



FUNDAÇÃO CALOUSTE GULBENKIAN



INSTITUTO GULBENKIAN DE CIÊNCIA

ANNUAL REPORT 2005

The complete version of this Report can be consulted at the
IGC website: <http://www.igc.gulbenkian.pt>

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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 2005 were:

President

Emílio Rui Vilar

Honorary President

Mikhael Essayan (retired July 2005)

Executive Trustees

Diogo de Lucena

Isabel Mota

Eduardo Marçal Grilo

Teresa Gouveia

Martin Essayan (from July 2005)

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 2005 were:

Board of Directors

Diogo de Lucena (Chairman)

João Caraça

Manuel Rodrigues Gomes

Manuel Carmelo Rosa

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 2005 were:

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

The Scientific Advisory Board met at the IGC on 22-24 May 2005.

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.

Filipa Alves (FCT)

Jean-Baptiste André (EU)

Carla Afonso (FCT)

Nuno Afonso (FCT)

Dulce Azevedo (FCT)

Jörg Becker (FCT)

José António Belo (UALG)

Marie-Louise Bergman (EU)

Natalia Beniers (EU)

Ana Cristina Borges (FCT)

Ana Sofia Cachaço (FCT)

Iris Caramalho (FCT)

Jorge Carneiro (Lab. Associado)

Margarida Carneiro (IGC)

Cristina Casalou (FCT)

Pierre-André Cazenave (Univ. Paris VI/Institut Pasteur/CNRS/FCT)

Ana Catarina Certal (FCT)
Youri Chanseaud (FCT)
Sukalyan Chatterjee (FCG)
Melvin Cohn (Salk Institute/FCT)
Rui Costa (Duke University/IGC)
Ana Paula Coutinho (FCT) – left May 2005
António Coutinho (CNRS/FCG)
Pedro Coutinho (FCT) – left May 2005
Jocelyne Demengeot (FCG)
Sérgio Dias (IPO/FCT)
Francisco Dionísio (FCT)
António Duarte (FMVUTL)
Paula Duque (FCT)
Rosa Elias (USP/CAPES)
Sabrina Epiphany (FCT)
José Faro (Univ. Salamanca)
José Feijó (FCUL)
Lisete Fernandes (ESTSL)
Ana Margarida Ferreira (FCT)
Carlos Alberto Ferreira (HUSM)
Constantin Fesel (FCT)
Cláudia Florindo (Univ. Heidelberg)
Victoria Gallego (Univ. Extremadura)
Rui Gardner (FCT)
Simone Gines (FCT) – left May 2005
Andreia Gomes (FCT)
Gabriela Gomes (FCT)
Carlos Penha Gonçalves (Lab. Associado)
Guilherme Gonçalves (Ministério da Saúde)
Alexis Gonzalez (FCG)
Isabel Gordo (FCT)
Luís Graça (FMUL)
Christophe Gregoire (FCT)
Isabel Pombo Gregoire (FCT)
Sérgio Gulbenkian (FCG)
Matthias Haury (FCG)
Domingos Henrique (FMUL)
Frank Hilker (EU)
António Jacinto (FCT)
Joaquin Rodriguez León (FCT)
Susana Santos Lopes (FCT)
Moises Mallo (Lab. Associado)
Cláudio Marinho (USP/CAPES/GRICES)
Moises Marinho (EU) – left June 2005
Gabriel Martins (FCUL/FCT)
Madalena Martins (FCT)

Erwan Michard (FCT)
Marta Moita (FCT)
Kalet Leon Monzon (Cent. Immunol. Mol. Cuba/FCT)
Maria Mota (FCT)
Sofia Oliveira (EU)
Maria Teresa Faria Pais (FCT)
Ana Maria Pamplona (FCT)
Michael Parkhouse (FCG)
Paula Parra (FCT)
Leonor Parreira (FMUL/IMM)
Ana Cristina Paulo (FCT)
Sylviane Pied (INSERM/Inst. Pasteur)
Ricardo Pimenta-Araújo (FMUL)
Miguel Prudêncio (EU)
Alessandro Ramos (FCT)
Luís Rocha (Indiana Univ.)
Gabriela Rodrigues (FCUL)
Marie-France Sagot (Univ. Claude Bernard)
Ana Paula Santos (FCT) – left May 2005
Leonor Sarmento (FCT)
Ana Catarina Moreira dos Santos Sarzedas (FCT/EMBO)
Leonor Tavares Saúde (FCT)
Elsa Seixas (FCT)
Gabriela Silva (FCT)
João Pedro Simas (FMUL)
Helena Soares (ESTSL)
Miguel Che Parreira Soares (Lab. Associado)
João Tiago Sousa (FCG)
Élio Sucena (FCT)
Álvaro Augusto Tavares (ISTUL)
Ana Teresa Tavares (FCT)
Vera Lucas Teixeira (FCT)
Henrique Teotónio (FCT)
Solveig Thorsteinsdottir (FCUL)
Maria de Jesus Trovoada (FCT)
Johann Truccolo (FCT)
Filipa Vala (FCT)
Tatiana Vassilevskaia (Emma)
Astrid Vicente (INSA-RJ)
Sheila Vidal (IGC)
Luisa Mota Vieira (HDES)
Ana Margarida Vigário (FCT)
Andrew Waters (Univ. Leiden)
Lisa White (Univ. Warwick/EU)
William Wood (FCT)

STUDENTS

Ph.D. Students

Ricardo Águas (ITQB/UNL/FCT)
Sónia Albuquerque (ICBAS/FCT)
Isabel Alcobia (FMUL/FCT) – completed PhD October 2005
Sílvia Almeida (ITQB/UNL/FCT)
Paulo Alves (FMUL/FCT)
Fernanda Bajanca (FCUL/FCT)
Marta Barreto (FCUL/FCT) – completed PhD September 2005
Rui Benedito (FMVUTL/FCT)
Leonor Boavida (FCUL/FCT) – completed PhD November 2005
Dinis Calado (FMUL/FCT)
Marta Campos (FMUL/FCT)
Marta Carapuço (ITQBUNL/FCT)
Ângelo António Chora (FMUL/FCT)
Jaime Combadão (ITQB/UNL/FCT)
Sofia Cordeiro (FCUL/FCT)
Catarina Correia (FCTUL/FCT)
Sílvia Correia (ITQB/UNL/FCT)
Vasco Correia (ICBAS/FCT)
Sofia Côrte-Real (FMUL/FCT)
Sílvia Costa (FCUL/FCT)
Margarida Courinha (ITQB/UNL/IPO)
Ana Margarida Coutinho (FCUL/FCT)
Andreia Cunha (ITQB/UNL/FCT)
Margarida Cunha (ICBAS/FCT)
Lígia Deus (ITQB/UNL/FCT)
Célia Domingues (FCUL/FCT)
João Duarte (FCTUC/FCT)
Ana Paula Elias (FCUL/FCT)
Mariana Faria (FCUL/FCT)
Elisabete Fernandes (FCUL/FCT)
Beatriz Fernandez (ITQB/UNL/FCT)
Manuela Ferreira (FCUL/FCT) – left August 2005
Catarina Figueiredo (ITQB/UNL/FCT)
Mário Rui Filipe (FCT/UNL/FCT)
Cláudia Florindo (FCT/UNL/FCT) – left April 2005
Francisca Fontes (FMUL/Hospital Egas Moniz/ Ministério da Saúde)
Rita Frago (FCUL/FCT)
Ana Rita França (FMUL/FCT)
Susana Godinho (IST/FCT)
Dinis Gokaydin (EU)
João Gonçalves (FCUL/FCT)
Mário Grãos (FCUL/FCT)

Cátia Igreja (FCUL/FCT)
Tiago Krug (FMUL/FCT)
Pedro Lares (FCUL/FCT)
Patrícia Leirião (IHMTUNL/FCT)
Loic Lhopitalier (ITQB/UNL/EU)
Ana Sofia Lopes (FCUL/FCT)
Sofia Marques (FMUL/FCT)
Maria Hortense Matos (ITQB/UNL/FCT)
Ana Lúcia Mena (ITQB/UNL/FCT)
Joana Monteiro (FCUL/FCT)
Filipa Moraes (ITQB/UNL/FCT)
Joana Moreira (FCUL/FCT)
Nuno Moreno (IGC/FCUL)
Rute Nascimento (FMUL/FCT)
Rita Neres (FFUL/FCT)
Hélia Neves (FMUL/CEBIP/FCT)
Sofia Nolasco (FCUL/FCT)
Vivian Oliveira (ITQB/UNL/FCT)
Tiago Paixão (ICBAS/FCT)
Nadja Pejanovic (IGC)
Lília Perfeito (ITQB/UNL/FCT)
Ana Margarida Prado (FCUL/FCT)
Ana Sofia Quina (FMUL/FCT) – left June 2005
Manuel Rebelo (FCUL/FCT) – completed PhD October 2005
Ana Luisa Reis (FMVUTL/FCT)
Cristina Rodrigues (FCUL/FCT)
Lénia Rodrigues (FMUL/FCT)
Paula Rodrigues (UNL)
Sofia de Albuquerque Rodrigues (ECSUM/FCT)
Maria do Rosário Sambo (FMUL/Hospital Pediátrico Luanda, Angola)
Margarida Santos (FMUL/FCT)
Ana Cecília Seixas (FCUL/FCT)
Mark Seldon (ICBAS/FCT)
Nuno Sepúlveda (IST/IGC)
Ana Cristina Silva (FCUL/FCT)
Susana Silva (FCUL/FCT) – left August 2005
Sérgio Simões (FCUL/FCT)
Laszlo Tokaji (ITQB/UNL/FCT)
Alexandre Trindade (FMVUTL/FCT)
Sander VanNoort (ITQB/UNL/EU)
Ana Sofia Veloso (ITQB/UNL/FCT)
Sónia Ventura (FMVUTL/FCT)
Ana Maria Vieira (FCUL)
Santiago Zelenay (FCUL/FCT)

M.Sc. Students

Ana Rita Alves (FCUL)
Rogério Candeias (FCUL)
Sofia Couceiro (FCUL)
Bruno Mateus (IST)
Catarina Moita (FCUL)
Ana Raquel Tomás (FCT, Univ. Coimbra)

B.Sc. Students

Cláudia Almeida (FCUL) – left October 2005
Teresa Barrio (FCUL)
Isabel Belo (Univ. Évora)
Pedro Campinho (FCUL)
João Carreira (FCUL)
Miguel Coelho (FCUL)
Renato Colaço (FCUL)
Ana Catarina Correia (Univ. Lusófona)
Ana Mónica Correia (FCUL) – left October 2005
Teresa Cruto (FCUL)
António Currais (FCUL)
Pedro Dias (FCUL)
Valter Duarte (Univ. Lusófona) – left October 2005
Joana Duarte (FCUL)
Célia Faria (Univ. Lusófona)
Célia Ferreira (Univ. Évora)
Nicolau Ferreira (FCUL)
Josina Filipe (FCUL) – left July 2005
Ana Gabriela Gomes (IST)
Marta Guimarães (Univ. Évora)
Ricardo Henriques (FCUL)
Maria João Lagareiro (Univ. Évora)
Carla Lopes (FFUL)
Lara Lourenço (FCUL)
Rui Martins (FCUL)
Raquel Mendes (Univ. Lusófona)
Carla Milagre (Univ. Lusófona)
Ricardo Sousa e Paiva (FCUL)
Raquel Penedos (IST)
Maria Inês Pereira (FMUL)
Ruben Ramalho (FCUL)
Daniel Reis (Univ. Évora)
Ana Catarina Ribeiro (FCUL)
Alexandre Rodrigues (FCUL)
Fredrico Rodrigues (FCUL)
Inês Rolim (FFUL)
Ana Bárbara Santos (FCUL)

Margarida Santos (FCUL)
Maria Emília Santos (FCUL)
Pedro Santos (FCUL)
Inês Sousa (FCUL)
Sandra Isabel Trindade (Univ. Évora)
Duarte Viana (Univ. Évora)
Tânia Vinagre (Univ. Lusófona)

Laboratory Technical Support

Ana Água-Doce (BTI/FCT)
Tânia Aires (BI/FCT)
Paulo Almeida (Lab. Associado)
Sofia Andrade (BI/FCT)
Raquel Antunes (BIC/FCT)
Carlos Araújo (IEFP)
Gilberto Bento (BI/FCT) – left September 2005
Margaret Bento (BI/FCT)
Paulo Bettencourt (BTI/FCG)
Cláudia Bicho (BI/FCT)
Joana Bom (BTI/FCT)
Dolores Bonaparte (BTI/FCG)
Rita Caldeira (Short-term visitor)
Sílvia Cardoso (IEFP)
Lara Carvalho (BTI/FCG)
Sara Carvalho (IEFP)
Ricardo Casimiro (Short-term apprentice)
João Coelho (IEFP)
Maria do Céu Conceição (BTI/FCG)
Joana Côrte-Real (IEFP)
Marta Costa (IEFP)
Tânia Cruz (IEFP)
Judite Dias (IEFP)
Teresa Dias (IEFP)
Cláudia Duarte (IGC/UE)
Joana Duarte (BI/FCT)
Lurdes Duarte (IEFP)
Francisco Esteves (IEFP)
Carla Fernandes (BTI/FCG)
Daniel André Ferreira (BTI/FCT)
Daniel Gil Ferreira (IEFP)
Inês Ferreira (BIC/FCT)
Sara Fidalgo (BI/EU) – left December 2005
Joana Figueiredo (IEFP)
Benedita Fonseca (BI/EU)
Lídia Fonseca (BTI/EU)
Ana Cristina Gaspar (BTI/FCG)

Gabriela Gomes (BIC/FCT)
Alexandre Gonçalves (BIC/FCT) – left May 2005
Lisa Gonçalves (BI/FCT)
Raquel Gonçalves (IEFP)
Raquel Lourenço (IEFP)
Diogo Manoel (BI/FCT)
Sara Marques (BI/FCT)
Inês Matos (IEFP)
Bruno Moreira (IEFP)
Joana Moreira (BTI/FCT)
Ester Morgado (IEFP) – left October 2005
Catarina Mota (BIC/FCT)
Ana Nóvoa (Lab. Associado)
Ana Oliveira (IEFP)
Isabel Peixeiro (BIC/FCT)
Sofia Rebelo (Astrazeneca)
Yara Reis (Short-term apprentice)
Pedro Rifes (BI/EU)
Joana Rodo (BI/FCT)
Ana Patrícia Rodrigues (IEFP)
Rui Rodrigues (Short-term apprentice)
Ana Salgueiro (BIC/FCT)
Ana Catarina Silva (BIC/FCT)
Gonçalo Silva (BI/FCT)
Mariana Simões (IEFP)
Patrícia Simões (IEFP)
Maria Helena Soares (IEFP)
Filipe Sousa (IEFP)
Flávio Sousa (BI/FCG)
Natacha Sousa (BTI/FCG)
Sara Sousa (IEFP)
Ana Teles (IEFP)
Tiago Vicente (BIC/FCT)
Iris Vilarés (Short-term apprentice)

Others

Pedro Asseisseiro
Rita Caldeira
João Garcia
João Lourenço
Paula Macedo

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

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
Vítor Santos
Abílio Simões
Teresa Maria Sousa
Lurdes Torres - retired August 2005

Laboratory Technical Staff

Ana Cristina Leitão Homem
Júlia Lobato
Isabel Marques
Nuno Moreno
Rosa Maria Santos

Technical Support Staff

António C. Ligeiro
João Carlos Lopes
Severino Matias
Carlos Nunes
António Sousa
Vítor 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.

INTRODUCTION

While unanimously recognized as the epic poet in Portuguese literature, Luis de Camões wrote many a love poem that most of us, locals, have learned by heart when coming of age. In a beautiful sonnet, Camões explains why Jacob submitted to serve Rachel's father for a whole 7 years: he wanted Rachel as a reward and, for that highest goal, was ready to serve for yet another 7 years. Interestingly, Camões gives no hint as to Jacob's performance, as if his full dedication sufficed to qualify him as deserving. Writing the 7th Annual report of the Instituto Gulbenkian de Ciência, I feel I must invoke the same benevolence in judgment, for full dedication there was, in attempting to establish material and intellectual conditions for the practice of good Science, to strengthen biomedical research and education in Portuguese institutions, to bring scientific values to the daily concerns of citizens and politicians. These were the missions that the Board of Administration of the Fundação Calouste Gulbenkian chose for this period of IGC's life, and, looking back, I must say that the Institute has walked some ground in these 7 years. And I must add that, in my metaphor of Camões sonnet, Jacob was not one but many, all of us shepherds and apprentices dreaming of our Rachel, ready to offer the best we have, in generosity and commitment, let alone our thoughts and minds.

After these 7 years, we all feel amply rewarded and are immodest enough to claim our part of responsibility in the unambiguous signs that Science has made it in this country to the interest of the public, to the headlines in the media, even to the current political discourse. If anything we did, we must also acknowledge the efforts of many others, at the Gulbenkian Foundation and elsewhere, who have given their best to call the attention of everyone to the values of scientific endeavor, and to claim the unique contribution of rationality and modern science to citizenship. I recall the excitement of meeting a few dozen people in the seminar room, years ago, when we first started with "science and society" sessions, only to rejoice with the nearly thousand visitors in the 2005 IGC's Open Day, and to thank all here who have invested in the necessary preparations. Science "sells" well these days in Portugal, and the contribution of the Gulbenkian Foundation towards this shift in societal concerns is undeniable. It is very good news that the recent "Einstein Exhibition" at the Foundation had over 60,000 visitors. I am well aware that this tremendous success was the deserved reward for the competence and devotion of those responsible for the exhibition, and the end-result of the initiatives of the Science Sector of the Foundation, most notably, the "Despertar para a Ciência" series who attracted hundreds every week for several years. But I would like to think that the thousands of secondary school students and the hundreds of teachers who have visited the IGC over the last 7 years were part of those 60,000. Not all the signs are positive, however, and the battle for deeper roots of science in society is just started: while science is today in the local political agenda, obscurantist practices still have the upper hand in various sectors of society, many new sects continue to proliferate in Portugal, and the major media continue to harbor astrology sections and advertisements for future-tellers and various types of "alternative medicine". Ironically, the statue of a major figure of Portuguese Medicine from the early XXth Century, proudly rising just in

front of the Lisbon Faculty for Medical Sciences, has now become a site for supernatural veneration of devotees, crowded with candles, flowers and marble dedication plates. We are indeed in a desperate need for better education, all the way from the primary schools, when the values of curiosity, diversity and tolerance have better conditions to take roots. Again, our duty is to promote the values of rationality in the quest for natural laws that is the essence of scientific enquire, even if we often have to concede to the interest-driven public curiosity in the results of what we do today and might be “useful” tomorrow. A significant contribution to this higher profile of science in the country has been the initiative of a number of young scientists and students, many of whom have passed or are at the IGC, who founded a new association for the advancement of science (“Associação Viver a Ciência”). I must acknowledge here, as before, their example of taking the future in their own hands, which will certainly encourage others to do the same.

Together with science, arts and charities, education is one of the statutory goals of the Gulbenkian Foundation, and we are all well aware that education is the necessary beginning of all forms of cultural transmission and of social progress at large. Accordingly, from the foundation of the Institute in the 1960’s, graduate education at the IGC represents perhaps its greatest contribution. In this respect, the “new IGC” is actually older than 7 years, as the first PhD program was launched in 1993. This program (Gulbenkian PhD Program in Biology and Medicine - PGDBM) has interviewed over a thousand applicants, engaged and promoted the best possible education of just over 100 PhD holders, as it was assumed in the original compromises. Many of these have proven their competence and dedication in laboratories in Europe and in the US, have consolidated the reputation of the Gulbenkian Foundation in science over the world, and more than 60 have now returned to Portugal. In spite of their youth and “out bred” characteristics in the local University system, their quality and specific scientific interests have prompted several Universities and other public institutions to recruit them, letting us hope for a significant contribution to the necessary reform of the national science and technology system in these areas. Most importantly, the PGDBM has served as a model to several similar programs in other Portuguese institutions, and was a critical factor in the decision of Foundation and Government to launch a new program (Gulbenkian PhD Program in Biomedicine - PGDB) that admitted the first students in 2000. Over the last 5 years, the PGDB recruited to science and sent abroad close to another 90 PhD students, and in the tradition of cooperation and openness, has initiated, together with two other national programs, annual retreats that now congregate all such students, promoting productive interactions and enlarging the basis for mobility, cooperation, and merit-based competition.

Yet, after 12 years of running successful programs of graduate education that were now replicated in other institutions, it was time to reflect over new needs and/or strategies, perhaps to initiate other activities that had remained unattended. Thus, along those years, prevalent conditions evolved, both at the IGC and elsewhere in the country, including the governmental policies for distribution of fellowships, as well as the marked increase in the number of laboratories of general biology with excellent conditions to accept our own students. While remaining committed to programs that promote international exchange, it would appear appropriate to try and equilibrate the flow of PhD

students, thus far almost exclusively based on sending our students abroad with national support. We have thus prepared, together with several prestigious European institutions that share scientific interests with the IGC, to launch, already in 2006, an international PhD program for equilibrated student exchange. Furthermore, we have structured and improved the IGC's "internal" PhD program (PDIGC), in order to make it attractive to foreigners as well. Finally, in 2005, the IGC has launched a new PhD Program in Computational Biology (PDBC), again in cooperation with the National Research Council (Fundação para a Ciência e a Tecnologia - FCT), but now also with the help of Fundação PT (Portugal Telecom) and FCCN (Fundação para o Cálculo Científico Nacional) and the decisive support of the Portuguese branch of Siemens. Siemens Portugal leads innovative investments in technology in this country, and holds great competence and broad interests in this area. This program also innovates by establishing the conditions to better anchor to Portuguese institutions both students and teachers. Thus, several University Departments are formally associated to the program, and the presence in Portugal of the visiting Faculty for longer periods was ensured by the establishment of the FLAD (Fundação Luso-Americana para o Desenvolvimento) Co-laboratorium in Computational Biology at the IGC. The first 12 PhD students in various fields of bioinformatics, mathematical and theoretical biology have been recruited and started working last autumn. Last but not least, the reorganization of working spaces following their renewal, together with the admission of new groups in this area, have also resulted in strengthening the ties with our neighbor institute, the ITQB (Instituto de Tecnologia Química e Biológica) of the Universidade Nova de Lisboa, which together with the IBET (Instituto de Biologia Experimental e Tecnológica), are IGC partners in the Oeiras Associated Laboratory at the Research Council. Thus, the research groups of both institutions now share facilities and have access to constant interaction.

Counting those engaged in graduate programs, the IGC has been responsible for the recruitment and, at least in part, for the education of **336** PhD students, over these years: 101 in the PGDBM, 87 in the PGDB, 85 in the PDIGC, 12 in the PDBC, together with another 51 who were individually accepted for thesis work in the laboratories at the Institute. If we add to these, the undergraduate students in the final year of their University degrees (111), a handful of Master students (8) and those engaged in the IGC's Bioinformatics educational programs (43), **498** youngsters had their scientific initiation at the Institute, hopefully in such a way that they will retain our collective "spirit", the intellectual freedom and scientific curiosity, but also the generosity and responsibility in the common adventure that are marks of the IGC. A strong indication that this is the case, was given by the First Gulbenkian Alumni Meeting (GAMeet), held by the initiative of PGDBM alumni in December 2005. They brought us good science, together with their career concerns and daily difficulties, but also the joy of being reunited, giving proof of their "esprit de corps" and commitment to the common project. They showed such a tremendous strength as a group that we can only trust a great promise for their future. Other than running graduate courses and programs sending students to other institutions around the world, the Institute has also started to contribute as a place for full graduate education, notably by hosting students who prepare their theses working in our laboratories, under the supervision of IGC's scientists. Thus far, as many as **111** University dissertations were prepared, at least in part, at the IGC, **41** of

which were PhD Theses successfully defended at various Universities in Portugal and elsewhere in Europe.

Clearly, however, initiatives on graduate education will have a higher impact in the Portuguese scientific community if they can be conducted under shared responsibilities with other local institutions, Universities in particular. To this end, efforts continued to be deployed attempting to establish a Graduate School of Biomedical Sciences in Lisbon, together with several other local institutions and the Universities. The enthusiastic support of all those involved would indicate its viability and justify our hopes that it will soon come true. This is all the more important as it could provide the necessary conditions to initiate PhD programs for medical doctors. Clinical research is perhaps the single area where investments in education are most rewarding, and certainly one of the least developed in this country. On the one hand, the recent shift of medicine from an empirical to a scientific basis imposes that medical personnel are educated in science and basic biology, in much broader and deeper ways than in the past. This can only improve the quality of medical care, accelerate the transfer of the most recent advances to daily clinical practice, and, more often than not, will result in considerable savings for the health system. Ignorance has never been the best way to save money. On the other hand, bringing medical doctors to scientific research will certainly contribute to reinforce organism-centered concerns and approaches, of which modern biology is in great need, given its concentration in component analyses along the last few decades.

The Institute's policy of openness and pro-active cooperation is also clear by the considerable number of formal cooperation agreements we have established with other institutions, dedicated to research and education and/or public health, in Portugal and abroad. Thus far, cooperation protocols have been established with the following Portuguese institutions: Faculdade de Medicina e Faculdade de Ciências da Universidade de Lisboa, Instituto Superior Técnico e Faculdade de Medicina Veterinária da Universidade Técnica de Lisboa, Instituto de Tecnologia Química e Biológica (ITQB) da Universidade Nova de Lisboa, Universidades do Algarve, Instituto de Biologia Experimental e Tecnológica (IBET), Instituto Nacional de Saúde Ricardo Jorge do Ministério da Saúde, Hospital do Divino Espírito Santo, and we have ongoing projects with the Fundação Luso-Americana para o Desenvolvimento. We are particularly pleased with the agreement that was established between the Spanish Research Council (Consejo Superior de Investigaciones Científicas) and the Gulbenkian Foundation, aimed at the transnational support of research projects. Not surprisingly, the most intimate cooperation is established with the other two institutions in the Oeiras campus with which we form the Associated Laboratory (ITQB and IBET). Thanks to the dedication of all scientists, students and technicians, as well as to the vision, competence and endurance of the Directors (Profs. Manuel Nunes da Ponte and Peter Lindley), the Associated Laboratory has now completed its first 5 years of activity, and we were happy to hear from the Minister of Science, Technology and Higher Education that our contract will be renewed for another 5 years period, letting us hope that, the recruitment policies being maintained, they will allow for the establishment of new independent groups under the leadership of young investigators. The relatively large investment in heavy equipment that was granted in 2005 by the FCT to the Associated Laboratory, demonstrates if necessary it was, the

competitive advantage of common scientific policies based on a strategic vision of technological development and on the sharing of facilities and competences. Another recent example is a particularly good token of inter-institutional cooperation within the Associated Laboratory: a young scientist who had gained a position at the ITQB was installed at the IGC, being convinced, just as both directors, that her activity would better “cross-fertilize” and provide for “institutional bridges” if conducted here, for a few years at least.

The process of bringing the IGC to an active and internationally respected institution has proceeded stepwise, and it is far from completed. A couple of laboratories in the basement were started in 1998 with a handful of courageous colleagues who had believed in this project and offered their generosity to bring it forth. Year after year, new laboratories were renewed, equipped, and occupied by newcomers, progressively increasing the IGC’s population to the current **389**¹. Along the way, the turnover of technicians, students, post-docs and group leaders was initiated, such that a total of **737** persons have worked at the Institute since 1998, all profiting from the Foundation’s investments in science. Most importantly for our mission, a considerable number of group leaders were installed at the IGC (**46**), most of which were coming from abroad to enrich the Portuguese scientific community. Perhaps the major concern in the original strategy for institutional reform, which was imposed by the impossibility of recruiting scientists for stable positions, was the doubtful capacity of the IGC to attract excellent scientists to set up their groups in a corner of Europe with little scientific tradition and shaky public or private support, for some years only, in addition. After 7 years, it looks as though we have grown out of these fears, as the Institute did attract and install a significant number of group leaders, most of which did very well indeed. Many were young scientists who had then the first opportunity to show their abilities to operate as “micro-entrepreneurs” in science by forming and supporting their own groups, while conducting fully independent research. Those who have been successful in proving their excellence were recruited to stable positions, the overwhelming majority in other institutions. The IGC has exported, thus far, a total of **15** group leaders to Universities and other public institutions, while **3** others have already been recruited and shall move out along the next year or so. Most of these remained in Portugal, some having returned abroad for lack of opportunities or appropriate conditions. These last figures also answer our second most important concern, namely, that once installed at the Institute, and even if marked by excellent performances, such young scientists would perhaps have to stay on for longer periods of time, “immobilized” here for lack of other alternatives in the country. This would have jeopardized the strategy of high turn over rates for the research groups, entirely devoted to give opportunities to as many as possible, and to offer a large number of “certified” group leaders to other institutions in the country. The experience indicates, however, that the national scientific system already represents a relatively active and open market for scientists, as also demonstrated by the considerable number of

¹ Not all these are at the IGC on a permanent basis. These numbers include the members of “external groups”, which are associated to the Institute and, for all scientific purposes, are an integral part of the IGC’s community. As they regularly participate in the Institute’s graduate programs, seminars and workshops, use facilities and services in the same conditions as those who are IGC-based, they are equivalent targets for the Foundations’ investments and contribute as well for the Institute’s life.

alumni from our PhD programs who are now working in other Portuguese institutions. Once again, the Foundation's decision of concentrating investments in its own Institute that enacts a strategy for "re-distributing" those investments over the scientific community, seems to pay off in a relatively short time span.

We were particularly pleased by the establishment, in 2005, of a protocol between APIFARMA (the Portuguese association of pharmaceutical companies) and the Gulbenkian Foundation, in which these organizations agree to share additional support for the establishment of the laboratories of group leaders who leave the IGC to other institutions in the country. This agreement, which also contemplates specific funding for "networking" amongst all those who have been at the Institute, supports the Foundation's policy of serving, through the IGC, other institutions in Portugal, along the ultimate aim of an "IGC without walls".

While the identification, education and incubation of new "certified" leaders to other institutions was the Institute's principal mission, we have always aimed at doing, on the way so to speak, the best science we could. After all, the first objective of a scientific institution is to produce science, and the scientific performance at the IGC in the conditions that were offered was, from the start, an issue of great concern. As all the institute's scientific output was left in the hands of young group leaders, it could well be that, however good their performance had been in excellent laboratories around the world, they would be far too busy with establishing their groups, recruiting collaborators and learning personnel management, with getting acquainted with administration, writing grants and gaining research support, with all it means to be fully autonomous in relative isolation of the big centers, such that they would not produce serious, competitive science. Again, this aspect of the Institute's activity has developed reasonably well, mostly in the last few years. Along this time, scientists at the IGC have published a total of **341** scientific papers, which together with the **111** theses and other articles under publication, amount to nearly **500** publications. While some of the scientific articles were invited contributions, **309** of those have appeared in refereed periodical journals, a whole **54** of which were published in the very best journals². Aware of the fact that it is still too common a practice to evaluate scientists by the sheer number of publications, we shall nevertheless continue to prefer quality, and encourage our scientists to avoid publishing useless papers. In addition, our students and investigators have contributed **938** communications in congresses, seminars and lectures throughout the world. While the scientific production at the IGC has much to improve, those figures are quite promising, particularly as this production essentially corresponds to the last couple of years, and as our young scientists are actually busy learning so many other things. As I look through the records, it is also very rewarding to realize that many of the Institute's contributions resulted from productive collaborations with colleagues and groups in other Portuguese and foreign institutions. As the Chairman of our Scientific Advisory Board often says, however, scientific performance should not be evaluated by published papers but by discoveries. While I am in a poor position to judge the relevance of IGC's discoveries, a handful of the Institute's contributions are significant enough to contribute already to

² The 100 journals with the highest "impact factor", ranked among nearly 6,000 "indexed" journals by the Institute for Scientific Information.

shape the respective fields, as it may be suspected by the recognition they received in the international community.

A major objective of the IGC's operation and investment has been to provide its students and scientists with the appropriate exposure to the best science around the world, while promoting the interest of every one in all ongoing projects at the Institute. Along these 7 years, the IGC has organized a total of **75** courses, workshops and meetings of various formats, nearly always open to students and colleagues in other institutions, often with an international faculty and/or attendance, whenever possible with the support of international organizations, such as EMBO. Surprisingly, the records show that a total of **2,439** speakers gave seminars and lectures at the IGC, in the context of the various graduate programs, of those workshops and meetings, or else, in our regular seminar series. As nearly all of these lectures and seminars were open to the participation of students and scientists from other institutions, which continue to be encouraged, we feel that this particular investment of the Foundation has had a significant impact in the internationalization, cross-fertilization and "maturation" of the Portuguese scientific community, much beyond the IGC. Our invited speakers were a continuous source of information and inspiration, such that nobody here should be able to blame failure on relative isolation from the main centers. Every time, we have tried to do better than the young editors of high-impact journals, attempting to distinguish, amongst potential lecturers, those who serve science from those who use it for rapid fame and career advancement. This is relevant since, although not specifically intended to foster collaborations, visits often do result in exchange, and we must protect young scientists and students from opportunistic figures. Likewise, we also profit of the student's annual retreats to expose them to exemplary figures, many of who we have elected as role models, thus attempting to demonstrate to them that the appropriate attitudes – e.g., openness, generosity and cooperation spirit, more often than not, are associated with a meaningful career.

In addition to the continuous support of the Gulbenkian Foundation, all that was done counted on moneys from various granting agencies. This rule offers to the Foundation one additional fail-safe mechanism to ascertain the quality and interest of the research conducted at the Institute. In those 7 years, **179** research contracts have been signed by the IGC with external agencies, mostly the national FCT, but also European and American, public or private, institutions or corporations. In addition, beyond integrating the Associated Laboratory from which we have obtained positions for 6 scientists and 2 technicians, the IGC has received two "program grants" from the FCT, supporting "R&D centers" in Infection & Immunity and Developmental Biology. Furthermore, the FCT and the EU/Marie Curie Program have attributed quite a large number of individual research fellowships to beginners, technicians, students and post-docs, as well as to a hand full of visiting scientists working at the IGC. For several reasons, most particularly its value as an example to local authorities throughout the country, I must underline the initiative of the Oeiras Municipality to establish a "Oeiras Chair of Scientific Excellence" that was first awarded to the IGC. Currently, the Gulbenkian Foundation contributes roughly half of the IGC's global budget: while the external funds are preferentially directed at running specific projects and at personnel

costs, the Foundation's support is spent, other than in salaries and fellowships, on laboratory equipments, operation of common platforms and services, courses, workshops and meetings, as well as in the installation and start-up of new groups. With few exceptions, the use of common facilities at the IGC continue to be free of charge to internal and "external" groups, and we would hope to be able to keep this policy for longer, even if adjusted to the continuous development of novel technologies and the progressive diversification of the Institute's activities. In 2005, we were granted a relatively large investment from the FCT, in the context of an exceptional National Program for heavy equipments. This will now allow us to install a state-of-the-art facility for genotyping and gene expression, to update our imaging and cell sorter equipment, to improve bioinformatics services and to re-structure the animal facility and the gene-targeting service. In this respect, the vision and additional support from the Gulbenkian Foundation and from the IBET's Board of Administration were most appreciated.

2005 marked the beginning of implementation of the 5-years development plan for the IGC that was approved by the Foundation's Board of Administration, with the support from the Scientific Advisory Board. This was a very important moment for the Institute, as it demonstrated the Foundation's commitment to our activities, and provided for a progressive increase in financial support that should allow for some strategic planning in time. This plan aims at completing the "growth phase" of the Institute, by re-enforcing theoretical/mathematical biology and opening a novel research program in neurosciences, bringing the steady-state operation to somewhat higher levels than the current activity. The plan also included completing the renewal of all laboratory spaces at the IGC. Accordingly, the renewal of the computational biology facilities was carried out last year, allowing for the start new PhD program. Furthermore, the first group in neurosciences was installed and equipped, though on a provisional basis, as the renewal of the wing where all such groups will be accommodated is only to start early in 2006.

The 5-years plan for the IGC represented the natural development of previous scientific policies, aimed at finding competitive advantage on transdisciplinarity, and thus based on the thematic diversity of small groups within an institution of a size and an operation model that foster productive cooperation. In this context, it was felt that the broad scientific diversity of IGC's research and the "transversality" of its operation could support, and simultaneously profit from re-enforcing mathematical-based "systems biology", as well as from neurosciences, provided research in this area would concentrate in "organism-centered" approaches. Thus, the IGC's "scientific policy" is not limited to serve all who want to work here, even if these are always selected by their individual qualities. New groups are also chosen by their research plans and how well they fit into the current institutional structure, activities, and overall interests. We know too well how tough competition is these days, and how little competitive the IGC's conditions may be for small groups, removed from the main centers, obliged to cope with limited and erratic support from a scientific environment that is far from fertile. It is our conviction that a relatively small institution, where every one knows and closely follows what all others are doing, can offer some advantage if it contains a wealth of diverse research topics. Thus, the frontiers between different "fields" are often left unattended, the major groups being occupied by the fierce competition for novelties in each one field. Comforting this

strategy, some of the most creative ideas, even a few of the most significant contributions at the IGC were indeed the result of collaborations between seemingly distinct areas of research. This was also a reason to insist on the strategic choice of fostering “organism-centered” approaches to current biological questions, for the organism is the common ground for all biologists and medical doctors alike. While all groups are free to engage in whatever they think best, in selecting group leaders the Institute has systematically given preference to processes and organisms, rather than to molecules, pathways and cells, having implanted a solid concern with evolutionary biology, with quantitative approaches, and with theoretical analyses. Accordingly, genetics of complex phenotypes in model organisms are investigated side by side with the genetics of complex human diseases and the genetics of resistance/susceptibility to infections, virology and parasitology search the interfaces between microbes and host, immunology and inflammation often approach (together, and on a strong theoretical basis) the same conditions that are investigated by the geneticists, developmental biology is concerned with very general processes, such as the biophysics of morphogenesis and regeneration. Yet, there is much left to do in this respect. For example, cancer research has never been a specific topic at the IGC, for most research in the field is still “cancer-centered”. Fuelled by the cell and molecular biology of host-cancer relationships, however, the time is ripe to engage in “organism-centered” approaches to the problem, and various groups at the Institute may now be classified as doing cancer research. A comparable situation may be described for epidemiology, essentially concerned, until quite recently, with infectious diseases. While dealing with populations, the “center” of all epidemiology, theoretical and field alike, has been the microbe and how it varies and spreads. Perhaps not by chance, the major contribution of epidemiologists at the IGC has addressed the “re-infection threshold”, a variable that is determined by both host organisms and microbes. Such general policies, as well as the choice of candidates to establish their own research groups at the IGC, obviously determine the success of the operation. We have been extremely privileged by the competence and generosity of our Scientific Advisory Board, who has given time and attention to our problems. I have no doubt that a large part of what was done owes to their vision and interest.

Yet, for all those who are concerned that our work should contribute to fight disease and pain, let alone those with a medical education, it is often frustrating to work so far from the clinical setting. This is much more than an emotional concern, however, as more often than not, the directions of research, the critical questions, the most relevant problems are brought about by the diseases, the patients and the clinical findings. Unfortunately, few medical doctors in this country have gained a scientific “drive” in their education, and even fewer seem to have the generosity to engage in a scientific career, infinitely less secure and well paid than medical practice. Left orphan of clinical problems, biomedical research is thus running the risk of a solipsistic evolution, increasingly concerned with questions that do not represent the most urgent and relevant problems, or the most profound and groundbreaking solutions. Biomedical research needs more medical doctors, just as much as medical doctors need more science.

Before closing this text, I must here acknowledge, most sincerely, the continuous support and guidance, but also the freedom of initiative and operation, that we enjoyed

along these years from the Gulbenkian Foundation's Board of Administration. It also wish to deeply thank Sydney Brenner and all other members of the Institute's Scientific Advisory Board, whose enormous competence and vision can only be matched by their generosity in advice and their patience with my questions. I must underline the critical importance that public money has had, mostly from the Fundação para a Ciência e a Tecnologia, through individual fellowships, project and program grants that were competitively distributed in all fairness. For those who are not aware of the true revolution in the levels and modes of distribution of Portuguese public money, this may sound surprising, but I must add here that nothing could have been done in the conditions that prevailed before the 1990's. Together with the clear progress in the opening of some Universities, these are all excellent signs of recovery. Owing to the feed-forward loops of the concerted interests of media and politicians, Science is "à la mode" in Portugal, certainly as a result of the work in a handful of institutions and to the enthusiasm and capabilities of many youngsters. Their quality and devotion promises a bright future, such that "we older amateurs had perhaps better sit back, waiting for the End".

António Coutinho

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, fish 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.

This Annual Report presents summaries of the individual research projects, their full description being available in the IGC's web page, following short introductive notes to the various areas of research. I thank all the colleagues who helped in the preparation of these summaries. As in previous years the report has been edited by Sérgio Gulbenkian with the help of Maria Matoso.

Experimental Evolution

The 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. "Experimental evolution" approaches are preferred, 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 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 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.

We hope that the Institute's efforts in this area will re-inforce evolutionary thinking in other programs and contribute to promote the study and public knowledge of evolution in Portugal.

The role of the social interaction in bacterial diversity and evolution.

Members: Francisco Dionisio, Isabel Gordo.

External Collaborators: Inês Conceição (Univ. Barcelona, Barcelona, Spain); Ana Cláudia Marques (Univ. Lausanne, Lausanne, Switzerland).

Commonly, resistance to antibiotics confers a cost to bacteria in the absence of antibiotics. Often, resistance genes are carried by DNA elements called conjugative plasmids that move between bacteria even of different species. We have studied the evolution of one of these elements in non-pathogenic *Escherichia coli* cells and found that the cost disappears rapidly. The presence of these evolved conjugative plasmids may even confer advantage to bacteria, including when they move to pathogenic cells of *Salmonella enterica*. This suggests that, not only non-pathogenic bacteria may be *helping* pathogenic cells to resist to antibiotics, but also that, if usage of antibiotics was halted, antibiotic-resistant bacteria would still persist.

Population Genetics of adaptation in *E. coli*.

Members: Isabel Gordo, Francisco Dionsio, Lisete Fernandes.

Students and Technicians: Lilia Perfeito, Catarina Mota.

External Collaborators: Doris Bachtrog (University of San Diego, California, USA).

All natural populations have to adapt to new environments. Knowledge of the genetics of adaptation should provide the centerpiece of a unified theory of evolution. Despite its extreme importance, the process of adaptation is far from being understood. For example: What is the rate at which positive Darwinian selection occurs in a natural population? What is the distribution of fitness effects throughout the adaptive process? are questions whose answer remains obscure to the scientific community. This is possibly because the rate of mutation to new beneficial alleles is low and the associated increase in fitness small, which make empirical observations on advantageous mutations extremely difficult. Moreover theoretical studies show that some generalities may underlie the long-term process of adaptation. These predictions have remained essentially untested. This, together with the biological importance of understanding adaptation, are the main reasons for developing a research project with the aim of measuring relevant biological parameters in the process of adaptation and testing theoretical predictions.

In this project, we use a modified version of a powerful marker system, recently developed, for identifying beneficial mutations in *Escherichia coli*. This new marker system allows us to estimate, with great accuracy, both the rate and the fitness effects of beneficial mutations under different environmental and genetical conditions. The empirical data obtained will allow us to develop more realistic theoretical models to understand the population genetics of adaptation.

Adaptive evolution in spatially structured asexual populations.

Members: Isabel Gordo.

External Collaborators: Paulo Campos (Universidade Federal Rural de Pernambuco, Brazil).

We study the process of adaptation in a spatially structured asexual haploid population. The model assumes a local competition for replication, where each organism interacts only with its nearest neighbors. We observe that the substitution rate of beneficial mutations is smaller for a spatially structured population than that seen for populations without structure. The difference between structured and unstructured populations increases as the adaptive mutation rate increases. Furthermore, the substitution rate decreases as the number of neighbors for local competition is reduced. We have also studied the impact of structure on the distribution of adaptive mutations that fix during adaptation.

Patterns of variation in subdivided asexual populations.

Members: Isabel Gordo.

Students and Technicians: Jaime Combadão.

In this project we are analyzing the levels and pattern of genetic diversity in asexual organisms or chromosomal regions with no recombination, such as the Y chromosome and mitochondria. The model assumes that species are subdivided into small populations called demes and the genomes are under constant weak purifying selection. We study the role of migration in the pattern of mutation accumulation and also the influence of different forms of geographical subdivision. We also study several statistics commonly used to describe genetic diversity and several standard statistical tests in population genetics and molecular evolution.

Muller's ratchet and Small world networks

Members: Isabel Gordo, Francisco Dionsio.

Students and Technicians: Jaime Combadão.

External Collaborators: Paulo Campos (Universidade Federal Rural de Pernambuco, Brazil).

Muller's ratchet is an evolutionary process that has been implicated in the extinction of asexual species, the evolution of non-recombining genomes, such as the mitochondria, the degeneration of the Y chromosome and the evolution of sex and recombination. Here we study the speed of Muller's ratchet in a spatially structured population, which is subdivided into many small populations (demes) connected by migration, and distributed on a graph. We studied different types of networks: regular networks, *small-world* networks and completely random graphs. We show that at the onset of the *small-world* behavior - high local connectivity among the populations but low average path length - the speed of the ratchet starts to decrease drastically. This result is independent of the

number of demes considered, but is more pronounced the larger the number of demes . Since *small-world* networks have been shown to describe well real contact networks among people we discuss our results in the light of the evolution of microbes and disease epidemics.

Recombination Activating Genes 1 and 2 and Vertebrate Genome Stability.

Members: Leonor Sarmento, Antonio Jacinto, Carlos Penha Gonçalves, Jocelyne Demengeot.

Students and Technicians: Paulo Almeida, Paulo Bettencourt.

We are investigating the impact of the recombination activating genes Rag-1 and 2 on the vertebrate genome evolution by making use of bio-informatic tools and genetically engineered organism. Somatic V(D)J recombination constitutes the molecular basis of the generation of a diverse repertoire of antigen receptors in jawed vertebrates and has no identified ancestors in other fila. It consists in the joining of V, D and J gene segments and depends on the recognition of recombination signal sequences (RSSs) flanking these segments by the RAG proteins. The hypothesis under test is that apparition of the Rag genes in an ancestor of jawed vertebrate induced a remodelling of the genome to purge it of cryptic RSS, either physically or by regulating their accessibility.

Given the degenerate sequence of these RSSs, we use a bio-informatic approach to search for RSS common properties that could be used to distinguish RSSs and constitute the basis of recognition by the recombination machinery. We also aim at identifying sequence differences in RSS subtypes (Ig and Tcr loci; V, D and J segments) that might account for the degeneracy. Using DNA conformation analysis tools we identify RSS-specific conformational patterns involving non-conserved spacer DNA and RSS flanking DNA and found that RSSs seem to share distinct properties, such as bendability and BZ transition. Using an algorithm based on nucleotide composition and internal sequence correlations within the RSS, we identified differences between RSSs in different species. Finally, machine learning techniques using nucleotide sequence and DNA bending information allowed us to differentiate Ig and Tcr RSSs. Current work aims at encoding RSS-specific conformational patterns into algorithms to identify RSSs among unrelated sequence to further evaluate cryptic RSS distribution in various genomes and sequences.

In parallel we further investigate in animal models whether ectopic expression of the Rag gene induces genomic modifications outside of the lymphocyte lineages. We previously reported that transgenic mice expressing Rag1 and Rag2 ectopically revealed the cellular toxicity of these genes. This year we generated new tools, such as inducible transgenes for controlled expression of Rag1 and or Rag2 in mouse and drosophila. In addition, retrovirus containing either Rag 1 or 2 sequences fused to fluorescent marker genes will allow the targeting of Rag expression to particular cellular subsets and lineages.

Towards a molecular understanding of Antagonistic Pleiotropy.

Members: Élio Sucena.
Students and Technicians Emília Santos.

Life-history trade-offs prevent different components of fitness, such as longevity and fertility, to be maximized simultaneously. Such trade-offs are often thought to be caused by the allocation of limited resources among competing traits such as reproduction, somatic growth and maintenance. Recent evidence suggests that such trade-offs may be genetically determined, a phenomenon referred to as Antagonistic Pleiotropy (AP).

Indeed, there is growing evidence life-history trade-offs hard-wired into the genome and some putative loci involved in this phenomenon have been identified. What is the molecular nature of this hard-wiring? How does the physiological and life-history phenomenon of trade-off translate into molecular terms?

The first step is to determine the transcriptional behaviour of these loci during the life time of the organisms. A description of such a profile will generate clearer hypotheses regarding the molecular nature of AP. For example, while an increase of gene expression with age or expression contrived to a given age are not expected to derive from AP genes, a bi-modal gene expression early and late in life is expected for AP genes.

We propose to investigate the expression profile of loci including members of metabolic pathways, such as the enzyme copper-zinc superoxide dismutase, and members of the insulin pathway, such as chico during ageing of flies (*D. melanogaster*) and nematodes (*C. elegans*) using RT-PCR, in controlled laboratory environments.

Vertical transmission of *Wolbachia* bacteria in *Drosophila*.

Members: Élio Sucena, Filipa Vala.

Endosymbiosis (the symbiotic interaction between a host and an intracellular symbiont) is a powerful evolutionary force because endosymbiosis is a source of novel metabolic functions. *Wolbachia* are cytoplasmic, vertically transmitted alpha-proteobacteria which require an eukaryotic cell to survive. The fact that closely related *Wolbachia* strains can establish both positive and negative interactions with their hosts makes it the ideal symbiont to study transition from (endo-)parasitism to (endo-)mutualism.

Specifically, associations with these bacteria provide a mean to study the mechanisms by which a host becomes dependent on its symbiont. To this goal, it is important to characterize the genetics underlying host-symbiont interactions. The transition from parasitism to mutualism involves a switch of selection forces acting on the host: while selection acting on *Wolbachia* always favors efficient vertical transmission (this is the only route by which it can colonize new hosts); selection on the host acts to increase vertical transmission of the endosymbiont once the latter has become important for survival and/or reproduction.

Our project aims at characterizing how *Wolbachia* finds its way into the reproductive tissues of a developing host. Presently, one single study describes the patterns of distribution of *Wolbachia* during oogenesis/early development. With regard to the distribution of bacteria in oocyte/syncytial embryo of *Drosophila*, that study shows these patterns fall into three categories: along a posterior to anterior axis, along an anterior to

posterior axis, and a uniform distribution along this axis. The former two resemble the distribution of *Drosophila* oocyte polarity determinants like *oskar* and *bicoid*, respectively. Also, three cytological studies suggest that *Wolbachia* associate to cell cytoskeleton components to migrate.

With this project we propose to identify the host developmental cues that the endosymbiont uses to reach gonad tissues, using a combination of immunostaining techniques for confocal microscopy analysis, ectopic expression of genes and mutant lines of candidate genes of *Drosophila melanogaster*.

The present proposal asks three questions:

1. Does the distribution of *Wolbachia* in early embryos rely on oocyte polarity determinants or their regulators;
2. Do *Wolbachia* associate with cytoskeleton components to create these patterns;
3. Do *Wolbachia* reach gonadal tissues by actively following the host endogenous pole cell migration cues or do they reach the gonads by hitchhiking in association with these cells?

A Populational look into *Drosophila* Development.

Members: Élio Sucena.

Students and Technicians: Gilberto Bento.

In the past twenty years we have learned a great deal about the Developmental Genetics of *Drosophila*, from its molecular cascades to the succession of cellular phenomena that shape the embryo. Nevertheless, few are the examples in which Development is viewed and studied as an integrated whole. Here, we look at Development as a succession of interdependent events, prone to variable degrees of plasticity at different stages and/or external perturbations.

Under this light, we have been measuring the phenotypic variance in Developmental time in six independent geographical isolates of *Drosophila melanogaster*. We have divided development into 4 distinct periods, defined by well-characterized morphological features. Each of these periods can broadly be associated with particular genetic events, such as early maternal-driven processes or segmentation. We observed that Variance does not change throughout Development. Such result challenges the notion of a constrained Phylotypic Stage.

We also, have taken advantage of the natural occurrence of *Wolbachia* in three of the six lines. We have cured the infection in the infected lines using Tetracycline and also infected one naïve line (Villeurbanne). Preliminary data shows that the presence of *Wolbachia* in normally infected and naïve populations, is totally innocuous to the normal progression of Development.

Ontogenic and evolutionary origin of cells specialized in the regulation of inflammatory responses

Members: Élio Sucena, Moises Mallo, Jocelyne Demengeot.
Students and Technicians: João Duarte, Santiago Zelenay.

The indication that organisms devoid of adaptive immune system are susceptible to systemic inflammatory pathologies call for a reassessment of the role of the specificity of the adaptive immune system for immuno-pathologies and their regulation. We choose to initiate an evo-devo approach where expression of genes known to be involved in lymphocyte regulation is tested during mouse development and in various species of vertebrates and invertebrates. We expect to establish i) whether FoxP3 gene assume other functions early in development, ii) whether regulatory T cells in fish and birds display similar functions as in mammals, iii) whether insects present cells specialized in the dampening of inflammatory responses, as a signature of Treg ancestors.

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

Members: Henrique Teotonio.
Students and Technicians: Tania Aires.
External Collaborators: Anthony 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. After a description of phenotypic evolutionary patterns and processes during the reverse evolution of laboratory adapted populations of *Drosophila melanogaster*, we now characterize patterns of molecular evolution. First, patterns of linkage disequilibrium at the *Copper-Zinc Superoxide dismutase (Sod)* and *Phosphoglucosmutase (Pgm)* loci are determined. Second, an association between the phenotypic and molecular trajectory at the *Sod* and *Pgm* loci during 50 generations of experimental reverse evolution is done for a few selected SNP markers in these candidate gene regions.

The experimental evolution of outcrossing in populations of *Caenorhabditis elegans*.

Members: Henrique Teotonio.
Students and Technicians: Diogo Manoel, Goncalo Silva, Sara Carvalho.
External Collaborators: Patrick Phillips (University of Oregon, Eugene, USA).

We are addressing the hypothesis that males are evolutionary maintained due to their role in promoting outcrossing since hermaphrodites cannot outcross amongst themselves in the nematode species *Caenorhabditis elegans*. Two evolutionary mechanisms will be studied: inbreeding depression and sorting of beneficial genetic variation. The proposed methodology make use of an experimental evolution approach with genetically variable and laboratory adapted populations of *Caenorhabditis elegans*. Mating system theory is tested by manipulating levels of outcrossing on one hand and mutational loads on the other, while studying the adaptation of populations to novel and varying environments.

Several phenotypic characters will be followed during evolution including competitive performance, inbreeding depression, male and hermaphroditic fertility. A study of the dynamics of genetic variability at the DNA sequence level, as measured by genome-wide levels of heterozygosity at microsatellites, will be done concurrently to experimental evolution.

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 intractable. One of these relates to the genetics of “complex” phenotypes, which do not follow classical mendelian 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, Type I diabetes, and brain stroke, while the mouse projects include the genetics of susceptibility to malaria and diabetes. Research in bioinformatics and statistical population genetics has also been launched, leading to the development of statistical methods that incorporate multiple parameters in phenotype definition, as well as methods assessing the contribution of multiple genes to specific quantitative phenotypes. A gene expression unit is now fully operational at the Institute, while public financing was competitively obtained for installing a technology platform for medium-throughput DNA sequencing and genotyping.

Stroke genetics and genomics (Strokenetics, Genoport and Genostroke projects).

Members: Sofia Oliveira.

Students and Technicians: Sara Fidalgo, Tiago Krug, Maria Benedita Fonseca.

External Collaborators: José Ferro e Liliana Gouveia (Hospital de Santa Maria, Lisbon, Portugal); Isabel Henriques, Rita Silva (Hospital Espírito Santo, Évora, Portugal); Miguel Viana-Baptista (Hospital Egas Moniz, Lisbon, Portugal) Ana Amélia Pinto, Rita Silva (Hospital Fernando Fonseca, Amadora Sintra, Portugal); Manuel Correia, Assunção Tuna, Gabriela Lopes (Hospital Geral de Santo António, Porto, Portugal); João Ramalho Fontes, Carla Ferreira (Hospital São Marcos, Braga, Portugal), Mário Rui Silva (Hospital São Pedro, Évora, Portugal) Ilda Matos (Hospital Distrital de Mirandela, Portugal).

Stroke is the third leading cause of death in the developed world. It is even more disabling than lethal, and the persistent neurological impairment and physical disability caused by stroke have a substantial socioeconomic cost. Stroke is a complex disease resulting from the interplay of environmental and genetic factors. Identification of genes

increasing susceptibility to stroke would have far-reaching public health impact, from enhancing motivation to make behavioral and lifestyle changes in susceptible individuals to providing basic biological and clinical information about the development, prevention and treatment of stroke.

We will use the novel genomic convergence approach that combines genomic screening (STROKENETICS), expression analysis (GENOSTROKE), and association studies (GENOPORT) to identify susceptibility genes for stroke. The STROKENETICS study aims at collecting biological samples and information from 800 individuals originating from 200 Portuguese multiplex families with stroke. These samples will then be used to perform a whole genome linkage screen. The GENOSTROKE project aims at identifying genes whose expression pattern implicates them in the pathogenesis of stroke. This will be achieved comparing the expression profiles in blood mononuclear cells of stroke cases and controls. Finally, the 500 cases and 500 controls collected through the GENOPORT project will be used together with the STROKENETICS samples to perform association studies on candidate genes and on genes differentially expressed and that map to linkage peaks.

Genetic, molecular and cellular study of Behçet's Disease.

Members: Sofia Oliveira, Jocelyne Demengeot, António Coutinho.

Students and Technicians: Maria Francisca Fontes, Tiago Krug, Maria Benedita Fonseca.

External Collaborators: Jorge Crespo (Hospital Infante D. Pedro, Aveiro, Portugal).

Behçet's disease (BD) is a complex disorder characterized by generalized vasculitis, the cause and pathogenesis of which are still unclear. Although there is evidence for environmental risk factors, epidemiological and family studies strongly support the existence of genetic risk factors. The only established genetic predisposition is the HLA-B*51 antigen, which accounts for about 19% of the overall genetic liability. A recent linkage study confirmed the existence of additional genes associated with BD. We will perform a whole genome linkage screen in one large Portuguese family with two individuals affected with BD and nine individuals partially affected to identify novel chromosomal loci linked with BD.

Since endothelial and immunological dysfunction appear to be the main pathological features of BD, and heme oxygenase 1 (HO1) has a critical role in inflammation and stress responses in the endothelium, we hypothesize that HO1 may have an important role in BD susceptibility. Therefore, we propose to create a BD biobank of at least 100 BD cases and 100 controls to analyze the association of several polymorphisms in HO1 with the risk for developing BD, and to assess if this association is dependent on HLA-B*51.

Furthermore, regulatory T cells (Treg) are reduced in patients with several autoimmune diseases. We propose to look at circulating Treg numbers and analyse the association of several polymorphisms of FOXP3, a master regulatory gene, with Treg numbers and the risk for developing BD.

Finally, we will perform gene profiling studies in BD patients and controls to identify genes differentially expressed in cases versus controls and in the active versus inactive phase of the disease.

By forming an integrated collaborative effort between clinicians, immunologists and geneticists, we hope, through this multifactorial approach, to help elucidate the molecular, cellular and genetic etiology of BD.

Genetics of malaria liver stage in mouse models.

Members: Carlos Penha Gonçalves.

Students and Technicians: Lígia Deus.

This project aims to identify genetic factors that confer resistance to malaria infection in mouse models. Unraveling the identification of the 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 has led to the identification and fine-mapping of the *Ber11* locus into a 10 Mb region on mouse chromosome 17.

Pregnancy and neo-natal murine malaria.

Members: Carlos Penha Gonçalves, Cláudio Marinho.

Students and Technicians: Rita Neres.

In *Plasmodium falciparum* endemic areas, pregnancy-associated malaria is an important health problem with an increasing risk of maternal anemia, fetal intrauterine growth retardation and low birth weight. Pregnancy-associated malaria is characterized by placental accumulation of *P. falciparum* infected erythrocytes. The clinical presentation of malaria during pregnancy ranges from asymptomatic to severe, life-threatening illness. However the reasons for the increased malaria susceptibility during pregnancy and the pathogenesis mechanisms leading to placental and fetal pathology are poorly understood. We initiated a project aiming to generate and analyze murine models for pregnancy malaria and *in utero* malaria infection. Three different methods to induce pregnancy malaria will be followed: (1) Analysis of malaria infection during pregnancy (2) Analysis of malaria recrudescence during pregnancy (3) Analysis of re-infection outcome in induced-malaria resistance models.

Analysis methods will include parasitemia follow-up, placenta pathology and isolation of parasite DNA in the fetal tissues and analysis the infection course in pups from malaria pregnancies. The establishment of these models will entail testing different mouse strains and different parasite strains, namely *P. berghei* and *P. chabaudi*.

Genetics of lymphocyte homeostasis.

Members: Carlos Penha Gonçalves.

Students and Technicians: Joana Rodo, Joana Corte-Real.

The goal of this project is to characterize the genetics of the homeostasis of the number of lymphocytes within the lymphoid organs. The workplan includes:

to study the establishment of lymphocyte homeostasis during the ontogenesis of the lymphoid organs in different laboratory mouse strains and **(2)** to genetically map and to identify the genetic factors involved in the homeostatic mechanisms that control the number of lymphocytes in the lymphoid organs of the mouse.

This project has identified a differential role for MHC class II molecules to efficiency in thymic positive selection and the size of the CD4 single positive thymic compartment.

This project has led to the mapping of a LPS response gene in the MHC region and explores the role of MHC class II genes in susceptibility of B cells to LPS. We also mapped the homeostatic control of serum IgM to Chromosome 13 and identified IRF4 as a candidate gene in controlling B cell maturation.

Effect of natural polyphenolic compounds on the pathogenesis of type 1 diabetes.

Members: Carlos Penha Gonçalves.

Students and Technicians: Sofia Couceiro.

External Collaborators: Catarina Duarte (IBET, Oeiras, Portugal).

Much of the research on type 1 diabetes prevention has been targeted to identify genetic components that predispose or modify the course of the disease pathogenesis. This has included the search for genetic predisposing factors aiming to facilitate the identification of type 1 diabetes at risk individuals. Non-genetic diabetogenic factors are considered either disease triggers or pathogenesis modifiers in genetically predisposed individuals. The discovery of non-genetic factors that interfere with disease pathogenesis are needed to develop preventing interventions to abrogate the disease process in such individuals.

This project aims to evaluate the effect of dietary polyphenolic compounds on the pathogenesis course of type 1 diabetes. The activity of flavonoid combinations in natural extracts is analyzed at the level of diabetogenesis and lymphocyte activity in the NOD mouse model of type 1 diabetes. Our work has shown that: (1) *In vivo* extract treatment had no significant effect on glycemia/diabetes but appear to partially restore defective NOD T cell activation. (2) *In vitro* quercetin treatment seems to induce T and B cell activation both in B6 and NOD strains.

Genetic epidemiology of autism.

Members: Astrid Vicente, Madalena Martins.

Students and Technicians: Ana Margarida Coutinho, Catarina Correia, António Currais, Inês Sousa, Isabel Peixeiro, Pedro Espada, Teresa Barrio.

External Collaborators: Guiomar Oliveira (Hospital Pediátrico de Coimbra, Coimbra, Portugal); Patricia Maciel (Universidade do Minho, Braga, Portugal); Michael Gill (Trinity College, University of Dublin, Republic of Ireland); Louise Gallagher (Trinity College, University of Dublin, Republic of Ireland); Jason Moore (Dartmouth Medical School, USA).

Autism is a neurodevelopmental disorder of unknown etiology. The impact of genetic factors in disease susceptibility has been established, but inheritance is complex and multiple genes are likely involved. Interaction between genes, or epistasis, is also expected to strongly influence disease risk. One of the most studied subphenotypes in autism is platelet hyperserotonemia, which is likely influenced by several genes in a non-additive manner. In our autism sample, we previously found an association of specific variants at the serotonin (5-HT) transporter gene (*SLC6A4*) with hyperserotonemia. This gene, together with the integrin $\beta 3$ chain precursor gene (*ITGB3*), maps to 17q11-21 where a *linkage* peak for both autism and 5-HT levels has been found, suggesting that the occurrence of hyperserotonemia in autism may be mediated by genetic variation at the *SLC6A4* and *ITGB3* genes, and that epistatic effects between these and other genes involved in the 5-HT system may play a role in autism. The Multifactor-Dimensionality Reduction (MDR) method was used to test for epistasis in autism between markers in seven candidate genes of the 5-HT system (*SLC6A4*, *HTR2A*, *HTR1D*, *HTR1A*, *HTR5A*, *TPHI*, and *ITGB3*), in a sample of 186 autistic patients and 181 controls. Epistasis underlying platelet 5-HT levels was tested using the Restricted Partition Method (RPM), in a sample of 109 autistic children and 38 age-matched controls. In autism, we found significant two- and three-*locus* interactions among markers in *ITGB3* and *SLC6A4*, and also in *HTR5A*. In the determination of 5-HT levels, we found significant two-*locus* interactions between *SLC6A4* and *TPHI*, *SLC6A4* and *HTR1D*, and between *HTR1A* and *ITGB3*. Our results indicate that the interaction of gene variants determining hyperserotonemia is also significantly associated with an increased risk for autism (AM Coutinho *et al*, *submitted*). Other observations in our patients suggest that neuroprotective factors are challenged in autism, namely the increased levels of serum BDNF, which in our sample we found not to be genetically determined by variation at the *BDNF* gene. An association between the *GADI* gene, which encodes a glutamine decarboxylase that converts glutamate into GABA, is associated with autism in our sample, and is involved in the determination of BDNF levels in these patients. These observations suggest that the balance between the main excitatory neurotransmitter in the brain, glutamate, and the inhibitory neurotransmitter GABA, play a role in autism symptomatology, while the observed changes in BDNF levels may be a consequence of increased glutamate (M Martins *et al*, *in preparation*). Interestingly, the *GADI* gene maps to a region of chromosome 2q which is linked to autism, and which we are fine mapping in collaboration with an research group at Trinity College, Ireland. The collaboration with

Autism Genome Project is further developing: the AGP has conducted a genome wide screen in multiplex families, and analysis of the results will soon identify which regions will be fine mapped in an additional sample that includes our patients. Funding for this Phase II of the project is being sought by the collaborative group.

Pharmacogenetics of risperidone therapy in autism spectrum disorders.

Members: Astrid Vicente, Madalena Martins.

Students and Technicians: Ana Margarida Coutinho, Catarina Correia, Pedro Espada, Teresa Barrio.

External Collaborators: Guiomar Oliveira (Hospital Pediátrico de Coimbra, Coimbra, Portugal).

The atypical antipsychotic risperidone is used to control disruptive behaviors associated with autism, with some improvement of typical symptoms. Risperidone is mainly metabolized by cytochrome P4502D6, while drug absorption and bioavailability is mediated by glycoprotein P, encoded by *MDR1* gene. The main objective of this study is the identification of genetic factors underlying the variability in individual response of autistic patients to risperidone; given the ethnic heterogeneity of *CYP2D6* and *MDR1* variants, prior determination of the frequency of pharmacologically relevant *CYP2D6* allelic variants and *MDR1* functional gene polymorphisms in the Portuguese population was required. *CYP2D6* *3, *4, *5, *6 alleles and gene duplication and *MDR1* C1236T, G2677T/A, C3435T polymorphisms were genotyped in 110 healthy individuals by PCR-ASA and RFLP. In ten autistic patients selected for risperidone therapy (3 females, 7 males), drug efficacy and tolerability was monitored at baseline and after one month, using the Autism Treatment Evaluation Checklist (ATEC) and assessing prolactin (PRL) levels and weight gain. The influence of *CYP2D6*, *MDR1*, *DRD2*, *HT2C* and *HT2A* gene variants in ATEC scores, PRL levels and weight gain was analysed. *CYP2D6* alleles are classified, according to protein activity, into Poor (PM), Intermediate (IM), Extensive (EM) and Ultrarapid Metabolizers (UM) and the determination of *CYP2D6* PM alleles *3, *4, *5, *6 and the UM gene duplication allows a correct prediction of enzyme activity. *CYP2D6* allele frequencies were 0.014 (*3), 0.133 (*4), 0.028 (*5), 0.018 (*6) and 0.035 (gene duplication), with five individuals classified as PM (4.9%) and three as UM (6.9%). These frequencies are in agreement with other European populations, reinforcing the previously suggested north/south gradient of non-functional allele frequencies. Allele frequencies for the *MDR1* polymorphisms were 0.52 (1236C and 3435T), 0.56 (2677T) and 0.03 (2677A); discrepancies found with frequencies in other Caucasians populations are difficult to interpret due to lack of data for Southern Europe. In patients undergoing treatment, the ATEC scores improved significantly after one month ($P=0.008$), particularly in the behavioural subscale ($P=0.002$). Weight and prolactin levels increased significantly from baseline ($P=0.004$ and 0.009, respectively). One patient had a PRL increase of clinical significance and carried the *DRD2* A1 allele, known to be associated with PRL increase. Two patients carried a *CYP2D6* gene duplication, associated with the UM phenotype, in one determining a complete lack of response and in the other a low improvement in ATEC score. Two patients homozygous

for the PM *CYP2D6**4 allele showed variable improvement in ATEC score but no evidence for increased side effects, possibly as a result of a combination of variants in other genes studied. In this small sample we found no overall correlation of ATEC scores, PRL levels or weight gain with *CYP2D6*, *MDR1*, *DRD2*, *HT2A* or *HT2C* genotypes. Our preliminary results indicate that response to medication is determined by a complex interplay of factors, pointing out the need for large samples and multiparametric approaches to dissect genetic influences in drug response.

Genetic Factors in Systemic Lupus Erythematosus.

Members: Astrid Vicente, Marta Barreto, Jocelyne Demengeot, Constantin Fesel.

Students and Technicians: Franscisca Fontes, Lara Lourenço.

External Collaborators: Carlos Ferreira; Carlos Vasconcelos, João Faro Viana, Berta Martins ((Associação de Doentes com Lupus, Lisbon, Portugal).

Naturally occurring CD4⁺CD25⁺ regulatory T cells (Treg) play a key role in suppressing self-reactive T cells and preventing autoimmunity. To investigate a putative involvement of Treg in Systemic Lupus Erythematosus (SLE) pathogenesis, we determined the frequency of Treg in a population consisting of 54 SLE families, including 76 patients and 166 relatives, and 117 healthy individuals. FACS analysis of PBMC showed a significantly lower percentage of Treg in SLE patients than in controls ($P<0.00001$). Treg frequency was negatively correlated with disease activity and with antinuclear autoantibody production, and changed with the development and remission of SLE-related symptoms. Interestingly, within the control group, the frequency of Treg was significantly lower in females. The distribution of Treg in SLE families was indicative of a genetic trait with an estimated heritability of 85%. We next searched for genetic factors influencing this trait by testing several genes involved in Treg generation and maintenance. We found an association of Treg frequency with the *CTLA4* and *TGFβ* genes but not with *FOXP3*, *IL2* or *CD25*. However, the *FOXP3* gene was associated to SLE, suggesting that this gene may confer susceptibility to the disease by mechanisms other than influencing Treg frequency. Furthermore, we established that the mechanism by which specific *CTLA4* and *TGFβ* variants determine the low frequency of Treg is independent of the level of gene expression in PBMC. The finding that SLE pathogenesis involves an inherited reduced frequency of Treg opens new perspectives for the research of pathogenic mechanisms and the design of preventive therapeutic approaches.

To further evaluate the association between the frequency of regulatory T cells and the development of SLE manifestations, we assessed Treg frequency in two patients before and after the onset of symptoms or a flare, and after treatment. We found a decrease in the frequency of regulatory T cells upon the acute onset of SLE symptoms and an increase in the frequency of this T cell population with disease remission. The frequency of Treg of a healthy woman, who developed SLE, dropped from 4.6% to 2.8%. A second case, a woman with an SLE flare who had a Treg frequency of 4.22%, had a significant increase in Treg frequency to 7.12% and an improvement in her clinical condition upon treatment with intravenous gammaglobulin (IVIg). Our findings suggest that the decrease in Treg frequency is associated with the development of SLE-related symptoms, and an

increase in the frequency of these cells is associated with disease remission. The results further suggest that IVIg can influence the generation or maintenance of Treg in the periphery, leading to the regulation of autoreactive T cell clones and consequently, to clinical improvement of the patients.

Genetic diversity of the Azorean population.

Members: Luisa Mota Vieira.

Students and Technicians: Claudia C. Branco, Raquel Palla, Silvia Lino and Ester Cabrol.

This project aims to characterize the genetic variability and ancestry of the Azorean population in order to carry out admixture and association studies. For this purpose, we analyzed 24 short tandem repeats (STRs) and 6 Alu markers in two cohorts: the Azorean and mainland Portugal populations. The results based on STR analysis show that the Azoreans presents lower average gene diversity (0.776), when compared with mainland (0.781), and higher inbreeding ($FIS=0.0531$). In both samples, the markers TPOX and D17S976 showed the lowest (≈ 0.6) and highest (≈ 0.9) values of gene diversity, respectively. The admixture coefficient (mY) reveals Portuguese as the major contributors to the genetic background of the Azoreans. This observation is corroborated by the dendrogram, in which Azores clusters with Portuguese, Spanish, Italians, and Belgians, apart from Moroccans and Cabo Verdeans. Concerning the Alu insertions, the data revealed that all markers are polymorphic in both populations studied. The less frequent insertions are PV92 and D1 in Azores and mainland, respectively. ACE and TPA-25 show the highest values of heterozygosity in both cohorts. Allele frequencies are very similar to those obtained in European populations. These results are validated by the Y-chromosome and mtDNA data, where the European represent the majority of the maternal and paternal lineages. Overall, these results are reflected in the phylogenetic tree, in which Azores and mainland Portugal branch with Catalans, Andalusians, Morrocans and Algerians. Taken together, the data complement the settlement history of the Azorean population and suggest that the Azores presents a particular genetic signature resulting from the admixture of several populations. [Ongoing project; CCB is a fellowship of FCT (SFRH/ BD/ 12254/ 2003); this work was funded by DRCT, Azores].

Hereditary hemochromatosis in São Miguel island (Azores): A population and clinical approach.

Members: Luisa Mota-Vieira.

Students and Technicians: Laura de Fez, Paula R. Pacheco, Rita Cabral.

External Collaborators: Graça Porto, Pedro Rodrigues, (IRIS, Iron genes and immune Systems, IBMC, Universidade do Porto, Porto, Portugal).

Hereditary hemochromatosis (HH, OMIM 235200) is an autosomal recessive disorder characterized by increased iron absorption. Three main mutations in the *HFE* gene (6p21.3) are implicated in HH: C282Y, H63D and S65C. Here, we estimated the

frequencies and geographic distribution of these mutations in São Miguel island population (131,609 inhabitants, 2001 Census). In total, 469 blood donors from the six municipalities of the island were analysed by PCR/RFLP method. The allele frequency was 5.01% for C282Y, 21.96% for H63D and 2.24% for S65C. We found nine genotypes: wild/wild (50.96%), wild/H63D (29.85%), wild/C282Y (7.68%), H63D/H63D (5.54%), wild/S65C (2.35%), C282Y/H63D (1.49%), H63D/S65C (1.49%), C282Y/S65C (0.43%) and C282Y/C282Y (0.21%). The six municipalities of the island show different values for the C282Y, the most severe mutation. To study the significance of these C282Y frequency values, we carried out a statistical analysis (Fisher's exact test). We observed a statistical difference ($p=0.048$) between Nordeste (9.8%) and the other five municipalities (4.6%). This result may be explained by the relative geographical isolation of Nordeste in the island, and by its reduced population (4% of total population). Considering São Miguel's settlement history, we are genotyping the HLA-A and HLA-B loci in order to characterize the haplotypes associated with *HFE* mutations in the island. [Ongoing project; this work is funded by DRCT, Azores].

Genetic and consanguinity of congenital heart disease in Azores.

Members: Luisa Mota-Vieira.

Students and Technicians: Rita Cabral, Paula R Pacheco, Laura de Fez.

External Collaborators: Rui Anjos (Hospital de Santa cruz, Carnaxide, Portugal), Carlos Pereira Duarte Carvalho, Clara Macedo (HDES, Ponta Delgada, Azores, Portugal).

In well-defined populations, parental consanguinity and familial aggregation suggest that genetic factors contribute to congenital heart disease (CHD), the most frequent of all clinically significant birth defects. Recently, we have shown that São Miguel Island (Azores, Portugal) has a high average prevalence (9.20 ‰) of CHD. This observation may be related to the population structure of the rural area, which has an average of 1508 inhabitants, and to the relatively high endogamy (60%). In order to investigate this hypothesis and for future molecular studies, we have currently (1) assessing the genetic contribution of children affected with complex CHD in São Miguel Island, through a structured family questionnaire, and (2) building a biobank of samples collected from patients (DNA, RNA and cells) and parents (DNA and RNA) only after their informed consent. In this study, all CHD children from São Miguel Island belong to the Azorean registry of CHD, which has complete clinical and personal information of 310 individuals until 2004. The family questionnaire includes queries for factors increasing the risk for CHD (maternal diabetes mellitus, alcohol and drug abuse by the mother during pregnancy, viral infections of the foetus and genetic conditions), and a detailed family history to construct the ascending genealogy of, at least, three generations. Until now we have assessed the familial aggregation, by genealogical research, of 50 CHD patients. Fifteen of them belong to simplex families and 35 patients to 14 multiplex ones (8, 5 and 1 families with 2, 3 and 4 affected patients, respectively). In 2/14 multiplex families parental consanguinity was identified. This preliminary data demonstrate that 50% of the analysed families are multiplex (more than one affected individual per family), suggesting a genetic factor involved in the susceptibility for CHD. Although the study

concerns an island population, the consanguinity was not apparently a major genetic factor. The analysis of microsatellite heterozygosity will be carried out in order to search a distant consanguinity in the CHD patients. To investigate the molecular CHD aetiology, we estimated one candidate polymorphism – the cytosine-to-thymine mutation at base 677 (677C→T) of the gene for methylenetetrahydrofolate reductase (MTHFR) – in 469 healthy controls. The homozygous *MTHFR* 677TT genotype was found in 84 out of 114 subjects (17.91%), whereas the other two genotypes 677CT and 677CC were identified in 221/469 (47,12%) and 164/469 (34,97%) respectively. The 677C→T polymorphism in the *MTHFR* gene, as well other candidate genes, will be analyzed in the CHD patient's group and subgroups based on the predominant cardiac lesion. [Ongoing project; this work is funded by FCT (POCTI/ESP/49236/2002) and DRCT, Azores].

Virology and 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 *functional* 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.

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

Members: R.M.E. Parkhouse, Ana Crespo.

Students and Technicians: Sílvia Almeida, Sílvia Correia, Rute Nascimento, Vivian Oliveira, Ana Luísa Reis.

The aim is to identify and exploit viral modifiers of cell biology and immunity as a potential source of novel health care pharmaceuticals for manipulation of immune responses and treatment of certain diseases. Such virus genes are being identified by nucleotide sequence and functional analysis of cloned viral ORFs of two large DNA viruses (African swine fever (ASFV) and Mouse herpes virus (MHV 68)). As a direct approach towards identifying novel virus evasion genes, which do not have homologies

in the database, the genes of these two viruses are being systematically screened in functional assays for their impact on cellular and immune 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 human α , γ herpesviruses homologues of gene inducing G2/M arrest have been studied and similar results were obtained in cells transduced with recombinant Lentivirus vectors expressing these genes. These results indicate an important and conserved function of this herpesviruses gene, which may contribute to the pathogenesis of the infection. The downstream cellular targets of these “evasion” genes are being identified, and the role of these genes in pathogenesis is being studied through the construction of deletion mutants. The deletion mutants provide a rational approach for the construction of attenuated, live virus vaccines.

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. Two particularly interesting transgenic mice have been constructed. In the first, we have established a metastasizing, angiogenic, transplantable thymoma through T cell restricted transgenic expression of a virus host evasion gene inhibiting activation of NFkB and NFAT. The other is a viral gene which interferes with function of the important signaling molecule vav.

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

Member: R.M.E. Parkhouse.

External 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, Bolivia and Venezuela, and, on occasions, clinical material in Spain. A new project in collaboration with our Venezuelan colleagues, is the development of a serological test to detect adult tapeworms carriers in the definitive human host

Finally, we have developed potential vaccines currently being tested for bovine and porcine cysticercosis, based on a recombinant oncospherical surface and secreted molecule. Interestingly this molecule is a functional adhesion molecule, possibly facilitating tissue invasion by the parasite in the intermediate host, and so constitutes a rational basis for a vaccine.

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, Lisbon, Portugal).

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 rational 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 in this project recombinant forms of these proteins have been produced as potential serological diagnostic probes. Their utility has now been assessed using sera from infected pigs and four antigens gave 100% sensitivity as diagnostic antigens.

Treatment of monocytes with IL-12 plus IL-18 stimulates monocyte survival, differentiation and the production of CXCL8, CXCL9 and CXCL10.

Member: R.M.E. Parkhouse.

External Collaborator: Margarita Bofill (Fundació IrsiCaixa, Fundació Germans Trias i Pujol Badalona, Spain).

During inflammation, interleukin (IL)-12 and IL-18 are produced by macrophages. Additional sources are neutrophils (IL-12) and keratinocytes and damaged endothelial cells (IL-18). The subsequent role of IL-12 and IL-18 in inflammatory innate immune responses was explored by investigating their impact on human monocytes and mature broncho-alveolar lavage (BAL) macrophages. IL-12 and IL-18 together, but not alone, prevented apoptosis and promoted clustering and subsequent differentiation of monocytes into macrophages, induced *de novo* secretion of CXCL9 and CXCL10 and significantly increased the production of CXCL8. These chemokines promote the recruitment of monocytes, activated T-cells (CXCL9 and CXCL10) and granulocytes (CXCL8) and are involved in the regulation of angiogenesis. In similar experiments with BALs, high levels of CXCL8 were observed, comparable to those present in IL-12 and IL-18 stimulated monocytes cultures, but there was no up-regulation when stimulated by IL-12 and IL-18. On the other hand the basal production of CXCL9 and CXCL10 by BALs was increased by 10 fold ($p < 0.001$) in the presence of either IL-12 or IL-18 alone and by 100 fold in the presence of both cytokines. In conclusion, our results indicate a relevant and autocrine role for IL-12 and IL-18 in the activation and resolution inflammatory immune responses.

Expression of aberrant forms of CD22 on B lymphocytes in *Cd22^a* lupus-prone mice affects ligand binding.

Member: R.M.E. Parkhouse.

External Collaborator: Shozo Izui (Department of Pathology and Immunology, University Medical Center, Geneva, Switzerland).

CD22 functions primarily as a negative regulator of B-cell receptor signaling. The *Cd22^a* allele has been proposed as a candidate allele for murine systemic lupus erythematosus. Here we show that *Cd22^a* B cells express aberrant forms of CD22, which can potentially deregulate B-cell signaling because of their decreased ligand-binding capacity, and this provides further support for *Cd22a* as a potential candidate allele for murine SLE.

Transcriptome analysis of germinal centre B cells during gammaherpesvirus latent infection.

Members: João Pedro Simas and Sofia Marques.

Students and technicians: Marta Alenquer.

The main objective of this project is to identify key cellular genes and biochemical pathways that are involved in cellular functions important for the control of gammaherpesvirus infection. We will use a virus, designated MHV68, as its pathogenesis can be readily investigated in the laboratory mouse.

This project will:

- assess if MHV68 establishes long term latent infection in longlived memory B cells following infection of germinal center (GC) B cells;
- analyze the transcriptome of GC B cells during the establishment of latent infection;
- determine the effect that recombinant viruses with specific gene deletions have on the transcriptome profile of GC B cells.

It is hoped that knowledge gained from this type of approach may not only help determining 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.

Modulation of NF- κ B transcriptional activity during gamma herpesvirus infection.

Members: João Pedro Simas, Miguel Soares.

Students and technicians: Bruno Almeida e Josina Corte Real.

The objectives of the proposed project are to characterise the biochemical pathways of ORF73 interference with NF- κ B activity, namely its interaction with SNIP1 and CBP, and investigate its biological significance in the context of MHV-68 infection in its natural host.

To this end, our strategy is:

- i) to perform competition assays to test if the inhibitory effect of ORF73 on NF κ B-dependent transcription is due to competition between p65 and ORF73 for CBP binding;
- ii) to determine at what level ORF73 is exerting its inhibitory effect on NF- κ B activity, namely interference with I- κ B-alpha phosphorylation or degradation or NF- κ B nuclear translocation or interference with NF- κ B DNA binding activity or interference with NF- κ B transactivation activity;
- iii) investigate the ORF73 role on apoptosis;
- iv) to identify minimal regions for protein interactions and assess the capacity of MHV-68 recombinant viruses with mutated ORF73 genes lacking binding activity towards SNIP1 and CBP to establish latent infection in wild type mice.

Herpesvirus modulation of B-lymphocyte function.

Members: João Pedro Simas, Marta Miranda.

Students and technicians: Lénia Rodrigues, Filipa Lopes.

External Collaborators: Xosé Bustello (Centro de Investigación del Cáncer/Cancer Research Center, University of Salamanca, Salamanca, Spain).

The objectives of the proposed project are:

- to further characterise the biochemical properties of the M2-Vav interaction;
- to investigate the biological significance of M2 binding to Vav in the context of MHV-68 infection in its natural host.

To this end, our strategy is:

1. to determine the molecular mechanism of M2 induced phosphorylation of Vav;
2. to determine the functional sub-cellular localization of M2, including the generation of a MHV-68 recombinant virus encoding the M2 gene fused to a myristylation signal;
3. to assess the ability of MHV-68 to establish latent infection in Vav deficient mice;
4. to assess the capacity of MHV-68 recombinant viruses with mutated M2 genes that lack binding activity to Vav to establish latent infection in wild type mice;

Given that Vav proteins play a crucial role in B cell differentiation and antigen triggered B cell activation, understanding how a virus modulates B cell function will not only contribute towards a better understanding of -herpesvirus pathogenesis but also potentially tell us how the immune system functions.

Inflammation and 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, rheumatisms, 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.

Thymic development and selection of immunoregulatory T cells.

Members: Marie Louise Bergman, Ricardo Araujo, Jocelyne Demengeot.

Students and Technicians: Manuel Rebelo, Santiago Zelenay, Francisca Fontes.

The recent finding that expression of the transcription factor Foxp3 is strictly necessary for regulatory T cells differentiation and function offers new tools to understand the mechanisms of Treg selection. We make use of anti-male antigen TCR transgenic models to establish the nature of the cells mediating Treg selection, by generating chimeric female/male mice where the cells presenting the selecting antigen originate either from BM or thymic epithelium. On the other hand, we expect that analysis of mice impaired in particular aspect of thymocyte development (including Foxp3 mutants) will allow the establishment of the stage of differentiation at which cells of the T lineage are selected to become Tregs.

Mechanisms of immune regulation by CD4 cells.

Members: Marie-Louise Bergman, Miguel Soares, Jocelyne Demengeot.

Students and Technicians: Santiago Zelenay, Angelo Chora.

CD4 regulatory T cells (Treg) have been implicated in the dampening of all aspect of an adaptive immune response, being protective or deleterious, directed at exogenous agents or self-components. In the past few years we set out to understand the nature of the triggering signal necessary for Treg function during inflammatory responses and concluded that inflammation per se and pro-inflammatory compounds together with TCR triggering define Treg activity. We next reasoned that the most likely common denominator to these various manifestations is innate inflammation and set out to test this hypothesis. We could evidence that Treg activity reduces the amount of TNF-alpha produced by peritoneal macrophages stimulated with the bacterial compound LPS. This inhibition requires TCR-MHC interaction and is IL-10, TGF-beta and IL-2 independent. Ongoing experiments are aiming at defining i) on the Treg side, the requirement for specific TCR-peptide interaction and ii) on the macrophage side, the molecular pathways triggered by Treg activity. The findings that Treg control inflammatory processes and that inflammation triggers Treg, lead us to speculate that Tregs may represent particular evolutionary intermediates between innate and adaptive immune cells. In this line of thoughts we tested whether Tregs make use of the innate anti-inflammatory enzyme HO-1 to exert their function. Functional analysis of various cellular subsets from HO-1 deficient mice in vitro and in vivo revealed that HO-1 expression by CD4 cells is not required for regulatory T cell function. Finally, we pursued our technological investment in setting the conditions to visualize in vivo the cellular interactions that take place during the process of immuno-regulation. Using multi-photon laser scanning microscopy enabled us to monitor simultaneously the motility of individual CD4 effector, Tregs and APC cells within inguinal and popliteal lymph nodes of live mice submitted to various inflammatory procedures.

Cellular immuno-regulation and autoimmune diseases.

Members: Iris Caramalho, Jocelyne Demengeot.

Students and Technicians: Francisca Moraes Fontes, Lurdes Duarte.

The findings that inflammatory and pro-inflammatory compounds trigger Tregs that dampen inflammatory responses, lead us to assess the effects of various pro-inflammatory and anti-inflammatory compounds on the incidence of autoimmune manifestations in various mouse models. We established that LPS treatment prevents the development of autoimmune diabetes in NOD mice by a mechanism that requires Tregs. In contrast, pertussis presumably by opening the Blood Brain Barrier allows the development of encephalomyelitis, a process we showed to be facilitated by Hydrocortisone. The cellular and molecular mechanisms involved in these contrasting regulations, by inflammatory and anti-inflammatory compounds, respectively, are under investigation. An extension of this work is the systematic analysis of the effects of one autoimmune disease (therefore ongoing inflammation) on the emergence of another.

Tolerance induction in autoimmunity: reprogramming the immune system with monoclonal antibodies.

Members: Luis Graça.

Students and Technicians: Ana Águas-Doce, Joana Duarte.

External Collaborators: Ruy Ribeiro (Los Alamos National Laboratory, NM, USA).

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease of unknown aetiology, afflicting about 1% of the world population, and characterized by synovial membrane inflammation in multiple joints. There is evidence for a role for arthritogenic CD4⁺ T lymphocytes in the pathogenesis of the disease, although other cell types such as B cells, fibroblasts and macrophages also seem to be involved in disease. The current treatment of RA is based on non-curative immunosuppressive and anti-inflammatory agents, including biological drugs such as monoclonal antibodies (mAbs) and soluble receptors targeting pro-inflammatory cytokines, like tumor necrosis factor- α (TNF- α). But these treatments do not work for all patients and, more importantly, can have severe side effects. Given the critical role of arthritogenic T cells in the pathogenesis of RA, alternative therapeutic strategies specifically targeting T cells, such as mAbs, have been proposed. However, until now, preclinical testing of mAbs relevant for RA has been hampered by the lack of animal models with spontaneous and chronic autoimmune arthritis. But recently mice were described, harbouring a mutated ZAP-70 gene leading to abnormal thymic T-cell selection, that spontaneously develop a chronic disease remarkably similar to human RA (SKG mice; Sakaguchi N et al; Nature 2003; 426:454).

We are using our experience with tolerogenic mAbs targeting T cells to investigate their ability to reprogram the immune system, and consequently treat arthritis in SKG mice. We have recently shown in mice that co-receptor blockade, co-stimulatory blockade, and more efficiently, a combination of both are effective to induce long-term tolerance to skin transplants (Graça L et al; PNAS 2004; 101:10122). We use anti-CD4, anti-CD40L and anti-OX40L mAbs, to investigate if these mAbs can lead to prevention and treatment of autoimmune arthritis rather than simply controlling inflammation (as with anti-TNF α therapy).

IL-10 and its role in regulation of immunological tolerance.

Member: Matthias Haury.

Students and Technicians: Dinis Calado, Rosa Maria Santos.

External Collaborators: Dan Holmberg (Univ. Umea, Umea, Sweden).

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.

Molecular mechanisms underlying the protective effect of HO-1 derived CO: interaction with the NF-Kappab signal transduction pathway.

Members: Miguel Soares, Gabriela Silva.

Student: Mark Pena Seldon.

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

Endothelial cell (EC) activation is a pre-requisite for effective immune responses to occur. However, it must be tightly regulated so that EC apoptosis and tissue injury do not occur. The transcription factor nuclear factor kappa B (NF- κ B) plays a pivotal role in this process. We have previously shown that NF- κ B activity can be regulated, in EC, by the expression of heme oxygenase-1 (HO-1), a stress responsive gene that degrades heme generating free Fe²⁺, inducing the expression of the Fe sequestering protein ferritin and activating a Fe-secreting ATPase pump. We asked whether modulation of the labile iron pool (LIP) by HO-1 in EC modulated NF- κ B driven gene expression. Intracellular Fe content, reporter assays and western blots were assayed using bovine aortic EC (BAEC). Transient over-expression of HO-1 in EC decreased the LIP by 16.6% (p=0.0039; n=9). By comparison, exposure of EC to the Fe chelator, desferoxmine mesylate (DFO)(125 μ M) decreased the LIP by 17.9% (p=0.0145; n=9). Expression of HO-1 inhibited TNF- α mediated NF- κ B mediated transcription, as assessed using a NF- κ B luciferase reporter assay. This effect was mimicked by DFO (125 μ M). Neither HO-1 expression nor Fe²⁺ chelation by DFO suppressed TNF- α mediated I κ B α degradation nor did they block NF- κ B (p65/RelA) nuclear translocation. Instead, Fe²⁺ chelation by DFO targets the N-terminal domain of RelA/p65, inhibiting its transcriptional activity, as assessed using chimeric RelA/p65 truncated mutants in a NF- κ B luciferase reporter assay. The effect of Fe chelation by DFO was specific to RelA/p65 as it did not interfere with cRel or p50 driven NF- κ B luciferase reporter transcription. Presumably inhibition of p65/RelA transcription occurs via modulation of its phosphorylation status. However, this does not seem to involve Ser536, a residue in the C-terminal region of p65/RelA that can regulate its activity. In conclusion, our present data suggests that HO-1 inhibits NF- κ B activity in EC by targeting specifically the N-terminal domain of p65/RelA. Presumably this inhibition of NF- κ B could account for the ability of HO-1 to modulate the expression of pro-inflammatory genes associated with EC activation.

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, Gabriela Silva.

Student: Andreia Cunha, Mark Pena Seldon.

External Collaborators: Leo Otterbein (Harvard Medical School, Boston, USA).

Heme oxygenase-1 (HO-1) protects endothelial cells (EC) from undergoing apoptosis. This effect is mimicked by carbon monoxide (CO), generated via the catabolism of heme by HO-1. The anti-apoptotic effect of CO was abrogated when activation of the p38 α and p38 β mitogen activated protein kinases was inhibited by the pyridinyl imidazol SB202190. Using small interfering RNA (siRNA), p38 β was found to be cytoprotective in primary EC while p38 α was not. When over-expressed in EC, HO-1 targeted specifically the p38 α but not the p38 β MAPK isoform for degradation by the 26S proteasome. This was also observed when HO-1 was induced physiologically by Fe protoporphyrin IX (hemin). Inhibition of p38 α no longer occurred when HO activity was inhibited by tin protoporphyrin, suggesting that p38 α degradation is mediated by an end product of heme catabolism by HO-1. In the absence of detectable HO-1, exogenous CO inhibited p38 α expression in EC, suggesting that it is CO that mediates this effect. The anti-apoptotic effect of HO-1 was impaired when p38 α expression was restored ectopically or when p38 α degradation was inhibited by the 26S proteasome inhibitor MG-132, demonstrating that the anti-apoptotic effect of HO-1 is dependent on the degradation of p38 α by the 26S proteasome.

Modulation of the pathogenesis of sepsis by HO-1.

Members: Miguel Soares.

Student: László Tokaji.

Septic shock remains a leading cause of death worldwide, affecting millions of individuals every year. Lethality, results from an uncontrolled inflammatory response culminating in the release of high mobility group box-1 (HMGB1) from activated leukocytes and necrotic cells. The cellular and molecular mechanisms regulating HMGB1 release remain elusive. We show hereby that heme oxygenase-1 (HO-1), a stress responsive gene that catabolizes heme into Fe, biliverdin and carbon monoxide, inhibits the systemic HMGB1 release that is normally associated with the development of septic shock. Cecal ligation and puncture (CLP) in BALB/c mice, a well established experimental model of septic shock, resulted in high levels of HO-1 expression by infiltrating peritoneal leukocytes. Mortality rate following CLP increased from 10% in wild type (ho-1+/+) mice to 90% in HO-1 deficient (ho-1-/-) mice (n=10-15 per genotype). Serum aspartate aminotransferase (AST), blood urea nitrogen (BUN) and creatine phosphokinase (CPK) after CLP were significantly ($p<0.05$) higher in ho-1-/- versus ho-1+/+ mice, showing that HO-1 is required to prevent multiple organ failure and lethality associated with the development of septic shock. Higher mortality rate of ho-1-/- versus ho-1+/+ mice correlated with a significant ($p<0.05$) increase in the number of infiltrating peritoneal leukocytes (3 fold) as well as peritoneal and serum HMGB1 (6 fold). Secretion of pro-inflammatory cytokines, i.e. TNF- α and IL-6 was not significantly ($p>0.05$) different in ho-1-/- versus ho-1+/+ mice. Peritoneal leukocytes isolated from ho-1-/- mice after CLP, released more HMGB1 in vitro than those isolated from ho-1+/+ mice. In conclusion, expression of HO-1 is part of a protective response that inhibits

systemic HMGB1 release by activated leukocytes. Most likely, this effect contributes to the ability of HO-1 to prevent lethality associated with the development of septic shock.

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

Member: Miguel Soares, Jocelyne Demengeot.

Students: Angelo Chora, Andreia Cunha.

External Collaborators: Paulo Fontoura (Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisbon, Portugal), Lawrence Steinman (Department of Neurology and Neurological Sciences, Beckman Center for Molecular Medicine, Stanford University, CA, USA).

Heme oxygenase-1 (HO-1) is a protective gene that generates carbon monoxide (CO) via the catabolism of heme. Deletion of ho-1 in C57Bl/6 mice resulted in exacerbated neuroinflammation, paralysis and mortality associated with experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis (MS). Induction of HO-1 by cobalt protoporphyrin IX (CoPPiX) reversed paralysis in C57Bl/6 and SJL/J mice with EAE, reducing i) central nervous system (CNS) demyelination, ii) major histocompatibility complex class II (MHC II) expression by microglia and dendritic cells, iii) T helper (T_H) cell proliferation and iv) interleukin-2 (IL-2) and interferon gamma (IFN- γ) production within the CNS. These effects were not observed in mice treated with zinc protoporphyrin IX (ZnPPiX), which does not induce HO-1. Inhaled CO mimicked broadly the protective effects of HO-1 induction. In conclusion, CO generated by HO-1 inhibits MHC II expression by antigen presenting cells, suppressing myelin-reactive T_H cell activation, and autoimmune neuroinflammation.

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

Member: Miguel Soares, Isabel Pombo Gregoire, Moises Mallo.

Student: László Tokaji.

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 central hypothesis of this project is that, when inhaled at low doses, inhaled carbon monoxide (CO) can mimic the anti-atherogenic effects of CO that is generated endogenously via the degradation of heme by heme oxygenase-1 (HO-1). We found that CO suppresses the development of atherosclerotic lesions such as they occur in response to hypercholesterolemia. We have tested this hypothesis in ApoE^{-/-} mice exposed to high cholesterol diet, a widely accepted experimental model of atherosclerosis. Inhaled CO (250 ppm) inhibits significantly the extent of atherosclerotic lesion formation and progression. CO did not inhibit the levels of total circulating cholesterol nor the ability of macrophages to uptake modified lipoproteins. Interestingly expression of vascular cell adhesion molecule 1 (VCAM-1)

and E-selectin were significantly inhibited in aortas of ApoE^{-/-} mice treated with CO. Moreover macrophage recruitment to the vessel wall was impaired in CO treated mice and TNF α expression in the vessel was reduced upon treatment with CO. In contrast, expression of both HO-1 and PPAR γ in the aorta was significantly higher in CO treated animals. We conclude that CO protects from atherosclerosis and that the mechanism underlying this effect possibly relies on the inhibition of EC activation and macrophage recruitment into lesion prone sites via a HO-1 and/or PPAR γ dependent mechanism.

Expression of heme oxygenase-1 in regulatory T cells.

Members: Miguel Soares, Jocelyne Demengeot.

Students: Santiago Zelenay, Angelo Chora.

CD4 regulatory T cells (T_R) ensure peripheral tolerance to self-antigens and limit the deleterious effects associated with inflammatory and immune responses by mechanisms that remain to be elucidated. Recently the heme-degrading enzyme Heme Oxygenase-1 (HO-1) and its product carbon monoxide have been implicated in human T_R function. In the present work, we used HO-1 deficient (HO-1^{-/-}) mice to study the putative role of HO-1 in T_R development and function. We have shown that CD25⁺Foxp3⁺ T_R occur with normal frequency in HO-1 deficient mice. Proliferation *in vitro* and expansion *in vivo* of CD4 T cells is equally controlled by CD4⁺CD25⁺ T cells whether or not HO-1 is expressed in the responder or the T_R cells. These findings demonstrate that HO-1 expression by T_R is neither required for their development nor for their activity.

Malaria and 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 5 years of age, representing nearly 10% of over 10 million children who die at these ages. 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.

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 yet to be fully explained. We currently lack an efficacious vaccine against malaria. This may be 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, several groups are 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. This work has led to the identification of several relevant chromosomal regions, and the isolation of the responsible genes is now underway, while mapping loci controlling hepatic infection. The extension of such genetic analyses to human populations has now been initiated in the Island of Príncipe, in a close collaboration with the Government of S. Tomé e Príncipe and the Cooperation Sector of the Gulbenkian Foundation. The availability of the complete genomes of several *Plasmodia*, on the other hand, makes it possible to search for molecules that activate “innate immunity”, or participate of other interactions with host cell receptors that are necessary for infection. Because inflammatory reactions can be pathogenic, cerebral malaria representing one such example, regulation of the acute responses to infection must be investigated.

The risk for malaria infection and disease varies wildly across Tropical Africa, and the overall results of therapeutic or environmental interventions also vary 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.

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”.

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- α , 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- κ B 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- κ B 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- κ B 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, 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 in the full development of the malaria parasite inside hepatocytes

Members: Maria Mota, Sabrina Epiphany, Miguel Prudêncio.

Students and Technicians: Cristina Rodrigues, Sónia Albuquerque.

External Collaborators: Cenix BioSciences, Dresden, Germany.

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 the clinical phase of infection. Because liver infection is the first obligatory step of infection, hepatocyte-*Plasmodium* interactions crucial for the establishment of infection, constitute an ideal target for potential anti-malarial vaccines or preventive treatments.

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 through its receptor MET, renders hepatocytes susceptible to infection. These results strongly suggest that the host cell has an important role on the success of infection. Additionally, *Plasmodium* sporozoites are able to enter any type of cell tested so far but only fully develop inside hepatocytes indicating a crucial role of the host cell in sustaining the growth and development of *Plasmodium*. This specificity is thought to be mediated by the unique ability of hepatocytes to provide an adequate environment for sporozoite development. The reason, however, why *Plasmodium* sporozoites are only able to develop within hepatocytes remains unknown. We propose to determine the host cell molecules and mechanisms required for proper parasite development inside hepatocytes. On one hand, we will focus on mechanisms that might be involved on the HGF/MET signaling. On the other hand, we will use two general methodologies to identify novel host cell factors crucial for infection. We expect to provide a significant contribution to the understanding of the mechanisms mediating *Plasmodium*/hepatocyte interactions, which might have important implications in the prophylaxis and/or treatment of malaria.

The role of hemeoxygenase-1 and its products in the course and pathology of a malaria infection.

Members: Maria Mota, Ana Pamplona, Sabrina Epiphanyo, Miguel Soares.
Students and Technicians: Margarida Cunha.

Malaria is a devastating disease that affects extensive areas of Africa, Asia, South and Central America, causing more than one million deaths per year in children under the age of five. Malaria is caused by the infection of the protozoan parasite *Plasmodium*, which belongs to the phylum Apicomplexa. Attempts to eradicate malaria have so far been unsuccessful. Their failure can be attributed to increasing resistance to insecticides in the mosquito vector and to anti-malarial drugs in the parasite. There is an urgent need of developing novel strategies against malaria.

Cerebral Malaria (CM) is a major cause of death by malaria infections. Although the nature of pathogenetic processes leading to the cerebral complications is poorly understood, activation of brain microvascular endothelial cells seems to play a pivotal role in this syndrome. When endothelial cells become activated they produce a series of pro-inflammatory molecules. To prevent an inflated activation that could lead to endothelial cell injury, the expression of these pro-inflammatory molecules needs to be tightly regulated. One of the mechanisms by which this occurs relies on the expression of “protective” genes. One such gene is the stress responsive gene heme oxygenase 1 (HO-1). Our preliminary results show that HO-1 activation protects susceptible mice from CM, which could be used in the endemic areas to protect children against this devastating syndrome. We now propose to elucidate the role of HO-1 and its products (carbon monoxide and biliverdin) in the course and pathology of malaria.

Expression of heme oxygenase-1 controls the pathogenesis of severe acute malaria (SAM).

Member: Miguel Soares, Maria Manuel Mota, Ana Pamplona.

Student: Angelo Chora.

Plasmodium, the causative agent of malaria is responsible for about 1.5 million deaths per year worldwide. During the blood stages of *Plasmodium* infection some individuals develop cerebral malaria (CM), the main cause of death from malaria. This stage of infection causes severe hemolysis and release of the pro-oxidant heme contained within hemoglobin, which up-regulates the expression of heme oxygenase-1 (HO-1), an enzyme that degrades heme into Fe^{++} , carbon monoxide (CO) and biliverdin. We found that susceptibility to murine CM is controlled by HO-1. CM did not develop in most of *P. berghei* ANKA infected BALB/c mice that up-regulated HO-1 very significantly following infection. CM developed promptly in all infected C57BL/6 mice that up-regulated HO-1 to a much lesser extent. Genetic deletion of HO-1 or inhibition of its enzymatic activity by zinc protoporphyrin IX (ZnPPiX) increased CM incidence in BALB/c mice to 58% and 77.5%, respectively. HO-1 induction by cobalt protoporphyrin IX (CoPPiX) reduced CM incidence in C57BL/6 mice to 10%. Inhaled CO (250 ppm; 24h) mimicked the protective effect of HO-1 in C57BL/6 mice, reducing CM incidence to 0%. HO-1 and/or CO did not affect parasitemia. CO inhibited blood brain barrier leakage, vascular congestion as well as lymphotoxin- α (LT- α), tumour necrosis factor- α (TNF- α) and interferon- γ (IFN- γ) expression in the brain, all of which mediate the pathogenesis of murine CM. In conclusion, HO-1 and presumably CO control susceptibility to CM and provide a new therapeutic approach to block CM pathogenesis.

T- Cell response in pathogenesis of malaria.

Members: Sylviane Pied, Margarida Vigario.

Students and Technicians: Tania Cruz.

External Collaborators: Virgilio do Rosario (CMDT Lisbon, Portugal); Antonio Bandeira (Institut Pasteur, Paris, France) Pierre-André Cazenave (Institut Pasteur, Paris, France).

Several observations in malaria patients and mouse experimental models suggest that T cells are implicated in cerebral malaria (CM) pathogenesis. Particularly, we and others have demonstrated in C57BL/6 mice infected with *Plasmodium berghei* ANKA (*PbA*), even if this model does not reproduce all the features of human CM, that the occurrence of the neuropathology in *PbA*-infected mice correlates with a significant increase of $\text{CD8}^+\text{TCRV}\beta 8^+$ cells in the peripheral blood and with an accumulation in the brain of activated CD8^+ T cells producing IFN- γ and TNF- α , two cytokines known to be mediators of CM. The exact mechanism by which these cells are involved is at present unknown. We first hypothesized that in CM susceptible mice the neuropathology could be, at least in part, the result of an inefficient control of pathogenic effector T cells by $\text{CD4}^+\text{CD25}^+$ regulatory T.

We initially showed in CM susceptible mice infected with *PbA* that the number of $\text{CD4}^+\text{CD25}^+$ T cells increased in the spleen during infection. These $\text{CD4}^+\text{CD25}^+$ Treg cells displayed an activated phenotype and showed an enhanced regulatory activity *in vitro*. By performing intracellular staining on the $\text{CD4}^+\text{CD25}^+$ population of Foxp3, a transcription factor expressed only by the subpopulation of regulatory T cells, we

confirmed that in day 6 infected mice 80% of these cells are Foxp3⁺ which confirms their regulatory nature (A. M. Vigario *et al.*, *submitted for publication*). We also analysed the kinetic of the regulatory T cells in mice infected with a different line of the same *Plasmodium* specie, *P. berghei* NK65, which does not induce CM. In this later case, we observed a similar increase of the CD4⁺CD25⁺Foxp3⁺ T cell subpopulation as well an increase on their activated *status* and their regulatory activity.

Taken together, these results exclude the possibility that the regulatory T cell population defined as CD4⁺CD25⁺Foxp3⁺ is implicated in protection against CM.

Furthermore, by analysing the brain of *Pb*NK65 infected mice, we surprisingly found an accumulation of an important number of CD8 T cells as observed in *PbA* infected mice developing CM. CD8 T cells accumulation in brain of non CM models of *Plasmodium* infection was never described before. Moreover, in contrary to the CM model the T cell infiltration was not followed by a BBB breakdown. This suggests that either other mechanisms control locally the pathology in these mice at a later stage of the infection or, that the brain infiltrated cells have a different behaviour in these two models. Experiments are undergoing to dissect these mechanisms.

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

Members: Sylviane Pied, Johann Truccolo, Margarida Vigario, Jorg Becker.

External Collaborators: Virgilio do Rosario (CMDT, Lisbon Portugal); Beatrice Reagnault (Institut Pasteur, Paris, France).

Both host and parasite *Plasmodium* factors play a role in mechanisms leading to the development of the neuropathology of severe malaria. However, mechanisms resulting from the interactions between the parasite, T lymphocytes and brain cells in cerebral malaria (CM) remain totally unknown. The aim of this project is to assess the cascade of events during malaria parasite - specific T-cells and brain-blood-barrier (BBB) interactions. Using an *in vitro* model of BBB, we analysed changes in astrocytes, endothelial and microglial cells genes transcription following exposure to 1) the parasite itself and 2) CD8⁺ T cells recruited in the brain of *Plasmodium berghei* ANKA (*PbA*) infected mice developing CM.

Two types of co-culture systems have been used. In co-cultures A, endothelial cells were directly in contact with astrocytes and microglial cells whereas in co-culture B, the endothelium was separated from the other cells by a membrane. The different types of cultures have been incubated or not with erythrocytic stages of *PbA* 48 hours before to add the lymphocytes. *PbA* infected red blood cells have been removed from the cultures and changes resulting from interaction with T-cells were assessed 6 hours and 2 days after the addition of CM⁺ mice brain CD8⁺ T cells. CD8⁺ T cells purified from spleen of non infected mice were used as control because few T lymphocytes are available in the brain under steady state conditions. These two models of culture allow differentiating between modifications induced in microglia and astrocytes through factors released by parasite activated endothelial cells from those associated to direct cell to cell contacts. For genomic analyses, mRNA purification was done from microglial cells and astrocyte

co-cultures after removing the T cells by successive washing. Data obtained were compared with those of naive microglial, astrocyte and endothelial cells. Non stimulated cell cultures were also used as controls. Gene expression analysis was done using the Affymetrix GeneChip mouse genome 430 2.0 microarray (up to 30000 genes). This strategy allows a global and objective comparison at the transcriptional level of changes induced in the BBB cultures after exposure to parasite-induced T-cells.

Data obtained from the analysis of the genes expression using different software such as S-Plus, Onto-Express or Cluster TreeView showed that stimulation of the different BBB cell cultures with *PbA* induced T cells leads to up or down-regulation of 5 to 13 genes. Some of these genes are involved in the immune response. A real-time PCR is undergoing to confirm at a quantitative level these results. In addition, a functional analysis will be performed to define whether these genes are involved *in vivo* in the neuropathology. In parallel to genomics, immunohistochemistry coupled to dynamic imaging will be used to study how these gene products interfere with mechanisms leading to CM.

Self-reactive antibodies produced during *Plasmodium falciparum* infection contribute to cerebral malaria and protection in asymptomatic patients.

Members: Sylviane Pied, Youri Chanseaud, Constantin Fesel.

Students: Vincent Guiyedi, Joana Duarte.

External Collaborators: Maryvonne Kombila (CHL, Libreville, Gabon); Gyan Mishra (NCCS, Pune, India) Abdelkader Namane, Institut Pasteur Paris, France).

Autoantibody production is a common feature of *Plasmodium* infections. However, the role of self-reactive antibodies in the development of cerebral malaria (CM) has not been studied. We used a combination of quantitative immunoblotting and multivariate analysis to compare the correlation between the reactivity of circulating IgG with a human brain protein extract and cytokine profile in cohorts of uninfected controls (UI) and *P. falciparum* (*Pf*)-infected Gabonese children developing uncomplicated malaria (UM), severe non-cerebral malaria (SNCM) or CM. The repertoire of brain antigens recognized by plasma IgGs was more diverse in infected than in UI individuals. Anti-brain reactivity was significantly higher in the CM group than in the UM and SNCM groups. IgG self-reactivity to brain antigens was also correlated with plasma IgG levels and age. We found that 90% of CM patients, 50% of SNCM patients, 44% of UI subjects and 39% of UM patients displayed reactivity to a high-molecular weight band. In addition, reactivity with this band was correlated with high TNF α concentrations in CM patients. No correlation with IFN γ and IL-10 levels was observed. These results strongly suggest that *Pf* infection induces an antibody response to brain antigens that may be associated with pathogenic mechanisms in patients developing CM (V. Guiyedi, C. Fesel, Y. Chanseaud *et al.*, *submitted for publication*). Protein(s) contained in this band is under identification using peptide mass fingerprinting with a matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometer. This work should also lead to the definition of new targets of potential value as markers of disease severity and of the risk of developing CM.

In parallel, we also analysed the IgE response during *Pf* malaria. IgE has been proposed to play different roles in malaria that still need to be clarified. IgE complexed to parasite were observed to be sequestered into brain microvessels of patients developing CM. A positive correlation between the levels of IgE, IgE immune complexes and levels of TNF- α , which is known to be involved in the development of severe disease, was also found in these patients. To assess whether the IgE response induced in *P. falciparum* infected patients is associated with malaria pathology, we first analysed the global repertoire of self-reactive IgE produced against a large panel of human cerebral antigens, in patients Indian and Gabonese patients with different clinical manifestations of malaria CM, SNCM and UM in comparison to asymptomatic and uninfected ones (J. Duarte, V. Guiyedi, C. Fesel, *et al.*, *manuscript in preparation*). We also studied the functionality of IgE recognizing brain antigens and look whether they correlate with development of CM by comparison with the other clinical groups. Data obtained showed that the asymptomatic infected group presented the highest auto-reactivity profile and the highest percentage of patients with functional IgE against brain when compared with the other clinical groups. Finally, we also found an association between IgE autoantibody repertoire, cytokine profile and parasitemia in the asymptomatics.

Role of microglia in neuropathogenesis.

Members: Sukalyan Chatterjee, Teresa Faria Pais.

Students and Technicians: Catarina Figueiredo, M^a Hortense Matos.

In the last two decades numerous studies both in humans and in animal models have shown that neurodegenerative disorders and infections of the CNS are concomitant with the activation of brain macrophages, historically known as microglial cells. Although, like other glial cells, brain macrophages may have a neuroprotective role their uncontrolled activation enhances the neuropathology associated with the different diseases. This deleterious effect is mediated by the release of inflammatory cytokines TNF- α , IL-1 α , IL-6 and IL-23 and also of neurotoxic molecules like NO and quinolinic acid.

Due to the key role of brain macrophages in the CNS pathology there has been an increasing interest in the characterization of the different types of cell activation and populations involved in brain inflammation and/or infection. Although the activation of brain macrophages is associated with both human and mouse cerebral malaria (CM) the relative contributions of the heterogeneous populations of brain macrophages to the disease are unknown. We have recently published the characterization of the activation phenotype of brain macrophage in mice developing CM taking into account the different cell subtypes and their origin. We have shown that the development of CM is associated with the accumulation and proliferation of CD8⁺ T cells in the brain. We have identified MHC class I and Sca-1 as activation markers of parenchymal brain macrophages during CM. The expression of MHC class I precedes the appearance of leukocytes in the brain suggesting a likely role for activated brain macrophages in the sequestration and activation/proliferation of CD8⁺ T lymphocytes. Thus, our results suggest that parenchymal brain macrophages are most likely contributing to the subsequent phases of

infection that culminate in death of mice with CM syndrome, rather than just being activated as a consequence of overwhelming brain inflammation.

We have also studied the interplay between cerebellar granule neurons (CGN) and microglial cells in the perspective of CGN survival to quinolinic acid (QA) induced cell death. QA is a neurotoxic molecule secreted by microglia and elevated concentrations of QA have been proposed as cause of cell death and subsequent pathology, such as Huntington's disease, HIV encephalopathy and cerebral malaria. We find CGN succumbs to QA challenge in a NMDA receptor dependent manner whereas microglia is resistant to QA due to the absence of functional NMDA receptor. We have determined the expression profile of NMDA receptor subunits in microglia and also show that the signaling cascade active in CGN culminating in cell death is inactive in microglia. Contrary to reports in the literature, in our system of cell culture microglia conditioned medium (MCM) is toxic for CGN, but it can be attenuated if microglia is pre-cultured with neuronal conditioned medium (NCM). QA mediated CGN death can be rescued either by mixing the two cell types or by using mixed culture conditioned medium (MCM). We present the putative identity of the survival factor and indicate the possible survival pathway operating in CGN. We propose neuronal secreted factor(s) which favours the secretion of survival factors from the microglia. Our work shows that neuron-microglia bidirectional signaling assures neuronal survival to excitotoxicity.

Developmental Biology in Animals and Plants

The search for the mechanisms that guide the affairs of an embryo in its way from fertilization to a full-grown organism is a major topic at the IGC, the variety and complexity of the underlying processes being reflected in the diversity of questions, approaches and biological models employed by our groups. 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.

Unquestionably, the Developmental Biology groups have had, and continue to have, a major part in the scientific outputs of the IGC and in building its international reputation of excellence. In addition, these groups and respective leaders play a critical role in driving the set-up, development and best usage of basic facilities at the Institute, such as the imaging, transgenic mice, and Affymetrix gene chip units.

Canonical Wnt signaling and its antagonist regulate anterior-posterior axis polarization by guiding cell migration in mouse visceral endoderm.

Members: José A. Belo.

Students and Technicians: Mário Filipe.

External Collaborators: Isao Matsuo (Riken Center for Developmental Biology, Hyogo, Japan).

The mouse embryonic axis is initially formed with a proximal-distal orientation followed by subsequent conversion to a prospective anterior-posterior (A-P) polarity with directional migration of visceral endoderm cells. Importantly, *Otx2*, a homeobox gene, is essential to this developmental process. However, the genetic regulatory mechanism governing axis conversion is poorly understood. Here, defective axis conversion due to *Otx2* deficiency can be rescued by expression of *Dkk1*, a Wnt antagonist, or following removal of one copy of the β -catenin gene. Misexpression of a canonical Wnt ligand can also inhibit correct A-P axis rotation. Moreover, asymmetrical distribution of β -catenin localization is impaired in the *Otx2*-deficient and Wnt-misexpressing visceral endoderm. Concurrently, canonical Wnt and Dkk1 function as repulsive and attractive guidance cues, respectively, in the migration of visceral endoderm cells. We propose that Wnt/ β -

catenin signaling mediates A-P axis polarization by guiding cell migration toward the prospective anterior in the pregastrula mouse embryo.

Identification of alternative promoter usage for the matrix Gla protein gene.

Members: José A. Belo.

Students and Technicians: Ana Cristina Silva.

External Collaborators: Leonor Cancela and Natércia Conceição (CCMAR, UAlg, Portugal).

Recent cloning of the *Xenopus laevis* (Xl) matrix Gla protein (MGP) gene indicated the presence of a conserved overall structure for this gene between mammals and amphibians but identified an additional 5'-exon, not detected in mammals, flanked by a functional, calcium-sensitive promoter, 3042 bp distant from the ATG initiation codon. DNA sequence analysis identified a second TATA-like DNA motif located at the 3' end of intron 1 and adjacent to the ATG-containing second exon. This putative proximal promoter was found to direct transcription of the luciferase reporter gene in the X. laevis A6 cell line, a result confirmed by subsequent deletion mutant analysis. RT-PCR analysis of XlMGP gene expression during early development identified a different temporal expression of the two transcripts, strongly suggesting differential promoter activation under the control of either maternally inherited or developmentally induced regulatory factors. Our results provide further evidence of the usefulness of non mammalian model systems to elucidate the complex regulation of MGP gene transcription and raise the possibility that a similar mechanism of regulation may also exist in mammals.

Collaborators:

Arteriogenesis – identification of novel members of the Notch pathway involved in arterial cell fate determination.

Members: Ana Cristina Borges, Antonio Duarte, Jorg Becker.

Students and Technicians: Alexandre Trindade.

Notch and its ligands participate in an evolutionary conserved signaling pathway that functions to modulate cell-fate decisions of a variety of cell types originating from all three germ layers.

Mutations in humans, mice and zebrafish demonstrated the importance of Notch signalling in the regulation of vascular development. In zebrafish, Notch signaling is required for arterial identity by suppressing the venous fate in developing artery cell. In mice, Notch4 and Dll4 are specifically expressed in arterial endothelial cells, suggesting a similar role. Knockout of the Notch4 receptor gave no obvious phenotype alone, although *Notch1/Notch4* double mutant embryos show severe vascular remodeling defects, also observed in *Hey1/Hey2* double mutants. In contrast, we have shown that the Dll4 ligand ALONE is required in a dosage-sensitive manner for normal arterial patterning in development. We observed lethal haploinsufficiency in *Dll4*^{+/-} embryos, with disrupted

vascular network and aortic atrophy. Dll4 overexpression, on the other hand, caused aortic hypertrophy and loss of vascular identity. In the Dll4 knockout embryos we observed ectopic expression of venous markers in the dorsal aortae whereas in the Dll4 overexpression transgenics arterial markers were expressed in the cardinal veins.

These two complementary phenotypes are suggestive of an important role for Dll4 in the development of the vascular bed, in particular in arteriogenesis, where it may be responsible for the activation of genes involved in the establishment of the arterial endothelial cell phenotype. Although there is compelling evidence for such a role, nothing is yet known on its mechanistic basis. It is this void which we will attempt to fill by discovering the downstream genes that are responsible for mediating the effect of Notch activation in the endothelial cells.

We propose to characterize the gene expression profile of endothelial cells in these two mutant strains by microarray analysis and expression pattern characterization in order to identify novel genes situated downstream of Notch signalling in mammalian arteriogenesis. We hope that our understanding of the molecular mechanisms by which Notch regulates arterial/venous specification may provide insights into the pathological angiogenesis that support cancer growth and tools with which we may induce arteriogenesis in ischemic tissues.

Use of transgenic conditional overexpression to address the function of a novel mammalian Delta homologue, mDll4.

Members: António Duarte.

Students and Technicians: Rui Benedito, Alexandre Trindade, Sónia Ventura.

The lethal haploinsufficiency displayed by Dll4 heterozygous mutants and constitutive overexpression transgenics makes it impossible to characterize Dll4 gain or loss-of-function phenotypes after E9.5. This precludes the study of the role of this ligand in the retinal neuroepithelium and thymus, where its expression is very abundant, as well as in adult endothelium. To circumvent this difficulty, we aim to produce conditional mutants using the cre/loxP system.

A genetic and molecular approach to the biophysics of cell-cell communication during sexual reproduction in plants.

Members: José Feijó, Leonor C. Boavida.

Students and Technicians: Ana Maria Vieira, Ana Margarida Prado.

The proposing team has built over the recent years a unique background of physiological and molecular tools to dissect the process of pollen tube growth, as a paradigm of cellular growth and morphogenesis of apical growing cells. Data acquired has been used to generate original models based on the general concept of ion dynamics, now boosted by large scale genomic analysis. All this data has been generated using the model properties on pollen tubes grown *in vitro*. We now propose to use the experience and knowledge

gained in this reductionist experimental environment to gain knowledge on the real evolutionary context of pollen tube growth, the pollen-pistil interaction that underlies sexual reproduction and seed set in higher plants. The regulation of interactions between pollen and pistil is poorly understood. It involves different check-points imposed at distinct levels and extremely well tuned: recognition and pollen hydration (metabolic activation), germination and stigma penetration, pollen tube guidance and feeding in the pistil. These events ultimately determine alone or simultaneously, if fertilization takes place or not. We intend to specifically explore the biological interactions and reactions occurring during the reproductive process in plants, with the aim to approach some of the fundamental questions of communication and cell-cell interaction, namely the type of responses from growing pollen tubes to external signals *in vivo*. We aim to identify and characterize molecular and/or biochemically the molecules involved. For a primary approach to this question, we intend to use two different and complementary strategies: (1) the identification and molecular characterization of functional mutants, performing a forward genetic screen in *Arabidopsis thaliana* for selection of mutants affected in the different check-points in the reproductive process; (2) using genome-wide DNA microarrays (Affymetrix GeneChip- full *Arabidopsis* genome), an already established technique in the proposing lab, to characterise gene expression profiles at different steps of the process of the generated mutants. Collaborations with 2 top labs in the world have been established for support on the genetics side. Concurrently, we intend to set a pioneer experimental approach, using non-invasive techniques, which minimize secondary signals not related with the system, resorting to advanced video microscopy techniques and to electrochemical detection of molecular fluxes in real time and *in vivo*. Using open-ovary floral mutants already available, or screened, and transgenic lines expressing GFP in pollen, we aim to experimental dissect the processes of metabolic activation in the male gametophyte, morphofunctional variations of pollen tube growth or guidance in the pistil, as well as detection of gradients of small molecules or ionic currents, which may take part into short or long range communication. Special emphasis will be given to (1) the study of the role of Nitric Oxide, for which good preliminary data indicates a crucial role in pollen tube growth and re-orientation and (2) the role of hydrogels in the control of hydration of pollen and metabolic re-activation. This approach is crucial for the strategy of the Plant Development Group at Instituto Gulbenkian de Ciência (IGC). It will build interfaces between the work at the level of molecular and physiological mechanisms of pollen tubes growing *in vitro*, and the understanding of the biological context in which pollen tubes evolved, through the interaction with the female tissues.

A comparative analysis of the arabidopsis sperm cell transcriptome to decipher the role of the male gametes in double fertilization.

Members: Jörg Becker, José Feijo.

Students and Technicians: Ana Maria Vieira, Nuno Moreno Gabriela Gomes.

External Collaborators: Sheila Mc Cormick (Berkeley, CA, USA).

Despite the fact that double fertilization in angiosperms was described more than a century ago, the way in which the male gametes participate in this process is still poorly

understood. Though they are passively transported to the embryo sac by the pollen tube, preferential fertilization and delayed sexual fusion to achieve cell cycle synchrony indicate that a complex program of interactions between the fusion partners must exist. In addition, there is accumulating evidence that sperm cells are not quiescent. This project aims at analyzing the transcriptome of Arabidopsis sperm cells on a full-genome level using Affymetrix Arabidopsis ATH1 GeneChips to decipher the genetic basis that underlies sperm cell functions in the fertilization process. A comparative analysis of the genes expressed in Arabidopsis sperm cells with the group's transcriptome data on vegetative tissues, pollen and pollen-pistil interactions as well as publicly available ATH1 data sets will lead to the identification of sperm enriched and selectively expressed transcripts. An Arabidopsis Reproductive Transcriptome Database will be created to make these data sets available to the research community. Detailed studies of sperm cell transcripts with a variety of molecular tools available for Arabidopsis thaliana might ultimately result in the identification of proteins mediating gamete recognition and fusion. More importantly, the identification and study of male gamete-specific promoter elements might eventually help to develop new tools for crop improvement.

Cell fate and cell polarity within the vertebrate embryonic neuroepithelium.

Members: Domingos Henrique, Evguenia Bekman, Cláudia Valente.

Students and Technicians: Rita Fior, Carolina Perdigoto, Alina Costa.

External Collaborators: Olivier Pourquié, Fernando Giraldez, Isabel Varela-Nieto, François Schweisguth, André LeBivic.

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 affect 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.

Wound repair using *Drosophila* as a model system

Members: António Jacinto, Will Wood.

Students and Technicians: Isabel Campos.

External Collaborators: Paul Martin (University of Bristol, Bristol, 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.

Cadherins and adhesion

Members: António Jacinto.

Students and Technicians: Sérgio Simões, Catarina Moita.

External Collaborators: James Castelli-Gair (CABD Seville, Spain).

The Cadherin superfamily is a diverse and multifunctional group of proteins with extensive representation across evolution that is involved in cell-cell communication and adhesion. We have performed a bioinformatics study to further understand and document this gene family and we have investigated experimentally the function of a small group of this family that had not been characterized before. We have used bioinformatics tools to retrieve present sequence knowledge about the complete repertoire of cadherins in *Anopheles gambiae*, an emerging model organism for the study of innate immunity and host-pathogen interactions, and compared it to *Drosophila*. In *Anopheles gambiae*, we have identified 43 genes coding for cadherin extracellular domains and have revised the *Drosophila* repertoire, which includes 17 genes.

In *Drosophila* there are 17 members of this superfamily, many of which are uncharacterised and lack a catenin binding domain, that is characteristic of classical cadherins, thus defining the non-classical subfamily. We've identified four novel non-classical Cadherins that are expressed dynamically during morphogenesis of the posterior spiracles, the major respiratory organ after larval hatching. Surprisingly, these Cadherins are expressed in subsets of posterior spiracle cells that show different morphogenetic behaviours. The most internal cells invaginate and connect to the tracheal dorsal trunk, acquiring a bottleneck shape while expressing cad96C, cad88C and cad74 according to their relative position along the formed chamber. We have evidence for two sorted subsets of bottle cells: a deeper subset that co-expresses cad88C and cad96C and a more superficial subset expressing cad74B. Moreover, cad86C is expressed by the most external cells that surround the invaginated bottle cells. These external cells intercalate in order to make a dome structure – the stigmatophore. These results indicate that non classical Cadherin expression may be a key factor in determining cell sorting during organ shaping. We are investigating whether differential adhesion mediated by homophilic cadherins is used to control cell shape and movement during morphogenesis, since differential Cadherin expression correlates with specific cell position and shape in the posterior spiracles.

Epithelial dynamics and adhesion during *Drosophila* dorsal closure.

Members: António Jacinto.

Students and Technicians: Beatriz Garcia, Ana Catarina Sarzedas.

External Collaborators: Alfonso Martinez Arias (University of Cambridge, Cambridge, UK).

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. 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.

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

Members: António Jacinto, Will Wood.

Students and Technicians: Célia Faria, Jennifer Geiger.

External Collaborators: Paul Martin (University of Bristol, Bristol, 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.

Hox genes in the development of the axial skeleton.

Members: Moisés Mallo.

Students and Technicians: Marta Carapuço, Tânia Vinagre, Ana Nóvoa, Joana Bom

External Collaborators: Nicoletta Bobola (Max-Planck Institute of Immunobiology, Freiburg, Germany).

Hox genes have been known for a long time to play a central role in defining the segmental identity of the different pieces of the axial skeleton (mostly the vertebrae and ribs) along the rostro-caudal axis. In the embryo, this skeleton derives from the somites, which are located at both sides of the neural tube all along the rostro-caudal axis of the embryo. Classically, it has been considered that Hox genes act on the differentiating somites to define the structures they are going to form. This view was often difficult to reconcile with a variety of experimental data, mostly that from gene inactivation experiments. For instance, recent genetic studies revealed that these genes are functionally relevant up to the thoracic/lumbar transition, but published expression patterns for the 3 Hox group 10 genes (*Hoxa10*, *Hoxc10* and *Hoxd10*) rarely extend to the corresponding somitic level, which in mice corresponds to somite 25, and seem to

differ correlating with the embryonic stage analyzed. We reevaluated the expression of the three Hox10 group at various embryonic stages to find that the Hox10 group expression domain indeed corresponds to the genetically defined functional domain but only at the stage at which the somites that correspond to the thoracic/lumbar transition are being formed in the presomitic mesoderm, which suggested that the activity of these genes is functionally relevant at this stage of somite development. We evaluated this hypothesis by comparing the activity of a Hox10 group gene in the presomitic versus the somitic paraxial mesoderm in transgenic mice. Similar experiments were performed with a Hox11 group gene. Our results show that the relevant function of Hox genes is provided in the presomitic mesoderm. We further sustain this conclusion with the finding that Gbx2, another homeobox-containing gene expressed in the presomitic and not in the somitic mesoderm, is required for proper patterning of the axial skeleton.

Hoxb4 in the proliferation of hematopoietic stem cells.

Members: Moisés Mallo, Leonor Parreira.

Students and Technicians: Ana Catarina Ribeiro.

It has been established that the Hoxb4 gene is able to expand hematopoietic progenitor cells. Most interestingly, these expanded hematopoietic progenitors are able to produce the whole complement of hematopoietic lineages without inducing leukemia. We are investigating the mechanisms mediating Hoxb4 activity in this process taking advantage of a embryonic stem cell (ESC) line that has been modified to allow controlled Hoxb4 expression by the tet-on system. We have found that Hoxb4 modulates Fgf signalling in the progenitor cells and that this modulation is mediated by a soluble factor. In fact, we have been able to produce expansion of hematopoietic progenitors using Hoxb4 conditioned media. We have also made progress in the identification of this active factor and we are testing its ability to produce functional expansion of hematopoietic progenitor cells obtained from the bone marrow. In addition, we have already identified potential Hoxb4 targets in the ESCs using a differential screening strategy based on Affymetrix GeneChip technology. We are currently testing the functional relevance of these target genes by producing ESCs in which expression of these genes can be modulated by the tet-on system. These cells will be used to test the effect of our candidate effectors in promoting hematopoiesis, using both in vitro (methylcellulose assays) and in vivo (repopulation of the hematopoietic system of lethally irradiated mice) approaches.

The role of the antisense transcript of Hoxb3 in mouse development.

Members: Moisés Mallo.

Students and Technicians: Victoria Gallego, Ana Nóvoa, Joana Bom.

We have recently found the production of an antisense transcript from the Hoxb3 locus in the mouse. This transcript (Hoxb3AS), which is non-coding and is produced as an intron-containing precursor, overlaps with the downstream exon of the Hoxb3 transcript

(Hoxb3S). Expression analysis using in situ hybridization revealed that Hoxb3AS has a defined expression pattern which is complementary to that of the normal Hoxb3 transcript. This finding suggests that Hoxb3AS might have a regulatory role in the expression of Hoxb3S. We are investigating this role using a combination of approaches to manipulate expression of Hoxb3AS in vivo. This includes the identification of the enhancer/promoter elements controlling the expression of Hoxb3AS and their ulterior inactivation in vivo. In addition, we are performing misexpression experiments to see the effects of the mature and immature Hoxb3AS transcripts on the expression of Hoxb3S.

Development of the heart outflow tract and the role of Tbx1.

Members: Moisés Mallo.

Students and Technicians: Filipa Moraes, Ana Nóvoa, Joana Bom.

The heart outflow tract from newborn and adult animals (i.e. the arteries that organize the distribution of the blood to the body as it leaves the heart chambers) is formed in the embryo by a complex morphogenetic process that involves the formation and restructuration of the embryonic aortic arches. The success of this process depends on complex interactions between the endoderm, ectoderm, mesoderm and neural crest cells within the branchial arches and requires the activity of many genes and signalling pathways. Tbx1 is one of those genes. We are studying how the embryonic aortic arches are formed both in normal and in Tbx1 mutant embryos and the role of the neural crest cells in this process. We are approaching these questions using several strains of transgenic mice. The formation of the aortic arches is being analyzed using a transgenic strain that expresses the green fluorescent protein (GFP) in the endothelia of the blood vessels. We have also introduced this transgene into the Tbx1 mutant background. The dynamics of this process, both in wild type and in Tbx1 mutant embryos, is being studied by live imaging in the confocal microscope. In addition, we have generated transgenic mice that express the red fluorescent protein (RFP) in the neural crest, to allow observation of the dynamics of neural crest migration in the context of the formation of the embryonic aortic arches in the wild type and Tbx1 mutant backgrounds. Our preliminary data indicates that the production of the different individual aortic arches might result from the separation of mesodermal progenitor cells into separate domains in the branchial arches, and that the endoderm could play a central role in this process.

The role of Bmp2 in the early steps of neural crest development.

Members: Moisés Mallo.

Students and Technicians: Catarina Correia, Marta Costa.

The neural crest cells (NCC) play a central role in vertebrate embryonic development, as they originate many of the tissues in the adult animal, such as most of the peripheral and autonomic nervous systems, the skeleton of the face and neck, the melanocytes and the smooth muscle cells of the vessels of the heart outflow tract. The NCCs originate at the

dorsal edge of the neural tube and migrate into different areas of the embryo where they differentiate to produce their diverse derivatives. We have previously shown that Bmp2, a member of the Tgfb family of signalling molecules, is essential for the early steps of neural crest formation. In the absence of this gene no migratory neural crest cells can be detected. However, it was not clear whether Bmp2 was required for the induction of NCCs or for the migration of induced NCCs. We have now addressed this issue by combining several experimental approaches. Using specific markers, we could determine that NCCs were indeed induced at the dorsal part of the neural tube in Bmp2 mutant embryos, to levels similar to those found in their wild type littermates, suggesting a role for Bmp2 in migration rather than in induction of NCCs. We have also devised a “mouse-into-chicken” grafting method to assay for activities that promote NCC migration. Experiments using this method also show that, while the dorsal neural folds of wild type animals can redirect NCC migration, a similar tissue from Bmp2 mutant embryos is unable to stimulate NCC migration. This data suggest a role of Bmp2 in inducing directional migration of NCCs. We are currently testing this hypothesis with the help of transgenic animals in which the cellular dynamics of NCCs can be observed in vivo.

Developmental signals in B Lymphocyte ontogeny and differentiation.

Members : Leonor Parreira, Antonio Coutinho, Jocelyne Demengeot.

Students: Margarida Santos, Lurdes Duarte.

It is well established that Notch signaling condition B cell lineage differentiation and that TLR signals leads to B cell activation and differentiation to Antibody Secreting Cells (ASC). We further investigated the role of these evolutionary defined developmental signals on the life of a B cell from its precursor to its last differentiation stage. We established that Notch signaling conditions the frequency of B cells differentiating to ASC upon activation through TLR or CD40, in a dose dependent and ligand dependent manner. Ongoing analysis of mice homozygotes for a null mutation of the Notch ligand Delta-1 support the physiological relevance of these findings. In parallel, throughout analysis of the B cell lineage during ontogeny of mice deficient for TLR2, 4, 9 and the signaling molecule MyD88, indicate that the TLR receptor family must be involved in early B cell differentiation, supporting the notion that in mammals as in invertebrates, endogenous ligands may operate through these receptors to control development and homeostasis.

Functional organization of chromatin in the nucleus of hematopoietic cells.

Members: Leonor Parreira.

Students: Isabel Alcobia, Hélia Neves, Andrea Gomes.

External Collaborators: Jacques Van Dongen and Frank Staal. (Erasmus University, Rotterdam).

This line of work was finished in 2005. The last contribution relates to the putative role of nuclear heterochromatic compartments formed by spatial association of telomeres in the epigenetic regulation of gene expression..

Summary: Positioning of genes relative to nuclear heterochromatic compartments is thought to help regulating their transcriptional activity. Given that human sub-telomeric regions are rich in highly expressed genes, we asked whether human telomeres are related to transcription-permissive nuclear compartments. To address this question we investigated in the nuclei of normal human lymphocytes, the spatial relations of two constitutively expressed genes (*ACTB* and *RARA*) and three nuclear transcripts (*ACTB*, *IL2RA* and *TCRB*) to telomeres and centromeres, as a function of gene activity and transcription levels. We observed that genes and gene transcripts locate close to telomere clusters and away from chromocentres upon activation of transcription. These findings, together with the observation that SC35 domains, which are enriched in pre-mRNA processing factors, are in close proximity to telomeres, indicate that telomere-neighboring regions are permissive to gene expression in human cells. Therefore, the associations of telomeres observed in the interphase nucleus might contribute, as opposed to chromocentres, for the establishment of transcription-permissive 3D nuclear compartments (work done by Ana Sofia Quina, PhD student).

Effects of Notch-ligands in early hematopoiesis.

Members: Leonor Parreira.

Students: Isabel Alcobia, Hélia Neves, Andrea Gomes.

External Collaborators: Jacques Van Dongen and Frank Staal. (Erasmus University, Rotterdam).

This line of work investigates the mechanisms underlying cell fate decision processes of normal hematopoietic stem cells. Specifically, the biological role of Notch receptors and their ligands, the Delta and Jagged proteins, is under analysis. Making use of retroviral transducing systems, the expression of these genes is experimentally induced and modified in vitro, using assays designed for the study of hematopoietic differentiation. Having previously shown that the differential effects of Notch signalling in lymphopoiesis are mediated by distinct Notch ligands, the study progressed for the analysis of the role of these Notch-ligands in a) human myelopoiesis, b) late-stages of B-cell development/maturation and c) embryonic hematopoiesis.

a) In human early hematopoiesis we observed that Delta1- and Jagged1-expressing stromal cells have distinct effects on the clonogenic and differentiation capacities of human CD34⁺CD38⁺ cells. Jagged1 increases the number of bi- (CFU-GM) and uni-potent progenitors (CFU-G and CFU-M), without quantitatively affecting terminal cell differentiation, whereas Delta1 reduces the number of CFU-GM and differentiated monocytic cells. Expression analysis of genes coding for Notch-receptors, Notch-targets and Notch-signaling modulators in supernatant CD34⁺ cells arising upon contact with Jagged1 and Delta1, unraveled previously unrecognized expression patterns of Notch-signaling-related genes exhibited by CD34⁺CD38⁺ cells as they develop in Jagged1 or Delta1-stromal cell environments. These patterns differ according to whether progenitors

were grown in the presence of Jagged1 or Delta 1 and appear to reflect sequential maturational stages of CD34+ cells into distinct cell-lineages (Neves et al submitted).

2) Late-stages of B-cell development/maturation (mouse) (J.Demengeot, IGC, collaborative work). See respective report.

3) Embryonic hematopoietic stem cells (HSC) (mouse) (collaboration with M. Mallo, IGC).

Recently, a number of in vitro models that appear to recapitulate the earliest events of embryonic hematopoiesis have been reported. All rely on the ability of Embryonic Stem Cells (ESC) to spontaneously generate HSC capable of differentiating into distinct hematopoietic lineages in vitro. These systems make it possible to rapidly obtain significant numbers of embryonic HSC and their immediate progenies. More recently, the analysis of ESC-derived HSC has been markedly refined by the discovery that the over-expression of HoxB4 in ESC (by retroviral transduction of ESC with HoxB4 under a tetracycline-inducible promoter), strongly enhances their hematopoietic potential without causing in vitro or in vivo HSC malignant transformation. We make use of these systems to address the role of Notch-signaling in embryonic hematopoiesis. Preliminary data indicate that the clonogenic potential of embryonic HSC is severely reduced by the action of γ -secretase inhibitors (which inhibit Notch-signaling) and, several Notch-signaling-related genes are dynamically and differentially expressed in ES-derived embryoid bodies undergoing hematopoietic commitment. In situ analysis of 3D-preserved EB over time further reveals that the emergence of HSC (c-kit+) occurs in discrete and compact clusters at day 4 of EB-development, in close contact with cells expressing mesodermal and ectodermal markers. Characterization of Notch-signaling pathways in embryonic HSC purified from EB overtime is in progress.

Role of the inhibitory Smad, Smad7, during limb development.

Members: Joaquín Rodríguez León.

Students and Technicians: Carla Milagre.

External Collaborators: Yasuhiko Kawakami, Juan Carlos Izpisua Belmonte (The Salk Institute for Biological Studies, La Jolla, CA, USA).

Vertebrate development is a process which has interested human kind since the beginning. There are many molecular cascades involved in limb development and in this study we focus on the molecular mechanisms controlling TGF β superfamily signaling, which is composed of Tgfs, BMPs, GDFs and activins. This family regulates multiple cellular responses via activation of Smad signaling. Thus, the main objective of this work was to understand how inhibitory Smads (Smad6 and Smad7) can regulate the development of the chick limb buds.

Using techniques like *in ovo* manipulation, *in vivo* electroporation and *in situ* hybridization we can observe, in chick, mouse and duck embryos that *smad7* is expressed in interdigital spaces, in cell that undergo apoptosis, surrounding the digits and in the tip of them when the last phalanx is forming. The expression pattern of *smad6* in chicken embryos that we obtained was similar to *smad7*, however in this case we didn't see an intense expression of *smad6* in the tips of the digits. In chick embryo, for both Smads we

observed that TGF β downregulates their expression in the interdigital spaces. In digits, TGF β can upregulate the expression of *smad7* and downregulates the expression of *smad6*. We also observed that BMPs potencies the expression of inhibitory smads also in interdigital space and in the tips of the digits.

Taken together these results, we can conclude that Smad6 and Smad7 are involved in modulating chondrogenesis. However, while *smad7* might be involved in controlling the size and the width of the digits, *smad6* only appear to be involved in controlling the digital width. Nevertheless, analyzing the results of overexpression, we observe that Smad7 can abolish chondrogenesis. Studies are also being carried for *smad7* RNAi in order to see the resulting phenotype.

We proposed that I-Smads act in a coordinated and synchronized way for the correct establishment of chondrogenesis and apoptosis pattern during development of the chick limb buds.

FGF signaling through Flrt3 co-receptor is responsible for AER integrity.

Members: Joaquín Rodríguez León.

Students and Technicians: Ana Raquel Tomás.

A signaling center is a group of cells located at specific areas of the primordia of an organ that patterns this structure for its proper morphogenesis. The formation and maintenance of a signaling center need to be tightly regulated, both at the spatial and temporal levels. The vertebrate limb is paradigmatic in the study of signaling centers. Limb outgrowth requires first, the formation and maintenance of different signaling centers and, second, a coordinated action among them to build the characteristic three-dimensional morphology of a limb.

During limb development, the apical ectodermal ridge (AER), a thickening of the limb epithelium at its distal tip, is pivotal for maintaining limb outgrowth. The importance and requirement of the AER, or its homologous structure in fish, is a conserved feature in the process of vertebrate limb development. Although extensive studies have been done, the molecular and genetic mechanisms that control initiation and maintenance of the AER activity in vertebrate organisms still needs further study.

The aim of this study is to unravel the role of *flrt3* during limb bud development. For this purpose we have described the expression pattern of *flrt3* during limb development, studied its relationship with different signaling molecules and performed functional studies involving silencing of *flrt3* expression.

Our results show that *flrt3* expression in limb buds is restricted to the AER and a small portion of the surrounding ectoderm, co-localizing its expression with *fgf8* and pERK activity. Moreover, we have observed that *flrt3* expression is not regulated by FGF activity, although ectopic *Wnt3a* is able to induce *flrt3* expression. Loss-of-function studies have shown that silencing *flrt3* affects the integrity of AER and, subsequently, its proper activity during limb bud outgrowth.

Based on our results we propose a working model for *flrt3* activity in the growing limb in which FGF10 from the mesenchyme, signals to the AER through *Wnt3a*, inducing *fgf8* and *flrt3* activity. FGF signaling, together with *flrt3* in the ectoderm induces ERK

activity, maintaining AER integrity. Simultaneously, FGF8 signals to the underlying mesenchyme via PI3K, inducing expression of *mkp3*, therefore inhibiting ERK phosphorylation therein and promoting survival of those cells.

***Fgf19* distribution and its role in the developing retina.**

Members: Joaquín Rodríguez León.

External Collaborators: Javier de Francisco Morcillo (Department of Cell Biology, Faculty of Sciences, University of Extremadura, Spain).

In this project we analysed, by non radioactive in situ hybridization (ISH), the expression pattern of *Fgf19* in the developing chick eye.

Fgf19 expression was first detected at HH16 (~E2.5) in the central region of the neural retina, in a region located dorsal to the optic nerve head, confined to scarce cells showing a migratory morphology. Analysis of BrdU incorporation showed that *Fgf19* mRNA expressing cells are postmitotic neuroblasts. By HH18 (E3) of labelled cells were located increased in number, preferentially in the temporo-central retina, and were evenly distributed throughout the retinal wall. Between HH21 (E3.5) and HH29 (E6) the neural retina thickened, and the number of labelled cells increased in number, and these cells progressively spread to more peripheral regions of the tissue. At HH27 (E5) labelled cells appeared unevenly distributed in the retinal wall and a population of cells accumulated in high number in the vitreal half, in close proximity to the presumptive ganglion cell layer (GCL). *Fgf19* labelling observed in the retina until HH29 was consistent with that expected for a gene involved in retinal neuronal differentiation.

At HH31 (E7) transcripts were principally restricted to a population of cells, arranged in a row, located in the outer region of the inner nuclear layer (INL). This change in the expression pattern proceeded peripherally as development progressed. By HH38 *Fgf19* expression was confined to the outer region of the INL except in the most peripheral region of the retina where bipolar shaped cells were still detected. At HH42 (E16) only a few elongated labelled cells could be distinguished in the most peripheral margins. From this stage on the strong expression detected in the outermost region of the INL was gradually lost and at P30, when the retina shows adult characteristics, most fields of view of the retina did not contain any *Fgf19* transcripts expression were not detected. The results described above indicated that *Fgf19* mRNA expression in the developing chick retina followed dorsal to ventral, temporal to nasal and central to peripheral (vitreal to scleral) gradients in the developing chick retina.

Thus, *Fgf19* mRNA expressing cells are likely to represent precursor cells that have completed their last division and migrate freely in the neuroepithelium.

Molecular and cellular characterization of segmentation in the chick embryo.

Members: Leonor Saúde.

Students and Technicians: Alexandre Gonçalves.

External Collaborators: Isabel Palmeirim (Escola de Ciências da Saúde/ Instituto de Investigação em Ciências da Vida e Saúde, Universidade do Minho, Braga, Portugal).

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? I subtraction screening of cDNA libraries from Medial *versus* Lateral chick PSM was performed in order to determine the genes differentially expressed in Medial-PSM. From the first round screening 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 in establishing the segmentation autonomy is being analysed. 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 and their relation with Fgf and Retinoic Acid pathways is under study.

The role of the transcription factor *terra* during vertebrate development.

Members: Leonor Saúde.

Students and Technicians: Raquel Lourenço and Alexandre Gonçalves.

External Collaborators: Isabel Palmeirim (Escola de Ciências da Saúde/ Instituto de Investigação em Ciências da Vida e Saúde, Universidade do Minho, Braga, Portugal).

The bilateral symmetry of the adult vertebrate body is evident at the level of the axial skeleton and the skeletal muscles. These structures derive from the somites. The formation of the somites is under the control of a molecular clock, revealed by the cyclic presomitic mesoderm expression of components of the Notch and Wnt signalling pathways. We have shown that in the absence of the transcription factor Terra, the onset of the segmentation clock is desynchronized between the left and the right presomitic mesoderm precursors. This implies that somite formation is not a bilaterally symmetrical process by default: rather its symmetry needs to be actively maintained by a mechanism that involves Terra and the recently implicated retinoic acid signalling. In addition, we have also shown that without Terra the embryos display a randomized expression of left-right markers in the lateral plate mesoderm, with a subsequent randomization of heart position. These studies have lead to the identification of the first molecular coordinator of two processes that are important to set up the vertebrate body plan: left-right asymmetry and presomitic mesoderm bilateral symmetry.

Myotome formation in the mouse: cell movements and cell-extracellular matrix interactions.

Members: Sólveig Thorsteinsdóttir, Gabriel G. Martins.

Students and Technicians: Fernanda Bajanca, Ana Sofia Lopes.

External Collaborators: Marilyn Duxson, (University of Otago, Dunedin, New Zealand); Margaret Buckingham and Shahrugim Tajbakhsh, (Pasteur Institute, Paris, France).

Early trunk skeletal muscle formation in vertebrates is a complex process involving the formation of the myotome and then fusion of myogenic cells into multinucleated myotubes, a process called primary myogenesis. We have shown that integrins are dynamically expressed during this process and our results indicate a role for the laminin receptor $\alpha 6 \beta 1$ integrin in epaxial myotome formation while the fibronectin and VCAM-1 receptor $\alpha 4 \beta 1$ appears to be important in hypaxial myotome development. The myogenic regulatory factor (MRF) Myf5 is essential for the proper morphogenesis of the myotome. In fact, myogenic precursor cells (MPCs) in Myf5-null embryos do not initially form a myotome and do not assemble a laminin matrix. Our results show that this failure of laminin matrix assembly and myotome organisation might be due to a lack of expression of the $\alpha 6 \beta 1$ integrin by the Myf5-null MPCs. Incubation of normal mouse embryos with a function blocking antibody against the $\alpha 6 \beta 1$ integrin gives a phenotype very similar to the one observed in Myf5-null embryos. Thus our results strongly suggest that Myf5 and $\alpha 6 \beta 1$ are both essential for myotome formation in the mouse.

Integrating signals in morphogenesis: the case of somitogenesis in the chick.

Members: Sólveig Thorsteinsdóttir, Gabriela Rodrigues, Gabriel G. Martins, Leonor Saúde.

Students and Technicians: Pedro Rifes, Pedro Campinho, Catarina Lopes.

External Collaborators: Isabel Palmeirim, (Escola das Ciências de Saúde, Universidade do Minho, Braga, Portugal); António Jacinto (Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Lisbon and IGC, Oeiras, Portugal).

In all segmented organisms, the proper place, size, and timing of the formation of segments, as well as their shape, has to be under tight control during embryogenesis. Somites are transient segments laid down early in vertebrate development. As a consequence of gastrulation, the mesoderm destined to form the somites becomes aligned on the two sides of the dorsal axis of the embryo, and is named the presomitic mesoderm (PSM). A continuous flow of cells populates this tissue from the gastrulation site, leading to its growth in the caudal direction. At the rostral portion of the PSM, cells are periodically incorporated into somites, occurring every 90 minutes in the chick embryo. Somitogenesis involves a complex morphological transition in that a group of undifferentiated, mesenchymal PSM cells aggregate and ultimately form a sphere of polarised epithelial cells with a few mesenchymal cells in the lumen. We propose to use high resolution 3D imaging/analysis techniques over time to characterise the locomotive, cytoskeletal and adhesive properties of chick PSM cells and the organisation of their surrounding ECM during this transition. We then experimentally manipulate the availability of ECM molecules and paracrine factors and determine the effect on PSM cell behaviour. Our aim is to produce an integrative working model on how paracrine factors and ECM cues collaborate to produce normal somites.

The role of VEGF and its receptors in tumour growth and angiogenesis.

Members: Sérgio Dias, Cristina Casalou.

Students and Technicians: Rita Fragoso, Ana Paula Elias, Andreia Mendonça.

External Collaborators: Shahin Rafii (Cornell University); Zhenping Zhu (ImClone Systems, NY, USA); Genentech (Napoleone Ferrara).

In this Project we have addressed several aspects of tumor angiogenesis, in the context of solid and liquid (hematologic) neoplasms. Our work focuses on the role and regulation of Vascular Endothelial Growth Factor (VEGF) and its receptor tyrosine kinases, working both as paracrine signaling partners as well as autocrine stimulators of neoplastic growth. As paracrine signals, we have found out that VEGF and its receptors are actively regulated within endothelial cells, and are now exploiting the mechanisms of VEGF splicing by neoplastic cells, under the influence of distinct environmental signals (ie, conferring some organ specificity to VEGF production). In addition, we have also described for the first time the existence of internal and external VEGF/VEGF receptor autocrine loops on malignant cells (leukemias). We described further that the activation of internal or external VEGF/VEGF receptor loops results in turn in the activation of distinct signaling pathways and as such different cellular functions. Finally, we have recently described a previously unrecognized role for VEGF receptor-1 in regulating the bone marrow localization of subsets of acute leukemias. VEGFR-1 stimulation (or blockade) results in the modulation of the localization of the leukemia cells within the bone marrow, which may have serious consequences for therapeutic efficacy in treating such diseases. We are now exploiting the molecular mechanisms that regulate leukemia cell movement within the bone marrow microenvironment, looking at lipid raft formation, receptor polarization and activation of specific signaling pathways. In parallel, we are evaluating the VEGF splicing patterns in different hematologic malignancies, when cells are exposed to different microenvironment signals, within the bone marrow microenvironment.

Endothelial progenitors: their role in health and disease.

Members: Sérgio Dias, Carla Real.

Students and Technicians: Cátia Igreja, Margarida Courinha.

External Collaborators: Shahin Rafii (Cornell University, NY, USA); Manuel Coelho (H. Curry Cabral); Julian Dye (Mount Vernon Hospital, UK).

The importance of angiogenesis for the progression of solid and hematologic tumors has been well documented. In addition, recent evidence suggests novel cellular pathways/mechanisms may regulate the onset of angiogenesis. One such mechanisms involves 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 and lymphoma growth and metastasis

formation. The results obtained to date suggest the 2 tumor types have distinct mechanisms to activate, recruit and incorporate EPC. In the case of lymphoma, we have discovered the co-existence of 2 EPC populations, circulating and biopsy-derived, characterized by common expression of endothelial specific markers but distinct CD133 (progenitor cell marker) isoforms expression. The relative contribution of both cell types to the process of lymphomagenesis is under investigation. Finally, we have described an association between the presence of prostate biopsy EPC and clinical correlates of prostate cancer. In detail, we are defining a threshold of EPC detection that may contribute to the stratification and classification of prostate lesions.

cDNA microarray technology in diagnosis and monitoring for oncology patients.

Members : Sérgio Dias, Jorg Becker.

Students and Technicians: Cátia Igreja, Margarida Courinha.

This project will seek to apply cDNA microarray chip technology for the study, monitoring and diagnosis of patients with different neoplasias. The project has a basic research component (ie, mechanisms of tumorigenesis and metastasis formation) and also a clinically-relevant part. This project started in January 2004.

The initial work has resulted in the creation of specific “circuits” to allow regular collection of tumor samples (for instance lymphomas, salivary gland tumors and gastrointestinal tumors, to be classified and studied further, and also the safe and detailed storage of such samples (the building of a tumour biobank). The first sets of samples analyzed by cDNA array technology were Thyroid, Salivary gland tumours and lymphomas (3 manuscripts in preparation).

Angiogenesis in cervical neoplasms: clinical and molecular correlates.

Members: Sérgio Dias, Ana Cachaço.

External Collaborators: Ana Félix (IPOFG, Lisbon, Portugal).

The involvement of HPVirus in cervical neoplasms carcinogenesis has been extensively studied. However, the importance of this agent in modulating the cellular and molecular microenvironment, resulting in changes in tissue architecture and neo-angiogenesis, has not been well documented.

In this project, we will study the importance of specific HPV types for the changes in extracellular matrix and angiogenic growth factor production in cervical neoplasms.

For this purpose, we will employ both cellular models as well as use clinical material, to study the molecular pathogenesis of these tumors.

Role of somatostatin during DRG chick embryo development.

Members: Matthias Haury, Paula Parra Bueno.

Students and Technicians: Duarte Viana, Nicolau Ferreira.

External Collaborators: Alfonso Fairen (University of Alicante. Alicante, Spain).

The aim of this project is to determine the function of somatostatin (SST) and its receptors (SSTRs) during the development of the chick embryo. In the developing mammalian brain SST and SSTR are expressed in developing neurons in a transient and variable manner. The transient expression of SST during late ontogenesis of the brain has been studied, and it has been suggested that SST and SSTR may be involved in the organization of the CNS. Moreover, high expression of the SSTR2 and SSTR3 genes in various embryonic neuroepithelia was detected, suggesting an involvement of those SSTR also in neurogenesis. On the other hand, it is known that SST have a modulatory role on the Ca^{2+} current, inhibiting several Ca^{2+} channel in dissociated chick DRG neurons. We have now identified chick sst and performed in-situ hybridization (ISH) studies to determine the distribution of sst mRNA in the chick embryo, and are currently investigating the functional role of SST in chick DRG embryonic development

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.

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. 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.

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.

Nature, origin and dynamics of regulatory T cells.

Members: Jorge Carneiro, Jocelyne Demengeot, Rui Gardner, Marie-Louise Bergman, Kalet Leon.

Students and Technicians: Tiago Paixão, Carline van den Dool.

Regulatory CD4⁺CD25⁺ T cells prevent autoimmune pathologies by suppressing other cells. We derived the first mathematical model of regulatory CD4⁺ T cell dynamics and cellular interactions. Using this model we have shown, based on first principles, that the persistence of regulatory T cell populations must depend on growth factors provided by the T cells they suppress, leading to a crosstalk hypothesis. Experimental evidence for this crosstalk hypothesis was provided both by us and others (for a recent review see Carneiro et. al. J. Comp. Appl. Math. 2005). Recently we have been further studying this crosstalk between regulatory and effector T cells along two lines. First, we are analysing the fine details of cell-to-cell interactions by comparing in vitro and in vivo confocal-imaging with the results of in silico Monte-Carlo like simulations of individual cells. This is leading us to advance several experimental and computational tools namely the three color imaging, and the algorithms for cell simulation as well as for automated feature detection and quantification of 2D and 3D images. Second, we are approaching the selection and dynamics of the repertoire of T lymphocytes by simulating many clones containing regulatory and/or effector T cells, as well as different subpopulations of APCs.

Quantification of antigen-receptor diversity in lymphocyte populations.

Members: Jorge Carneiro, Rui Gardner.

Students and Technicians: Nuno Sepúlveda.

External Collaborators: Ana Cristina Espada de Sousa (IMM, Lisbon, Portugal), Marília Cascalho (Mayo-Clinic, USA).

The quantification of antigen receptor diversity in a lymphocyte pool is a key issue in immunology, and many ways an outstanding unsolved problem. Recently, a promising technique based on microarray technology was developed (Ogle et al 2003) to measure lymphocyte diversity. This technique quantifies the hybridization of samples of antigen receptor-specific RNAs with the oligonucleotides in a DNA gene chip and relates it to the hybridization of samples of synthesized RNAs of known diversity. However, this technique lacks an appropriate statistical analysis to measure the error associated with the estimates of diversity. In this context, we set out to develop a mathematical and statistical description of the processes involved in this technique; this description is key to evaluate both accuracy and precision of the diversity estimates. We have tackled this problem using different modeling approaches, from simulation of models based on complementary sequence hybridization to statistical models based on the biophysical properties of the hybridization. Although these models allowed us to dissect some of the processes involved in the diversity estimation, further developments of the theoretical framework will be necessary in order to provide good error measurement estimates. Nevertheless, the analysis already performed allows to improve the technique by

reducing the number of oligonucleotides needed to estimate diversity to not more than a few hundreds instead of the hundred thousands typically available in the commercial DNA chips.

Polar growth, orientation and morphogenesis in pollen tubes.

Members: Jorge Carneiro, José Feijó.

Students and Technicians: Ramiro Magno, Tiago Paixão.

The pollen tube is a cell with polar growth that delivers the male gametocyte to the female oocyte, navigating through the female tissues in the flower. Aiming to better understand the mechanism by which the pollen tube navigates through the female tissues we modeled its polar growth, orientation and morphogenesis. We have shown that the shape, growth and orientation of the pollen tube in three dimensions can be described by the spatio-temporal orchestration of the rates of growth of the rigid cell wall surface and of the incompressible cytoplasm volume. The simulation of these processes is computationally efficient making it possible to simulate thousands of pollen tubes simultaneously and also to assess different hypotheses on the mechanisms of navigation. Using this model we tested different mechanisms of chemotropic responses showing that realistic behavior regarding chemoattractants and repellents requires an adaptive signaling mechanism, which might be key for pollen navigation in the female tissues.

Regulation of the (auto) immune response by gonadal hormones.

Members: Maria Margarida Souto Carneiro, José Faro.

Students and Technicians: Célia Ferreira.

External Collaborators: José António Pereira da Silva (Department of Rheumatology, University of Coimbra, Portugal); Claudia Berek (German Center for Rheumatism Research, Berlin, Germany); Veit Krenn (Division for Molecular Pathology of the World Health Organization Center for Rheumatic Pathology, Trier, Germany).

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease causing polyarthropathy and joint destruction. As in most autoimmune diseases, there is a significant gender disparity in the age range of 15-55 years, with women being 3 times more prone to develop the disease than men. It is known that alterations in the metabolism and excretion of androgens is present in patients with rheumatoid arthritis. Additionally, estrogens are known to diminish regulatory T cells' activity, leading to autoreactive cell' survival and activation at the naïve state, and play a major role in B cell apoptosis and activation. Moreover, raised levels in corticotrophin-releasing hormone transgenic mice leads to abnormal GC formation after primary immunization. Therefore, all these results suggest a close involvement of sex hormones in the modulation of the (auto)immune response. However, it is very difficult to understand the multilevel complexity of the immune response and the role that gonadal hormones and tissue differences play in the process simply by experimental methods alone. To overcome

those difficulties this project will bring together mathematical modeling and experimental studies to create a reliable body of information correlating gender differences and disease development.

Development of the inflammatory infiltration and ectopic germinal center formation in a murine model of rheumatoid arthritis.

Members: Maria Margarida Souto Carneiro, José Faro.

Students and Technicians: Maria João Lagareiro.

External Collaborators: Claudia Berek (German Center for Rheumatism Research, Berlin, Germany) Veit Krenn (Division for Molecular Pathology of the World Health Organization Center for Rheumatic Pathology, Trier, Germany).

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease causing polyarthropathy and joint destruction. In about 25% of RA patients with long-standing disease ectopic germinal centers can be detected in synovial biopsies taken from the inflammation sites. These ectopic germinal centers present similar structural characteristics to the germinal centers found on secondary lymphoid organs. However, it is widely accepted that the ectopic germinal centers allow a local autoantigen-driven immune response, while perpetuating the disease and breaking tolerance mechanisms. Usually, the presence of ectopic germinal centers in the rheumatoid synovium is associated with a rapid progression and bad prognosis of the disease. Recently, the description of the SKG mice (Zap 70 deficient) and the K/BxN mice that develop chronic (and spontaneous in the K/BxN) arthritis very much reproducing the characteristics of human disease, made available potent tools to study the formation of ectopic germinal centers. In the present study we will follow the progression of the inflammatory infiltrate in K/BxN mice and analyze the formation of germinal center like structures in the synovium, and by comparison with human data try to develop a theoretical model for the formation and maintenance of ectopic germinal centers in Rheumatoid Arthritis.

The germinal center reaction after primary immunization in CD25 depleted mice.

Members: José Faro, Maria Margarida Souto Carneiro, Jocelyne Demengeot, Jorge Carneiro.

Students and Technicians: Isabel Belo, Ana Água-Doce.

Previous reports have suggested that the absence of regulatory T (T_R) lymphocytes at the time of a primary immunization can cause the regular humoral response to be disrupted leading to the production of autoantibodies. However, it is still unknown how the absence of T_R lymphocytes influences the different phases, the structure and the cell populations involved in the germinal center (GC) reaction. Furthermore, previous studies did not contemplate the depletion of T_R lymphocytes in more advance phases of the immune response and its consequences. In the present project we intend to carry out a detailed analysis on the influence of T_R lymphocytes in the GC reaction, using both an

experimental and theoretical approach. We have immunized with ovalbumin (OVA) two groups of Balb/c mice, one in which CD25⁺ T cells were depleted by pre-treatment with a cytolytic Ab anti-CD25 and a control group. The kinetics of different lymphocyte populations was followed both in the spleen and in lymph nodes (LN) by flow cytometry. As expected, GC CD4⁺ T_R cells (defined as CXCR5⁺CD25⁺CD4⁺) were barely detectable in CD25-depleted mice. In contrast, those lymphocytes follow a typical GC kinetics in control mice, both in the spleen and in LNs. Surprisingly, while in the spleen of CD25-depleted mice the number of GC B cells (PNA⁺) is slightly reduced at late times of the response compared to control mice, in the LN of CD25-depleted mice there is an apparent enhancement of GC B cells at the peak of the response. In contrast, in respect to T cells, in the spleen of CD25-depleted mice the number of GC T cells (CXCR5⁺CD4⁺) is reduced along the GC reaction compared to control mice; in the LNs, however, GC T cell numbers are very similar to those of control mice. When those results are analyzed in the framework of our GC modeling previously developed, the clear difference between GC B and T cell kinetics in the spleen vs LNs of CD25-depleted mice and the strong dependency of the GC reaction on T cell kinetics predicted by our models are not easily reconciled. As a tentative hypothesis, that discrepancy can be interpreted as indicating that in the spleen there are many more preactivated CD25⁺ Ag-specific T cells as compared to the LNs, which upon treatment. We are currently analyzing this and other possibilities.

Theoretical estimation of the repertoire size of murine CD4⁺CD45RB^{high} and CD4⁺CD25⁺ T cells.

Members: José Faro.

Students and Technicians: Nuno Sepúlveda.

External Collaborators: Antonio Bandeira (Institut Pasteur, Paris, France); Kalet Leon (CIM, Cuba).

We have undertaken the estimation of the repertoire size of mature peripheral CD4⁺ T cell populations (conventional and regulatory T [T_R] cells) from limited experimental information on the repertoire determined for relatively small FACS-purified T-cell subpopulation samples expressing given TCR- or TCR- gene subfamilies. To this end a novel mathematical approach was developed. Two alternative models were implemented to cope with data derived from different experimental settings. These models rely on the following assumptions: 1) all rearrangements have an equal probability of being sequenced; 2) sequence obtention is a random draw with replacement, i.e., it does not alter the probabilities to observe the following sequences; 3) all rearrangements have a similar number of transcripts; 4) cell sampling is a draw without replacement, i.e., it alters the probabilities to observe the following cells; 5) cells in each sample are from different clones. In each model variant a maximum likelihood estimator was derived to calculate in a given splenic population the number of distinct CDR3 sequences. Our present results suggest that the CD4⁺ T_R population, being in the spleen ~10-fold smaller than conventional CD4⁺ T cell population, has a diverse repertoire that is proportionally larger than that of conventional CD4⁺ T cells (estimated TCR repertoire of T_R cells about

half that of conventional CD4⁺ T cells). In addition, an extension of our theoretical model allows us to estimate from the data the expected degree of overlap of TCR- repertoires between both splenic populations.

Criticality in epidemiology.

Members: Nico Stollenwerk, Frank Hilker, Lisa White, Cristina Paulo, Guilherme Gonçalves, Gabriela Gomes.

Students and Technicians: Paula Rodrigues, Ricardo Águas, Dinis Gökaydin.

External Collaborators: Ana Nunes (CFTC–FCUL); Kevin Marsh, Robert Snow (KEMRI Wellcome – Kenya); Chris Dye (WHO); Fonseca Antunes (DGS).

Epidemiology has a long tradition of importing concepts from statistical physics (Grassberger P. & de la Torre A. 1979, *Annals of Physics* 122, 373) based on analogies between disease transmission and the dynamical systems describing chemical reactions. Particularly useful to intervention design are situations where small changes in controllable factors lead to major transitions in system behaviour. Understanding the associated critical behaviour and establishing its implications to epidemiology is the objective of this project.

Recent data analysis has shown that mass vaccination against measles maintains this disease near the elimination threshold in some countries (Jansen V.A.A. et al. 2003, *Science* 301, 804). The notion of self-organized criticality explains the huge fluctuations that are often observed in measles time series. This is a general phenomenon prone to occur in the course of disease eradication.

Another aspect of this project is motivated by the observation of major variability in the effectiveness of vaccination against tuberculosis (Gomes M.G.M. et al 2004, *Proc. R. Soc. Lond. B* 271, 617). This infectious disease sustains two distinct *per capita* rates of infection – a baseline rate affects individuals that are immunologically naïve; and a reduced rate affects individuals that have been previously infected or vaccinated and, therefore, acquired some partial immunity. We have recently demonstrated that these combined processes can maintain radically different system behaviours depending on whether transmission is occurring below or above a reinfection threshold (Gomes M.G.M. et al. 2005, *J. Theor. Biol.* 236,111). Most notably, increasing transmission across the reinfection threshold leads to a sharp increase in the prevalence of infection by two orders of magnitude and a drastic fall in the effectiveness of vaccination programmes. We are applying techniques from statistical physics to investigate the critical fluctuations expected to occur in association with the reinfection threshold.

On other grounds, evolutionary biologists have observed that pathogen mutation and immune selection can induce huge fluctuations in disease incidence. In the case of meningococcal disease, we model the infection process with different variants of the pathogenic bacteria obtaining power law behaviour at a critical state of small pathogenicity (Stollenwerk N. 2003, *Physics Letters A* 317, 87) and the evolution towards this critical state. We also observe such signs of criticality in data (Stollenwerk N. et al. 2004, *Proc. Natl. Acad. Sci. USA* 101, 10229), rejecting previous models of meningococcal disease dynamics on a statistical basis. This case study of meningococcal

infection may serve as a paradigmatic system for a much wider phenomenon coined “theory of accidental pathogens” holding for a variety of bacterial as well as viral infections.

Criticality in epidemiology is a transversal project that tackles a number of diseases – measles, varicella, tuberculosis, hepatitis C, pertussis, malaria, meningococcal diseases, respiratory syncytial virus, influenza.

Pathogen diversity in disease epidemiology and vaccine research.

Members: Jean-Baptiste André, Lisa White, Isabel Gordo, Gabriela Gomes.

Students and Technicians: Sander van Noort, Loïc Lhopitalier, Dinis Gökaydin

External Collaborators: Viggo Andreasen (Roskilde University – UK); Ben Cooper (HPA London – UK); Julia Gog, Olivier Restif (Cambridge University – UK); David Conway (MRC – Gambia); Thierry Van-Effenterre (GSK Bio – Belgium); Kevin Marsh, Pete Bull (KEMRI Wellcome – Kenya).

Viruses, bacteria and parasitic pathogens have evolved multiple strategies to evade immune responses, facilitating transmission, permitting the establishment of chronic and recurrent infections, and often hampering the development of effective vaccines. Antigenic variation of immune response targets is one such pathogen strategy that can only be confronted with the support of a highly specialised research, development and public health programme combining basic and applied research. This project involves a network of collaborators integrating basic research in infection, population genetics, immunology and mathematical epidemiology, with a range of practical aspects from field epidemiology to vaccine development and evaluation and the mathematical modelling of the impact of vaccination programmes.

The overall research strategy is to unravel the molecular bases and epidemiological significance of immunity in order to guide vaccine development and evaluation. The specific objectives are to:

- 1) Assess to what extent pathogen diversity affects epidemiology and acquisition of immunity.
- 2) Consider host heterogeneity as a potential determinant of pathogen evolution (Gandon S. 2004, *Evolution* 58, 455).
- 3) Evaluate the evolution of mutation rates and switching rates associated with pathogen antigens (André J.B. et al. 2005, *Genetics*, in press).
- 4) Explore the epidemiological and evolutionary consequences of existing and potential vaccines (Gomes et al. 2004, *J. Theor. Biol.* 228, 539)
- 5) Determine the practical implications of this research for vaccine design.

This is a transversal project that tackles a number of pathogens – influenza viruses, respiratory syncytial viruses, rotavirus, *Bordetella pertussis*, meningococcus, pneumococcus, malaria parasites.

Disease propagation in real time and space.

Members: Gabriela Gomes, Cristina Paulo.

Students and Technicians: Sander van Noort, Dinis Gökyaydin, Loïc Lhopitallier, José Lourenço, Cláudia Duarte, Rita de Salles Caldeira, Paula Macedo, Sofia Cordeiro.

External Collaborators: Carl Koppeschaar (De Grote Griepmeting – The Netherlands); Francisco George, Mário Carreira (DGS); Ana Noronha, Mafalda Lapa (Ciência Viva); Sofia Galvão (Publico); Alexandra Rebelo (Novis); Flávio Coelho (Fundação Oswaldo Cruz – Brazil); Portuguese population.

The world is in the eminence of another influenza pandemic. A pandemic virus, once it emerges, is likely to spread worldwide within 3 months. Rates of infection and severity of disease are likely to be high as we have no immunity against the new virus. The demand for medical care will supersede what is normally available, and the supplies of vaccines and antiviral drugs will be inadequate. This is a summary of the WHO projections, which comes to enforce the importance of efficient surveillance and accurate modelling. In this project we do both:

1) Surveillance in real time and space. We have implemented, this season of 2005/06, a fast system to monitor the spread of influenza. The system consists of a site – www.gripept.net – where every person resident in Portugal can complete a weekly questionnaire concerning symptoms associated with influenza. The information is compiled in real time. The site is also a rich source of general information about influenza – scientific, medical, historical – and is constantly updated with the progress of the current epidemic. Educational activities for children and adolescents, and debates with the general public are implemented.

2) Simulation and intervention planning. Computational platforms are used to simulate the spread of pandemic influenza in a population with the realistic Portuguese demography. Possible scenarios will be constructed with reference to parameters that characterize the hypothetical emerging virus. The output of this study is informative with regards to the planning of possible interventions.

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 sense to separate the various “specialities” or areas of interest, as done here for reasons of commodity 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.

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.

The evidence pointing to the cross talk between the cell cycle and apoptosis are sparse but not lacking, although the details are still largely unknown. Cells have developed surveillance mechanisms that control the progression through the cell cycle by assuring that the initiation of one event only occurs after successful completion of the previous one. Recent reports on communication between cell cycle regulators and the apoptotic machinery led us to the hypothesis that competence for self-destruction of cells might change along the cycle. The regulation of survival and cell death is a key determinant of cell fate. We have published that BimEL (a BH3-only member of the Bcl-2 family of proteins) is phosphorylated in mitosis. Our results show that, in mitosis, Bim is phosphorylated downstream of growth factor signalling in a MEK-dependent manner with FGF signalling playing an important role. We suggest that phosphorylation of Bim is a decisive step for survival of proliferating cells. Although the mechanisms by which P-Bim is restrained from causing apoptosis are still not understood, P-Bim has been described to be targeted for proteasome degradation. However, other mechanisms responsible for loss of pro-apoptotic properties of Bim, such as relocation or revised interaction with interacting pro- or anti-apoptotic partners cannot be excluded. We speculated that Bim may get displaced from MTs during the spindle formation and pose danger of turning on apoptosis due to release and relocation, hence, the need to phosphorylate to incapacitate the molecule.

We have evidence now to claim that in mitosis phospho-Bim is indeed delocalized from the microtubules and resides in the cytosolic fraction. We further report that when Bim is dephosphorylated it revises location to the mitochondria which also correlates with cell death. These results support our hypothesis that the phosphorylation of Bim in mitosis is required to attenuate its pro-apoptotic activity and may play a decisive step for the survival of proliferating cells. In order to elucidate the fate of the phosphorylated form of Bim in mitosis, we investigated its possible degradation. Our results show that if we inhibit *de novo* protein synthesis in mitosis, there is a decrease in the phosphorylated

form of Bim that correlates with decreased levels of cyclin B1 and mitotic exit, even though cells were arrested in pre-metaphase with nocodazole. These cells undergo adaptation and became interphase cells. In the presence of an inhibitor of proteasome the phosphorylated form of Bim is restored and cells remain in mitosis suggesting that Bim phosphorylation is specific to mitosis, and during mitotic exit Bim is dephosphorylated.

Characterization of the Mob1-like proteins in higher eucariotes.

Member: Álvaro Tavares.

Students: Claudia Florindo, Célia Domingues, Inês Ferreira.

Collaborators: Jonathon Pines (CRC Cambridge, UK), Didier Fesquet (Centre de Recherche de Biochimie Macromoléculaire, CNRS, Montpellier, France), Rui Gomes, (FCUL, Lisbon, Portugal). Maria Arménia Carrondo (ITQB, Oeiras, Portugal).

Mob1 is a recently described essential gene in *S. cereveiseae*. Mutations in this gene cause an arrest in late anaphase with an elongated spindle and segregated DNA, indicating a failure in cytokinesis. The Mob1 gene is highly conserved among eucariotes. We have isolated and cloned four different genes coding for Mob-1 like proteins in *Drosophila* and human cells. These genes (HsMob1, 2, 3 and 4) have an homology with the yeast ortologue ranging from 44% to 20%. We have determined the intracellular localization of the human Mob-like proteins by indirect imunofluorescence, and found that they are centrosomal proteins throughout the cell cycle. Interestingly, the Mob4 protein seems to accumulate between the two centrioles up until telophase, when then it is associated only with the mother centriole. Western blots with synchronized HeLa cell extracts show that the levels of HsMob4 protein do not change from mitosis to interphase. The depletion of Mob4 by RNAi, results in a premature separation of the two centrioles and in an increased number of binucleated cells and tetranucleated, indicating a role in the execution of cytokinesis. Most interestingly we have shown that clonal mutants of the Mob4 gene in *Drosophila* results in the formation of tumors. In addition, null alleles of *Drosophila* Mob4 die early during development with problems in the central nervous system formation. This deficiency seems to be due to the association of Mob4 with the kinase NDR. We have also shown that Mob4 is a positive regulator of the NDR kinase activity. Taken together these results indicate that Mob4 is a new tumor supressor gene with essencial functions during development.

Study of mitotic kinases in *Drosophila melanogaster*.

Member: Álvaro Tavares.

Students: Mariana Faria, Susana Godinho, Inês Ferreira.

Collaborators: David Glover (CRC Cambridge, UK), Rui Gomes, FCUL, Lisbon, Portugal).

Polo-like kinases (Plks) are essential for progression through mitosis. The activity of these kinases peak during M phase and this activation has been attributed to

phosphorylation. In order to study the regulation of Polo activity we identified and cloned a *Drosophila melanogaster* kinase belonging to the ste20 ser/thr family that presents a close sequence homology with xPlkk1 and SLK. We termed this kinase dPlkk and showed that dPlkk associates with and phosphorylates Polo in vitro, resulting in the activation of the latter. On the other hand when dPlkk is depleted from S2 cells, Polo activation does not seem to be impaired, suggesting that other kinases are involved in regulating Polo activity in vivo. Additionally we found that a percentage of dPlkk depleted cells fail to form a proper actin ring at the end of mitosis, leading to a failure in the assembly of the cleavage furrow and to the formation of binucleated cells. The detected accumulation of dPlkk in the contractile ring late in anaphase reinforces the idea that this kinase plays a role in cytokinesis.

We assessed the ability of mouse Polo-like kinase 1 (Plk1) to perform the multiple mitotic functions of Polo kinase, by expressing a Plk1-GFP fusion in *Drosophila*. Consistent with the previously reported localization of Polo kinase, Plk1-GFP was strongly localized to centrosomes and recruited to the centromeric regions of condensing chromosomes during early mitosis. However, in contrast to a functional Polo-GFP fusion, Plk1-GFP failed to localize to the central spindle midzone in both syncytial embryo mitosis and the conventional mitoses of cellularized embryos and S2 cells. Moreover, unlike endogenous Polo kinase and Polo-GFP, Plk1-GFP failed to associate with the contractile ring. Expression of Plk1-GFP enhanced the lethality of hypomorphic *polo* mutants and disrupted the organization of the actomyosin cytoskeleton in a dominant-negative manner. Taken together, our results suggest that endogenous Polo kinase has specific roles in regulating actomyosin rearrangements during *Drosophila* mitoses that its mammalian counterpart, Plk1, cannot fulfill. Consistent with this hypothesis, we observed defects in the cortical recruitment of myosin and myosin regulatory light chain in Polo deficient cells.

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

Members: Lisete Fernandes.

Students and Technicians: Cláudia Bicho, Joana Monteiro.

The Yap family of bZIP transcriptional factors in *Saccharomyces cerevisiae* contains members which are central players in cellular responses to stress challenges such as: Yap1 in oxidative and cold signals, and Yap4 in response to compounds affecting cytoskeleton as well as in cold. 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 mediated-signaling cascades. From this point of view, Yap constitutes an excellent tool to address the cross-talk of signalling pathways, in particular, the cross-talk of cold and oxidative signals.

In this context, 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.

Our data does not support a link between Yap1 and processes described in literature as the basis for cold-phenotypes such as defects in cytoskeleton, and decrease mRNA stability; the potential function of Yap1 as transcriptional activator of oxidative-cold response is under conclusion. The study of 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 a mutant that reverts *yap4* cold-tolerance by affecting endocytosis.

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

Members: Lisete Fernandes.

Students and Technicians: Joana Monteiro.

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 study of GTFs as specific targets of oxidative signals generated by hydrogen peroxide. In this context, we have selected GTFs which should be primary targets of Yap1 and thus targets of oxidative stress. We found that SUA7 gene expression is highly regulated at the level of transcription and mRNA stability under oxidative stress. Contrary to our predictions, the levels of respective protein, TFIIB, do not follow the mRNA abundance showing that the later molecule is the primary target of oxidative stress. The fact that cells guarantee TFIIB abundance under oxidative environments indicates the relevance of such GTF in cell survival.

Molecular mechanisms bridging protein folding and transcription activation.

Members: Lisete Fernandes.

Students and Technicians: João Coelho.

The co-chaperone prefoldin, composed by 5 different subunits, engages folding of different proteins including actin, tubulin, and WD40 repeat proteins. Nevertheless, new additional functions for the subunits, designated as Gim, has been questioned. Indeed, an unconventional prefoldin subunit has been suggested to be an integrate component of the transcriptional machinery. In this context, the role of prefoldin subunits in transcriptional regulation of stress response is being addressed by characterizing *gim* mutant yeast cells.

Study of the function and the regulation of tubulin cofactor a in mammalian cells and during mouse development.

Members: Helena Soares.

Students and Technicians: Sofia Nolasco and João Gonçalves.

External Collaborators: Juan Zabala (Facultad de Medicina, Departamento de Biología Molecular, Universidad de Cantabria, Spain).

Microtubules are polarized polymers of α/β -tubulin heterodimers participating in a wide range of both essential and specialized cell functions. A multi-step process involving distinct molecular chaperones and cofactors produces new tubulin heterodimers competent to polymerize. In vitro cofactor A (TBCA) interacts with β -tubulin in a quasi-native state behaving as a molecular chaperone. In vivo requirements of mammalian tubulin cofactors are poorly understood due to the lack of loss-of-function studies. There are only a few studies reporting the role of TBCA in vivo. Therefore, not much is known about its role especially in multicellular organisms that possess complex tubulin gene families expressing distinct tubulin isoforms. Consequently, we have investigated the phenotypic effects of TBCA knockdown in two mammalian cell lines. We have used siRNA to silence TBCA expression in HeLa and MCF-7 cell lines. We observed that TBCA is essential for cell viability and its knockdown produces a decrease in the amount of soluble tubulin, modifications in microtubules and G1 cell cycle arrest. In MCF-7 cells, cell death was preceded by a change in cell shape resembling differentiation. We have also observed in vivo that free TBCA is not detected except when cells were treated for 1h with colchicine. Interestingly, experiments using microtubule depolymerizing agents (i.e. nocodazole, colchicine and cold-shock) showed that steady-state levels of TBCA/ β -tubulin dimer change in the response to an increase of native tubulin heterodimers. This suggests that components of tubulin folding pathway may regulate the pool of tubulin heterodimers competent to polymerize.

Our results also showed that there are two expressed genes encoding TBCA in mouse. Interestingly, these genes have different patterns of expression during testis maturation: the mRNA levels of one of them increases through spermatogenesis while the other decreases.

Taken together our results contribute to a better understanding of the TBCA functions in vivo, setting up new questions concerning the role of this protein in the tubulin folding pathway and its involvement in regulation of microtubule dynamics.

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

Members: Helena Soares.

Students and Technicians: Cecília Seixas and Miguel Coelho.

External Collaborators: Jacek Gaertig (Department of Cellular Biology, University of Georgia, Athens, USA), Luis Viseu Melo and Pedro Brogueira (Departamento de Física, Instituto Superior Técnico, Lisboa, Portugal).

The cytosolic chaperonin, CCT or TriC, is a hetero-oligomeric complex of about 900 kDa which mediates folding of several types of proteins including tubulins. Unfolded alpha and beta-tubulins interact with CCT where they acquire their tertiary structures. Once released from CCT, alpha and beta-tubulins are delivered to a set of proteins named tubulin cofactors that enable the formation of assembly-competent α/β tubulin dimers. We have been assuming that the folding of tubulins destined to cilia, including their dimerization, occurs in the cell body of *Tetrahymena* and that the IFT pathway transports tubulin dimers or oligomers to cilia. Surprisingly, by indirect immunofluorescence we observed that the *Tetrahymena* CCT alpha CCT delta, CCT epsilon and CCTeta-subunits co-localize with microtubules in cilia, basal bodies, cytoproct and contractile vacuole pores. In cilia, the CCT subunits were found mainly in the membrane-matrix fraction, suggesting that CCT is involved in either folding or transport of ciliary proteins. Biochemical studies revealed that deciliation/reciliation affects the oligomeric state of CCT subunits. While in growing cells tubulins were primarily associated with a 900 kDa CCT complex, in cilia-regenerating cells tubulins were preferentially associated with smaller CCT oligomeric species of about 500 kDa (so called microcomplexes). We have partially purified and characterized a complex of ~500 kDa containing alpha-tubulin. This complex is composed of at least 8 distinct proteins with molecular masses ranging from ~30 to 150 kDa. Besides tubulin, one of these proteins was identified as CCTalpha. To clarify the role of CCT subunits in assembly/maintenance of cilia we constructed germline knockout strains for CCTalpha and CCTdelta genes. Both CCTalpha and CCTdelta are essential genes and progenies of their knockout heterokaryons died shortly after separation of conjugants. CCTalpha null cells lacked intracytoplasmic microtubules, indicating a decrease in the pool of assembly-competent tubulin dimers. Experiments are in progress to rescue the lethality of mating heterokaryons with CCT genes containing random mutations to identify mutant alleles which do not affect the basic folding activity of the chaperone that is essential for cell growth, but do affect the ciliary functions.

The role of the cytoskeleton of the apicomplexa *Besnoitia besnoiti* protozoa on the initial steps of host cell invasion.

Members: Helena Soares.

Students and Technicians: Yara Reis, Isabel Marques.

External Collaborators: Alexandre Leitão (Instituto de Investigação Científica Tropical, CIISA, Lisbon, Portugal), Helder Cortes, (Laboratório de Parasitologia. Núcleo da Mitra. Universidade de Évora, ICAM, Portugal), Luis Viseu Melo, (Departamento de Física, Instituto Superior Técnico, Lisbon, Portugal).

The coccidia *Besnoitia besnoiti* (Apicomplexa: Sarcocystidae) is a cyst-forming protozoa parasite responsible for bovine besnoitiosis. In this work we investigated the role of protozoa microtubule (Mt) cytoskeleton in the first steps of host cell invasion. Using indirect immunofluorescence we observed that isolated *B. besnoiti* possesses a set of subpellicular Mts spirally arranged. These Mts radiate from the apical end of the parasite cell and extend for more than 2/3 of the cell body towards the posterior edge. Upon interaction with the host cell *B. besnoiti* undergoes dramatic modifications of its shape

and surface, as revealed by atomic force microscopy. These alterations are accompanied by a re-organization of the protozoa Mt cytoskeleton, characterized by the loss of the subpellicular Mts giving rise to tubulin globular-like structures. In the host cell, Mt cytoskeleton also re-organizes around the entering parasite. The treatment of extracellular tachyzoites with the anti-mitotic agent nocodazole does not affect their subpellicular Mts while maintain their capacity to invade the host cells. Depolymerization of host cell Mts by the same agent does not inhibit the invasion by the protozoa. Our results strongly suggest a cross-talk between the parasite Mt cytoskeleton and that of host cell during active host cell invasion.

Study of physical properties of microtubules by AFM techniques.

Members: Helena Soares.

Students and Technicians: Ruben Ramalho.

External Collaborators: Luis Viseu Melo (Departamento de Física, Instituto Superior Técnico, Lisbon, Portugal).

Microtubules are dynamic polymers that present a hollow cylindrical structure with an approximate outer diameter of 30 nm (in the case of a 13 protofilament microtubule). They are composed of α - β -tubulin heterodimers arranged in linear protofilaments (with α - and β -tubulin alternating along each protofilament), which, in turn, are disposed side by side to form the tubular structure. Microtubules are one of the components of the cytoskeleton and, thus, ubiquitous in eukaryotic organisms, where they perform a great variety of vital roles (e.g. cytoplasmic transport and chromosomal segregation).

Several theoretical studies have concluded that the tubulin heterodimers have a permanent electric dipole, with a component parallel to their longitudinal axis. Therefore, microtubules themselves should have a significant electric dipolar moment, also parallel to their axes. Therefore an electrical field of sufficient magnitude should be capable of causing microtubules to align themselves parallel to the field direction. Should this hypothesis be correct, it might help clarify the cellular functions of microtubules and even identify new ones – for example, it is possible that the electrical properties of microtubules have some bearing on the control of motor proteins.

To test our hypothesis, we have used atomic force microscopy (AFM) to observe samples of microtubules submitted to an electrical field.

Our work this far has included the purification of tubulin heterodimers from mouse brains, their polymerization to obtain microtubules, the optimization of microtubule sample preparation for observation in AFM and the study of the effects of electric fields on the placement and orientation of microtubules within each sample.

We have been able to obtain highly pure tubulin heterodimers, which were easily polymerized into microtubules and stabilized with taxol, and have established a reliable method of adsorbing microtubules to poly-L-lysine-covered glass and cleaning these samples in order to make them viable for electric field alignment assays.

The alignment of microtubules by electric fields was attempted at first with fields of up to 6 kV/m. No significant difference in the orientations of the microtubules was observed between samples exposed to electric fields and control samples not exposed to field.

The same experiment was then performed with larger electric fields of 2 MV/m. Preliminary results show the samples of microtubules exposed to the electric fields to have an approximately normal distribution of angles, centered in the field direction. Experiments are in progress to assert different field and buffer conditions. In a subsequent stage the consequences of these properties will be explored.

Behavioral Neuroscience

Following a proposal that was supported by the Institute's Scientific Advisory Board, the Board of Trustees of the Gulbenkian Foundation approved a novel program in Neurosciences at the IGC. We aim at installing up to 5-6 groups, dedicated to studying the molecular, cellular and systemic bases of behavior in mice and rats. We expect that the practice of recent methods allowing for the *in vivo* recording of cellular activity, together with genetics manipulation on a solid basis of developmental and evolutionary biology will result in significant contributions,

One wing of the Institute's building will soon be renewed and adapted to specific requirements for this work and the computational/theoretical sector of the IGC will also come to include groups in computational neurosciences.

The role of A kinase anchoring proteins in the regulation of synaptic activity.

Members: Marta Moita.

Students and Technicians: Marija Spasikova, Daniel Ferreira.

In this project, we intend to investigate part of the intracellular signaling cascades that lead to changes in synaptic activity. The PKA signaling pathway plays an essential role in regulating synaptic activity, namely in synaptic plasticity and memory storage. As with all other kinases, PKA activation leads to the phosphorylation of a variety of cellular targets, so that manipulations involving activation or inhibition of PKA make it hard to understand which downstream event is responsible for the effects observed. A family of scaffold proteins that bind PKA (A-kinase anchoring proteins, AKAP), which regulate the phosphorylation of several proteins by PKA, has been recently discovered. AKAP proteins determine the subcellular localization of PKA, thereby conferring specificity to PKA. The binding of PKA onto AKAPs has been shown to be essential for the phosphorylation of several proteins implicated in synaptic plasticity and memory formation. However, very few studies have addressed the role of the AKAP proteins in these processes. In this project, we intend to access directly the role of these protein complexes in regulating synaptic plasticity, by blocking PKA binding to AKAPs, using a well known peptide (Ht-31) and testing its effects on long term potentiation (LTP) and long term depression (LTD) *in vitro*. Since this peptide competes for the PKA binding site, which is conserved across all AKAP proteins, it is impossible to know which target is being affected. Thus, we intend to develop new tools to block the binding of specific

AKAPs to their corresponding unique targets. We chose to start by the protein Yotiao, which binds the NMDA receptor, a molecule crucial for most forms of synaptic plasticity and learning. Specifically, we will develop virally expressed dominant negative peptides that block the binding of yotiao to the NMDA receptor. Once we have these peptides, we will test the role of yotiao in the regulation of NMDA receptor function, and on the activation of signaling cascades that are activated upon calcium entry via the NMDA receptor.

The role of the auditory cortex in associative learning.

Members: Marta Moita.

Students and Technicians: Raquel Antunes.

In this project we intend to study the role of a particular structure in the brain, the auditory cortex, in associative learning, which involves learning about the association between two or more events. We will use classical fear conditioning as a behavioral paradigm. During fear conditioning a previously neutral stimulus, such as a tone, is paired with an aversive stimulus, such as footshock, so that the tone and shock co-terminate. Animals quickly learn that the tone predicts shock delivery. After conditioning the tone elicits fear responses, such as freezing, where animals stop all movement except for respiratory movements. The amygdala, a structure in the temporal lobe, is crucial for the acquisition and storage of fear memories. Information about the tone reaches the amygdala, either directly from the auditory thalamus, or indirectly via the auditory cortex. Lesioning each pathway alone does not affect the acquisition of auditory fear conditioning, however lesioning both impairs this form of learning. These two input pathways differ in important ways. For example, the mechanisms underlying synaptic plasticity of these two inputs to the amygdala are different. How each pathway contributes to the acquisition of auditory fear conditioning remains unclear. We would like to study the role of the auditory cortex, that is the indirect route of auditory input to the amygdala, during auditory fear conditioning. To test the role of activity of auditory cortical neurons, we intend to inactivate pharmacologically the auditory cortex during fear conditioning. Furthermore, we intend to record activity in the auditory cortex to look at fear induced changes in the representation of sound in this part of the brain. Finally, we would like to study fear induced changes in the representation of sound in areas downstream of primary auditory cortex, such as secondary auditory cortices and the lateral nucleus of the amygdala, and how these changes might depend on activity in primary auditory cortex.

The role of the amygdala in trace fear conditioning.

Members: Marta Moita.

Students and Technicians: Marta Guimarães.

In this project we are interested in understanding the mechanisms underlying learning of an association between stimuli separate in time. To this end we will use auditory trace fear

conditioning. In this paradigm a previously neutral tone is presented several seconds before an aversive shock.. Animals learn that the tone predicts shock even though there is a temporal gap between the tone and shock.. Trace fear conditioning engages several structures that are not required for delay fear conditioning (when tone and shock co-terminate). What is the role of these structures and where in the brain the association between the tone and shock is formed remains unclear. Until now it has been assumed that this association is formed in the amygdala, since this structure has been shown to be critical to all forms of fear conditioning tested. However, the role of the amygdala in trace fear conditioning has never been directly tested. We will thus study the role of the amygdala in trace fear conditioning, by performing pre- and post-training lesions of this structure and test their effect on the acquisition and expression, respectively, of this form of conditioning.

What are the rules governing synaptic plasticity in the amygdala?

Members: Marta Moita.

Students and Technicians: Patrícia Simões.

In this project we will study mechanisms of synaptic plasticity in the amygdala. It has long been shown that synaptic efficacy between two neurons can change in response to specific activity patterns of these neurons. When the activity between the pre and post-synaptic cell is correlated, then synaptic efficacy between the two is potentiated. When activity is uncorrelated, then, the synaptic efficacy will be depressed. These changes are long lasting and are thought to underly the formation of memories in the brain. Until now the focus has been on the temporal contiguity in activity between the two cells. Recently it has been found that in order to see a potentiation or depression of a synapse, activity in the pre and post synaptic neuron has to occur within a narrow time window (in the order of 100msec). This finding has raised enormous interest in the neuroscience community and the mechanisms underlying this *spike timing dependent plasticity*, are now being intensively explored.

Importantly, however, the contiguity between two stimuli is not the only requirement for animals to learn their association, being the probability of one stimulus occurring before the other crucial for learning their association. That is, even if two stimuli overlap in time, if this overlap has only a fifty percent chance of occurring, then animals will not learn the association between them. It has recently been shown that synaptic plasticity in the amygdala is sensitive not only to the contiguity between pre and post-synaptic activity, but also to the probability of the pre-synaptic cell being active before the post-synaptic cell. How are cells computing the probability of coincident firing remains unknown. Therefore, we will study the mechanisms underlying this phenomenon, using *in vitro* electrophysiology in amygdal slices.

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 2004 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/BIA-BCM/61270/2004

Jörg Becker

A comparative analysis of the Arabidopsis sperm cell transcriptome to decipher the role of the male gametes in double fertilization.

POCTI/MGI/46477/2002

Jorge Carneiro

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

POCTI 36312/1999

Jorge Carneiro

HIV2 infection as a model for the investigation of AIDS pathogenesis.

POCTI/ CBO/47565/2002

Sukalyan Chatterjee

Transcriptional regulation of CD34 antigen in stem cells and its role in development.

POCI/SAU-MMO/58192/2004

Jocelyne Demengeot

Inflammatory components in the biology of regulatory T cells and autoimmune diseases prevention.

POCTI/CBO/38391/2001

Sérgio Dias

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

POSI/55758/2004

Francisco Dionisio

The role of the social interaction in bacterial diversity and evolution.

POCTI/CVT/56015/2004

António Duarte

Arteriogenesis – identification of novel members of the Notch pathway involved in arterial cell fate determination.

POCTI/CVT/48766/2002

António Duarte

Use of transgenic conditional overexpression to address the function of a novel mammalian Delta homologue, mDll4.

POCTI/BCI/46453/2002

José Feijó

A genetic and molecular approach to the biophysics of cell-to cell communication during sexual reproduction in plants.

POCTI/BCI/37862/2001

Lisete Fernandes

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

PDCT/BIA-BCM/55501/2004

Lisete Fernandes

Molecular mechanisms bridging protein folding and transcription activation.

POSI/SRI/47778/2002

Pedro Fernandes

BioGrid - Parallel Algorithms for Gene Annotation.

POCTI/MGII/40478/2001

Pedro Fernandes

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

POCTI/MAT/58528/2004

Gabriela Gomes

Reinfection thresholds and the management of recurrent infections.

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*.

POCI/SAU-MMO/55974/2004

Luís Graça

Tolerance induction in autoimmunity: reprogramming the immune system with monoclonal antibodies.

POCI/SAU/MMO/61652/2004

Matthias Haury

Regulation of IL10 Allelic Expression.

POCTI/NSE/48782/2002

Domingos Henrique

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

POCTI/BIO/46695/2002

Domingos Henrique

Expansion and Differentiation of Neural Stem Cells in Bioreactors with stirring.

POCTI/BCI/41909/2001

Antonio Jacinto

Epithelial dynamics and adhesion during *Drosophila* dorsal closure.

POCI/BIA-BCM/58389/2004

Antonio Jacinto

Inflammation and Chemotaxis in *Drosophila* embryos.

POCTI/MGI/46337/2002

Moisés Mallo

Reversible gene inactivation in the mouse.

POCTI/BIA-BCM/60420/2004

Moisés Mallo

The function of the antisense transcript of the *Hoxb3* gene during mouse development.

POCTI/SAU-MMO/60419/2004

Moisés Mallo

Hoxb4 in the formation of hematopoietic stem cells.

POCTI/BIA-BCM/56938/2004

Marta Moita

Study of the role of A kinase anchoring proteins in the regulation of synaptic activity.

POCTI/SAU-NEU/56935/2004

Marta Moita

Study of the role of auditory cortex in associative learning.

POCI/SAU-MMO/58413/2004

Luisa Mota-Vieira

Study of genetic diversity of the Azorean population.

POCTI/ SAU-NEU/56986/2004

Teresa Pais/Sukalyan Chatterjee

Papel da activação dos macrófagos do cérebro em modelos animais de doenças neurodegenerativas.

POCTI/MGI/45100/2002

Michael Parkhouse

Viral modulation of cell division, apoptosis and interferon responses.

POCTI/SAU-MMO/59444/2004

Michael Parkhouse

Mecanismo e aplicação de um novo gene indutor de apoptose de Herpes Virus.

POCI/SAU/NEU/56627/2004

Paula Parra-Bueno

Role of somatostatin during DRG embryo development.

POCTI/MGI/44111/2002

Leonor Parreira

Delta1 and Jagged1 genes in normal hematopoietic differentiation.

POCTI/BCI/37953/2001

Leonor Parreira

The role of Notch ligands Delta1 and Jagged1 in lymphoid differentiation.

POCTI/MGI/46070/2002

Carlos Penha Gonçalves

Genetics and Pathogenesis of Type 1 diabetes in Murine Models.

POCI/SAU-MMO/62964/2004

Carlos Penha Gonçalves

Genetics of innate immune response in murine type 1 diabetes.

POCTI/SAU-MMO/57955/2004

Carlos Penha Gonçalves

Diabetes Tipo 1 : Immunopathology and genetic susceptibility.

POCTI/SAU-IMI/61057/2004

Carlos Penha Gonçalves

Genetic determinants of resistance to hepatic infection in murine malaria models.

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/SAU-MMO/63284/2004

Joaquin Rodriguez-Léon

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Leonor Saúde

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Pedro Simas

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Helena Soares

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Miguel P. Soares

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Filipa Vala/Elio Sucena

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Astrid Moura Vicente
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EUROPEAN UNION PROJECTS

QLRI-CT-2001-01363
Pedro Fernandes
EMBCORE – The Core European Bioinformatics Research Infrastructure in the Life Sciences.

MEXT-CT-2004-14338
Gabriela Gomes
Reinfection thresholds and the management of recurrent infections.

LSHG-CT-2003-503494
Domingos Henrique
FunGenES- Functional Genomics in Engineered ES cells.

LSHM-CT-2003-504468
António Jacinto/Joaquin Rodriguez Leon/ Isabel Palmeirim/ Leonor Saude/ Solveig Thosteinsdottir.
Cells into Organs: Functional genomics for development and disease of mesodermal organ systems.

MERG-CT-2004-510174
Maria Mota
GPIs and malaria.

MIRG-CT-2004-513760
Sofia Oliveira
Stroke genetics.

QLK3-CT-2001-00422
Miguel Soares
Targeting Heme-Oxygenase-1 (HO-1) or its molecular mediators: a new therapeutic approach for treatment of inflammation.

Marie Curie Research Training Network (RTN) /FP6 - EU
Álvaro Tavares
Understanding the dynamics of cell division.

OTHER PROJECTS

cDNA Microarray Technology in Diagnosis and Monitoring for Oncology patients.
Sérgio Dias
Fundação Calouste Gulbenkian.

Angiogenesis in Prostate Cancer.
Sérgio Dias
Portuguese Society of Urology and from Abbott Pharmaceuticals.

Mechanisms of Endothelial Differentiation from Endothelial Progenitors.
Sérgio Dias
Liga Portuguesa Contra o Cancro and Crioestaminal.

Angiogenesis in Leukemias and Myelodysplastic syndromes.
Sérgio Dias
Liga Portuguesa Contra o Cancro and Novartis.

Gripept.net – Viagens de um vírus.
Gabriela Gomes and Sander van Noort
Fundação Calouste Gulbenkian.

Heterogeneidade geográfica da incidência da tuberculose em Portugal.
Gabriela Gomes and Cristina Paulo
Direcção Geral de Saúde.

FEDER/Saúde XXI/I/1.1/1960
Guilherme Gonçalves
Vacinação contra a difteria, o tétano e a rubéola em Portugal. Estudos seroepidemiológico para avaliar estratégias em curso e permitir decisões baseadas na evidência.

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.
António Jacinto
069880 Wellcome Trust, UK.

WellcomeTrust- African Swine Fever Virus
Michael Parkhouse
Development of African Swine Fever Virus vaccines.

Liga Portuguesa Contra o Cancro - Terry Fox
Michael Parkhouse and Rute Nascimento

Elimination of a murine BCL1 lymphoma through specific antibody mediated delivery of liposomes containing MHV-68 gene VGAP mediating apoptosis.

PAL+

Sylviane Pied

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

GEMI Fund AgaLinde Healthcare

Miguel P. Soares

Inhaled CO and Multiple Sclerosis.

Phillip Morris External Research Program

Miguel P. Soares

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NIH RO1 Grant No: HL67040-01.

Miguel P. Soares

Regulation of endothelial cell apoptosis by HO-1 derived CO apoptosis.

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Álvaro Tavares/Thomas Surry (Heidelberg)

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2. André J-B. and Gandon S. Vaccination, within-host dynamics, and virulence evolution. *Evolution*.
3. André J-B. and Godelle B. Within-host evolution and virulence in microparasites. *J Theor Biol*.

4. Boavida L., Becker J.D. and Feijó J.A. Cell-to-cell communication during sexual plant reproduction. *Int J Dev Biol*.
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12. Moreira J. and Faro J. The hypothesis of recycling in the germinal center: agreement of mathematical models with experimental data. *WSEAS Trans Biol Biomed*.
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Articles in books and other articles in press

1. Bendahmane M. and Karlsen K.H. Uniqueness of entropy solutions for doubly nonlinear anisotropic degenerate parabolic equations. *AMS Contemporary Mathematics*.
2. Bendahmane M. and Saad M. Entropy solutions of anisotropic reaction-diffusion systems with L1 data. *Revista Matematica Complutense* .
3. Bettencourt-Dias M., Coutinho A.G. and Araújo S.J Strategies to Promote Science Communication: Organisation and Evaluation of “Comunicar Ciência”, a Workshop to Improve the Communication Between Portuguese Researchers, the Media and the Public. *Comunicação e Sociedade*.

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9. Teotónio H., M.R. Rose and S. Proulx S. in press. Phenotypic plasticity and evolvability: an empirical test with experimental evolution. In: *Insect Phenotypic Plasticity*, Eds. D. Whitman and T.N. Ananthakrishnan, Science Publishers, Inc. Plymouth, UK.

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1. Cohn M. What are the commonalities governing the behavior of humoral immune recognitive. (2006) 30:19-42.
2. Dionisio F. and Gordo I. The tragedy of the commons, the public goods dilemma and the meaning of rivalry and excludability in evolutionary biology. *Evol Ecol Res* (2006) 8:1-12.
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SEMINARS AT THE IGC

January

Susana Lima (Yale University, Yale, USA)

Reconstitution of animal behaviors through genetically targeted photostimulation of neuronal activity.

Margarida Barroso (Albany Medical Center, Albany, USA)

Fluorescence resonance energy transfer (FRET) reveals different organizations of endocytic receptors (pIgA-receptor and transferrin-receptor) in polar.

Olga Tounghousova (Faculty of Medicine, Oslo University, Oslo, Norway)

Tuberculosis caused by bacilli of the W-Beijing genotype.

Ron H. Schwartz (NIH Lab., National Institute of Allergy and Infectious Diseases, Bethesda, USA)

Immune tolerance.

Ana Sofia Oliveira (IGC)

Stroke genetics.

William Scott (Duke University Medical Center, Durham, USA)

Genetics of Parkinson disease.

Thiago Lopes-Carvalho (University of Alabama at Birmingham, USA)

Origin and function of marginal zone B cells.

Angus Buckling (University of Bath, Bath, UK)

Bacterial cooperation, spite and virulence.

February

Isabel Campos (IMM/IGC)

Insights into Gli3 role during zebrafish development.

Eduardo Eyras (Biomedical Informatics, Pompeu Fabra University, Barcelona, Spain)

Completing the catalogue of human genes.

Fernando Roch (Centre de Biologie du Développement, Université Paul Sabatier, Toulouse, France)

Cell biology in drosophila discs: a genetic approach.

Stephen Wilson (Cold Spring Harbor Laboratory, NY, USA)
Left and Right - Genetic, neuroanatomical and behavioral approaches to CNS asymmetry in Zebrafish.

Bruno Lemaitre (Centre de Genetique Moleculaire, Gif-sur-Yvette, France)
Genetic and genomic approaches of the drosophila immune response.

Peter Scheiffele (Center of Neurobiology and Behavior, Columbia University, New York, USA)
Molecular mechanisms of synaptic differentiation.

Mike Crair (Baylor College of Medicine, Houston, Texas, USA)
Nature vs. Nurture in sensory map development.

Rui Alves (University of California Davis, USA)
Reconstructing metabolism and investigating design principles in molecular biology.

Paulo Campos (Federal Rural University of Pernambuco, Brazil)
Spatially structured model for ecosystems dynamics.

Rui Costa (Duke University, Durham, USA)
Cognitive and sensorimotor dysfunction: from molecules to systems in mouse models.

Maria Manuel Mota (IMM/IGC)
Malaria functional genomics host factors that contribute for liver infection.

Hernando del Portillo (Instituto de Ciências Biomédicas, São Paulo University, Brazil)
Molecular approaches to the study of chloroquine resistance and virulence in plasmodium vivax.

Irwin Cohen (Weissman Institute, Rehovot, Israel)
Regulatory networks in the immune system.

March

Gabriel Martins (FCUL/IGC)
Imaging cell migration and extracellular-matrices in 3D.

Michael Rose (UCI, Irvine, California)
Aging and immortality.

Ana Cristina Paulo (IGC)
Mathematical models of transmission and control - childhood diseases.

Fran Balkwill (Cancer Research UK, Translational Oncology Laboratory, UK)
Inflammation and cancer.

Nicolas Gompel (Howard Hughes Medical Institute, Madison, USA)
The evolution of morphological traits in insects.

Angelo Chora (IGC)
Effect of heme oxygenase expression in multiple sclerosis.

Lars Hviid (The Centre for Medical Parasitology, Rigshospitalet and Univ. Copenhagen, Copenhagen, Denmark)
Evidence-based progress towards syndrome-specific vaccines against plasmodium falciparum malaria.

Francisco Dionisio (IGC)
The tragedy of the commons, the public goods dilemma, and the significance of rivalry and excludability in evolutionary biology.

April

Malcolm Love (TV and Radio Producer, Bristol, United Kingdom)
Gods, boffins and Dr. Strangelove - popular images of science and scientists.

Karina Xavier (Princeton University, Princeton, USA)
Quorum sensing and inter-species communication in bacteria.

Alexander Kel (SVP Research and Development, BIOBASE GmbH)
From composite patterns to pathways: prediction of key regulators of gene expression.

Ana Claudia Zenclussen (Reproductive Immunology Group, Inst Medical Immunology, Charite, Medical University Berlin, Germany)
Immunotolerance during pregnancy.

Andreia Gomes (IGC)
HOXB4 in hematopoiesis.

Luciano Milanesi (Institute for Biomedical Technologies, National Research Council, Milano, Italy)
Context-dependence mutation spectra analysis of the nucleotide sequences in gene.

Carlos F. Barbas (The Skaggs Institute for Chemical Biology and The Scripps Research Institute, CA, USA)
Designer transcription factors: developing strategies to control and discover genes.

Fedor Kolpakov (Design Technological Institute of Digital Techniques, Novosibirsk, Russia)

BioUML-Open source extensible workbench for systems biology.

Tom Gridley (Jackson Laboratory, Bar Harbor, Maine, USA)

Notch signaling in mice.

Astrid Moura Vicente (INSA-RJ/IGC)

Autism genes.

Leonor Boavida (IGC)

Genetic dissection of cell-to-cell interactions in sexual reproduction in arabidopsis thaliana.

John F. Kearney (Tumor Institute, University of Alabama at Birmingham, Birmingham, USA)

Marginal zone B cells: development and function.

May

Miodrag Grbic (University of Western Ontario, Ontario, Canada)

Evolution of arthropod development: from ancestral developmental program to developmental novelties.

Vojislava Grbic (University of Western Ontario, Ontario, Canada)

Natural variation in shoot patterning in arabidopsis.

Anthony Bishopp (Institute of Biotechnology at the University of Helsinki, Helsinki, Finland)

Cytokinin regulates vascular patterning in the arabidopsis root.

Simon Rumpel (Department of Anatomy and Developmental Biology, University College London, London, UK)

How many neurons takes to form a memory?

Malcolm Logan (National Institute for Medical Research, London, UK)

Constructing the forelimbs and hindlimbs of the vertebrate embryo.

Nuno Afonso (IGC)

Specificities of the caudal neural tube in the chick embryo.

Robert Kelsh (University of Bath, Bath, UK)

Making neurons, painting fish_fate specification as a common theme for sox10 gene function in the neural crest.

Leslie Vosshall (Laboratory of Neurogenetics and Behavior, The Rockefeller University, New York, USA)

Olfaction in drosophila: from receptors to perception.

Tobias Bonhoeffer (Max-Planck Institute of Neurobiology, Munich, Germany)

Activity dependent plasticity in the mammalian brain: new insights into functional and morphological changes on the synaptic level.

June

Ricardo Azevedo (Houston University, Houston, USA)

Sexual reproduction selects for robustness and negative epistasis in artificial gene networks.

Gabriela Silva (IGC)

Degradation of p38a mitogen activated protein kinase (MAPK) isoform mediates the anti-apoptotic effect of heme oxygenase-1 in endothelial cells.

John Runions (School of Biological and Molecular Sciences, Oxford Brookes University, UK)

Using photoactivatable GFP to study dynamics of the plant secretory pathway.

Lena Alexopoulou (Centre d'Immunologie de Marseille-Luminy, CNRS-INSERM, Marseille, France)

The role of toll-like receptors in immunity: lessons from knockout mice.

Ana Teresa Tavares (IGC)

Transcriptional regulation of Caronte asymmetric expression.

Antonio Jacinto (IMM/IGC)

In vivo cell migration: deciding where to go?

Manuel Rebelo (IGC)

Interleukin-7 in CD4 T cell homeostasis.

July

Ari Waisman (Inst Med Klinik und Poliklinik, Clinic of the Johannes Gutenberg, University of Mainz, Germany)

Antigen presentation by B-cells regulatory effect in a mouse model for multiple sclerosis.

Alvaro Tavares (IGC)

Cell cycle progression and cancer: flying into new genes.

Marie-Anne Felix. (Institut Jacques Monod, Paris, France)
Evolution of vulva development in C.elegans and closely related nematodes.

Nico Stollenwerk (Faculdade de Ciências, Universidade do Porto, Porto, Portugal)
Criticality and its self-organization in epidemiology, models and data.

Marion Muehlen (Robert Kosh Institute, Berlin, Germany)
Diversity and multiplicity of plasmodium falciparum in pregnant women.

Caetano Reis e Sousa (Immunobiology Lab, Cancer Research UK, London)
Infection sensing by dendritic cells.

September

Flávio Coelho (Fundação Oswaldo Cruz, Rio de Janeiro, Brazil)
Modeling the spread of a directly transmitted disease in a real, complex geographical network.

Sander van Noort (IGC)
The influenza project: measuring the dutch influenza epidemic via the internet.

Paul Fine (London School of Hygiene and Tropical Medicine, London, UK)
Polio eradication - problems of the endgame.

João Xavier (University Delft, Netherlands / ITQB)
Studying the activity and structure of microbial biofilms by mathematical modelling.

Jen Sheen (Massachusetts General Hospital and Dept Genetics, Harvard Med School, Boston, USA)
The Plant MAPK cascade signaling network.

Elisabetta Padovan (Dept Clinical Research, Bern University Hospital, Bern, Switzerland)
Adjuvancy of TLR agonists: structure-function relationship.

António Duarte (FMVUTL/IGC)
Activation of notch signalling by delta-like 4 determines arterial identity.

Karine van Doninck (Harvard University, Boston, USA)
The unique histone H2A variants and histone cluster organization of the bdelloid rotifer Philodina roseola.

October

Luis Graça (IMM/IGC)

From transplants to allergy, and from allergy to autoimmunity: chasing regulatory T cells around the world.

Joseph Kunkel (Univ. Massachussets, Massachussets ,USA)

Application of non-invasive scanning microprobes to biological systems.

Helena Soares (IGC)

Microtubules and tubulin folding crossroads.

Rod Ceredig (University of Basel, Basel, Switzerland)

Interleukin-7, an elixir for T and B lymphocytes.

André Valente (National Cancer Institute, USA)

The yeast protein interaction network.

Jonathan Ewbank (Centre d'Immunologie de Marseille-Luminy, France)

Specificity in C.Elegans innate immunity.

Maria Manuel Mota (IMM/IGC)

Malaria in circulation.

Salvatore Adinolfi (Division of Molecular Structure, National Institute for Medical Research, London, UK)

Frataxin: An iron lover or a pretender?

November

Dipa Natarajan (MRC National Institute for Medical Research, London, UK)

Isolation of enteric nervous system progenitors from embryonic and postnatal gut; developing new therapies for human Hirschsprung's disease.

Henrique Teotónio (IGC)

Evolution of breeding systems in Caenorhabditis elegans.

Ueli Grossniklaus (Insitute of Plant Biology, University of Zürich, Zurich, Germany)

Sex, parental conflict and infanticide.

Isabel Gordo (IGC)

Adaptation in structured populations.

Mark Ptashne (Memorial Sloan-Kettering Cancer Center, New York, USA)

Genes and signals: an overview.

Richard Ransohoff (Lerner Research Institute, Cleveland, Ohio, USA)

Chemokines and chemokine receptors: regulators of inflammation and development in the vertebrate nervous system.

Eberhard Voit (Medical University of South Carolina, USA)
Trends in complex systems biology.

Rui Reis (University of Minho, Braga, Portugal)
New multidisciplinary approaches for the tissue engineering of human.

Jennin Metcalfe (Econnect, Australia)
Making it big on TV.

Vincent Moulton (School of Computing Sciences, University of East Anglia, UK)
Networks from genomes and metabolomes.

Alain Debec (UMR 7009 CNRS/Université Pierre et Marie Curie, Paris, France)
Characterization of new mitotic proteins in drosophila.

Sólveig Thorsteinsdóttir (FCUL/IGC)
Integrin $\alpha6\beta1$ -laminin interactions regulate early myotome formation in the mouse embryo.

December

Fritz Bach (Lewis Thomas Distinguished Professor, Harvard Medical School, Boston, USA)
Heme Oxygenase-1: an anti-inflammatory mediator of the pleiotropic effects of statins.

Constantin Fesel (IGC)
Systemic lupus erythematosus, autoantibodies and T-cell regulation.

Joy Hirsch (Neurological Institute, Columbia University, USA)
Neuroimaging: a new view of brain and mind.

António Coutinho (IGC)
A non-disquisitional conversation on malaria immunity.

Ana Margarida Coutinho (IGC)
Gene interaction in autism and hyperserotonemia.

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 (PGDB)

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Olga Carina de Oliveira Fernando

Jennifer Geiger - left February 2005
Sarah Keusch
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Ana Margarida Abrantes Mateus
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Roni Lara Moya - left September 2005
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Gulbenkian PhD Programme in Biomedicine for 2005

3-12 Jan: Developmental Biology

Organiser: Alfonso Martinez-Arias (University of Cambridge, UK)
Faculty: Patrick Lemaire (LGPD/IBDM, Marseille, France)
António Jacinto (IGC, Oeiras, Portugal)
Domingos Henrique (IHE-FMUL, Lisboa/IGC, Oeiras, Portugal)
François Schweisguth (École Normale Supérieure, Paris, France)
Marcos Gonzalez Gaitan (Max-Planck Institute, Dresden, Germany)

13-25 Jan: Immunology

Organiser: Jocelyne Demengeot (IGC, Oeiras, Portugal)
Faculty: Fredrik Ivars (Immunology Unit, University of Lund, Sweden)
Vasco Barreto (Rockefeller University, NY, USA)
Thiago Carvalho (University of Alabama at Birmingham, USA)
António Coutinho (IGC, Oeiras, Portugal)
Carlos Penha-Gonçalves (IGC, Oeiras, Portugal)
Constantin Fesel (IGC, Oeiras, Portugal)
Miguel Soares (IGC, Oeiras, Portugal)
Salvatore Valitutti (INSERM, Toulouse, France)

26-28 Jan: Virology

Organiser: Michael Parkhouse (IGC, Oeiras, Portugal)
Faculty: Paul Digard (Dept. Pathology, University of Cambridge, UK)
Jonh McCauley (Institute for Animal Health, Newbury, UK)
Linda Dixon (Institute for Animal Health, Woking, UK)
Jane Greatorex (Univ. Cambridge, UK)

31 Jan -4 Feb: Host-Pathogen Interaction

Organiser: Michael Parkhouse & Maria Mota (IGC, Oeiras, Portugal)
Faculty: Christoph Tang (Imperial College, London, UK)
Deborah Smith (Imperial College, London, UK)

Murray Selkirk (Imperial College, London, UK)

7-16 Feb: Neurobiology I

Organiser: Oscar Marín (Instituto de Neurociencias, Alicante, Spain)

Faculty: Stephen Wilson (University College London, London, UK)

Peter R. Scheiffele (Columbia University, New York, USA)

Michael Crair (Baylor College of Medicine, Houston, USA)

18 Feb and 28 Feb - 4 Mar: Neurobiology II (21-25 Feb - to be decided)

Organiser: Ricardo Gil da Costa (Harvard University, USA)

Faculty: Maria Ida Gobbini (Princeton University, USA)

William Kyle Simmons (Emory University, USA)

Miguel Castelo-Branco (IBILI, Coimbra, Portugal)

Rui Costa (Duke University, USA)

7-11 Mar: Tumorogenesis, angiogenesis & Cancer

Organiser: Sérgio Dias (IPOFG, Lisboa & IGC, Oeiras, Portugal)

Faculty: Frances Balkwill (Institute of Cancer at Barts, London, UK)

Branca Cavaco (IPOFG, Lisboa, Portugal)

14-18 Mar: Epidemiology & Evolution

Organiser: Francisco Dionísio (IGC, Oeiras, Portugal), Isabel Gordo (IGC, Oeiras, Portugal) and Gabriela Gomes (IGC, Oeiras, Portugal)

Faculty: Pedro João Neves e Silva (FCUL, Lisboa, Portugal)

Lars Hviid (Centre for Medical Parasitology, Copenhagen, Denmark)

Guilherme Gonçalves (IGC, Oeiras, Portugal)

21- 24 Mar: Theoretical Biology, Genomics & Bioinformatics

Organiser: Jorge Carneiro (IGC, Oeiras, Portugal)

Faculty: Stan Marée (Univ. Utrecht, The Netherlands)

Rui Alves (Depart. Ciències Mèdiques Bàsiques, Universitat de Lleida, Lleida, Spain)

José Fernando Mendes (Univ. de Aveiro, Portugal)

Pedro Coutinho (IGC, Oeiras, Portugal)

29-31 Mar: Science Communication

Organiser: Ana Godinho Coutinho (IGC, Oeiras, Portugal)

Faculty: Malcolm Love (Bristol, UK)

Annual Meeting of PGDB in Tomar 18-21 December 2005

After the success of last year's experience, the Annual Meeting gathered again the three Portuguese doctoral programmes on Biomedical Sciences. The 2nd Joint Annual Meeting was this time held in Tomar. From the 18th to the 21st December students from GABBA (Graduate Program in Areas of Basic and Applied Biology, University of Porto), PDBEB (Doctoral Programme on Experimental Biology and Medicine, University of Coimbra) and PGDB (Gulbenkian PhD Programme in Biomedicine), were together to present their work, exchange their experiences and discuss science.

Oral presentations (51 from PGDB students in a total of 103) were held in simultaneous sessions and PGDB-5 students presented posters (16 in a total of 39) 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 them.

Members of the PGDB research review committee (António Jacinto, Leonor Saúde, Luis Graça, Miguel Godinho Ferreira, José Leal and Rui Costa) followed closely all the presentations and provided feedback to the PhD programme Executive.

There were two keynote conferences by invited speakers: Professor Spencer Wells, National Geographic Society, on "The Genographic Project", and Professor Allan Bradley, Sanger Institute, Cambridge, UK, on "Tumour suppressor mouse knockouts: A gene and a genome based analysis".

Professor Nuno Crato was also invited to talk on a special session on "Science Communication", leading to fruitful discussion.

By decision of the Board of Trustees of the Gulbenkian PhD Programme in Biomedicine (PGDB), there were no admissions to the Programme in 2005. A number of developments concerning IGC's activities in doctoral education are currently under preparation and these will soon be announced.

PhD PROGRAMME IN COMPUTATIONAL BIOLOGY

Progress in life sciences is increasingly dependent on the research and development in Computational Biology. In recent years, this interdisciplinary research area underwent a great development at an international scale that was not encompassed by a corresponding development in Portugal.

The Fundação para a Ciência e a Tecnologia, the SIEMENS SA Portugal, and the Fundação Calouste Gulbenkian decided to join efforts to promote a pilot PhD Programme in Computational Biology.

The PhD Program in Computational Biology aims to ensure the training of a limited number of PhDs in this area at an internationally competitive level. This program is organized by the Instituto Gulbenkian de Ciência in cooperation with several national and international institutions, and runs in close interplay with a Collaboratorium in Computational Biology.

The PhD Program in Computational Biology is a four-year program divided into one year of full-time courses, workshops and projects on the main aspects of computational biology from the biological, computational, mathematical, chemical and physical points of view, and three years of research training in a recognised laboratory anywhere in the world. The choice of laboratory is left to the student but must meet the standards set by the Program Direction and Scientific and Pedagogic Advisory Board.

The objectives of the Program are:

Introducing the inter-disciplinary field of computational biology by giving students a wide culture on most of its aspects;

offering in-depth research training in one area of computational biology chosen by each student during the first year of the Program.

A critical spirit and an open mind from the part of the students are nurtured and strongly encouraged. The courses during this first year are given mostly by renowned invited researchers from Europe and beyond.

Direction

Marie-France Sagot , INRIA, France (Program Director)

Jorge Carneiro, IGC, Oeiras (Program Deputy-Director)

Luis Rocha, Indiana University, USA (Collaboratorium Director)

Board of Trustees

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Fernando Lopes da Silva, UVA, NL
Alexandre Quintanilha, IBMC, Porto
Amílcar Sernadas, IST, Lisboa

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Jonas de Almeida MUSC, USA
Rui Alves , UC Davis, USA
António Baptista , ITQB, Oeiras
José Bártholo Pereira Leal , MRC, UK
Pedro Coutinho , IGC, Oeiras
Francisco Dionísio, IGC, Oeiras
José Faro, IGC, Oeiras
Ana Teresa Freitas , IST and INESC, Lisbon
Gabriela Gomes, IGC, Oeiras
Isabel Gordo , IGC, Oeiras
Paulo Martel , ITQB, Oeiras and University of Algarve
Eduardo Rocha , Pasteur Institut, France
Claúdio Soares , ITQB, Oeiras
Raquel Tavares, University of Lyon, France

Organs and Committees

The Program has a Board of Trustees and a Scientific and Pedagogic Advisory Board, as specified in the Convention signed by the three Patrons: Fundação para a Ciência e a Tecnologia, SIEMENS SA Portugal, and Fundação Calouste Gulbenkian.

The Board of Trustees will follow the operation of the Program and will ensure that its goals are fulfilled. To this end the Board of Trustees will appoint committees of external expert evaluators that will regularly assess the quality of the Program. Scientific and pedagogic advise both to the Program Direction and to the Board of Trustees is of the responsibility of the Scientific and Pedagogic Advisory Board.

Internal Program Committee

Besides these Boards created in the context of the Convention signed by the Patrons, the Program has an Internal Program Committee, composed of experts in computational biology who are aware of the portuguese scientific community and who were invited by the Direction to overview the general planning of the Program, offering criticisms and suggestions for its improvement. To this purpose, the members of the advisory committee may have access to every document referring to the Program and its management, are invited to the retreats organized during the Program, and have the possibility to privately interview the students and instructors.

Students of the Phd Programme in Computational Biology 2005

Ana Paula Santos Botelho Oliveira Leite
Bruno Emanuel Ferreira de Sousa Correia
Guilhermina Isabel dos Santos Duarte
Liliana da Conceição Monteiro Salvador
Luís Filipe Domingos Pereira de Figueiredo

Jacinto José Fonseca Pereira
Márcio Duarte Albasini Mourão
Nuno Miguel Dias Mendes
Pedro Tiago Gonçalves Monteiro
Ramiro Emanuel Magno Morgado
Rogério Paulo Pelaio Candeias
Sara Vieira da Silva

PhD Programme in Computational Biology for 2005

Introductory Module October 4th-28th, 2005

Basics on the Theory of Evolution

Isabel Gordo, Francisco Dionisio, Henrique Teotonio, Elio Sucena

Statistics

Dinis Pestana, Susana Vinga and Nuno Sepulveda

Basics of Biology

Jorge Carneiro, Jose Feijo, Alvaro Tavares, Helena Soares, Isabel Palmeirim, Moises Mallo, Marta Moita, Jocelyne Demengeot, Sukalyan Chatterjee

A Primer in Computer Science: Algorithms

Ana Teresa Freitas

Logics

Pavão Martins

Introduction to Programming (Python)

António Leal

Molecular Sequences and Structures 1 October 31st- November 25th, 2005

Theory of evolution and population genetics

Brian Charlesworth

General Introduction to Algorithms

Eric Tannier

Sequence alignment, from pairwise to multiple, from genes to genomes

Laurent Duret

Introduction to graphs and to graph optimisation problems

Eric Tannier

Multiple alignment and whole genome sequence alignment

Burkhard Morgenstern

Introduction to algorithmic complexity

Nadia Pisanti

Introduction to Phylogenetic Trees

Vincent Moulton

Text algorithms and indexes

Julien Allali

Trends in Complex Systems Biology

Eberhard Voit

Constructing Phylogenetic Trees

Katharina Huber

New Directions in Phylogenetic Networks

Katharina Huber

Molecular Sequences and Structures 2 (DNA, RNA and proteins)
November 28th-December 16th, 2005

Structural Bioinformatics and Molecular Simulation

Protein Structures

Margarida Archer

Carbohydrate Structures

Phil A Jackson

Lipids and Membrane Structures

Manuel Prieto

Algorithms

Eric Tannier

Nucleic Acid Structures

Alvaro Tavares

Determination of Biomolecular Structures .1. X-Ray Crystallography

Isabel Bento

Determination of Biomolecular Structures .2. NMR

Pedro Lamosa.

Overview of Structure Bioinformatics and Biomolecular Simulation

Claudio Soares

Visualisation of Structural Information

Antonio M. Baptista, Diana Lousa, Sara Campos.

The role of statistical mechanics in Biomolecular Modeling

Antonio M. Baptista.

Principles of Molecular Mechanics and Dynamics

Claudio Soares.

Practical on Molecular Mechanics and Dynamics

Claudio Soares, Nuno Micaelo, A. Sofia F. Oliveira.

Practical on Continuum Electrostatics

Antonio M. Baptista, Vitor H. Teixeira, Miguel Machuqueiro

Principles of Molecular Docking .1.

Claudio Soares, Bruno Victor, Carlos A. Cunha

Principles of Molecular Docking .2.

Claudio Soares, Bruno Victor, Carlos A. Cunha

Methodologies in Drug Design .1.

Roderick E. Hubbard.

Practical on Protein Structure Prediction.

Claudio Soares, Bruno Victor, A. Sofia F. Oliveira

Wrapping up structural bioinformatics and molecular simulation.

Claudio Soares

INTERNAL PhD PROGRAMME (PDIGC)

Director

Sérgio Gulbenkian

Staff

Maria Matoso

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

Annual Meeting of PDIGC

17-20 October 2005

The third annual PDIGC meeting took place at the IGC from 17th to 20st October 2005. The scientific sessions included presentations from 58 students, 7 post-docs and 2 principal investigator and plenary lectures given by Prof. Miguel Seabra (Imperial College, London, UK) who talked about “Rab GTPases and the intracellular organisation of membranes in health and disease” and Prof. Jonathan Howard (Univ of Cologne, Cologne, Germany) who talked of “Non-linearities in the evolution of immune mechanisms” and about his life in science.

THE GULBENKIAN TRAINING PROGRAMME IN BIOINFORMATICS **(GTPB)**

An entry level Bioinformatics training course took place from July 11th to July 15th. Faculty: David P. Judge, Department of Genetics, University of Cambridge, Cambridge, UK, Lisa Mullan, European Bioinformatics Institute, Hinxton, UK, Pedro Fernandes (IGC) and Isabel Marques (IGC).

THE FCUL/IGC POST-GRADUATE PROGRAMME IN BIOINFORMATICS **(PGBIOINF)**

The fourth edition of the programme started on the 5th of September. 10 candidates were admitted. The Introduction to Bioinformatics course bi-101 was taught at the IGC. Faculty: Pedro Fernandes and Mário G. Silva .

Earlier in 2005, Part B of the third edition was taught in the IGC. 14 advanced seminars took place and the 14 students of the 2004/2005 (third) edition completed the course.

Courses:

January 24 - 28

Statistical Methods in Bioinformatics

Faculty: Lisete Sousa, FCUL, Lisboa, Portugal

January 31 - February 4

Gene Prediction and Identification

Faculty: Charles Chapple, IMIM, Barcelona, Spain

Jan Jaap Wesselslink, IMIM, Barcelona, Spain

Eduardo Eyras, IMIM, Barcelona, Spain

February 15 – 18

Quantitative Human Genetics

Faculty: Astrid Vicente, IGC, Oeiras, Portugal, INSARJ, Lisboa, Portugal

Constantin Fesel, IGC, Oeiras, Portugal

Ana M. Coutinho, IGC, Oeiras, Portugal

Catarina Correia, IGC, Oeiras, Portugal

Marta Barreto, IGC, Oeiras, Portugal

Isabel Marques, IGC, Oeiras, Portugal

February 21 – 25

Population Genetics

Faculty: Mark Beaumont, U. Reading, Reading, UK

Lounes Chikhi, U P.Sabatier, Toulouse, France

March 7 – 11

Population Dynamics and Epidemiology

Faculty: Gabriela Gomes, IGC, Oeiras, Portugal
Manuel Carmo Gomes, FCUL, Lisboa, Portugal

March 29 – April 1

Genetic Expression and Microarrays
Faculty: Joaquin Dopazo, Valencia, Spain
Juan M. Vaquerizas, Valencia, Spain

April 4 – 8

Proteomics, Transcriptomics and Metabolomics
Faculty: Luciano Milanesi, ITB, Milano, Italy
Alexander Kel, BIOBASE, Wolfenbüttel, Germany

April 11 – 15

Functional and Comparative Genomics
Faculty: Luciano Milanesi, ItalyB, Milano, Italy
Alexey Lagunin, IBC, Moscow, Russia
Fedor Kolpakov, DTIDT, DOTE, Novosibirsk, Russia

April 18 – 22

Data Warehousing and Mining
Faculty: Christian Blaschke, ALMA Bioinf., Madrid, Spain
Jose Maria Fernandez , CNB, Madrid, Spain

May 2 – 6

Protein Structure and Function Prediction
Faculty: Michael Tress, CNB, Madrid ,ES
Manuel J Gómez, C. Astrobiologia, Madrid,ES

May 9 -13

Gene Ontology
Faculty: Robert Stevens, U. Manchester, Manchester, UK
Helen Parkinson, EBI, Hinxton, UK
Amelia Ireland, EBI, Hinxton, UK
Nick Drummond, Manchester, UK

May 16 – May 20

Biologically Inspired Algorithms
Faculty: Carlos Lourenço, FCUL, Lisboa, Portugal
Luis Correia, FCUL, Lisboa, Portugal
Ana Respício, FCUL, Lisboa, Portugal
Sara Silva, CISUC, Coimbra, Portugal
Miguel Lupi Alves,FCUL, Lisboa, Portugal

May 23 – 26

Phylogenetics and Molecular Evolution

Faculty: Hernan Dopazo, CIPF, Valencia, Spain

May 30 - June 3

Limits and Expectations in Bioinformatics

Faculty: Terri Attwood, U. Manchester, Manchester, UK

Pedro Fernandes, IGC, Oeiras, Portugal

Ana Sofia Figueiredo, IGC, Oeiras, Portugal

SCIENCE AND SOCIETY

The IGC has been developing a Science Communication Programme in the past few years that aims at promoting scientific culture, by involving scientists and the public in activities that stimulate scientific curiosity. Several activities have now become a tradition in the IGC, namely the organization of conferences for science teachers (Biology in Modern Times) and school visits. Also, in 2005, an important collaboration was started with the Oeiras City Hall, in the form of a programme called Oeiras Vive a Ciência (*Oeiras Lives Science*). This programme was a collaborative effort of 3 research institutes in the Oeiras campus (IGC, ITQB – Instituto de Tecnologia Química e Biológica and EAN – Estação Agronómica Nacional) within which a number of activities aimed to showcase the research carried out at these institutions as well as provide an opportunity for scientists to interact directly with the public of the campus district.

Although specific activities are developed, tailored to each audience, the underlying rationale to the entire programme is the promotion of public *engagement* in science, through direct, two-way communication, that is, dialogue.

In 2005, the following activities were organized by the IGC Science Communication Office, detailed according to the target group:

Media

Press Office duties

These include liaison with the media, preparing and sending out press releases and organising press conferences. The aim is to provide journalists with media subsidies, in the form of press releases, to aid in their task of reporting the research undertaken at the IGC. Selected press releases have been placed online at AlphaGalileo, the European science news agency.

From January through to the end of November 2005, a total of 10 press releases were prepared and put out, on different topics, ranging from published papers to meetings and awards. As a direct result of these press releases, IGC research was covered in several of the leading national and weekly newspapers and magazines in Portugal (including Público, Diário de Notícias, Jornal de Notícias, Capital, Expresso, Focus, Visão), and also on television (2010).

Scientists and Journalists: a clash of cultures?

23rd November, IGC, as part of National Science and Technology Week.

The one morning seminar/debate, held at the IGC, was directed at science communicators and science journalists but also at all interested scientists. Its aim was to discuss how to breach the cultural barriers between scientists and journalists in order to improve the relationship between the two classes.

Organisers: Sofia Cordeiro (IGC) and Sheila Vidal (IGC)

Speakers: António Granado (*Público* national newspaper), Jenni Metcalfe (*Econnect Communication* Pty Ltd, Australia) and Toss Gascoigne (Director of CHASS, *Council for the Humanities, Arts and Social Science*, Australia).

Participants and evaluation: In total, 26 scientists and/or science communicators and 2 journalists were present. Participants rated the event 4,5 (out of 5).

Science Teachers

Biology in Modern Times Seminars

These seminars are targeted at secondary school teachers. The main aims are to update teachers on the latest developments in life science research both in terms of concepts and methods and to foster the teachers' interest and enthusiasm in scientific research and in science in general and to consolidate a network of scientists (from graduate students to senior scientists) and school teachers, looking to future collaborations.

Table 1 summarizes the sessions held during the 2004/05 school year. Each session was made up of three seminars, by three different scientists, grouped by general themes. Between 40 to 60 teachers were present in each session.

Table 1. Biology in Modern Times seminars during the 2004/05 school year.

Date	Theme	Titles
19th Nov 04	Host-pathogen interaction	Viruses as disease agents (Pedro Simas, IGC) Bacterial evolution, focusing on antibiotic resistance and pathogenicity (Francisco Dionísio, IGC) Control of parasites in the nematode family through vaccination and diagnosis (Michael Parkshouse)
27th Jan 05	Hereditarity and genetics	Autism Genetics (Astrid Vicente, IGC) Genetics of Resistance and Disease Susceptibility (Carlos Penha, IGC) Stem cells: Promises and Reality (Leonor Parreira, IGC)
15th Mar 05	Techniques and technology of gene function study	Immunology Day – a challenge for teachers (Jorge Carneiro, IGC) Using genetically manipulated mice in Biology: how and why (Moises Mallo, IGC) RNAi (interference RNA) – how to make a protein disappear from cells without using genetics (Álvaro Tavares, IGC) Macrophage dynamics in transgenic fly embryos (António Jacinto, IGC)

Students

School visits to the IGC

18 schools visited the IGC in 20 visits, in a total of 532 students, from Lisbon, Amora, Vila Franca de Xira, Almeirim, Coimbra, Faro and several local schools (Carcavelos and Parede).

Science at meal-time

As part of the *Oeiras Vive a Ciência* Programme, this initiative comprised the conception, design, production and distribution of placemats for school cafeterias. There were 4 major themes: Genetics, Ecology and Environmental Conservation, Mathematics, Chemistry and Physics and for each theme, scientists at the organising institutions developed contents for 3 different placemats (primary schools, early secondary schools and secondary schools). The place mats were then distributed in 35 primary schools and 17 secondary schools in the Oeiras district. Evaluation is now proceeding.

Scientists

“Science Communication: skills, approaches and context” module for graduate students of the PGDB programme

29-31 March, IGC

This module was organised as part of the PGDB series of courses and focused on written and oral presentation skills, and introduced students to the history and contexts of communicating with the public, with special emphasis on promoting dialogue.

Speakers: Ana Coutinho (IGC) and Malcolm Love (Freelance Producer and Birkbeck College, UK)

2nd Science Communication Workshop

1-4 Setembro, IGC

This workshop, held for the second time at the IGC, aimed at improving communication between scientists and non-technical audiences.

Organisers: Ana Godinho Coutinho (IGC), Sofia Jorge Araújo (Instituto Biologia Molecular de Barcelona, Espanha), Mónica Bettencourt-Dias (Cambridge University, UK), Associação Viver a Ciência

Speakers: Ana Godinho Coutinho (IGC), Sofia Jorge Araújo (Instituto Biologia Molecular de Barcelona, Espanha), Mónica Bettencourt-Dias (Cambridge University, UK), Frank Burnet (Univ. West England, UK), Malcolm Love (Freelance Producer and Birkbeck College, UK), Steve Miller (Univ. College London, UK), Elizabete Caramelo (Chief Press Officer of the Presidency of the Republic, Portugal), Rosália Vargas (Ciência Viva, Portugal), Ana Noronha (Ciência Viva, Portugal), António Granado (Público newspaper), Ana Correia Moutinho (GAI, Univ. Lisboa), Bruno Afonso (Instituto Superior Técnico, Portugal), Greta Martins (IGC)

Participants: 16 researchers (from several scientific areas) and communication officers

Scientists and Journalists: a clash of cultures?

23rd November, IGC, integrated in the National Science and Technology Week.
(see above in the *Media* section)

Public

Consensus Conference on Genetic Manipulation of Plants

15-16th January 2005, Vila Flor

This consensus conference was a collaboration between two IGC staff members (Ana Coutinho and Jose Mario Leite) and the Vila Flor City Council.

Coordinator: Ana Godinho Coutinho (Instituto Gulbenkian de Ciência)

Steering Committee: Alfredo Teixeira (President, Instituto Politécnico de Bragança), Anabela Martins (Quercus – Núcleo de Bragança), António Monteiro (Direcção Regional de Agricultura de Trás-os-Montes), Gracinda Peixoto (Câmara Municipal de Vila Flor), Margarida Oliveira (Instituto de Tecnologia Química e Biológica)

Moderator: José Mário Leite (Instituto Gulbenkian de Ciência)

Lay panel: 12 members of the public, selected by

Expert Panel: Alexandre Quintanilha (IBMC), Margarida Silva (Quercus and Univ Católica), Jaime Piçarra (Associação Portuguesa dos Industriais de Alimentos Compostos para Animais), Mário Frota (Associação Portuguesa do Direito do Consumo)

The consensus report prepared by the lay panel, after two days of questioning the experts, discussing amongst themselves, and negotiating a consensus, is available on the website of the Vila Flor City Council. The aim of this consensus conference was to experiment with an innovative form of dialogue between experts (scientific and others) and the public in Portugal, one that would give the public a leading role in setting the agenda of the conference and in reaching the final decision on a controversial topic in science.

Science goes to the Cinema

Several dates between January-April 2005, ITQB Auditorium, as part of the *Oeiras Vive a Ciência* Programme

Movies with a underlying scientific theme or one of scientific interest were screened, followed by a question and answer session with a panel of scientists.

21st January, A Beautiful Mind (Theme: Mathematics and Neuroscience)

Invited scientists: Alexandre Caldas and Nuno Crato; Chairperson: António Lopes

18th February, Medicine Man (Theme: Biodiversity Conservation and Phytochemistry)

Invited scientists: Maria do Céu Madureira; Chairperson: Alexandra Lima

11th March, Jurassic Park (Theme: Ecology and Genetics)

Invited scientists: Galopim de Carvalho; Chairperson: Cristina Borges

15th April, GoldenEye (Theme: Technology and Nuclear Energy)

Invited scientists: João Caraça, Agostinho Silva; Chairperson: Ana Coutinho

Scientist for a day, the IGC Open Day

9th April, IGC, as part of the *Oeiras Vive a Ciência* Programme

For one day, the IGC opened its doors to the public, allowing people to experience what the scientific activity is like, how science is done and why and to stimulate direct contact with the scientists. This was achieved by a series of activities, namely, an informal seminar with the IGC director, a round-table about the life of scientists, an interactive bioinformatics exercise, screenings of documentaries on IGC research, exhibition of pictures and movies made by IGC scientists and an interactive showroom of all the labs at the IGC, comprising demos and simple experiments.

Between 900-1000 people visited the IGC on this day. All of them took home IGC souvenirs (*scientist for a day* t-shirts, a complementary *scientist for a day at the IGC* canvas bag, an activity passport for which stamps were available in each activity) and the results of some experiments and activities (DNA extracted from strawberry, a “cell” made of play-dough, a “brain-hat” painted by the user, several drawings, etc.).

Science Factory

28th May, Fábrica da Pólvora de Barcarena, as part of the *Oeiras Vive a Ciência* Programme

With similar activities to the ones tested at the open days of the research institutions involved, the Science Factory aimed at showing science in everyday life - in the kitchen, on the street, etc - and at stimulating scientific curiosity in children and their families, regular visitors to the Fábrica da Pólvora gardens.

About 300 people visited Science Factory and all of them rated the event “Good” or “Very Good” (out of 5 categories, “Very Good” being the highest).

Science writing

In the Newsletter of the Calouste Gulbenkian Foundation, under the item ‘Story of a Scientific Discovery’:

- “Zebrafish proteins help in understanding human disease”, *Fundação Calouste Gulbenkian Newsletter*, **Issue 64**, June 2005, Fundação Calouste Gulbenkian, Lisbon
- “The Instituto Gulbenkian de Ciência Open Day: the wonder of being a scientist for a day”, *Fundação Calouste Gulbenkian Newsletter*, **Issue 63**, May 2005, Fundação Calouste Gulbenkian, Lisbon
- “New ways of controlling human tapeworm parasites”, *Fundação Calouste Gulbenkian Newsletter*, **Issue 62**, April 2005, Fundação Calouste Gulbenkian, Lisbon
- “The Instituto Gulbenkian de Ciência: European excellence award is cherry on top of this year’s cake”, *Fundação Calouste Gulbenkian Newsletter*, **Issue 60**, February 2005, Fundação Calouste Gulbenkian, Lisbon

SYMPOSIA, CONFERENCES AND MEETINGS ORGANISED BY THE IGC

Control of tuberculosis: Scientific bases

Instituto Gulbenkian de Ciência

2 June and 14 August 2005

Organisers: Gabriela Gomes, Cristina Paulo, Guilherme Gonçalves (IGC, Oeiras, Portugal), Fonseca Antunes (DGS, Lisbon, Portugal)

National series of meetings designed to promote dialogue and interaction between scientists, mathematicians, clinicians and public health doctors engaged in tuberculosis research. The meetings were successful with an attendance around 20, and will be resumed in 2006.

EMBO Course on Advanced Light Microscopy in Living Cells

Instituto Gulbenkian de Ciência

9 – 17 June 2005

Organiser: Nuno Moreno (IGC, Oeiras, Portugal)

2005 was the third year in which a course on Light Microscopy in Living Cells had been run at the Instituto Gulbenkian de Ciência, and the first year in which it was almost fully supported course of EMBO. Based on previous years' experience, this year it was extended from a week to 9 days. The course was hugely over subscribed, and it was necessary to select the 16 students who could be accepted from 160 applicants. The final student makeup (see attached list) was 8 males and 8 females. Two attendees (12.5%) were from outside Europe (USA and Brazil), three were from Eastern Europe (Russia, Estonia and the Czech Republic) and the remaining 11 were from countries of the European Union. Three of these (18.75%) were from the host country, Portugal.

The format of the course was based on intensive teaching (both lectures and hands-on training) in the first 4 days (with Sunday being free), followed by three days in which the students had formal instruction in the mornings and worked on specific projects in the afternoon and evening. Monday included a manufacturers' forum session – a very useful feature since the manufacturers were much more forthcoming about new technical information than at a normal conference. Students were divided into four groups for their projects, and gave presentations on their results on the final afternoon. Students were invited to suggest or provide projects for this part of the course; some of the projects finally assigned did originate with students and others were provided by the faculty. Projects were all based on living cells and included techniques such as calcium imaging, GFP transfection, Förster resonant energy transfer (FRET) and fluorescence lifetime. While there were inevitably technical difficulties in carrying out many of these projects, the learning experience of carrying through a complex experiment was very useful to the students and most groups produced very impressive reports on the final day.

XX International Conference for Physics Students
Instituto Gulbenkian de Ciência
16 August 2005

Organiser: Pedro Fernandes (IGC, Oeiras, Portugal)

How to Use Bioinformatics to Understand Biology: a brief introduction to Bioinformatics for students from other areas.

Bioinformatics consists of using biological information to solve biological problems. In other words, a gain in knowledge is obtained by a clever usage of the biological information that we have access to. In the Bio-Medical area, information is massively accumulated and massively produced by high throughput experiments, for example. Access to this enormous amount of information is possible in a wide scale, and this continuously opens new challenges to bioinformaticians. Moreover, Biology is arguably the richest source of information that man has direct access to, in number and in complexity. Aside from the fundamental research aspects, Bioinformatics reveals its power in industries (food, pharmaceuticals, biotechnology, etc.) and in decision making (both in academia and in administration).

The Instituto Gulbenkian de Ciencia is involved in Bioinformatics since the early 1990's. The aim of this talk is to explain why Bioinformatics is challenging, also for people that have developed their studies in other areas, in this case Physics. Extra skills have to be put in place, naturally.

This session was attended by over 90 participants.

Tuberculosis: Scientific Basis for Control
Instituto Gulbenkian de Ciência
7-9 September 2005

Organisers: Gabriela Gomes, Cristina Paulo, Guilherme Gonçalves (IGC, Oeiras, Portugal), Fonseca Antunes (DGS, Lisbon, Portugal)

International advanced course covering a wide spectrum of topics in tuberculosis: history of infection, microbiology, immunology, vaccination, epidemiology, economics and mathematical modelling. The course had around 50 participants.

Exploring Pathogen Diversity in Disease Epidemiology and Vaccine Research

Instituto Gulbenkian de Ciência
10-13 September 2005

Organisers: Gabriela Gomes (IGC, Oeiras, Portugal)

Workshop bringing together 7 teams from Europe and Africa to complete an application to the European Commission to fund a Marie Curie Research Training Network. The stage 1 application was submitted on the 28 September, and the result of evaluation is expected this December 2005.

Patenting in Biotech and Life Sciences
Instituto Gulbenkian de Ciência
26-29 September 2005

Organiser: Matthias Haury (IGC, Oeiras, Portugal)

International Patent Workshop Series on 'Patenting in Biotech and Lifesciences' with the participation of the European Patent Office

Flowcytometry and Microscopy workshop
Instituto Gulbenkian de Ciência
24-28 October 2005

Organiser: Nuno Moreno (IGC, Oeiras, Portugal)

Despite of the international courses organized in the institute, there is the need to bring up knowledge to a broader range of people that don't need such a deep knowledge about a specific technique. In that sense we organize an open course for people belonging to the associated laboratory (IGC, ITQB and IBET).

GAMeets - Gulbenkian Alumni Meeting
Instituto Gulbenkian de Ciência
27 December 2005

Organisers: António Jacinto, Sheila Vidal, Manuel Rebelo and Greta Martins (IGC, Oeiras, Portugal)

The Gulbenkian alumni organised the first Gulbenkian Alumni Meeting (GAMeets) with the objective of getting together a large number of young scientists that are, or were, connected to the IGC. These meetings are thought to become an annual event to stimulate scientific discussion and networking among Portuguese scientists that work in any part of the world. This meeting was also a great opportunity for the students of the Gulbenkian PhD programmes.

In this first edition of the GAMeets we had a very exciting scientific programme with four seminars and a closing session with a debate on the conditions that Portugal offers for young scientists to return and do their research here. The Ministry of Health, the Secretary of State for Science and Technology and the presidents of the Champalimaud Foundation and the Gulbenkian Foundation were also present in this discussion.

THESES

PhD Theses

Isabel Alcobia “Organização espacial da heterocromatina no núcleo: Uma visão funcional de cromocentros em células humanas”, University of Lisbon, Lisbon, Portugal, October 2005.

Marta Barreto “Identification and characterization of genetic susceptibility factors for Systemic Lupus Erythematosus”, University of Lisbon, Lisbon, Portugal, September 2005.

Leonor Boavida “Genetic dissection of cell-to-cell interactions in sexual reproduction in *arabidopsis thaliana*”, University of Lisbon, Lisbon, Portugal, November 2005.

Ana Sofia Cachaço “ $\beta 1$ integrins in mouse (*Mus musculus*) skeletal muscle development”, University of Lisbon, Lisbon, Portugal, February 2005.

Patrícia Madureira “Caracterização da proteína de latência M2 do herpesvirus-gama 68 de murganho”, University of Lisbon, Lisbon, Portugal, January 2005.

Sofia Marques “Murine gammaherpesvirus-68 latency in B cells”, University of Lisbon, Lisbon, March 2005.

Leonor Orge “Caracterização da estirpe de BSE em Portugal e estudo da susceptibilidade genética dos ovinos às TSEs”, Trás-dos-Montes University, Covilhã, Portugal, September 2005.

Manuel Rebelo “Lymphocyte homeostasis and development: role of Notch and Interleukin-7”, University of Lisbon, Lisbon, Portugal, October 2005.

MSc Theses

Lídia Fonseca “O estudo da função da proteína codificada pelo ORF73 do MHV68”, University of Lisbon, Lisbon, Portugal, March 2005.

Bruno Douradinha Mateus “The role of Pb36p protein during the hepatic stage of malaria infection”, Instituto Superior Técnico, Universidade Técnica de Lisboa, Lisbon, Portugal, January 2005.

Catarina Susana Ferreira Moita “Computational analysis of the Cadherin superfamily in *Drosophila melanogaster* and *Anopheles gambiae*”, University of Lisbon, Lisbon, Portugal, January 2005.

Ana Raquel Viegas Tomás “Role of Flrt3 in the control of Apical Ectodermal Ridge activity during limb bud development”, Faculty of Sciences and Technology, University of Coimbra, Coimbra, Portugal, December 2005.

BSc Theses

Cláudia Alexandra Ribeiro de Almeida “Modulation of NF- κ B-dependent pro-inflammatory genes in Endothelial Cells by the Stress-responsive Enzyme Heme Oxygenase-1”, University of Lisbon, Lisbon, Portugal, October 2005.

Pedro Campinho “Inhibition of fibrillar collagen synthesis in the chick (*Gallus gallus*) embryo affects the epithelialisation of the lateral presomitic mesoderm”, University of Lisbon, Lisbon, Portugal, October 2005.

Miguel Coelho “Estudo da montagem dos cílios no ciliado Tetrahymena”, University of Lisbon, Lisbon, Portugal, September 2005.

Ana Mónica Pais Correia “Atheroprotective Effect of Inhaled Carbon Monoxide”, University of Lisbon, Lisbon, Portugal, September 2005.

Catarina Correia “The role of Bmp2 in early neural crest development”, Universidade Lusófona de Humanidades e Tecnologias, Lisbon, Portugal, September 2005.

António Currais “Autismo e fenótipos associados: estudos de expressão e associação com genes candidatos”, University of Lisbon, Lisbon, Portugal, October 2005.

Valter Duarte “Mediterranean: Função e alvos em *Drosophila melanogaster*”, Universidade Lusófona de Humanidades e Tecnologias, Lisbon, Portugal, September 2005.

Josina Côrte-Real Filipe, “Modulation of Nuclear Factor κ B by Murine γ -herpesvirus 68” University of Lisbon, Lisbon, Portugal, September 2005.

Dinis Gökyaydin “O limiar de reinfeção e o padrão evolutivo da gripe”, University of Lisbon, Lisbon, Portugal, July 2005.

Rui P. Martins “Evolução de um plasmídeo conjugativo”, University of Lisbon, Lisbon, Portugal, October 2005.

Ines Matos “Cloning, expression, purification and study of the mitogenic activity of HSP90 derived from *Plasmodium berghei*”, Évora University, Évora, Portugal, January 2005.

Carla Sofia Curado Milagre “Expression, regulation and action of Smad7 during digit development”, Universidade Lusófona de Humanidades e Tecnologias, Lisbon, Portugal,

June 2005.

Isabel Peixeiro “Genética e neuroinflamação em crianças autistas com elevados níveis da neurotrofina BDNF”, University of Lisbon, Lisbon, Portugal, July 2005.

Ruben Ramalho "Estudos das propriedades biofísicas dos microtúbulos: implicações nas suas funções *in vivo*", University of Lisbon, Lisbon, Portugal, September 2005.

Ana Catarina Ribeiro "A via de sinalização dos FGF na regulação hematopoietica mediada por Hoxb4", University of Lisbon, Lisbon, Portugal, July 2005.

Pedro Espada Santos “Factores genéticos subjacentes à variabilidade individual na resposta ao tratamento com risperidona”, University of Lisbon, Lisbon, Portugal, October 2005.

Inês Sousa “Análise de genes candidatos para dois subfenótipos associados com o autismo”, University of Lisbon, Lisbon, Portugal, October 2005.

Tânia Vinagre "O papel do gene Gbx2 no desenvolvimento embrionário de murganho", Universidade Lusófona de Humanidades e Tecnologias", Lisbon, Portugal, September 2005.

PARTICIPATION IN ACADEMIC COMMITTEES

José António Belo

Member of the Jury of the Ph.D Thesis of Ana Sofia Cachão, Faculdade de Ciências da Universidade de Lisboa, Lisbon, Portugal , February 2005.

Jorge Carneiro

Member of the Jury of the Ph.D Thesis of Francisco Rodrigues Pinto, Universidade Nova de Lisboa/ITQB, Oeiras, Portugal, December 2005.

Member of the Jury of the Ph.D Thesis of Sara Pinto Garcia, Universidade Nova de Lisboa/ITQB, Oeiras, Portugal, December 2005.

Jocelyne Demengeot

Member of the Jury of the Ph.D Thesis of Leonor Sarmiento, Faculdade de Medicina da Universidade de Lisboa, Lisbon, Portugal, June 2005.

Member of the Jury of the Ph.D Thesis of Stephanie Louis, Faculté de Médecine, Université de Nantes, Nantes, France, June 2005.

Member of the Jury of the Ph.D Thesis of Manuel Rebelo, Faculdade de Ciências da Universidade de Lisboa, Lisbon, Portugal, October 2005.

Francisco Dionisio

Member of the Jury of the BSc Thesis of Rui P. Martins, University of Lisbon, Lisbon, Portugal, October 2005.

Pedro Fernandes

Member of the Executive Committee of PGBIOINF: FCUL/IGC Post-Graduate Programme in Bioinformatics

Appointed representative of the Faculdade de Medicina da Universidade de Lisboa for the shared co-ordination, with the Instituto Superior Técnico, of the Bioinformatics course integrated in LEB, the BSc course in Biomedical Engineering.

Member of the Jury of the MSc. Thesis of Catarina Moita , University of Lisbon, Lisbon, Portugal, January 2005.

Gabriela Gomes

Member of the Jury of the BSc Thesis of Dinis Gökaydin, University of Lisbon, Lisbon, Portugal, July 2005.

Member of the Jury of the BSc Thesis of Bárbara Parreira, University of Lisbon, Lisbon, Portugal, July 2005.

António Jacinto

Member of the Jury of the Ph.D. Thesis of Luís Manuel Vall Teixeira, University of Lisbon, Lisbon, Portugal, January 2005.

Member of the Jury of the Ph.D. Thesis of Ana Isabel Domingos, University of Lisbon, Lisbon, Portugal, May 2005.

Member of the Jury of the Ph.D. Thesis of Catarina Lidia de Almeida Rodrigues Lemos, University of Porto, Porto, Portugal, June 2005.

Member of the Jury of the Ph.D. Thesis of Alexandra Sanfins, University of Lisbon, Lisbon, Portugal, September 2005.

Moisés Mallo

Member of the Jury of the Ph.D. Thesis of Catarina de Moura Elias de Freitas, University of Lisbon, Lisbon, Portugal, January 2005.

Member of the Jury of the Ph.D. Thesis of Carla Sofia Fernandes do Amaral Real Afonso, University of Lisbon, Lisbon, Portugal, March 2005.

Member of the Jury of the Ph.D. Thesis of Nina Trokovic, University of Helsinki, Helsinki, Finland, March 2005.

Member of the Jury of the Ph.D. Thesis of Nuno Miguel Duarte Afonso, University of Paris 6, Paris, France, March 2005.

Member of the Jury of the Ph.D. Thesis of Teresa Vasconcelos Costa, Universidade Nova de Lisboa, Oeiras, Portugal, December 2005.

Gabriel G. Martins

Member of the Jury of the BSc Thesis of Célia Faria, Universidade Lusófona, Lisbon, Portugal, September 2005.

Marta Moita

Member of the Jury of the Ph.D Thesis of Susana Lima, University of Lisbon, Lisbon, Portugal, December 2005.

Maria Mota

Member of the Jury of the MSc Thesis of Sónia Pimentel, University of Lisbon, Lisbon, Portugal, September 2005.

Luisa Mota-Vieira

Member of the jury of the Ph.D Thesis of Marta João Ribeiro Barreto, University of Lisbon, Lisbon, Portugal, September 2005.

Michael Parkhouse

Member of External Review Board de la Red de Investigación de Centros de Enfermedades Tropicales (RICET), Madrid, Spain, September 2005.

Leonor Parreira

Member of Jury of Ph.D Thesis of Luis Manuel Valla Teixeira. University of Lisbon, Lisbon, Portugal, January 2005.

Member of Jury of Ph.D Thesis of Patrícia Saraiva Madureira. University of Lisbon, Lisbon, Portugal, January 2005.

Member of Jury of Ph.D Thesis of Fátima Lopes Veríssimo. University of Lisbon, Lisbon, Portugal, February, 2005.

Member of Jury of Ph.D Thesis of Sofia Pinto Guia Marques. University of Lisbon, Lisbon, Portugal, March 2005.

Member of Jury of Ph.D Thesis of Carlos Calhaz Jorge. University of Lisbon, Lisbon, Portugal, May 2005.

Member of Jury of Ph.D Thesis of Maria Leonor Morais Sarmento. University of Lisbon, Lisbon, Portugal, June 2005.

Member of Jury of Ph.D Thesis of Alexandra Sanfins. University of Lisbon, Lisbon, Portugal, September 2005.

Member of Jury of the Agregação of Cecília Maria Pereira Rodrigues. University of Lisbon. Lisbon, Portugal, September 2005.

Member of Jury of Ph.D Thesis of Manuel Silva Rebelo. University of Lisbon, Lisbon, Portugal, October, 2005.

Member of Jury of Ph.D Thesis of Isabel Alcobia Principe Henriques. University of Lisbon, Lisbon, Portugal, October 2005.

Member of Jury of Ph.D Thesis of Floor Weerkamp. University of Rotterdam, Rotterdam, The Netherlands, November 2005.

Gabriela Rodrigues

Member of the Jury of the BSc Thesis of Tânia Vinagre, Universidade Lusófona, Portugal, September 2005.

Member of the Jury of BSc Theses of the following students: Member of the Jury of Licenciatura Theses of the following students: Guilherme Costa, Catarina Catela, Maria Inês Sequeira, Catarina Pimentel,, Viviana Durão, Luís Marques, Ana Dias, Ana Catarina Rita, Ana Rita Marques, Sara Pimentel and Ana Margarida Charouco.

Leonor Saúde

Member of the Jury of the Master Thesis of Yuri Weber, Universidade Lusófona, Lisbon, Portugal, October 2005.

Miguel Soares

Member of the Jury of the Ph.D Thesis of Juliette Fitau, Universite de Nantes, Nantes, France, November 2005.

Álvaro Tavares

Member of the Jury of the Ph.D Thesis of Tânia Reis de Almeida Bastos, Instituto de Ciências Biomédicas de Abel Salazar, Porto University, Porto, Janeiro 2005.

Member of the Jury of the Ph.D Thesis of Patrícia Madureira, University of Lisbon, Lisboa, January 2005.

Member of the Jury of the MSc Thesis of Lídia Fonseca, University of Lisbon, Lisbon, May 2005.

Member of the Jury of the MSc Thesis of Bruno Douradinha, Instituto Superior Técnico, Universidade Técnica de Lisboa, Lisbon, January 2005.

Sólveig Thorsteinsdóttir

Member of the Jury of the Ph.D Thesis of Catarina Freitas, Faculdade de Ciências da Universidade de Lisboa, Portugal , January 2005.

Member of the Jury of the Ph.D Thesis of Ana Sofia Cachaço, Faculdade de Ciências da Universidade de Lisboa, Portugal , February 2005.

Member of the Jury of the Masters Thesis of Susana Magrito, Universidade Lusófona, Portugal, October 2005.

Member of the Jury of BSc Theses of the following students: Guilherme Costa, Catarina Catela, Maria Inês Sequeira, Catarina Pimentel, Viviana Durão, Luís Marques, Ana Dias, Ana Catarina Rita, Ana Rita Marques, Sara Pimentel, Francisco Freire Esteves and Pedro Campinho, Faculdade de Ciências da Universidade de Lisboa, July-October 2005.

Astrid Vicente

Member of the Jury of the Ph.D Thesis of Judith Conroy, University of Dublin, Dublin, Ireland, December 2005.

Margarida Vigário

Member of the Jury of the Master Degree Thesis of Graça Maria Miranda, Universidade de Lisboa, Faculdade de Medicina, Lisbon, Portugal September 2005.

Member of the Jury of the BSc Thesis of Rita Alexandra Moura, Universidade de Lisboa, Faculdade de Ciências, Lisbon, Portugal. December 2005.

HONOURS AND AWARDS

Núcleo de estudos das doenças autoimunes (NEDAI) Investigação em Auto-imunidade, Sociedade Portuguesa de Medicina Interna.

Marta Barreto and Astrid Vicente

Identification and characterization of genetic susceptibility factors to Systemic Lupus Erythematosus (SLE).

Pulido Valente – Ciência, 2005: Immunologia – Ciência Básica e Clínica

Íris Caramalho, Thiago Lopes-Carvalho, Dominique Ostler, Santiago Zelenay, Matthias Haury and Jocelyne Demengeot

Regulatory T Cells selectively express Toll-like receptors and are activated by lipopolysaccharide”. The Journal of Experimental Medicine, Vol. 197, Number 4, February 17 (2003), 403-411.

Pfizer de Investigação Básica

Marta Carapuço, Ana Nóvoa e Moisés Mallo

Ter ou não ter costelas: quando e onde os genes *Hox* dão informação.

Leonor Saúde, Raquel Lourenço, Alexandre Gonçalves, Isabel Palmeirim

Terra é um gene que promove a assimetria esquerda-direita ao mesmo tempo que é necessário para a sincronização esquerda-direita do relógio da segmentação.

Will Wood, Célia Faria e António Jacinto

Mecanismos distintos regulam a quimiotaxis de macrófagos durante a cicatrização e o desenvolvimento em *Drosophila*.

European League Against Rheumatism (EULAR) Young Investigator Awards

Maria Margarida Souto Carneiro

Cellular and Molecular Dynamics of Ectopic Germinal Center Formation in Rheumatoid Arthritis.

Medalha de Prata da Escola Superior de Tecnologia da Saúde de Lisboa

António Coutinho

Marie Curie Excellence Team (EXT)

Maria Gabriela Gomes

Reinfection Threshold and the Management of Recurrent Infections.

Sociedade Portuguesa de Genética Humana 2005

Sara Maria Lopes Marques

The activity of the Nodal antagonist Cerl-2 in the mouse node is required for correct L/R body axis (2004). Sara Marques, Ana Cristina Borges, Ana Cristina Silva, Michelangelo Cordenonsi & José António Belo. *Genes & Development* 18, 2342-2347.

Pfizer de Investigação Clínica

Guiomar Oliveira, Assunção Ataíde, Carla Marques, Teresa S. Miguel, Ana Margarida Coutinho, Luísa Mota Vieira, Luís Diogo, Carla Domingues, Esmeralda Gonçalves, Nazaré Mendes Lopes, Luís Borges, Vítor Rodrigues, Henrique Carmona da Mota, Astrid Moura Vicente

Epidemiologia das Perturbações do Espectro do Autismo em Portugal: prevalência, caracterização clínica e condições médicas associadas numa população infantil.

Medalhas de Honra L'Oreal Portugal para Mulheres na Ciência

Ana Catarina Santos Sarzeda

Identifying genes required for wound healing in *Drosophila* and conserved in Humans.

Marie Curie Research Training Network (RTN) /FP6 - EU

Álvaro Tavares

Understanding the dynamics of cell division.

Excelência em Imunologia: Mário Arala Chaves

Santiago Zelenay, Ângelo Chora, Miguel Soares and Jocelyne Demengeot
Best poster in the XXXI Reunião da Sociedade Portuguesa de Imunologia.

PARTICIPATION OF IGC PERSONNEL IN CONFERENCES, SEMINARS AND SCIENTIFIC MEETINGS

January

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

Subtractive cloning of differentially expressed genes in chick heart/hemangioblast precursor cells (H/HPC).

Poster at I Encontro da Sociedade Portuguesa de Células Estaminais e Terapia Celular, Lisbon, Portugal.

Coutinho A.

Opening conference for the new resident MD's.

Curso para Internos, Hospital Fernando da Fonseca, Amadora/Sintra, Portugal.

Coutinho A.

O ensino nas áreas da saúde.

Celebração do dia do Instituto Politécnico de Lisboa.

Escola Superior de Tecnologia da Saúde de Lisboa, Lisbon, Portugal.

Marques S., Borges A.C., Silva A.C., Cordenonsi M. and Belo J.A.

Study of genetic function by gene targeting using the embryonic stem cell technology.

Poster at I Encontro da Sociedade Portuguesa de Células Estaminais e Terapia Celular, Lisbon, Portugal.

Moraes-Fontes M.F.

Autoimunidade nas doenças reumatológicas.

XX Semana de Medicina Interna do Hospital do Santa Marta, Lisbon, Portugal.

Parreira L.

Como se atinge a excelência em investigação científica?

Actualizações em Oncologia. Faculdade de Medicina de Coimbra. Coimbra, Portugal.

February

Belo J.A.

Disposição assimétrica dos órgãos: o papel do gene Cerl-2.

NEBi 2005, FERN, Algarve University, Portugal.

Dias S.

Molecular mechanisms regulating angiogenesis.

International Roche Symposium on angiogenesis and lymphangiogenesis in clinical practice, Porto, Portugal.

Pais T.F.
Microglia activation in neuropathologies
Graduate Programme in Basics and Applied Biology (GABA), Porto, Portugal.

Parreira L.
Investigação em células estaminais. Os porquês e as limitações.
Post-graduate course on Genética, genoma e genómica: da clínica à saúde pública. Escola Nacional de Saúde Pública, Lisbon, Portugal.

Saúde L.
The dual function of *Terra*.
University College London, London, UK.

March

Belo J.A.
Animais modelo de doenças genéticas humanas.
IX ENEB Faro, Algarve University, Portugal.

Fernandes P.
Bioinformaticians from raw ingredients
Instituto de Medicina Molecular. Lisbon, Portugal.

Figueiredo A.S., Fernandes P.L., Pissarra P. and Ferreira A.
Integration of software tools for the in silico design of metabolic pathways using flux balance analysis.
FEBS SysBio2005 Advanced course on systems biology: from molecules and modeling to cells, Gosau, Austria.

Graça L.
Using monoclonal antibodies to induce regulatory T cells and tolerance.
Cellular Therapy 2005. Regensburg, Germany.

Mallo M.
Of face and vertebrae: determination of segmental identities by Hox genes.
Institute of Biotechnology, Helsinki, Finland.

Mallo M.
Determination of segmental identities by Hox genes.
Faculté de Médecine Pitié-Salpêtrière, Paris, France.

Marques I.
Introdução à Bioinformática

Workshop on Biologia Molecular, Unidade de Biologia Molecular, Instituto de Higiene e Medicina Tropical, Lisbon, Portugal.

Parkhouse R.M.E.

Control of cystercois.

Higher Education Links Programme, British Council, Merida, México.

Soares M.P.

Heme-Oxygenase-1 and the destruction of “globules rouges”.

Congres Annuel de la Société Française d’Hématologie, Paris, France.

Tavares A.

MOB1-like genes in drosophila melanogaster.

46th Drosophila Research Conference, San Diego, USA.

Truccolo J., Rodrigues R., Chene A., Soulard V., Vigario M., Becker J. and Pied S.

A genomic approach to study astrocytes, microglial cell and P. berghei ANKA interactions.

Poster at the First Annual BioMalpar conference on the Biology and pathology of the malaria parasite. EMBL, Heidelberg, Germany.

Parreira L.

Células estaminais. enigmas e promessas.

Sociedade de Ciências Médicas. Lisbon, Portugal.

Vigário A.M., Georgette O., Cruz T., Dujardin H., Bandeira A. Six A., Pied S.

Regulatory CD4+CD25+ T cells are expanded during Plasmodium infection but do not prevent cerebral malaria.

Poster at the First Annual BioMalpar Conference on the Biology and Pathology of the Malaria Parasite. EMBL, Heidelberg, Germany.

April

Correia S., Crespo A., Goodbourn S. and Parkhouse R.M.E.

Modulation of cell division and interferon responses by an African Swine Fever Virus gene.

Poster at the Society for General Microbiology (SGM) Meeting, Edinburgh, UK.

Demengeot J.

Regulation at the frontier of innate and adaptive immunity.

Riken research center for Allergy and Immunology, Yokohama city, Japan.

Fidalgo S.V., Krug T., Oliveira S.A.

Genetic factors controlling risk and age-at-onset of common neurological diseases.

20th Meeting of the Cerebrovascular Diseases Group and 1st Meeting of the Portuguese Stroke Society. Sesimbra, Portugal.

Graça L.
Indução de tolerância imunitária em transplantação.
Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal.

Graça L.
A importância da tolerância!
International Immunology Day, Instituto de Medicina Molecular, Lisbon, Portugal.

Jacinto A.
Epithelial movements during *Drosophila* dorsal closure.
BSDB/BSCB Spring Meeting 2005. University of Warwick, UK.

Mallo M.
Genes Hox y regulación de identidades esqueléticas.
Instituto de Investigaciones Biomedicas Alberto Sols, Madrid, Spain.

Nascimento R., Crespo A., Parkhouse R.M.E.
Conserved α , γ and β herpesviruses protein inducing G2/M arrest and apoptosis.
Society for General Microbiology (SGM) Meeting, Edinburgh, UK.

Neves H.
Delta1 and Jagged1 in early human hematopoiesis.
EMBO Workshop. Rome, Italy.

Oliveira S.A.
Genetic factors controlling risk and age-at-onset of common neurological diseases.
University of Helsinki, Helsinki, Finland.

Palanniapan S.
Bioinformatics exploration of human whole saliva.
Aveiro University, Aveiro, Portugal.

Reis A.L., Correia S., Crespo A., Goodbourn S., Leitão A. and Parkhouse R.M.E.
Modulation of interferon response by an ASFV gene.
Poster at the Society for General Microbiology (SGM) Meeting, Edinburgh, UK.

Santos M.
Notch ligands Delta-like-1 and Jagged-1 exert distinct effects on B lymphocyte differentiation to Ig secreting plasmacytes.
Poster at the EMBO Workshop on Notch Signalling, Development and Cancer, Rome, Italy.

Saúde L.

Terra is a left-right asymmetry gene required for left-right synchronization of the segmentation clock.

European Network of Excellence “Cells into Organs” Plenary Meeting, Braga, Portugal.

Soares H., Nolasco S, Seixas C, Coelho M., Ramalho R., Reis Y., Cortes H., Leitão A., Viseu-Melo L. and Brogueira P.

Os microtúbulos polímeros dinâmicos das células eucariotas.

Congresso de Saúde de Bragança, Bragança, Portugal.

Tavares A.

Bioquímica, uma ciência de interacção.

II Encontro Nacional de Estudantes de Bioquímica, FCUL, Lisbon, Portugal.

Teotónio H.

The role of deleterious mutations in the maintenance of males in *C.elegans*.

Institut Jaques Monod, Paris, France

Wood W. and Jacinto A.

Drosophila macrophages require phosphoinositide 3-Kinase to chemotax towards a wound but not for developmental migrations.

Keystone Symposia 2005 on Cell Migration and Adhesion, Snowbird, Utah, USA.

May

Branco C.C., Pacheco P.R., Cabral R., de Fez L., Peixoto B.R. and Mota-Vieira L.

Genetic diversity of the Azorean population revealed by 13 short tandem repeats.

Annual Meeting of the European Society of Human Genetics, Praga, Czech Republic.

Branco C.C., Pacheco P.R., Cabral R., de Fez L., Peixoto B.R. and Mota-Vieira L.

Genetic background of the Azorean population assessed by 13 microsatellite loci.

2nd International Meeting on Genetics of Complex Diseases and Isolated Populations, Paestum, Salerno, Italy.

Cabral R., Anjos R., Cymbron T., Macedo C., Duarte C.P. and Mota-Vieira L.

Genetic evaluation of Congenital Heart Disease by genealogical research in São Miguel Island, Azores (Portugal).

40th Annual Meeting of the Association for European Paediatric Cardiology, Copenhagen, Denmark.

Caramalho I.

Regulatory CD4 T cell function and dynamics in inflammation.

INSERM Unit 563, Institut Claude de Préval, Toulouse, France.

Carneiro J.

From cellular to systems immunology.

Instituto de Medicina Molecular, Lisbon, Portugal.

Carneiro J.

Know thy self: modeling the basic cognitive properties of the immune system.

Instituto de Sistemas e Robotica. Instituto Superior Técnico, Lisbon, Portugal.

Cortes H., Marques I., Reis Y., Waap H, Vidal R, Pereira da Fonseca I, Fazendeiro I, Ferreira ML, Caeiro V, Soares H and Leitão A.

Analysis of the 18S, ITS1, 5.8S, ITS2 and 28S rDNA sequence from a Portuguese isolate of *Besnoitia besnoiti*.

Poster at 2nd Annual Workshop (COST Action 857) Apicomplexan Biology in the post genomic era, Beatenberg near Interlaken, Switzerland.

Coutinho A.

Roundtable on “Bio-Ciências”

Fundação Luso-Americana para o Desenvolvimento, Lisbon, Portugal

Coutinho A.

Precisamos de mais médicos em investigação médica.

Award Ceremony “Prémio Pulido Valente Ensino 2005”, Aula Magna da Faculdade de Medicina de Lisboa, Lisbon, Portugal

Coutinho A.

A relevância e o custo da infeção hospitalar.

Chair of the conference. “Saúde sem fronteiras”, Forum Gulbenkian de Saúde 2005, Fundação Calouste Gulbenkian, Lisbon, Portugal.

de Fez L., Pacheco P.R., Branco C.C., Cabral R., Peixoto B.R. and Mota-Vieira L.

HFE mutations in the São Miguel’s population: genetic frequencies and geographic distribution.

Annual Meeting of the European Society of Human Genetics, Praga, Czech Republic.

Dias S.

Angiogenesis and leukemia: basic research and therapeutic perspectives.

Sociedade de Ciências Médicas, Lisbon, Portugal.

Fernandes P.

Bioinformática – genética molecular humana.

BSc in Biochemistry, Lisbon University, Lisbon, Portugal.

Fidalgo S.V, Krug T., Oliveira S.A.

Genomic convergence approach applied to the study of stroke genetics.

9^a Reunião da Sociedade Portuguesa de Neurociências, Luso, Portugal.

Graça L.

Imunoterapias: qual o seu funcionamento?

Annual Congress of the Portuguese Internal Medicine Society. Braga, Portugal.

Lino S., Palla R., Branco C.C., Pacheco P.R., Cabral R., de Fez L., Peixoto B.R. and Mota-Vieira L.

Alu insertion polymorphisms in Azores (Portugal).

2nd International Meeting on Genetics of Complex Diseases and Isolated Populations, Paestum, Salerno, Italy.

Mallo M.

What do Hox genes have to say to the face and the vertebrae?

CABD, Sevilla, Spain.

Mallo M.

Hox genes determine skeletal identities in the mouse.

IBMC, University of Porto, Porto, Portugal.

Mallo M.

Heads and ribs: what do Hox genes have to do with them?

IMM, University of Lisbon, Lisbon, Portugal.

Matos M.H., Pais T.F. and Chatterjee S.

Activation of microglia in the adult brain by GM-CSF using a tumor mouse model.

Poster at the VII European Meeting on Glial Cell Function in Health and Disease, Amsterdam, The Netherlands.

Moraes-Fontes M.F.

Definir denominadores comuns nas doenças auto-imunes.

Pré-11º Congresso Nacional de Medicina Interna Immunology Course, Braga, Portugal.

Mota-Vieira L., Pacheco P.R., Almeida M.L., Cabral R., Carvalho J., Branco C.C., Matos C., de Fez L., Loura M., Peixoto B.R., Araújo A.L. and Mendonça P.

The DNA bank from the population of Sao Miguel Island (Azores): a resource for genetic diversity studies.

Annual Meeting of the European Society of Human Genetics, Praga, Czech Republic.

Pacheco P.R., Branco C.C., Lismond A., de Fez L., Cabral R., Peixoto P.R. and Mota-Vieira L.

Sao Miguel: the genetic history of an Azorean island described by HLA Class I and Class II genes.

Annual Meeting of the European Society of Human Genetics, Praga, Czech Republic.

Palla R., Lino S., Branco C.C., Pacheco P.R., Cabral R., de Fez L., Peixoto B.R. and Mota-Vieira L.

Azorean ancestry assessed by Alu insertion polymorphisms.

Annual Meeting of the European Society of Human Genetics, Praga, Czech Republic.

Parkhouse R.M.E

Vaccines and immunological memory.
Medical School, London, UK.

Reis Y., Cortes H., Viseu-Melo L., Leitão A. and Soares H.
Besnoitia besnoiti undergoes remarkable microtubule cytoskeleton rearrangement during initial steps of host cell invasion.
2nd Annual Workshop Apicomplexan Biology in the post-genomic era, Beatenberg, Switzerland.

Seixas C., Coelho M., Melo L.V., Brogueira P., Gaertig J. and Soares H.
Molecular chaperones involved in tubulin folding are candidates for tubulin transport and axoneme assembly during *Tetrahymena* cilia recovery.
Poster at FASEB Summer Research Conferences. Ciliate Molecular Biology, Lucca, Italy.

Seixas C., Melo L.V., Brogueira P. and Soares H.
Cilia assembly: the construction of a cellular nanomachine.
Current Trends in Nanoscience - From Materials to Applications at the upcoming E-MRS 2005 Spring meeting, Strasbourg, France.

Soares M.P.
Modulation of free iron by heme oxygenase-1 inhibits the activation of the transcription factor nuclear factor kappa B in endothelial cells.
First Congress of the International BioIron Society; Prague, Czech Republic.

Tavares A.
Identifying new tumour suppressors using *Drosophila*.
Paterson Institute for Cancer Research, Manchester, UK.

Tavares A.
Jury member for the Cientific Ideas Contest , XI Encontro de Jovens investigadores, Fundão, Portugal.

Tavares A.
Clonagem e manipulação de DNA.
XI Encontro de Jovens investigadores, Fundão, Portugal.

Teotónio H.
The role of deleterious mutations in the maintenance of males in *C.elegans*.
Department of Zoology, Oxford University, UK.

Zelenay S
Regulatory T cells. Differential requirement for innate and adaptive immune cells inhibition in vitro.
Poster at the FOCIS 5th Annual Meeting, Boston, USA.

June

Becker J.

From transcriptome profiling to novel hypotheses: what genechips can tell us about sexual plant reproduction.

CeBiTec Kolloquium, Center for Biotechnology, University of Bielefeld, Germany

Coutinho A.

Precisamos tanto de médicos-cientistas como de cientistas que são médicos.

Instituto de Investigação em Ciências da Vida e Saúde, Escola de Ciências da Saúde da Universidade do Minho, Braga, Portugal.

Coutinho A.

Ciência e desenvolvimento da cultura científica.

Seminar on Ciência e Educação em Ciência – Situação e Perspectivas, Conselho Nacional de Educação, Lisbon, Portugal.

Coutinho A.

La santé et ses sciences.

Centre Culturel Calouste Gulbenkian, Paris, France.

Demengeot J.

TLRs and regulatory T cells.

Euroconference The interactions between innate and adaptive immunity in mammalian defense against bacterial infections, Joachimsthal, Brandenburg, Germany.

Demengeot J.

Adaptive immune regulation uses innate components.

Faculté de Médecine, Université de Nantes, Nantes, France.

Deus L.

Innovative Mouse Models.

Poster at the Leiden University Medical Centre, Leiden, The Netherlands.

Feijó J.A.

The control of apical cell growth and morphogenesis by ion dynamics.

Padova, Italy

Feijó J.A.

Live cell imaging methods: new tools and old tricks.

L'imaging dalla cellula alla coscienza", PhD. on pharmacology, chemotherapy and toxicology, Università di Milano, Istituto di Neuroscienze, Milano, Italy.

Feijó J.A.

The control of apical cell growth and morphogenesis by ion dynamics.

Siena, Italy.

Figueiredo A.S.

Integration of software tools for the in silico design of metabolic pathways using flux balance analysis.

Poster at the BKDB2005 Bioinformatics: knowledge discovery in biology, Lisbon, Portugal.

Fragoso R. and Dias S.

A role for FLT-1 in acute lymphoblastic leukemia.

10th Congress of the European Hematology Association. Stockholm International Fairs, Sweden.

Gomes G.

The reinfection threshold and its consequences to vaccination.

Evolutionary considerations of vaccine use, DIMACS, New Jersey, USA.

Mallo M.

Los genes Hox en la determinación de la identidad esquelética.

Faculty of Biology, University of Badajoz, Badajoz, Spain.

Marques I.

Introdução à Bioinformática

Lusófona University, Lisbon, Portugal.

Moita C. and Fernandes P.

Comparison of cadherin repertoires in *D. melanogaster* and *A. gambiae*.

BKDB2005 Bioinformatics: knowledge discovery in biology, Lisbon, Portugal.

Parkhouse R.M.E.

Host-pathogen interaction: a two-edged sword.

50 Years Immunology Meeting at the Trudeau Institute, USA.

Parkhouse R.M.E.

Control of human, porcine and bovine cystercosis.

University of Carabobo, Maracay, Venezuela.

Parreira L.

Conselho Nacional de Ética para as Ciências da Vida. Audição sobre aspectos éticos da clonagem.

Reis Y., Cortes H., Melo L.V., Soares H. and Leitão A.

Besnoitia besnoiti undergoes remarkable microtubule cytoskeleton rearrangement during initial steps of host cell invasion.

Poster at Scanning probe microscopy, Sensors and nanostructures Conference, Cancun, Mexico.

Tavares A.
Interaction between tubulin and kinetochore proteins.
18th Symposium of the Protein Society, Barcelona, Spain.

Teles J.
Rheugulation DB, a Bioinformatics tool for the study of rheumatoid arthritis.
Poster at the BKDB2005 Bioinformatics: knowledge discovery in biology, Lisbon, Portugal.

Vigário A.M.
CD4+CD5+ regulatory T cells during murine malaria infection.
Instituto de Medicina Molecular, Lisbon, Portugal.

July

Águas R., Gökyaydin D., Rodrigues P.
The reinfection threshold and its implications to epidemiology.
Poster at the ECMTB05 – European Conference of Mathematical and Theoretical Biology, Dresden, Germany.

Caramalho I.
Linking inflammation and immunoregulation: a role for regulatory T cells.
Instituto de Medicina Molecular, Lisboa, Portugal.

Carneiro J.
Moments to cherish.
VI European Conference of Mathematical and Theoretical Biology, Dresden, Germany.

Coutinho A.
Closing ceremony academic year.
Auditório Municipal Eunice Munõz, Oeiras, Portugal.

Coutinho A.
Ensino e investigação em medicina – novos desafios.
Centro de Ciência Viva do Algarve, Universidade do Algarve, Campus de Gambelas, Faro, Portugal.

Dionisio F.
Microbial Population Biology.
Gordon Research Conferences, NH, USA.

Feijó J.A .
The control of apical cell growth and morphogenesis by ion dynamics.
XVII International Botanical Congress, Viena, Austria

Figueiredo A.S., Forte J, Fernandes P. and Ferreira A.

Integration of open source software tools for the in silico design of metabolic pathways using flux balance analysis.

Poster at the BioSysBio – Bioinformatics and systems biology conference, Edinburgh, UK.

Gardner R. and Carneiro J.

Analysis of lymphocyte receptor diversity based on gene chip hybridization technology.

Poster at the VI European Conference of Mathematical and Theoretical Biology, Dresden, Germany.

Magno R., Paixão T., Feijo J., and Carneiro J.

A model of pollen tube morphogenesis, growth and orientation.

Poster at the VI European Conference of Mathematical and Theoretical Biology, Dresden, Germany.

Paixão T. and Carneiro J.

Why are protein distribution in cell populations distributed as a lognormal?

Poster at the VI European Conference of Mathematical and Theoretical Biology, Dresden, Germany.

Prado M.

Nitric oxide a new signal in pollen tube growth regulation and re-orientation.

SEB Main Meeting, Barcelona, Spain

Ramos A., Feijó J.A., Costa S., Antão J., Cordeiro S., Novaes L., Façanha A.R.

Differential activation of the root surface H⁺ fluxes in medicago truncatula as a function of the phosphate supply.

XXXIV Reunião Anual da SBBQ, Reunião Brasileira de Bioquímica e Biologia Molecular 2005, Águas de Lindóia, Brazil.

Ramos A., Feijó J.A., Façanha A.R.

Modulação da atividade de fosfohidrolases em fluido apoplástico de raízes de trevo colonizadas com fungos micorrízicos arbusculares.

VI mostra de pós-graduação da UENF, 2005, Campos dos Goytacazes, Brazil.

Saúde L. Lourenço R., Gonçalves A. and Palmeirim I.

Terra is a left-right asymmetry gene required for left-right synchronization of the segmentation clock.

Poster at Society for Developmental Biology 64th Annual Meeting, San Francisco, USA.

Sepúlveda N., Serrano C., Espada de Sousa A., Victorino R., and Carneiro J.

Why is progression of HIV infection to AIDS so slow?

Poster at the VI European Conference of Mathematical and Theoretical Biology, Dresden, Germany.

Soares M.P.

Protective genes in ischemia reperfusion injury.

Innate immunity in transplantation: from scientific roots to clinical practice. The Royal Society of Medicine, London, UK.

White L.

The Role of Reinfection in the Transmission and Control of Infectious Disease.

London School of Hygiene and Tropical Medicine, London, UK.

August

Carvalho S., Silva G. and Teotónio H.

From phenotypes to genotypes in hybrids of *C. elegans* wild isolates.

Xth Congress of the European Society for Evolutionary Biology, Krakow, Poland.

França A.R., Mota M.M. and Coutinho A.

Toll-like receptors in the hepatic stage of malaria infection.

Poster at Gordon Research Conference on Malaria, Queen's College, Oxford, U.K.

Seixas C., Melo L.V., Brogueira P. and Soares H.

Tetrahymena cilia are assembled from pre-built blocks at an early reciliation stage.

FASEB Summer Research Conferences. Ciliate Molecular Biology, Lucca, Italy.

Sepúlveda N., Paulino C.D. and Penha-Gonçalves C.

New statistical models for joint action of two loci in complex binary traits and their analysis by MCMC methods.

Joint Statistical Meetings, Minneapolis, USA.

Tavares A.

Centrosomal protein Mob4 is a new tumor suppressor.

19th European Drosophila Research Conference, Eger, Hungary.

Teotónio H. and Manoel D.

Genetics and selection for outcrossing in *C. elegans*.

Xth Congress of the European Society for Evolutionary Biology, Krakow, Poland.

September

Afonso N.

Involvement of Smad8 during vertebrate limb development and programmed cell death. Poster at the European Life Science Organisation, Dresden, Germany.

Becker J.

From transcriptome profiling to novel hypotheses: what genechips can tell us about sexual plant reproduction.

Congresso Nazionale di Biotecnologie, Siena, Italy.

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

Subtractive cloning of differentially expressed genes in chick heart/hemangioblast precursor cells (H/HPC).

Poster at ELSO 2005 Meeting, Dresden, Germany.

Branco C.C., Pacheco P.R., Cabral R., de Fez L., Peixoto B.R. and Mota-Vieira L.

Autosomal microsatellite analysis of the Azorean population.

21st congress of International Society for Forensic Genetics, Ponta Delgada, Azores, Portugal.

Campinho P.

Inhibition of fibrillar collagen synthesis in the chick (*Gallus gallus*) embryo affects the epithelialisation of the lateral presomitic mesoderm.

International post-graduate course on Different tissues, same strategies: common molecules in different developmental systems, Braga, Portugal.

Carapuço M.

Hox genes specify vertebral types in the presomitic mesoderm.

Poster at 6th EMBL Mouse Molecular Genetics Meeting. Heidelberg, Germany.

Coutinho A.

XI Encontro Nacional de Educação em Ciências, Porto, Portugal.

Coutinho A.

Presentation of Nicole Le Douarin's book "Quimeras, Clones e Genes".

Fundação Calouste Gulbenkian, Lisbon, Portugal.

Coutinho A.

Os meus anos em Basileia.

ECOS, Forum Roche, Amadora, Sintra, Portugal.

Deus L.

Genetic control of malaria liver infection: fine mapping of Ber11 locus with subcongenic strains.

Poster at the 6th EMBL Mouse Molecular Genetics Meeting, Heidelberg, Germany.

Faria C., Wood W. and Jacinto A.

Distinct mechanisms regulate hemocyte chemotaxis during wound healing and development in *Drosophila*.

ELSO Annual Meeting 2005, Dresden, Germany.

Feijó J.A.

The control of apical cell growth and morphogenesis by ion dynamics.
Soc.Exp.Biol. meeting on Plant Channels, Glasgow, UK.

Feijó J.A.

The control of apical cell growth and morphogenesis by ion dynamics.
Wageningen University, Wageningen, The Netherlands.

Fernandes E.B., Sepúlveda N., Canto e Castro Loura L. and Penha-Gonçalves C.
Mapeamento de fenótipos quantitativos usando a abordagem por penetrâncias alélicas.
XIII Annual Congress of Portuguese Society of Statistics, Ericeira, Portugal.

Figueiredo C., Pais T.F. and Chatterjee S.

Mechanism of quinolinic acid mediated neuronal cell death.

Poster at the Meeting on programmed cell death, Cold Spring Harbour Laboratory, New York, USA.

Graça L.

Transplantes de órgãos: rejeição e tolerância!

Encontro Juvenil de Ciência, Lisbon, Portugal.

Martins G.G.

Visualização 3D multimodal e morfodensitometria in situ a partir de imagens de microscopia confocal de embriões de vertebrados.

Encontro Nacional de Visualização Científica, Espinho, Portugal.

Mena A and Chatterjee S.

Regulatory mechanism of phosphorylation of Bim in mitosis.

Poster at the Meeting on programmed cell death, Cold Spring Harbour Laboratory, New York, USA.

Moita C. and Fernandes P.

A comparative study of cadherins opens way to research on functional roles.

Poster at the ECCB2005, Madrid, Spain.

Mota-Vieira L., Pacheco P.R., Almeida M.L., Cabral R., Carvalho J., Branco C.C., de Fez L., Peixoto B.R., Araújo A.L. and Mendonça P.

Human DNA bank in Sao Miguel Island (Azores): a resource for genetic diversity studies. P184.

21st congress of International Society for Forensic Genetics, Ponta Delgada, Azores, Portugal.

Pacheco P.R., Branco C.C., Cabral R., de Fez L., Araújo A.L., Peixoto B.R., Mendonça P. and Mota-Vieira L.

The Y-chromosome in the Azores Islands: phylogeny and diversity. P210.

21st congress of International Society for Forensic Genetics, Ponta Delgada, Azores, Portugal.

Saúde L.

Asymmetrical on the inside, symmetrical on the outside.

Workshop Different Tissues, Same Strategies: common molecules in different developmental systems, Braga, Portugal.

Simões S. and Jacinto A.

Drosophila posterior spiracles as a model to study tubulogenesis.

ELSO Annual Meeting 2005, Dresden, Germany.

Soares M.P.

Carbon monoxide and multiple sclerosis.

Scientific Seminars on Gas Enabled Medical Innovations. The GEMI Fund grant award ceremony. Stockholm. Sweden.

Soares M.P.

Heme-oxygenase-1 counters pro-inflammatory signaling in endothelial cells.

Heme oxygenase. The III International Congress, Boston, USA.

Tavares A.

Centrosomal protein Mob4 is a new tumor suppressor.

EMBO Conference on Centrosomes and Spindle polo Bodies, EMBL, Heidelberg, Germany.

Tavares A.T., Andrade S. e Belo J.A.

Transcriptional regulation of Caronte asymmetric expression.

Poster at 15th International Society of Developmental Biologists Congress, Sidney, Australia.

Thorsteinsdóttir S. and Bajanca F.

Integrin $\alpha 6 \beta 1$ -laminin interactions regulate early myotome formation in the mouse embryo.

EMBO/FEBS Workshop on the Molecular and Cellular Mechanisms Underlying Skeletal Muscle Formation and Repair. Fontevraud, France.

Tomás A.R.

Role of Flrt3 in the control of apical ectodermal ridge activity and limb outgrowth during vertebrate development.

International Post-Graduate Course: Different tissues, same strategies: common molecules in different developmental systems, Braga, Portugal.

Veloso A. and Chatterjee S.

Mechanism of relocation of pro- apoptotic protein Bim in mitosis.

Poster at the Meeting on programmed cell death, Cold Spring Harbour Laboratory, New York, USA.

Vinagre T.

The role of Gbx2 in the development of the mouse neural system.

Poster at 6th EMBL Mouse Molecular Genetics Meeting, Heidelberg, Germany.

White L.

Dynamical systems in Biomedical Engineering.

University of Warwick, Warwick, UK.

October

Barreto M., Ferreira R., Lourenço L., Fesel C., Demengeot J., Martins B., Andreia R., Viana JF., Vasconcelos C., Mota-Vieira L., Ferreira C., Vicente A.M.

Association of *FOXP3* gene variants with systemic lupus erythematosus (SLE).

Annual Meeting of the American Society of Human Genetics, Salt Lake City, USA.

Branco C.C., Pacheco P.R., Cabral R., de Fez L., Peixoto B.R. and Mota-Vieira L.

Assessment of global genetic diversity of the Azorean population by 24 microsatellite loci.

55th Annual Meeting of the American Society of Human Genetics, Salt Lake City, USA.

Correia C., Oliveira G., Santos P., Almeida J., Coutinho A.M., Bento C., Marques C., Ataíde A., Miguel T.S., Vicente A.M.

Pharmacogenetics of risperidone therapy in autism.

Annual Meeting of the American Society of Human Genetics, Salt Lake City, USA.

Costa G.

Structural characterization of drosophila kinetochore.

Spindle Dynamics Workshop, Santorini, Greece.

Coutinho A.

A idade de ouro da Física e as origens da Biologia Molecular.

Conference “A Física e a Vida”, Centro de Congressos dos Hospitais da Universidade de Coimbra, Coimbra, Portugal.

Coutinho A.

A co-constituição de ciência e tecnologia.

III Forum da Química “Ciência, Tecnologia e Inovação”, Faculdade de Ciências e tecnologia da Universidade de Lisboa, Monte da Caparica, Portugal.

Coutinho A.M., Oliveira G., Sousa I., Morgadinho T., Fesel C., Macedo T.R., Bento C., Martins M., Marques C., Ataíde A., Miguel T.S., Borges L. and Vicente A.M.

Epistatic effects in hyperserotonemia and autism.

Annual Meeting of the American Society of Human Genetics, Salt Lake City, USA.

Fernandes E.B., Sepúlveda N., Canto e Castro Loura L. and Penha-Gonçalves C.
Mapping of quantitative phenotype using the allelic penetrance framework.
Poster at the Workshop of Statistics in Genomics and Proteomics, Estoril, Portugal.

Fernandes P.
Systems biology approaches based on biological information.
Workshop on statistics in genomics and proteomics, Estoril, Portugal.

Fernandes P.
Creating, describing and sharing medical databases.
Workshop on the long term curation of medical databases. Fundação Calouste Gulbenkian, Lisbon, Portugal.

Gomes G., Águas R., Lhopitalier L., van Noort S., White L.
Theoretical epidemiology at the IGC.
KEMRI Wellcome Research Programme, Kilifi, Kenya.

Haury M.
EMBO Neuroscience Sectoral Meeting.

Krug T. Fonseca M.B., Oliveira S.A.
Strokenetics e genoport: genética de AVCs.
2º Encontro da Sociedade Portuguesa do AVC, Curia, Portugal.

Mallo M.
Hox genes and the mouse skeleton.
Umea Center for Molecular Medicine, University of Umea, Umea, Sweden.

Moraes-Fontes M.F.
Auto-imunidade.
XVIII Curso de Doenças Hepato-Biliares, Coimbra, Portugal.

Pacheco P.R., Pereira M., Maurício L., Baptista M., Quental A., Paiva C. and Mota-Vieira L. HLA risk haplotypes segregating in a multiplex family affected with systemic lupus erythomatosus (SLE).
55th Annual Meeting of the American Society of Human Genetics, Salt Lake City, USA.

Paixão T. and Carneiro J.
Explaining lognormal distributions of constitutively expressed proteins.
Poster at the VI International Conference in Systems Biology, Boston, USA.

Parkhouse R.M.E.
The reciprocity of host-pathogen interaction.
University of Carabobo, Maracay, Venezuela.

Parkhouse R.M.E.

Pathogen host evasion strategies.

Instituto Nacional de Investigaciones Agropecuarias Veterinarias, Maracay, Venezuela.

Paulino C.D., Sepúlveda N. and Penha-Gonçalves C.

Modelos de Interação entre dois genes em fenótipos binários complexos.

1st Congress of Statistics and Operational Research of Galiza and of North of Portugal, Guimarães, Portugal.

Reis Y., Cortes H., Viseu Melo L., Leitão A. and Soares H.

O citoesqueleto de microtúbulos de besnoitia besnoiti e da célula hospedeira sofre alterações profundas durante o processo de invasão.

Congresso de Ciências Veterinárias 2005, Vale de Santarém, Portugal.

Saúde L.

Asymmetrical on the inside, symmetrical on the outside.

IMM Seminars, Lisbon, Portugal.

Saúde L.

Asymmetrical on the inside, symmetrical on the outside.

Inaugural Meeting of “Sociedade Portuguesa de Biologia do Desenvolvimento”, Lisboa, Portugal.

Sepúlveda N., Paulino C.D. and Penha-Gonçalves C.

Two-gene interaction models in complex binary traits.

Poster at the Workshop on Statistics in Genomics and Proteomics, Estoril, Portugal.

Silva A.C., Filipe M., Marques S., Vitorino M., Becker J.D., Steinbeisser H. and Belo J.A.

Expression pattern of xenopus orthologs of novel genes expressed in the mouse AVE.

5th German-Italian Xenopus meeting, Lovenno di Menaggio, Italy.

Soares M.P.

Genes That Protect Transplanted Organs From Rejection.

Congresso da Sociedade Portuguesa de Transplantação, Óbidos, Portugal.

Tavares A.

TCTP, a new essential gene in drosophila, and its correlation with cancer.

Spindle Dynamics Workshop, Santorini, Greece.

White L.

Measuring the epidemiologically relevant parameters of immunity to malaria.

KEMRI Wellcome Research Programme, Kilifi, Kenya.

November

Azzoni A.R., Tavares A., Monteiro G.A. and Prazeres D.M.F.
Generation of plasmid DNA vectors with improved resistance to nucleases by in vitro recombination.
Biotec-2005 Congress, Braga, Portugal.

Bergman M.L.
Generation of antigen-specific regulatory T cells in the thymus.
Poster at the XXXI Meeting of the Portuguese Society for Immunology, Lisbon, Portugal.

Branco C.C., Pacheco P.R., Cabral R., de Fez L., Peixoto B.R. and Mota Vieira L.
Caracterização da diversidade genética da população Açoriana através da análise de 13 microssatélites.
9ª Reunião da Sociedade Portuguesa de Genética Humana, Cascais, Portugal.

Carneiro M.M.S.
Animal models of arthritis: how can they help us understand rheumatoid arthritis.
XXXI Meeting of the Portuguese Society for Immunology, Lisbon, Portugal.

Côrte-Real J., Rodo J., Almeida P., Coutinho A., Demengeot J., Penha-Gonçalves C.
Genetic mapping of the homeostatic control of serum IgM in the mouse.
Poster at the XXXI Meeting of the Portuguese Society for Immunology, Lisbon, Portugal.

Couceiro S., Matias A., Serras A., Duarte C., Catarino M., Penha-Gonçalves C.
Influence of natural polyphenolic compounds on the pathogenesis course of Type 1 diabetes.
Poster at the XXXI Meeting of the Portuguese Society for Immunology, Lisbon, Portugal.

Coutinho A.
Global Health Experts Workshop – Glossary Terms
European Partnership for Global Health, Fundação Calouste Gulbenkian, Lisbon, Portugal.

Coutinho A.
Tratamento dos dados pessoais e genética.
Congresso Luso-Hispano de Direito dos Seguros, Hotel Altis, Lisbon, Portugal.

Coutinho A.
Moderator of the conference: Portuguese Radiation-Oncology: what present, what future?
Organized by Serviço de Radioterapia, Hospital Santa Maria and Sociedade Portuguesa de Radioterapia Oncologia.
Fundação Calouste Gulbenkian, Lisbon, Portugal

Deus L., Almeida P., Mota M., Penha-Gonçalves C.
Genetic control of malaria liver infection: fine mapping of Ber11 locus with subcongenic strains.

Poster at the XXXI Meeting of the Portuguese Society for Immunology, Lisbon, Portugal.

Gökaydin D. and Gomes G.
Gripept.net – Viagens de um vírus.
Câmara Municipal de Vila Flor, Vila Flor, Portugal.

Gomes G.
Gripept.net – viagens de um vírus.
Fundação Calouste Gulbenkian, Lisbon, Portugal.

Gomes G.
Há gripes e gripes.
Pavilhão do Conhecimento – Ciência Viva, Lisbon, Portugal.

Graça L
Monoclonal antibodies for the induction of tolerance and regulatory T cells.
Instituto de Biologia Molecular e Celular, Porto, Portugal

Igreja C. and Dias S.
Evidence for distinct phenotypic/molecular stages during endothelial progenitor differentiation.
8th Imperial College London Symposium- Vascular Endothelium: role in disease pathogenesis and as a therapeutic target, London, UK.

Moraes-Fontes M.F
The effect of one autoimmune disease over another: does inflammatory bowel disease protect from encephalomyelitis?
Poster at the XXXI Meeting of the Portuguese Society for Immunology, Lisbon, Portugal.

Oliveira S.A.
Genética de AVCs.
Annual Meeting Faculdade de Medicina de Lisboa, Hospital Santa Maria, Lisbon, Portugal.

Pacheco P.R., Branco C.C., Lismond A., de Fez L., Cabral R., Peixoto B.R. and Mota Vieira L.
A perspectiva dos sistema HLA na caracterização genética da ilha de São Miguel, Açores.
9^a Reunião da Sociedade Portuguesa de Genética Humana, Cascais, Portugal.

Parreira L.

Studies of hematopoietic precursors. A view from Notch.
Department of Immunology. University of Rotterdam, Rotterdam, The Netherlands.

Ramos A., Feijó J.A., Façanha A.R. and Canellas L.P.
O fluxo extracelular de prótons das raízes é estimulado pelos ácidos húmicos: evidências obtidas com a microsonda vibrátil seletiva ao H⁺.
VI Encontro Brasileiro sobre Substâncias Húmicas, Rio de Janeiro, Brazil.

Rodo J.
Mechanisms of T cell recognition and activation.
IBMC, Porto, Portugal.

Tavares A.
Identifying new tumour suppressors using drosophila.
CBME, Universidade do Algarve, Portugal

Teotónio H.
The role of deleterious mutations in the maintenance of males in *C.elegans*.
ConGen. ESF Meeting on Conservation Genetics, Santiago de Compostela, Spain.

Vicente A.M.
Genetic and immune factors in autism.
XXXI Meeting of the Portuguese Society for Immunology, Lisbon, Portugal.

Zelenay S.
HO-1 expression by CD4⁺ T cells is not necessary for regulatory T cells.
Poster at the XXXI Meeting of the Portuguese Society for Immunology, Lisbon, Portugal.

December

André J.B.
Innovation in the dark. The example of mutation rate in asexuals.
"Entretiens de Bures" Innovation in Biological Systems: Mechanisms, Evolution and Consequences, IHES, Bures-sur-Yvette, France.

Coutinho A.
Chairman of the conference "Indústria Farmacêutica – melhor saúde, mais competitividade".
Hotel Ritz, Lisbon, Portugal.

Coutinho A.
Closing Lecture, Advanced Immunology Course.
Institut Pasteur, Paris, France.

Coutinho A.

Chair of the conference “Fazer novo em tempos difíceis” by Giovanni Cerri and Manuela Ferreira Leite. Ao Encontro da Medicina conferences.

Fundação Calouste Gulbenkian, Lisbon, Portugal.

Coutinho A.

Comments on the conference “From humanitarian to comprehensive medicine” by A. Carpentier. Forum Gulbenkian de Saúde.

Fundação Calouste Gulbenkian, Lisbon, Portugal.

Duarte J.H.

FoxP3 expression in T cell ontogeny – from embryonic development to vertebrate evolution.

Poster at the 2nd Joint Meeting of Graduate Programmes on Biosciences , Tomar, Portugal.

Fragoso R., Casalou C. and Dias S.

VEGF regulates leukemia migration via FLT-1, involving Pi3 kinase, RhoA and Rac1 activation and lipid rafts formation.

Poster at 47th Annual Meeting of The American Society of Hematology. Atlanta Georgia, USA.

Gökaydin D. and Gomes G.

Gripept.net – viagens de um vírus.

Câmara Municipal de Odivelas / Instituto Superior de Ciências da Educação, Odivelas, Portugal.

Marques I.

Uma viagem pelo genoma humano.

Alfredo dos Reis Silveira Secondary School, Seixal, Portugal.

Tavares A.

TCTP, a new essential gene in drosophila and its correlation with cancer.

IV Portuguese Drosophilists Meeting, IBMC, Porto, Portugal.

Tavares A.

Cell division and tumor supressor genes: life without cell abscission.

Instituto de Medicina Molecular, Lisbon, Portugal.

Teixeira M.C., Fernandes A.R., Mira N., Santos P.M., Viegas C.A., Becker JD, Sá-Correia I.

Global responses to stress imposed by the herbicide 2,4-D in the eukaryotic experimental model *saccharomyces cerevisiae*.

Poster at the Micro'05-Biotec'05, Póvoa de Varzim, Póvoa de Varzim, Portugal.