, the resident macrophages of the brain, constitutes 5-20% of the cell population of the central nervous system (CNS). Although in the normal CNS microglia are in a "resting state" and have architectural and reparative functions they promptly respond to physical trauma and microbial infection. Microglia migrate to the site of injury where it can proliferate and/or get activated. Activated microglia have up-regulated surface receptors and produce cytokines which initiate a local immune

response. Several studies have suggested a role for activated brain macrophages in neurodegenerative diseases, brain injury and in several infections of the CNS. It is, however, difficult to distinguish between different populations of brain macrophages, namely the relative contribution of resident microglia versus infiltrated macrophages that derive from blood monocytes. Moreover, the function and phenotype of brain macrophages whether or not activated are far from being characterized. *Plasmodium falciparum* is the infectious agent of cerebral malaria (CM) in humans, a severe cerebral complication characterized by reversible encephalopathy. There are evidences that microglia are activated very early on after infection. Whether activation of resident microglia is a key event in causing neuronal damage that culminates in CM or the result of a strong immune response with disruption of the blood-brain barrier is still a matter of debate. This project is addressing these issues in the context of other models of neuronal degeneration. The signaling cascades, mechanisms initiating microglial activation and the consequences of activation are largely unknown. Microglia in the central nervous system are both protective and cytotoxic. A controlled balance of which needs to be maintained to prevent pathogenesis. Here we will address the signaling mechanisms in activated microglia by infection (extracts of parasitized erythrocytes) and dying neurons adopting a molecular approach to identify secreted entities like cytokines, intracellular signaling cascades and mechanisms of transcriptional regulation. This may elucidate the crux of the balance between cytotoxic and protective role of microglia. The results will contribute to the understanding of brain inflammation and associated neurological diseases. Moreover, the project will investigate the role of quinolinic acid secreted by activated microglia in the neuropathogenesis of CM. Interestingly, unlike neurons which succumb to quinolinic acid challenge microglia is remarkably resistant to death caused by this agent. The mechanism(s) of this resistant and its in vivo consequence is under current investigation.

Malaria & immunity

Malaria remains the most devastating parasitic disease worldwide. In any given year, nearly ten per cent of the global population will suffer from malaria — 500 million clinical cases — and more than 1 million will die. In Africa, the disease kills one child in twenty before five years of age. In addition, malaria has a major negative impact in economic development and stability of many developing countries. Various attempts at eradicating malaria have thus far failed.

After the bite of an infected *Anopheles* mosquito, the first obligatory step is the infection of hepatocytes, which is free of symptoms. A few days later, each parasite has developed in thousands of new parasites, which then reach the bloodstream and infect erythrocytes, accounting for all the symptoms and complications associated with the infection. Most fatal cases of malaria occur in this acute phase of previously uninfected individuals, particularly in young children, by mechanisms that involve both host immune system and parasite factors. A fraction of severe malaria cases succumb to neurological complications (Cerebral Malaria), the pathogenesis of which is yet to be fully explained. The central problem with malaria is the lack of an efficacious vaccine, and many recent attempts have had no success. In turn, this is explained by the fact that malaria infection leaves little or no “immunity” such that the infection becomes chronic or the individual is recurrently re-infected. Hence, it seems that vaccine development requires prior understanding of this unusual immunological behavior. At the IGC there are 5 groups dedicated to study distinct

but complementary aspects of the interactions between the malaria parasite (*Plasmodium*) with its vertebrate hosts, and how the disease spreads in populations. In turn, each of these groups collaborates with others in the Institute (and elsewhere), such that malaria has come to occupy a considerable fraction of our research.

One of our approaches is genetics-based, aiming at identifying factors that confer resistance to malaria infection and its severe complications. The work was initiated using *P. berghei* in mouse models, and it has led to the identification of 2 loci controlling resistance to severe malaria, and 2 more chromosomal regions imparting resistance to high levels of parasitemia. Isolation of the genes responsible for these effects, while proceeding to the genetic mapping of loci controlling hepatic infection, are the next steps in this approach. Genetic analysis of host resistance should soon be extended to human populations, taking advantage of the information on the genomes of man and mouse. The availability of the complete genomes of several *Plasmodia*, on the other hand, makes it possible to search for putative molecules that activate “innate immunity”, and might be responsible for differential resistance/susceptibility to malaria infection and its severe consequences. Several such parasite “mitogens” have been isolated, capable of evoking “acute phase responses” in a variety of host cell types. Variation in the severity of these responses in different genetic backgrounds is now analysed, and various control strategies are tested. Yet an alternative approach is to understand the interactions between parasite molecules and host cell receptors, which are necessary for infection. Thus, *Plasmodium* can invade many types of cells but only fully develops inside hepatocytes, indicating a critical role of the host cell in sustaining parasite growth and development. These studies have led to identifying critical host molecules that support hepatocyte infection and may offer novel targets for therapeutic intervention, notably through blocking/activating the respective signaling cascades. Similar analyses are conducted for the interactions of infected erythrocytes with receptors on other host cells. As seen above, inflammatory reactions can be pathogenic, and cerebral malaria provides one such example. Hence, regulation of the acute responses to infection is investigated, also because absence of effective immunity can be thought to result from excessive regulation. The expansion of a particular population of T cells and its accumulation in the brain of infected mice, have been correlated with cerebral malaria. Current work aims at the control of such pathogenic lymphocytes by Regulatory T cells, and at dissecting the effects of pathogenic lymphocytes on endothelial, astro and microglial cells of the brain, using genomics and proteomics and hoping to identify new predictive markers for the infection outcome. The risk for malaria infection and disease varies wildly across Tropical Africa, and the overall results of therapeutic or environmental interventions also very widely, suggesting unexpected thresholds in transmission. Furthermore, to be effective, interventions in malaria need not be radical, as they might bring prevailing conditions across those thresholds. By developing mathematical models, we aim at a better understanding of malaria epidemiology and control. We have recently shown that variations in the “reinfection threshold” that is intrinsic to the population dynamics of recurrent infections may explain those discrepancies.

The role of Toll-like receptors in cerebral malaria

Member: Christophe Gregoire, Elsa Seixas, Antonio Coutinho and Andrew Waters
Student: Vasco Correia

Despite intensive research, the pathogenic mechanisms of cerebral malaria – the major cause of death in *P. falciparum* infection - are not fully characterised. Known central features are the T cell-dependence of the process in murine malaria, a key role of TNF-alpha, and the sequestration of mature forms of parasitized erythrocytes and ring stages within the microvasculature of the major body organs, following interactions between surface molecules on parasitized red blood cells and host receptors.

Toll-like receptor 4 (TLR-4) is known to be a mediator of cellular activation and production of proinflammatory cytokines. TLR signalling operates through the release of cytoplasmic NF-kB and its translocation to the nucleus, which we have now demonstrated occurs in endothelial cells of the brain microvasculature in animals undergoing cerebral malaria.

We have now investigated whether brain inflammation in cerebral malaria involves TLR-4 signalling, by comparing the course of *P. berghei* infection in wild-type and TLR-4 mutant mice. The results show that TLR-4 “null mutants” (C57BL/10.Sc.Cr) mice do not develop cerebral malaria when inoculated with *P. berghei*, and do not translocate NF-kB into the nucleus of endothelial cells in the brain microvasculature. In contrast, C57BL/6 controls develop cerebral malaria at a very high frequency, and show readily detectable NF-kB translocation in brain endothelia.

As B10.Cr mice carry a second mutation in the gene encoding one IL-12 Receptor chain, we used the C57BL/10.SN mouse strain to confirm that resistance to cerebral malaria is imparted by the TLR-4 mutation. Moreover, by producing hemopoietic bone-marrow chimeras in the B10.Cr/B6 combination, we show that the presence of TLR-4 on hematopoietic cells, but not on endothelial cells, is required for the development of cerebral malaria in this genetic background.

Interestingly, C3H/HeJ (carrying a TLR-4 point mutation that results in a complete loss of function phenotype) and C3H/HeN (wild-type) mice do not differ in susceptibility to cerebral malaria, indicating variability in the respective molecular pathogenesis. These alternative mechanisms are currently under investigation.

In conclusion, the present results support the notion that TLR-4, at least in some “backgrounds”, plays an important role in the development of cerebral malaria. Innate immunity, however, does not provide all the critical factors in the pathogenic process, as demonstrated by the complete resistance of Rag-1 ^{-/-} mice. Hence, cerebral malaria represents a novel form of pathology resulting from the interaction of innate and adaptive immunity.

In search of malaria mitogens

Member: Elsa Seixas, Christophe Gregoire, Antonio Coutinho and Andrew Waters
Students: Margarida Cunha, Vasco Correia and Dominique Ostler

Using three inbred mouse strains and *P. berghei* as well as *P. chabaudi chabaudi*, we have now analysed in detail the alterations in cellular composition and state of activation of lymphocytes in representative secondary lymphoid organs, bone marrow and thymus. The results confirm that malaria infection is accompanied by a marked

polyclonal activation of T and B lymphocytes and serum hypergammaglobulinemia. We initiated, therefore, the search for “malaria mitogens”, following two complementary approaches: on the one hand, we screen plasmodium products for activating lymphocytes and/or dendritic cells; on the other hand, we use the information contained in the recently completed plasmodium genome sequence in order to identify “candidate” molecules with this ability.

Innate immunity in malaria infection: interactions of Dendritic Cells (DC) and other antigen presenting cells with blood stages *P. chabaudi*

Member: Elsa Seixas

Student: Dominique Ostler

Primary infection of mice with *P. chabaudi chabaudi* is characterized by a rapid inflammatory response where IL-12, TNF- α and IFN- γ are produced in the spleen and are transiently present in the plasma. The cells involved in this early response are unknown. Previous results (Seixas et al. 2001), however, have shown that interaction of bone marrow-derived DC (BMDC) with schizont-stage parasites leads to production of TNF- α , IL-6, and IL-12, and to up-regulation of MHC class II, CD86 and CD40, as well.

Such a DC response could explain the rapid cytokine production upon infection, and the preferential activation of TH1 cells that occurs early in the primary infection with *P. chabaudi chabaudi*, but this needs to be established in more physiological conditions. Accordingly, this work aims at investigating the “innate response” of splenic DC to blood stages of the parasite, and to establish the molecular basis of DC activation.

Accordingly, we conduct in vivo studies of splenic DC (characterized by the differential expression of surface markers and production of cytokines) during the malaria infection, and study DC responses in mutant mice carrying selective defects at each of the known TLRs. The first results show that TLR-4 is not essential for malaria innate immunity.

The innate immune response during the hepatic stage of infection in malaria

Members: Christophe Gregoire, Elsa Seixas, Maria Mota and Antonio Coutinho

Students: Ana Rita França

The innate immune response is the first line of defense against infectious diseases. Responsiveness to microbial products requires expression of TLRs and their associated accessory molecules. Gene expression for all nine TLRs and related molecules, like MyD88, was identified in both human primary hepatocytes and in the human hepatoma cell line HepG2. Given that liver cells express all known microbial recognition and signaling molecules and also that the hepatic stage is the first crucial step of the infection, it can be hypothesized that the liver is probably the major mediator of innate immunity against *Plasmodium* spp.

A greater appreciation of the mechanisms of innate immunity during the infection should provide critical clues on how manipulation of the immune system may best be achieved. Thus we propose to characterize the cellular and molecular mechanisms

underlying innate immune processes induced by *Plasmodium* sporozoites and to clarify whether these innate mechanisms are beneficial or not to the parasite and/or host. The ultimate goal is to elucidate the role and the significance of TLRs in hepatocytes, and to clarify the pathways initiated by recognition of sporozoites in both the initial interaction and during the development of an immune response.

The role of host cell factors during the hepatic stages of a malaria infection

Members: Maria Manuel Mota and Sabrina Epiphany

Students: Daniel Carapau, Cristina Rodrigues and Margarida Cunha

Collaborators: Ana Rodriguez (NYU, USA) and George Dimopoulos (JHSPH, USA)

Plasmodium is the causative agent of malaria, one of the most prevalent and severe human infectious diseases. *Anopheles* mosquitoes inject sporozoites into the host, which rapidly migrate to the liver, invade hepatocytes and develop into merozoites that are released to the blood stream initiating repeated cycles of schizogony responsible for the disease symptoms. Because liver infection is the first obligatory step of the disease, hepatocyte-*Plasmodium* interactions that are crucial for the establishment of infection, constitute an ideal target for potential anti-malarial vaccines or preventive treatments. Parasites have developed a remarkable ability to survive in their hosts. It is becoming evident that intracellular parasites are masters at manipulating the host cell pathways for their own benefit, to create a more hospitable environment. Our aim is to identify hepatocyte genes that are differentially expressed at the transcript level as a result of *Plasmodium* development. Studies of pathogen-induced changes in host cells gene expression have relied on examination of the expression of a limited number of specific gene products. While these studies can be quite valuable ascertaining the role of known genes, the approach fails to provide information about genes for whom a functional role is not intuitive. The recent development of high-throughput methods for the analysis of gene expression under various conditions provides an opportunity to examine host-pathogen interaction, in an unbiased way. To achieve our aim, we will prepare cDNA probes from infected hepatocytes (at different times after infection) that will be hybridized to microarrays containing 15,000 mouse cDNAs. As a control for the baseline expression, we will use non-infected hepatocytes cDNA. This screen is expected to identify a large number of host cell genes whose transcript levels are significantly increased in response to a *Plasmodium* infection.

Sporozoites traverse the cytosol of several hepatocytes before the final infection. During this migration, *Plasmodium* sporozoites disrupt the host plasma membranes. Our recent results show that host cell wounding by sporozoite migration induces the secretion of hepatocyte growth factor (HGF), which renders hepatocytes susceptible to infection. Infection depends on the activation of HGF receptor (MET). The malaria parasite exploits MET as a mediator of signals that make the host susceptible to infection. HGF is known to induce alterations on cell cytoskeleton, vesicular trafficking, lipid metabolism and apoptosis. We propose to determine the contribution of any of these effects on *Plasmodium* infection. We will also use mutated MET, which are able to separate the various signalling cascades.

The interactions of infected erythrocytes with host cells during malaria infections

Members: Maria Manuel Mota and Ana Pamplona

Students: Patricia Leirião, Margarida Cunha, Bruno Mateus, Susana Silva and Sónia Albuquerque

Collaborators: Ana Rodriguez (NYU, USA) and Silvia Giordano (IRCC, Italy)

Most morbidity and mortality from malaria is caused by infection with *P. falciparum*. Falciparum malaria is not simply a major public health problem but it is even considered to restrain economic growth in many parts of the world (7). The major reason for the lethality caused by this Plasmodium spp. is the fact that their infected erythrocytes express some adhesions able to mediate the adhesion of infected erythrocytes to host receptors on endothelium, uninfected erythrocytes, and platelets. In addition, it also know that during these stages infected erythrocytes also interact with other host cells such as dendritic cells and macrophages. Our aim is to study the role of there interactions during infections.

T- Cell response in pathogenesis of malaria

Members: Sylviane Pied, Margarida Vigario.

Students: Ricardo Ataíde, Tania Cruz.

Collaborators: Virgílio do Rosario, CMDT Lisbon, Portugal, Pierre-André Cazenave, Institut Pasteur Paris, France.

Cerebral malaria (CM) is one of the most severe complications of *Plasmodium falciparum* infection. Several observations in patients with malaria suggested that T cells are implicated in CM pathogenesis. Rodent models allowed to demonstrate the involvement of CD4 and/or CD8 T cells in CM pathology. A link between an expansion of CD8⁺ T cells bearing V β 8⁺ T cell receptor (TCR) chains in the blood and an accumulation of CD8⁺ T cells in the brain of mice developing CM was found during *Plasmodium berghei* ANKA infection, a parasite strain able to induce in susceptible mice a neurological syndrome mimicking human CM.

Pathology could be the result of an inefficient control of the pathogenic CD8 T cells. Regulatory CD4⁺CD25⁺ (Treg) cells are a subset of T cells with the capacity to inhibit the expansion of CD4 and/or CD8 effector T cells. We analyzed this population of Treg cells during the infection with *Plasmodium* in order to establish if they could participate in mechanisms leading to protection against CM pathology. To address this question a murine model of CM was used : C7BL/6 mice infected with *Plasmodium berghei* (PbA). The pathology was induced either by inoculation of parasitized erythrocytes (PE) (the most used model of CM) or (to better reflect the Human disease) by inoculation of sporozoites (spz) (a new model of CM).

The kinetic of CD4⁺CD25⁺ T cell subpopulation was followed during PbA infection. An expansion of both Treg and effector CD25⁺ cells was observed in the spleen of infected mice. In addition, the CD4^{int}CD25^{high} cells were sorted at different time points and their TCR repertoire was analyzed using a high throughput CDR3 spectratyping based on a molecular approach linked to statistical multivariate methods to determine whether these cells are restricted in their TCR gene usage. Furthermore, functional activity of Treg cells expanded during infection was studied by testing there capacity to inhibit *in vitro* proliferation of naive non-specifically stimulated CD4⁺CD25⁻ T cells. Results obtained showed that Treg cells isolated from PbA

infected mice do not lose their capacity to inhibit non specific proliferation of either CD4⁺ or CD8⁺ T cells. Finally, no difference on CM development was found between CD25 depleted and non depleted mice. However, mice depleted in CD25⁺ cells developed lower parasitemia than control mice (Vigario M., et al., manuscript in preparation).

T lymphocytes, astroglial, microglial and endothelial cell interactions during malaria neuropathology

Members: Sylviane Pied, Johann Truccolo, Jorg Becker

Students: Rui Rodrigues,

Collaborators: Abelkader Namane, Proteomic platform Institut Pasteur Paris, France

The pathogenesis of Cerebral Malaria (CM) is a complex process involving both host immune system, in particular T lymphocytes, and parasite factors (1). The goal of this project is to dissect the cascades of events taking place during malaria parasite - specific T-cells and brain-blood-barrier (BBB) interactions. This is assessed by the use of an in vitro model of BBB, genomics/proteomics and immunochemistry in order to *i*) investigate the changes in astrocytes, endothelial and microglial cells genes transcription and protein expression following exposure to CD8⁺ T cells recruited in the Brain of *Plasmodium berghei* ANKA infected mice developing cerebral malaria (CM) and *ii*) describe their input in the immune mechanisms contributing to the development of the neuropathogenesis during malaria.

We have developed an in vitro model of the Brain-Blood-Barrier to study interactions between T lymphocytes astroglial, microglial and endothelial cells during CM. Gene expression analysis using the GeneChip murine genome U74v2 microarray (up to 6000 genes), 2-D PAGE proteomic coupled to mass spectrometry and statistic algorithms is used to allow a global and objective comparison at the transcriptional and post-transcriptional level of changes in the BBB cultures after exposure to parasite-induced T-cells.

In parallel to genomic and proteomic analyses, immunohistochemistry coupled to confocal microscopy analyses is used to look for the expression of several markers on microglia and astrocytes and endothelial cells.

Do IgE autoantibodies play a role in pathogenesis of *Plasmodium falciparum* malaria?

Members: Sylviane Pied, Vincent Guiyedi, Constantin Fesel

Students: Joana Duarte

Collaborators: Maryvonne Kombila, CHL, Libreville, Gabon and Gyan Mishra, NCCS, Pune, India.

Despite much clinical and scientific effort, the physiopathology of pernicious malaria remains obscure. The general objective of the project is to understand better the role of the immune system in the development of *P. falciparum* infection and especially in cerebral malaria. In order to elucidate these phenomena, we studied *P. falciparum*-infected patients from different cohorts:

- 1) from an endemic region in India comprising 120 adults aged of 20 to 72 years presenting non-severe clinical manifestations, severe forms without cerebral affection, and cerebral malaria.

- 2) from Gabon, more than 300 children under 5 year-old are included in the study. They constitute several group: endemic control, asymptomatic, acute non severe, severe non cerebral and cerebral malaria groups.

For each patients, sera were taken on day of arrival at the hospital, 7 and 30 days after treatment.

Sera level of cytokines such as IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IFN γ , TNF α , TGF β , specific IgE antibodies against *Plasmodium falciparum* and total IgE antibodies were quantified by Elisa. A correlation was found between high level of total IgE and severity of the disease.

Global profiles of brain autoreactive IgE antibodies produced during infection is studying with PANAMA-BLOT method that allows to describe thousands of specificities simultaneously and will be analysed statistically on a basis of principal component analysis to see whether there is an association between cytokine profiles, specific and non specific IgE levels, brain reactivity and pathogenesis.

This study could allow to identify new markers with predictive value for the expected development of the infection.

Integrated research in malaria

Members: Gabriela Gomes, Mostafa Bendahmane

Students: Ricardo Aguas, Paula Rodrigues

Modelling malaria is a fascinating grand challenge. This project is a coordinated effort to integrate laboratory, modelling and field research to promote new ideas for malaria control. This involves several groups at the IGC, UK, France, Denmark, and Kenya. In theoretical epidemiology, Paula Rodrigues is developing within-host models, and Ricardo Aguas and Mostafa Bendahmane are developing epidemiological models

Developmental Biology (DB) in animals and plants

One of IGC's scientific focuses is the search for the mechanisms that guide the affairs of an embryo in its way from fertilization to a full-grown organism. The variety and complexity of the underlying processes is also reflected in the diversity of questions, approaches and biological models employed by the different groups on DB. A common theme in biology, however, is that similar cellular or molecular mechanisms are used once and again to control specific processes in different organisms and within different areas of the same embryo. We learned this in the evolution of species, often resulting from small variations in developmental processes, or even in disease where, for instance, tumor metastasis results from abnormalities in the physiological mechanisms that control formation of tissues and organs. This basic concept has a variety of theoretical and practical implications. The knowledge gained in one particular system can be of enormous relevance for the understanding of another, apparently unrelated, problem. This allows for choosing the particular experimental model that offers the best technical possibilities to approach specific questions, while addressing very general questions. In addition, it leads to interactions among groups working in apparently distinct areas, which may result in very fruitful collaborations.

The importance of this interplay of complementary interests emerges from the work performed at the IGC in 2003. An excellent example is the apparent lack of ontogenetic or phylogenetic barriers for signalling strategies. Thus, nitric oxide (NO), long known as a vasoactive factor, was now shown to be involved in the guidance of pollen tubes across the female plant organs. Calcium signalling was also in the spotlight. Calcium fluxes had long been established as a major signalling mechanism controlling cellular behavior, and the role of calcium in early animal development was known for some years. We have now shown that calcium is a key ion in the control of polarized growth in plants and participates in the first detectable event on egg activation after fertilization. The expertise on “ion biology” accumulated by the “plant group” at the IGC proved very helpful for progress in one apparently unrelated field: the control of right/left asymmetry in vertebrate embryos. As it turned out, left side-specific accumulation of extracellular calcium seems to be among the earliest mechanisms controlling laterality in chicken embryos.

Last years’ contributions to left/right asymmetry breaking were not over with the calcium bursts. Other groups analyzing developmental processes were able to extend our understanding on how some internal organs are asymmetrically positioned. It had previously been determined that the Nodal and Shh pathways are involved in these processes, but at a rather late patterning stage. A regulator of Nodal signalling, *Cerl2*, turned out to be part of this signalling network. In addition, the link between these late events and the early stages of asymmetry breaking (including calcium accumulation) seems to be provided, at least in part, by members of another well known signalling pathway: Notch and its associated factors. Interestingly, the key experiments leading to these findings emerged from studies aimed at understanding a different developmental process, most notably, anterior-posterior axis formation, further highlighting the phylogenetic and ontogenetic conservation of genetic mechanisms.

Further understanding of the regulation of cellular responses to signalling molecules in the proper building of an embryo was also achieved in 2003. Thus, we now know that the selective deactivation of a specific downstream network mediating FGF signalling is essential to properly modulate the cellular responses triggered by these growth factors, eventually resulting in accurate development of areas as different as the limb bud and the brain. Also, it turned out from research at the IGC, that control of mesenchymal responses to these same factors (Fgfs) is, at least in part, a mechanism used by Hox genes to specify segmental identities.

Finally, we experienced last year a rich harvest of data in the search for the genetic control of a variety of morphogenetic processes. These include epithelial dynamics and adhesion, both in physiological processes and during wound healing; the growth and morphogenesis of plant cells and the control of interaction between gametes; the morphogenesis of the ear and of branchial arch structures; the induction of neural crest cells; the ventro-medial control of somitogenesis; the specification of the anterior posterior axis in vertebrate embryos; the understanding of hematopoietic cell lineage specification. The variety of processes studied reflects, again, the wide spectrum of research efforts at the IGC directed at understanding how a single cell generates a complex organism. Of special mention in this section is the increasing use of the Affymetrix gene chip technology in these studies, which has proven very powerful to obtain candidate genes for the control of a wide variety of patterning, growth and morphogenetic processes. Together with the imaging unit and the transgenic mouse facility, an international reputation of excellence has been achieved, mostly driven by the DB groups and their questions.

The role of Tbx1 in embryonic development

Members: Moisés Mallo

Students: Filipa Moraes, Ana Nóvoa

Collaborators: Virginia Papaioannou, Columbia University, New York, USA

The Tbx1 gene belongs to the T-box-containing family of transcription factors. In humans, it is included within the microdeletion on chromosome 22 that has been associated with the DiGeorge syndrome. A variety of genetic analyses, both in mice and humans, indicate that among the more than 20 genes included within this deletion, Tbx1 is the major responsible for the pathological manifestations observed in DiGeorge patients.

Mutant mice for the Tbx1 gene contain a variety of malformations, including abnormal branching of the heart outflow tract, agenesis of the thymus and parathyroid glands, facial abnormalities and strong hypomorphism of the three ear compartments (i. e. outer, middle and inner ears). We are studying the role of Tbx1 in the development of the structures affected in the mutant mice. Our main focus during 2003 has been to understand the role of Tbx1 on ear development.

In Tbx1 mutants the outer ear is very small or absent, most likely as a consequence of the failure of the second branchial arch to develop. The middle ear is also affected. One of the ossicles, the stapes, is completely absent, and the incus is reduced to a small cartilaginous nodule. The third ossicle, the malleus, is also slightly affected, but its structure is still conserved. The tympanic ring and the eardrum are smaller but still recognizable. The defects in the middle ear are the consequence of both the failure of the second arch to develop and the misrouting of some of the neural crest cells normally fated to populate the second arch, that migrates into the first arch in the Tbx1 mutants.

The inner ear is strongly hypomorphic in the absence of Tbx1. The whole ear is reduced to a small hollow cartilaginous structure with no signs of cochlear or vestibular elements. However, even in such affected inner ears, the endolymphatic duct and the vestibuloacoustic ganglion developed almost normally. Analysis of inner ear development at earlier stages indicated that growth and patterning processes are normal in Tbx1 mutant otocysts until day E10.5, when the vesicles are smaller and the spatial regionalization was lost. These results indicated that Tbx1 is not required for the induction or early patterning of the otic vesicle, but it is essential for maintaining these patterns. We are now analyzing the molecular mechanisms mediating this function of Tbx1. In addition, we are studying the role of Tbx1 in the development of the heart outflow tract.

Molecular mediators of Hoxa2 activity during embryonic development

Members: Moisés Mallo

Students: Marta Carapuço, Diogo Pimentel

Collaborators: Nicoletta Bobola, MPI, Freiburg, Germany, Annette Neubueser, IMP, Vienna, Austria, Jacques Drouin, IRCM, Montréal, Canada

Hoxa2 is one of the members of the Hox family of transcription factors. Hox genes are involved in a large variety of developmental processes, and their dysregulation has been often associated with the development of tumors. In the case of Hoxa2, we and others have shown that it is essential for proper development of the anterior region of

the hindbrain and of the second branchial arch. In the second branchial arch, Hoxa2 is required to define the identity of the skeleton that develops from this area. In the Hoxa2 mutants, the second arch skeleton develops abnormally to produce elements that resemble those typically developing from the first arch. We are interested in understanding how this gene performs this function. Therefore, we set to identify the genes that function downstream of Hoxa2 using a differential screening with Affymetrix gene chips. For this screening, we concentrated on the second branchial arch, as it is a major area of Hoxa2 expression and the region where the mutant phenotype is most prominent. From this screening we obtained several clones differentially expressed in the second branchial arches of mutant embryos versus their wild type littermates. This differential expression was confirmed by in situ hybridization for several clones and the analysis of the functional relevance of this differential gene expression has already been started for several of them. Detailed analysis was already performed for Ptx1. In normal embryos, this gene is expressed in the first but not the second arch. In the Hoxa2 mutant embryos, Ptx1 is also expressed in the second branchial arches. A variety of studies, both in vivo and in vitro, indicated that Hoxa2 blocks Ptx1 expression by interference with Fgf signalling. We were able to show that Ptx1 is under the control of Fgf8 and that Hoxa2 interferes with this signalling by modulating the ability of the mesenchymal cells to respond to this signal. The upregulation of Ptx1 in the second arches of Hoxa2 mutant embryos is functionally relevant, because part of the Hoxa2 phenotype was rescued in Hoxa2/Ptx1 double mutants.

We are now analyzing how Hoxa2 modulates Fgf signalling. In addition, we are studying other Hoxa2 targets isolated in our screen for their functional relevance downstream of Hoxa2. Finally, we are trying to understand if other Hox genes also use this mechanism to determine regional specificity in other areas of the developing embryo.

Spatial and temporal requirements for hoxa2 during embryonic development

Member: Moisés Mallo

Students: Vanessa Cristão, Ana Novoa

We have developed a genetic method to inactivate genes in a reversible fashion. This method uses the regulatory elements of the bacterial tetracycline operon. This operon contains two basic elements: a DNA sequence (the tetracycline operator, tetO) located within the promoter of the operon, and a repressor (tetR), a protein that binds tetO to block transcription of the genes included in the operon. tetR binding to tetO (and thus transcriptional repression) is tetracycline dependent: it binds in the absence of the antibiotic and is released from the DNA upon administration of tetracycline or one of its analogs. We have incorporated these elements into the locus of Hoxa2 using gene recombination and transgenic approaches, to produce a Hoxa2 allele that is able of external regulation by the administration or not of doxycycline. With the aid of this mouse strain we set to determine the temporal requirements of Hoxa2 function during mouse embryonic development. Preliminary experiments provided surprising results that open new insights into the spatial and temporal mechanisms of the regulation of Hoxa2 expression in the neural crest cells populating the second branchial arch. These results indicate that activation of this gene requires the combined activity of several transcription factors in the neural crest cells before they migrate out of the hindbrain. However, maintenance of its expression requires only a subset of these factors.

We have also started to determine the when and where is Hoxa2 required for correct patterning of the anterior hindbrain. These experiments take advantage of the possibility of expressing tetR in specific embryonic areas with the use of tissue-specific promoters. The spatial specificity, combined with the modulation of the temporal activity of the molecule with administration of doxycycline will give us insights into when and where is Hoxa2 required for normal mouse development.

The role of Hoxb4 in hematopoiesis

Members: Moisés Mallo, Leonor Parreira

Student: Ana Cristina Santos

Hox genes are transcription factors involved in a wide range of patterning and differentiation processes. Recent data has implicated some members of this gene family, most particularly Hoxb4) in the production of hematopoietic stem cells. Controlled expression of this gene in embryonic stem (ES) cells results in their differentiation into hematopoietic stem cells when they are cultured in the presence of specific growth factors. These cells are able to produce a full range of lineages in the hematopoietic lineage when induced to differentiate in vitro, and to repopulate lethally irradiated mice.

We are interested in understanding the molecular mechanisms mediating Hoxb4 activity in the formation of hematopoietic stem cells. For this, we are comparing the mRNA contents of ES cells that are induced to produce cells in the hematopoietic lineage in the presence and absence of Hoxb4. In these experiments we are following a double strategy. First, we are looking for specific candidates to be regulated by Hoxb4, based on previously published data or on knowledge obtained in our laboratory with other Hox genes. Second, we are undertaking a more systematic approach by analyzing the transcripts of the induced ES cells in the presence and absence of Hoxb4 using the Affymetrix chip technology. Functional analyses with the obtained candidate genes will follow to gain insights into how Hoxb4 controls the formation of hematopoietic stem cells.

The role of Bmp2 during early neural crest development

Members: Moisés Mallo

Student: Emília Almeida

Bmp2 is a secreted protein of the Tgf β family. A variety of in vivo and in vitro experiments have implicated this molecule in different processes, including the production of migratory neural crest cells, septation of the heart and differentiation of neurons of the peripheral nervous system. In our laboratory we have shown, with the use of a transgenic approach, that Bmp2 is essential for the production of neural crest cells in the cranial region of the embryo. Inactivation of this molecule resulted in the absence of neural and skeletogenic derivatives, together with agenesis of the thymus and parathyroid glands and alterations in the formation of the heart outflow tract. Our goal is now to determine how Bmp2 controls the early steps of neural crest development. We are performing experiments aimed at understanding whether Bmp2 is required for the production of neural crest cells or for their migration after they have been induced in the dorsal neural tube. In addition, we are performing a screen to

identify the genes operating downstream of Bmp2 during this process. Finally, we are generating mice containing Bmp2 alleles whose expression can be modulated in a timely and spatially controlled fashion. These mice will allow evaluating the time frame for Bmp2 functional activity and its in vivo requirements for processes like kidney development that cannot be investigated with the use of mice in which this gene was ubiquitously inactivated.

Endogenous Cerberus activity is required for anterior head specification in *Xenopus*.

Member: José A. Belo

Students: Ana Silva and Mário Filipe

Collaborators: Klaus-Michael Kuerner and Herbert Steinbeisser, Max-Planck-Institut für Entwicklungsbiologie, Tuebingen, Germany

We analyzed the endogenous requirement for Cerberus in *Xenopus* head development. “Knock down” of Cerberus function by antisense morpholino oligonucleotides did not impair head formation in the embryo. In contrast, targeted increase of BMP, Nodal and Wnt signaling in the Anterior-Dorsal-Endoderm (ADE) resulted in synergistic loss of anterior head structures, without affecting more posterior axial ones. Remarkably, those head phenotypes were aggravated by simultaneous depletion of Cerberus. These experiments demonstrated for the first time that endogenous Cerberus protein can inhibit BMP, Nodal and Wnt factors in vivo. Conjugates of Dorsal Ectoderm (DE) and ADE explants in which Cerberus function was “knocked down” revealed the requirement of Cerberus in the ADE for the proper induction of anterior neural markers and repression of more posterior ones. This data supports the view that Cerberus function is required in the leading edge of the ADE for correct induction and patterning of the neuroectoderm.

Mkp3 is the strongest direct negative feedback modulator of Fgf8 signaling in the mammalian isthmic organizer

Members: José A. Belo and Vera Lucas-Teixeira

Student: Sara Marques

Collaborators: Diego Echevarria and Salvador Martínez, Instituto de Neurociencias. University of Miguel Hernández (UMH-CSIC), Alicante. Spain

The pivotal mechanisms that govern the correct patterning and regionalization of the distinct areas of the mammalian brain are driven by key molecules that emanate from secondary organizers at neural plate and tube stages. FGF8 is the candidate morphogenetic molecule to pattern the mesencephalon and rhombencephalon in the Isthmic organizer (IsO). Recognizable relevance has been given to the intracellular pathways by which *Fgf8* is regulated and modulated. Recently, it has been demonstrated that the dual mitogen activated protein kinase phosphatase-3 (*Mkp3*), plays a role as a negative feedback modulator of the MAPK/ERK FGF8 signaling in chick limb bud development.

We have investigated the role of the mouse *Mkp3* and its functional relationship with the Fgf8 signaling pathway in the mouse IsO using gene transfer micro-electroporation assays and protein-soaked-bead experiments. Here we demonstrate

that *Mkp3*, beyond any other known modulators, has a fast, direct and strong negative action on the MAPK/ERK-mediated FGF8 signaling in the mouse neuroepithelium.

Emergence of the anterior-posterior axis after implantation relates to the re-orienting symmetry of the mouse embryo rather than the uterine axis

Member: José A. Belo

Student: Mario Filipe

Collaborators: Daniel Mesnard and Magdalena Zernicka-Goetz, Wellcome Trust/Cancer Research Institute, Cambridge, UK

When the anterior-posterior axis of the mouse embryo becomes explicit at the time of gastrulation it is almost perpendicular to the long axis of the uterus. This has led to the belief that the uterus could play a key role in positioning the major future body axis.

We demonstrate that when the anterior-posterior axis first emerges it does not respect the axes of the uterus, but rather the morphology of the embryo itself. Unexpectedly, the emerging anterior-posterior axis is initially aligned not with the long, but rather with the short morphological axis of the embryo. Then whether the embryo is allowed to develop either *in vitro* or in the uterus, the anterior-posterior axis becomes aligned with the long morphological axis of embryo just prior to gastrulation. Of three mechanisms that could account for this apparent shift in orientation of the anterior-posterior axis - cell migration, spatial change of gene expression or a change in embryo shape - lineage tracing studies on E6.0 embryos favor a change in shape accompanied by a restriction of the expression domain of anterior markers. This property of the embryo must be modulated by some interactions with the uterus as ultimately the anterior-posterior axis and long axis of the embryo become aligned with the left-right uterine axis.

In conclusion, the emerging anterior-posterior axis relates to the morphology of the embryo rather than that of the uterus. The apparent shift in its orientation to become aligned with the long embryonic axis and at the same time with the uterus is associated with a change in embryo shape and a refinement of anterior gene expression pattern. This suggests a fine inter-dependence between the expression of anterior-posterior markers, the shape of the embryo and the axes of the uterus.

Study of the genetic interaction between *cerberus-like* and *cripto*

Member: José A. Belo

Student: Ana C. Borges

Collaborators: Giovanna Liguori and Graziella M. Persico, International Institute of Genetics and Biophysics, Naples, Italy

Nodal encodes a TGF- β superfamily ligand essential for mesoderm specification and anterior-posterior (A-P) axis formation in the mouse embryo (for review see Shier and Shen, 2000). Nodal signals by binding to type I receptor ALK4 and the type II receptors Activin receptor (ActR) IIA and ActR IIB. Cripto is an EGF-CFC protein located extracellularly and bound to the membrane through a GPI anchor (Minichiotti et al, 2000). Cripto directly binds nodal and interacts with ALK4 and ALK7, both type I serine/threonine kinase receptors, permitting in the first case and enhancing in the second the ability to respond to nodal signal. *cripto* null mutants display severe

gastrulation defects, resembling a “head without a trunk” (Ding et al, 1998). In these mutants early markers of the AVE (*hex* and *cer-1*) are mislocalized in the distal visceral endoderm and early markers of the primitive streak (*fgf-8* and *brachyury*) are mislocalized proximally. So, *cripto* seems essential for the correct localization of the anterior and posterior organizing centers and, consequently, rotation of anterior-posterior axis fails to occur.

In this project we will identify and characterize genetic interactions between members of nodal pathway by producing double mutant mice.

Both *cer-1* and *cripto* are actively involved in Nodal transduction pathway: *cripto* modulates the signaling and *cer-1* inhibits it, since the binding of *cer-1* and Nodal prevents the activation of the Nodal receptors. Our hypothesis is that the inactivation of the Nodal inhibitor *cer-1* in a *cripto* null background could result in a less severe phenotype than the *cripto*^{-/-} one and our proposal is to generate a double null mutant *cer-1;cripto* to test it.

The novel secreted factor *cerberus-like2* is involved in the genetic pathway determinating the left-right asymmetry in the mouse

Member: José A. Belo

Students: Sara Marques, Ana C. Borges and Ana Silva

By sequence homology analysis, we have identified a novel mouse gene of the *Cerberus-like* family, that we designated *cerberus-like 2* (*cerl-2*). The genomic region and full-length cDNA were cloned and sequenced. The mouse *cerl-2* gene encodes a 185 amino acid protein that contains a typical hydrophobic signal sequence at its amino terminus and at the carboxy terminus, a cysteine-rich domain closely related to the *Xcerberus* and mouse *cerberus-like* ones. This secreted molecule is only expressed at the perinodal region between E7.0-8.0. In *Xenopus* assays, *cerl-2* mRNA is able to fully mimic the activity of *Xcer* including the induction of ectopic head-like structures as *Xcer* does.

Inactivation by homologous recombination in ES cells demonstrated that *cerl-2* is involved in the correct establishment of the left/right body asymmetry during embryonic development. Expression of *nodal* and *Lefty2* in the left LPM is absent in *cerl-2*^{-/-} mutants, whereas *Pitx2* is randomized. These defects in gene expression lead to *sinus inversus* of abdominal organs and to morphological and laterality defects of the heart. Biochemical, functional and genetic analyses uncovered the *cerl-2* mechanism of activity, which is crucial for the genetic pathway determinating the left-right asymmetry.

Transcriptional regulation of *Caronte* during embryonic development

Members: José A. Belo and Ana T. Tavares

Students: Sara Marques

Xenopus Xcer, mouse *Cer-1* and chick *Car* are expressed in equivalent embryonic structures such as the anterior endomesoderm, anterior visceral endoderm, and hypoblast, respectively. In mouse and chick embryos, these genes are also expressed in the anterior definitive mesendoderm. However, at later stages, *Xcer* transcripts are no longer detected, mouse *Cer-1* RNA is found in the rostral domain of nascent

somites and presomitic mesoderm, and chick *Car* is expressed in the left lateral plate and paraxial mesoderm. The general aim of this project is to dissect the transcriptional regulatory mechanisms that establish these similarities and differences in the expression patterns of the *Cerberus-like* gene family.

In this second year, the specific steps taken to pursue this project were the following:

- (1) To identify the transcriptional *cis*-regulatory elements that control *Car* expression in the different tissues of the chick embryo;
- (2) To investigate the regulation of *Car* reporter constructs by signalling molecules known to repress or activate *Car* asymmetric expression (*i.e.*, BMP4 and SHH);
- (3) To analyse the evolutionary conservation of *Cerberus-like* gene regulation in cross-species experiments. In order to determine if the regulatory mechanisms that control *Car* expression are conserved in mouse embryos, we have generated and analysed reporter gene expression in transgenic mice carrying chick *Car* regulatory regions (Car2.5-EGFP).

In short, we have obtained the following major results:

- (1) A left-side enhancer is located between -360 and -280-bp.
- (2) The FoxH1 and Smad-binding sites found in the *Car cis*-regulatory region are necessary for the activation of *Car* expression on the left-side mesoderm.
- (3) In concert with the reported regulation of *Car* expression, *Car cis*-regulatory regions are inhibited by BMP4 and activated by SHH proteins.
- (4) The upstream regulators and the *cis*-regulatory elements that activate *Car* and *Cer-l* expression in the anterior mesendoderm have been conserved.
- (5) *Car cis*-regulatory regions contain the elements required for the activation of expression in the presomitic mesoderm.
- (6) The upstream regulators of *Car* asymmetric expression are present on the left side of the mouse embryo.

Epithelial dynamics and adhesion during *Drosophila* dorsal closure

Members: Antonio Jacinto

Students: Beatriz Garcia Fernandez, Sérgio Simões, Anja Hagemann, Pedro Laires

Collaborators: Alfonso Martinez Arias

The movement and adhesion of epithelial sheets are fundamental morphogenetic processes that occur throughout embryogenesis and whenever a tissue is wounded in the adult organism. In humans, defects in epithelial movement and adhesion can be the cause for clinical conditions such as spina bifida and palate clefts in newborns. Dorsal closure is a morphogenetic movement during *Drosophila* development that provides a genetically tractable model of cell spreading, cell-cell recognition and adhesion. During this process two opposing epithelial fronts move dorsally to form a neat seam closing over the dorsal surface of the embryo REF. We are combining *Drosophila* genetics and advanced imaging techniques to investigate dorsal closure: (1) to analyse mutant phenotypes at the cellular level to further elucidate the function of *Drosophila* genes already known to be involved in this process; (2) to test the function of adhesion/recognition related candidate genes, such as members of the *cadherin* gene family; (3) to develop genetic screens to identify new genes involved in cell-cell recognition and adhesion during (4) to identify novel components of the cell-cell recognition and adhesion system during dorsal closure using proteomics and genomics.

Analysis of the molecular cascade for mesodermal limb chondrogenesis: *Sox* genes and BMP signaling

Members: Joaquin Rodríguez-León

External Collaborators: J. Chimal-Monry (Universidad Autónoma de México, México), J.A. Montero (Universidad de Cantabria. Spain), Y. Gañan (Universidad de Extremadura, Spain), D. Macias (Universidad de Extremadura, Spain), R. Merino (Universidad de Cantabria. Spain) and J. Hurle (Universidad de Cantabria. Spain).

The formation of cartilage is an essential process during skeletogenesis in vertebrates. During embryonic development, the formation of the endochondral bones is preceded by the formation of a transitory cartilaginous template. The sequence of molecular and morphological events leading to chondrogenesis has not been fully defined, but the formation of the chondrogenic aggregates involves changes in cell shape and growth kinetics, the production of a specific extracellular matrix, and the expression of adhesion molecules. We are studying how *Sox* genes and BMP signaling are functionally coupled during limb chondrogenesis. Using the experimental model of TGF1-induced interdigital digits, we dissect the sequence of morphological and molecular events during in vivo chondrogenesis. Our results show that *Sox8* and *Sox9* are the most precocious markers of limb cartilage, and their induction is independent and precedes the activation of BMP signaling. *Sox10* appears also to cooperate with *Sox9* and *Sox8* in the establishment of the digit cartilages. In addition, we have observed that experimental induction of *Sox* gene expression in the interdigital mesoderm is accompanied by loss of the apoptotic response to exogenous BMPs. *L-Sox5* and *Sox6* are respectively induced coincident and after the expression of *Bmpr1b* in the prechondrogenic aggregate, and their activation correlates with the induction of *Type II Collagen* and *Aggrecan* genes in the differentiating cartilages. The expression of *Bmpr1b* precedes the appearance of morphological changes in the prechondrogenic aggregate and establishes a landmark from which the maintenance of the expression of all *Sox* genes and the progress of cartilage differentiation becomes dependent on BMPs. In addition, we have observed that *Ventropin* a novel BMP antagonist, precedes *Noggin* in the modulation of BMP activity in the developing cartilages.

MKP3 mediates the cellular response to FGF8 signalling in the vertebrate limb

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During the development of any given structure or organ, several signalling mechanisms cooperate in providing positional information to cells in the corresponding developmental fields. In many cases, this cross-talk of signalling pathways involves interactions between different tissues of the developing embryo, with sequential transfer of positional information from one tissue to another. One of the best examples of signalling molecules involved in the complex cross-talk mechanisms that pattern developing embryos is the FGF superfamily of secreted factors. FGFs are essential during embryonic development, including the regulation of

cellular proliferation and differentiation. The mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) and phosphatidylinositol-3-OH kinase (PI(3)K)/Akt pathways are involved in the regulatory mechanisms of several cellular processes including proliferation, differentiation and apoptosis. We have shown that during chick, mouse and zebrafish limb/fin development, a known MAPK/ERK regulator, *Mkp3*, is induced in the mesenchyme by fibroblast growth factor 8 (FGF8) signalling, through the PI(3)K/Akt pathway. This correlates with a high level of phosphorylated ERK in the apical ectodermal ridge (AER), where *Mkp3* expression is excluded. Conversely, phosphorylated Akt is detected only in the mesenchyme. Constitutively active *Mek1*, as well as the downregulation of *Mkp3* by small interfering RNA (siRNA), induced apoptosis in the mesenchyme. This suggests that MKP3 has a key role in mediating the proliferative, anti-apoptotic signalling of AER-derived FGF8.

Notch activity acts as a sensor for extracellular calcium during vertebrate left-right determination

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Notch activity plays an important role in the early phases of LR determination by directly regulating Nodal expression around the node in mouse and zebrafish embryos. The activity of the Notch signalling pathway depends on the complex interplay of several receptors, ligands and modulators. In an effort to identify factors that regulate Notch activity around the node, we have characterized the dynamics of gene expression and interactions among relevant genes and proteins of this signalling pathway during early embryo gastrulation, and have generated a mathematical model to help us pinpoint those factors that are essential in LR determination. During vertebrate embryo development, the breaking of the initial bilateral symmetry is translated into asymmetric gene expression around the node and/or in the lateral plate mesoderm. The earliest conserved feature of this asymmetric gene expression cascade is the left-sided expression of Nodal, which depends on the activity of the Notch signalling pathway. We have identified the source of the asymmetric activation of Notch as a transient accumulation of extracellular calcium, which in turn depends on left-right differences in H1/K1-ATPase activity. Our results uncover a mechanism by which the Notch signalling pathway translates asymmetry in epigenetic factors into asymmetric gene expression around the node.

Intracellular pathways controlling outgrowth during vertebrate limb development

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Intracellularly, Wnt activity is transferred through, at least, three different pathways. The canonical pathway involves β -catenin activation, which eventually results in

transcriptional activation of target genes in the nucleus. The planar cell polarity pathway triggers Rho GTPases activity, thus inducing JNK activation and eliciting cytoskeletal rearrangements. Finally, the Calcium pathway increases the levels of intracellular calcium and decreases the level of intracellular cyclic guanosine monophosphate (cGMP). This last action is mediated by the activation of intracellular phosphodiesterase (PDE). The role of cGMP in the last pathway is the less understood. In this pathway, Wnts seem to be a regulator of the ratio of intracellular cGMP which is synthesised after guanylyl cyclase activation by Nitric Oxide (NO). These different Wnt intracellular pathways are activated by different Wnt molecules that bind to several receptors, named frizzleds, what introduces an additional level of complexity in the pathway. There are evidences that support that Wnt-Calcium-cGMP pathway antagonises the wnt β -catenin pathway but other show a crosstalk between both intracellular responses.

Wnt signaling plays a major role during limb development. It is responsible for AER formation, p-d outgrowth, chondrogenesis and muscle development. However, it has been reported that Wnt molecules acting through a non- β -catenin mediated pathway also play a role in limb outgrowth. In particular, inactivation of the *wnt5a* gene resulted in shortened limbs. We now want to better understand the role of Wnt5a in limb development. We want to focus on the action of Calcium, NO and cGMP during limb bud development by using the chicken embryo as a model. We are characterising by in situ hybridization and immunohistochemistry the expression pattern of the different components of the Wnt Calcium-cGMP pathway, including Prickle proteins, Calcium calmodulin dependent protein Kinase II (CamKII) and Siah family members. In addition, inhibitors for guanylyl cyclase and PDEs are also available and can be delivered in different limb areas by bead application followed by the study of the limb phenotype and different genetic markers.

Time control during vertebrate embryonic development

Members: Isabel Palmeirim

Students: Catarina Freitas, Sofia Rodrigues

External Collaborators: Marie-Aimée Teillet (Institut d'Embryologie Cellulaire et Moléculaire – Nogent sur Marne - France); Martin Catalá (Laboratoire d'Histologie, Embryologie et Cytogénétique, Faculté de Médecine Pitié-Salpêtrière, Université Paris VI, France)

Somites constitute the basis of the segmental pattern of the vertebrate embryo, giving rise to vertebrae, intervertebral disks, epaxial muscles and ribs, and impose a segmental pattern upon peripheral nerves, ganglia and vascular primordia. Somitogenesis occurs periodically, in a cranial-to-caudal sequence, at the anterior tip of the presomitic mesoderm (PSM), while new cells are added at the posterior end of this tissue, arising from the primitive streak. The temporal and spatial regulation of somitogenesis requires an intrinsic molecular oscillator, which is translated into cyclic waves of expression of Notch-related genes in the PSM cells, denominated cycling genes.

In this study, we demonstrated that the expression of several cyclic genes is already dynamic at the level of the prospective somitic territory, and that medial and lateral prospective somitic cells located in distinct territories express these genes in an asynchronous way. These observations led us to conclude that the segmentation clock is providing cellular positional information not only in the anterior/posterior but also

in the medial/lateral PSM axis. Moreover we showed that medial and lateral PSM cells are differently committed to the segmentation programme: in contrast to medial cells, lateral PSM cells isolated from their medial counterpart are neither able to form morphological segments nor to express segmentation genes, including the cycling genes. In the anterior third of the PSM, immediately before somites are formed, the information for somite formation is restricted to the medial cells.

We are currently performing microsurgical assays using quail-chick grafting as well as *in ovo* insertion of barriers at different levels in the chick embryo to uncover when, during development, the information for segmentation becomes restricted to the medial PSM cells. Unpublished data from our group raises the possibility that Hensen's node might be responsible for conferring segmentation autonomy to prospective medial PSM cells. We are undertaking a series of experiments in order to unveil the nature of this molecular signal.

New aspects on coordinating limb bud development

Members: Isabel Palmeirim

Students: Susana Pascoal (PhD Student/UM)

External Collaborators: Juan Carlos Izpisua-Belmonte (Salk Institute, San Diego, USA); Joaquín Rodríguez León (IGC); Delphine Duprez (CNRS, UMR 7622, Biologie du développement -Université Pierre et Marie Curie, Campus de Jussieu)

The vertebrate limb originates from a dual contribution of lateral plate and somitic mesoderm. Through differential proliferation of the flank, specific regions of the lateral plate protrude and form the limb buds. Shortly thereafter, cells from the lateral edges of nearby somites migrate into the limb. All adult limb muscles derive from these migratory cells. The limb bud is enveloped by an overlying ectoderm, which distal tip forms a specialized epithelial structure, the apical ectodermal ridge (AER). Cells directly under the AER remain in an undifferentiated state, forming the progress zone (PZ), while bone formation initiates in proximal limb regions. Among the three cardinal limb axis (P/D, antero-posterior (A/P) and dorso-ventral (D/V) the mechanisms that lead to cell fate specification along the P/D axis is the least understood. One paradigm, largely unmodified since its conception more than 20 years ago, is the progress zone model. According to this model, cell fate along the P/D axis is specified by the amount of time spent in the progress zone. Nevertheless, until now, no evidence has been provided to explain how cells “know” the time they spend in the progress zone. In a completely different system – somitogenesis - Palmeirim et al., (1997) presented evidence for the existence of a molecular clock underlying the process of chick somitogenesis by describing the dynamic pattern of expression of the *hairy1* gene, and later the *hairy2*, in presomitic mesoderm (PSM) cells.

In this study, we analysed the expression pattern of the cycling gene *hairy2* during limb development. We found that *hairy2* transcripts are detected in the ZPA and AER signaling centers and we investigated the regulation of *hairy2* expression in those structures. Surprisingly, we found that *hairy2* expression oscillates not only along the p-d axis of the distal mesenchyme, but also along the d-v axis, in the dorsal and ventral mesenchymal masses of the limb. We propose that the molecular clock underlying the process of somitogenesis is not an exclusive property of PSM cells, but, instead, a more general way to count time and to provide cellular positional information during vertebrate embryonic development.

Molecular basis of medio-lateral presomitic specification

Members: Isabel Palmeirim, Leonor Saúde

Students: Alexandre Gonçalves

The discovery that segmentation and somite formation is a process guided by Medial-PSM raises the question: What are the genes responsible for the segmentation autonomy of Medial-PSM?

To address this problem, we have performed a subtraction screening of cDNA libraries from Medial versus Lateral chick PSM in order to determine the genes differentially expressed in Medial-PSM. We have already done a first round screening and one new gene (clone 43) was selected for study based upon its differential pattern of expression in the Medial-PSM using whole-mount/cross section *in situ* hybridisation techniques. The role of clone 43 and other selected Medial-PSM genes in establishing the segmentation autonomy will be analysed by gain and loss-of-function experiments using *in ovo* electroporation, soaked beads, transfected COS cells, virus RCAS and RNA interference. Additionally, from the first round screening, two new genes (clone 28 and clone 45) were selected based on their specific pattern of expression in anterior PSM and their absolute complementary expression to the secreted factor *fgf8*. These genes are therefore good candidates for the establishment of the maturation territory in anterior PSM.

Molecular and temporal characterisation of the rostral-most somites in early somitic stages of the chick embryo

Members: Isabel Palmeirim

Students: Sofia Rodrigues

In zebrafish and mice, several knock-outs of genes related to somitogenesis specifically disrupt the most posterior somites without affecting the most anterior ones. These mutants can be categorised in two groups: (1) the Wnt/FGF pathway which includes mutations in the genes *T/Brachyury*, *wnt5*, *Wnt3a*, *tbx6*, *FGFR1alpha* isoforms and the *TCF1/Lef1* double mutant and (2) the Notch signalling pathway which includes mutations in the genes *DeltaD*, *Dll1*, *Dll3*, *Notch1*, *PS1*, *RBP-jk*, *Mesp2* and *lunatic fringe* (reviewed by Pourquié, 2001). Surprisingly, the number of anterior somites not affected in these mutations is not always the same and it is highly variable. Therefore, the fact that the most anterior somites are never disrupted in these mutations lead us to evaluate whether the anterior somites are genetically different from the most posterior ones. By using the chick embryo as a model to our study, we started by analysing the expression pattern of several genes related to somite formation in order to better characterise their formation and specification. Then, we have examined whether the mechanism regulating somite periodicity is the same for the first and more posterior somites. Then, we checked whether the establishment of AP polarity in the rostral PSM is different between embryos ranging from 1 to 10 somites and older embryos. And finally, we are currently performing a time-lapse analysis that shows precisely the time of formation of the most rostral somites, allowing a direct correlation with the oscillations of the cycling genes in the PSM.

Mechanisms of plant cell growth and morphogenesis

Member: José Feijó

Students: Ana Catarina Certal, Ana Catarina Silva, Ana Margarida Prado, Jörg Becker, Sílvia Costa, Sofia Cordeiro

We are developing a systematic approach to the basic phenomena underlying cell growth and morphogenesis. We intend to tackle some of these issues by means of state-of-the-art biophysical approaches aimed at understanding some of the fundamental physiological regulatory loops in growing pollen tubes grown in vitro, a paradigmatic model for studying apical growth. Data gathered with electrophysiology and imaging techniques is to be integrated on a coherent theoretical background by established collaborations with *physicists and* theoretical biologists. On the other hand a systematic molecular approach will now be started to establish the molecular counterparts of the physiological models.

Pollen stigma interaction and sexual plant reproduction

Member: José Feijó

Students: Ana Catarina Silva, Ana Margarida Prado, Ana Maria Vieira, Jörg Becker and Leonor Boavida

Sexual Plant Reproduction represents the evolutionary context in which pollen tubes evolved and fit in. We aim to apply the knowledge on the mechanisms that control growth to a better understanding of the complex communication and guidance behaviour of pollen tubes within the female tissue. This objective will imply development of a number of fluorescent tags for pollen tubes and advanced imaging inside living pistils using multi-photon microscopy. On the other end mutants of *Petunia* and *Arabidopsis* defective on reproductive steps will be screened, and characterized in terms of the inherent physiological deficiencies.

A lateral effort is being made on the establishment of sexual cycles in a number of non-studied species, especially with forestry or fruticulture interest. Besides the immediate applied interest of the results, this effort has repeatedly guided us into interesting basic research projects.

Notch signaling and lymphocyte regulation

Members : Jocelyne Demengeot, Leonor Perreira

Students: Manuel Rebelo, Margarida Santos

Notch receptors and their ligands are evolutionarily conserved trans-membrane proteins that regulate cell fate decisions during development and postnatal life. Notch signaling is also known to differentially affect the development of lymphoid B- and T-cell throughout life. We are investigating the role of Notch ligands in controlling latter stages of B and T cell physiology. Monitoring responses of B cell subpopulation to various stimuli in the presence of Notch receptor ligands, we evidenced that Notch triggering by specific ligand determine the frequency of B entering differentiation to Ig secreting plasma cells. This findings are now examined in relation to the differential expression of Notch ligands in substructures of lymphoid tissues In

parallel, we evidenced that Deltex, a mediator of the Notch signaling pathway, is highly expressed by naïve (CD45RB^{high}) but not by naturally (CD45RB^{low}) nor *in vitro* activated cells. However, naïve CD4 cells injected into lympho-deficient animals (Rag-2^{-/-}) expand and acquire an apparent activated phenotype (CD45RB^{low}) but maintained high level of Deltex expression. We interpret these results as an indication that naïve CD4 cells and cells under homeostatic proliferation are constrained by Notch engagement, while activated T cells are relaxed from this control. Ongoing experiments aim at testing this hypothesis directly.

Apoptosis vs. differentiation – the cell-fate of Cajal Retzius cells

Members: Matthias Haury and Paula Parra Bueno

Students: Helena Nunes Cabaço, Susana Silva

Collaborators: Alfonso Fairen (University of Alicante, Spain), Lisa Marubio (Baylor College, Houston, USA), Dan Holmberg (Umea University, Sweden).

We are studying a developmental cell fate decision during hippocampus development. The investigation of the mechanisms of cell fate decision (differentiation vs apoptosis) in the developmental pathway of Cajal Retzius cells in the hippocampus is investigated in a joint project with Dr. Sukalyan Chatterjee, and carried out by the PostDoc Dr. Paula Parra Bueno, who has generated several new GFP transgenic mouse with specific expression patterns in the brain, and we are now in the process of characterizing these strains to allow the in-vivo study of the apoptosis and differentiation processes using multiphoton confocal imaging and patch-clamp electrophysiology.

Molecular cross talk between cell-cycle and apoptosis in cell fate decisions

Member: Sukalyan Chatterjee

Students: Mário Grãos, Ana Gírio, Ana Lucia Mena, Ana Alexandra Almeida

Cell fate decisions, a highly regulated mechanism that coordinates self-renewal, proliferation, differentiation and apoptosis are crucial for development, physiological homeostasis and pathology. The driving force for such fate decision is the continuous assessment of the “state-of-the-cell” in the perspective of arising signals resulting from interactions within the cell and with neighbouring cells. Depending on the tissue type cells have the option of self-renewal, differentiation, proliferation or apoptosis. The underlying mechanism of fate decisions is centred around cell cycle and apoptosis which is intricately & intimately achieved by cross talk between both.

Bim is a pro-apoptotic BH3-only domain Bcl-2 family member. The Bcl-2 family comprises of both anti- and pro-apoptotic members, which by transcriptional and post-translational regulation is known to control the outcome of several apoptotic signals. Bim has been described to be sequestered to the microtubules (MTs) as a complex with dynein. Under certain stimuli, this complex gets released and Bim potentially interacts with Bcl-2 in the mitochondria, leading to cytochrome-c release and subsequent activation of caspases. The ongoing work in the group shows that in NIH-3T3 cells, two isoforms of Bim (Bim-L and Bim-EL) are phosphorylated at early mitosis (M), but not in other phases of the cell-cycle. We observed that inhibiting cdc-

2 at M phase causes loss of phosphorylation of BimL/EL, which correlates with loss of mitochondrial potential. This indicates that at mitosis, dephosphorylated Bim is pro-apoptotic, whereas phosphorylated Bim is not. We hypothesize that phosphorylation of Bim attenuates the pro-apoptotic potential of the protein in a context of M-phase, such that it can be rapidly reactivated by dephosphorylation in case the cell decides to enter apoptosis instead of mitosis. MEK/Erk pathway has been described to play a role in the cell cycle as well as survival. We have observed that levels of activated Erk1/2 in M phase cells are higher than in those in interphase. Inhibition of this pathway at mitosis leads to complete loss of phosphorylation of Bim. Moreover, stimulation of MEK/Erk pathway by bFGF causes a rapid phosphorylation of Bim in a cell-cycle independent manner, which can be reverted by pre-treatment with MEK inhibitor. We suggest that at mitosis, cell-cycle (cdc-2) and survival signals (MEK/Erk pathway) cross-talk to drive cells to take decisions in terms of proliferation/survival/apoptosis. These signals converge upon Bim, inducing its phosphorylation and consequent attenuation. If the survival/proliferative signal is disrupted, Bim regains its pro-apoptotic features and triggers cell death. Furthermore, levels of FGF receptor 3 levels is drastically upregulated in mitosis compared to control & G1/S arrested cells while the levels of receptor 1 are also modestly up regulated 3-4 fold in mitosis where as receptor 2 is largely unaffected and if anything slightly reduced in levels. It is likely that these increased levels of the receptors may be responsible for activated ERK pathway leading to the phosphorylation of Bim and experiments are running to elucidate the mechanistic details. To identify the cognate kinase a directed two hybrid system has been established with the putative partners and with a potential for screening a G2/M phase enriched activation domain fusion library.

To elucidate the events downstream of the phosphorylation of Bim, its localization by subcellular fractionation in G1/S and G2/M-synchronized cells as well as in UV-irradiated cells are being pursued by another PhD student. Our results indicate that there is a population of Bim present at the mitochondria and endoplasmic reticulum (ER), being unphosphorylated in G1/S cells and phosphorylated in G2/M cells, while upon UV treatment it was observed to be unphosphorylated at the mitochondria but phosphorylated at the ER. Our data suggest a role of Bim in ER stress, which we intend to investigate. We are also performing microtubule isolation to clarify if in mitosis Bim itself or in bound form with α LC8, gets translocated from the microtubules to the cytosol. This biochemical approach will be supported by immunofluorescence analysis using fusion fluorescent reporters. Though sparse but there are reports that integrins are pertinent for the M phase of the cell cycle. Integrins are also integrally involved in shape mediated signalling. Furthermore, integrins have a positive compensatory function in upregulation & consequent survival through various growth factor receptors. A student in the group is beginning to address this issue in M phase of the cell cycle under physiological conditions. Cell detachment mediated signalling contributing to survival or death may harness cross talk between adhesion molecules & growth factor receptors which will be elucidated in this students' dissertation project. This project will also encompass cross talk between differentiation & apoptosis in the model cell type keratinocyte because when keratinocytes are detached unlike other cell types they don't die but undergo differentiation. It is obvious that in contrast to other cell types in these cells the apoptotic machinery needs to be tightly restrained. Hence, we are beginning to address whether Bim is constitutively phosphorylated and also investigating the role of other pro & anti apoptotic factors in preventing cells from apoptosis. The growth factors receptors involved and the related relevant adhesion molecules that induce

differentiation and control the shutdown of the apoptotic machinery will be focussed on with the long term aim to characterize the detailed mechanisms of these cross talks.

The Role of VEGF and its receptors in tumour growth and angiogenesis

Members: Sérgio Dias, Susana Constantino, Cristina Casalou

Student: Rita Fragoso

Collaborators: Shahin Rafii, Cornell University, ImClone Systems, Zhenping Zhu,, Genentech, Napoleone Ferrara.

Neo-vascularization (angiogenesis) plays a key role in tumor growth and may contribute to the formation of metastasis. Tumors, by releasing angiogenic growth factors such as VEGF, stimulate their newly formed vasculature, which in turn provides the necessary support for the expanding tumor mass. Such paracrine pathways between endothelium and tumor cells are critical for tumor growth, as shown in murine models of human solid tumors, where blocking endothelial proliferation resulted in tumor growth delay and regression. On the contrary, demonstration that angiogenesis is important for the growth of liquid tumors such as lymphoma and acute leukemias has still not been provided. Recent studies have shown that increases in bone-marrow vascularization accompany leukemia progression. Similar to solid tumor growth, in response to leukemia-derived VEGF, the expanding bone marrow endothelial mass may support leukemia in a paracrine fashion, by releasing cytokines such as GM-CSF, IL-6, among others. However, certain leukemias not only produce angiogenic factors such as VEGF, that stimulate bone marrow endothelial proliferation, but also express VEGF receptors, generating an autocrine loop that supports leukemia survival. Such a VEGF/VEGF receptor autocrine loop appears critical for the growth of subsets of leukemias, as shown in vitro and in murine models, but its significance in terms of disease progression or clinical outcome in humans is not known. Likewise, although VEGF is produced by most liquid and solid tumors, the precise contribution of VEGF receptors in tumor angiogenesis and progression has not been well defined.

Angiogenesis and prostate cancer

Member: Sérgio Dias

Students: Cátia Igreja, Margarida Courinha

External Collaborators: Shahin Rafii, Cornell University, ImClone Systems, Zhenping Zhu.

The importance of angiogenesis for the progression of solid tumors has been well documented. However, precise contribution of such a neo-vascularization process, namely its relative importance throughout the different stages of tumorigenesis, has not been well shown. In addition, recent evidence suggests novel cellular pathways/mechanisms may regulate the onset of angiogenesis. One such mechanisms is the active recruitment of Endothelial Progenitors (EPC), from the bone marrow, to the peripheral circulation and ultimately into sites of active angiogenesis.

In the present project we investigate the relative contribution of angiogenesis (classical pathway) and EPC towards prostate cancer growth and metastasis

formation. The obtained results will be studied in the context of tumor development and eventual resistance to conventional therapies.

Role of β 1-integrin in skeletal myogenesis: analysis of integrin β 1D knock-in mouse embryos.

Members: Sólveig Thorsteinsdóttir

Students: Ana Sofia Cachaço, Carlos Pereira, Ana Margarida Nunes

Collaborators: Susana Chuva de Sousa Lopes and Christine L. Mummery (Hubrecht Laboratory, The Netherlands); Arnoud Sonnenberg (Netherlands Cancer Institute, The Netherlands).

Integrins are extracellular matrix receptors composed of α and β subunits involved in cell adhesion, migration and signal transduction. The β 1 subunit has two isoforms, β 1A ubiquitously expressed and β 1D restricted to striated muscle. They are not functionally equivalent. Replacement of β 1A by β 1D (β 1D knock-in) in the mouse leads to midgestation lethality on a 50% Ola/50% FVB background (Baudoin et al., Genes Dev. 12:1202-1216, 1998).

We have recently shown (Cachaço et al. Development 130:1659-71, 2003) that when the β 1D knock-in line is crossed into a less penetrant genetic background, there is an attenuation of the midgestation lethality, revealing a second period of lethality around birth. We showed that midgestation lethality is caused by impaired development of the labyrinth of the placenta, leading to placental failure and embryonic death in 2/3 of the homozygous β 1D knock-in embryos analysed. The remaining 1/3 developed until birth, but exhibit severely reduced skeletal muscle mass. Quantification of myotube numbers showed that substitution of β 1A by β 1D impairs primary myogenesis with no direct effect on secondary myogenesis. Furthermore, primary myotube survival is affected in β 1D knock-in embryos. Together these results demonstrate for the first time distinct roles for β 1 integrins in primary versus secondary myogenesis and that the β 1A and β 1D variants are not functionally equivalent in this process.

We are currently addressing the origin of the observed defect in primary myogenesis of β 1D knock-in embryos. Preliminary results show a dramatic reduction in the number of myocytes present in E11.5 myotomes of β 1D knock-in embryos. Furthermore, the reduced amount of myogenic cells observed in E11.5 mutant embryos, is also observed in E13.5 mutants, suggesting that these individuals do not recover. These results suggest that the defect in primary myogenesis observed in β 1D knock-in embryos can be traced to the earliest phases of muscle development.

Formation of the myotome in the mouse: cell movements and cell-extracellular matrix interactions.

Member: Sólveig Thorsteinsdóttir

Students: Fernanda Bajanca, Marta Luz, Ana Sofia Cachaço

Collaborators: Shahragim Tajbakhsh and Margaret Buckingham, Pasteur Institute, France; Arnoud Sonnenberg, Netherlands Cancer Institute, The Netherlands, Marilyn Duxson, University of Otago, New Zealand

In vertebrates, the cells that will form the skeletal muscles are primarily derived from the somites. The first myogenic precursor cells express transcription factor Myf5 and then undergo an epithelium-mesenchyme transition and migrate from the dermomyotome portion of the somites to the underlying space where they organize themselves into a parallel array of mononucleated myocytes, called the myotome. With the development of the embryo, several waves of myogenic precursors arise from the dermomyotome, which together ultimately give rise to the definitive skeletal muscles of the embryo.

Very little is known about the cell-extracellular matrix interactions involved in early skeletal muscle development. In this project, we have described the expression patterns of the $\alpha 1$, $\alpha 4$, $\alpha 5$, $\alpha 6$ and $\alpha 7$ integrin subunits in the mouse myotome and correlate them with the expression of several differentiation markers. Our results indicate that these integrin subunits may be involved in different phases of myogenic determination and differentiation. A detailed characterisation of the myogenic cell types expressing the $\alpha 4$ and $\alpha 6$ subunits showed a regionalisation of the myotome and dermomyotome based on cell-adhesion properties. We conclude that $\alpha 6\beta 1$ may be an early marker of epaxial myogenic progenitor cells. In contrast, $\alpha 4\beta 1$ is upregulated in the intercalated myotome after myocyte differentiation. Furthermore, $\alpha 4\beta 1$ is specific for the hypaxial dermomyotome and is maintained by early hypaxial MPCs colonising the myotome.

Myf5-null mouse embryos do not initially form a myotome (Tajbakhsh et al., Nature 384:266, 1996). Our results using these embryos show that Myf5-null cells do not express the $\alpha 6\beta 1$ integrin and fail to assemble a laminin-containing basement membrane. These findings suggest that (1) Myf5 promotes normal laminin assembly by upregulating the expression of $\alpha 6\beta 1$ on myogenic precursor cells at this stage, and (2) that an organised laminin matrix may have a role in myotome formation.

Extracellular matrix and somitogenesis: causes and consequences

Members: Sólveig Thorsteinsdóttir, Isabel Palmeirim, Gabriela Rodrigues
Students and Technicians: Lara Carvalho, Pedro Rifes

Somites are transient epithelial segments of the paraxial mesoderm that are formed in a rostral-to-caudal progression during vertebrate embryogenesis. Ectoderm is believed to be crucial for the proper epithelialisation of somites, since cultured explants of the presomitic mesoderm (PSM) only form somites in its presence, but the extracellular matrix (ECM) has also been implicated in somite epithelialisation.

In order to investigate a possible relationship between the role of the ectoderm and the ECM in somitogenesis, explants of chick PSMs were cultured in the presence and absence of ectoderm and assayed for the expression of ECM proteins. Furthermore, some explants are treated with enzymes that specifically remove certain ECM molecules and the epithelialisation monitored. An analysis of the results obtained suggests that the presence of a fibronectin matrix promotes somite epithelialisation. Thus the ECM synergises with the ectoderm in promoting the epithelialisation of somites in the chick.

The role of cell-fate decision genes in human hematopoietic differentiation

Member: Leonor Parreira. In collaboration with Jocelyne Demegeot, IGC.

Student: Hélia Neves

The choice between alternative cell-differentiation pathways is regulated by direct intercellular contacts mediated by trans-membrane proteins expressed by adjacent and apparently equivalent stem cells. Two protein families involved in this process are the Notch receptors and their ligands, the Delta and Jagged proteins. Both protein families are phylogenetically conserved and involved in several developmental scenarios including the decision processes underlying the functional divergence of CD4/CD8 T lymphocytes and the choice between $\alpha\beta$ and $\gamma\delta$ T-cell receptors in mouse thymocytes. Using a cell coculture assay we have recently observed that the Notch ligand Delta-1 completely inhibits the differentiation of human hematopoietic progenitors into the B-cell lineage while promoting the emergence of cells with a phenotype of T-cell/natural killer (NK) precursors. In contrast, Jagged-1 did not disturb either B- or T-cell/NK development. Furthermore, cells cultured in the presence of either Delta-1 or Jagged-1 can acquire a phenotype of NK cells, and Delta-1, but not Jagged-1, permits the emergence of a de novo cell population co-expressing CD4 and CD8 (*Jaleco et al, J Exp Med, 2001, 194:991-1001*). Recently, the effects of these Notch ligands on myeloid development were investigated. A long-term culture assay was used, where bone marrow stromal cells transduced with human Delta-1 or Jagged-1 cDNAs, were co-cultured with normal human progenitors, followed by the analysis of their differentiation potential in methylcellulose clonogenic assays. We observed that Delta1 and Jagged1, when acting in similar microenvironmental conditions, have different effects on the myeloid clonogenicity of CD34⁺ cells, both at the level of mature bi- and uni-potent progenitors, and differentially regulate the balance between the granulocytic and monocytic cell compartments of myelopoiesis. Microarray analysis of gene expression profiles of CD34⁺ cells, emerging upon contact with Delta1 or Jagged1, show that the observed phenotypic and functional differences are associated with distinct gene transcription programs in those cells. The results indicate that both Delta1- and Jagged1-mediated Notch signalling may play an important role in the homeostatic equilibrium of distinct myeloid-cell lineages in the bone marrow.

Cell fate and cell polarity within the vertebrate embryonic neuroepithelium

Members: Domingos Henrique, Evguenia Bekman

Students and Technicians: Cristina Afonso, Rita Fior, Gonçalo Neto, Alina Costa

External Collaborators: Olivier Pourquié (Stowers Institute, Kansas City, USA), Fernando Giraldez (Universidad Pompeu Fabra, Barcelona, Spain), Isabel Varela-Nieto (Instituto Alberto Sols, Univ. Autónoma, Madrid, Spain), François Schweisguth (École Normale Supérieure, Paris, France)

A major research focus in our lab concerns how cell polarity is established in the neural epithelium and how it contributes to cell fate decisions during neural development. In *Drosophila*, it has been shown that neural progenitors can divide asymmetrically, generating daughter cells with different fates. In vertebrates, it has been suggested that similar mechanisms can control the acquisition of different fates by dividing neuroepithelial cells during neurogenesis. We have investigated this issue in the chick embryo, looking at the orientation of mitotic spindles in dividing

neuroepithelial cells of the spinal cord, using fixed specimens and time-lapse microscopy. No correlation between the axis of division and the fate of the daughter cells could be inferred: at any time, the great majority of neuroepithelial cells divide within the plane of the epithelium, with only a very minor fraction dividing along the apico-basal axis. Also, chick homologues of *Drosophila* polarity proteins, which are asymmetrically segregated during neuroblast divisions, don't reveal a polarized apico-basal distribution in dividing neuroepithelial cells. Our findings, therefore, do not support a role for intrinsically controlled, asymmetric divisions during vertebrate neurogenesis.

We have also found that the PAR polarity complex (PAR3, PAR6 and aPKC) is specifically localized at adherens junctions of neuroepithelial cells. Misexpression of the PAR3 protein in the chick embryonic neural tube leads to a profound disturbance of the neuroepithelium, with loss of normal apico-basal polarity and appearance of "ectopic" cell junctions. This leads to the formation of characteristic neuroepithelial aggregates ("rosettes") within the neuroepithelium. We are currently trying to understand how this phenotype develops and how this affects the normal process of neurogenesis.

Another research program of the lab aims to investigate the basic molecular mechanisms that regulate the production of nerve cells in vertebrate embryos, in particular the role of the Delta/Notch signalling pathway in cell fate decisions within the developing nervous system. We are studying how different Notch ligands contribute to the regulation of neuronal production in the embryo, from neural precursor division until the acquisition of the differentiated characteristics of the neuronal cells. We are studying these processes in the chick neural retina, taking advantage of our experience with manipulating Notch activity in this tissue, using both retrovirus and electroporation.

Finally, we are studying the process of neuronal commitment and differentiation using mouse ES cells, trying to understand the molecular controls that regulate the *in vitro* production of neurons from ES cells.

Theoretical and Computational Biology

Given the scientific interests of the IGC on "systems biology", and our preference for organism-centered approaches, it makes sense to dedicate a significant fraction of the Institute's activity to the theory of complex systems and organisms. This is the objective of the Oeiras Advanced Studies (OAS): to provide theoretical, statistical and computational support to the empirical research at the Institute, conducting research on Mathematical and Computational Biology, and promoting this field in Portugal.

Molecular biology had a notorious success in identifying molecular components and mechanisms of relatively simple biological processes, providing molecular explanations for genetic or infectious diseases. Greater challenges are posed by complex systems and diseases, which involve the simultaneous interplay between many processes at molecular, cellular, individual, and populational levels. Developing new quantitative modeling frameworks that help bridging the gaps between these different levels of biological organisation is the agenda of mathematical and computational biology research at the OAS. One of our originalities resides in using mathematical models and simulation as tools for designing and analysing quantitative, bench experiments. Several such complementary research programs are carried out at

the IGC. In immunology, they relate to signal transduction in lymphocytes, maturation of immune responses and lymphocyte population dynamics, notably, in diseases of the immune system. In evolutionary biology, mathematical modelling and experiments were combined to address, in simple systems using bacteria and plasmids, host-parasite co-evolution, emergence of antibiotic resistance, and the evolutionary forces responsible for the generation and maintenance of diversity in populations. Last years' advances included the findings that regulatory T cell populations, which prevent autoimmunity, expand as a function of disease-causing T cells in the same individual. These and other results suggest that individuals undergoing diverse subclinic infections gain "strength" in regulatory T cell populations, thus explaining the increased incidence of autoimmune diseases and allergies in countries with a reduced prevalence of infectious diseases. The epidemiology of such conditions is alarming, as it predicts that more than half of human populations in the developed world will suffer from allergy or autoimmune diseases in this Century.

Functional genomics also combines computational and experimental biology, as it makes use of computation to extract novel information from the very extensive genomic and proteomic databases now available in a variety of living organisms. The IGC's agenda is to analyse genomes in search of unnoticed structural signatures of how biological systems operate, how diseases emerge, or how hosts and parasites co-evolve. In this context, a transcriptome-wide computational method was developed last year, for the identification of genes that code for proteins under pressure to vary. This method has been successfully tested on several malaria parasite species, for proteins that might represent optimal target candidates for therapeutic intervention.

Genome-scale technology creates statistical and computational problems on its own, as novel sources of biological information accumulate, notably with high-throughput screening methods, such as gene-chip technologies. An example of such emerging problems is the comparison of the expression patterns of thousands of genes. A new graphical representation to visualize and make pairwise comparisons of gene expression profiles from different tissues was developed and the utility software made available.

Mathematical biology is also increasingly relevant to epidemiology, particularly to accurately represent the natural dynamics of recurrent and persistent infections, and to predict the impact of interventions. While attempting to derive quantitative frameworks, researchers at the IGC developed a strong, novel concept: reinfection threshold is a notion that was introduced in the quantification of pathogen transmission, and is gaining an increasing number of applications. The variable efficacy of the BCG vaccine against pulmonary tuberculosis, as well as the unusual patterns of influenza diversity and evolution, both seem to be better explained using that concept.

The extraordinary relevance of computational approaches in systems biology and in medicine imposes special efforts to re-inforce this area of research at the IGC, particularly in doctoral education. Owing to the reduced number of specialists in Portugal, the IGC is studying the possibility to launch a Doctoral Program and a Collaboratorium in Mathematical and Computational Biology. The Collaboratorium aims to foster and support the networking of scientists in the field, in Portugal and abroad, by providing infrastructures and intellectual environment at the IGC, where collaborative research can be carried out by visitors who will also participate in the graduate teaching.

Nature, population dynamics, and mechanism of action of regulatory CD25+ T cells: A biomathematical and experimental approach

Members: Jorge Carneiro, Kalet Leon, Jocelyne Demengeot Matthias Haury
Students: Íris Caramalho and Andreia Lino

Regulatory CD4+CD25+ T cells have been implicated in the prevention of autoimmune pathologies by suppressing other cells. The mechanism of action and population dynamics of these cells is still unclear. We have shown, by first principle modeling, that the regulatory function of these cells cannot be explained by competition alone, and requires that regulatory cells actively inhibit disease-causing cells and use the latter as a growth factor (Leon et al. J 2000, Theor Biol; Leon et al. 2001 J Immunol). Together with Demengeot's group (IGC, Oeiras), we gave experimental support to this crosstalk mechanism (Leon, 2002 PhD thesis). On theoretical grounds, we have shown that the same crosstalk mechanism could allow for an efficient self-nonself discrimination (Leon et al. 2003 J. Theor Biol) and could also explain the epidemiological observation that autoimmune disease incidence is inversely correlated with the overall prevalence of infections (Leon et al, J Autoimmunity in press).

Modeling T cell activation, differentiation and commitment

Member: Jorge Carneiro
Students: João Sousa, Tiago Paixão and Andreia Lino
External Collaborators: Dejan Milutinovic (PhD student, IST)

We study the T-cell receptor (TCR) signaling mechanism, which is critical for T-lymphocyte function and population dynamics. Analysis of published data on antigen-mediated TCR-down-regulation allowed us to identify several kinetic constraints (Sousa et al. 2000 Eur J Immunol) that we used to probe candidate TCR-signaling mechanisms. Previous models of signal transduction were unrealistic since they assumed continuous antigen dynamics. We have improved significantly these models by taking into account the discrete antigen dynamics resulting from intermittent contacts between the T-cell and the antigen-presenting cell (Sousa et al, 2003 PhD. Thesis; Milutinovic et al, 2003 Proc Med03). In essence these models follow the probability density functions (pdfs) of the activities of cell-associated molecules (membrane TCR density, kinases and phosphatase activities downstream the TCR) in whole cell-populations. The predictions these models make about the evolution of the pdfs of TCR density were tested based on histograms of TCR expression measured by flow cytometry.

Mechanisms involved in the germinal center reaction: a biomathematical and experimental approach

Member: Jose Faro

Students: Joana Moreira, Ana Agua-Doce, Lurdes Duarte, Rui Gardner

Collaborators: África González-Fernández, Univ. Vigo, Vigo, Spain, Antonio Bandeira, Institut Pasteur, Paris, France and Michal Or-Guil, Institute for Theoretical Biology, Humboldt University, Berlin, Germany.

Germinal Centers (GC) are short-lived, dynamically organized microenvironments that form transiently during T cell-dependent immune responses within follicles. There intense proliferation, apoptosis, and V(D)J hypermutation takes place, as well as some affinity-based selection of B-cells. Also, GCs are essentially involved in generation of memory B cells. Yet, there is little knowledge on how the molecules and cells involved operate at a cell population level to drive and regulate the GC reaction. The present project aims to clarify current controversies on the mechanism(s) involved in the peculiar rise-and-fall dynamics of GCs. For that, we have started a multidisciplinary approach involving biocomputational and experimental work. Initial experiments are designed to calibrate the models. In particular, it will be assessed: (a) the apparent oligoclonality of GCs at different time points; (b) the amount of Ag-Ab complexes on FDCs; (c) the kinetics of hypermutations increasing antibody affinity; (d) the kinetics of GC T-cell help for centrocytes; and (e) phenotypic changes in FDCs and Th cells influencing centrocyte differentiation. Different mechanisms potentially accounting for the GC behavior are currently being investigated theoretically by mathematical modeling describing the population dynamics of those variables. Once calibrated, the behavior of the models will be compared to results of specifically designed experiments. So far our theoretical results allow us to discriminate qualitatively between the different model variants. In addition, we find that the rate of proliferation of T cells is the parameter having the largest influence on the global dynamics of GCs in all model variants. In respect to the hypermutation process, our model results suggest that specific mutational hotspots are not required to explain the distribution of mutations observed experimentally at days 14 and 21 after immunization of mice with phOx-OVA.

Tuberculosis transmission and control

Member: Gabriela Gomes

Students: Ana Franco, Inês Mota

Collaborators: Manuel Gomes, FCUL Lisbon, Portugal, Graham Medley, Univ. Warwick, UK.

The aim of this project is to provide quantitative frameworks within which the impact of specific vaccines and treatment programmes can be assessed, and targets for future interventions can be set in terms of their impact on pulmonary tuberculosis (TB). The bacille Calmette-Guérin (BCG) is the only vaccine in current use against tuberculosis, but its usefulness is debatable. Efficacy ranges from 0 to 80%, and low vaccine efficacy appears associated with high prevalence of disease. We propose an explanation based on three postulates: (1) the potential for transmission varies between populations; (2) exposure to mycobacteria induces partial protection against reinfection; (3) this protection is not significantly improved by vaccination. These

postulates combine into a mathematical model reproducing observed trends. The variabilities are promoted by a reinfection threshold that could be manipulated by more potent vaccines, suggesting a powerful role in global control. Within our framework, vaccine development and use can be combined with alternative prevention measures (e.g. detection and treatment of latent TB) to rationally design TB control in the high burden regions. We find that high coverage programmes directed at early latency appear generally effective, but the benefit will be greater where prevalence is high. A widespread treatment strategy extended to late latency has a moderate effect in the long term, but it shows a capacity to induce large amplitude oscillations and appear temporarily more beneficial where prevalence is low. The implementation of such strategies alone should be carefully followed, and efficacy measures should be carefully interpreted. We plan to proceed with more detailed models and closer interaction with public health and vaccine development professionals.

Modelling the efficacy of immune protection

Member: Gabriela Gomes

Student: Inês Mota

Collaborators: Lisa White, Univ. Warwick, UK, Graham Medley, Univ. Warwick, UK

The mechanisms described in the TB project are essentially applicable to other pathogens characterized by recurrent infections. Population patterns of infection are determined largely by susceptibility to infection. Infection and vaccination induce an immune response that, typically, reduces susceptibility to subsequent infections. This immunity is highly effective in the some viral infections (e.g. smallpox, measles, mumps, rubella) but more generally, protection is only suboptimal combining three basic modes of action: partial; all-or-none; temporary. The partial protection corresponds to what we proposed as the tuberculosis scenario: all previously exposed individuals have their susceptibility reduced by some factor. In this case, a reinfection threshold emerges, above which reinfection is the principal mode of transmission and, consequently, infection levels are much higher and vaccination fails. The alternative that infection or vaccination inducing fully protective immunity in some individuals and none in others leads to significantly different results and we are currently investigation the more realistic situations between these two extremes. Temporary immunity is an important determinant of inter-epidemic periods, and therefore has a predictive role in the recurrence of epidemics.

Viral evolution and epidemiology

Members: Gabriela Gomes, Isabel Gordo, Jorge Carneiro, Francisco Dionisio, Jose Faro.

Students: Jose Nuno Oliveira Martins

External Collaborators: Alessandro Margheri, CMAF, Univ. Lisbon, Portugal, Carlota Rebelo, CMAF, Univ. Lisbon

Many pathogens (viruses in particular) exhibit antigenic diversity and elicit strain-specific immune responses. This potential for cross-immunity structure in the host motivates the development of mathematical models, stressing competition for

susceptible hosts in driving population dynamics and genetics. We have previously established that under certain model formulations, the pathogen population self-organizes into clusters as long as transmissibility is above a certain threshold. More recently we have used the resulting hierarchical structure to develop a framework within which different evolutionary patterns can be compared. In particular, we use the model to simulate influenza A shift evolution.

Epidemiology and control of leishmaniasis

Members: Gabriela Gomes, Abdelkader Lakmeche
Collaborators: Orin Courtenay, Univ. Warwick, UK

Visceral leishmaniasis (VL) is a vector-borne disease of humans and dogs caused by infection with the protozoan *Leishmania infantum* and transmitted by sandflies. Dogs are the main reservoir. Leishmaniasis is endemic in Mediterranean countries, including Portugal, where domestic dogs are the important reservoir with an estimated 2.5 million of 15 million dogs infected. There are no effective VL control methods available, and recovery of dogs following treatment is transitory with an 80% relapse rate. Recent interest in the application of pyrethroid-impregnated dog collars to protect dogs against sandfly bites (i.e. transmission), has been shown to reduce human and canine infection in small field trials. However, the effectiveness of this approach as a community-wide control method will depend on knowing (i) the critical proportion of the dog population which requires coverage with a collar, and (ii) the minimum time period required that the population is covered relative to local variations in transmission rates. This new project aims to clarify these issues.

Constructing mendelian phenotypes by multivariate analysis

Member: John Stewart

Characterisation of the EGF/Laminin superfamily

Members: Pedro Coutinho and António Jacinto
Students: Joana Ribeiro
Collaborators: Sarah Teichmann, LMB, Cambridge, U.K.

The objective of this project is to better understand the dynamics of cell signalling of the evolution of genomes and protein families. The protein superfamily that we are using to address these questions is the EGF/Laminin.

The EGF/laminin superfamily comprises all proteins with at least one EGF/Laminin domain. These domains are defined by structural homology to a sequence of about 40 amino acids that is significantly homologous to the epidermal growth factor (EGF). There are three EGF/Laminin structural domains: The EGF-like domain, the Calcium-binding EGF-like domain and the Laminin-type EGF domain (LE) (which has four pairs of conserved cysteines, unlike the other two types of domains that have just three). The common structure of these structural domains consists of two anti-parallel β -strands and (three or four) pairs of conserved cysteines.

The rationale to choosing this superfamily was that it is one of the most diverse superfamilies both in terms of structural architectures and functional annotation. Furthermore, a vast number of EGF/laminin proteins have been shown to play key roles in signalling, in contexts such as development and cancer.

The initial steps of the project consist in the identification and structural characterisation of the EGF/laminin superfamily in model species, including *C. elegans*, *D. melanogaster*, *D. rerio* and *H. sapiens*. We will then try to correlate this information with available functional data, in order to better understand the basic rules and constraints of structural architectures generation.

In silico* identification of G-protein coupled receptors in *Plasmodium

Members: Pedro Coutinho and Maria Mota

Collaborators: Ana Rodriguez, NYU, USA

Experimental work from the groups of M. Mota, A. Rodriguez and others indicate that G-protein coupled receptors (GPCRs) must exist in *Plasmodium*. In addition, there is strong evidence that they play important roles in the development of these parasites. An analysis of the available annotation from *Plasmodium* species does not show any GPCR. Therefore we have decided to use the most up-to-date bioinformatic tools in order to define putative GPCR for *P. falciparum* and *P. yoellii*, the two *Plasmodium* species for which the genomes have been published.

We used two parallel approaches, one relying on sequence similarity and another relying on structural properties (QFC algorithm). The results are quite promising since the putative GPCRs are quite distinct from the previously known Human GPCRs. Therefore these proteins are optimal targets for drug therapeutics.

Genome wide characterisation of gene relative volatility

Members: Pedro Coutinho, Maria Mota, Isabel Gordo

A recent study in *Influenza A* virus has shown that genes that code for proteins under immune pressure are specifically more diverse in their antigenic regions. This work is based on the comparison of cDNA alleles, which is not available for the majority of species, in particular for other parasites such as *P. falciparum*.

The lack of allele sequences led us to develop new bioinformatic tools to analyse codon variability, at a genome wide level. Our initial efforts have identified a gene property, the relative volatility, which we use to successfully distinguish the three highly variable *P. falciparum* gene families: *var*, *stevor* and *rif*. Furthermore, we can use this property to select other genes that are putatively under strong immune pressure as well. When we apply the property to *P. falciparum*, we obtain a set corresponding to approximately 4% of all putative genes.

We are extending these results to other parasitic and non-parasitic species.

Genome-wide analysis of transcription regulation in eukaryotes

Members: Pedro Coutinho, António Jacinto

Students: João Duarte, Pedro Lares

We are developing and using bioinformatic tools to improve our knowledge of gene network structure and transcriptional regulatory mechanisms, for model organisms such as *D. melanogaster* and *D. rerio*.

We are developing a tool for fast identification of putative direct transcriptional targets for DNA binding transcription factors, with inferred/known binding sites. This will be extremely useful for functional genomics approaches that may follow.

In addition, we are also generating tools to study particular gene networks, the Ci/Gli networks. We aim to infer putative elements of these networks from genomic data. To accomplish this we will perform genome-wide screens of putative targets, selecting the ones that are conserved across species. After testing these predictions experimentally, and depending on the robustness of the inference, we will then apply these tools to other networks.

BioCASE - A Biological Collection Access Service for Europe

Member: Pedro Fernandes

Technical work: Ana Maya

Progress in this project was achieved by surveying the portuguese holders of biological collections and inserting about 40 new or updated records in the BioCASE central database. Contacts were made in order to prepare a national meeting in 2004.

EMBER - The European Multimedia Bioinformatics Educational Resource

Members: Pedro Fernandes and Isabel Marques

Help in gathering new materials for the project was provided, namely in two case-studies and a demonstration. The IGC is now prepare to hold a trial course, scheduled for early 2004.

EMBCORE - The Core European Bioinformatics Research Infrastructure in the Life Sciences

Members: Pedro Fernandes and Isabel Marques

The two IGC members participate in governing structures of the organization. Pedro Fernandes is the secretary of the Publications and Public Relations Committee, while Isabel Marques is a member of the Education and Training Committee.

PROCURA - Portuguese Proteomics Network

Member: Pedro Fernandes

Collaborators: Deborah Penque, INSRJ and Margarida Botelho, INSRJ

The main task was the establishment of the 2D Gel database as a service. More contacts were established with the portuguese community of Proteomics data generators and consumers, and initial steps towards establishing links to European resources were taken.

Cell Biology: Mitosis, Cytoskeleton, and Stress

From a unifying evolutionary theory and a strong basis of cell and molecular biology, modern biological sciences reached unity in concepts, approaches even in semantics. Today, it makes little sence to separate the various “specialities” or areas of interest, as done here for reasons of comodity of the reader. A good example of this contention is the fact that this sector of the IGC’s activities could well be “dissolved” in several others, or else, include various projects listed under other headings (Stress and Inflammation, Developmental Biology, etc.). Yet, this grouping aims at underlining that several apparently diverse interests converge in cytoskeleton structure, dynamics and functions.

Regulation of proteolysis during mitosis

Member: Álvaro Tavares

Student: Mariana Faria

Collaborators: Rui Gomes, FCT/UNL, Peter Deak, Hungary

The ubiquitin-proteosome pathway is a major proteolytic system acting in various cellular processes (Hershko and Ciechanover 1998). In this system, the proteins are tagged with multi-ubiquitin chains and are then degraded by the 26S proteosome. The 26S proteosome is made up of two subcomplexes: the 20S proteosome and the regulatory complex. The regulatory complex consists of 18 subunits with molecular masses of 28-110kDa, including 6 putative ATPases (Rtp1-Rtp6) and 12 non-ATPase subunits (Rpn1-11) (Finley et al 1998, Holzl et al 2000). One level of control is obviously at the step of ubiquitination, and in fact most mutants described are components of the different ubiquitin-ligase complexes (eg. Glotzer and Dechant 2002). The 26S proteosome is believed to be constitutively active and has not attracted much attention as a regulatory molecule. However, according to recent progress in structural analysis of the proteosome the specificity of proteolysis pathway may well be modulated by the 26 proteosome. Phosphorylation of proteosome components C8 and C9 seems to increase proteolytic activity, but the responsible kinase has not been identified. In this project we study the effects of mutations in a gene coding for a component of the regulatory complex, the protein Rpn9. Surprisingly, we have found that mutations in this gene result only in abnormal mitosis, suggesting that it is required to selectively direct the degradation of proteins during this stage of the cell cycle. We wish to determine the nature of these proteins, and for such we will use a combined approach of RNAi and MALDI mass

spectrometry. In addition, the detailed characterization of the mutant phenotype, using classical genetics and confocal microscopy, will help to clarify the role of those proteins during mitosis (or better say, what happens if they're not degraded) and of the proteosome itself.

Role of mammalian Mob-like proteins in determining the timing of cytokinesis

Members: Álvaro Tavares and José António Belo

Student: Claudia Florindo

Collaborators: Jon Pines, Cambridge, UK, Maria Arménia Carrondo, ITQB/UNL

The aim of this project is to study the cell division mechanism, in particular the aspects regulating the formation of a bipolar mitotic spindle. More specifically, we intent to study the human Mob1-like proteins (HsMobs). Our results indicate that HsMobs are centrosomal proteins essential for mitotic spindle assembly and for cytokinesis, and RNAi experiments resulted in an increase of multinucleated cells. The four different HsMob proteins, although co-localising at the centrosomes during mitosis, seem to have different functions. We wish to precise the function of each HsMob protein and define if they are all essential for spindle assembly, cytokinesis, or both.

For such, we are applying a concerted approach using molecular biology, protein crystallography, and mouse genetics, congregating three different groups. We explore the in vivo function of the HsMob in tissue culture cells by preventing the expression of each protein, using RNAi protocols. To this end we observe fixed or live cells by confocal microscopy, after transfection with dsRNA. We use time-lapse microscopy and serial section EM to define the in vivo behavior of centrosomes during the vertebrate somatic cell cycle, expecting this way to define the role of each HsMob during the different phases of the cell cycle. Time-lapse will also be used to characterise the dynamic intracellular localization of each HsMob, tagged with GFP- or CFP-proteins. Finally, interacting proteins will be identified by phage-display.

In order to analyse the function of this class of proteins throughout development, in particular if their suppression leads to tumour formation, we will generate conditional knockout mice. We will use the Cre-recombinase loxP system in order to avoid possible early lethal phenotypes, and to be capable of determining the gene function in specific tissues.

Another important objective is to determine the three-dimensional structure of the Mob1-like proteins by X-ray diffraction. Few centrosomal proteins have been crystalised to date and we hope this way, together with the information gathered in the other tasks, to be capable of establishing a relationship structure-function for these centrosomal proteins. This will allow the design of new molecules capable of interfering with HsMob function with potential therapeutic applications, namely prevent cell proliferation. The identification of HsMob molecular partners can also give important clues to about cancer progression.

Mitotic roles for the Plkk and Mps1 kinases

Member: Álvaro Tavares

Students: Paulo Alves, Mariana Faria, Dinis Gokaydin.

In a typical somatic cell cycle, M-phase comprises mitosis and cytokinesis. M-phase progression is largely controlled by protein phosphorylation, and several protein kinase families have already been implicated in such control. The aim of this project is to study the cell division mechanism, more specifically, we intend to study the function of the proteins kinases DPlkk and DMps1. The kinase Plkk was originally in *Xenopus* described as the activator of the mitotic kinase polo, and Mps1 protein is an essential *S. cerevisiae* kinase involved in the mitotic checkpoint and in the duplication of the spindle pole body. In preliminary work conducting to this project we have cloned the *Drosophila* homologues of the kinases Plkk and Mps1. We will address the function of these kinases following a molecular biology and genetics concerted approach. We already have a DPlkk mutant allele created by insertion of a P-transposable element into the gene. The mutant is lethal in the early embryos stages, much like strong alleles of polo. As there is no mutant alleles of DMps1 we will analyse, in *Drosophila* S2 cells, the effects of a lack of function brought about by transfection with double-stranded RNA (dsRNA) although the construction of such a mutant is simultaneously being done. One of the major breakthroughs we expect with this project is the finding of physiological kinase substrates. We will look for substrates of the DPlkk and DMps1 in preparations of centrosomes and study how phosphorylation by these kinases can modify the function of the substrates. Very powerful *in vitro* systems have been developed in *Drosophila* extracts that allow biochemical and biophysical approaches to the function of the centrosome (eg. Carmo Avides and Glover 1999). We will utilise this *in vitro* system to study the roles of both kinases in centrosomal microtubule nucleation (as done for the kinase polo, Carmo Avides et al 2001). We will also look for DPlkk and DMps1 interacting proteins, using affinity columns to isolate protein complexes capable of binding these proteins. Our approach will be complemented by database searches for human ESTs to identify the human orthologues of novel proteins identified in *Drosophila*. We hope with these different approaches to advance the discovery of new centrosome and spindle proteins and elucidate their function in the regulation of chromosome segregation.

Molecular and biochemical analysis of centrosome components in *Drosophila melanogaster*

Member: Álvaro Tavares

Students: Susana Godinho, Célia Domingues

Collaborators: David Glover, Cambridge, UK

The accurate segregation of chromosomes at mitosis is essential for the provision of genetic material to ensure cell viability. Defects in any stage of this process can lead to cell death or, in higher organisms, the development of cancer. Multipolar spindles have often been observed in human cancers *in situ* as well as an abnormal number of centrosomes. Identification of the molecular targets of centrosome kinases and elucidation of the pathways that regulate centrosome duplication, separation and function provide novel opportunities for therapeutic intervention. We previously showed that the protein kinase polo is a centrosomal kinase, and that is required for the formation of a bipolar spindle and for the proper execution of cytokinesis. We wish to understand how the activity of the polo protein kinase is regulated and how it functions at the level of the centrosomes. We previously found that polo proteins, either from *Drosophila* embryo extracts or from *Xenopus* egg extracts, bind to several proteins forming different stable complexes. We are now on the process of identifying

the complex's components in total embryo extracts and in centrosome preparations. We want to characterize these proteins, sorting which are polo substrates and which are activators. Taking advantage of *Drosophila* genetics we have also searched and isolated new genes required for spindle assembly and centrosome function, some of which coding for proteins with high degree of homology with the *Saccharomyces cerevisiae* proteins. We are now on the process of characterising these genes.

Transactivator Yap1 – crossroad of cold and oxidative stress signaling pathways in *Saccharomyces cerevisiae*

Members: Lisete Fernandes, Helena Soares

Students: Alexandra Almeida, Ana Neves Costa, Cláudia Bicho, Joana Monteiro

Eukaryotic cells respond to both stimuli, like oxidative stress and temperature fluctuations, by activating cascades which are diverse but with discrete specificity. How does the cell keep track of multiple cascades? What are the cross-talks between such cascades and how are they regulated?

The Yap family of bZIP transcriptional factors in *Saccharomyces cerevisiae* contains members which are central players in cellular responses to stress challenges as: Yap1 in oxidative and cold signals, Yap4 in response to compounds affecting cytoskeleton as well as in cold-sensitivity of yap1-deleted cells, and Yap8 in arsenite response. Although it has been previously suggested that each Yap family member plays distinct biological function, the involvement of Yap proteins in different phenomena emphasizes putative overlapping among the respective signaling cascades. From this point of view, Yap constitutes an excellent tool to address the cross-talk of signalling pathways and, in particular, it will also provide clues on the cellular mechanisms for adaptation to low temperatures.

Our major aim has been focussed on understanding the crossroad of cold and oxidative signaling pathways mediated by Yaps. The involvement of Yaps, as the target regulatory proteins, in both phenomena has been ascertained by addressing (a) the specific role of Yap1 under cold signals as well as (b) the specific role of YAP4 under equivalent conditions.

Understanding the specific role of Yap1 under cold signals has been primarily addressed by determining the type of transcriptional activity of this regulator and by studying cellular transcriptional pattern putatively mediated by this factor in those conditions.

The specific role of YAP4 under same conditions was approached by determining the type of transcriptional activity of this regulator as well as by developing a genetic screening to identify genes essential to the cold-phenotype of yap4 cells. This loss-of-function genetic screening has generated few mutants that revert yap4 cold-sensitivity. The identification of such mutations by complementation assays are being undertaken.

Mechanism of activation by Yap1:-signaling through RNA polymerase II basal machinery

Member: Lisete Fernandes

Students: Diogo Fonseca, Joana Monteiro, Marcos Pinho

Yeast *Saccharomyces cerevisiae* as well as other fungi contains a set of non classical AP-1 factors, the Yaps, that are similar in structural motifs yet distinct in their amino acid sequences. Yaps are described as key proteins in cellular response to specific stress signals. Transactivators, like Yap1, stimulate gene expression by binding regulatory *cis*-elements, contacting directly or indirectly components of the RNA polymerase II basal machinery (GTFs as TFIIA, TFIIB, components of TFIID) or the Mediator, as well as by recruiting the nucleosome-remodeling complexes.

In order to understand the specific signaling downstream of Yap1, we are addressing the role of GTFs as specific targets under oxidative conditions generated by hydrogen peroxide. In this context, we have selected a GTF as primary target for Yap1 activity, and we are studying the mechanism that regulates its gene expression as well as the biological relevance of such regulation under oxidative contexts. To further explore the relevance of the GTF to Yap1-mediated transcription, *gtf* mutants with impaired oxidative stress response (but with normal physiological behaviour) have been generated and the mutations are currently being identified. Further characterization will address the relevance of such mutation on Yap1 activity.

Study of the role and regulation of the mammalian cofactor a. implications in tubulin folding, microtubules biogenesis/dynamics and signalling pathways

Member: Helena Soares

Student: Sofia Nolasco

Collaborators: Juan Carlos Zabala, Departamento de Biología Molecular, Facultad de Medicina, Universidad de Cantabria, Santander, Spain

The generation of new tubulin heterodimers is a multistep process involving several chaperones. Nascent α - and β -tubulin chains first interact with prefoldin that delivers them to the cytosolic chaperonin CCT. Afterwards tubulins follow distinct folding pathways; α -tubulins are captured by cofactor B, while β -tubulin by cofactor A. Then cofactors E and D replace B and A, respectively. The two pathways converge to create a super-complex (α -tubulin/E+ β -tubulin/D). Subsequently, cofactor C binds to this complex and upon GTP hydrolysis assembly-competent α/β -tubulin heterodimers are released. It has been suggested that cofactor A plays a double role inside of the cell, enhancing the rate of β -tubulin dimerization and serving as a reservoir of excess β -tubulin.

We have characterized the genes encoding cofactor A in mouse genome. Interestingly, we found, besides only one cDNA sequence encoding the cofactor A was described in literature, two genes encoding cofactor A are expressed in several mouse tissues. This small multigenic family is also composed of several pseudogenes that seem to be originated by DNA duplication over evolution. In human genome (chromosomes 5 and X) we have identified the two genes that are the respective mouse counterparts. However, one of these genes contains a stop codon in frame being probably a pseudogene in humans. We are investigating if the identified pseudogenes in mouse and humans are transcribed and if may have functional roles as recently described for several mammalian pseudogenes.

We are also studying the cofactor A role under conditions that increase the pool of native tubulin heterodimers *in vivo*. HeLa cells treated with Mt depolymerizing agents or submitted to cold-shock for 15 minutes have a microtubule network completely depolymerized, as shown by indirect immunofluorescence. In parallel cofactor A that

is spread over the cytoplasm in control cells is concentrated in a region nearby the nuclei. These data suggest that disorganization of Mt cytoskeleton affects the distribution of cofactor A in the cytoplasm. The levels of cofactor A mRNA were analysed by Northern blot and Real-time PCR in cells submitted to microtubule depolymerizing agents (cold-shock, colchicine and nocodazol). The patterns of expression of CCT β and CCT ϵ and factors involved in translation were also studied. The results revealed a decrease in steady-state levels of cofactor A mRNA under these treatments. Interestingly, cofactor A gene expression is induced in 50% of the cells obtained from gastric cancers that in general express specific tubulin isotypes. Additionally the amount of cofactor A protein did not change in cells with microtubules depolymerized. This last observation seems to be related with the half-life of the cofactor A protein (>24h under physiological conditions) and suggest that the amount of cofactor A seems to be sufficient to deal with the increased amount of tubulin heterodimers generated by microtubule depolymerization. However, the amount of cofactor A/ β -tubulin complex transitory decrease in response to an increase of native tubulin heterodimers upon cold-shock or nocodazol and colchicine treatment. Therefore the amount of this complex is affected by the amount of free native tubulin heterodimers. Assuming that the tubulin-folding pathway is reversible our results indicate that probably this may be not reversible up to cofactor A but only up to the super complex (α -tubulin/E+ β -tubulin/D). Studies are in progress to clarify this hypothesis, to investigate the alternative roles of cofactor A in mammalian cells and to establish a relationship with the translational status of the cell and other components of the folding pathway.

The role of the cytosolic chaperonin cct in mature cilia and during cilia biogenesis

Member: Helena Soares

Student: Ana Cecília Seixas

Collaborators: Luis Viseu Melo and Pedro Brogueira Departamento de Física, Instituto Superior Técnico, Lison, Portugal

Cilia and flagella are cellular polar compartments composed of microtubules, polymers of α - and β -tubulin heterodimers, in complex arrangements with more than 250 distinct polypeptides. Mature cilia and flagella are dynamic structures presenting a continuous transport of axonemal particles (intraciliary/intraflagellar transport) and seem to play prominent roles during embryonic development and as sensory organelles.

In ciliate *Tetrahymena* exponentially growing cells we observed by indirect immunofluorescence that the cytosolic chaperonin CCT-subunits, TpCCT α , δ , ϵ and η - are associated with mature cilia. CCT is a hetero-oligomeric complex of about 900 kDa composed of two back-to-back rings, each containing eight different, although related, gene products. This chaperonin mediates the folding of actin and tubulin. During *Tetrahymena* reciliation TpCCT-subunits localization was affected. Morphological alterations occurring during the first 90 minutes of reciliation, and the indirect localization of TpCCT-subunits using the antibodies against CCT subunits in mature isolated cilia were observed by tapping modeTM atomic force microscopy (AFM). At 15 min reciliation AFM measurements combined with immunofluorescence microscopy showed that small axonemes are growing, but the

recruitment of CCT-subunits to these structures is limited. As reciliation proceeds the typical organization of 9 pairs of Mt in the axonemes becomes very clear. At this time the characteristic cap structure present in the tip of mature cilia is not visible neither the Mt central pair. In these structures the content of CCT-subunits increases. At 90 minutes reciliation, small cilia already contain the cap structure and antibodies directed to CCT-subunits label them over their entire length. The AFM images in mature isolated cilia revealed that CCT-subunits are localized on the axoneme. These data are in agreement with preliminary results obtained by biochemical fractionation of cilia. Our results strongly suggest that CCT proteins are involved in tubulin transport and axoneme assembly, and give clues for a structural model of their localization in cilia structure.

On the other hand combined biochemical techniques revealed that reciliation affects the oligomeric state of TpCCT-subunits being tubulin preferentially associated with smaller CCT oligomeric species in early stages of reciliation. Using different chromatography strategies we were able to partially purify one of these complexes of about 500 kDa. This complex is composed of at least 12 different proteins with molecular masses ranging from 30~150 kDa. Two of these proteins were identified by immunoblot and immunoprecipitation as corresponding to the proteins CCT α and CCT ϵ . We are in process of a large-scale purification of the referred complex in order to characterize by protein sequence analysis of the other unidentified polypeptides.

C-the role of the cytoskeleton in the activation of NF- κ B by H₂O₂

Member: Helena Soares

Students: Nuno Pedroso

Collaborators: Luisa Cyrne and Fernando Antunes, Departamento de Química e Bioquímica, Faculdade de Ciências de Lisboa, Portugal

Long-term goal of the studies is to understand the mechanism of cellular adaptation to oxidative stress. The main focus of this study is to elucidate the nature of the interaction between the redox state of the cell, cytoskeleton and NF- κ B activation. The NF- κ B activation pathway is a crucial signal transduction pathway to a variety of essential cellular processes. Therefore is our aim to establish regulatory links between the generation of oxidants by the cell, microtubule disulfides, and cytoskeleton functionality, and how this could affect NF- κ B migration to the nucleus. In this context we focused on the induction of the transcription factor NF- κ B and its interaction with the microtubules, one of the components of the cytoskeleton, in response to oxidative stress generated by H₂O₂ steady-state delivery. The ensuing changes in the redox state of the cell were assessed by a kinetic and thermodynamic analysis.

We showed in MCF-7 cell line that there is a moderate NF- κ B induction when cells are challenged with low steady-state H₂O₂ concentrations (25 μ M). Besides inducing NF- κ B by promoting its nuclear translocation, H₂O₂ also induced a progressive depolymerization of the microtubules. However, when a microtubule depolymerising agent (nocodazole) was added simultaneously with H₂O₂ there was a decrease of the NF- κ B induction as well as a decrease in the loss of cell viability. Moreover, the treatment of cells with the Mt polymerizing agent taxol did not affect the activation of NF- κ B by H₂O₂.

From these results we concluded that the polymerisation/depolymerization of microtubules is not involved in NF- κ B activation and that probably the Mt depolymerization has a repression effect.

The functional organization of chromatin in the nucleus

Members: Leonor Parreira, Ana Paula Santos

Students: Isabel Alcobia, Ana Sofia Quina

This research line investigates the functional implications of the 3-D organization of chromatin in the nucleus of hematopoietic cells. The spatial positions of specific genes as well as heterochromatic centromeric regions are analysed in 3D preserved cells using in situ hybridisation and confocal microscopy. We have shown that genes commonly involved in chromosomal translocations in leukaemia have an intrinsic spatial dynamics that is established early in hematopoiesis, and perpetuated differentially in distinct cell lineages, what may facilitate their collision and reciprocal recombination at subsequent stages of hematopoietic differentiation (*Neves et al, Blood, 1999, 93:1197*), a phenomenon which may be mechanistically relevant for the occurrence of oncogenic gene rearrangements in human leukemia. As to the spatial organization of heterochromatic regions, we observed that chromocenters (associations of centromeres) present in quiescent lymphoid and non-lymphoid peripheral blood cells represent cell-type-specific arrangements of centromeric heterochromatin (*Alcobia et al, Blood, 2000, 95:1608*). These observations were followed by the analysis of the dynamics of these spatial arrangements during hematopoietic differentiation. The spatial patterns of association of different centromeres were analysed in CD34+ cells and compared with those in early-B, early T cells, mature B and T lymphocytes. Those patterns were shown to change during lymphoid differentiation, with major spatial arrangements taking place at different stages during T and B-cell differentiation. Heritable patterns of centromere association are observed, which can occur either at the level of the common lymphoid progenitor, or in early-T or early-B committed cells. A correlation of the observed patterns of centromere association with the gene content of the respective chromosomes, further suggests that the variation in the composition of these heterochromatic structures may contribute to the dynamic relocation of genes in different nuclear compartments during cell differentiation, what might have functional implications for cell-stage-specific gene expression (*Alcobia et al, Experimental Cell Research, 2003*).

RESEARCH CONTRACTS

The research activities at the IGC are supported to a significant level by the National Research Council (Fundação para a Ciência e a Tecnologia, FCT), but also by the European Union and by a few private corporations. All research contracts signed by the IGC that pertained, at least in part, to 2003 or later are listed below. The support from the FCT comes through various mechanisms, all awarded on the basis of competitive applications: (1) institutional support, as positions for scientists and technicians, in the frame of the Laboratório Associado ITQB/IBET/IGC; (2) institutional support, in the frame of the Unidade de Investigação FCT on “Tolerância Natural”; (3) individual support, as fellowships to visiting scientists, post-doctoral

fellows, PhD students and technicians (these are indicated in the lists of people at the Institute); (4) group support, as research contracts (listed below); (5) sporadic support for the organization of scientific meetings, as indicated. We are pleased to acknowledge this support as it has become absolutely essential to the activities of the Institute.

FCT PROJECTS

POCTI/BCI/41725/2001

Jörg Becker

Whole genome approach to ion dynamics regulation of pollen tube growth and morphogenesis

POCTI/BCI/46392/2002

José A. Belo

O papel multifuncional da endomesoderme anterior no processo da formação da cabeça em vertebrados.

POCTI/NSE/46420/2002

José A. Belo

Regulação intracelular durante o desenvolvimento do cérebro, da via de sinal FGF8 pela fosfatase MKP3.

POCTI/CBO/46691/2002

José A. Belo

Identificação e análise funcional do transcriptoma de células precursoras comuns do coração/hemangioblasto.

POCTI/BME/46257/02

José A. Belo and Ana T. Tavares

Vertebrate left/right asymmetry: analysis of the transcriptional regulation of chick Caronte during embryonic development.

POCTI/NSE/39166/2001

Paula Parra Bueno

Differentiation vs. Apoptosis – The Cell-fate of Cajal-Retzius Cells

POCTI/BCI/42249/2001

Sukalyan Chatterjee

Molecular mechanisms of microtubule mediated signalling under oxidative stress

POCTI/CBO/47565/2002

Sukalyan Chatterjee

Transcriptional regulation of CD34 antigen in stem cells & its role in development

POCTI/43063/MGI/2001

Jocelyne Demengeot

Control of acute inflammatory responses by regulatory T cells: Characterization of the cellular and molecular mechanisms

POCTI/43411/ BCI/ 2001

Jocelyne Demengeot

Effects of the Recombination Activating Genes 1 and 2 on the Vertebrate Genome Stability: Consequences at the Cellular and at the Organism level.

POCTI/CBO/38391/01

Sérgio Dias

The Role of VEGF and its Receptors in Tumour Growth and Angiogenesis.

POCTI 34772/1999: 2001-2003

José Feijó

Genetic characterization of ion dynamics modulators in pollen tubes

POCTI 33201/1999

José Feijó

The role of extensin peroxidases and extensin deposition in plant development

POCTI/39906/FCB/2001

José Feijó

Resposta inflamatória e sinalização astrócito-neurónio na lesão celular causada por sepsis, hipóxia-isquémia e hiperrubilinémia

POCTI/36413/1999

José Faro

Mechanisms involved in the Germinal Center Reaction: a biomathematical and experimental approach.

POCTI/BCI/37862/2001

Lisete Fernandes

Transactivator Yap1 – crossroad of cold and oxidative stress signaling pathways in *Saccharomyces cerevisiae*.

POCTI/MAT/47510/2002

Gabriela Gomes

Epidemiology and evolution of infectious diseases: Influenza A and Malaria.

POCTI/BSE/46856/2002

Isabel Gordo

Population genetics of adaptation in *Escherichia coli*.

POCTI/P/BIO/10091/1998

Matthias Haury

IL-10 GFP Transgenic mice

POCTI/BCI/41909/2001

António Jacinto

Epithelial dynamics and adhesion during *Drosophila* dorsal closure

POCTI/BCI/48577/2002

António Jacinto

Cell-cell recognition and sorting during *Drosophila* morphogenesis

POCTI/MGI/43466/2001

Moisés Mallo

Molecular mediators of Hoxa2 function during mouse development.

POCTI/MGI/46337/2002

Moisés Mallo

Reversible gene inactivation in the mouse.

POCTI/38563/MGI/2001

Maria Manuel Mota

Host-Parasite Interactions during the Hepatic Stages of Malaria Infections

POCTI/MGI/44517/2002

Maria Manuel Mota

Mechanism of action of HGF released by sporozoite-wounded hepatocytes during malaria infection.

POCTI/ ESP/ 49236/ 2002

Luisa Mota-Vieira

Genetic and consanguinity of congenital heart disease in Azores.

POCTI/MGI//36369/1999

Sylviane Pied

T- Cell response in pathogenesis of malaria

POCTI/MGI/46719/2002

Sylviane Pied

A genomic and proteomic approach to study T lymphocytes, astroglial, microglial and endothelial cell interactions during malaria neuropathology.

POCTI/34240/1999

J. Pedro Simas

Molecular interactions in Murine Herpesvirus 68 Latent Infection of B-Lymphocytes.

POCTI/46378/2002

J. Pedro Simas

Transcriptome Analysis of Germinal Centre B cells During Gammaherpesvirus Latent Infection.

POCTI/BIA 10097/98

Helena Soares

Possible role of the Tetrahymena cytosolic-chaperonin CCT in the biogenesis and dynamics of microtubules: an attempt to correlate with the effects of antimitotic agents.

POCTI/BCI/34405/99

Álvaro Tavares

Isolation and characterization of protein complexes containing the mitotic proteins polo and Dmob.

POCTI/BME/33221/99

Álvaro Tavares

Full-length cDNA cloning and biochemical characterization of a novel human protein kinase.

POCTI/BCI/41735/2001

Álvaro Tavares

Functional characterisation of the mitotic kinases DPlkk and DMps1.

POCTI/CBO/39099/2001

Álvaro Tavares

Structure and function of the centrosomal proteins HsMob in cell division.

POCTI/BCI/40754/2001

Sólveig Thorsteinsdóttir

Extracellular matrix and somitogenesis: causes and consequences.

POCTI/BCI/47681/2002

Sólveig Thorsteinsdóttir

Formation of the myotome in the mouse: cell movements and cell-extracellular interactions.

POCTI/MGI/40478/2001

Pedro Fernandes

In search of new molecular targets for the development of novel therapeutic strategies for Cystic Fibrosis

POCTI/32576/BCI/2000

Leonor Parreira

The functional organization of the chromatin in the nucleus.

PRAXIS SAU/14000/1998

Leonor Parreira

The role of cell-fate decision genes in human hematopoietic differentiation.

POCTI/MGI/46477/2002

Jorge Carneiro

Nature, population dynamics, and mechanism of action of regulatory CD25⁺ T cells: A biomathematical and experimental approach.

POCTI 36312/99

Jorge Carneiro

HIV2 infection as a model for the investigation of AIDS pathogenesis

POCTI/MGI/36403/99

Michael Parkhouse

The potential and application of virus host evasion genes that modify apoptosis and cytokine responses.

POCTI/MGI/45100/2002

Michael Parkhouse e Ana Crespo

Viral modulation of cell division, apoptosis and interferon responses.

POCTI/ESP/39636/2001

Astrid Vicente

Genetic Epidemiology of autism

POCTI/FCB/44706/2002

Astrid Vicente

Pharmacogenetics of risperidone therapy in autism spectrum disorders

POCTI 36392/99

Carlos Penha Gonçalves

Genetics of Malaria in wild mouse models

POCTI/BME/36192/99

Juan Carlos Izpisua Belmonte

Specification of vertebrate limb bud

POCTI/BSE/48228/2002

Henrique Teotónio

Experimental evolution and the genetic basis of adaptation: analysis of candidate genes during reverse evolution

POCTI/1999/BCI/34599

Isabel Palmeirim

Time counting system during vertebrate embryonic development

POCTI/2001/BCI/42040

Isabel Palmeirim

New aspects on coordinating limb bud development

POCTI/BCI/45914/2002

Leonor Saúde

Molecular and cellular characterisation of segmentation in the chick embryo

POCTI/NSE/34622/1999

Domingos Henrique

Notch signalling and the generation of neuronal diversity in the vertebrate neural retina

POCTI/NSE/48782/2002

Domingos Henrique

Characterization of vertebrate prickly-like genes and their functional role during embryonic development

FCT-MGI/37296/01

Miguel Soares

Molecular mechanism by which carbon monoxide generated by heme oxygenase-1 suppresses endothelial cell apoptosis.

GRICES Projects

GRICES/CNR/4.1.1/03

José A. Belo

Identification and characterization of genetic interactions between members of nodal pathway by double mutant mouse analysis.

GRICES/BC 2003

Gabriela Gomes

Epidemiology and evolution and control of leishmaniasis.

EU Projects

EU-QLK2-CT-2002-00810

J. Pedro Simas

Antiviral peptides blocking herpes simplex virus type 1 entry into cells.

EU FP6 Proposal number 504468

António Jacinto (Project Head: Tony Durston, Netherlands Institute for Developmental Biology, Utrecht, The Netherlands)
Cells into organs.

EU EVR1-CT-2001-40017

Pedro Fernandes

BIOCASE – A Biological Collection Access for Europe

EU IST 1999-20469

Pedro Fernandes

EMBER - Design of a European Biocomputing Educational Resource

EU QLRI-CT-2001-01363

Pedro Fernandes

EMBCORE – The Core European Bioinformatics Research Infrastructure in the Life Sciences

EU QLK3–2000–00362

Michael Parkhouse

The potential and application of virus host evasion genes that modify apoptosis and cytokine responses.

EU QLK2–CT–2001–02216

Michael Parkhouse

African swine fever (ASF): improved diagnostic methods and understanding of virus-host epidemiology and virus-host interaction.

EU QLG3-CT-2000-01224

Domingos Henrique

Neural Stem Cells and stem cell-based therapies

EU QLK3-CT-2001-00422

Miguel Soares

Targeting Heme-Oxygenase-1 (HO-1) or its molecular mediators: a new therapeutic approach for treatment of inflammation

Other Projects

Portuguese Society of Urology

Sérgio Dias, Manuel Xavier Coelho

Angiogenesis in Prostate Cancer

Wellcome Trust, UK

Travel-Collaborative grant 069880

António Jacinto

A gain of function screen to identify the molecular basis of epithelial adhesion, contact inhibition and cell:cell matching during *Drosophila* dorsal closure and wound repair

PAL+ (Malaria Network, French Ministry of Research)

Sylviane Pied

Réponses immunes induites par *Plasmodium falciparum*: Rôle dans la genèse des formes graves notamment le neuropaludisme

AstraZeneca

Miguel Soares

In vivo Delivery of Tat-fusion Proteins to Inhibit the Activation of the Transcription factor Nuclear Factor kappa B (NF- κ B) in vivo

The Pfizer Atorvastatin Research Awards Program

Miguel Soares

Protective effects of heme oxygenase-1 in vascular injury

NIH RO1 Grant No: HL67040-01

Miguel Soares

Regulation of endothelial cell apoptosis by HO-1 derived CO

FCG

Projectos transversais e inovadores

António Coutinho

Malária Mitogens

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SEMINARS AT THE IGC

January

Susana Campino (IGC)
Genetic Control of Murine Malaria.

Dan Holmberg (IGC/Univ. Umea, Sweden)
Genetic analysis of Type I diabetes.

Jorg Becker (IGC)
Profiling Sex in Arabidopsis - the Male Contribution.

Linda Dixon (Institute Animal Health, Pirbright Lab, Surrey, UK)
Subversion of macrophage function by African swine fever virus.

Christian Doerig (INSERM U511 team, Wellcome Centre for Molecular Parasitology, The Anderson College, Glasgow, UK)
Cell proliferation in Plasmodium: fundamental and applied aspects.

Claudia Florindon (IGC)
Human cell division: the directors cut.

February

Susana Nery (New York University, USA)
Tangential migration in the telencephalon: where do cortical interneurons come from?

Jocelyne Demengeot (IGC)
FlowCytometry, before going to the machines.

Miguel Vaz Afonso (Cellular and Systems Neurobiology Department, Max-Planck-Institute of Neurobiology, Munich, Germany)
Observing neurons in their natural habitat: long-term in vivo 2-photon microscopy in the mouse visual cortex.

Rosalina Fonseca (Cellular and Systems Neurobiology Department, Max-Planck-Institute of Neurobiology, Munich, Germany)
Competitive Interactions Between Potentiated Synapses.

Marta Moita (Cold Spring Harbor Laboratory, USA)
Putting fear in its place.

Joseph Mascarenhas (University at Albany, USA)
Reflections on some issues concerning science policy and science funding.

Allen Braun (National Institute on Deafness and Other Communication Disorders, USA)
Sleep and Wakefulness.

Maria Mota (IGC)
Hepatocyte Growth Factor and its receptor are required for malaria infection.

March

Vania Braga (Imperial College, UK)
Cadherin adhesion and Rho GTPases.

Jan Andersson (IGC)
The Biology of NK cells.

Minus van Baalen (Institut d'Ecologie, UMR 7625 Université P. et M. Curie, Paris, France)
Common language or Tower of Babel? On the evolutionary dynamics of signals and their meaning.

Helia Neves (IGC)
Differential Role of Notch Ligands, Delta-1 and Jagged-1 in Human Hematopoietic Progenitor Cell Differentiation.

Stan Marée (Univ. British Columbia, Vancouver, Canada)
Modeling an organism.

Miguel Soares (IGC)
Modulation of inflammation cell proliferation and apoptosis by the protective gene heme oxygenase-1.

Rita Abranches (ITQB/UNL)
Molecular farming: Plants as factories for biopharmaceuticals.

April

Thomas Boehm (Dept. Dev. Immunol., Max-Planck Institute of Immunobiology, Germany)
Genetic dissection of thymus development in mice and zebrafish.

Marcus Peter (The Ben May Institute for Cancer Research, Univ. Chicago, USA)
Novel activities of CD95.

Lynne E. Maquat (Dept. Biochemistry and Biophysics, School of Medicine and Dentistry, Univ. Rochester)
Nonsense-mediated mRNA decay in mammalian cells: Evidence for a pioneer round of translation.

Helen Dawe (Lab Molecular Cell Biology and Cell Biology Unit, University College London, UK)

ADF I cofilin controls cell polarity during fibroblast migration.

Fernando Casares (IBMC)

Control of Drosophila organ growth and identity by wingless and homothorax.

Shohei Hori (Research Center for Allergy and Immunology, The Riken Institute, Japan)

Control of regulatory T cell development by the transcription factor Foxp3.

Mario Grãos (IGC)

Cell-cycle regulation of Bim.

Francisco Dionísio (IGC)

Public Goods Dilemma or the Tragedy of the Commons: general considerations and the case of Antibiotic Resistance.

Miguel Soares (IGC)

The Inflammation Laboratory at the IGC: An Overview of ongoing projects after one year.

Teresa Faria Pais (IGC)

Response of microglia to neuronal cell death.

Margarida Amaral (FCUL)

CFTR processing and folding inside the cell: implications for Cystic Fibrosis.

May

Carlos Penha Gonçalves (IGC)

The quest for type 1 diabetes genes.

Sergio Dias (IPO/IGC)

Our first year in angiogenesis.

Thiago Carvalho (IGC)

Santa Claus, The Easter Bunny and Suppressor T-cells.

Robert Elston (Case Western Reserve University, USA)

The analysis of case-control data to detect candidate genes.

Robert Elston (Case Western Reserve University, USA)

Biometrical Genetics: past, present and future.

John Stewart (Univ. Compiègne, France/IGC)

Constructing Mendelian phenotypes by multivariate analysis.

Miguel Ramalho Santos (Harvard Univ., Boston, USA)

Stemness: transcriptional profiling of embryonic and adult stem cells.

Manuel Santos (Univ. Aveiro)
The molecular mechanisms of evolution of alternative genetic codes.

June

Suzanne Bourgeois (The Salk Institute, USA)
Cancer around the world: what you should know.

José Carlos Machado (IPATIMUP)
Helicobacter pylori and host genetic variability: role in gastric carcinogenesis.

Martin Weigert (Dept. of Molecular Biology, Princeton University, USA)
Autoimmunity and Free Light Chains.

Jordan Raff (Wellcome/Institute of Cancer and Developmental Biology, University of Cambridge, UK)
Centrosomes and cancer: lessons from a TACC.

Melvin Cohn (The Salk Institute, USA/IGC)
Divining the Logic used by "Evolution" to regulate Immune Responsiveness.

Max Coope (Howard Hughes Medical Institute, The Univ. of Alabama at Birmingham, USA)
Search for the Roots of the Adaptive Immune System in the Sea Lamprey.

Luis Graça (Oxford University, UK)
Regulatory T cells and transplantation tolerance.

Daniela Finke (University of Lausanne, Switzerland)
Hematopoietic/mesenchymal crosstalk during lymphoid organ development.

July

Margarida Archer (ITQB/UNL)
What can we learn from x-ray crystallography.

Alberto Nobrega (Universidade Federal do Rio de Janeiro, Brazil)
Natural antibodies and B cell immunoglobulin repertoires.

Mariana Faria (IGC)
Nobody degrades proteins for mitotic exit.

Joana Santos (IGC)
Discussion of a recent paper about RNAi on Nature Biotech.

Pedro Domingos (The Rockefeller University, NY, USA)
The role of the transcription factor Spalt during photoreceptor development in the Drosophila eye.

Helena Vieira (ITQB, UNL)
Mitochondria and apoptosis.

Guy Cox (University of Sidney, Australia)
Fluorescent proteins in the wild: the glowing colours of corals.

Leonor Sarmiento (IMM/FML and Division of Experimental Hematology, Aids Research Center, USA)
Notch1 promotes cell cycle progression by inducing SKP2-dependent p27KIP1.

Sofia Rodrigues (IGC)
First somites first.

Will Wood (IGC)
Wound healing in Drosophila.

September

Leonor Saude (IGC)
Genespotting in the presomitic mesoderm.

J.C. Marinoni (European Patent Office (EPO), Munich, Germany)
The European Patent System.

A. Chakravarty (European Patent Office (EPO), Munich, Germany)
Patenting Biotechnology.

Hiroshi Hamada (Graduate School of Frontier Biosciences, Osaka University)
Generation and transfer of asymmetric signals for left/right patterning.

Frank Burnet (Univ. West England, UK)
Pride and prejudice: whatever happened to the publics love affair with science.

Joaquin Rodriguez Leon (IGC)
Right or left? Check your Calcium.

João Caraça (Serviço de Ciência, FCG)
A science-based innovation system.

Davor Solter (Max-Planck Institute of Immunobiology, Freiburg, Germany)
Genetics and epigenetics in early mammalian development.

Maria Manuel Mota (IGC)
Malaria Cell Biology lab: an overview.

Adriano Henriques (ITQB/UNL)
Coupling cell division to chromosome segregation in Bacillus subtilis.

Joerg Fritz (InterCell Biomedical, Vienna , Austria)
The artificial antimicrobial peptide KLKLLLLLKLK induces adaptive immunity to co-injected antigens.

October

Alexandre Castro Caldas (FMUL)
Os genes, o cérebro e o comportamento.

M^a Cecilia Angulo (Lab de Neurophysiologie, ESPC-INSERM EPI)
Central role of astrocytes in regulating both neuronal activity and vessels in the brain.

Filippo Rusconi (Muséum National d' Histoire Naturelle)
Functional complementation of RNAi mutants in trypanosomes.

Frank Uhlmann (Cancer Research UK, London Research Institute)
Orchestrating chromosome segregation during mitosis.

Julie Cooper (Telomere Biology Laboratory Cancer Research, UK London Research Institute, UK)
On and off the edge of the chromosome: multiple roles for telomeres in protecting genome stability.

Viggo Andreasen (Roskilde University, Denmark)
Epidemiology and natural selection in Influenza A.

Purnima Bhanot (New York University Medical School, USA)
Studies on the cell biology of Plasmodium sporozoites.

Frank Staal (Dept. Immunology of the Erasmus Medical Center, The Netherlands)
Functional genomics of T cell development in the thymus.

Antonio Jacinto (IGC)
Cadherins and morphogenesis in Drosophila.

Manuel Sobrinho Simões (IPATIMUP)
Mitochondria in neurodegenerative disorders and cancer.

Lee Segel (Department of Computer Science and Applied Mathematics, The Weizmann Institute of Science, Israel)
How success-dependent distributed feedback via information-laden signalling chemicals modulates immunological response.

Jocelyne Demengeot (IGC)
Immune Regulation: Our current approaches.

Ariane Abrieu (Centre de Recherches de Biochimie Macromoléculaire, Montpellier, France)
Kinetochore dependent checkpoint - Detecting Chromosome to spindle attachment.

Michael Glotzer (Institute Molecular Patology, Viena, Austria)
The central spindle: assembly, regulation, and function in cytokinesis.

November

Scott Kaufmann (Mayo Clinic and Medical School, Minnesota, USA)
Caspases and the response to anticancer drugs.

Ana Paula Santos (IGC)
3D Interphase chromosome organization in plants and animals - similarities and differences.

Michael Ferenczi (Imperial College London, UK)
When in the actomyosin ATPase cycle is muscle force being produced?

Daniel Cutler (MRC Laboratory for Molecular Cell Biology and Cell Biology Unit, University College London)
Biogenesis of Weibel-Palade Bodies: Organelles at the interface of inflammation and haemostasis

Margarida Prado (IGC)
When pollen tubes say no to NO.

Marcus Thelen (Institute for Research in Biomedicine, Switzerland)
Regulatory circuits in chemokine receptor-mediated signal transduction.

December

Silvia Costa (IGC)
How is the polarization of extracellular proton fluxes regulated in pollen tubes?

Luis Archer (UNL)
Do DNA à sequência do genoma humano, 50 anos de História.

Roderic Guigo (Genome Bioinformatics Research Lab, IMIM - Institut Municipal d'Investigació Mèdica Barcelona, Spain)
Comparative gene finding. Helping to complete the catalogue of human genes.

André Pires da Silva (Max-Planck Institute, Tuebingen, Germany)
Sex determination as a model for studying the evolution of developmental pathways.

Jorge Carneiro (IGC)
CD25-positiveness explained. Insights into the nature, origin and function of regulatory T cells.

Mario Gomes Pereira (Institute of Biomedical and Life Sciences, Univ. Glasgow, UK)
DNA repeats, replication and repair: the good, the bad and the ugly.

SYMPOSIA, CONFERENCES AND MEETINGS ORGANISED BY THE IGC

EMBO Pratical Course

Gulbenkian Biology Course

Plant Development: Molecular and Cellular Basis

Instituto Gulbenkian de Ciência

31 March-16 April 2003

Organisers: José Feijó (IGC/FCUL, Portugal) and Margarida Oliveira (IBET/UNL)

With the sequencing of several flowering plant genomes, molecular and genetic tools are becoming available that will allow integration with organism-centered approaches. Thus the time has come for putting the organism together again and this will require close monitoring of in vivo properties and follow-up of the extended processes that each gene or group of genes controls.

This course aims to provide the basis for post-genomic work, with a solid theoretical background and practical training on the most utilised and versatile molecular tools and approaches, fostering interdisciplinary interfaces with biophysical and imaging techniques, and grounded in a strong sense of integrative biology.

Workshop on Molecular and Genetic Basis of Autoimmune Diseases: SLE and RA

Fundação Calouste Gulbenkian

Centre for International Meetings on Biology

Co-Sponsored By Instituto Gulbenkian de Ciência

European Molecular Biology Organization

7-9 April 2003 Lisbon

Organizers: António Coutinho (IGC, Oeiras, Portugal), Werner Haas (IGC, Oeiras, Portugal), C. Martínez-A. (Centro Nacional de Biotecnología, Madrid, Spain)

For their prevalence in the western world, autoimmune diseases (AID) represent a major public health problem. AID raise basic scientific questions on natural tolerance to body tissues and its relationship to infections. Yet, there is no diagnostic of AID prior to target lesion, and no rational, curative therapies are available. Over the last years, progress was achieved on the molecular basis of complex processes in immunology and in cell and tissue biology, on the genetic basis of susceptibility/resistance to AID, and on the clinical evaluation of novel, "biology-based" therapies. These are the topics that will be dealt with at this workshop, specifically focused on systemic lupus erythematosus (SLE) and heumatoid arthritis (RA).

S.A.G.E. – Statistical Analysis for Genetic Epidemiology.
Instituto Gulbenkian de Ciência
27-29 May 2003

Organisers: Astrid Vicente, (IGC, Oeiras, Portugal); **Professor Robert Elston** (Case Western Reserve University, USA)

The Statistical Analysis for Genetic Epidemiology (S.A.G.E.) software provides researchers with tools necessary to perform various types of statistical genetic analysis, including study of family correlations, segregation analysis and association analysis. In this course, genetic epidemiology terminology and concepts were overviewed, and the theory and applications of the S.A.G.E. program package for statistical analysis of family data was fully covered.

Advanced Light Microscopy in Living Cells
Instituto Gulbenkian de Ciência
17-18 June 2003

Organiser: Nuno Moreno (IGC, Oeiras, Portugal)

Speakers: António Jacinto (IGC, Portugal), Guy Cox (University of Sydney, Australia), José Braga (Instituto de Medicina Molecular, Portugal), José Feijó (IGC/Universidade de Lisboa, Portugal), José Rino (Instituto de Medicina Molecular, Portugal), Jan Willem (Wageningen University, Netherlands), Miguel Vaz Afonso (Max Planck Institute, Germany).

Advances in Microscopy and electronics have opened new windows for viewing live cells. New tools like spectral confocal, two-photon, fast scanning system and deconvolution are making it possible to learn from biological systems as never before. This course will focus on imaging living cells, one of the most demanding applications in microscopy.

Oeiras Mathematical and Computational Biology Workshop
Instituto Gulbenkian de Ciência
20 June 2003

Organisers: Luis Rocha (LANL), and Jorge Carneiro (IGC, Oeiras, Portugal)

One of these initiatives of the Oeiras Advanced Studies at IGC was the launching of a multi-institutional *Mathematical and Computational Biology Collaboratorium*. We envision this collaboratorium as an open organization, designed to enable intense cooperation with national and international institutions: the center hub of a collaborative network of research institutions. Its chief aims are to identify and provide suitable facilities for visiting scientists, and hosting informatics technology to enable continuing off-site collaboration and research in Mathematical and Computational Biology. The other main goal of the collaboratorium is to use its research collaborations to ensure the attraction and education of quality students.

The objective of this workshop was to bring together those interested in this area in Portugal so that we can map the community of researchers and institutions working in Portugal who are interested in this collaboratorium.

Taxonomic Databases Working Group (IUBS) Meeting 2003
Challenges and Solutions in Biodiversity Informatics: Taxonomic Names and beyond
Instituto Gulbenkian de Ciência
21 – 28 October 2003

Organizer: Pedro Fernandes (IGC, Oeiras, Portugal)

87 members were present and held several sectorial meetings, conferences and a General Assembly. Two practical courses “BioCASE Providers Training Workshop” and “Regional GBIF DiGIR Training Workshop” were held in the Bioinformatics Training Room of the IGC.

2nd Meeting of the Immunology Groups at the IGC: MIG-IGC03
EvoraHotel, Evora.
23-25 November 2003

Organisers: Jocelyne Demengeot (IGC, Portugal) , **Dinis Calado** (IGC, Portugal), **Margarida Santos** (IGC, Portugal)

This event was organized as a retreat with an explicit goal of rejoining all IGC immunologists for a brain storming on the state of Immunology in the word and the position of the Institute in the field. A total of 53 people attended the seven sessions distributed from the 23rd evening to the 25th afternoon. Numerous presentations of ongoing work followed by discussion during and after the sessions allowed a rather complete tour of the activities at the IGC. This meeting will help refining common lines of investigation in the filed of Immunology at the IGC.

Comparative gene finding. Helping to complete the catalogue of human genes
Instituto Gulbenkian de Ciência
5 December 2003

Organizers: Pedro Fernandes (IGC, Oeiras, Portugal),

Speaker: Roderic Guigo (Genome Bioinformatics Research Lab, IMIM - Institut Municipal d'Investigació Mèdica, Barcelona)

While substantial progress has been made towards the completion of a highly confident version of the human genome sequence, the debate remains on the number of human protein coding genes and the specific amino acid sequences encoded by them. Comparative analysis of the human and rodent genomes - based on the underlying assumption that conservation of sequence often reflects conservation of function - has certainly contribute to a better understanding of the human gene complement, but it is far from solving definitively the controversy. Sequencing projects underway across the whole eukaryotic phylogenetic spectrum will help towards such a goal, but still a number of problems are likely to require specific treatement. Among these, we can cite: alternative splicing, non-canonical splicing, selenoprotein genes, intronless genes, genes within introns, species-specific genes, and fast evolving genes. During my talk, I will focus specifically on the identification of selenoprotein genes.

Epidemiology and control of leishmaniasis
Instituto Gulbenkian de Ciência
5 December 2003

Organisers: Gabriela Gomes (IGC, Portugal) and Orin Courtenay (University of Warwick, UK)

This meeting had the objective of setting new collaborations in leishmaniasis research, involving field epidemiology in Portugal and Brasil, and mathematical modelling provided by the theoretical epidemiology group at the IGC.

The Thinking of Art
Representations of the World
Instituto Gulbenkian de Ciência

Organiser: Leonor Parreira (FML/IGC, Portugal)

The Instituto Gulbenkian de Ciência has launched a series of conferences entirely devoted to Art, which took place at the premises of the Institute during the year 2003. The aim was to offer to all IGC scientists, a wider vision of the World and Man, as seen, thought and felt, by some of our most distinguished specialists in different forms of Art. The title “*The thinking of Art. Representations of the World*” embodies what has been the founding idea: that in Art, just like in Science, the heart of the matter is the process of thinking though, as opposed to Science, the realm of Art is representation, rather than explanation, of that very same World.

Fine Arts

Manuel Castro Caldas (ArCo)

Not What it Seems - modern art and the quest for the invisible.
6 March 2003

Dance

Maria José Fazenda (Escola Superior de Dança)

Entre a narrativa e a abstracção, ou as formas como a dança se refere ao mundo.
27 March 2003

Music

Rui Vieira Nery (Fundação Calouste Gulbenkian)

Racionalidade e emoção na experiência musical.
10 April 2003

Photography

Jorge Calado (Instituto Superior Técnico)

Fotografia à luz da Música e da Escultura.
5 June 2003

Architecture

Paula Cadima (Faculdade de Arquitectura de Lisboa)

Sustainable Approach to Architecture.
3 July 2003

Cinema

João Mário Grilo (Faculdade de Ciências Sociais e Humanas, Universidade Nova de Lisboa)

O homem imaginado, uma ciência para o cinema.

30 October 2003

Art and Science

Nuno Crato (Instituto Superior de Economia e Gestão, Universidade Técnica de Lisboa)

Painting through eyes of science.

6 November 2003

TEACHING

POST-GRADUATE EDUCATION

Post-graduate education has always been a strong valence of the IGC, and this tradition has been maintained through the establishment of the Gulbenkian Programme in Biology and Medicine which ended in 1999 and was followed by the Gulbenkian Programme on Biomedicine.

GULBENKIAN PhD PROGRAMME IN BIOMEDICINE

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Rita Margarida Morais Tavares
Tibor Zoltán Vag
Marta Sofia Pereira Castro Vitorino

Gulbenkian PhD Programme in Biomedicine for 2003/2004

15-16 Sept: Techniques I - Biochemistry

Ricardo Pires (ITQB, Oeiras, Portugal)
Sukalyan Chatterjee (IGC, Oeiras, Portugal)
Margarida Archer (ITQB, Oeiras, Portugal)
Cristina Silva Pereira (ITQB, Oeiras, Portugal)
Teresa Catarino (ITQB, Oeiras, Portugal)
Isabel Correia (ITQB, Oeiras, Portugal)
Pedro Lamosa (ITQB, Oeiras, Portugal)

17-19 Sept: Techniques II – Recombinant DNA

Lisete Fernandes (IGC, Oeiras, Portugal)
Sukalyan Chatterjee (IGC, Oeiras, Portugal)
Joana Monteiro (IGC, Oeiras, Portugal)

22-24 Sept: Techniques III – Cell Biology I

Célia Domingues (IGC, Oeiras, Portugal)
Paulo Alves (IGC, Oeiras, Portugal)
Susana Godinho (IGC, Oeiras, Portugal)
Patrícia Madureira (IGC, Oeiras, Portugal)

25-29 Sept: Curia Meeting

30 Sept: Techniques III – Cell Biology II + III

Nuno Moreno (IGC, Oeiras, Portugal)

Sérgio Gulbenkian (IGC, Oeiras, Portugal)

1-3 Oct: Techniques IV - Genetics

António Jacinto (IGC, Oeiras, Portugal)

Rui Gomes (FCUL, Lisboa, Portugal)

José Eduardo Gomes (Institute of Molecular Biology, Univ. of Oregon, USA)

Francisco Dionísio (IGC, Oeiras, Portugal)

Jorg Becker (IGC, Oeiras, Portugal)

Moises Mallo (IGC, Oeiras, Portugal)

Sérgio Simões (IGC, Oeiras, Portugal)

6-8 Oct: Replication, Recombinant and Telomere Biology

Miguel Godinho (Telomere Biol. Lab., Cancer Research UK, London, UK)

Karim Labib (Paterson Institute of Cancer Research, Manchester, UK)

Julia Promisel Cooper (Telomere Biol. Lab., Cancer Research UK, London, UK)

Frank Uhlmann (Chromosome Segregation Lab., Cancer Research UK, London, UK)

9-14 Oct: Transcription

Sukalyan Chatterjee (IGC, Oeiras, Portugal)

Thomas Oelgeschlager (Transcription Lab., Marie Curie Research Institute, Surrey, UK)

Colin Goding (Signalling & Development Lab., Marie Curie Research Institute, Surrey, UK)

Reiner Veitia (Université Denis Diderot/Paris VII, Paris, France)

Ana Sofia Quina (IGC, Oeiras, Portugal)

João Ferreira (IHEFM-UL, Lisboa, Portugal)

14-17 Oct: mRNA Biogenesis

Margarida Gama-Carvalho (IMM, Lisboa, Portugal)

Olga Calvo (University of Columbia, USA)

Oliver Muhleman (University of Bern, Switzerland)

Nuno Morais (IMM, Lisboa, Portugal/Cambridge, UK)

José Rino (IMM, Lisboa, Portugal)

20-24 Oct: Translation & Advanced Protein Chemistry

Organiser: Sukalyan Chatterjee (IGC, Oeiras, Portugal)

Paul O'Shea (Medical School, University of Nottingham, Nottingham, UK)

27-31 Oct: Cell Cycle

Álvaro Tavares (IGC, Oeiras, Portugal)

Ariane Abrieu (CNRS, Montpellier, France)

Iain Hagan (Univ. of Manchester, UK)

Jonathon Pines (Wellcome/CRC Cambridge, UK)

Michael Glotzer (IMP, Viena, Austria)

Susana Godinho (IGC, Oeiras, Portugal)

Mariana Faria (IGC, Oeiras, Portugal)

Cláudia Florindo (IGC, Oeiras, Portugal)

3-7 Nov: Apoptosis

Yuri Lazebnik (Cold Spring Harbor Laboratory, Cold Spring Harbor, USA)
Michael Hengartner (IMB, University of Zurich, Switzerland)
David Vaux (The Walter and Eliza Hall Institute, Victoria, Australia)
Scott Kaufmann (Mayo Clinic, Rochester, USA)

10-14 Nov: Cytoskeleton Motors

Miguel Seabra (Imperial College School of Medicine, London, UK; IGC, Oeiras, Portugal)
Mike Ferenczi (Imperial College London, UK)
Klemens Rottner (GBF, Germany)
Irina N. Kaverina (Dep. Cell Biology, IMB, Austrian Academy of Sciences, Salzburg, Austria)

17-21 Nov: Membrane Traffic

Dan Cutler (UCL, London, UK)
Thierry Galli (Membrane Traffic & Neuronal Plasticity, INSERM U536, Paris, France)
Adam Linstedt (Carnegie Mellon University, Pittsburg, USA)
Graça Raposo (Institut Curie, Section de Recherche, CNRS UMR 144, Paris, France)

24-28 Nov: Signal Transduction

Marcus Thelen (Institute for Research in Biomedicine, Bellinzona, Switzerland)
Philip Hawkins (Babraham Institute, Cambridge, UK)

1-4 Dec: Motility & Cell Adhesion

Vânia Braga (Imperial College School of Medicine, London, UK)
Anne Ridley (Ludwig Institute, London, UK)
Kairbaan Hodivala-Dilke (Queen Mary's School of Medicine & Dentistry, London, UK)

9-19 Dec: Developmental Biology

Alfonso Martinez-Arias (University of Cambridge, UK)
António Jacinto (IGC, Oeiras, Portugal)
Patrick Lemaire (LGPD/IBDM, Marseille, France)
Marcos A. González-Gaitán (Max-Planck Institute, Dresden, Germany)
James Castelli Gair (Centro Andaluz de Biología del Desarrollo, Univ. Pablo Olavide, Sevilla, Spain)

5-16 Jan: Immunology

Jan Andersson (IGC, Oeiras, Portugal)
Thiago Carvalho (Univ. of Alabama at Birmingham, USA)
António Coutinho (IGC, Oeiras, Portugal)
Jocelyne Demengeot (IGC, Oeiras, Portugal)
Werner Haas (IGC, Oeiras, Portugal)
Matthias Haury (IGC, Oeiras, Portugal)
Miguel Soares (IGC, Oeiras, Portugal)
Gabriela Gomes (IGC, Oeiras, Portugal)
Jorge Carneiro (IGC, Oeiras, Portugal)

19-29 Jan: Host Pathogen Interaction

Mike Parkhouse (IGC, Oeiras, Portugal)
John Skehel (National Institute for Medical Research, London, UK)
Tony Minson (University of Cambridge, Cambridge, UK)
John McCauley (Institute for Animal Health, Berkshire, UK)
Linda Dixon (Institute Animal Health Pirbright Lab, Surrey, UK)
Covadonga Alonso (INIA, Madrid, Spain)
Antonio Alcami (University of Cambridge, Cambridge, UK)
Pedro Simas (IGC, Oeiras, Portugal)
Murray Selkirk (Imperial College of Science, Technology & Medicine, London, UK)
Jennefer Blackwell (Wellcome Trust Centre, Cambridge, UK)
Sylviane Pied (Institut Pasteur, Paris, France/IGC, Oeiras, Portugal)
Margarida Vigário (IGC, Oeiras, Portugal)
Maria Mota (IGC, Oeiras, Portugal)

2-5 Feb: Neurobiology I- Development of neurons, synapses and circuits

Gordon Fishell (Skirball Institute, NYU Medical Center, NY, USA)
Oscar Marín (Instituto de Neurociencias, Univ. Miguel Hernández, Alicante, Spain)

6-13 Feb: Neurobiology II- Sensory systems: signal processing systems

Miguel Vaz Afonso (Max-Planck Institute, Martinsried, Germany)
Rosalina Fonseca (Max-Planck Institute, Martinsried, Germany)
Guoping Feng (Duke University, Durham, USA)
Justin Crowley (Carnegie Mellon Univ., Pittsburg, USA)
Karim Nader (McGill University, Montreal, Canada)

16-20 Feb: Neurobiology III- Sensory systems: Cerebral Cortex and Perception

Ricardo Gil da Costa (Harvard University, Cambridge, USA)
Allen Braun (NIH, Bethesda, USA)
Alex Martin (NIH, Bethesda, USA)
William Tecumseh Fitch (Univ. of St. Andrews, Fife, UK)
Miguel Castelo-Branco (IBILI, Faculdade de Medicina, Coimbra, Portugal)

23-27 Feb: Molecular Basis of Disease I - Genetics

Carlos Penha Gonçalves (IGC, Oeiras Portugal)
Gonçalo Abecasis (Center for Statistical Genetics, Ann Arbor, USA)
Susana Campino (IGC, Oeiras, Portugal)

1-5 Mar: Molecular Basis of Disease II – Cancer

Sérgio Dias (IPOFG, Lisboa & IGC, Oeiras, Portugal)
Chris Marshall (Institute of Cancer Research, London, UK)
Fionna Watt (Keratinocyte Lab., London Research Institute, London, UK)
Paula Gameiro (IPOFG, Lisboa, Portugal)

8-12 Mar: Molecular Basis of Disease III – Neurodegeneration and gene therapy

Maria João Saraiva (Amyloid Unit, IBMC, Porto, Portugal)
Darren Monckton (Div. Molecular Genetics, Univ. of Glasgow, Glasgow, UK)
Luigi Naldini (San Raffaele Telethon Institute for Gene Therapy, Milan, Italy)
Arnold Munnich (Hopital Necker Enfants Malades, Paris, France)

15-17 Mar: Evolution

Francisco Dionísio (IGC, Oeiras, Portugal)
Jonathan Howard (Institute of Genetics, Cologne, Germany)
Isabel Gordo (IGC, Oeiras, Portugal)

18-19 Mar: Epidemiology

Gabriela Gomes, Isabel Gordo (IGC, Oeiras, Portugal)
Kevin Marsh (Wellcome Trust Research Labs, Kilifi, Kenya)
Lisa White (Univ. of Warwick, UK)

22-26 Mar: Theoretical Biology

Jorge Carneiro (IGC, Oeiras, Portugal)
Stan Marée (Utrecht, The Netherlands)

29 Mar-2 Apr: Genomics & Bioinformatics

José Pereira-Leal (MRC-Laboratory of Molecular Biology, Cambridge UK)
Dag Ahren (EMBL-European Bioinformatics institute, Cambridge, UK)
Pedro Fernandes (IGC, Oeiras, Portugal)
Isabel Marques (IGC, Oeiras, Portugal)
Eduardo Rocha (Institute Pasteur, Paris, France)

6-7 April: Science Communication

Ana Godinho Coutinho (IGC, Oeiras, Portugal)
Malcolm Love (UK)

14-16 April: Chemical Genetics

Rodney Kiplin Guy (Univ. of California, San Francisco, USA)
Mårten Grötlén (Göteborg University, Göteborg, Sweden)
Jamie Moore (University of California, San Francisco, USA)

**Annual Meeting of PGDBM/PGDB in Curia
25-29 September 2003**

According to the tradition, Curia hosted the Annual Meeting of the PGDBM/PGDB from 25th to 29th September. The scientific sessions included a poster session presented by the PGDB-III students as part of their requirements for passing the first year of the graduate programme. The posters were kept for the whole duration of the meeting, enabling frequent daily discussions around the posters. In addition there were oral presentations from 50 students, which stimulated lots of discussions. A research review committee constituted by ex-students of PGDBM (Isabel Palmeirim, António Jacinto, Miguel Castelo-Branco, Miguel Godinho Ferreira, Vasco Barreto and Francisco Dionísio) followed closely all the presentations and provided feedback to the PhD programme Executive.

There were three keynote conferences by invited speakers: Professor Martin Raff, University College London, UK, on "A life in science - in retrospect"; Professor Andrew Robertson, University of Surrey, Guildford, UK, on "International competitiveness and innovation in the national research effort; a cooperation between universities, funding agencies, government and industry"; and Professor Frank

Gannon, EMBO, Germany, on "The estrogen receptor: a paradigm for transcription factors?"

Many important visitors kindly participated in the Annual Meeting's session on Sunday, including the Secretary of State Assistant to the Minister for Science and Higher Education, Professor José Manuel Pinto Paixão, the President of FCT (Foundation for Science and Technology), Professor Ramôa Ribeiro, the Director-General for Higher Education, Dr. Luis Requincha Ferreira, and Professor Diogo de Lucena (FCG board member).

The official ceremonies on Sunday included a presentation on "Future challenges and opportunities for science in Europe" delivered by Professor Frank Gannon, followed by an open session of discussion about science policy in Portugal held as a round table composed of Professor Ramôa Ribeiro, Professor Pinto Paixão and Prof. Diogo Lucena.

The new format of the meeting, starting on a Thursday and finishing on a Monday, provided for a more condensed and intense meeting.

IGC INTERNAL PHD PROGRAM (PDIGC)

Director

Sérgio Gulbenkian

Staff

Maria Matoso

In 2003 the Doctoral Programme of the Instituto Gulbenkian de Ciência (PDIGC) was launched. The aim of the PDIGC is to provide a research training environment at the IGC so that PhD students can develop the skill and knowledge to contribute to research as professionals. The Programme encourages creativity, critical reflection, conceptual development and professional competence, judgement and confidence.

The Programme provides the necessary conditions and standards at the IGC for the satisfactory execution of the student's doctoral project, in accordance with the programme of work and methods presented by the student and by the supervisor. A Theses Committee monitors the progress of the student's work up to its conclusion. The Programme also organises graduate thematic courses in order to provide the doctoral students with the "common language" of modern biology by exposing them to some of the most active research areas. Each year, during their stay at the IGC, the students must attend three weeks of thematic courses in various scientific areas by taking advantage of the Gulbenkian PhD Programme in Biomedicine (PGDB). The themes covered by the courses include: protein chemistry and structural biology, cell and molecular biology, developmental biology, genetics and evolution, and more specialised subjects such as neuroscience, immunology and human diseases. In addition, all PhD students, in the presence of their supervisors, must present the evolution of their work at an annual meeting.

Annual Meeting of PDIGC

The first PDIGC meeting took place at the IGC from 18 to 20 September. The scientific sessions included presentations from 61 students and plenary lectures given by Prof. João Caraça (Fundação Calouste Gulbenkian) entitled "A science-based

innovation system” and Prof. Davor Solter (Max-Planck Institute of Immunology, Freiburg, Germany) entitled “Genetics and epigenetics in early mammalian development”. Also at this meeting, Prof. Ramôa Ribeiro (Fundação para a Ciência e Tecnologia) talked to the students about national scientific policies.

THE GULBENKIAN TRAINING PROGRAMME IN BIOINFORMATICS (GTPB)

Organizers: Fernandes P.L. and Marques I.

Support: Ana Maya

The Bioinformatics Unit of the IGC, which maintains the Portuguese Node of the European Molecular Biology Network (EMBNET), develops a specific educational program to train scientists in proper use of Bioinformatics tools and techniques.

Analysis of Biological Sequences

Faculty: **David Judge** (*University of Cambridge, UK*) and **Lisa Mullan** (*HGMP-RC, Hinxton, Cambridge, UK*)

March 2003

Bioinformatics: An Introductory Course

Faculty: **David Judge** (*University of Cambridge, UK*)

December 2003

PGBIOINF

The FCUL/IGC Post-Graduate Programme in Bioinformatics

2003 was the first year of PGBIOINF. Classes started at FCUL and IGC on January 6th.

PGBIOINF is a specialization course that picks-up students at the graduate level, both from the life sciences and from the mathematics/engineering/computing areas, and gives them the necessary skills to become professional Bioinformaticians. The course is student-centred, i.e. individual student follow-up is guaranteed. Naturally it is problem-oriented, as Bioinformatics is problem-solving in Biology using biological information and computing. It is also designed for an academic path, as it provides the platform required for access to thesis work both in the MSc and PhD degrees in Bioinformatics.

The course has two main periods: The first one consists of cross-disciplinary short courses, taken in parallel at FCUL, that aim at levelling the students so that their initial knowledge differences is faded and that they become fairly comfortable to take advanced seminars in the second part. In this period they also take an Introduction to Bioinformatics course, mainly at the IGC where they also take contact with the research activities in Oeiras. The second part consists of a series of advanced seminars, taken at the IGC, taught by active scientists in Bioinformatics.

Six of the initial nine students finished the specialization course. Evaluation takes several forms: written exams, essays, home and in-class assignments and journal clubs. The overall contents of the course have been inspected regularly by the teaching staff.

The students that have completed PGBIOINF in 2003:

Pedro Antunes
Ana Sofia Figueiredo
Pooja Jain
Mário Pulquério
Catarina Moita
Eugénia Nogueiro

The following courses, totaling more than 500 hours of tuition, were held at the IGC:

<u>Course</u>	<u>Lecturers</u>
<u>Introduction to Bioinformatics</u>	Pedro Fernandes (IGC) Mário G Silva (FCUL) Margarida Amaral (FCUL) Ana Coelho (ITQB) Jorg Becker (IGC) João Cunha (FCUL) Francisco Couto (FCUL) Manuela Regala (ITQB) Isabel Marques (IGC)
<u>Population Genetics</u>	Lounes Chikhi, Université Paul Sabatier, Toulouse, FR Mark Beaumont, University of Reading, UK
<u>Phylogenetics and Molecular Evolution</u>	James McInerney, Nat University of Ireland, Maynooth, IE
<u>Gene Prediction and Identification</u>	Genis Parra, IMIM, Barcelona, SP Sergi Castellano, IMIM, Barcelona, SP
<u>Genetic Expression and Microarrays</u>	Javier Herrero, CNIO, Madrid, SP
<u>Proteomics, Transcriptomics and Metabolomics</u>	Rita Casadio, Universita di Bologna, IT Luciano Milanese, CNR, Milano, IT
<u>Gene Ontology</u>	Robert Stevens, Univ. of Manchester, UK Amelia Ireland, EBI, Hinxton, UK Helen Parkinson, EBI, Hinxton, UK
<u>Population Dynamics and Epidemiology</u>	Gabriela Gomes, IGC, PT Manuel Carmo Gomes, FCUL, PT
<u>Data Warehousing and Data Mining</u>	Christian Blaschke, CNB, Madrid, SP José Maria Gonzalez, CNB, Madrid, SP
<u>Limits and Expectations in Bioinformatics</u>	Martin Bishop, HGMP, Hinxton, UK Pietro Lio, EBI, Hinxton, UK

SCIENCE AND SOCIETY

CIÊNCIA VIVA

“Ciência Viva” is a programme of the Ministry of Science and Higher Education for the promotion of science in society. The IGC participates in several of its initiatives.

Project “Scientific occupation of teenagers during holidays”

The programme “scientific occupation for teenagers during holidays”, started in 1997 and has provided secondary education students with the opportunity of being in close contact with the reality of scientific research work, through their participation in training sessions in public and private laboratories, research centers and entities for the promotion of science. In the context of this programme the IGC, in 2002, participated with the following training sessions:

Como é que o parasita da Malária interage com as nossas Células?

Maria Mota

15-31 July 2003

Pesquisa bibliográfica on-line de factores de transcrição e respectivas sequências genómicas de ligação.

Pedro Coutinho

1-15 August 2003

National week for the disclosure of scientific activities:22-28 November 2003

The IGC contributed with the following activities:

School visits

The aim of the school visits held during the first 2 days was to bring back to the IGC Ciência Viva high-school students that had previously worked in some of the labs. Ciência Viva called this initiative “Returning to the Lab” and asked all participating institutes to perform a visit during this week in order to allow those students’ classmates to get to know the research labs to.

Practical workshops: What is DNA?

In this workshop, participants could extract and look at their own DNA. Time would be set aside for discussion on the advances of life science research, since the discovery of the structure of DNA, 50 years ago.

Round Table: Come meet the scientists

A round table debate with scientists at different stages of their careers (undergraduates, post-docs, group leaders and directors), where participants would be able to learn about and share in what being a scientists involves: the driving force, the difficulties and joys, prospects for the future of scientific research, etc.

INITIATIVES OF THE IGC

Activities for Science Journalists

Scientists-Journalists Club 2003

Aims:

- To discuss, within context, the background and most recent findings in biomedical research
- To establish a network of journalists and scientists, thus fostering communication links and better understanding between the two groups
- To discuss the social, ethical and political implications of biomedical research

<u>Date</u>	<u>Topic</u>	<u>Scientists</u>
January	Genomes: why all the fuss?	Pedro Coutinho (IGC) Susana Campino (IGC)
February	Malaria	Maria Mota (IGC) Margarida Vigário (IGC) António Coutinho (IGC)
March	Visit to IGC Labs	Morphogenesis Lab Malaria Cell Biology Lab
April	Neurodegenerative Diseases	Alexandre Mendonça (IMM) Cristina Costa (Hosp. Fernando Fonseca)
May	Biotechnology applications in healthcare: from the lab to the marketplace	Nuno Arantes-Oliveria (ATGC Portugal and Alfama) Hélder Cruz (ECBio) Pedro Cruz (ECBio) Luís Amado (Biotechnol, APBio)
June	Developmental Biology/Embryology	Leonor Saúde (IGC) Joaquín León (IGC)
October	Genetic epidemiology: autism and other diseases	Astrid Vicente (IGC)
	Epidemiology of infectious diseases	Gabriela Gomes (IGC) Manuel do Carmo Gomes (FCUL; Com. Técnica Vacinação) Leonor Sasseti (Com. Técnica Vacinação) Margarida Ferreira (C.Técnica Vacinação)

Activities for Scientists

Patenting In Europe Seminar, 8-9 September 2003

IGC, 8th September

Fundação Calouste Gulbenkian, Lisbon, 9 September

Organisers: Ana Paula Godinho Coutinho (IGC), Maria Teresa Sommerfeld (EPO, Germany), Ana Eiró (FCT, Portugal), Francisca Moura (FCG)

Speakers: Werner Haas (Alfama, Lda), Siobhán Yeats (EPO, Germany), Ashok Chakravarty (EPO, Germany), Jean-Christophe Marinoni (EPO, Germany)

Aims:

- To establish new and strengthen existing relationships between the European Patent Office and researchers in Portugal
- To discuss and clarify the concepts underlying and the procedures for patent applications in Europe

Science Communication Workshop, 10-12 September 2003

Organisers: Ana Paula Godinho Coutinho (IGC), Sofia Jorge Araújo (King's College London, UK), Mónica Bettencourt-Dias (Cambridge University, UK)

Speakers: Frank Burnet (Univ. West England, UK), Malcolm Love (Producer, UK), Helen Pilcher (Nature, UK), Rosalia Vargas (Ciência Viva, Portugal), Ana Correia Moutinho (CISEP/ISEG, Portugal), Cláudia Magalhães (Unimagem, Portugal), Elisabete Caramelo (Press Officer, Portugal)

Aims:

- To make scientists more proficient in communicating and discussing scientific research and knowledge with the public and the media
- To motivate and empower scientists to become pro-active in developing science communication activities, for the public and the media
- To introduce scientists current theories and perspectives on public understanding and engagement in science
- To familiarize scientists with the methodology and day-to-day running of the media

Science Communication Conference And Debate, 12 September 2003

Organizers: Ana Paula Godinho Coutinho (IGC), Sofia Jorge Araújo (King's College London, UK), Mónica Bettencourt-Dias (Cambridge University, UK)

Speaker: Frank Burnet (Univ. West England, UK)

Panellists: Jorge Wemans (FCG, Portugal), Rosália Vargas (Ciência Viva, Portugal), António Coutinho (IGC, Portugal), Jorge Buescu (IST, Portugal), João Caraça (FCG, Portugal), Fernando Ramôa Ribeiro (FCT, Portugal), Helena Mendonça (Journalist)

Aims:

- To discuss attitudes and approaches to science communication in Portugal

Activities for Science Teachers

Biology in Modern Times Conferences 2003

Aims:

- To update teachers on the latest developments in biomedical research: knowledge and methods.
- To foster teachers' interest and enthusiasm in scientific research and science
- To build a network of scientists (from post-graduate students to senior scientists) and secondary school teachers, looking to future collaborations

Date	Titles	Speakers	Teachers
March	Malaria: a parasite's journey Viral genes – the drugs of tomorrow Cancer and new blood vessels formed in tumours	Maria Mota (IGC) Ana Crespo (IGC) Sérgio Dias (IGC, IPO)	Approx. 60
June	DNA: from birds and peas to the double helix...and beyond Demystifying cloning Gene therapy: what is it and what is it's use?	PGDB-3 students	Approx. 60
November	The cells that make up DNA sequences Autoimmunity: naturally occurring and disease-provoking Inflammation and the immune response	António Coutinho (IGC) Jorge Carneiro (IGC) Miguel Soares (IGC)	Approx. 60

Activities for schools

School visits to the IGC

Organizer: Sofia Rodrigues (IGC)

Aims:

- To give students (mainly 15-18 year olds) direct contact with state-of-the-art biomedical research
- To allow students to meet scientists, and together discuss issues surrounding research and careers

School Visits at the IGC

Esc. Salesiana Manique	25 students
Esc. Salesiana Estoril	23 students
Esc. Salesiana Estoril	26 students
Esc. Salesiana Estoril	22 students
Externato de Penafirme	18 students
Esc.Sec.Matias Aires -Cacém	24 students
Colégio Marista de Carcavelos	26 students
Esc.Sec.Gama Barros-Cacém	24 students
Esc.Sec.Gama Barros-Cacém	24 students
Esc.Sec.Júlio Dantas-LAGOS	58 students
Esc.Sec.de Viriato -Viseu	47 students
Esc.Sec. Linda-a-Velha	20 students
Col.Sagrado CoraçãoMaria-Lx	24 students
Esc. Sec. de Trofa	18 students
Col.Sagrado CoraçãoMaria-Lx	24 students
Colégio Marista de Lisboa	24 students
Colégio Marista de Lisboa	24 students
TOTAL 14 schools	451 students

Seminars at local schools

Aims:

- To promote greater interaction between scientists, school teachers and students
- To take to schools real-life experiences of scientific research
- To foster students' enthusiasm for science through discussion of up-to-date research projects

Date	Title	Speaker	School/Audience
May	Viral genes – the drugs of tomorrow Cancer and newblood vessels formed in tumours)	Ana Crespo (IGC) Sérgio Dias (IGC, IPO)	Esc. Sec. Carcavelos/11º ano students and teacher
TBC	Viral genes – the drugs of tomorrow	Ana Crespo (IGC)	To be confirmed
October	Cloning: science, technology, hopes and fears	Ana Coutinho (IGC)	Esc. Sec. D. Diniz, Lisbon/ Teachers, few students

Activities for the Public

Celebrating the 50th Anniversary of the DNA Double helix

At the Benfica-Sporting football match, 4th May 2003, Estádio Nacional

- Spectators were given a key-ring and bookmark with a selection of “Ways that DNA changed the world”.
- The players from both teams came onto the pitch wearing DNA 50th Anniversary T-shirts

- A DNA 50th Anniversary banner was paraded around the pitch, before the match.
- “What is DNA?” text on IGC webpage**
- In Media and Public section; to be kept updated
- Year-round conferences, art exhibitions and photography competition**
- In collaboration with the FCUL; coordinated by Pedro Fernandes at the IGC
 - Events:

DNA, junk and chromosomes

Fundação Calouste Gulbenkian

25 June 2003

Organiser: Pedro Fernandes (IGC, Oeiras, Portugal)

Speaker: Howard J Cooke (MRC Human Genetics Unit, Edinburgh, U.K)

DNA50 Celebrations

Organizers: Pedro Fernandes (IGC, Oeiras, Portugal), **Margarida Amaral** (FCUL, Portugal) and **Graça Fialho** (FCUL, Portugal)

Arqueogenética: escavações nos genomas

Faculdade de Ciências da Universidade de Lisboa

3 July 2003

Speaker: António Amorim (IPATIMUP, Porto, Portugal)

Os genes que são precisos para fazer uma célula e uma planta

Faculdade de Ciências da Universidade de Lisboa

11 September 2003

Speaker: José Feijó (FCUL-Lisboa / IGC-Oeiras, Portugal)

O DNA e o cancro

Faculdade de Ciências da Universidade de Lisboa

23 October 2003

Speaker: Manuel Sobrinho Simões (IPATIMUP, Porto, Portugal)

As células que inventam sequências de DNA

Faculdade de Ciências da Universidade de Lisboa

20 November 2003

Speaker: António Coutinho (IGC, Oeiras)

Os genes, o cérebro e o comportamento

Faculdade de Ciências da Universidade de Lisboa

11 October 2003

Speaker: Alexandre Castro Caldas (FMUL, Lisboa, Portugal)

Do DNA à sequência do genoma humano - 50 anos de História

Fundação Calouste Gulbenkian

4 December 2003

Speaker: Luis Archer (UNL, Lisboa, Portugal)

O impacto da genómica na sociedade

Fundação Calouste Gulbenkian

4 December 2003

Speaker: Luis Archer (UNL, Lisboa, Portugal)

Round Table with: José David Ferreira (UL, Lisboa, Portugal), **Fernando Catarino** (UL, Lisboa, Portugal), **Humberto Rosa** (UL, Lisboa, Portugal), **José Rueff** (UL, Lisboa, Portugal), **Michel Renaud** (UNL, Lisboa, Portugal)

Organization of Art exhibitions

DNA and the secret of life

By graduates from the Faculdade de Belas Artes de Lisboa

DNA: the twists of a molecule

By members of the Oficina de Cores e Pintores

DNA and Man

Photography contest (open until Feb 2004)

Poster Exhibition

DNA: The impact of a discovery in different scientific areas

Faculdade de Ciências da Universidade de Lisboa

Weekend conference with IGC researchers and the public of Oeiras

Organizer: Ana Coutinho (IGC)

Participants: Astrid Moura Vicente (Group leader; Genetic Epidemiology Lab), Moisés Mallo (Group leader; Neural Crest Lab), Francisca Fontes (Researcher; Lymphocyte Physiology Lab), Ana Crespo (Post-doc; Infections and Immunity Lab), Pedro Coutinho (Group leader; Functional Bioinformatics Group), Álvaro Tavares (Group leader; Mitosis Lab), Sérgio Dias (Group leader; Neoangiogenesis Lab), Sofia Araújo (Chairperson; Post-doc; King's College London, UK)

Aims:

- To allow the public of Oeiras to meet the scientists of the IGC and learn more about the research and running of the institute
- To promote direct, two-way communication between the public and scientists, with a view to fostering public engagement in science (rather than public understanding of science)

Participations in public discussions/events organized by others

Weekly afternoon discussion group (tertúlia) of retired Oeiras professionals, 22 October

Speakers: Ana Coutinho (IGC), Jorge Carneiro (IGC)

Media

Press releases

- Scientific papers by IGC researchers – 7
- Meetings – 3
- Other events - 8

Consultancy

“A 2” Programme “Etiqueta” for teenagers

- Scientific consultancy and participation in two episodes, on Cloning and Cancer

THESES

The following Theses were prepared in part at the IGC and were presented in 2003:

PhD Theses

Ricardo Ataíde. Papel das células TCD4+CD25+ na regulação da reposta imune a uma infecção de *Plasmodium berghei*, University Lusófona, Lisbon, Portugal.

Susana Campino. Genetic analysis of murine malaria, University of Umea, Sweden.

Thiago Lopes Carvalho. The role of regulatory CD4 T cells in inflammation, ICBAS, Porto, Portugal.

Cristina Casalou. A chaperonina Citoplasmática CCT do Eucariota ciliado *Tetrahymena pyriformis*: estudos durante a regeneração ciliar, FCUL, Portugal, July 2003.

Martyn Parker. Forcer symmetry breaking of Euclidean equivariant partial differential equations, pattern formation and Turing instabilities, Mathematics Institute, University of Warwick, UK.

Maria Gabriela Figueiredo Rodrigues. Culturas primárias de células epiteliais da vesícula seminal do hamster – um modelo para o estudo da actividade secretora, FCUL, Lisbon, Portugal.

João Tiago Santos Caldas Sousa. Modeling the antigen and cytokine receptors signaling processes and their propagation to lymphocyte population dynamics, Faculty of Sciences, University of Lisbon, Lisbon, Portugal.

MSc Theses

Hélia Cunha e Silva. Pesquisa pelo método do Híbrido duplo de parceiros moleculares da proteína humana Mob1, UBI, Covilhã, Portugal.

BSc Theses

Ana Alexandra Almeida. Establishing conditions to identify putative kinases that phosphorylate the BH3-only protein Bim, FCUL, Lisbon, Portugal.

Catarina Correia. Genetic epidemiology of autism, Faculdade de Ciências da Universidade de Lisboa.

Vanesa Cristão. Estudo da regulação espacial e temporal da expressão de Hoxa2 e suas consequências biológicas, FCUL, Lisbon, Portugal.

Ricardo Ferreira. Identification of immune-related genetic traits in Systemic Lupus Erythematosus, Faculdade de Ciências da Universidade de Lisboa.

Alexandre José Correia Gonçalves. Searching for genes involved in early organ differentiation by screening of a cDNA library of total chick embryos from stage 5 to 10 HH, Department of Animal Biology, Faculty of Sciences, University of Lisbon, Portugal.

Ana Martins. New aspects on *Colloid-like1* function during chick somitogenesis, Faculty of Sciences, University of Minho, Braga, Portugal.

Jose Nuno Barros de Oliveira Martins. Modelização de epidemiologia do vírus Influenza A, Biology Department, University of Evora, Portugal.

Nuno Miguel Vieira Pedroso. Função do Citoesqueleto na activação do NF- κ B pelo H₂O₂, FCUL, Lisbon, Portugal.

Rui Peixoto. Microglia activation by necrotic neurons is mediated by detergent resistant membrane microdomains, FCT, UC, Coimbra, Portugal,

Carlos Pereira. β 1 integrins during primary myogenesis in the mouse embryo, FCUL, Lisbon, Portugal.

Joana Domingues Ribeiro. Caracterização Funcional de Proteínas da Superfamília EGF/Laminina em *Drosophila melanogaster*, FCUL, Lisbon, Portugal.

PARTICIPATION IN ACADEMIC COMMITTEES

José A. Belo

Member of the Jury of the Ph.D Thesis, Maria Alexandra Capela, ICBAS, UP, Porto, Portugal.

Member of the Jury of the M.Sc. Thesis, Hélia Cunha e Silva, University of Beira Interior, Covilhã, Portugal.

Jorge Carneiro

Member of the Jury of the Ph.D Thesis, João Tiago Santos Caldas Sousa, University of Lisbon, Lisbon, Portugal.

António Coutinho

Member of the Jury of the PhD Thesis, Luís Maranga, Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Lisbon, Portugal

Member of the Jury of the PhD Thesis, Thiago Lopes Carvalho, Instituto de Ciências Biomédicas de Abel Salazar da Universidade do Porto, Porto, Portugal.

Member of the Jury for “Professor Coordenador”, Escola Superior de Tecnologia da Saúde de Lisboa, Lisbon, Portugal.

Gabriela Gomes

Member of the Jury of the BSc Thesis, Jose Nuno Barros de Oliveira Martins, University Evora, Portugal.

Matthias Haury

Member of the Jury of the Ph.D Thesis, Veronica Carvalhal, UNL, Lisboa, Portugal,

Isabel Palmeirim

Member of the Jury of the Ph.D Thesis, Sónia Pinho, University of Porto, Portugal, Supervisor: Prof Claudio Stern.

Michael Parkhouse

Member of the Jury of the Ph.D. Thesis, Elizabeth Ferrer, at Instituto Carlos III, Madrid, Spain.

Helena Soares

Member of the Jury of the Ph.D Thesis, Cristina Maria Tavares Lino Casalou, FCUL.

Miguel P. Soares

Member of the Jury of the Ph.D Thesis, “Insights into TRIP and NOD2 signalling” by Vasco Andre Machado de Oliveira, Instituto de Ciências Médicas Abel Salazar, University do Porto, Porto, Portugal.

Álvaro Tavares

Member of the Jury of McS Thesis, Hélia Cunha e Silva, UBI, Covilhã, Portugal.

Examiner final year project, Catarina Amaro Serra, IST/UTL, Lisbon, Portugal.

Examiner final year project, Maria Margarida Carvalho Negrão Serra, IST/UTL, Lisbon, Portugal.

Sólveig Thorsteinsdóttir

Member of the Jury of the M.Sc Thesis, Filomena Delgado, FCUL, Lisbon, Portugal.

Member of the Jury of the B.Sc. Theses of Joana Vaz Pato Osório, Carlos Pereira and Paulo Navarro Costa, FCUL, Lisbon, Portugal.

Honours and awards**António Coutinho**

- Distinguished with the Grande Oficial da Ordem do Infante D. Henrique
- Elected Vice-Chairman of the EMBO Council
- Elected Chairman of the Sociedade das Ciências Médicas de Lisboa
- Elected Foreign Member of the Academia Brasileira das Ciências
- Nominated member of the Advisory Council of the COTEC Portugal (Associação Empresarial para a Inovação)
- Nominated member of the AC IDE (Alto Conselho de Investimento Directo Estrangeiro)

José Feijó

- Elected Director of the Vegetal Biology Departament of the Faculdade de Ciências da Universidade de Lisboa

Maria Mota

- EMBO Young Investigator Award

Leonor Parreira and Helia Neves

- Pfizer Prize for Research

Helena Soares

- Elected Director of the Biology Departament of the Escola Superior de Tecnologia da Saúde de Lisboa

Tavares A.T., Filipe M. and Belo J.A.

Best Poster Prize of Fundacion Juan March Conferences, Madrid, Spain.
Transcriptional regulation of Cerberus-like genes during Embryonic Development.
Fundacion Juan March Conferences “The dynamics of morphogenesis: regulation of cell and tissue movements in development.

Nascimento R., Crespo A. and Parkhouse R.M.E.

Prémio de excelência em Imunologia, Mário Arala Chaves, Portuguese Society of Immunology, XXIX Annual Meeting, Porto, Portugal.

vGAP a novel viral protein inducing cell cycle arrest and apoptosis.

The following IGC scientists were elected to the Direction of the Sociedade Portuguesa de Imunologia

Jocelyne Demengeot (President)

Jorge Carneiro (Vice-President)

Carlos Penha Gonçalves (General Secretary)

João Pedro Simas (Treasurer)

Francisca Fontes and Michael Parkhouse (Scientific Secretaries)

PARTICIPATION OF IGC PERSONNEL IN CONFERENCES, SEMINARS AND SCIENTIFIC MEETINGS

January

Coutinho A.

From innate to adaptive immunity

Fundamental Immunology Course 2002-2003, Institut Pasteur, Paris, France

Coutinho A.

Autoimunidade – O Estado da Arte

I Annual Meeting Medinterna “Doenças Autoimunes”, Seminário de Vilar, Porto, Portugal

Fernandes P.L., Marques, I.M.

Bioinformática

A one day session in the MSc course “Metodologia de Investigação em Microbiologia Clínica” of FMUL, Portugal

Neves H.

Notch ligands Delta1 and Jagged1 in early human myelopoiesis.

Department of Immunology, Erasmus University Medical Center Rotterdam, The Netherlands

February

Coutinho A.

Autoimunidade em Medicina Interna

2as Jornadas de Medicina Interna do Hospital de Egas Moniz

Instituto de Higiene e Medicina Tropical, Lisbon, Portugal

Coutinho A.
Ora então, vamos à vida!
Conferences FCT/FCG, “Despertar para a Ciência”, Fundação Calouste Gulbenkian,
Lisbon, Portugal

Dionisio F.
Cooperação entre Seres Vivos: Altruísmo, Egoísmo e Divisão de Tarefas
Faculdade de Engenharia da Universidade Católica Portuguesa, Lisbon, Portugal

Fernandes P.L., Marques, I.M.
Interim Meeting of the EMBnet Committees, BMC, Uppsala, Sweden

Mallo M.
Reversible gene inactivation in the mouse.
Roche, Basel, Switzerland.

Parreira L.
Bioética na investigação em biomedicina.
Mestrado em Bioética. Faculty of Medicine, Lisbon University, Portugal

March

Faria M., Parelho V., Deak P., Glover D., Gomes R. and Tavares, A.
Nobody, a regulatory subunit of the 26S proteasome important for mitotic exit.
44th Annual Drosophila Research Conference, Chicago, USA. (Poster)

Gomes, G.
Epidemiologia da Tuberculose
Universidade Nova de Lisboa, Lisbon, Portugal

Marques I.M.
Aplicações de Bioinformática em Biologia Molecular
Instituto de Higiene e Medicina Tropical, Lisboa Portugal

Marques I.M.
Computação em Biociências
Instituto Superior de Ciências da Saúde Sul, Monte da Caparica, Portugal

Parkhouse M.
Estrategias racionales para el diagnóstico y desarrollo de vacunas.
Biomed, Univ. Carababo, Venezuela

Rodrigues, G.
Culturas primárias de células epiteliais da vesícula seminal do hamster – um modelo
para o estudo da actividade secretora.
Encontros de Scientia, Faculty of Sciences, University of Lisbon, Lisbon, Portugal

Vicente, A.M..
Epidemiology of Autism Spectrum Disorders (ASD) in Portugal.
The Social Brain, Goteborg, Sweden

April

Caramalho I.

Regulatory T cells selectively express toll like receptors and are activated by lipopolysaccharide.

Juan March workshop on Molecular and Genetic basis of autoimmune diseases: SLE and RA. FCG, Lisbon, Portugal. (Poster)

Chatterjee S.

Specificity, complementarity and molecular biology techniques.

IV Encontro Nacional de Jovens Biotecnólogos, Univ. Minho, Portugal

Chatterjee S.

Cell cycle, apoptosis and cell fate decisions.

I Encontro Nacional de Estudantes Bioquímicos, Coimbra, Portugal

Coutinho A.

EMBO Council Meeting

Heidelberg, Germany

Coutinho A.

Self-nonsel self discrimination: physiology and pathology of autoreactivity

EMBO/Instituto Juan March de Estudios e Investigaciones/Instituto Gulbenkian de Ciência Workshop on Molecular and Genetics Basis of Autoimmune Diseases: SLE and RA. Fundação Calouste Gulbenkian, Lisbon, Portugal

Coutinho A.

An outsiders view on the problems of vaccination

Keynote lecture. Conference on "The future of vaccines", Vienna, Austria

Coutinho A.

Investigação do Genoma Humano – Perspectiva de um Cientista

XXIV Congresso Português de Cardiologia, Sociedade Portuguesa de Cardiologia, Funchal, Madeira, Portugal

Coutinho A.

Ciência e Vida

Conference at Ar.Co, Centro de Arte e Comunicação Visual, Lisbon, Portugal

Demengeot J.

Function and dynamic of regulatory T cells during inflammatory responses

Juan March workshop on Molecular and Genetic basis of autoimmune diseases: SLE and RA. FCG, Lisbon, Portugal

Fernandes P.L.

Bioinformática: o que é? Para que serve?

VI Jornadas de Engenharia Biológica, Universidade do Minho, Vila do Gerês, Portugal

Fontes F.
The negative effects of steroids and cyclophosphamide on regulatory T cells.
Juan March workshop on Molecular and Genetic basis of autoimmune diseases: SLE and RA. FCG, Lisbon, Portugal. (Poster)

Mallo M.
Reversible gene inactivation in the mouse.
IBMC, University of Porto, Portugal

Marques I.M.
Computação em Biociências
Instituto Superior de Ciências da Saúde Sul, Monte da Caparica, Portugal

Parkhouse M.
Estrategias racionales para el diagnóstico y desarrollo de vacunas.
Centro de Recerca en Sanidad Animal, Univ. Autonoma de Barcelona, Spain

Vigário, A.M.
Modelos animais na investigação da malária cerebral.
Congresso Português de Parasitologia. Lisbon, Portugal

May

Cachaço A.S.
The role of $\beta 1$ integrins in skeletal muscle development in the mouse.
Encontros de Scientia , Faculty of Sciences, University of Lisbon, Portugal

Caramalho I.
LPS directly activates Regulatory T cells through engagement of toll like receptor-4.
FOCIS Paris, France

Carapau D., Cunha M., Rodriguez A., Dimopoulos G. and Mota M.
Plasmodium-driven transcriptional reprogramming of dendritic cells.
Federation of Clinical Immunology societies (FOCIS) Meeting, Paris, France. (Poster)

Coutinho A.
A governação das Universidades
Workshop. Fundação Calouste Gulbenkian, Lisbon, Portugal

Coutinho A.
Innate Immunity, Inflammation, Regulatory T Cells and Autoimmunity
FOCIS 3rd Annual Meeting, La Maison de la Chimie, Paris, France

Coutinho A.
Tolerância e Auto-imunidade
9º Congresso Nacional de Medicina Interna, Funchal, Madeira, Portugal

Coutinho A.
Concluding Remarks
Colloque L'Immunité Innée, De la Drosophile à l'homme, Institut de France,
Académie des Sciences, Paris, France

Dionisio F.
Tragedy of the Commons, the Public Good Dilemma and Evolutionary Biology.
Workshop of the European Science Foundation. Montpellier, France

Dionisio F.
Spread of conjugative plasmids among different bacterial strains and species
Workshop of the European Science Foundation. Montpellier, France

Fontes F.
The negative effects of steroids and cyclophosphamide on regulatory T cells.
FOCIS Paris, France. (Poster)

Fragoso R. and Dias S.
A role for FLT-1 in Acute Lymphoblastic Leukemia biology.
B cells in Health and Disease: Microenvironments and B cell Development, ESF
Conference, Acquafredda di Maratea, Italy. (Poster)

Godinho S. and Tavares, A.
Studying the role of POLO kinase in the spindle checkpoint using RNAi
EMBO World Programme Practical Course on Imaging Biological Functions in Cells
and Organisms. São Paulo, Brazil

Gomes, G.
Epidemiologia da Tuberculose
Dept. Pure Mathematics, University Porto, Porto, Portugal

Marques I.M.
Computação em Biociências
Instituto Superior de Ciências da Saúde Sul, Monte da Caparica, Portugal

Parkhouse M.
Estrategias racionales para controlar a los patógenos.
Instituto Carlos III, Madrid, Spain

Santos M.
Control of mouse B-cell differentiation by the notch ligands delta-1 and jagged-1.
Euresco conference on B cells in Health and Disease- Microenvironments and B Cell
Development, Italy

Tavares A.
Da mosca ao homem – Investigação básica em Portugal
II Conferências de Engenharia Biológica, Instituto Superior Técnico, Lisbon, Portugal

Tavares A.T., Filipe M. and Belo J.A.
Transcriptional regulation of Cerberus-like genes during Embryonic Development.
Fundacion Juan March Conferences “The dynamics of morphogenesis: regulation of cell and tissue movements in development. Madrid, Spain. (Poster)

June

Coutinho A.
Chair of the session: *Flow of Innovation*
XX IASP World Conference on Science and Technology Parks “Habitats of Excellence, Managing and Promoting Innovation”, Estoril, Portugal

Coutinho A.
As promessas da genética moderna na medicina
Congresso da Associação Portuguesa de Urologia, Sintra Caesar Park, Penha Longa Golf Resort, Sintra, Portugal

Coutinho A.
T-cell tolerance: where we stand today
EULAR 2003 Congress, Annual European Congress of Rheumatology, Lisbon Congress Center, Lisbon, Portugal

Coutinho A.
A beleza da vida
Conferences “Biologia na noite”, Centro Cultural e de Congressos de Aveiro, Universidade de Aveiro, Aveiro, Portugal

Demengeot J.
Are regulatory T cells important in autoimmunity?
15th European immunology congress.EFIS2003, Rhodos, Greece

Feijó J.A.
Ion dynamics and the control of pollen tube growth and morphogenesis.
Conference on Plant Gametophyte 2003: Evolution, development and function. Ascona, Switzerland

Feijó J.A.
The Confocals - The right tool for the right experiment. Advanced Light Microscopy in Living cells.
Instituto Gulbenkian de Ciência, Oeiras, Portugal

Fernandes P.L.
Percorso Cibernético da Consciência
in Percursos da Consciência, Fundação CulturSintra, Palácio da Regaleira, Sintra, Portugal

Mallo M.
Hoxa2 and the hindbrain.
8th Meeting of the Portuguese Society of Neurosciences, Curia, Portugal

Marques I.M.
Computação em Biociências
Instituto Superior de Ciências da Saúde Sul, Monte da Caparica, Portugal.

Pascoal S.
Is the molecular clock playing a role during limb development
EMBO workshop: Boundaries in development: 30 years of progress, Heidelberg, Germany. (Poster).

Rebelo M.
Role of Notch in the peripheral immune system
15th European immunology congress.EFIS2003, Rhodos, Greece. (Poster)

Rodrigues S.
Somitogenesis runs differently in the first formed somites
Workshop no Instituto Juan March, Madrid, Spain (Poster)

Silva A., Filipe M., Kuerner K., Steinbeisser H. and Belo J.A. Endogenous Cerberus activity is required for anterior head induction in *Xenopus*.
Fundacion Juan March Conferences “Developmental mechanisms in vertebrate organogenesis”. Madrid, Spain. (Poster)

Silva A., Filipe M., Kuerner K., Steinbeisser H. and Belo, J.A. Endogenous Cerberus activity is required for anterior head induction in *Xenopus*.
Gordon Research Conference “Developmental Biology”. Proctor Academy, Andover, New Hampshire, USA. (Poster)

Silva A., Filipe M., Kuerner K., Steinbeisser H. and Belo J.A. Endogenous Cerberus activity is required for anterior head induction in *Xenopus*.
Gordon Research Conference “Developmental Biology”. Proctor Academy, Andover, New Hampshire, USA. (Poster)

Vigário A.M., Voegtli D., Casimiro C., do Rosário V., Cazenave P-A and Pied S.
Do regulatory T cells play a role on cerebral malaria?
15th European Immunology Congress. Rhodes, Greece

July

Alcobia I., Quina A.S., Neves H., Clode N., Parreira L.
The spatial organisation of centromeric heterochromatin during normal human lymphopoiesis.
Alan Wolffe EMBO Workshop “Chromatin and Epigenetics”. Heidelberg, Germany. (Poster)

Casalou C. Melo L.V, Nolasco S., Seixas C., Brogueira P and Soares H. Subunits of the chaperonin CCT area associated with *Tetrahymena* microtubule structures and are involved in cilia biogenesis.
FASEB Summer Research Conferences on Ciliate Molecular Biology. Saxtons River, Vermont, USA

Gomes G.
Reinfection Thresholds
Workshop on “Biology, mathematics and statistics of recurrent infections”, Wellcome
Trust Research Labs, Kilifi, Kenya

Monteiro J., Fernandes L.
Role of TFIIB under specific non-physiological cellular contexts.
Gordon Research Conference “Stress-Induced Gene Expression”. Queen’s College,
Oxford, UK. (Poster)

Parkhouse M.
Estrategias racionales para controlar a los patógenos.
Colegio de Veterinarios, Santa Cruz de la Sierra, Bolivia

Quina A.S., Parreira L.
Functional role of nuclear heterochromatic domains on housekeeping gene expression
in human lymphocytes.
Alan Wolfe EMBO Workshop “Chromatin and Epigenetics”. Heidelberg, Germany.
(Poster)

Seixas, C.
Subunits of the chaperonin CCT area associated with *Tetrahymena* microtubule
structures and are involved in cilia biogenesis.
FASEB Summer Research Conferences on Ciliate Molecular Biology. Saxtons River,
Vermont, USA (Poster)

August

Dionisio F.
Evolution of Conjugative Plasmids under Antibiotic Pressure.
IX Congress of the European Society for Evolutionary Biology. University of Leeds,
UK

Gordo I., Marques A.C.R., Fernandes L., Dionisio F.
Unbeatable Mutators: a polymorphism for the mutation rate.
IX Congress of the European Society for Evolutionary Biology. University of Leeds,
UK. (Poster)

September

Belo J.A.
The role of the Cerberus-like gene family in the establishment of the L/R asymmetry
in vertebrate development.
Institute for Human Genetics, University of Heidelberg Medical School, Germany

Belo J.A.
The novel secreted factor *cerberus-like2* is involved in the genetic pathway
determinating the left-right asymmetry in the mouse.
5th EMBL “Mouse Molecular Genetics Meeting”, Heidelberg, Germany

Casalou C., Constantino Rosa Santos S. and Dias S.
The VEGF activation of the MAP kinase pathway is mediated by the microtubular cytoskeleton.
ELSO 2003 International Conference, Dresden, Germany. (Poster)

Cavalcante M., Vasilevskaya T., Tokaji L. Gregoire C., Fernandes B., Balla J. and Soares MP.
Generation and characterization of chimeric form of heme oxygenase-1 that can enter cells “spontaneously”
Heme Oxygenase: Regulation, Functions & Clinical Applications Conference. Uppsala, Sweden. (Poster)

Coutinho A.
A Unidade da Biologia Moderna enquanto ciência explicativa da natureza
X Encontro Nacional de Educação em Ciências, Faculdade de Ciências de Lisboa, Lisbon, Portugal

Coutinho A.
A Comunicação de Ciência em Portugal
Round Table, Workshop “Comunicar Ciência”, Instituto Gulbenkian de Ciência, Oeiras, Portugal

Coutinho A.
Embo Council Meeting, Heidelberg, Germany

Demengeot J.
Function and dynamic of regulatory T cells during inflammatory responses.
34th annual meeting of the German society of Immunology, Berlin, Germany

Feijó J.A.
Ion dynamics and the control of pollen tube growth and morphogenesis.
Iberian Meeting of the Spanish and Portuguese Societies of Plant Physiology. Palma de Maiorca, Spain

Figueiredo C. and Chatterjee S.
Resistance of Microglia to Quinolinic Acid Mediated Cell Death VI.
European Meeting on Glial Cell Function in Health and Disease. Berlin, Germany (Poster)

Florindo C., Perdigão J., Fesquet D., Pines J. and Tavares A.
Human Mob1-like proteins Mob4A and Mob4B are required for cytokinesis
The Cell Biology of Cancer, Oxford, UK

Freitas C.
When do medial PSM cells acquire autonomy for segmentation?
Ist joint meeting of the British and French Societies for Developmental Biology, Nice, France

Grãos M. and Chatterjee S.

Cell cycle dependent regulation of Bim.

ELSO 2003 International Conference, Dresden, Germany. (Poster)

Igreja C., Casalou C., Constantino Rosa Santos S., Clode N. and Dias S.

Evidence for distinct phenotypic stages during Endothelial progenitor Differentiation.

ELSO 2003 International Conference, Dresden, Germany. (Poster)

Jacinto A. and Garcia-Fernandez B.

The role of Dpp during *Drosophila* dorsal closure.

ELSO 2003, Dresden, Germany. (Poster)

Jacinto A. and Simões S.

Drosophila non-classical cadherins and posterior spiracles morphogenesis.

ELSO 2003, Dresden, Germany. (Poster)

Marques I.M.

17th AGM of the EMBnet, Warsaw, Poland

Nolasco S., Malta-Vacas J. Brito M., Zabala J.C. and Soares H.

Regulation of tubulin Cofactor A expression in mammalian cells submitted to microtubule depolymerising agents.

EMBO/FEBS workshop on Frontiers in Cytoskeleton Research. Gosau, Austria

Pais T. F., Peixoto R. and Chatterjee S.

Response of microglia to neuronal cell death.

VI European Meeting on glial cell function in health and disease.

Berlin, Germany. (Poster)

Palmeirim I.

Is the molecular clock playing a role during limb development

Ist joint meeting of the British and French Societies for Developmental Biology, Nice, France. (Poster)

Parkhouse M.

Host evasion by pathogens

Univ. Oxford, UK

Rodríguez-León J.

MKP3 mediates the cellular response to FGF8 signalling in the vertebrate limb

ELSO 2003 International Conference, Dresden, Germany. (Poster)

Saúde L.

Subtraction Screen to Identify Medial Presomitic Mesoderm Genes

ELSO 2003 Meeting, Dresden, Germany (Poster)

Seixas C, Melo L.V, Brogueira P. and Soares H.

The cytosolic chaperonin CCT are involved in *Tetrahymena* cilia biogenesis: studies by AFM and immunofluorescence microscopy.

EMBO/FEBS workshop on Frontiers in Cytoskeleton Research. Gosau, Austria

Seldon MP., Pombo I., Vassilevskaia T., Berberat P., Yu J., Tsui T., Bach F. and Soares MP.

Heme Oxygenase-1 Modulates the Expression of Adhesion Molecules Associated with Endothelial Cell Activation.

Heme Oxygenase: Regulation, Functions & Clinical Applications Conference. Uppsala, Sweden. (Poster)

Seldon MP and Soares MP.

Heme Oxygenase-1 inhibits activation of the transcription factor NF- κ B in endothelial cells.

Heme Oxygenase: Regulation, Functions & Clinical Applications Conference. Uppsala, Sweden.

Silva G., Pombo I., Brouard S., Chora A. and Soares MP.

Anti-Apoptotic effect of Heme Oxygenase-1 (HO-1): Degradation of the p38-MAPK isoform by the 26S proteasome.

Heme Oxygenase: Regulation, Functions & Clinical Applications Conference. Uppsala, Sweden

Soares MP.

Heme oxygenase-1 a stress responsive gene that controls inflammatory reactions: mechanisms of action

Heme Oxygenase: Regulation, Functions & Clinical Applications Conference. Uppsala, Sweden. (Invited Lecture)

Tavares A.

Human Mob1-like proteins Mob4A and Mob4B are required for cytokinesis.

The Cell Biology of Cancer, Oxford, UK

Tavares A., Alves P. and Godinho S.

DPlkk1, a Drosophila Polo kinase kinase, is required for cytokinesis

ELSO 2003 International Conference, Dresden, Germany

Tokaji L.

Anti-atherogenic effect of inhaled carbon monoxide: Assessment of mechanism of action and possible therapeutic applications.

Annual meeting of the PGDB, Curia, Portugal. (Poster)

October

Bajanca F., Luz M., Tajbakhsh S., Buckingham M. and Thorsteinsdóttir, S.

Differential expression of integrins during myotome development in the mouse: a potential role for $\alpha 6 \beta 1$ in epaxial myotome formation.

European Research Conferences: Sixth Research Conference on Molecular Biology of Cellular Interactions: Cell Adhesion Molecules and Receptor Cross Talk. Obernai, France. (Poster)

Belo J.A.

Endogenous Cerberus activity is required for anteriorhead specification in *Xenopus*.

IV Italian-German *Xenopus* Meeting at Lovenno di Menaggio, Italy

Belo J.A.
Culturas celulares e manipulação genética dirigida.
Instituto Superior Técnico (IST), Universidade de Lisboa, Portugal

Caramalho I.
Innate and adaptive inflammation promotes regulatory T cells expansion and activation.
Portuguese Society of Immunology, XXIX Annual Meeting, Porto, Portugal.(Poster)

Cavalcante M., Vasilevskaia T., Tokaji L., Gregoire C., Fernandes B., Balla J. and Soares M.
Generation and characterization of chimeric form of heme oxygenase-1 that can enter cells “spontaneously”.
Heme Oxygenase: Regulation, Functions and Clinical Application Conference.
Uppsala, Sweden. (Poster)

Correia S., Mar Alba M., Crespo A. and Parkhouse R.M.E.
Characterisation of a putative Pim Ser-Thre kinase homologue of ASFV.
Portuguese Society of Immunology, XXIX Annual Meeting, Porto, Portugal.(Poster)

Coutinho A.
Immunology: where we are today
XXVIII Congresso da Sociedade Brasileira de Imunologia “30 anos de Imunologia no Brasil”, Mangaratiba, Brazil

Coutinho A.
Ciência: Pilar da democracia e raiz de todo o progresso sócio-económico
IV Encontro Nacional das Tecnologias da Saúde, Escola Superior de Tecnologia da Saúde de Lisboa, Lisbon, Portugal

Coutinho A.
Imunologia: Tolerância, transplantes e autoimunidade
Autoimunidade e Doença Renal. XVII Congresso da Sociedade Portuguesa de Nefrologia
Pavilhão de Congressos do Centro de Congressos de Lisboa, Lisbon, Portugal

Dias S.
VEGFR-1 and -2 cross-talk on endothelial cells.
Third Angiogenesis Meeting of the European School of Hematology, Dublin, Ireland

Dias S.
Angiogenesis Pathways in bone marrow dysfunction.
Third Angiogenesis Meeting of the European School of Hematology, Dublin, Ireland

Fontes F.
Negative effects of setroids on regulatory T cells.
Portuguese Society of Immunology, XXIX Annual Meeting, Porto, Portugal.(Poster)

Igreja C.

Evidence for Distinct Molecular/Phenotypic stages during Endothelial Progenitor Differentiation.

Third Angiogenesis Meeting of the European School of Hematology, Dublin, Ireland

Leirião L., Corso S., Rodriguez A., Giordano S. and Mota M.

Anti-apoptotic signaling by HGF/MET leads to a successful malaria infection.

11th Euroconference on apoptosis. Ghent, Belgium

Mallo M.

Reversible inactivation of Hoxa2: implications for its expression in the neural crest.

I Forum da Química, UNL, Lisbon, Portugal

Malta-Vacas J. Nolasco S., Costa P., Carmona B., Gonçalves J., Monteiro C. Soares H. and Brito M.

Aplicação do PCR em tempo real na análise de tumores. Resultados preliminares em cancro gástrico.

IV Encontro das Tecnologias da Saúde. Lisbon, Portugal

Nascimento R., Crespo A. and Parkhouse R.M.E.

vGAP a novel viral protein inducing cell cycle arrest and apoptosis.

Portuguese Society of Immunology, XXIX Annual Meeting, Porto, Portugal.(Poster)

Neves H.

Stem-cells, hematopoiesis e transcriptomas de diferenciação.

II workshop de Engenharia de Biomédica. Instituto Superior Técnico, Universidade Técnica de Lisboa, Lisbon, Portugal

Oliveira V., Mar Alba M., Crespo A. and Parkhouse R.M.E.

Inhibition of Toll-like Receptor signaling by African Swine Fever Virus.

Portuguese Society of Immunology, XXIX Annual Meeting, Porto, Portugal. (Poster)

Pais T. F. and Chatterjee S.

Characterization of brain macrophage cell populations in experimental cerebral malaria.

Portuguese Society for Immunology, XXIX Annual Meeting. Porto, Portugal

Parkhouse M.

Host-pathogen interactions

Portuguese Society of Immunology Symposium, Porto, Portugal

Peres A.

Assessing tumor-induced reinforcement of Natural tolerance to self mediated by CD4⁺CD25⁺ regulatory T cells. Portuguese Society of Immunology, XXIX Annual Meeting, Porto, Portugal.(Poster)

Rebelo M.

Age dependent variation of Notch signaling pathway in Thymocyte development.

Portuguese Society of Immunology, XXIX Annual Meeting, Porto, Portugal.(Poster)

Santos M.

Control of mouse B-cell differentiation by the notch ligands delta-1 and jagged-1.
Portuguese Society of Immunology, XXIX Annual Meeting, Porto, Portugal

Seldon M.P., Pombo I., Vassilevskaia T., Pascal O. Berberat J-Y., Tung-Yu T., Bach F.H. and Soares M.P..

Heme Oxygenase-1 Modulates the Expression of Adhesion Molecules Associated with Endothelial Cell Activation.

Heme Oxygenase: Regulation, Functions and Clinical Application Conference. Uppsala, Sweden. (Poster)

Seldon M. P. and Soares M.

Heme Oxygenase-1 inhibits activation of the transcription factor NF-kB in endothelial cells.

Heme Oxygenase: Regulation, Functions and Clinical Application Conference. Uppsala, Sweden. (Poster)

Silva G. Pombo I., Brouard S. Chora A. and Soares M.P.

Anti-Apoptotic effect of Heme Oxygenase-1 (HO-1): Degradation of the p38 α MAPK isoform by the 26S proteasome Heme Oxygenase: Regulation, Functions and Clinical Application Conference. Uppsala, Sweden. (Poster)

Soares M.

Heme oxygenase-1 a stress responsive gene that controls inflammatory reactions: Mechanisms of action.

Heme Oxygenase: Regulation, Functions and Clinical Application Conference. Uppsala, Sweden

Soares M.

Protective Resposes of Endothelial Cells in Organ Transplantation.

Luso-Brazilin Congress of Organ Transplantation. Fortaleza, Brazil

Soares M.

Heme oxygenase-1: a regulatory gene that modulates inflammatory and immune responses

30 Anos de Imunologia no Brasil - XXVIII Congress of The Brazilian Immunological Society. Margaritiba, Rio de Janeiro, Brazil

Zelenay S.

Appearance and persistence of autoantibodies after CD4⁺ CD25⁺ regulatory T cells transient depletion.

Portuguese Society of Immunology, XXIX Annual Meeting, Porto, Portugal

November

Coutinho A.

As células que inventam sequências de DNA

Conferences FCUL/IGC, "DNA 50", Faculdade de Ciências da Universidade de Lisboa, Lisbon, Portugal

Coutinho A.
Concretizar o impensável
Conferences “Cultura: travão ou dinamismo da inovação tecnológica?”, Institut
Franco-Portugais, Lisbon, Portugal

Demengeot J.
Regulatory T cells and EAE.
13th annual workshop Immune regulatory Networks, Center for the study of
inflammatory bowel disease. Boston, USA

Fernandes, P.L.
Bioinformática
in Workshop sobre Bioinformática, V Jornadas de Biologia Aplicada, Universidade
do Minho, Braga, Portugal

Fernandes, P.L.
Bioinformática
in "Do Genoma Humano às Nanotecnologias - Novas Estratégias de Intervenção
Terapêutica", IV Simpósio do Núcleo de Estudantes de Ciências Farmacêuticas do
ISCS-Sul, Monte da Caparica, Portugal

Fernandes P.L.
Bioinformatics in the Igc
In "Functional Genomics And Proteomics", 1st Annual Meeting Of Portuguese
Proteomic Network- Procura, Inst. Nacional De Saúde Dr Ricardo Jorge, Lisboa,
Portugal

Fernandes P.L.
Bioinformática, uma breve introdução para alunos do Ensino Secundário
Instituto Gulbenkian de Ciência, Oeiras, Portugal

Parkhouse M.
Rational strategies for pathogen control
Sociedade Portuguesa de Ciências Veterinárias, Lisboa , Portugal

Vicente, A.M
Evidence for the involvement of the BDNF gene in autism and relation to family
history of depression.
ASHG Annual Meeting, Los Angeles, USA

Vicente, A.M.
Epidemiology of Autism Spectrum Disorders (ASD) in Portugal.
International Autism Europe Congress, Lisbon, Portugal

December

Coutinho A.

Comentator on Lewis Wolpert's Conference "Time in the developing embryo"

Colóquio Tempo e Ciência, Auditório da Universidade de Coimbra, Coimbra, Portugal

Coutinho A.

Ora então, vamos à vida!

Auditório do Centro Cultural de Vila Flor, Vila Flor, Portugal

Coutinho A.

Uma visão global do sistema imune

Curso básico de Imunologia para clínicos 2003. Curso de Imunologia Clínica do Hospital Geral Santo António, Hotel Meridien, Porto, Portugal

Coutinho A.

Imunossuppression or imunostimulation

Imunologia Clínica 2003, Hospital Geral Santo António, Hotel Meridien, Porto, Portugal

Fernandes P.L.

Meeting of the EMBnet Publications and Public Relations Committee

Instituto Gulbenkian de Ciência, Oeiras, Portugal

Fernandes P.L.

The IGC

in "ERPANET/CODATA International Archiving Workshop on the Selection, Appraisal, and Retention of Digital Scientific Data" Biblioteca Nacional, Lisboa, Portugal

Leirião P., Mota M. And Ana Rodriguez

Hepatocyte Apoptosis during Malaria Infection and Phagocytosis by dendritic cells.

43rd American Society for Cell Biology Annual Meeting, San Francisco, USA (Poster)

Marinho H.S., Martins L., Fernandes L., Cyrne L.

Oxidative stress and regulation of oxidant enzymes gene expression in *S. cerevisiae*.

6th Portuguese Congress on Free Radicals in Chemistry, Biology and Medicine. Coimbra, Portugal

Parkhouse M.

Estrategias racionales para controlar a los patógenos.

Centro Nacional de Estudios Avanzados, Mexico

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