



FUNDAÇÃO
CALOUSTE
GULBENKIAN

INSTITUTO GULBENKIAN DE CIÊNCIA ANNUAL REPORT 2007

The complete version of this report is available to download from the IGC website at <http://www.igc.gulbenkian.pt>

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BOARD OF ADMINISTRATION

The Fundação Calouste Gulbenkian, established by Calouste Sarkis Gulbenkian through his will dated 18th June 1953, is a private institution of general public utility, endowed with legal personality. The statutory aims of the Foundation are in the fields of charity, art, education and science. The members of the Board of Administration in 2007 were:

PRESIDENT

Emílio Rui Vilar

HONORARY PRESIDENT

Mikhael Essayan

EXECUTIVE TRUSTEES

Diogo de Lucena

Isabel Mota

Eduardo Marçal Grilo

Teresa Gouveia

Martin Essayan

NON-EXECUTIVE TRUSTEES

André Gonçalves Pereira

Eduardo Lourenço

Artur Santos Silva

Instituto Gulbenkian de Ciência

BOARD OF DIRECTORS

The Board of Directors of 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 2007 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 oversees the scientific progress and education programmes, as well as recruitment of new staff and research activities of established 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 2007 were:

Sydney Brenner (Chairman)
Jonathan Howard
Nicole Le Douarin
Martin Raff
Kai Simons
Susumu Tonegawa
Gines Morata*
David Sabatini*
Richard Axel*
Jean-Pierre Changeux*
Terrence Sejnowsky*

*In the context of the normal turnover of the Board members, Gines Morata and David Sabatini replaced Philippe Kourilsky and Lewis Wolpert in the SAB. Due to the recent development in neuroscience research at the IGC, three new members were appointed: Richard Axel, Jean-Pierre Changeux and Terrence Sejnowsky.

The Scientific Advisory Board met at the IGC on 1-3 May and on 28-30 October 2007.

STAFF

DIRECTOR

António Coutinho

DEPUTY DIRECTORS

Sérgio Gulbenkian

José Mário Leite

RESEARCH MEMBERS

The IGC is not divided into departments, and its scientific activities are carried out within relatively small groups. Research is autonomously conducted by individual scientists and small groups, free, and indeed, strongly encouraged, to associate and collaborate in projects.

It should be noted that the vast majority of scientists at the IGC are either affiliated with institutions other than the Foundation, or supported by national or international organisations, indicated in brackets below. Some of those listed below were present at the IGC for only part of the year.

Alessandro Ramos (FCT)
Álvaro Augusto Tavares (ISTUTL)
Ana Catarina Certal (FCT)
Ana Catarina Santos (IMM/FCT)
Ana Cristina Borges (FMVUTL/FCT)
Ana Cristina Paulo (EU)
Ana Elisabete Pires (FCT)
Ana Margarida Ferreira (FCT)
Ana Margarida Vigário (Univ. Madeira/FCT)
Ana Maria Pamplona (IMM/FCT)
Ana Sofia Cachaço (IPO/FCT)
Ana Teresa Tavares (FCT)
Andrea Gomes (IMM/FCT)
António Coutinho (CNRS/FCG)
António Freitas Duarte (FMVUTL)
António Jacinto (IMM)
Astrid Vicente (INSARJ)
Elsa Abranches (IMM/FCT)
Elsa Seixas (FCT)
Erwan Michard (FCT)

Beatriz Garcia Fernandez (FCT)
Beatriz Garcia Fernandez (FCT)
Carla Real (IPO/FCT)
Carlos Penha Gonçalves (Laboratório Associado)
Clara Reis (Association for International Cancer Research)
Claudia Florindo (FCT)
Cláudia Valente (IMM/FCT)
Claúdio Marinho (CAPES)
Constantin Fesel (FCT)
Cristina Casalou (IPO/FCT)
Cristina João (FCT/Associação Portuguesa contra a Leucemia)
Domingos Henrique (IMM/FMUL)
Dulce Azevedo (IMM/FCT)
Élio Sucena (FCUL/FCT)
Elisabetta Padovan (FMUL)
Manuel Rebelo (IGC)
Maria de Jesus Trovoadá (FCT)
Maria João Leão (FCT)

Fernanda Bajanca (Univ. Minho/FCT)
 Filipa Alves (FCT)
 Florence Janody (FCT)
 Francisco Dionísio (FCUL/FCT)
 Frank Hilker (EU)
 Gabriel G. Martins (FCUL/FCT)
 Gabriela Gomes (EU)
 Gabriela Rodrigues (FCUL)
 Gabriela Silva (FCT)
 Gareth Weedall (EU)
 Greg King (FCUL/FCG/EC)
 Helena Soares (ESTSL)
 Henrique Teotonio (Laboratório Associado)
 Inês Crisóstomo Ramos (EU)
 Iris Caramalho (FCT)
 Isabel Alcobia (IMM/FCT)
 Isabel Campos (IMM/FCT)
 Isabel Gordo (FCT)
 Isabel Pombo Gregoire (FCT) – left August 2007
 Ivo Chelo (FCT)
 Jacinta Serpa (IPO/FCT)
 Jennifer Rowland (EU)
 João Pedro Simas (IMM/FMUL)
 Joaquin Rodriguez Leon (CMBR)
 Jocelyne Demengeot (FCG)
 Johann Truccolo (FCT) – left April 2007
 Jorg Becker (FCT)
 Jorge Carneiro (Laboratório Associado)
 José A. Feijó (FCUL)
 José António Belo (Univ. Algarve)
 José B. Pereira-Leal (Laboratório Associado)
 José David-Ferreira (FMUL/IGC)
 Jose Faro (Univ. Vigo)
 Karina Bivar Xavier (Laboratório Associado)
 Madalena Martins (INSA/FCT)
 Sofia Marques (IMM/FCT)
 Sofia Nolasco (FCT)
 Sofia Oliveira (FCT)
 Sólveig Thorsteinsdóttir (FCUL)
 Sukalyan Chatterjee (FCG)

Maria Margarida Souto Carneiro (FCT)
 Maria Mota (IMM/FMUL)
 Maria Teresa Faria Pais (FCT)
 Marie-Louise Bergman (FCT)
 Marion Muehlen (EU)
 Mark Seldon (IGC)
 Marta Barreto (INSA/FCT)
 Marta Miranda (IMM/FCT)
 Marta Moita (FCT)
 Marta Monteiro (IMM/FCT)
 Melvin Cohn (Salk Institute)
 Michael Parkhouse (IGC)
 Michal Michal-Bejerano Sagie (IGC)
 Miguel Godinho Ferreira (FCT)
 Miguel Prudêncio (IMM/EU)
 Miguel Seabra (Imperial College School of Medicine/IGC)
 Miguel Soares (Laboratório Associado)
 Moisés Mallo (FCG)
 Mónica Bettencourt-Dias (FCT)
 Natalia Mantilla-Beniers (EU) – left November 2007
 Natalia Moncaut (FCT)
 Nico Stollenwerk (EU)
 Nuno Afonso (IMM/FCT)
 Paula Duque (FCT)
 Pierre-André Cazenave (Institut Pasteur)
 Rasmus Larsen (FCT)
 Ricardo Gil da Costa (IGC)
 Rita Fior (FCT)
 Rosa Elias (CAPES/IGC)
 Rosalina Fonseca (Hospital Júlio de Matos/FCT)
 Rui Costa (Laboratory for Integrative Neuroscience)
 Rui Gardner (FCT)
 Rui Gonçalo Martinho (FCT)
 Rui Oliveira (ISPA)
 Rute Conceição do Nascimento (FCT)
 Tatiana Vassilevskaia (EMMA)
 Tiago Carneiro (Association for International Cancer Research)
 Vanessa Oliveira (IMM/FCT)
 Vera Teixeira (FCT)

Susana Lopes (FCT)
Sylviane Pied (INSERM/Institut Pasteur)

Victoria Gallego (FCT)
William Wood (IMM/FCT)

PHD STUDENTS

PDIGC PHD PROGRAMME

Ana Cecília Seixas (FCUL/FCT)
Ana Cristina Silva (FCUL/FCT)
Ana Luísa Reis (FMVUTL/FCT)
Ana Paula Elias (FCUL/FCT)
Ana Rita França (FMUL/FCT)
Ana Rita Marques (ITQB/UNL/Marie Curie Grant)
Ana Sofia Veloso (ITQB/UNL/FCT) – finished July 2007
Andreia Cunha (ITQB/UNL/FCT)
Ângelo Chora (FMUL/FCT)
Catarina Figueiredo (ITQB/UNL/FCT) – finished December 2007
Catarina Sim-Sim Pereira (ITQB/UNL/FCT)
Catia Igreja (FCUL/FCT) – finished October 2007
Dusan Djokovic (FMVUTL/FMUL/FCT)
Fátima Pereira (ISTUTL/FCT)
Filipa Moraes, (ITQB/UNL/FCT)
Helena Costa (ITQB/UNL/FCT)
Hugo Almeida (Association for International Cancer Research)
Ines Matos (FMUL/FCT)
Jaime Combadão (ITQB/UNL/FCT)
Joana Corte-Real (ITQB/UNL/FCT)
Joana Monteiro (FCUL/FCT) – finished March 2007
Joana Moreira (FCUL/FCT) – finished January 2007
Joana Rodo (ITQB/UNL/FCT)
João Duarte (FCTUC/FCT)
João Gonçalves (FCUL/FCT)
Lígia Gonçalves Deus (ITQB/UNL/FCT)
Lília Perfeito (ITQB/UNL/FCT)
Mafalda Silva (ITQB/UNL/FCT)

Margarida Santos (FMUL/FCT) – finished January 2007
Maria do Rosário Sambo (FMUL/Hospital Pediátrico de Luanda)
Maria Francisca Moraes Fontes (FMUL/Hospital Egas Moniz/Ministério da Saúde)
Marta Campos (IMM/FCT) – finished July 2007
Marta Carapuço (ITQB/UNL/FCT) – finished February 2007
Marta Guimarães (ITQB/UNL/FCT)
Nadja Pejvanovic (ITQB/UNL/FCT)
Nuno Moreno (FCG/FCUL)
Nuno Sepúlveda (ICBAS/FCT)
Patrícia Simões (IEFP)
Paula Rodrigues (FCUL/UNL/FCT)
Pedro Rifes (FCUL/EU Network of Excellence)
Raquel Antunes, (ITQB/UNL/FCT)
Raquel Carvalho (ITQB/UNL/IEFP)
Raquel Lourenço (ITQB/UNL/FCT)
Ricardo Águas (ITQB/UNL/FCT)
Rita Neres (ITQB/UNL/FCT)
Rita Rasteiro (ITQB/UNL/FCT)
Ruben Ramalho (ITQB/UNL/FCT)
Sander van Noort (ITQB/UNL/EC)
Sara Carvalho (ITQB/UNL/IGC)
Sílvia Costa (FCUL/FCT) – finished April 2007
Sílvia Maurício Correia (ITQB/UNL/FCT)
Sofia Carvalho (ITQB/UNL/Marie-Curie)
Sofia Cordeiro (FCUL/FCT)
Tânia Vinagre (ITQB/UNL/FCT)
Tiago Krug (FMUL/FCT)

Tiago Paixão (ICBAS/FCT) – finished
October 2007
Vasco Correia (ICBAS/FCT)

Vitor Conde Sousa (FCUL/FCT)
Zita Santos (ITQB/UNL/FCT)

OTHER PHD STUDENTS

Alexandre Trindade (FMVUTL/FCT)
Ana Lucia Mena (**PGDB**/ITQB/UNL/FCT)
Ana Raquel Tomás (Univ. Coimbra/FCT)
Ana Rodrigues-Martins (University of
Cambridge/FCT)
Bruno Raposo (University of Lund/FCT)
Catarina Correia (INSA/FCUL/FCT)
Cristina Rodrigues (IMM/FCUL/FCT) -
finished November 2007
Emiliano Barreto Hernandez (FCUL/EU)
Filipa Lopes (IMM/FCT)
Filipe Vilas-Boas, (IMM/FCT)
Francisco Caiado (IMM/FCT)
Ivonne Wollenberg (IMM/FCT)
Jennifer Geiger (IMM/FP6)
Joana Duarte (IMM/FCT)
Lazlo Tokaji (**PGDB**/ITQB/UNL/FCT)
Lénia Rodrigues (IMM/FCT) – finished
March 2007
Lisa Gonçalves (UAlg/FCT)
Luciana Ferrari (Federal University of
Rio de Janeiro/CAPEs)

Margaret Bento (UAlg/FCT)
Margarida Cunha-Rodrigues (IMM/FCT)
Mariana Simões (IMM/IEFP/FCT)
Marta Alenquer (IMM/FCT)
Marta Caridade (IMM/FCT)
Marta Vitorino (UAlg/FCT)
Patricia Leirião (IMM/IHMTUNL/FCT)
Rita Fragoso (IPO/FCUL/FCT)
Rui Benedito(FMVUTL/FCT) – finished
November 2007
Sara Mauricio Sousa (IMM/FCT)
Sandra Trindade (Univ.Evora)
Sérgio Simões (IMM/FCUL/FCT) –
finished April 2007
Sílvia Portugal (IMM/FCT)
Sónia Ventura (FMVUTL/IBET/FCT)
Susana Rocha, (IMM/FCT)
Tânia Ferreira (FCT)
Vivian Leite de Oliveira
(**PGDB**/ITQB/UNL/FCT) – left December
2007

IGC PHD PROGRAMMES 2007

PhD Programme in Computational Biology 2007

Ana Vieira dos Santos Cruz
Daniel Gil Gonçalves Ferreira
Hélder António Martins Pedro
Hélio Ernesto Coronel Machado Pais
Inês de Santiago Domingos de Jesus
João André Coutinho Viana Alpedrinha
João Filipe da Custódia Dias

José Alexandre Teles
Nuno Miguel Martins Tenazinha
Patrícia M. Simões
Pedro Pintado Jorge Gonçalves
Rita Martins Rocha
Sandra Cristina Milheiro Cabral Botelho

Gulbenkian PhD Programme 2007

Ana Inês da Cunha Ferreira
Ana Teresa dos Santos Avelar
Barbara Jezowska
Barbara Vreede
Clara de Fátima Alves Pereira

Ivo Marguti
Mariana Coelho Correia da Silva
Migla Miskinyte
Patrícia Inácio
Ricardo de Sousa e Paiva

Gulbenkian/Champalimaud PhD Programme in Neuroscience 2007

Ana Margarida Lago Agrochão
Iris Margarida Donga Vilarés
José Joaquim Fernandes
Maria Inês Alves Vicente
Maria Isabel Santos Lestro Henriques

Mariana Marcelino Belchior Cardoso
Patrícia Marçal Alves Correia
Patrício Manuel Vieira Simões
Pedro Nuno Galvão Ferreira
Rodrigo Manuel Abril de Abreu

MSC STUDENTS

Ana Catarina Correia (BIC/FCT)
André Mendonça (ISTUTL)
André Rosa (IEFP)
Eliane Cortês (IGC)
Hugo Soares (Univ. Coimbra)
Íris Vilarés (ISAUTL/FCT)
Joana Louçã (UVA, Amsterdam)
João Marques (IEFP)
Maria Emília Santos (FCUL/IEFP)

Maurícia Vinhas (IEFP)
Nuno Oliveira (IEFP)
Nuno Miguel Ribeiro Palha (FCUL)
Pedro Miguel Gaspar (IGC)
Pedro Saavedra (IMM)
Rita Amândio (FCUL)
Rita Mateus (IMM/IEFP)
Sofia Raquel Rebelo (IEFP)
Vanessa Borges (IEFP)

BSC STUDENTS

Ana Filipa Barahona (Univ. Évora)
Nuno Morgado (Univ. Évora) – left
October 2007
Patrícia Lopes (FMVUTL)
Pedro Dias (FCUL)

Pedro Ferreira (Univ. Lusófona)
Raquel Mendes (Univ. Lusófona)
Renato Alves (IST/UTL)
Rui Buzaco (Univ. Évora) – Left October
2007

LABORATORY TECHNICAL SUPPORT

Alexandra Duarte (Short Term Apprentice) – left December 2007
Alexandre Rodrigues (IEFP) – left May 2007
Ana Água-Doce (IMM/BTI/FCT)
Ana Catarina Silva (BIC/FCT)
Ana Gaspar (FCUL/EU Network of Excellence)
Ana Nóvoa (Laboratório Associado)
Ana Salgueiro (UALG/BI/FCT)
Ana Sofia Leocádio (BTI/IGC/FCG)
Ana Sofia Oliveira (BTI/IGC/FCG) – left May 2007
Benedita Fonseca (Marie Curie/IEFP)
Cláudia Marques (BIC/FCT) – left December 2007
Dolores Bonaparte (BTI/FCG) – left September 2007
Duarte Viana (BIC/FCT)
Elsa Guilherme (IEFP) – left October 2007
Inês Rolim (FCT)
Isabel Belo (IEFP) Left November 2007
Joana Bom (FCT)
Joana Freire Monteiro (IEFP)
João Garcia (BTI/IGC/FCG)
José Afonso (Short-term apprentice)
Lara Carvalho (IMM/FCT)
Lurdes Duarte (BTI/FCT)
Maíra Aguiar (EC)
Sílvia Cardoso (Phillip Morris)
Sofia Rebelo (Astrazeneca)
Teresa Cruto (IEFP) – left December 2007

Mariana Simões (IMM/FCT)
Maria João Lagareiro (IEFP) – Left November 2007
Marisa Pardal (BTI/IGC/FCG)
Miguel Coelho (Short Term Apprentice) – left October 2007
Miguel Roque (BTI/FCT) – left December 2007
Miguel Simões (FCT)
Miguel Teixeira (Short Term Apprentice)
Natacha Sousa (IMM/FCG)
Nuno Cláudio (Short Term Apprentice)
Paulo Almeida (Laboratório Associado)
Paulo Bettencourt (BTI/IGC/FCG) – left August 2007
Patricia Lopes (BTI/UE) – left December 2007
Pedro Patraquim (FCUL)
Pedro Sanches (IEFP) – left September 2007
Renato Colaço (IPJ) – left November 2007
Rui Martins (IEFP) – left February 2007
Rui Tostões (IEFP) – left July 2007
Sara Marques (UALG/BI/FCT) – left December 2007
Sara Violante (IEFP) – left September 2007
Sílvia Batista (Short Term Apprentice) – left December 2007
Vítor Faria (FCUL)

OTHERS

Ines Nisa Rato (Ectopia)

Inês Rebelo (Ectopia)

José Lourenço (Informatics
Technician/EU)

Maria Manuela Lopes (Ectopia)

Marta de Menezes (Ectopia/FCT)

Paula Macedo (Administrative
Personnel/EC)

Rita Cachao (Ectopia) – left September
2007

Simon Frederick (Ectopia)

Vitor Faustino (Science
Communication/FCT)

ADMINISTRATIVE, SECRETARIAL AND TECHNICAL STAFF

The administrative, secretarial, and technical staff of the IGC provide support to the research and teaching activities. All listed below were at the IGC for all or part of 2007.

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

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
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 researchers at the IGC and elsewhere on campus, while open to others in Portugal and abroad.

ANIMAL FACILITY

Jocelyne Demengeot

BIOINFORMATICS

José Pereira-Leal/Pedro Fernandes

CELL IMAGING

José Feijo

HISTOLOGY AND HISTOPATHOLOGY

Sérgio Gulbenkian/Miguel Soares

INFORMATICS

João Tiago Sousa

LIBRARY AND SCIENTIFIC INFORMATION

Sérgio Gulbenkian

Science and Society

Sofia Cordeiro

SEQUENCING AND GENOTYPING

Carlos Penha-Gonçalves

Theoretical and Computational Biology

Jorge Carneiro

TRANSGENIC UNIT

Moises Mallo

Zebrafish Facility

Leonor Saúde

The activities of all Units and Services are accompanied and adjusted to current needs by Users Committees which include a significant proportion of all scientists at the Institute.

Foreword by the Director

To promote science and to serve the Portuguese research community, using the independence and flexibility of a private organization that can take the risks of innovation, are the first principles of the Foundation's Science Sector. Translated into the Institute's missions ten years ago, this meant producing a new generation of leaders in biomedical science, who could integrate various other institutions, enacting the values of science and the principles of individual autonomy, while pursuing modern biomedical research in both content and technological basis. Attaining this objective required introducing novel modes of institutional structure, operation and financing, as well as building a strong "esprit de corps" that should help to anchor an international education base at the IGC, and should serve as criterion of identity. "On the way" to these goals, and as part of the necessary method to achieve them, the Institute should produce internationally competitive science.

Culture has a Lamarckian mode of transmission, as it leaves no inherited imprint in individuals. Education, perhaps the noblest of all human endeavors, thus represents the only strategy to "improve" upon biological evolution. Institutions are cultural constructions and, therefore, are necessarily marked by their history, all the more when devoted to an educational mission. Graduate education is a strong tradition at the Gulbenkian Institute, ever since 1969, and this has been maintained over the last 10 years, a period corresponding to a remarkable national effort in this area. With the continued support of the National Research Council (Fundação para a Ciência e a Tecnologia - FCT), the Institute has launched 5 PhD Programs, attracting and identifying a large number of youngsters interested in science. A total of 416 PhD students were admitted and educated, in part at least, at the IGC. The first Program (PGDBM - 1993 to 1999) has now been closed for enough time, such that "long term" results for the country can be estimated: of the 103 students that completed with success the graduate course program at the IGC, 101 have obtained a degree at Portuguese or foreign Universities. Of these, 61 have returned to Portugal (and several others are preparing to do so), a considerable number (35) today holding positions of responsibility in the scientific community, as research group leaders, university professors, CEOs of start-ups in biotechnology. Interestingly, a number of these have chosen a career with "lateral mobility", remaining nevertheless associated with science: as MDs in hospitals, as grant administrators and officers of science communication in local institutions, as dedicated personnel in NGOs abroad. Those who remained somewhere else in the world continue in close contact with the IGC and colleagues in Portugal, often helping in various ways to their activities. It would be tempting to conclude that the generous vision of both the government and the Foundation, which invested their money to send abroad our best students, was an excellent decision.

The programs that followed the PGDBM were designed on the basis of the accumulated experience, but also in order to adapt the intervention to the precise needs of the country, a fast-growing and ever-changing landscape when it comes to science. For

another 5 years, while maintaining a program essentially dedicated to educate abroad our best students (PGDB – 2000 to 2004), a parallel program was experimented with similar criteria of excellence, but in which the students also produced their thesis work in local laboratories. Thus, the foundation of new research institutions in biomedical sciences in the country, the establishment of a number of internationally competitive groups, the general evolution of the local scientific community in quality and numbers, all made it possible to enter a more mature phase of science education: to keep some of our best PhD students here, thus strengthening the local groups and saving public money, while encouraging them to go abroad as post-docs. Furthermore, as expected if it would prove successful, the model of the Gulbenkian Programs was adopted by a number of institutions in Portugal that launched their own PhD programs. In short, the IGC could now concentrate in the education of its own students. A new program was, therefore, formalized in 2007 (PGD) that is devoted to recruit and educate PhD students in the Institute's groups. This provided the first opportunity to attract foreign students to local programs and laboratories, as well as the preferred pathway for entering the IGC. In parallel, it was time to pay attention to specific areas of research that, for one reason or another, were lagging behind. In 2005, following an exemplary decision of Siemens Portugal, the FCT, Siemens Academia, and the IGC launched a new PhD program in Computational Biology (PDBC), much on the same model as the initial programs (multidisciplinary recruitment, international faculty, most students sent abroad after one year of graduate courses), but now anchored in local groups throughout the country, through the Co-laboratorium in Computational Biology supported by the Fundação Luso-Americana para o Desenvolvimento. With less than 3 years' operation, the PDBC embodies already a network of many groups in Portugal and abroad. Also in 2007, the Champalimaud Foundation, the FCT, and the IGC founded a program in Neuroscience with many of the same characteristics, which attracted, from its first call, a considerable number of applications, and has already brought to Oeiras a large number of international leaders in the field.

In all three programs, the basic principles remain the same: provide the students with graduate courses given by the best faculty we can find in the world, so that they gain critical knowledge and inspiration; encourage that their motivation and commitment result in hard work; let them decide, if they are good enough, on what to do and with whom to do it. As before, this scheme provides for two major advantages. First, it relies on a Faculty that is renewed every year, "recruited" amongst the very best active scientists in the world, providing for excellent teaching and many choices for an eventual thesis supervision. Second, it solves a persistent problem in PhD education: usually, students do not decide on their own theme or subject of research, but are "given" a ready-made "project" by one of the very few supervisors they may approach. Most naturally imposed by constraints in the distribution of public money, the current rules for fellowship funding actually strengthen the "passive" attitude of the students, for they require submission of "project, supervisor and university registration" at the time of application. This severe limitation in the system may be solved by the distribution of "studentship packages" to formal programs, the Research Council delegating to the program's directions the selection and evaluation of the students.

The IGC has gained with the continuing vision of the FCT Presidents who decided to follow precisely the latter alternative.

Currently, the IGC recruits some 40 PhD students every year, most of which through stringent selection after public calls for the programs. They “enter” education by being exposed to over a hundred professors, each criteriously selected by the program directors to talk about the subjects of their own choice, to provide inspiration, advice and contacts, eventually, a laboratory for thesis work. Over the last few years, we have experimented with three months of common education for all students, irrespective of the program they belong. As expected, this strengthens the “esprit de corps” and, most importantly, consolidates multidisciplinary – a major principle in the IGC’s education, given the “unicity” of modern biomedical sciences. From the start, students are encouraged to take time to visit laboratories, at the Institute and elsewhere, to get familiar with real life, to obtain information on their research activities, and to establish personal contacts with the group leaders, whom eventually will serve as thesis advisors. The majority of these students do go abroad for their theses, but an increasing number of Portuguese and all the foreigners remain in local laboratories. The full operational autonomy of the groups at the Institute, on the other hand, ensures group-leaders have full freedom to recruit students who are not in any of the programs; these are naturally integrated with all others in graduate courses, retreats and in the students’ life, providing a strong link between education and research at the Institute. I hear often that, for programs of this quality, for the extraordinary reputation that the “Gulbenkian students” have gained over the world, the Institute ought to do better in promoting their image and in attracting the best students in Europe, offering additional competitive conditions for groups at the IGC. This may well be correct. Yet, such strategic choice involves another critical aspect: thus, the likelihood to keep in (or attract back to) Portugal the young investigators generated by the programs is lower for foreigners than for Portuguese residents, such that full internationalization of the IGC’s PhD programs, even if better for the Institute, would amount to giving up, or at least diminish, the effort of providing new leaders to the local scientific community. Hence, an eventual shift of strategy will have to await decisions of the Foundation’s Board of Administration, as to the priorities of the Institute’s missions.

Along these years, other educational objectives were met. For example, PhD programs are now the rule in many Portuguese institutions, providing a strategy for a fair and efficient distribution of public fellowships, for a better selection and education of incoming students, for the internationalization of science in the respective institutions, for the excellent reputation of Portuguese students abroad. The hundreds of students who were educated at the IGC now embody a network of alumni that meets every year in Oeiras and provides an extraordinary source of contacts and “inside” information from many institutions and laboratories in the world. More importantly, as they are admitted to this network, incoming students enter a real, lively school that is ready to ensure the defense of its own values and criteria.

The mission of identifying, educating, sending abroad and attracting back to Portugal some of our best minds, required to adopt an institutional mode of operation that would allow for offering full autonomy to young scientists. These are given the opportunity to establish their own groups and lines of research, but also the responsibility to find the necessary financial resources: their own salaries and those of their collaborators, as well as the money to develop their projects. The institutional resources can thus be used to “start them up” and to provide the necessary support in technology (platforms, equipments, animal facilities) and services (administrative and financing, grant administration, communication), as well as the best possible intellectual conditions (programs of seminars, workshops, conferences, and long-term visitors). As the strategy aimed at contributing to consolidate the Portuguese scientific community, the IGC has attracted since 1998, a total of 56 group leaders, of which 53 moved here from abroad. After some 3-7 years at the IGC, most of these have left or will leave to other institutions, taking with them, we hope, the spirit of inquire in freedom, cooperation and commitment that they lived at the Institute. While 8 of these have, again, moved abroad, 18 research groups have been “exported” (or are in the process of moving) to other Portuguese institutions, bringing to full fruition the investments of the Gulbenkian Foundation in their education and “incubation”. The interest in giving opportunities to the highest number implied two operational requirements: first, the small size of research groups, and, second, a high rate of group turnover. Both of these seemed to impinge negatively in the objective of producing “internationally competitive science”. How much can it be done today by a couple of students and a post-doc, particularly if the PI starts alone and, soon after starting, has to look out for where to move to next? On the other hand, these structural requirements forced a set of conditions that turned out to be extremely favorable. First, it became necessary to release the groups from all concerns and investments in technology, allowing for a more economical, rational and productive set-up of platforms and services. Second, it fostered the spirit of priority to the common interest, the communal sense of owing and using everything together, resources, equipments and space. Third and very relevant, it forced the groups to engage in collaborations, creating conditions for transversality in the scientific questions and approaches, as well as reinforcing the notion that all problems are better addressed through a “multidisciplinary” spirit of cooperation. Fourth and most importantly, it allowed to ascertain, perhaps develop, the uniqueness of each individual investigator, thus promoting diversity as the institution’s major value.

The essence of a science institution is to produce science, and to educate new scientists. At the IGC, the latter has been set as its principal mission, thus determining our strategic choices: first, in the process to educate abroad incoming students; then, on the model of operation for the internal groups, as the students started to be educated in Oeiras. The quality of the scientific process to which the students are exposed is essential in graduate education; hence, the IGC’s scientific policy was set aiming at excellence in the research process itself, based on rigor and creativity, but on full openness, exchange and cooperativity, as well. The priority being given to the process rather than to the end product, it was natural that scientific production at the IGC aimed at excellence rather than volume. The Institute has undoubtedly gained a reputation of quality, internationally and at

home, but it would seem appropriate to assess whether this reputation is justified by “objective” measurements of scientific production. In other words, it seemed pertinent to produce a bibliometric analysis of the IGC’s production in the period of 2000-2007 (see “supplementary information”). In short, this analysis indicates that our initial goals were achieved, but let me first make a strong point of criticism to this particular way of measuring scientific contributions.

Bibliometric analyses, or any other “objective” measure of scientific outputs for that matter, are but an extreme simplification, reducing a very complex process to a few numbers, ignoring the historical process and the variable difficulties imposed by very diverse environments upon individual scientists’ performances, as well as “peer respect” and other incentives that scientists, young as they might be, often praise. The problem is thus general, the “objective” analysis of scientific production suffering, like so many other aspects of modern life, of a progressive “reduction” to a few “indicators”, bibliometric in this case. The damage this is causing to science is enormous, most evident at the level of individual scientists and their careers. The evolution in the nature of such “indicators” brings no promise of better times. Initially used almost exclusively by administrations – as the scientists themselves “know of each other” and can always tell who is good or not by direct personal interactions¹ – the only “indicator” was, for some time, the number of published papers. Several factors made it little interesting to rank scientists, institutions and countries by this parameter, however: in years of “prime production”, the publication records of some investigators grew to levels that would make us wonder if the author had time to only read them all; the multiple cases of plagiarism and other forms of fraud in the construction of such long lists; the proliferation of journals that brought us to the current situation, when publication essentially only requires to write in English a manuscript with the standard form of a scientific paper. In other words, driven by market rules (in this case the opportunity of business for publishers and editors), the number of journals publishing scientific papers has also grown to many thousands, such that no paper, irrespective of novelty or quality, should remain unpublished. Obviously, there must be some difference to publish in a major, credited journal, or in the Municipal Archives of Molecular Proctology². The development of databases of “scientific information”, however, rapidly came to rescue the administrators, with the derivation of “impact factors” (IF) for each journal. Actually, a novel category of administrators was born, fully dedicated to produce or “analyze” scientific information, often for governments, institutions and grant-givers. The IF of a journal represents the average number of times that papers published in that particular journal were cited in the world literature along the year following publication. This obviously represents the relevance of the papers, but many other aspects as well. First of all, the circulation and availability of the journals, with a clear advantage of those with a “generalist” profile that are available at every scientific institution, and are read by essentially everyone in science; in other words, these papers might well not be the best,

¹ In times prior to bibliometric data-bases, my mentor Gøran Møller had two lists of scientists names that he proposed to the interested student: we should read every paper published by those in one of the lists, and none of those authored by the other set (however relevant they might appear and wherever they had been published, by the way).

² Journal title imagined by Ricardo Brentani, University of S. Paulo, Brazil.

but they are those that everyone reads. Institutions with tight budgets, forced to decide on a limited number of journals, take the most “famous” only. In addition, faced with the extreme proliferation of publications, the scientists more often than not “delegate” to the editors of journals the choice of what they read ... and cite. A second factor is the “reputation” of the journal, often driven by excellent promotional campaigns, and largely built on a feed-forward process of large audiences, correspondingly high indexes of citations and interest of the scientists to publish, large numbers of manuscripts submitted, necessity for increased levels of selection and difficulty in “getting the papers published”... all re-enforcing the notion of exceptional quality and the tendency to cite such papers: if it published in one of the “high IF journals”, it must be true and important. It is clear to those involved, on the other hand, that other factors often inform the selection criteria in some such journals: editors and publishers are no longer active research scientists, driven by the best for science. These are entrepreneurs who are driven by profit and eventually, like any corporation, by the wish to “grow” and occupy an increasing fraction of the market. These cannot but be the most relevant factors in the selection of what is published. The fact that science has gained, not always by the best reasons, the attention of the general public, has come to complicate matters even further. Hence, criteria of selection seems to include not only the “impact” in the scientific community, but in the general news as well, justifying publication of papers on ethically or socially controversial topics, or simply on subjects that are “à la mode”. In addition, a “big name” always sells better, as it is also the case for the “big” institutions, which will always compete favorably with remote, unknown places. For all the respect we scientists have for the process of “peer review” that, more than a method, embodies the very foundation of science and ensures its progress, we all know of stories of review incidents and extremely controversial editorial decisions. It would be equally unfair to blame the poor editors for all the misgivings of today’s science literature: they, themselves, are evaluated by the IFs of the papers they accept and will keep their jobs only if these are above averages. In short, as to the evaluation of scientific production, we seem to be in a regimen that reminds us of Churchill’s famous statement on representative democracy. The current system is perhaps the least bad we have, but here and there, serious scientists, often some of the best, speak of the value of papers published in “VLI (very low impact) journals” or launch campaigns “against IF”. The recent initiatives on “open access journals” might be a solution, if partial, as they might bring back the interest of publication to science itself, and reattribute its responsibility and eventual gains to the scientists themselves.

Coming back to bibliometry, scientific production may be quantitated in at least two alternative manners: the relevance of papers can be estimated by the IFs of the journals where they were published, or by the real impact they have had, that is, by the number of times they are cited in the literature. While both suffer from some of the limitations above, the second would seem more appropriate, for IFs are only a “promise” of future relevance, actually built upon the relevance of the work of all others who have previously published in the same journal... and on all those strategies of publishers and editors. Number of citations, on the other hand, is at least independent of judgments (including those of editors and referees) other than that of the peers who write other papers. Incidentally,

comparing those two figures may give indication on the fairness (or lack of it) of the editor's decisions. Number of citations per article, some say, has a major drawback, namely that they are "conservative" and favour past performance: individual scientists (or institutions) may rest on a few great publications in the past to maintain high scores, while doing little for many years. This, however, is not a problem when dealing exclusively with the last 7 years of the institution, as is the case here.

The volume of scientific production at the IGC has steadily increased over the period of 2000-2007, and so has, naturally, the number of citations of IGC publications. The average number of citations of papers that were published by the IGC in this period allows for comparisons with other institutions, independently of the institution's size and number of investigators, and to place its impact in the context of Portugal and of the world. A few conclusions can be derived from these numbers. First, the IGC has produced science well above the country's level and is second to no other institution in Portugal, as to the global impact of the research produced here³. Second, scientific production at the IGC is not at the levels of the best institutions in Europe. This sobering result should not hide, however, that the IGC produces science with an average impact that is comparable or even superior to that of many European institutions of solid reputation, often given as examples of excellence and sought after by Portuguese students and post-docs. In short, we are doing very well as to the country's standards, but only reasonably well internationally, such that there is a lot of room for improving the current situation.

These large volumes of information may be further analyzed in various manners. Given the specificity of different institutions in various areas of life/biomedical sciences, each with a distinct "market value" and, thus, commanding differential numbers of citations, it has become customary to conduct these analyses by "category", roughly representing the various topics of research. This reduces total numbers and makes it possible to also compare, countries and "systems". From 2000-2007, the IGC has produced science with an impact that, in some areas, is much superior to that of the country's average (see "supplementary information"). Keeping in mind that the IGC's budget depends, to more than 80%, on Portuguese sources (the Gulbenkian Foundation included), this should be a very useful comparison for decisions on the distribution of science funding in the country. The favorable ratio between the impact of scientific production at the IGC *versus* the rest of Portugal is particularly true for Immunology, Plant Sciences, Biochemistry & Molecular Biology, even Cell Biology and Mathematical & Computational Biology. Most clearly, the differential of impact between the IGC and the rest of scientific production with an address in Portugal is highest in the "transversal" category Medicine, Research & Experimental, and it remains high in Multidisciplinary Sciences. If flattering, it remains surprising that a small institution such as the IGC, which produced 0.17% of all "articles" with an address in Portugal, accounts for a full 7% of the most cited papers. Incidentally, the most cited article with a "Portugal only" address published in the last 7 years was from the Institute. In

³ Interestingly, possibly deserving some analysis, according to the searches that we conducted, the second best institution in Portugal is the IPATIMUP, of a size and "coherence" that are similar to the IGC's.

contrast, the IGC is not doing better than the rest of the country in some other areas of institutional investment, such as, Developmental Biology, Genetics & Heredity, Evolutionary Biology and Parasitology. This is likely to reflect, at least in some areas, the progressive installation of the respective groups at the IGC, the very first being the most favored, as the numbers represent citations that were accumulated over time. Furthermore, in areas that are less developed in Portugal, scientific production at the IGC cannot be very different from the whole country, as so little is done elsewhere and the Institute contributes the bulk of all local publications. It could be argued that, having introduced some of these areas in Portugal and contributed to the recent period of accelerated growth in the local scientific community, the IGC might now serve the purpose of establishing standards of quality that should become, rather than volume, our next common aim.

Relevant as they may be to the Institute's survival, comparisons within Portugal are of local interest only. We have also calculated, therefore, the average number of citations for all articles in those categories published all over the world in the period 2003-2006 (the only ones that are readily available in the data-bases). These show that, in most subjects, the IGC ranks well above the world average. It is as well clear, however, that institutional benchmarking is favorable to the IGC in Portugal only. Thus, according to this indicator, the IGC ranks in the mid-to-low range of the known world institutions and it is still far from the level of excellence that could compare to the best institutes in Europe (see supplementary information).

An interesting aspect of the rapid progress in life sciences and biomedicine is the requirement for a constant adjustment of the institutional program in the pursuit of an active and interesting, eventually productive, intellectual environment. Nothing is more depressing and sad than a whole institution occupied with "no-problems" or small problems, be these most fashionable, for there is no excitement, no challenge, no fun, really. Obviously, no "institutional programs" can ever be successfully implemented "top-down", to the dismay of politicians and science policy-makers. An institution is a live organism, which breathes the rhythms of the concerns and excitements of each group, of every scientist and every student, the pulsation of their productive interactions. If the policy is to have these as diverse as possible, the "institutional program" will emerge from this dynamics, enriched by a dense network of interactions within and without the institution itself. Institutions may promote such an emergence by ensuring diversity, by investing in intellectual exchange and provocation, by caring about the human quality of interactions. All the rest is programmatic research, which may as well be conducted by functionaries of pharmaceutical multinationals who are better paid for that. In turn, this strategy involves the identification of the central questions, as well as the perception of what has been solved and which new questions and approaches have emerged.

One consistent difficulty in current biology is its patchwork description of the reality in the living world. A good friend brought to my attention a paper by Freeman Dyson on the future of biotechnologies, in one of the last issues of the New York Review of Books.

Quoting Carl Woese, Dyson speaks about the “obsolescence of reductionist biology as it has been practiced for the last hundred years”, with a candor that is most often absent in the discourse of “professional” biologists. The reaction of the latter is predictable: “emergent patterns of organization” and other jargon of specialists on “complexity” are mere “philosophy” that has little to do with the reality of discovery in the laboratories, let alone publication in “serious” scientific journals. Yet, many examples may be invoked to justify the discomfort with “the assumption that biological processes can be understood by studying genes and molecules”. Above all, the concern arises from the fact that, rarely if ever, we see alternatives to the analyses of genes and molecules in “normal” science. With few exceptions, genetics is done following “one gene by one gene” approaches, to use a telling expression that Melvin Cohn applied to ascertain that the understanding of the immune system, yet another area of patch working, could never have a “one cell by one cell” solution. Concurring with Dyson and Woese, this seems to be a natural consequence of the success story of the molecular biology’s agenda of component analysis over the last 50 years or so. Yet, it is surprising that novel, global approaches have not entered “normal science”, as we now have at hand many more whole genomes in machines than those we can accommodate in our theories. All the more so as there is increasing, if unsuspected, evidence for widespread non-linear effects that could be designated by “epistatic”, and for large blocks of linkage in more complex genomes, both of which remaining to be fully understood and precisely questioning the “one gene by one gene” approach. We go as far as to speak of “gene networks” and “developmental modules”, but empirical “network approaches” to genomes continue to be the exception, and our experimental systems often ignore that perturbations of the genetic network, small as they might be, in individuals or in the germ-line, should be expected to impinge in large areas of genetic operation. Systems Biology is in its infancy, and essentially looked at as a branch of computational biology, not as the access to organism-centered biology, not as common ground upon which we all design our experiments at the bench. It seems that we need robust, quantitative multiparametric approaches, to try to understand genomes rather than genes. Ironically, methods are already available that measure expression of all genes, but genomics, transcriptomics and proteomics, are mostly used as preparative, screening techniques to “isolate the genes of interest”. It would also seem that current limitations lie on “phenotyping” rather than on the genotyping, perhaps because the latter are carried out in automatic machines while the former still require man-years of detailed observation. The historical problem with genetics has been, as some say, the exclusive consideration of genes and phenotypes, thus ignoring the intervening semantics of physiology. This has been in part solved, but the progress in developmental biology and “evo-devo” might not be sufficient, and better methods for “automated, multiparametric, quantitative phenotyping” should be developed. Thus, complex problems of this kind require, one would think, organism-centered approaches in good synergy with component analyses: solid knowledge on molecular and cellular mechanisms, but a better understanding the organization of healthy and diseased systems in the whole organism.

The rapid and sustained progress of developmental biology has been rich and all encompassing. Yet, it is often stated that this field lacks a general theory, and all aspects

related to morphogenesis, to shape and size of cells, organs and organisms, but also to the variation in developmental times and life spans, remain quite far from a solution. On the other hand, these provide some of the most exciting, if extremely difficult, topics of research in this area. Unfortunately, it is surprising that, given the wealth, in numbers and quality, of the scientists in this field, that so few are fully dedicated to these questions, and prefer to concentrate on signaling pathways, even if these may well provide the stepping stones for a real solution.

Similar difficulties have been apparent in Immunology for decades. Ever since Jerne introduced the Darwinian principles of variation and selection in this field, and Burnet opened modern Immunology with his clonal selection theory, a large number of dedicated specialists accumulated and extraordinary volume of observations. After the resolution of the central question on the genetic origin of Variable region diversity, the field had wobbled between problems of cell commitment and differentiation, and the cellular and molecular analysis of lymphocyte responses. For many years, it has seemed to some, Jerne included, that we would better “sit back and wait for the end”. Yet, when it comes to solving practical problems, modern immunology shows up to this day a depressing, if surprising, inability to deal with essentially all: we have no rational and curative treatments for autoimmune diseases and allergy, all progress in clinical transplantation owes to advances in the pharmacology of immunosuppression (rather than to “translating” a better understanding of tolerance), no effective vaccines are available to chronic infections such as AIDS, malaria or tuberculosis, and there is nothing in sight as to the immunological therapies of cancer. Again here, it seems that we know everything about components (e.g., allergic reaction) but little about the whole organism (e.g., we continue to treat allergy as we did 60 years ago). This evident lack of progress might well be attributed to the prevalence of core ideas that turned out to be wrong, though only quite recently. Thus, the classical view of Ehrlich on the “disteleology” of autoreactivity have persisted for a whole century in the form of “recessive tolerance” by deletion of all autoreactive lymphocytes, in modern times also comforted by the equally wrong conviction that the problem could have a “one cell by one cell” solution. In other words, also here, the mistake seems to be of the same (epistemological) nature: to reduce to single components (cells or molecules) a property (tolerance) that pertains to whole organisms. In the last 10 years or so, however, two major concepts have made their way, both of which bring forth notions of “systemic properties”. The first is the principle of “dominant tolerance”, now widely accepted after decades of resistance: a tolerant state remains when a non-tolerant immune system is admixed with a tolerant one, demonstrating that tolerance is a systemic property that supersedes those of individuals cells. The second step of progress is somehow related to the former. Thus, classical views of “recessive tolerance” “isolated” the immune system from the body, while it has become clear that many other physiological systems modulate and determine immune activities: responses to cellular stress induce expression of cytoprotective genes, the products of which show a variety of interactions with lymphocytes and other immune cells, either inhibiting or amplifying the respective activities. This is the most ancient level of “protective responses” that pertains to all cells in the organism and is intimately connected to the next in evolutionary terms: innate immunity, mediated by

mobile (phagocytic), inflammation-inducing cells, which use receptors for invariant microbial molecules ... and for products of the stress response. Since lymphocytes also express such receptors and respond to interactions with “innate” cells, these three levels are integrated to an extent that was not anticipated only a few years ago. Tissue protective responses also participate in various other physiological systems (e.g., oxygen transport, coagulation, tissue remodeling and angiogenesis), deeply rooting immune responses and their outcome to the overall physiology of other body systems. More interestingly for historians, perhaps, is the fact that, for decades, the literature contained a host of both observations and theory on these matters.

In spite of major investments all over the world, genetics of complex traits and diseases in human populations is taking time to deliver. Many reasons may be invoked to justify that investment and the initial hopes, as well as the apparent difficulties. One of the latter was invoked above, namely that unsuspected epistatic effects may make it such that essentially any gene might show up in our lists for a given complex trait (now designated by “modifier genes”). The nonlinearity of those effects casts doubts on our ability to generalize and ever come to a biological theory of the genetic basis for development and physiology. Furthermore, unanticipated but extensive linkage may also come to complicate the matters, and this may well vary with the populations under study. Perhaps both hopes and difficulties are related: on the one hand, the hope was grounded on the notion that identification of susceptibility/resistance genes would open the way to novel discoveries on mechanisms, eventually, on novel means of preventive or therapeutic intervention. In other words, genetic discoveries could help progressing on physiopathology. Conversely, it may be argued that it is precisely our current limitations on physiology and respective pathological alterations that hampers real progress in this area of great significance. We seemingly need more theory and mathematical techniques applied to biology, but it would also help to gather the genetic history of populations, including the fossile record, and to better organize the construction and access to databases of human phenotypes, as produced by MDs, who are precisely educated to be professional “phenotypers”.

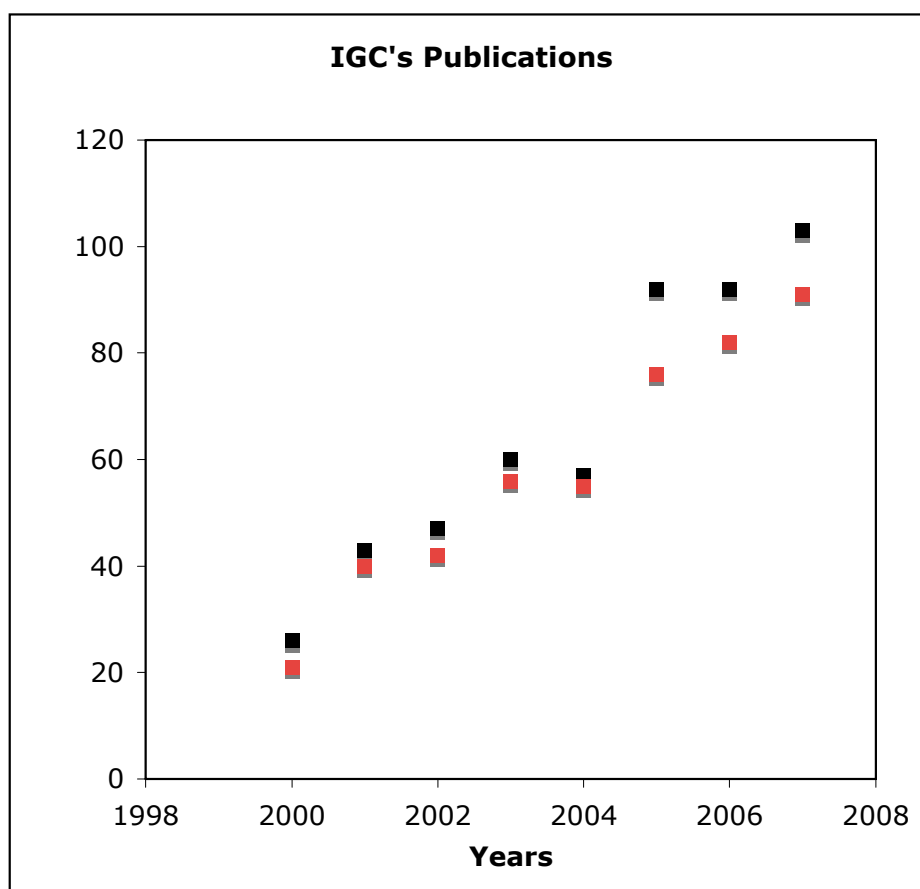
Shear money will not solve any of these limitations. Since the “war on cancer” launched by the White House decades ago, we have learned, several times already, where such “top-down” approaches may lead. Large investments on AIDS and malaria vaccines, and on brain degenerative diseases worldwide, have essentially failed, and there are logical reasons for such failures, which pertain to market rules. Abundance of support following top-down decisions for investment attracts a wealth of new players into the “target” area. These, in most cases, one would think, “go where the money is”. Obviously, such newcomers either had no problem at all before, or were occupied with small problems that could readily be dropped. Excellent scientists, on the other hand, are usually extremely committed to the problems they have identified as “the most interesting” and are actively trying to solve, such that they do not “sell their souls” to the first offer. In addition, excellent scientists are usually already well supported and do not need to move where the

money is. Rather, they bring the money to where the problem is. In short, policy-makers and the public in general (including interest groups and stake-holders) must understand that massive investments will buy only mercenaries who are not necessarily the best performers. Many battles have been lost, History tells us, by recruiting mercenaries. In other words, it is always good to put money on good people who will solve the problems, and it never works to put the money on the problems, for this will not attract the best people.

The past year marked the launching of the Champalimaud Neuroscience Program at the IGC. The first group leaders were recruited and their installation in a newly renovated, specially designed wing of laboratories begun. The Program counts today with three PIs and two “fellows”, and we expect to recruit a few more in 2008. The common theme to these groups is “systems neuroscience”, and several of them have experience with multi-electrode recording in live rodents going about their business in the behavioral tests the scientists invent for them. The workings of the brain is a topic of enormous promise, ridden by the corresponding difficulty. The most complex of all known structures in the universe, approachable at so many multiple levels, it is not surprising to see large institutions with hundreds of scientists, entirely dedicated to try to understand the brain. From the molecular and cell biology of neurons and glia, to the development of its architecture, each of its structures and the setting-up of circuits, to the chemistry of synaptic transmission, to learning & memory and consciousness, let alone its evolution from flies to men, the field can certainly accommodate specialists of all kinds. The major difficulty may be to ask the “right questions”. Thus, the overwhelming volume of descriptions on various aspects of brain structure and function, which are constantly being produced and published in the “best journals”, often leave the impression that little progress is achieved in real “understanding”. The “right questions” are often substituted either by those that, if critical, are too general or vague for current knowledge and possible approaches (e.g., consciousness), or else, by too small questions (e.g., chemical details of signaling). Some say that there is no theory of brain function at the hinge where the “mind” and “higher” brain functions emerge from the chemistry of individual cells. This is precisely the agenda for the Champalimaud Neuroscience Program at the IGC. Our expectations concerning the work of these groups and this intimate collaboration between the Gulbenkian and the Champalimaud Foundations are enormous.

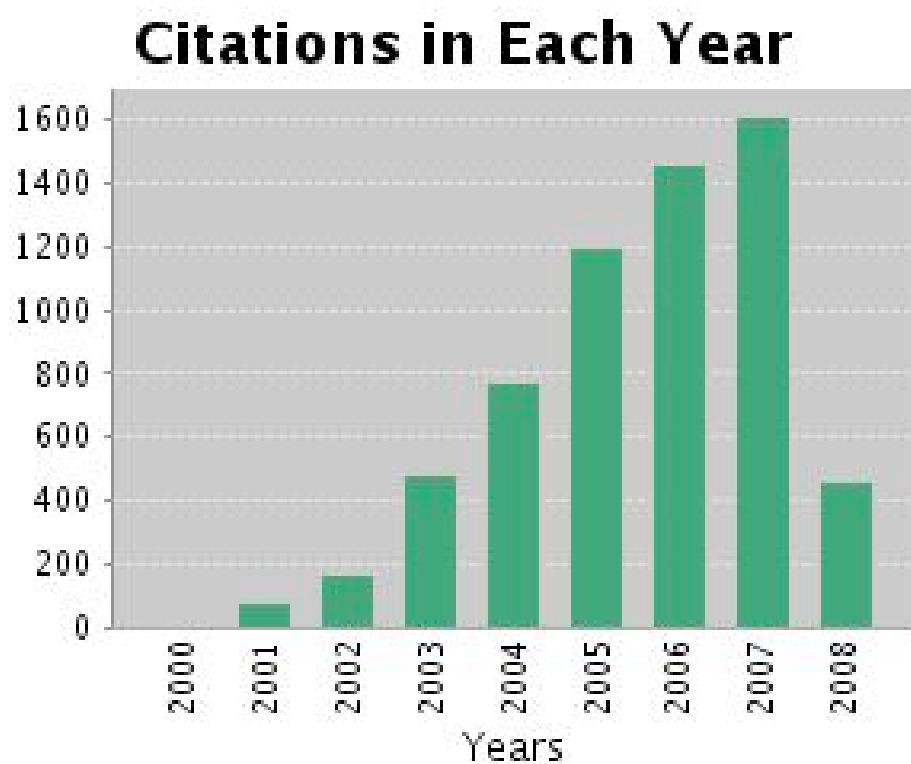
1. Scientific production at the IGC during 2000-2007.

Black Symbols: Total publications
Red symbols: ISIindexedpapers



2. Citations to IGC's publications during 2000-2007.

2.1. Total papers



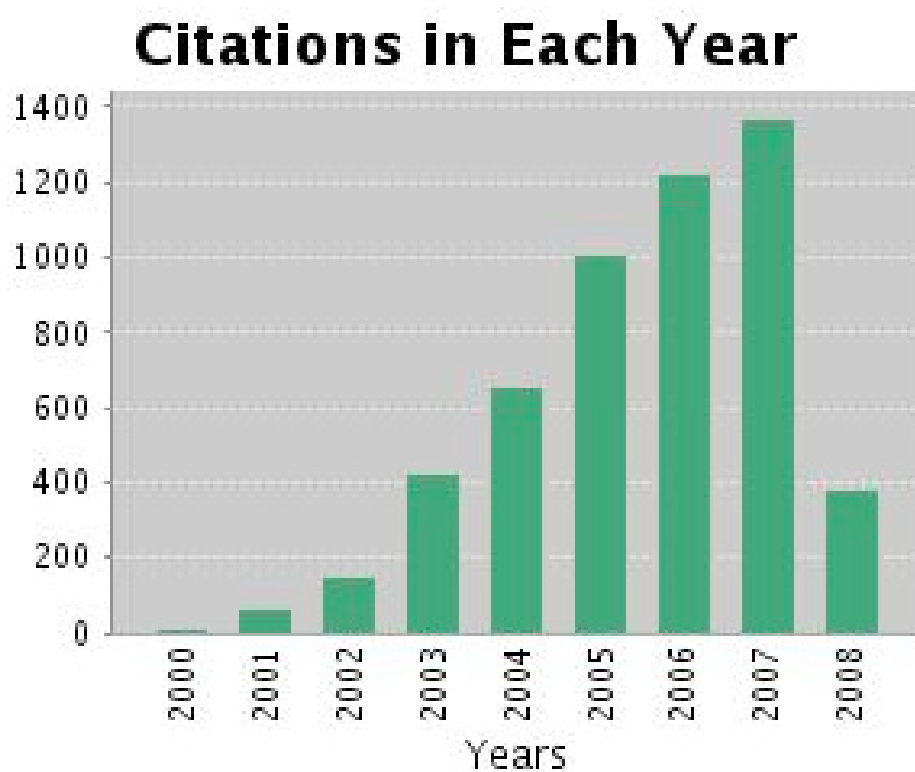
Results found: 509

Times Cited: 6,248

Average Citations per Item: **12.28**

h-index: **41**

2.2. Articles



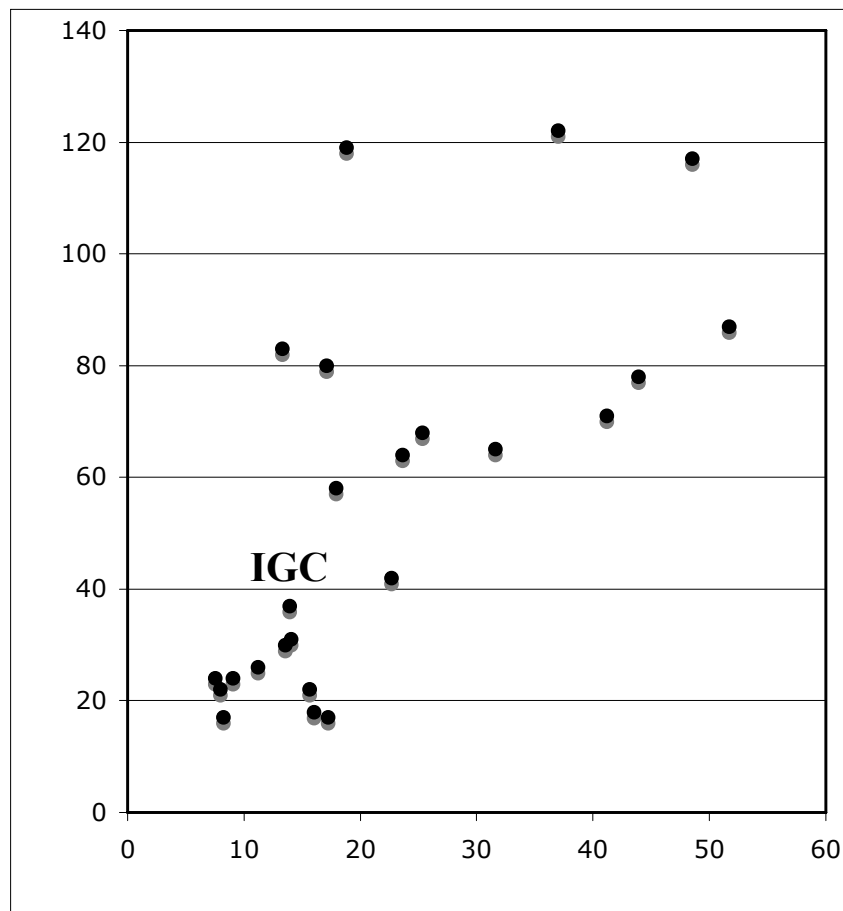
Results found: 381
Sum of the Times Cited: 5,308
Average Citations per Item: **13.93**
h-index : **37**

3. Comparison of IGC citations/papers with the rest of Portugal in various areas of activity (IGC contribution to the country's figures is not deducted)

2000-2007 IGC and PORTUGAL indexed *articles* by field:
citations/paper

2000- 2007 Articles only	N° Articles IGC	N° Articles PORTU	Times Cited IGC	Times Cited PORTU	Average Citations per item IGC	Average Citations per item PORTU	h- index IGC	h-index PORTU
Total Papers	367	37312	4879	----	13.29	----	36	----
Immunology	60	398	1315	2584	21.92	6.49	14	23
Biochem & Mol Biol	52	1997	973	18861	18.71	9.44	17	50
Dev Biol	43	76	580	831	13.49	10.93	15	16
Cell Biol	42	494	928	5805	22.10	11.75	16	38
Genetics & Herdity	42	693	262	7263	6.24	10.48	9	38
Biology	29	213	307	1401	10.59	6.58	9	17
Parasitology	20	155	54	634	2.70	4.09	4	12
Biotechnol & Applied Microbiol	19	1117	172	6616	9.05	5.92	8	28
Multidisciplinary Sciences	18	195	491	2501	27.28	12.83	10	28
Evolutionary Biol	15	211	49	1786	3.27	8.46	4	22
Mathematical & Comput Biol	15	67	119	250	7.93	3.73	7	8
Plant Sciences	15	700	282	3596	18.80	5.14	9	25
Virology	15	91	146	715	9.73	7.86	7	13
Neurosciences	14	584	121	4565	8.64	7.82	6	29
Ecology	13	619	46	4668	3.45	7.54	4	30
Medicine, Research & Exp	13	197	780	1216	60.00	6.17	7	19
Tropical Med	11	68	46	390	4.18	5.74	4	12
Hematology	9	182	99	2132	11.00	11.71	5	24

4.1. Plot of the results above (in 4.)



5. Comparison of IGC citations/paper with the rest of the world in various areas of activity.

2003-2006 IGC and WORLD indexed publications by field:
average Citations/paper

2003- 2006 Articles only	Nº Articles World	Times Cited World	Average Citations World	Nº Articles IGC	Times Cited IGC	Average Citations IGC
Immunology	70.216	244.762	3.48	34	629	18.50
Biochem & Mol Biol	191.245	679.031	3.55	31	617	19.90
Cell Biol	78.885	364.559	4.62	30	670	22.33
Dev Biol	15.279	62.634	4.09	29	411	14.17
Genetics & Herdity	57.805	210.366	3.63	26	173	6.65
Biology	23.356	51.233	2.19	21	239	11.38
Neurosciences	104.682	317.553	3.03	11	104	9.45
Biotechnol & Applied Microbiol	65.102	152.762	2.34	9	102	11.33
Parasitology	10.130	15.228	1.50	9	40	4.44
Virology	19.348	59.212	3.06	9	131	14.56
Evolutionary Biol	13.441	38.893	2.89	8	49	6.12
Ecology	43.525	90.055	2.06	7	47	6.71
Hematology	39.026	163.104	4.17	6	94	15.67
Tropical Med	5682	8299	1.46	6	24	4.00
Medicine, Research & Exp	40.490	116.311	2.87	5	506	101.20
Multidisciplinary Sciences	41.014	286.914	6.99	5	213	42.60
Plant Sciences	56.860	103.877	1.82	5	139	27.80

Research

RESEARCH PROGRAMMES

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 characteristics.

This Annual Report presents summaries of the individual research projects, preceded by short introductory notes to each areas of research. Full descriptions are available on the IGC's website (www.igc.gulbenkian.pt). Acknowledgements to all 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 and Ana Godinho.

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 at 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.

Fitness effect of mutations *E. coli*.

MEMBERS: Francisco Dionisio.

STUDENTS AND TECHNICIANS: Claudia Marques.

We use *E. coli* as a model organism to study the fitness effects of spontaneous mutations in housekeeping genes.

The tragedy of the commons among bacteria.

MEMBERS: Francisco Dionisio.

STUDENTS AND TECHNICIANS: Rui Pimpão.

The Tragedy of the Commons is a condition where individuals cooperate or everyone loses; yet each individual has incentive not to cooperate. This project aims at testing the existence of the *tragedy* among bacteria (in particular, with *Escherichia coli*).

The role of plasmids in bacterial cooperation.

MEMBERS: Francisco Dionisio.

STUDENTS AND TECHNICIANS: Claudia Marques and Ana Margarida Duarte.

Each bacterial cell often co-inhabit with billions of other bacterial cells, usually belonging to tens or hundreds of species. Many tasks performed by bacteria require that many cells cooperate. This project studies the role of plasmids in this cooperation.

Population Genetics in *E. coli*.

MEMBERS: Isabel Gordo, Sara Magalhães.

STUDENTS AND TECHNICIANS: Catarina Mota, Sandra Trindade.

COLLABORATORS: Lisete Fernandes (IGC, Oeiras, Portugal).

We use *E. coli* as a model organism to ask questions about mutation rates, fitness effects of spontaneous mutations and test theoretical predictions about the evolution of mutation rates, the genetics of adaptation and the role of population structured in evolution.

Experimental evolution in *Tetrahymena thermophila*.

MEMBERS: Isabel Gordo.

STUDENTS AND TECHNICIANS: Elsa Guilherme.

COLLABORATORS: Helena Soares (IGC, Oeiras, Portugal).

This project aims at estimating some key evolutionary parameters in the species *Tetrahymena thermophila*. This estimates, together with a population genetics study of the species diversity in natural populations, will shed some light into understanding its ecology and evolution.

Genetic diversity in pathogen populations.

MEMBERS: Isabel Gordo.

STUDENTS AND TECHNICIANS: Daniel Reis.

EXTERNAL COLLABORATORS: Paulo Campos (Universidade de Pernambuco, Brasil) and Gabriela Gomes (IGC, Oeiras, Portugal).

The analysis of genetic variation in populations of infectious agents may help us understand their epidemiology and evolution. In this project we study models for assessing the levels and patterns of genetic diversity in pathogen populations. The pathogen population is assumed to be a metapopulation, composed of many small subpopulations, which correspond to their hosts. These are connected according to a specific type of contact network. Working on the grounds that the correct null model for understanding pathogen genetic diversity is the neutral metapopulation model, we investigate the best statistical test for identifying genes under selection in natural pathogen populations.

Evolution by gene duplication: structure and functions of the FoxP gene family.

MEMBERS: Élio Sucena.

STUDENTS AND TECHNICIANS: Maria Emília Santos.

FOXP proteins (Fork head box p) play an essential role in development and immunity of vertebrates. There are four Foxp proteins in vertebrates while in invertebrates there is only one ortholog (CG16899, in *Drosophila melanogaster*). This fact argues for gene duplication after the split from invertebrates. We determined the genealogy of this gene class showing that CG16899 is more related to Foxp1 than to any other Foxp of vertebrates. Analysis of the *Drosophila melanogaster* ortholog led to the cloning of two alternative isoforms of CG16899. We report the conservation of this pattern of alternative splicing in FoxP1 of *Mus musculus*. These findings suggest that the Foxp1 maintain its original function while the other three duplicates evolved a new function (neofunctionalization). In examining CG16899 expression profile during ontogeny we found that the two isoforms are expressed in all life stages. The preliminary functional analysis shows that the knock out of the second isoform (FoxpAlt2) causes a delay in development.

Mechanisms of Vertical and Horizontal Transmission of *Wolbachia* in *Drosophila*.

MEMBERS: Élio Sucena.

STUDENTS AND TECHNICIANS: Joana Louçã, Pedro Patraquim, Vitor Faria.

In order to expand current understanding of the ecology of the interaction of *Wolbachia* with *Drosophila melanogaster*, we conducted experiments to compare the effect of *Wolbachia* on the flies' fecundity and egg survival under optimal and stress conditions. Overall, high temperature had a negative impact on egg survival, but pooling all lines showed no significant differences. However, when analysing the lines separately, this negative effect was also found in uninfected crosses, hence we could not reach any conclusion regarding the effect of *Wolbachia* infection under these stress conditions.

Regarding the existence of CI, it was tested checking for differences among crosses within the same temperature treatment. Pooling all lines showed no significant differences. When analysing the lines separately, in fecundity, no differences were found. In egg viability, most lines also showed no differences. However, one line (1037) showed differences in both treatments, and another (187) showed differences in the control treatment. But the results in these two lines are very different, and the differences are probably host genotype dependent. In both treatments of line 1037, the crosses UxU and WxW had higher egg viability than the others. In the control treatment, these were significantly different from the cross UxW, and in the high temperature treatment from the cross WxU. However, in the control treatment of line 187, the cross of WxW had significantly lower egg viability than all the others. These scattered results do not allow us to extrapolate but again we are convinced that the experiment should be repeated with a larger sample size.

In addition to this, we studied whether the patterns of distribution of *Wolbachia* depend on the genetic background of the host, *Wolbachia*, or both. We had an introductory approach to this matter by trying to transinfect lines of *D. melanogaster* infected with *Wolbachia* infecting its sister species *D. simulans*. The success rate we got from the methodology used by Frydman and colleagues (2006) was extremely low when infecting *D. melanogaster* with *Wolbachia* infecting the same strain, and we did not manage to get any transinfection of *Wolbachia* from *D. simulans*. It is therefore not clear whether the negative result in transinfection was due to a small sample size, or to other factors, namely the induction of an immune response; to clarify this doubt, the experiment will be repeated with a larger sample size.

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

MEMBERS: Henrique Teotonio, Ivo Chelo.

EXTERNAL COLLABORATORS: Anthony Long (University of California Irvine, USA).

The reversibility of evolution has been empirically studied at the phenotypic level, both experimentally and using a comparative approach. Studies addressing the reversibility of evolution at the molecular level, and its relationship to phenotypic reverse evolution, are however still rare, in particular for sexual and outbred organisms. The present project studies the reversibility in 20 laboratory populations of *Drosophila melanogaster* while evolving in their common ancestral environment for 50 generations. Phenotypic reverse evolution has been described for life-history characters, while keeping frozen samples of

individuals of each population throughout experimental evolution. These samples constitute the material basis of this project.

The project is divided into two phases. During the first phase the goal is to describe patterns of gametic linkage disequilibrium (LD) in several genomic regions encompassing candidate gene loci for life-history evolution (Sod, Pgm, Fatty CG4852 and Ftless). This information will then be used for the second phase where DNA polymorphisms that evolve independently, i.e. that are at linkage equilibrium, will be used as molecular markers of reverse evolution in all 25 populations for the 50 generations of study. The extent of LD with genetic distance is contingent on the previous evolutionary history of the populations studied, because of such processes as genetic drift and natural selection. It is unknown how experimental evolution, with usually strong selection and modest population sizes, will determine patterns of LD. The second phase has the goal of describing the molecular evolutionary trajectories of the molecular markers that may have hitch-hiked with the actual genetic factors involved in reverse evolution, and then to correlate these trajectories with those previously obtained for life-history phenotypes. The mode of molecular evolution will be determined by describing whether 50 generations of reverse evolution will allow the attainment of new genetic equilibria or if there is the fixation of certain variants correlated with reversion.

Experimental evolution of outcrossing in *Caenorhabditis elegans*.

MEMBERS: Henrique Teotonio.

STUDENTS AND TECHNICIANS: Sara Carvalho, Miguel Roque.

EXTERNAL COLLABORATORS: Patrick Phillips (University of Oregon, Eugene, USA).

The evolutionary mechanisms explaining the origin and maintenance of diversity in breeding systems have long been of interest to biologists. Androdioecy in particular is a rarely occurring mixed breeding system where males co-exist with hermaphrodites in the same population. Why do males persist if selfing has associated evolutionary benefits? In this project we are addressing the hypothesis that males are evolutionarily maintained due to their role in promoting outcrossing since hermaphrodites cannot outcross amongst themselves.

Two evolutionary mechanisms are studied: inbreeding depression and sorting of beneficial genetic variation. The proposed methodology makes use of an experimental evolution approach with genetically variable and isogenic, both laboratory adapted, populations of *Caenorhabditis elegans*. Breeding 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. Since cryogenics are possible, ancestral and derived states are compared at the same time.

The main goals of this project are to test the role of inbreeding depression in the evolutionary dynamics of outcrossing; to test the role of advantageous mutations and their interaction with deleterious mutations in the evolutionary dynamics of outcrossing; and to describe the dependency of genetic variability dynamics with the evolution of outcrossing.

Sex and the maintenance of diversity in heterogeneous habitats.

MEMBERS: Henrique Teotonio, Sara Magalhães.

STUDENTS AND TECHNICIANS: Patricia Lopes.

This project aims at understanding the ecological and genetic mechanisms that underlie the maintenance of diversity in heterogeneous environments. To provide new insights on these mechanisms, we study the evolution of populations of *C. elegans*, under two breeding systems, as they face two novel environments under global and local population density regulation.

The experimental design encompasses six selection environments, with populations being replicated 6 times, for a total of 72 populations: (1) original habitat (*E. coli*); (2) hab1; (3) hab2; (4) each habitat offered in predictable alternation, each generation, simulating temporal heterogeneity ; (5) each habitat simultaneously and such that density regulation occurs locally on each habitat, implying spatial heterogeneity and soft selection; and (6) each habitat simultaneously and such that density regulation is global, implying spatial heterogeneity and hard selection. Local regulation will be achieved by selecting equal number of individuals from each habitat at each selection round. Global regulation will be mimicked by collecting individuals from each habitat proportionally to local densities.

The main goals of this project are to test for trade-offs in adaptations to different environments; to measure the genetic cost of adaptation; to assess whether exposure to a heterogeneous environment leads to the evolution of generalists or favors the evolution of specialists; to test the hypothesis that frequency dependence generated by local density regulation will lead to the maintenance of polymorphisms; and to test the role of breeding system in adaptation to novel environments.

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.

Genetic diversity of the Azorean population.

MEMBERS: Luisa Mota Vieira.

STUDENTS AND TECHNICIANS: Claudia Castelo Branco, Marta São Bento and Paula Ribeiro e Pacheco.

EXTERNAL COLLABORATORS: Astrid Moura Vicente (IGC, oeiras, Portugal and INSRJ, Lisbon, Portugal).

This project, which is in its last year, aimed to characterize the genetic variability, ancestry and linkage disequilibrium (LD) extension of the Azorean population, in order to carry out admixture and association studies. Taking into consideration the geographical and settlement history differences of the archipelago, we assessed the genetic diversity pattern

and the internal migration of the Azorean population, based on the analysis of 15 STR loci in 592 unrelated individuals. The results reveal that Terceira displays the highest value of gene diversity (0.7979) and Corvo the lowest (0.7717). Gene flow analysis indicates that Corvo has the lowest values of migration, 23.35, whereas São Miguel and Terceira present the highest values of emigration, 108.14 and 87.66, respectively. The extent of LD in the Azores and mainland Portugal populations was evaluated in the Xq13.3 region by genotyping 8 STR markers spanning 20.9 Mb. Standardized multiallelic disequilibrium coefficient (D') analysis indicates that the Western Azorean group presents higher values when compared with the Central and Eastern groups. However, all islands group's show values of D' lower than 0.33, suggesting no extensive LD in these populations. The same results were obtained for the São Miguel Island population, where a comparison between LD in HLA, Xq13.3 and NRY regions was performed. Overall, the data indicate that the Azorean population can be analysed as a homogeneous genetic group, which possibly, can present the same drug-reaction profile. In terms of genomic medicine, these results will have a significant impact in the design of future genetic and pharmacogenomic studies in the Azorean population.

Genetic and consanguinity of congenital heart disease in Azores.

MEMBERS: Luisa Mota-Vieira.

STUDENTS AND TECHNICIANS: Rita Cabral, Paula Ribeiro e Pacheco, Filipe Serrano Tejero, and Claudia Castelo Branco.

EXTERNAL COLLABORATORS: Rui Anjos (Hospital de Santa Cruz, Carnaxide, Portugal) and Carlos Pereira Duarte (HDES, Ponta Delgada, Azores, Portugal).

Congenital heart defects are among the most common birth defects, and the leading cause of birth defect-related deaths. Recently, we demonstrated that in São Miguel Island the CHD prevalence is relatively high: 9.16 *per* 1000 live births. We performed a structured family questionnaire which includes: 1. queries for CHD risk factors (maternal diabetes mellitus, alcohol and drug abuse by the mother during pregnancy, viral infections of the foetus and genetic conditions), and 2. a detailed family history to construct the ascending genealogy until the 3rd generation. To that end, 195 CHD families were contacted by phone and/or letter, 109 (55.9%) of which accepted to participate. We identified 39 (35.8%) multiplex families (with 2 to 5 patients), 5 (4.6%) consanguineous families, and 5 (4.6%) multiplex families with consanguinity. These data suggest a genetic factor involved in the susceptibility for CHD. However, although the study concerns an island population, the consanguinity was not apparently the major genetic factor. Using the biobank (DNA and RNA) build in the scope of this project after written informed consent, we are analyzing the microsatellite heterozygosity in order to search a distant consanguinity in the CHD patients, and the candidate genes (*MTHFR*, *NKX2.5*, *TBX5* and *GATA4*) for heart malformations. The first variants screened were the C677T and A1298C of the *MTHFR* gene in 469 healthy individuals from São Miguel, being respectively, 84 (18.0%) homozygous for the variant (TT) and 223 (47.5%) heterozygous (CT), and 28 (6.0%) homozygous for the variant (CC) and 177 (37.7%) heterozygous (AC). A comparative analysis is being performed for these variants in the CHD patients and their mothers.

The study of inbreeding, recessive mutations and genomic homozygosity in the Azorean population.

MEMBERS: Luisa Mota-Vieira.

STUDENTS AND TECHNICIANS: Paula Ribeiro e Pacheco, Claudia Castelo Branco, Francisc Sigalat Savall, Helena Polena and Maria José Brilhante.

EXTERNAL COLLABORATORS: Cirurgic (Dr. Victor Santos) and Anatomic Pathology (Dr. Vitor Carneiro) Departments, Hospital of Divino Espirito Santo of Ponta Delgada, EPE, Azores, Portugal.

This ongoing project aims to study recessive mutations in health and disease. During this year, we analysed the association between the mutations in *HFE* and breast cancer. Hereditary hemochromatosis (HH) is an autosomal recessive disorder of iron metabolism that increases iron absorption and results in excessive iron accumulation. This iron overload may be carcinogenic because it can catalyse the formation of free radicals, suppress the host immune system and increase the growth of tumour cells. In order to investigate if heterozygosity for *HFE* mutations was related to the risk of breast cancer in the Azorean population, we determined *HFE* C282Y and H63D genotypes in 86 female breast cancer patients and compared results with the *HFE* genotype distribution in 183 healthy women. The C282Y allele frequency in the breast cancer group was 4.07%, higher than in the control group, 3.28%; with an OR=1.25 (95% CI, 0.48 – 3.24). Regarding the H63D mutation, the allele frequency in the breast cancer group was 21.51%, very similar to the frequency found in the control group, 21.04%; OR=1.03 (95% CI, 0.66 – 1.6). The mean age at diagnosis for breast cancer patients was 60.4 yr (range, 33-87) and 54.8 yr (range, 31-84) in the healthy control group. As age is known to influence breast cancer risk and thus could be a confounding factor, we stratified the breast cancer and the healthy control group into three age stratum, according to the menopausal status (<48, 49-58 and >59 yr). Odds ratio for breast cancer risk associated with the H63D mutation was 1.29 (95% CI, 0.54 – 3.13) in women bellow 48 yr; 0.69 (95% CI, 0.24 – 2.01) in the range of 49-58 yr, and 0.97 (95% CI, 0.53 – 1.78) in women above 59 yr. On the other hand, odds ratio for breast cancer risk associated with the C282Y mutation increased with age, from 1.14 (95% CI, 0.29 – 4.52) in women bellow 48 yr to 2.03 (95% CI, 0.44 – 9.27) in women above 59 yr. The risk for breast cancer was higher in older women bearing the C282Y mutation than in healthy controls, although this was not statistically significant. In conclusion, the results of this case-control study suggest that C282Y and H63D mutations do not appear to be associated with an increased risk for breast cancer in the Azorean female population. Presently, we are analysing other *HFE*-interacting genes such as the *TFR* (transferrin receptor) gene, to extend the search for a supposed BC susceptibility for the compound *HFE-TFR* genotypes.

Development of a molecular test for human *leptospirosis*.

MEMBERS: Luisa Mota-Vieira.

STUDENTS AND TECHNICIANS: Cidália T. Gomes and Paula Ribeiro e Pacheco.

EXTERNAL COLLABORATORS: Margarida Collares-Pereira, Maria Luisa Vieira and Ana Teresa Gonçalves, Unidade de Leptospirose e Borreliose de Lyme (ULBL, Instituto de Higiene e Medicina Tropical da Universidade Nova de Lisboa, Lisbon, Portugal).

The aim of this study, carried out in the scope of the Azores Leptospirosis Project (Scientific Cooperative Agreement N°. 58-4001-3-F185, USA), was to implement the diagnosis of leptospirosis using the PCR, in order to offer a laboratory response to clinicians and patients with early clinical suspicion of leptospirosis. We analysed paired samples of blood and urine collected from 178 patients, with a median of 6 (sd=4.64) days after the onset of symptoms, at their first admission to the Hospital. DNA was extracted from these samples and hydrated during 24 hours. For *Leptospira* DNA detection, two consecutive PCR amplifications – *nested* PCR – were carried out, using two sets of genus-specific primers (gene *rrs*, sub-unit 16S). The PCR analysis revealed that 73 (41%) patients tested positive for *Leptospira* sp. whereas 105 (59%) were negative. The positive pattern was the following: 38 (52%) patients were both positive in the serum and urine (S+/U+); 18 (25%) were negative in the serum and positive in the urine (S-/U+); and 17 (23%) were serum positive and urine negative (S+/U-). Being MAT the reference method for leptospirosis, 77 PCR results were matched with those from serology in 55 (71%) examined patients. It was possible to confirm some of the PCR results, namely: 21 (45%) patients were both PCR and MAT positives and none of the patients with negative PCR had a positive MAT. The 21 patients with positive MAT (52% in the first sample) had three different PCR patterns: S+/U- (n=3, 14.3%), S+/U+ (n=11, 52.4%) and S-/U+ (n=7, 33.3%). In addition, the PCR technology enabled the early detection of *Leptospira* DNA, in the absence of a concomitant positive serology, as it was shown in 10 (21%) cases, positive by MAT only in further samples. In conclusion, the Azores Leptospirosis Project allowed the implementation of the molecular diagnosis (research phase) for this disease at the UGPM in the HDES. This approach is based on a sensitive and quick PCR test, which has an earlier potential diagnostics than MAT in the very acute stage (1-3 days after the beginning of symptoms). So far, the majority of the PCR results can be communicated to the clinicians within 48 hours, favouring a quick lab diagnostics for a severe public health problem in the São Miguel and Terceira Islands. Furthermore, an upgrade of this technique by Real-Time PCR is necessary for early diagnosis.

Study of the ancestry of the HFE C282Y mutation in the São Miguel island population.

MEMBERS: Luisa Mota-Vieira.

STUDENTS AND TECHNICIANS: Cidália T. Gomes and Paula Ribeiro e Pacheco.

Hereditary hemochromatosis (HH) is an autosomal recessive disorder of the iron metabolism. It is typically associated with homozygosity for the C282Y mutation of the *HFE* gene, which is located on the HLA region (6p21.3). Generally, this mutation lies within the celtic ancestral HLA-A*03-B*07 haplotype. Here, C282Y mutation was selected as a model to study the diversity and origin of recessive mutations in a geographic isolated population. A total of 130 individuals from Sao Miguel Island (Azores) were genotyped for HLA-A and -B by PCR-SSP, and for *HFE* mutations (C282Y, H63D and S65C) by PCR-RFLP. Data

analysis was performed using Arlequin v3.1 and Graphpad Prism v5.0 softwares, after dividing the sample in two groups: 48 homozygous or carriers for the C282Y mutation and 82 with no mutations. Statistical analysis revealed that four alleles – HLA-A*03 (21%), HLA-A*26 (2%), HLA-B*29 (10%) and HLA-B*45 (9%) – protrude in the C282Y mutation group, when compared to the control subjects ($p < 0.05$). Moreover, three haplotypes, identified by computational inference, were found to be significantly associated with the C282Y: HLA-A*02-B*58 (5%; OR=19.78, 95% CI: 1.08-362.0), HLA-A*03-B*07 (5%; OR=8.96, 95% CI: 1.03-77.84) and HLA-A*29-B*45 (7%; OR=27.57, 95% CI: 1.56-488.70). Another haplotype HLA-A*24-B*15 (3%) was also identified by direct inference in an individual homozygous for HLA region and C282Y mutation. Although São Miguel island has a small population, these four haplotypes corroborate with its diverse genetic ancestry.

Stroke genetics and genomics.

MEMBERS: Sofia Oliveira, Astrid Vicente.

STUDENTS AND TECHNICIANS: Tiago Krug, Benedita Fonseca, Helena Manso, Sara Violante, Alexandra Rosa, João Sobral.

EXTERNAL COLLABORATORS: L. Gouveia, J. Ferro (Hospital de Santa Maria, Lisboa, Portugal); G. Lopes, M. Correia (Hospital de Santo António, Porto, Portugal); I. Matos (Hospital Distrital de Mirandela, Mirandela, Portugal); J.P. Gabriel, M.R. Silva (Hospital São Pedro, Vila Real, Portugal); C. Ferreira, J. Fontes (Hospital São Marcos, Braga, Portugal); A.A. Pinto (Hospital Fernando Fonseca); M. Viana Baptista (Hospital Garcia de Orta); M. Rodrigues (Hospital São Bernardo).

Stroke, a “brain attack” cutting off vital blood to the brain cells, is the second cause of death worldwide but the leading cause of death in Portugal. Stroke is even more disabling than lethal and requires more effective prevention and treatment strategies. It is a complex disease resulting from the interplay of environmental and genetic factors, but very few genetic factors for the common form of stroke have been identified. We investigated the association of several candidate genes (e.g. PDE4D, ALOX5AP, mitochondrial genome) and are conducting the novel “genomic convergence” approach which combines data from whole-genome linkage screens with data from gene profiling analyses to determine which genes will be tested in association studies.

Parkinson’s disease microRNomics and proteomics.

MEMBERS: Sofia Oliveira.

STUDENTS AND TECHNICIANS: Alexandra Rosa, Madalena Martins, Benedita Fonseca, Sara Violante.

EXTERNAL COLLABORATORS: Joaquim Ferreira, Leonor Correia Guedes, etc (Hospital Santa Maria, Lisboa, USA); Ana Varela Coelho (ITQB, Oeiras, Portugal); Deborah Penque (INSA, Lisboa, Portugal); Jeffery M. Vance (Miami Institute for Human Genomics, University of Miami, USA).

Since there are currently no biomarkers for the antemortem diagnosis of degenerative parkinsonian disorders such as Parkinson’s disease (PD), dementia with Lewy bodies

(DLB), progressive supranuclear palsy (PSP), multiple system atrophy (MSA), and corticobasal degeneration (CBD), diagnosis currently relies upon the presence and progression of clinical features and confirmation depends on neuropathology. Clinicopathologic studies have shown significant false-positive and false-negative rates for diagnosing these disorders, and misdiagnosis is especially common during the early stages of these diseases. Patients with parkinson-plus syndromes typically have a worse prognosis than those with PD, and parkinson-plus syndromes respond poorly to the standard anti-parkinson treatments. A good biomarker should be precise and reliable, distinguishable between normal and disease, and differential between different diseases. It is believed that biomarkers have great potential in predicting chances for diseases, aiding in early diagnosis and treatment, and setting standards for the development of new remedies to treat diseases. To identify biomarkers for PD that fulfill the clinical criteria of sensitivity and specificity, we are conducting proteomic studies and microRNA profiling.

Behçet's disease genetics.

MEMBERS: Sofia Oliveira.

STUDENTS AND TECHNICIANS: Joana Xavier, Tiago Krug, Benedita Fonseca, Sara Violante.

EXTERNAL COLLABORATORS: José Vaz Patto, Filipe Barcelos (Inst. Port. De Reumatologia, Lisboa, Portugal); J. Crespo, G. Jesus, S. Cavadas, M. Coutinho, C. Neves, A. Oliveira, S. Rodrigues, M. de Sousa (Hospital Infante D. Pedro, Aveiro, Portugal); M. Salgado (Hospital Pediátrico de Coimbra); MJ. Serra (Hospital dos Capuchos); Graham Wallace (University of Birmingham, UK); F. Shahram, A. Nadji, C. Chams-Davatchi, H. Shams, N. Shafiei, F. Davatchi (Teheran University for Medical Sciences, Teheran, Iran).

Behçet's disease (BD) is a chronic inflammatory condition involving several organs, such as skin, mucous membrane (oral and genital aphtae), eye, joint, lung and gastrointestinal and central nervous system. Ankylosing spondylitis (AS) is a prototype of seronegative spondyloarthritis, and a chronic systemic inflammatory disorder of the axial skeleton, mainly affecting the sacroiliac joint and spine but also frequently involving the eyes, heart, lungs and gastrointestinal system. They are complex diseases, with strong genetic factors but also environmental factors implicated in their etiology. The only genes that have been strongly associated worldwide with these diseases belong to the major histocompatibility complex: HLA-B*51, which contributes to 19% of the risk to BD, and HLA-B*27 which has an attributable risk of 20-40% for AS. The frequent association between BD and AS, their familial aggregation in a Portuguese family and the fact that both are chronic inflammatory disorders derived from an unusual immunologic response, led to the assumption that BD and AS may have a common genetic factor. We conducted a whole genome linkage in a Portuguese family with 7 individuals affected with BD and AS and identified a linkage peak on chromosome 8. We are currently performing a mutation search in the genes under this peak.

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

MEMBERS: Carlos Penha-Gonçalves.

STUDENTS AND TECHNICIANS: Ligia Gonçalves.

EXTERNAL COLLABORATORS: Maria Mota (IMM, Lisbon, Portugal).

This project aims to identify genetic factors that confer resistance to *Plasmodium* hepatic infection in mouse models. Unraveling the identity of genetic host factors controlling resistance to the hepatic stage of malaria is an important element to the understanding of malaria pathogenesis and may lead to the identification of naturally selected mechanisms of disease resistance. By choosing the hepatic stage of the disease this project elects the liver as the target organ of investigation. This will offer clear advantages in the identification and evaluation of candidate resistance genes and is enabling the scrutiny of the individual liver cell types as mediators of the resistance mechanisms.

The workplan entails the genetic mapping of liver resistance to malaria infection, proceeds through the fine genetic mapping by construction of congenic and subcongenic mouse strains, towards the identification of the positional candidate genes of malaria resistance. At this point the project has identified the *Belr1* locus in mouse chromosome 17 and a search for positional candidate genes is underway.

In addition, we will aim to isolate the cellular components of liver resistance and to test whether candidate genes are associated with the control of molecular pathways involved in liver response to infection. The project starts by the genetic analysis of a phenotype of liver resistance to infection by *Plasmodium berghei*, a parasite known to be highly pathogenic in the mouse.

Generation of mouse models for pregnancy-associated malaria: pathology and immunological characterization.

MEMBERS: Carlos Penha-Gonçalves, Cláudio Marinho.

STUDENTS AND TECHNICIANS: Rita Neres.

Every year at least 50 millions pregnant women are exposed to malaria infection leading to life threatening conditions for the mother and the developing fetus. Pregnancy-associated malaria (PAM) courses with parasite sequestration in the maternal placental with consequent maternal anemia, decreased fetal viability and infant low birth weight due to both prematurity and intra-uterine growth retardation. Although, PAM has recently attracted many research efforts, the specific pathologic bases for these outcomes are poorly understood and many difficulties are posed to study PAM in humans. This project aims to establish and analyze mouse models that show pathologic features of human PAM. This was initiated by characterizing pregnancy outcome and placenta pathology in mouse PAM and will proceed to investigate malaria recrudescence and PAM preimmunity. These pathology models will be used as tool to study gestational malaria and will offer opportunities to investigate the transplacental transmission of disease and vertical transmission of disease resistance.

Innate responses and B cell reactivity in the non-obese diabetic mouse.

MEMBERS: Carlos Penha-Gonçalves.

STUDENTS AND TECHNICIANS: Joana Corte-Real.

The Non-Obese Diabetic (NOD) mouse spontaneously develops a form of autoimmune diabetes that closely resembles the human Type 1 diabetes (T1D). The disease is caused by an autoimmune reaction that targets the β -cells in the pancreatic islets of Langerhans and leads to their progressive destruction and to overt diabetes. The autoimmune process is preceded by islet infiltration of mononuclear cells; an inflammatory process called insulinitis. While the T cell compartment of the NOD mouse has been intensively studied, it is uncertain whether the NOD specific B cell reactivities play a role in the development of diabetes. This project proposes a genetic approach to study the involvement of the B cell repertoire in the pathogenesis of the autoimmune process evolving in the NOD mouse. We will focus on the B cell compartments generating natural antibody repertoires and search for correlated responses to TLR ligands in the NOD mouse. Genetic analysis of such phenotypes will provide an etiological link to the disease. In addition, we will evaluate if absence of functional TLR response has an impact on diabetes incidence, insulinitis severity and B cell repertoire development. These results will shed light on innate mechanisms operating at B cell level that condition the autoimmune process developing in the NOD mouse and will contribute to the dissection of the polygenic component of T1AD in the NOD mouse

Type 1 Diabetes: associated immunopathology and genetic susceptibility.

MEMBERS: Carlos Penha-Gonçalves.

STUDENTS AND TECHNICIANS: Inês Rolim.

EXTERNAL COLLABORATORS: Mamuela Catarino (Fac. Farmácia, Universidade de Lisboa, Lisbon, Portugal), Rosário Sancho, Dário Ligeiro (Centro Histocompatibilidade do Sul, Portugal), José Manuel Boavida, Rui Duarte (Associação Protectora dos Diabéticos de Portugal), Guilhermina Fonseca, Rosa Pina (Hospital D. Estefânia, Lisbon, Portugal), Maria Manuela Madeira, Lurdes Sampaio (Hospital de Santa Maria, Lisbon, Portugal).

Immune mediated type 1 diabetes (T1AD) is a common disease of multifactorial nature. Although genetic susceptibility to T1AD has been attributed to a fairly high number of chromosomal regions, their pathogenic role is not understood and to date just three loci have been confirmed and considered as true diabetogenic factors (MHC, CTLA4 and INS). This proposal represents a multidisciplinary effort to study the genetic basis of the immunopathology associated to T1AD in the Portuguese population. Affected child families will be ascertained among recently diagnosed children/adolescents attending the major T1AD clinical services in the Lisbon region. This family collection will be investigated for compliance with the T1AD diagnosis criteria, for inheritance of impairments related to immunological functions and for frequency of autoimmune disorders concurrent with T1AD. In parallel, allele frequencies at T1AD susceptibility loci will be determined namely for HLA and CTLA4 genes.

This project aims to collect data to test the hypothesis that organ-specific autoimmunity entails susceptibility genetic factors conferring general predisposition to autoimmunity. To this end, genetic association tests will be performed to search for genotypic combinations of T1AD susceptibility HLA and CTLA4 alleles, that control immuno-regulatory functions, patterns of auto-antibody occurrence and expression of immuno-mediator molecules. Furthermore, we will investigate the involvement of such genetic factors in the occurrence of other organ-specific autoimmune thyroiditis, celiac disease and multi-organ autoimmunity associated to T1AD. This work will contribute to elucidate the pathogenic mechanisms operated by the combination of genetic factors that confer susceptibility to T1AD and to evaluate their role in clinical, silent or undiscovered concurrent autoimmune disorders of T1AD patients in the Portuguese population.

Genetics of human malaria.

MEMBERS: Carlos Penha-Gonçalves, Maria de Jesus Trovoadá.

STUDENTS AND TECHNICIANS: Rosário Sambo.

EXTERNAL COLLABORATORS: Centro Nacional de Endemias, S. Tomé e Príncipe.

The goal of this project to carry out fine genetic mapping of regions of the human genome to find genetic determinants affecting the disease and to determine the causal basis of associations between candidate genes polymorphisms and susceptibility to malaria. The projects unfolds in two levels:

1. A whole population-based study in the S. Tome Island. The epidemiological and clinical monitoring of a genetic and geographically isolated human population in the island of Principe provide an ideal scenario to identify host genetic factors that confer resistance to malaria, control the clinical course of the disease or affect the outcome of therapeutic interventions.
2. A case-control association study in Angola. This study will focus on Cerebral Malaria and is aimed to identify genetic factors conferring CM susceptibility as compared to other clinical forms of disease and to healthy controls.

Genetic epidemiology of autism.

MEMBERS: Astrid Vicente, Marta Barreto, Inês Cabrito, Tiago Magalhães.

STUDENTS AND TECHNICIANS: Catarina Correia, Ana Filipa Sequeira.

EXTERNAL COLLABORATORS: Guiomar Oliveira, Hospital Pediátrico de Coimbra, Portugal; Michael Gill, Louise Gallagher, Sean Ennis, Trinity College, University of Dublin, Republic of Ireland; Mette Gilling, University of Copenhagen, Denmark; Autism Genome Project International Consortium for the Genetics of Autism.

Autism is a chronic disorder that in most cases represents a permanent and complete disability, with patients requiring social, medical and economic support all their lives. The familial aggregation of autism is striking, with multiple as yet unidentified genes likely to contribute to disease onset. Understanding the etiology of this disorder will be fundamental for the development of effective therapies and its prevention.

Given the probable genetic heterogeneity of autism, we favour an approach directed at the characterization of disease-associated traits, which is expected to facilitate the identification of susceptibility loci and the definition of impaired biological pathways. It also provides an opportunity to identify biomarkers that may prove useful for early detection of autism and therefore impact on both drug development and early intervention. In this context, we previously found that BDNF plasma levels in autistic children were significantly increased compared with control children, showed an heritability of 30% and were positively correlated with serotonin levels. We therefore sought to identify genetic factors that might regulate BDNF distribution both in the periphery and in the brain. Several candidate genes were assessed, including *BDNF* and its receptor *NTRK2* genes; the *HTR1A* gene region, since the 5-HT_{1A} serotonin receptor regulates serotonin levels; the *GAD1* gene, as it encodes a key regulator of glutamate which, neurotoxic when in excess, induces *BDNF* expression as a neuroprotective mechanism, and *SLC1A2* which encodes a glutamate transporter; and *CADPS2*, located in the 7q31 candidate region for autism, which is known to modulate BDNF release. Tag SNPs covering these candidate genes were tested for association with autism and with the BDNF distribution in patients. We found a borderline association of markers in the *HTR1A* genomic region with BDNF levels, all showing a strong association with autism in the subset of patients with high BDNF and one strongly associated with autism in the overall population. Unexpectedly, these markers were located within *RNF180*, a recently identified gene mapping in the vicinity of *HTR1A*, and encoding a protein implicated in the ubiquitination signal pathway. Several SNPs in the *CADPS2* gene showed associated with autism but provided no evidence for an involvement of in BDNF level distribution. Four markers in *TrkB* were also mildly associated with autism. The *BDNF*, *GAD1* and *SLC1A2* genes were not associated with autism or BDNF levels. Replication of the above findings in an independent population sample is ongoing.

Following on our previous observation of a strong interaction between the serotonin transporter gene and a $\beta 3$ integrin gene associated with hyperserotonemia, we are developing an *in vivo* system in RN46A neuronal cell lines to evaluate the functional role of the interaction of specific variants in these genes in the serotonin system, assessing alterations in serotonin production, release, reuptake and degradation.

The collaboration with *Autism Genome Project* (AGP) has further developed. Fine mapping of linkage regions identified by the genome wide scan on Phase I is ongoing, as well as the collaborative replication of WGA results by other groups, namely the BROAD institute study. Meanwhile genotyping of autism trio families for Whole genome association using a 1million SNP array and the Illumina platform has been initiated in several of the collaboration sites, including genotyping of the Portuguese population sample.

Pharmacogenetics of risperidone therapy in autism spectrum disorders.

MEMBERS: Astrid Vicente.

STUDENTS AND TECHNICIANS: Catarina Correia, Ana Filipa Sequeira.

EXTERNAL COLLABORATORS: Guiomar Oliveira (Hospital Pediátrico de Coimbra).

The atypical antipsychotic risperidone is used to control disruptive behaviors associated with autism, with some improvement of typical symptoms. The main objective of this study is the identification of genetic factors underlying the observed variability in individual response of autistic patients to risperidone. The final goal of recruiting 50 patients diagnosed with autism, assessed for developmental, cognitive and adaptive profile, and with clinical indication for risperidone therapy is close to being reached, with 41 patients currently enrolled in the study. Risperidone efficacy and tolerability are monitored at baseline and monthly after starting the therapy, using the Autism Treatment Evaluation Checklist (ATEC) and assessing prolactin (PRL) levels, extrapyramidal movements and weight gain. Behavior alterations and adaptive behavior, as well as language skills are evaluated after completing six months of therapy. In patients undergoing treatment, the ATEC scores improved significantly after one month, particularly in the behavioural subscale. Weight and prolactin levels also increased significantly from baseline. Risperidone is mainly metabolized by cytochrome P4502D6, while drug absorption and bioavailability is mediated by glycoprotein P, encoded by *MDR1* gene. We find that the presence of *CYP2D6* UM and PM alleles have an impact on the efficacy and tolerability of the drug which is, however, modulated by specific alleles at *DRD2* and *MDR1*. *MDR1* variants were associated with improvements in the cognitive and behavioural domains of the ATEC, as well as with the occurrence of hyperprolactinemia. *BDNF* variants also have a mildly significant contribution to the increase of BMI, which is more pronounced early in treatment. Further analysis is progressing.

Genetic factors involved in susceptibility to stroke and in outcome after 3 and 12 months.

MEMBERS: Astrid Vicente, Sofia Oliveira.

STUDENTS AND TECHNICIANS: Helena Manso, João Sobral, Tiago Krug.

EXTERNAL COLLABORATORS: José Mourão Cabral Ferro (IMM/FML, Lisbon, Portugal)
Isabel Albergaria, Gisela Gaspar (INSA, Lisbon, Portugal).

Stroke is a major cause for morbidity and mortality in developed countries. Given the increased life expectancy of populations, finding ways of preventing the disease and adequate treatment is a priority task. For this purpose, it is warranted that risk factors, genetic and environmental, are properly characterized, both for disease susceptibility and for disease outcome. The objective of the present project is the identification and characterization of genetic factors predisposing to stroke and influencing disease outcome and patient's prognosis. For this purpose, association analysis of candidate genes with stroke susceptibility and recovery parameters is ongoing and a high resolution whole-genome association study in pooled DNA samples will be carried out.

Genetic variation in 24 candidate genes for risk and recovery has been assessed in 685 stroke patients, for which an extensive clinical characterization and outcome assessment 3 and 12 months after the stroke episode are documented, and 530 ethnically and gender matched controls. Association of these genes with disease recovery and outcome measures at 3 and 12 months was evaluated, namely the mortality rate, the recurrence rate of stroke or other vascular events, the Rankin disability scores and with composite

measures of these parameters defined by multivariate analysis. Genetic variability was assessed covering the whole gene or functional regions with *tag* SNPs. In this population sample we find a strong association of a gene on chromosome 3 with stroke risk. Dense coverage of SNPs in the *PDEA4* gene, which maps to chromosome 5q in the single strongly significant linkage region for stroke, provided only weak evidence for association with disease susceptibility in our sample, and thus we do not replicate the previously reported contribution to stroke risk. Specific variants in this gene were, however, significantly associated with stroke outcome. The *MMP2* gene, which encodes a metalloproteinase previously found to have a role in stroke recovery in neuronal tissue in vitro, was also found to be significantly associated with patient recovery. Further analysis of these candidate genes is ongoing, and funds have been secured for further research through an FCT grant which will include a Whole Genome Association scan in pooled samples.

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.

STUDENTS AND TECHNICIANS: Sílvia Almeida, Sílvia Correia, Rute Nascimento, Vivian Oliveira, Ana Luísa Reis, Lara Santos, Pedro Ferreira e Hugo Soares.

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 eight novel viral genes inhibiting interferon responses. Of these, 5 inhibited both the induction of type I IFN and the response of cells to type I and type II IFN's, one uniquely inhibited the induction of type I IFN, one inhibited the cellular response to type I IFN and another inhibited the response to type II IFN. One gene from the murine herpesvirus MHV-68 induced cell cycle arrest/apoptosis. Finally, one gene inhibiting some, but not all, toll receptor-like signaling pathways has also been identified in ASFV. The mechanisms and 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 human α , γ herpesviruses homologues of the murine MHV-68 herpesvirus 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. In particular, we are studying the human cytomegalovirus (HCMV) homologue (UL76). The objectives are to understand how UL76 favors the virus and to identify the downstream target(s) of UL76 by determining the impact of UL76 on the host cell cycle and transcription profiles. This project may provide a rational basis for the construction and patenting of attenuated herpes virus vaccines and novel approaches for the manipulation of cell division in health and disease.

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 and which impacts on germinal center B cell apoptosis in the transgenic mice.

Control of African swine fever (ASF) through improved diagnosis.

MEMBER: R.M.E. Parkhouse.

STUDENT: Ana Luísa Reis.

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

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

devastating. The rationale of this project is to improve diagnostic methods to enable rapid implementation of prophylactic measures.

Our previous work has identified the 12 principle serological determinants of ASFV and 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. Of these, two have potential for the diagnosis of recent infections through detection of IgM antibodies.

Secretion of Interferon gamma (IFN- γ) by human macrophages demonstrated at the single cell level after co-stimulation with Interleukine (IL) -12 plus IL-18.

MEMBER: R.M.E. Parkhouse.

COLLABORATOR: Prof Margarita Bofill (Irsicaixa.Ctra Canyet snBadalona 08916, Spain).

The interferon gamma (IFN- γ) component of the immune response plays an important and essential role in infectious and non-infectious diseases. Induction of IFN- γ secretion by human T and NK cells through synergistic co-stimulation with interleukin 12 (IL-12) and IL-18 in the adaptive immune responses against pathogens is well known, whereas a similar activity by macrophages is still controversial, largely due to criticisms based on contamination of macrophages with NK or T cells in the relevant experiments. The possible contribution of macrophages to the interferon response is, however, an important factor relevant to the pathogenesis of many diseases. To resolve this issue, we have determined the production of IFN- γ at a single cell level by immunohistochemistry and enzyme-linked immunosorbent spot (ELISPOT) analysis and unequivocally demonstrated that human monocytes differentiated to macrophages *in vitro* through the combined stimulation of IL-12 and IL-18 or with macrophage colony stimulating factor (M-CSF) were able to produce IFN- γ when further stimulated with a combination of IL-12 and IL-18. In addition, naturally activated alveolar macrophages immediately secreted IFN- γ upon treatment with IL-12 and IL-18. Therefore, human macrophages in addition to lymphoid cells contribute to the IFN- γ response, providing another link between the innate and acquired immune response.

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

MEMBER: R.M.E. Parkhouse.

COLLABORATORS: Dr. T. Garate (Instituto de Salud Carlos III, Centro Nacional de Microbiología, Madrid, Spain), Dr. L. Harrison (University of Edinburgh, Department of Tropical Animal Health, Centre for Tropical Veterinary Medicine, Scotland), Dr. E. Sciutto (Universidad Nacional Autónoma de México, Instituto de Investigaciones Biomédicas, México), Prof. 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 *Echinococcus*); 2) Synthetic peptide based assays to detect antibodies to *Taenia* parasites 3) An ELISA assay which detects secreted metacestode antigens and thus viable metacestode parasites in pigs, cattle and man and 4) An ELISA assay, detecting adult parasite “coproantigen” in human faeces in order to detect human carriers of the adult worm and thus, by treatment, interrupt the cycle of transmission to man and pig (with our Venezuelan collaborator). These diagnosis tools are all now being applied in endemic areas, principally Mexico, Peru, Bolivia and Venezuela, and, on occasions, clinical material in Spain. In Venezuela, the application of the coproantigen test in rural areas has led to the identification and treatment of adult *Taenia* carriers, a strategy that should reduce the endemicity of cysticercosis. 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 has been shown to be a functional adhesion molecule for both *T. solium* *T. saginata*, possibly facilitating tissue invasion by the parasite in the intermediate host, and so constitutes a rational basis for a vaccine.

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

MEMBERS: J. Pedro Simas, Lénia Rodrigues.

Analysis of genomes from gammaherpesviruses reveals the presence of large blocks of co-linearly arranged conserved genes interspersed with virus specific ORFs and cellular homologues. Hence, there are two classes of putative viral host control proteins, namely those encoded by genes with and without sequence similarity to cellular genes. The existence of viral homologues to cellular genes suggests that during co-evolution viruses have ‘hijacked’ host genes that were subsequently modified for the benefit of the virus. Virus specific ORFs may represent novel structures with functional activities homologous to cellular proteins or could simply be an example of proteins for which the host homologues have not yet been identified.

Our objectives are focused in trying to reveal the molecular function that these ORFs and cellular homologues have in a context of infection, that result in evasion of the host immune response and life-long latency. To this end we use a gammaherpesvirus

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

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

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

Herpesvirus modulation of B-lymphocyte function.

MEMBERS: J. Pedro Simas, Sofia Marques, Marta Miranda.

STUDENTS AND TECHNICIANS: Marta Alenquer, Filipa Lopes.

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: to determine the molecular mechanism of M2 induced phosphorylation of Vav; 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; to assess the ability of MHV-68 to establish latent infection in Vav deficient mice; 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.

Herpesvirus-encoded microRNAs as novel regulators of host gene expression: molecular function during latency in B-lymphocytes.

MEMBERS: J. Pedro Simas, Teresa Carlos.

MicroRNAs (miRNAs) are small (21 to 23 nucleotides long) non-coding RNA molecules that regulate the stability or the translational efficiency of complementary mRNAs. They were first recognized in the nematode *Caenorhabditis elegans* and were later found to be common in other species throughout the animal and plant kingdoms. Initially considered a biological sideshow, it is now recognized that miRNAs are a considerable part of the transcriptional output of living organisms, offering an additional layer of post transcriptional control, which must be understood if we are to decipher the complexities of expression and

the regulatory potential of the genome. The discovery of miRNAs in viruses, namely herpesviruses, highlights the importance of these novel molecules as regulators of gene expression. Moreover, it indicates that viruses have evolved to exploit RNA interference for the regulation of both host and viral gene expression with the additional advantage that unlike viral proteins, miRNAs are not antigenic. For the past few years a wealth of miRNAs have been identified and confirmed in many species including viruses. Now, the primary focus of many research groups lies with the challenge to identify host mRNA targets to elucidated miRNA function. The recent discovery that murine γ -herpesvirus 68, encodes 9 miRNAs, offers a unique opportunity to address the function that these novel molecules play in vivo. The reasons for this lie within the fact murine γ -herpesvirus 68 is a natural pathogen of laboratory mice, which has been widely used as a model system for the study of γ -herpesvirus pathogenesis, providing the opportunity to address the function of the virally encoded miRNAs by the utilization of genetically modified viruses. Our research group has gained considerable expertise in the study of this model system. Thus, here we propose to address what is the function that these novel miRNAs play towards the establishment of latent infections in B lymphocytes leading to life-long persistent infections and associated pathologies. To this end, our strategy involves the determination of miRNA expression during infection, the identification of host mRNA targets for miRNA regulation, and the generation of recombinant viruses with disrupted miRNA expression and their phenotypic characterization upon infection of laboratory mice.

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, rheumatism, multiple sclerosis), many of which are autoimmune and continue to represent a serious therapeutic challenge. To be effective and, yet, not provoke disease, inflammatory reactions must thus be regulated. The molecular basis of inflammation and respective controls are, therefore, of utmost importance in biomedicine. Several groups at the IGC are concerned, directly or indirectly, with these questions, analyzing cellular and molecular mechanisms regulating inflammation. The specificity of our research relates to the complementarity of approaches (disease genetics, cell and molecular biology, immunology, theoretical biology), and to common concerns with Regulatory T cells, tissue-protective genes and mechanisms, which the IGC groups have helped to establish. Genetic analysis of inflammatory processes can provide relevant information on the molecular mechanisms involved. This approach has been undertaken in man and mouse, studying either patients and families, or various mouse strains and their crosses, in order to identify genes that are associated with susceptibility to inflammatory disease.

Dynamics and function of regulatory T cells during inflammatory pathologies.

MEMBERS: Marie Louise Bergman, Santiago Zelenay, Jocelyne Demengeot.

STUDENTS AND TECHNICIANS: Francisca Fontes, João Duarte, Catarina Martins.

Our original finding that regulatory T cells (Treg) prevent deleterious inflammatory responses and limit also protective immune responses has prompted a major group effort in the past years to identify Tregs activating signals. Using various TCR transgenic models, various inflammatory triggers and housing conditions (including Germ-free) and various mouse mutant strains we further explored the contribution of spontaneous and induced inflammatory signals in the generation, expansion and/or activation of Treg. This line of investigation has evidenced a robust feed back mechanism whereby maintenance, expansion and *de novo* generation of Tregs in adult is continuously adapted to the extent of inflammatory immune responses, whether directed at self or non-self components. The development of this project also drives us to further investigate the selective event involved in Treg development and pursue the analysis of the parameters conditioning Treg phenotype stability.

Effector molecules in adaptive immune regulation.

MEMBERS: Jocelyne Demengeot and Miguel P. Soares.

STUDENTS AND TECHNICIANS: Santiago Zelenay, Angelo Chora.

CD4 regulatory T cells (Treg) ensure peripheral tolerance to self-antigens and limit the deleterious effects associated with inflammatory and immune responses by mechanisms that remain to be fully understood. The enzyme heme oxygenase-1 (HO-1), through its known anti-inflammatory activity, is a candidate for a functional role in Treg activity. We compared wild type and HO-1 deficient (hmx-1^{-/-}) mice in order to assess the role of HO-1 in mouse Treg development and function under physiologic conditions. The frequency of CD25⁺ and Foxp3⁺ Treg was similar in hmx-1^{-/-} and hmx-1^{+/+} mice. More importantly, CD4⁺CD25⁺ Treg purified from either hmx-1^{-/-} or hmx-1^{+/+} mice were equally efficient in controlling the proliferation *in vitro* and the expansion *in vivo* of CD4⁺CD25⁻ T cells, whether or not these responder cells expressed HO-1. In addition, induction of expression of HO-1 *in vivo* did not affect Treg suppressor function. As shown before, expression of HO-1 was higher in Treg than in naïve T cells, however, naturally activated Foxp3⁺ T cells displayed equal amount of HO-1 mRNA as Treg. We concluded that under physiological conditions in mice, Treg development, maintenance and function are independent of HO-1 activity.

The role of Notch signal in tuning the differentiation of B lymphocytes to antibody-secreting cells.

MEMBERS: Jocelyne Demengeot, Leonor Parreira, Leonor Sarmento, Manuel Rebelo, Elia Neves.

STUDENTS AND TECHNICIANS: Margarida A. Santos, Ana Agua Doce

EXTERNAL COLLABORATORS: Freddy Radtke (SIECR, Switzerland); Warren Pears (U. Penn, USA).

Notch signaling regulates B and T lymphocyte development and T cell effectors class decision. We tested whether Notch activity affects mature B cell activation and their differentiation to antibody secreting cells (ASC). We showed increased frequency of ASC in cultures of splenic B cells activated with lipopolysaccharide (LPS) or anti-CD40 when provided exogenous Notch ligand Delta-like-1 (Dll1). Our results indicated that Notch–Dll1 interaction releases a default pathway that otherwise inhibits Immunoglobulin (Ig) secretion upon B cell activation. Thus Dll1 enhanced spontaneous Ig secretion by naturally activated Marginal Zone B and B1 cells and reversed the inhibition of ASC differentiation mediated by BCR crosslinking during LPS stimulation. Moreover, suppression of Notch signaling in B cells by either expression of a dominant negative mutant form of Mastermind-like 1 or null mutation of Notch-1 not only prevented Dll1 mediated enhancement of ASC differentiation, but also reduced dramatically LPS induced Ig secretion. Finally, we show that Dll1 and Jagged-1 are differentially expressed in discrete areas of the spleen and that the effect of Notch engagement on Ig secretion is ligand specific. These results indicated that Notch ligands participate in the definition of the mature B cell microenvironment that influences their terminal differentiation.

Impact of the RAG genes activity on lymphocyte homeostasis and genomic instability.

MEMBERS: Jocelyne Demengeot, Leonor Sarmiento, Carlos Penha Gonçalves, António Jacinto.

STUDENTS AND TECHNICIANS: Paulo Bettencourt, Catarina Martins, Paulo Almeida, Ana Novóia.

Recombination-Activating-Genes (RAG) 1/2 are responsible for the somatic DNA rearrangements that generate antigen receptor diversity in lymphocytes. Reduced Rag activity in humans is the cause of the Omen syndrome, a severe immune deficiency that results in multiorgan autoimmunity. We established Rag Tg animals that constitutively express low level of either Rag1 or Rag2. Genetic complementation of the corresponding Rag null mutation allowed the development of a novel model of lymphopenia induced systemic auto-immunity resembling human Omen syndrome. On the other end, RAG activity has been proposed to participate to genomic instability and tumor development. To formally test qualitatively and quantitatively the contribution of Rag activity to lymphoid and non lymphoid cell transformation, we generated transgenic mice with ubiquitous, inducible RAG activity. Moreover, we developed a novel fluorescent reporter of RAG activity that will serve to identify non-lymphoid cells that underwent RAG mediated recombination, either naturally or upon forced expression of the Rag genes. We are also investigating the impact of Rag-1 and 2 on the vertebrate genome evolution by introducing these genes in invertebrate organisms and by a bio-informatic approach.

Effects of therapeutic Ivlg on adaptive immune regulation in SLE patients.

MEMBERS: Constantin Fesel, Francisca Fontes, Jocelyne Demengeot.

STUDENTS AND TECHNICIANS: Ana Monica Gabriel.

EXTERNAL COLLABORATORS: C. Vasconcelos (Associação dos Doentes com Lupus, Lisbon, Portugal), C. M. Ferreira (Hospital Santa Maria, Lisbon, Portugal), J. Martins, (Hospital dos Marmeleiros, Funchal, Madeira, Portugal), J. Matos-Costa (Hospital Distrital de Santarem, Santarem, Portugal), B. Martins (Instituto de Ciências Biomédicas Abel Salazar, Porto, Portugal).

SLE patients display reduced frequency of activated regulatory T cells. The intravenous administration of high doses of immunoglobulins pooled from the plasma of healthy donors (IVIg therapy) has beneficial effects in patients with a variety of autoimmune disorders including SLE. The question whether Ivlg may be beneficial for SLE as a consequence of restoration of the Tregs pool or enhancement of their function has never been directly addressed. However, theoretical grounds for this hypothesis exist: i) animal deficient in B cells have reduced number of Tregs, ii) the inherent diversity of reactivities contained in Ivlg is likely to encompass that of receptors expressed by Treg, iii) the effects of Ivlg administration resemble in many aspect those monitored after adoptive transfer of Tregs (modification of endogenous Ig repertoire, general physiological equilibrium, inhibition of innate cell activity). We are conducting a longitudinal study on SLE patients treated or not with Ivlg. Blood samples have been collected before, during and after a 6 month therapy. Parameters followed include Regulatory T cells frequency, concentration and phenotype.

Systemic lupus erythematosus.

MEMBERS: Constantin Fesel and Jocelyne Demengeot.

Apart from publishing results from previous work with S.Pied (on malaria), three projects were followed in 2007:

the study of T-cell regulation and its relation to autoantibody repertoires in human Systemic Lupus Erythematosus (SLE) and mouse models, applying a parallel strategy of human and mouse studies;

differential aptamer selection against specific antibodies, with the aim of distinguishing patterns of SLE-associated autoantibodies;

study of the effect of ivlg therapy on T-cell regulation in human SLE (led by J. Demengeot).

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

MEMBERS: Luís Graça.

STUDENTS AND TECHNICIANS: Joana Duarte, Ana Água-Doce.

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. 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 have confirmed anti-CD4 mAbs are efficient in preventing the onset of disease, as well as in preventing disease progression in overtly arthritic mice.

Co-receptor and co-stimulation blockade for the induction of regulatory T cells in allergic airways disease.

MEMBERS: Luís Graça, Vanessa Oliveira.

STUDENTS AND TECHNICIANS: Ana Água-Doce, Ivonne Wolenberg, Marta Caridade.

EXTERNAL COLLABORATORS: Patrick G. Holt and Phillip Stumbles (Telethon Institute for Child Health Research, Perth, Australia); Herman Waldmann (University of Oxford, UK); Shohei Hori (RIKEN, Yokohama, Japan).

The control of deleterious immune responses causing diseases, such as allergy, autoimmunity and transplant rejection, has been a major objective of immunologists. Non-depleting CD4 monoclonal antibodies (mAbs) have been successfully used to induce immunological tolerance in animal models of transplant rejection and autoimmune disease. Using a well established murine model of allergic airways disease (OVA-alum sensitization of BALB/c mice followed by intranasal OVA challenge), we here show that anti-CD4

treatment at the time of sensitization is effective in preventing airways eosinophilia, goblet cell hyperplasia, production of OVA-specific IgE and IgG1, production of Th2 cytokines in the lung, and importantly abrogation of airway hyperreactivity to inhaled methacholine. We are currently investigating the cellular and molecular mechanisms leading to allergen-specific tolerance.

Immune recovery after autologous stem cell transplantation - modulation by Ig and potential clinical application.

MEMBERS: Cristina João, Elisabete Pires, Maria Gomes da Silva.

STUDENTS AND TECHNICIANS: Ana Queirós e Ana Filipa Barahona.

EXTERNAL COLLABORATORS: Adrien Six (Paris, France), Luis F. Porrata (Mayo Clinic College of Medicine, Rochester, MN, USA) and Svetomir Markovic (Mayo Clinic College of Medicine, Rochester, MN, USA).

In spite of the increased survival and cure rates obtained by conventional chemotherapy for non-Hodgkin's lymphoma (NHL), once relapsed, the probability of cure is less than 20%. Autologous stem cell transplantation (ASCT) has been shown to be superior to salvage chemotherapy in prolonging survival of patients with relapsed, chemosensitive aggressive NHL. However, post-transplant relapse rates still range between 40% to 70%. The observed relapse rate after ASCT is attributed to the failure of the high-dose chemotherapy in fully eradicating residual lymphoma and to the lack of graft versus tumor effect observed in allogeneic stem cell transplantation (allo-SCT). Thus, one possible way to improve the clinical outcomes following ASCT is to attempt to mimic the anti-tumor efficacy of graft versus lymphoma effect of allo-SCT by facilitating early immune reconstitution. Post-ASCT immune-recovery studies have shown that T and B cell functions are not fully recovered during the period in which most lymphomas recurrences are detected. A faster reconstitution of numbers and function of anti-tumor elements of the immune system may allow a more efficient eradication of the residual tumor. We have recently reported that the absolute lymphocyte count (ALC), as a surrogate marker of immune recovery, at day 15 post-ASCT is a powerful prognostic factor for survival in NHL. The superior clinical outcome observed in ASCT with an ALC > 500 cells/ml at day 15 is explained by the fact that the immune system may protect against minimal residual progression post-ASCT, similar to the graft versus tumor effect seen in the allogeneic stem cell transplantation setting. Likewise, our work has revealed that polyclonal immunoglobulin (Ig) may contribute diversity of the T cell repertoire, which in turn has been related to increased T cell-mediated immunity. Thus, we propose to define the role of specific therapeutic strategies using immunoglobulin to enhance immunity after ASCT in a lymphoma mouse model (A20) of autologous transplantation. Experiments will focus on the effect of the infusion of immunoglobulin and immunoglobulin derivatives in optimally promoting immune reconstitution, T cell development and anti-tumor immune cells stimulation post-transplant, directed at achieving maximal anti-lymphoma efficacy. Understanding the function of immunoglobulin in T cell immune reconstitution following ASCT in vivo will potentially create an opportunity to modulate this process in patients after

ASCT (e.g. intravenous immunoglobulin therapy or IVIG derivative products) and to facilitate post-ASCT T cell immune recovery.

Adjuvanticity of microbial-derived particles and synthetic analogs *in vitro*.

MEMBERS: Elisabetta Padovan.

STUDENTS AND TECHNICIANS: Richard Kamgang Kenmoe, Mascia Ghielmetti (University of Bern, Bern, CH); Lurdes Duarte, Inês Ramos (IGC, Oeiras, Portugal).

EXTERNAL COLLABORATORS: Clemens Dahinden (Bern Institute for Immunology, Bern, CH), Marina Freudenberg (Max Planck Institute for Immunobiology, Freiburg, D), Jorge Carneiro (IGC, Oeiras, Portugal).

Engagement of Toll-Like Receptors (TLR) on Antigen Presenting Cells (APC) by molecular patterns expressed by natural pathogens or live/attenuated vaccines is crucial for the development of long-term immunoprotection. On the contrary, antigen formulations that lack the capacity to signal through TLR are poorly immunogenic. These observations have generated a strong demand for the rational design of synthetic analogs of TLR agonists suitable for complementing the activity of subunit vaccines. As new compounds become available, there is need to develop applicable screening methods predicting adjuvanticity and safety of those molecules. We have developed a systematic study that provides the rational for the screening and subsequent development of adjuvants suitable for use in human vaccines based on responses observed on human T cells and APC. Our results will help to refine most current methods for adjuvant selection that are based on *in vivo* studies using a variety of animal models not necessarily representative of human reactivity.

By monitoring proliferation and differentiation of CD4⁺ T cells, as well as APC responses in short-term *in vitro* cultures, in the presence of standard TLR2, TLR4 and TLR3 we have define molecular signatures of adjuvanticity and pyrogenicity, restricted to human DC and monocyte, respectively. Based on our observations we propose to use pre-screening tests assessing the production of TNF- and/or CXCL10 by human DC, and IL-1 release by CD14⁺ monocytes in order to narrow down the number of compounds that need to be assessed for enhanced immune protection and lack of toxicity *in vivo*.

When applied to large-scale chemical libraries this method can facilitate the selection of candidate adjuvants prior *in vivo* testing, thus reducing the need of costly, demanding and potentially irrelevant animal studies.

How toll-like receptors signaling controls T lymphocytes activity.

MEMBERS: Elisabetta Padovan, Salvatore Valitutti, Iris Caramalho.

STUDENTS AND TECHNICIANS: Lurdes Duarte, Inês Ramos.

EXTERNAL COLLABORATORS: Jocelyne Demengeot, Marie Louise Bergman (Instituto Gulbenkian de Ciência).

The induction of CD4⁺ T cell responses results from TCR interaction with MHC-II expressed depends on dendritic cells (DC) and the outcome of this process is influenced by the engagement of Toll-like Receptor (TLR). During activation, a A differentiating naive CD4⁺ T cell can receive an instructive TLR signal indirectly (in trans) or directly (in cis).

Activation in trans is provided by cytokines released by DC that have been stimulated through TLR. Trans-activating signals are known to induce T cell proliferation and differentiation into cytokine-producing T helper cells²¹. Recent reports indicate that Hence, naive T cells that have been activated through the T cell receptor (TCR) complex also express functional TLR molecules. Direct TLR stimulation on differentiating T cells enhances T cell proliferation and cytokines secretion. These observations have generated the general belief, suggesting that TLR act as costimulatory receptors on T cells. However, Very recent studies have described novel functions and regulatory mechanisms induced by PRR. However, sso far no rationale attempt has been made to understand whether these TLR activation in cis events occurs during T cell-DC interaction antigen recognition and how naive T cells integrate trans- and cis-activating signals into their differentiation process remains to be assessed.

We intend toare currently addressing these questions by comparing the direct and indirect activation induced by TLR signaling in mouse co-cultures of bone marrow-derived DC from TLR2-deficient mice and naïve CD4⁺CD25⁻CD45RB^{hi} T cells purified from TLR4-deficient animals in the presence of specific TCR and TLR antigen and cognate TLR agonists.

The role of the CD27 co-receptor in and Foxp3⁺ regulatory T cell development and function.

MEMBERS: Bruno Silva-Santos, Julie Ribot.

STUDENTS AND TECHNICIANS: Ana de Barros.

EXTERNAL COLLABORATORS: Daniel J. Pennington, Queen College Medical School, London, UK, Adrian C. Hayday, King's College London, UK, Jannie Borst, The Netherlands Cancer Institute.

During their development, thymocytes receive signals from multiple surface receptors that either promote their survival and further differentiation, or induce a program of cellular death. CD27 is a Tumor Necrosis Factor Receptor (TNFR) family member, whose interactions with its ligand CD70 have been suggested to control the survival of activated CD4⁺ and CD8⁺ T cells, by providing non-redundant co-stimulatory signals that complement those of CD28 (J. Hendriks et al. 2003, J. Exp. Med. 198: 1369).

Although CD27 is highly expressed in thymocytes following their acquisition of surface (pre-)TCR complexes, its specific role in T cell development has not been examined in detail. We have used CD27-deficient mice to address this question, and demonstrated that CD27 signals are critical for the differentiation of T cells into Th1-like (IFN- and TNF-producing) lymphocytes. Thus, in the absence of CD27, the production of those cytokines is severely impaired. Moreover, CD27-deficient T cells show a reduced proliferative capacity in response to TCR signals, when compared to wild-type thymocytes. We are now investigating the T cell developmental pathway associated with CD27 expression.

Our results also suggest that the signal transduction mechanism downstream of CD27 involves the up-regulation of the anti-apoptotic gene Bcl-2 A1, which is highly expressed in two specific thymocyte subsets: TCR ⁺ and CD4⁺ CD25⁺ T cells. We are now employing RNA-interference to investigate the role of Bcl-2 A1 in the differentiation of these T cell

lineages. Interestingly, the early expression of Bcl-2 A1 in their common CD4⁻ CD8⁻ CD44⁺ CD25⁺ (Double Negative stage 2, DN2) progenitors appears to depend on the developmental influence of CD4⁺ CD8⁺ (Double Positive, DP) thymocytes, which are responsible for a trans-conditioning process we and our collaborators have previously described (B. Silva-Santos et al. 2005, Science 307: 925).

We are currently assessing the impact of CD27 signalling on Foxp3⁺ CD4⁺ CD25⁺ regulatory T cell function both in vitro and in vivo.

Mechanisms of tumour cell recognition by T lymphocytes.

MEMBERS: Bruno Silva-Santos, Anita Q. Gomes.

STUDENTS AND TECHNICIANS: Daniel V. Correia, Francisco d'Orey, Natacha Sousa.

EXTERNAL COLLABORATORS: Adrian C. Hayday (King's College London, UK).

Human T cells are potent killers of a variety of tumour cell lines, and mice lacking T cells suffer from high incidence of experimentally induced tumours. However, the molecular mechanisms mediating tumour cell recognition and specific T lymphocyte activation remain largely unknown. We aim at identifying potential tumour antigens and co-stimulation molecules expressed in ex vivo tumours and in tumour cell lines that activate human T cells for tumour cytotoxicity.

As immune evasion mechanisms that down-regulate tumour antigens may operate *in vivo*, we are initially identifying candidates from human tumour cell lines that constitute *in vitro* cytotoxicity targets for V α 9/V β 2⁺ lymphocytes, the major population of T cells in the human blood. We have therefore screened a panel of 26 lymphoma and leukaemia cell lines, and determined which are 'targets' or 'non-targets' of V α 9/V β 2⁺ cells. Based on the tumour characteristics (phenotype and genotype), we have selected 4 of those cell lines (2 targets and 2 non-targets) for cDNA microarray analysis. The objective is to compare the full transcriptomes of target versus non-target cell lines. We have produced cDNA of the tumours and initiated the microarray procedure for the analysis of differentially expressed genes; the results are due in January 2008. In the next step of the analysis, the differentially expressed genes of interest (according to structural features and known biological data) will be validated by real-time PCR, and then candidates will be selected for a knock-down (RNA interference) functional screening.

As a complementary approach, we have been conducting genome-wide bioinformatics searches, aiming at defining molecules that share structural similarities with MHC class I and class IIb proteins, which are crucial for cytotoxic responses by other lymphocyte lineages (CD8⁺ and NK cells). We reason that MHC class I/IIb-like proteins, expressed by tumour cells, may play important roles in the activation of T cells. We use a homology algorithm to search for proteins (including novel ones) that share homology with classical (class I) and non-classical (class IIb) MHC molecules. The latest versions of the human gene predictions of the Ensembl database are analyzed using the algorithm that identifies sequence and conformational similarities among proteins, developed by our collaborator Dr. Bernard De Bono at the European Bioinformatics Institute (Cambridge, UK).

Using this strategy, we have identified a novel MHC-like protein, conserved in all vertebrates, that also shares structural homology with members of the Immunoglobulin

sub-family B7. Cellular localization (confocal microscopy) studies have shown that the murine protein encoded by this gene is expressed both in the cytosol and in the cell membrane. The expression of this gene in mouse cell lines is highly inducible by physical stress, such as heat-shock at 42°C and ultraviolet radiation. Furthermore, papillomas and carcinomas extracted from mice express the gene at levels much above those of control (non-transformed) cells.

The candidate gene is also over-expressed in human tumour cell lines, particularly in lymphomas and leukemias that constitute killing targets for human V α 9/V β 2+ T cells. For example, the Daudi lymphoma cell line, a preferential target of T cells, expresses the gene 20-fold above the level of normal (healthy donors) peripheral blood lymphocytes. We are currently knocking-down its expression in human lymphoma cell lines to then assess the impact on T cell-mediated tumour cell lysis.

In addition to identifying tumour antigens responsible for human T cell recognition, we are also exploring the stimulatory potential of non-peptidic phosphoantigens (intermediates of the biosynthesis of isoprenoids) for the anti-tumour function of V α 9/V β 2 lymphocytes. These can be expanded both *in vitro* (from 5-10% of all peripheral blood T cells to >95% in two weeks) and *in vivo* by such low molecular weight compounds. We have been studying the molecular mechanisms behind the stimulatory effect of 4-hydroxy-3-methyl-but-2-enyl-pyrophosphate (HMB-PP), the most potent phosphoantigen known to date, with a picomolar bioactivity on V α 9/V β 2 cells (ref). We have compared HMB-PP to *bona fide* T cell receptor complex signalling, as mimicked by anti-CD3 monoclonal antibody (CD3) *in vitro* treatment. We have shown that HMB-PP and TCR/CD3 signalling lead to identical levels of T cell activation, as measured by the secretion of effector cytokines and the terminal killing of lymphoma and leukaemia target cell lines. Furthermore, the two stimuli achieve this by sharing common signal transduction cascades, activating p56Lck, Akt, and the Jak/Stat and the MAP kinase pathways. In accordance, chemical inhibition of these pathways prevents the activating effect of the two stimuli in a strikingly similar way, as evaluated by cytokine production or the expression of cellular activation markers.

In order to dissect the more distal consequences of HMB-PP signalling, we have employed cDNA microarrays to analyse the full transcriptome of V α 9/V β 2 cells after 20 hours of activation with the phosphoantigen, and compared it with CD3 treatment. Whereas both stimuli induce over 4-fold changes in the expression of nearly 2000 genes, the two gene expression profiles are virtually indistinguishable.

In spite of reproducing all the tested TCR/CD3 signalling effects on V α 9/V β 2 cells, HMB-PP clearly does not induce the internalization of the TCR complex, suggesting that it acts by an unknown, non-crosslinking, mechanism. We will therefore continue to investigate the nature of such a process, in order to understand and apply the potentiation of T cell activity by phosphoantigens for cancer immunotherapy.

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

MEMBERS: Miguel Soares, Gabriela Silva and Isabel Pombo Gregoire.

STUDENTS: Mark Pena Seldon, Nadja Pejanovic.

EXTERNAL COLLABORATORS: Josef Anrather (University of Cornell, New York City, USA). Heme oxygenase-1 (HO-1) catalyzes the degradation of free heme into biliverdin, via a reaction that releases iron (Fe) and carbon monoxide (CO). Under this project we found that HO-1 down-regulates the proinflammatory phenotype associated with endothelial cell (EC) activation by reducing intracellular non-protein bound Fe (labile Fe). EC isolated from *Hmox1*^{-/-} mice have higher levels of intracellular labile Fe and reactive oxygen species (ROS), as compared to EC isolated from *Hmox1*^{+/+} mice. Basal and TNF-induced expression of vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecule 1 (ICAM-1) and E-selectin were increased in *Hmox1*^{-/-} versus *Hmox1*^{+/+} EC, an effect reversed by Fe chelation using deferoxamine mesylate (DFO). Fe chelation inhibits TNF-driven transcription of *Vcam-1*, *Icam-1* and *E-selectin*, as assessed using luciferase reporter assays. This inhibitory effect is associated with inhibition of the transcription factor nuclear factor kappa B (NF- κ B), via a mechanism that is not associated with inhibition of I κ B phosphorylation/degradation, NF- κ B (i.e. RelA) nuclear translocation while affecting modestly NF- κ B binding to DNA κ B consensus sequences in the *Vcam-1*, *Icam-1* or *E-selectin* promoters. HO-1 inhibits NF- κ B (i.e. RelA) phosphorylation at Ser276, a phosphoacceptor that is critical to sustain TNF-driven NF- κ B activity in EC. This effect was mimicked by Fe chelation as well as by anti-oxidants (N-Acetylcystein). In conclusion, we demonstrate a novel mechanism via which HO-1 down modulates the proinflammatory phenotype of activated EC, i.e. inhibition of RelA phosphorylation at Ser276. Such an effect should contribute to the ability of HO-1 to downregulate inflammatory reactions and prevent the pathogenesis of inflammatory diseases.

Identification of kinases and phosphatases controlling the transcriptional activity of nuclear factor kappa B (NF- κ B) p65/RelA in endothelial cells.

MEMBERS: Miguel Soares.

STUDENTS: Nadja Pejanovic.

EXTERNAL COLLABORATORS: Josef Anrather (University of Cornell, New York City, USA), Antonio Jacinto (IGC/IMM, Portugal) and Buzz Baum (MRC laboratory for Molecular Cell Biology and Cell Biology Unit, UK).

The transcription factor nuclear factor kappa B (NF- κ B) is central to the regulation of inflammation and immunity. Phosphorylation of the NF- κ B family member p65/RelA controls NF- κ B transcriptional activity in a variety of cell types. Under this project we aim at identifying and characterizing novel kinases/phosphatases involved in phosphorylation of p65/RelA. Given that the NF- κ B system is highly conserved throughout evolution we are using an RNA interference approach in *Drosophila* S2 cells to identify kinases/phosphatases controlling the activity of *Drosophila* p65/RelA homologues, i.e. Dorsal and Dorsal related immunity factor (Dif). We have set-up an assay allowing to monitor Dorsal and Dif activity based on the activation of a Drosomycin-luciferase reporter. We have shown that mutation of specific phospho-acceptors in Dorsal and Dif can modulate their activity, indicating that activity is phosphorylation-dependent. So far, targeting upstream atypical protein kinase C (aPKC) or cAMP-dependent protein kinase (PKA), which have been shown to regulate p65/RelA activity failed to control Dorsal and

Dif activity. However, we have recently targeted a kinase that unexpectedly modulates both Dorsal and Dif transcriptional activity, as revealed by the very significant drop in Drosomycin-luciferase reporter expression. Aside from identifying a new pathway targeting NF- κ B activity, this result serves as a “proof of concept” that the approach we have set-up can be used to identify upstream kinases/phosphatases that modulate NF- κ B activity via RelA (Dorsal/Dif) phosphorylation. We now aim at performing a “loss of function gene-screen targeting all Drosophila kinases and phosphatases, thus enabling the identification of those regulating Dorsal and/or Dif phosphorylation/activity.

Gammaherpesvirus modulation of NF- κ B.

MEMBERS: Miguel Soares, Pedro Simas.

STUDENTS: Josina Filipe, Lidia Fonseca, Bruno Almeida, Mark P.Seldon.

EXTERNAL COLLABORATORS: Josef Anrather (University of Cornell, New York City, USA). Many viruses can target the transcription factor nuclear factor κ B (NF- κ B). For herpesviruses, it is possible that this strategy could contribute to the establishment of latency. We therefore tested this hypothesis focusing on the g-herpesvirus 68 (MHV-68) open reading frame 73 (ORF73) a gene required for the establishment of latency of this murine g-herpesvirus. We found that this was indeed the case. ORF73 binds to the p65/RelA Rel homology domain (RHD), as assessed by co-immunoprecipitation assays. When over-expressed in the human kidney embryonic cell line 293, ORF73 inhibited TNF- α driven NF- κ B transcriptional activity, as assessed using a NF- κ B reporter assay. ORF-73 did not interfere with I κ B α phosphorylation/degradation, NF- κ B nuclear translocation and/or NF- κ B DNA binding. This suggested that ORF73 might target directly DNA bound nuclear Rel proteins, a finding consistent with the nuclear localization of ORF73. When co-expressed with RelA/p65, p50 or c-Rel, ORF-73 suppressed equally well the transcriptional activity of these NF- κ B family members, as assessed using a NF- κ B reporter assay. This inhibitory effect was dependent on RHD, as assessed using chimeras in which the N-terminus domain of p65/RelA was fused to the VP-16 transactivation domain. In conclusion, ORF73 targets the RHD of NF- κ B family members to suppress their transcriptional activity, a finding consistent with its involvement in the establishment of latency in B cells.

Modulation of the pathogenesis of sepsis by HO-1.

MEMBERS: Miguel Soares, Rasmus Larsen.

STUDENTS AND TECHNICIANS: Silvia Cardoso, Sofia Rebelo.

Severe sepsis remains a major cause of death worldwide. Heme oxygenase-1 (*Hmox1*/HO-1) is a stress responsive gene that prevents the deleterious effects of inflammatory reactions. The mechanisms underlying the protective effects of HO-1 remain elusive. Under this project we found that severe sepsis, induced in mice by cecal ligation and puncture (CLP), led to the expression of HO-1 in infiltrating peritoneal leukocytes, kidney and liver. Mortality rate of CLP increased from 20% in wild type (*Hmox1*^{+/+}) mice to 87% in HO-1 deficient (*Hmox1*^{-/-}) mice. Following CLP, *Hmox1*^{-/-} but not *Hmox1*^{+/+} mice developed

end-stage multi-organ failure, revealed by increased levels of circulating aspartate aminotransferase (AST), blood urea nitrogen (BUN) and creatine phosphokinase (CPK). Mortality of *Hmox1*^{-/-} mice was associated with increased peritoneal leukocyte infiltration but not with increased pro-inflammatory cytokine secretion, e.g. tumor necrosis factor alpha (TNF- α) and interleukin 6 (IL-6) were equivalent in *Hmox1*^{-/-} and *Hmox1*^{+/+} mice. Circulating levels of high mobility group box 1 (HMGB1), a central mediator of the pathogenesis of severe sepsis, were 6-9 fold higher in *Hmox1*^{-/-} versus *Hmox1*^{+/+} mice following CLP. HMGB1 outward nuclear translocation, an event that precedes cellular HMGB1 release from necrotic cells, was also more pronounced in the liver and kidney of *Hmox1*^{-/-} versus *Hmox1*^{+/+} mice. HMGB1 release from murine monocyte/macrophages activated *in vitro* by bacterial LPS and IFN- γ , was 3 fold higher when HO-1 was suppressed using siRNA, as compared to control cells. In conclusion, HO-1 expression in response to microbial infection prevents HMGB1 release associated with necrosis and monocyte/macrophage activation, thus suppressing the development of severe sepsis.

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

MEMBERS: Miguel Soares, Sofia Rebelo, Silvia Cardoso.

STUDENTS AND TECHNICIANS: Angelo Chora and Andreia Cunha.

COLLABORATORS: Paulo Contour (Universidade Nova de Lisboa, Lisbon, Portugal), Lawrence Steinman, Peggy P Ho, Lowen Y Lee (Department of Neurology and Neurological Sciences, Beckman Center for Molecular Medicine, Stanford University, CA, USA). Raymond A. Sobel (Department of Pathology, Stanford University School of Medicine, Stanford, CA, USA).

Heme oxygenase-1 (*hmx-1*/HO-1) dampens inflammatory reactions via the catabolism of heme into carbon monoxide (CO), iron and biliverdin. Under this project we found that expression of HO-1 can dictate the pathologic outcome of experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis (MS). Induction of EAE in C57BL/6 *hmx-1*^{-/-} mice led to enhanced central nervous system (CNS) demyelination, paralysis and mortality, as compared to *hmx-1*^{+/+} mice. Induction of HO-1, by cobalt protoporphyrin IX (CoPPIX) administration after EAE onset, reversed paralysis in C57BL/6 and SJL/J mice and disease relapse in SJL/J mice. These effects were not observed using zinc protoporphyrin IX, which does not induce HO-1. CoPPIX protection was abrogated in *hmx-1*^{-/-} C57BL/6 mice, indicating that CoPPIX acts via HO-1 to suppress EAE progression. The protective effect of HO-1 was associated with inhibition of major histocompatibility complex class II expression by antigen presenting cells and inhibition of T_H and CD8 T cell accumulation, proliferation and effector function within the CNS. Exogenous CO mimicked these effects, suggesting that CO contributes to the protective action of HO-1. In conclusion, HO-1 or exposure to its end product CO counters autoimmune neuroinflammation and thus might be used therapeutically to treat MS.

Regulation of autoimmune neuroinflammation by protective genes expressed in the central nervous system.

MEMBERS: Miguel Soares.

STUDENTS: Andreia Cunha, Ângelo Chora, Eliane Cortez.

COLLABORATORS: Ingo Bechmann (Johann Wolfgang Goethe-University, Frankfurt), Paulo Fontoura (Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisbon, Portugal).

Multiple sclerosis (MS) is an autoimmune neuroinflammatory disease that targets the central nervous system (CNS). The aim of this project is to test whether “protective genes” expressed in oligodendrocytes under the control of the nuclear factor erythroid 2-related factor 2 (Nrf2) family of transcription factors can modulate the onset and/or the pathogenesis of murine experimental autoimmune encephalomyelitis (EAE), a model of MS. Our hypothesis is that when Nrf2 activity is suppressed, oligodendrocytes might become susceptible to undergo apoptosis, a critical event in the pathogenesis of EAE. We found that expression of a dominant negative mutant of Nrf2 (Nrf2^{DNM}) in oligodendrocytes renders these cells more susceptible to undergo TNF- α or Fas-ligand-mediated, apoptosis as compared to control cells. This finding suggests that inhibiting Nrf2-driven transcription abrogates the expression of protective genes that suppress oligodendrocytes from undergoing apoptosis. We have cloned and expressed the Nrf2^{DNM} under the control of oligodendrocyte-specific, i.e. myelin oligodendrocyte glycoprotein (MOG) promoter allowing for Nrf2^{DNM} expression specifically in oligodendrocytes of transgenic mice. We expect that these transgenic mice will have exacerbated EAE, suggesting that expression of protective genes in oligodendrocytes may control the pathogenesis of autoimmune neuroinflammation.

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

MEMBERS: Miguel Soares, Isabel Pombo Gregoire.

STUDENTS AND TECHNICIANS: Silvia Cardoso.

EXTERNAL COLLABORATORS: Anne Marie Noordeloos and Eric Duckers (Erasmus Medical Center, Rotterdam, Holland).

Heme oxygenase-1 (encoded by *Hmox1*) is a ubiquitous stress-responsive enzyme that catabolyzes heme into biliverdin, Fe and the gas carbon monoxide (CO). HO-1 and its end-products, i.e. biliverdin and CO, counter vascular remodeling associated with the development of arteriosclerotic lesions in mice. HO-1 also counters the development of lipid-mediated atherosclerosis in mice. Under this project we have demonstrated that biliverdin/bilirubin mediate this later effect. Lipid-mediated atherosclerosis was induced in apolipoprotein E deficient mice (ApoE^{-/-}) fed high (1.25%) cholesterol diet (HCD). CO inhalation (250 parts per million), starting either before or simultaneously to HCD, suppressed by 30-50% the extent of atherosclerotic lesions in the aorta (quantified by red oil O staining and by histology), as compared to air treated controls ($p < 0.05$). A similar effect was observed when exposure to CO was initiated two months after HCD, suggesting that CO may be used therapeutically to arrest the progression of atherosclerosis. The

protective effect of CO was mimicked by the administration of biliverdin (70mg/kg/day). Neither CO nor biliverdin affected plasma cholesterol concentration or distribution. CO inhibited macrophage recruitment into vascular lesions as well as the expression of several pro-inflammatory genes associated with macrophage and endothelial cell activation and involved in the pathogenesis of atherosclerosis, as quantified by real time quantitative RT-PCR. In addition, CO induced the expression of HO-1 in the vessel wall and its protective effect was lost when *Hmox1* expression was impaired, i.e. *ApoE^{-/-}Hmox1^{-/-}* mice. This observation suggests that CO acts via a positive feed back amplification loop in which it induces the expression of HO-1 and thus the production of biliverdin/bilirubin that act as the “effector arm” responsible for the anti-atherogenic effects of HO-1. The molecular mechanism via which biliverdin/bilirubin exert anti-atherogenic effects are under investigation and shall be discussed.

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

MEMBERS: Miguel Soares, Ana Ferreira.

STUDENTS: Angelo Chora, Margarida Cunha-Rodrigues, Silvia Portugal, Cristina D. Rodrigues.

COLLABORATORS: Maria Manuel Mota, Ana Pamplona, Sabrina Epiphany, József Balla, Viktória Jeney, György Balla (Department of Medicine and Neonatology, Medical and Health Science Center, University of Debrecen, Hungary).

Cerebral malaria (CM) claims more than one million lives per year. Under this project we found that heme oxygenase-1 (HO-1 encoded by *Hmox1*) prevents the development of experimental CM (ECM). *Plasmodium berghei*-ANKA infected BALB/c mice up-regulated HO-1 expression/activity and did not develop ECM. *Hmox1* deletion or pharmacological inhibition of HO activity increased ECM incidence to 83% and 78%, respectively. Infected C57BL/6 mice up-regulated HO-1 to a lower extent than BALB/c mice and developed ECM (100% incidence). Pharmacological induction of HO-1 or exposure to the end-product of HO-1 activity carbon monoxide (CO), reduced ECM incidence in C57BL/6 mice to 10% and 0%, respectively. HO-1 and/or CO did not affect parasitemia while preventing blood-brain barrier (BBB) disruption, brain microvasculature congestion and neuroinflammation, including CD8⁺ T cell brain sequestration. The mechanism underlying these effects relies on the ability of CO to bind hemoglobin, prevent its oxidation and subsequently the generation of free heme, shown hereby to trigger the pathogenesis of ECM.

Induction of HO-1 during *Plasmodium* liver infection protects infected hepatocytes by modulating the inflammatory response.

MEMBERS: Maria Manuel Mota, Sabrina Epiphany, Ana Pamplona and Miguel Soares.

STUDENTS: Lígia A. Gonçalves, Sónia Albuquerque, Silvia Portugal, Sofia Rebelo.

COLLABORATORS: Hans-Peter Vornlocher (Roche Kulmbach GmbH, Kulmbach, Germany), Michael Goldberg (Department of Chemistry, Massachusetts Institute of Technology, USA).

The clinically silent malaria liver stage is an obligatory step in the establishment of infection and disease. We report here that expression of heme-oxygenase-1 (HO-1, encoded by *Hmox1*) is significantly up-regulated in the liver following infection by *Plasmodium berghei* sporozoites and is required for the establishment of infection. HO-1 overexpression in the liver leads to a proportional increase in parasite liver load. Conversely, deletion of *Hmox1* leads to the complete resolution of infection. In the absence of HO-1 the levels of chemokines that sustain the establishment of inflammatory foci are significantly increased, which in turn leads to increased levels of inflammatory cytokines involved in liver infection control. These findings establish definitively that, while stimulating inflammation, the liver stage of *Plasmodium* also induces the expression of HO-1 that modulates the host inflammatory response and thus protects the infected hepatocytes.

Heme oxygenase-1 underlies the “protective trait” afforded by sickle cell disease against severe malaria.

MEMBERS: Miguel Soares, Ana Ferreira.

COLLABORATORS: Laboratoire de Biochimie, Assistance Publique/Hôpitaux de Paris, Hôpital Saint Louis, Paris, France.

Sickle cell disease (SCD) is a genetic disorder caused by a single amino acid substitution in the hemoglobin (Hb) β -globin chain that leads to the synthesis of sickle Hb (HbS). The molecular mechanisms linking the ensuing red blood cell (RBC) and vascular dysfunction and variations in clinical severity are not well understood. SCD is triggered by HbS polymerization associated with RBC deoxygenating. This leads to micro-vascular occlusion, hemolysis and heme release from oxidized Hb. When exposed to heme, endothelial cells up-regulate the expression of heme oxygenase-1 (Hmox1/HO-1), the rate-limiting enzyme in the catabolism of heme into iron, carbon monoxide (CO) and biliverdin. Expression of HO-1 plays a central role in the regulation of inflammatory conditions including those associated with hemolytic diseases as well as with the development of severe acute malaria. Presumably, this is also the case for SCD, a prototypical hemolytic disease that is associated with a chronic induction of HO-1 expression in peripheral blood mononuclear cells, vascular endothelial and smooth muscle cells. Furthermore, induction of HO-1 expression or administration of end-products of heme catabolism by HO-1 prevents the pathologic outcome of SCD in mice. While sickle cell disease affects millions of people throughout the world, the presence of the HbS mutation in the heterozygous form (sickle cell trait) affords significant protection against severe forms of malaria, including cerebral malaria (CM), a lethal outcome of malaria infection that we have recently shown to be suppressed by HO-1 expression or by exposure to CO. Though the results published to date have not been conclusive, it was postulated that the protection against CM afforded by the sickle cell trait was inherent to the intracellular environment triggered by HbS that would be deleterious to *Plasmodium*, the causative agent of malaria. Our data suggests that other mechanisms might be involved in this process, namely that up-regulation of HO-1 mediates the protection afforded by the sickle cell trait against the development of CM. Our data shows that unlike their wild type littermates, SAD mice, a transgenic mouse strain

expressing a modified human sickle Hb that develops mild features of SCD, i) express high levels of HO-1 constitutively and ii) do not develop CM.

Ferrylhemoglobin, an oxidized form of hemoglobin that acts as a potent pro-inflammatory agonist in vascular endothelial cells.

MEMBERS: Miguel Soares, Gabriela Silva.

COLLABORATORS: Miguel Teixeira, József Balla, Viktória Jeney, György Balla (Departments of Medicine and Neonatology, Medical and Health Science Center, University of Debrecen, Hungary).

Inflammation can be associated with hemolysis and subsequently with release of (Ferrous) hemoglobin (Hb) from RBC. When exposed to polymorphonuclear cell-driven H_2O_2 , cell-free Hb oxidizes rapidly into methemoglobin (MtHb) and eventually into FerrylHb. Vascular endothelial cells (EC), in the lumen of blood vessels, are the most likely cellular targets for the biologic effects of cell-free Hb. It is well-established that when exposed to pro-inflammatory agonists, EC can up-regulate the expression of pro-inflammatory genes, e.g. adhesion molecules (E-selectin (CD62), intracellular adhesion molecule-1; ICAM-1 (CD54) and vascular cell adhesion molecule 1; VCAM-1 (CD106)), cytokines or chemokines. These play a critical role in recruiting leukocytes into sites inflammatory sites. Under this project we found that FerrylHb, but not FerrousHb or MtHb, can induce the expression of pro-inflammatory genes associated with EC activation, including the adhesion molecules E-selectin, ICAM-1 and VCAM-1. This effect is similar in “strength” to that of bacterial lipopolysaccharide (LPS), a potent and well-established pro-inflammatory agonist. The pro-inflammatory effect of FerrylHb is not mediated by a putative exogenous (i.e. endotoxin or H_2O_2) or endogenous (i.e. heme) contaminant. Expression of pro-inflammatory genes in response to FerrylHb, is abrogated by the transcription inhibitor actinomycin D, suggesting that this response is regulated at the transcriptional level, via a mechanism that we are actively investigating. To assess whether FerrylHb acts as a pro-inflammatory agonist *in vivo*, naïve C57BL/6 mice received an intraperitoneal “bolus” of FerrylHb, FerrousHb or MtHb. Contrary to FerrousHb or MtHb, FerrylHb triggers potent inflammatory response, revealed by the accumulation of polymorphonuclear cells in the peritoneal cavity. We now aim at identifying putative receptors involved in the recognition of FerrylHb as well as the signal transduction pathway triggered by these receptors and leading to the transcription of pro-inflammatory genes.

Characterization of the protective effects of a chimeric heme oxygenase-1 protein.

MEMBERS: Miguel Soares, Rasmus Larsen, Tatiana Vassilevskaia, Isabel Pombo Gregoire.

STUDENTS: Nadja Pejanovic.

The pro-inflammatory phenotype of activated macrophages ($M\phi$) contributes critically to the pathogenesis of inflammatory diseases. This phenotype, is thought to be controlled physiologically via the expression of heme oxygenase-1 (HO-1), the rate-limiting enzyme in the catabolism of heme into Fe, carbon monoxide (CO) and biliverdin. Both CO and

biliverdin have known anti-inflammatory properties in M ϕ as well as cytoprotective properties in non-hematopoietic cells. Presumably, these underlie to at least some extent, the ability of HO-1 to prevent the deleterious effects of inflammatory conditions such as severe sepsis, atherosclerosis, autoimmune neuroinflammation and cerebral malaria. Under this project we hypothesized that the protective effect of HO-1 might be used therapeutically to prevent the development of these conditions. To this end, we took advantage of the transacting transcriptional activator (TAT) protein transduction domain (PTD) derived from the human immunodeficiency virus. Proteins fused to this PTD can be efficiently incorporated into virtually any cell type and tissue *in vitro* and *in vivo*. HO-1 cDNA was re-cloned in an *E. coli* expression vector containing an N-terminal hexa-histidine-tag followed by the TAT PTD. The resulting recombinant protein, i.e. His-TAT-HO-1, was isolated to high purity via affinity chromatography. Purified His-TAT-HO-1 retained HO enzymatic activity and was readily taken-up by cells *in vitro*. His-TAT-HO-1 suppressed TNF, IL-6 and IL-10 production associated with M ϕ activation *in vitro*, an effect reversed by zinc protoporphyrin (ZnPPiX), a specific HO inhibitor. In addition His-TAT-HO-1 also afforded potent cytoprotective effects in non-hematopoietic cells, i.e. hepatocytes. When administered to BALB/c mice, before bacterial lipopolysaccharide challenge (5mg/kg), or just after infection by *Plasmodium berghei* ANKA, His-TAT-HO-1 (14-18mg/kg) reduced death rate to 10%, as compared to 80-90% in control mice treated with PTD-green fluorescent protein. The ability of His-TAT-HO-1 to suppress the pathogenesis of autoimmune neuroinflammation, i.e. experimental autoimmune neuroinflammation (EAE) and atherosclerosis in apolipoprotein deficient mice are being tested.

Statin-mediated cytoprotection of human vascular endothelial cells: a role for Kruppel-like factor 2-dependent induction of heme oxygenase-1.

MEMBERS: Miguel Soares.

COLLABORATORS: Faisal Ali, Shahir S Hamdulay, Anne R Kinderlerer, Joseph J Boyle, Elaine A Lidington, Dorian O Haskard, Anna M Randi, Justin C Mason (Bywaters Centre for Vascular Inflammation and Histopathology Section, Imperial College London Hammersmith Hospital, Du Cane Road, London, UK).

Heme oxygenase-1 (HO-1), by exerting anti-inflammatory, anti-proliferative, antiapoptotic and anti-oxidant effects in the vasculature, protects against atherosclerosis and post-transplant vasculopathy. We noted the overlap between the effects of HO-1 and those attributed to 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins). This led to an investigation of the role of HO-1 in statin-mediated cytoprotection in primary human endothelial cells (EC), and the ability of Kruppel-like factor 2 (KLF2) to regulate HO-1 function. Treatment of human umbilical vein and aortic EC with atorvastatin significantly upregulated HO-1 promoter activity, mRNA and protein expression, increasing HO-1 enzymatic activity as evidenced by raised intracellular bilirubin IX. This effect was indirect, dependent upon inhibition of HMG-CoA and geranylgeranylation, and independent of nitric oxide or changes in mRNA stability. Atorvastatin protected EC against the generation of reactive oxygen species and H₂O₂-induced injury. HO-1 inhibition, with small

interfering RNA (siRNA) or zinc protoporphyrinIX, abrogated atorvastatin-mediated cytoprotection. Atorvastatin upregulated KLF2 expression, while KLF2 siRNA attenuated statin-induced HO-1 and its associated anti-oxidant cytoprotective effects. Iron chelation, adenoviral-mediated over-expression of ferritin or supplementation of culture media with biliverdin, reversed the inhibitory effects of HO-1 and KLF2 siRNA, suggesting bile pigments and ferritin mediate the anti-oxidant actions of statin-induced HO-1. In conclusion, we have identified a novel link between KLF2 and HO-1 in human vascular EC, demonstrating that atorvastatin-mediated HO-1 upregulation, and its associated anti-oxidant effect, is KLF2-dependent. The relationship between KLF2 and HO-1 is likely to represent an important component of the vasculoprotective profile of statins.

Heme-oxygenase-1 expression enhances vascular endothelial resistance to complement-mediated injury through induction of decay-accelerating factor.

MEMBERS: Miguel Soares, Isabel Pombo Gregoire, Gabriela Silva.

COLLABORATORS: Anne R Kinderlerer, , Rivka Steinberg, Shahir Hamdulay, Faisal Ali, , Dorian O Haskard, Justin C Mason (Bywaters Centre for Vascular Inflammation and Histopathology Section, Imperial College London Hammersmith Hospital, Du Cane Road, London, UK).

Catabolism of free heme by heme oxygenase-1 (HO-1) generates carbon monoxide, biliverdin and free Fe. Presumably, these end-products are responsible for much of the biologic activity of HO-1 including its anti-inflammatory, anti-apoptotic, anti-proliferative and anti-oxidant effects. We have identified an additional cytoprotective action, the regulation of complement activation, mediated via induction of decay-accelerating factor (DAF). Pharmacological inhibition of HO activity by zinc protoporphyrin IX prevented tumor necrosis factor- α and vascular endothelial growth factor from inducing DAF expression in human endothelial cells (EC). The HO-1 agonist cobalt protoporphyrin IX significantly increased DAF expression in EC, reflecting an increase in steady-state daf mRNA. Adenoviral mediated over-expression of HO-1 or exposure to bilirubin increased DAF expression and enhanced protection against C3 deposition as well as complement-mediated lysis, which was reversed by DAF inhibitory mAb 1H4. Likewise, bilirubin, carbon monoxide, iron chelation or over-expression of the heavy chain ferritin (H-ferritin) all induced DAF expression in EC. Analysis of cardiac EC from *Hmox1*^{-/-} revealed a 60% reduction in DAF expression as compared to *Hmox1*^{+/+} EC and enhanced sensitivity to complement activation. We propose that modulation of complement activation, through induction of DAF, represents an important component of the overall cytoprotective effects of HO-1 against vascular injury associated with post-transplant vasculopathy, allograft rejection and ischemia-reperfusion injury.

Delayed killing of melanoma cells by CTL: multiple hits must be provided for tumor cell annihilation.

MEMBERS: Salvatore Valitutti, Iris Caramalho.

It is well established that cytotoxic T lymphocytes (CTL) against tumor antigens expand at relatively high frequency in blood and malignant tissues of cancer patients. However, the effector function of these naturally occurring CTL is often insufficient to achieve tumor remission even upon specific immunotherapy. We are investigating whether an impaired interaction between CTL and tumors results in defective delivery of lethal hit and/or CTL activation.

We have examined the extent of T cell activation in antigen-specific CTL conjugated with HLA-A2⁺ melanoma or conventional targets (EBV-transformed B cell lines) pulsed with the antigenic peptide. We observed that CTL undergo similar TCR down-modulation, IFN- γ secretion, Lamp-1 exposure and perforin release when conjugated with melanoma or B cells, yet melanoma cells are resistant to CTL mediated cytotoxicity. Detection of active Caspase 3 in target cells and Tunnel assays confirmed that melanoma cells are resistant to enter apoptosis when interacting with fully activated CTL.

In spite of this poor cytotoxicity, time-lapse microscopy experiments demonstrated that melanoma cells indeed undergo $[Ca^{2+}]_i$ increase upon contact with CTL indicating that they receive the hit of cytotoxic granules by activated CTL.

Our results show that although melanoma cells receive multiple hits by specific CTL, they significantly resist the CTL-mediated attack. The “delayed” capacity of CTL to lyse tumor cells may explain the limited capacity to control tumor growth *in vivo* and, most importantly, may favor the development of tumor variants resistant to immunotherapy

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 the age of five, representing nearly 10% of over 10 million children who die at these ages. In addition, malaria has a major negative impact on the 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.

The role of Heme oxygenase-1 and its products in the course and pathology of malaria infection.

MEMBERS: Ana Pamplona.

STUDENTS AND TECHNICIANS: Cristina D. Rodrigues.

COLLABORATORS: Miguel Soares (IGC, Oeiras, Portugal).

The malaria parasites are transmitted by the bite of a mosquito (*Anopheles* spp.). After the mosquito injects the parasite, it enters the liver for a first cycle of replication. This phase is clinically silent and is part of the incubation period. After the parasite is released from the liver cells it invades red blood cells (RBC), where it multiplies during 48-72 hours, depending on the species. At the end, the RBCs rupture and release the new generation of parasites that invades other RBCs and the cycle begins again. This erythrocytic phase of exponential parasite growth is responsible for the clinical picture.

Plasmodium parasite causes extensive hemolysis. About 40% of hemoglobin (Hb) contained within each infected red blood cell can be released and readily oxidized (reviewed by Francis et al, 1997). This leads to the generation of free heme (reviewed by Balla et al, 2005), a molecule cytotoxic to the parasite when generated within red blood cells and to the host when released into the circulation (Balla et al, 1994). *Plasmodium* has developed strategies to cope with free heme generated within red blood cells, polymerizing it into hemozoin (Egan, 2008). When exposed to free heme, host cells (human or rodent) up-regulate the expression of heme oxygenase-1 (HO-1), a stress-responsive enzyme that catabolizes heme into iron (Fe), biliverdin and carbon monoxide.

Using the malaria rodent model described above, Pamplona *et al.* showed that HO-1 plays a crucial role in the control of malaria pathology, as HO-1 expression/activity controls susceptibility to cerebral malaria in mice HO-1 was found to be up regulated to a lesser extent in infected C57BL/6 mice, all of which developed ECM, than in infected BALB/c mice, which did not develop ECM. Moreover, deletion of *Hmox1* or inhibition of HO activity in BALB/c mice increased ECM incidence. Subsequently, it was shown that exposure to inhaled CO protects mice against ECM. Whereas HO-1 and CO did not affect parasitemia, both prevented blood-brain barrier (BBB) disruption, brain microvasculature congestion and neuroinflammation, including CD8⁺ T-cell brain sequestration.

The role of CO during the course of malaria infection.

MEMBERS: Ana Pamplona, Sabrina Epiphanyo.

COLLABORATORS: Miguel Soares (IGC, Oeiras, Portugal).

After *Plasmodium* sporozoites have been deposited in the skin, by the bite of a mosquito (*Anopheles* spp.), they rapidly travel to the liver, where a sporozoite migrates through several hepatocytes before invading a final one in the hepatocyte, inside a parasitophorous vacuole, the sporozoites initiates a developmental program, which results in the production of 10,000-30,000 merozoites, inside a single hepatocyte. . This phase is clinically silent and is an obligatory step in the establishment of infection and disease. The hepatocyte-*Plasmodium* interactions during liver stages constitute an ideal target for intervention strategies aiming to block *Plasmodium* life cycle and infection. Despite the powerful prophylactic potential of host factors that influence liver infection,

they remain poorly understood. A microarray-based analysis of host cell genes altered during the course of sporozoite infection identified heme-oxygenase-1 as one of the genes that is significantly up-regulated (2-fold increase in expression *versus* non-infected controls) in hepatocytes following *Plasmodium* sporozoite infection (Albuquerque *et al.*, unpublished). We report that heme-oxygenase-1 (HO-1, encoded by *Hmox1*) is significantly up-regulated in the liver following infection by *Plasmodium berghei* sporozoites and is required for the establishment of malaria infection. HO-1 over-expression in the liver leads to a proportional increase in parasite liver load. Conversely, deletion of *Hmox1* leads to the complete resolution of the infection. In the absence of HO-1, the levels of chemokines - that help in the establishment of inflammatory foci - are significantly increased. These, in turn, lead to increased levels of inflammatory cytokines known to be involved in liver infection control. These findings show definitively that, liver stage of *Plasmodium* stimulates inflammation and, consequently, HO-1 is induced, suppressing the host inflammatory response and, thus, protecting the infected hepatocytes.

Cellular pathogenesis of malaria.

MEMBERS: Miguel Seabra, Elsa Seixas.

STUDENTS AND TECHNICIANS: Mafalda Silva, Carolina Matos.

EXTERNAL COLLABORATORS: Maria Mota (IMM, Lisbon, Portugal), Robert Sinden (Imperial College London, London, UK), Oliver Billker (Imperial College London, London, UK).

We are interested in understanding the molecular mechanisms of parasite invasion in host cells. The first important host-cell interaction in malaria infection is between the sporozoite and liver cells. We plan to dissect the mechanism by which the sporozoite induces the formation of a parasitophorous vacuole, where it grows into a merozoite and eventually ruptures the infected cell. Once in the circulation, the merozoite invades red blood cells and also induces the formation of a parasitophorous vacuole. Important questions to investigate include: Where do the vacuolar membranes derive from? The host, the parasite, or both? What are the components (proteins and lipids) present in these membranes? How is the vacuole transported to the center of the cell? What are the optimal conditions inside the vacuole that allow the parasite growth? What are the differences between liver cells and red blood cells when dealing with the parasite infection? How can the process of parasite invasion/growth and vacuole maturation be interrupted?

DEVELOPMENTAL BIOLOGY IN ANIMALS AND PLANTS.

The search for the mechanisms that guide the affairs of an embryo 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 again 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 played, and continue to play, 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.

Isolation and study of novel head-inducing genes expressed in the anterior visceral endoderm.

MEMBERS: José A. Belo and Alvaro Tavares.

STUDENTS AND TECHNICIANS: Marta Vitorino, Ana Salgueiro, Sara Marques, Lisa Gonçalves, Ana C. Silva.

EXTERNAL COLLABORATORS: Herbert Steinbeisser (University of Heidelberg, Germany).

Several reports point to an involvement of the mouse Anterior Visceral Endoderm (AVE) in early anterior neuroectoderm induction. Secreted antagonists of the BMP4, Nodal and Wnt8 pathways, like Cer-I, Lefty1 and Dkk1, are expressed in the AVE (Belo et al., 1997; Glinka et al., 1997). This region underlies the prospective anterior neuroectoderm and was shown to play an important role in head formation.

In order to further characterize the molecular mechanisms that play a role in the early forebrain induction and organogenesis, a transgenic mouse line was generated in which EGFP is expressed in the AVE, under the control of the promoter region of the Cer-I gene (Mesnard et al., 2004). Gene expression profiling using GeneChips® (Affymetrix®) identified several differentially expressed transcripts at the very early stages of A-P axis

establishment (Mário Filipe, unpublished observations; Filipe et al 2006; Silva et al 2006; Salgueiro et al, 2006).

One of the novel genes identified, expressed in the AVE, was denominated ADTK1 (Anterior Distal Tyrosine kinase1) due to its expression in anterior endoderm and encodes a 493 a.a. protein containing a predicted dual-specificity Ser/Thr/Tyr kinase catalytic domain, which may play essential roles in the embryonic development, switching on and off signalling pathways through phosphorylation of key substrates, as for example Nodal and Wnt signaling pathways. However, this novel gene is the first one known with expression in the AVE and little is known about their role in the AVE. *ADTK1* has also a singular expression pattern during organogenesis.

Using the ADTK1 sequence to search NCBI databases, we identified 2 potential *Xenopus* orthologs with close homology to the mouse clone. The spatio-temporal expression pattern of these genes was briefly analyzed by in situ hybridization.

XADTK1-L1 encodes a 489 a.a. protein and contains a predicted Serine/Threonine protein kinase catalytic domain. At the beginning of gastrulation *XADTK1-L1* is expressed exclusively in the ADME (Figure 2E-H). As gastrulation proceeds *XADTK1-L1* mRNA start being expressed also in the axial mesoderm.

XADTK1-L2, is a second *Xenopus* ortholog of *ADTK1* that encodes a 449 a.a. protein with the region of higher identity corresponding to the predicted Ser/Thr protein kinases catalytic domain. Whole-mount *in situ* hybridization using *Xenopus* embryos shows that the expression is restricted to the dorsal blastopore lip (Figure 2I-L) and to the ADME. Tailbud stages, *XADTK1-L2* expression is restricted to the ventral body wall, foregut and head region.

An in-depth study of these proteins, as the one presented in this current application, will help unravel the roles and the mechanisms of this family of genes in signal transduction signaling pathways.

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

MEMBER: José A. Belo.

STUDENTS AND TECHNICIANS: Ana Cristina Silva, Marta Vitorino.

EXTRERNAL COLLABORATORS: Leonor Cancela and Natércia Conceição (CCMAR, UAIG, 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. This putative proximal promoter was found to direct transcription of the luciferase reporter gene in the X. laevis A6 cell line. 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. Currently we are conducting up-regulation and Knock-down experiments using the *Xenopus* system in order to try to further characterize the genetic cascade involved in chondrogenesis in vertebrates.

Transcriptional regulation of *Caronte* during embryonic development.

MEMBERS: José A. Belo and Ana T. Tavares.

STUDENTS AND TECHNICIANS: Sara Marques, Ana C. Silva.

The TGF- β -related molecule Nodal plays an essential and conserved role in left-right patterning

of the vertebrate embryo. Previous reports have shown that zebrafish and mouse Cerberus-related

proteins Charon and Cerberus-like-2 (Cerl-2), respectively, act in the node region to prevent Nodal signal from crossing to the right side, whereas chick Cerberus (cCer) has an unclear function in the left side mesoderm. In this study, we investigate the transcriptional regulation and function of chick Cer in left-right development. By analyzing the enhancer activity of *cCer* 5' genomic sequences in electroporated chick embryos, we identified a *cCer* left-side enhancer that contains two FoxH1 and one SMAD binding site. We show that these Nodal-responsive elements are necessary and sufficient for the activation of transcription in the left side mesoderm. In transgenic mouse embryos, *cCer* regulatory sequences behave as in chick embryos, suggesting that the *cis*-regulatory sequences of *Cerberus-related* genes have diverged during vertebrate evolution. Moreover, our findings from *cCer* overexpression and knockdown experiments indicate that cCer is a negative feedback regulator of Nodal asymmetric signaling. We propose that chick Cer and mouse Cerl-2 have evolved distinct regulatory mechanisms but retained a conserved function in left-right development, which is to restrict Nodal activity to the left side of the embryo.

Genetic and biochemical control of Cerl-2 in during early development.

MEMBERS: José A. Belo and Jorge Carneiro and Salomé Almeida.

STUDENTS AND TECHNICIANS: Sara Marques, Ana C. Silva, Ana Carolina Borges.

We have characterized the function of Cerl-2, a Nodal antagonist, which displays a unique asymmetric expression on the right side of the mouse node. Cerl-2 knockout mice display multiple laterality defects including randomization of the L/R axis. Our results demonstrated that Cerl-2 plays a key role in restricting the Nodal signaling pathway toward the left side of the mouse embryo by preventing its activity in the right side.

We intend to study the regulation of *Cerl2* gene expression, and for that end, transgenic approaches will be used to map *cis*-regulatory elements responsible for spatially and temporally restricted *Cerl2* expression. We intend to see if *Cerl2* perinodal domain of expression is Delta1 pathway dependent, like *Nodal* (Raya et al, 2003; Krebs et al, 2003), by doing whole mount *in situ* hybridization for *Cerl2* in Delta1 mutant embryos. We also intend to assess in mutants of other candidate transcription factor(s) the presence or absence of *Cerl2* mRNA.

In *iv/iv* and *inv* mutants *Nodal* expression domains in the node are altered. We are also studying changes in *Cerl2* asymmetrical domain of expression in the node of these mutants. *Cerl2* mRNA is upregulated in the right side of the node and gives rise to a secreted protein that is produced when the 'nodal flow' is active and determinant. A key question that we want to answer is where does *Cerl2* protein exert its activity, is it displaced towards the left influenced by the fluid flow, or does it remain upregulated in the right. We propose to address this question by seeing *Cerl2* protein localization by confocal microscopy in WT and in node motility flow-challenged embryos (*iv/iv*).

Study of novel genes expressed in the H/HPC for a correct control of heart organogenesis.

MEMBERS: José A. Belo.

STUDENTS AND TECHNICIANS: Margaret Bento, Ana Ledo, Ana R. Serralheiro and Elizabeth Correia.

We have isolated a 2.5 kb genomic fragment upstream the ATG of chick Cerberus homolog, Caronte, that is able to drive the expression of EGFP into the cell populations that express cCar. Furthermore, a large population of EGFP positive cells, defining the heart and the hemangioblast precursor cells can be clearly visualized as early as Stage 3⁺. In order to identify and study novel genes expressed and involved in the correct development and differentiation of the vertebrate heart/hemangioblast precursor cell (HPC) lineages, a differential screening using Affymetrix GeneChip system technologies was performed (M. Bento et al., *in preparation*).

Remarkably, this screening led to the identification of more than 200 new genes potentially expressed in these haematopoiesis, angiogenesis or cardiogenesis precursor lineages. The aim of this project is to accomplish a detailed study of some of these genes, by performing developmental, genetic, biochemical and functional studies in chick and mouse models. This will provide novel insights about important genes in heart development.

One of the novel genes identified, expressed in the early cardiogenic region, was initially denominated CHEP36 (Chick Heart/Haemangioblast Progenitor #36) due to its expression in anterior cardiac endomesoderm precursor cells and encodes a 449 a.a. protein, containing a predicted dual-specificity Ser/Thr/Tyr kinase catalytic domain, which may play essential roles in the embryonic development. *CHEP33* encodes a novel 308 a.a. protein and contains a predicted Zinc-finger motif.

An in-depth study of these proteins, as the one presented in this current application, will help unravel the roles and the mechanisms of these of genes in early vertebrate heart/hemangioblast induction and organogenesis.

The Role of VEGF and its receptors in normal and malignant bone marrow/the importance of the bone marrow vascular compartment in homeostasis and in disease.

MEMBERS: Sérgio Dias, Cristina Casalou, Ana Sofia Cachaço, Ana Costa, Tânia Carvalho.

STUDENTS: Rita Fragoso, Ana Paula Elias, Catarina Osório, Ana Gomes.

EXTERNAL COLLABORATORS: Shahin Rafii (Cornell University, NY, USA), Zhenping Zhu, Yan Wu (ImClone Systems, NY, USA), Genentech (Napoleone Ferrara), Maria Gomes Silva (IPOFG, Lisbon, Portugal).

This Project 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. We have found out that VEGF and its receptors are actively regulated (transported, internalized, etc: published in *Experimental Cell Research*) on endothelial cells. More recently, we have begun to exploit the mechanisms of VEGF splicing by neoplastic cells, under the influence of distinct environmental signals. Concerning the role of bone marrow VEGF in regulating neoplastic growth, we described a previously unrecognized role for VEGF receptor-1 in regulating the bone marrow localization of subsets of acute leukemias (this study was published in *Blood*). Along the same lines, we have found that VEGF actively suppresses lymphoid (B) differentiation *in vitro* and *in vivo* (manuscript submitted).

We have also been exploiting the role of the extracellular matrix (ECM) in Bone marrow physiology and in disease. Normal and malignant hematopoietic cells release ECM components and express some of its receptors (integrins), which may be important for several cellular functions (survival, migration). Within the aims of the Project, research into the mechanisms by which cells differentiate, are distributed within, and finally exit the bone marrow is being actively pursued.

Finally, a putative role for cholesterol metabolism in the regulation of normal and malignant bone marrow function has begun to be elucidated. We have preliminary evidence that cholesterol levels control hematopoietic cell differentiation and recruitment/mobilization, and are just starting to dissect the signaling pathways and the specific cholesterol receptors involved in these effects.

Endothelial progenitors: their role in health and disease.

MEMBERS: Sérgio Dias, Carla Real, Tânia Carvalho.

STUDENTS: Cátia Igreja, Francisco Caiado, Catarina Osório.

EXTERNAL COLLABORATORS: Shahin Rafii (Cornell University, NY, USA), Antonio Duarte (F. Medicina Veterinária/IGC), José Boavida (Associação Protectora dos Diabéticos de Portugal), Ana Bastos (Serviço Oftalmologia, Hospital Santa Maria), Perpétua Pinto do Ó (IBMC), Julian Dye (Raft Institute of Surgery and Plastic Surgery, NHS, Northwood, UK).

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 this Project, we have been defining in detail the cDNA micro array Profile of umbilical cord and bone marrow-derived EPC in different physiological and pathological angiogenesis processes.

In detail, we defined an EPC profile by a set of genes/gene categories, which are expressed in a temporal and functionally-coordinated manner (manuscript accepted for publication). Members of the Delta/Notch signaling are examples of such genes. We are currently assessing the importance of Delta4:Notch signaling on tumor vessels and EPC during angiogenesis and tumor growth.

In addition to the studies in tumor angiogenesis, we are also exploiting the hypothesis that defects/reduced expression of specific genes/gene families may be the underlying molecular problem for the vascular defects seen in many diseases. In this context, we have characterized the gene expression profile of EPC in diabetic patients and those with ophthalmology-related complications (macular degeneration, diabetic retinopathy).

We have exploited also the possibility that bone marrow-derived EPC may play a role in maintaining tissue (bone marrow) homeostasis, while in situations of neoplastic transformation (leukemia onset) they may contribute towards the expansion of the bone marrow vasculature. We are studying this concept in murine models of bone marrow disease and also in samples from myelodysplastic patients. More recently, we have begun to study the importance of EPC and EPC-related signaling pathways during wound healing and in the modulation of cardiac recovery following stroke/Ischemic insult.

Angiogenesis in cervical neoplasms: clinical and molecular correlates.

MEMBERS: Sérgio Dias, Ana Cachaço.

EXTERNAL COLLABORATORS: Ana Félix (Anatomia Patológica, 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 are currently studying 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. We have optimized immunohistochemical staining protocols and are in the process of analysing a large collection of endometrial cancers.

Microenvironment regulation of tumor angiogenic properties.

MEMBERS: Sérgio Dias, Jacinta Serpa.

STUDENTS: Ana Paula Elias.

In this Project we are exploiting the hypothesis that metabolic adaptations/selection of cancer cells regulates the angiogenic properties (more or less angiogenesis and invasive properties) of tumors. We have started by determining the importance of acidic microenvironment (low pH) in the regulation of VEGF alternative splicing using endometrial cancer cells as models. More recently we have started to study the metabolic adaptation of colon cancer cells in response to butyrate levels and the net output in VEGF production.

Notch signaling in vascular development and angiogenesis.

MEMBERS: António Duarte, Ana Cristina Borges.

STUDENTS AND TECHNICIANS: Alexandre Trindade, Sónia Ventura, Dusan Djokovic, Patrícia Rodrigues, Joana Quaresma.

EXTERNAL COLLABORATORS: Parkash Gill (University of Southern, California, USA), Adrian Harris (Oxford University, UK), Ralf Adams (Cancer Research UK, UK), Freddy Radtke (Swiss Institute for Experimental Cancer Research, Switzerland), Anne Eichmann, (Collège de France, France).

Notch signalling is a conserved pathway that functions to modulate fate decisions of a wide variety of cell types. Mutations in humans, mice and zebrafish demonstrated the importance of this pathway in the regulation of vascular development . In particular, we have recently shown that the Notch ligand Delta-like 4 (Dll4) is required in a dosage-sensitive manner for normal arterial patterning during mammalian development. Furthermore, ongoing work in our laboratory suggests a novel role for Dll4 in the regulation of vessel branching during vascular development in the mouse retina by suppressing endothelial tip cell identity.

The great sensitivity of the embryonic and retinal vasculature to Dll4 levels raises the possibility that it may constitute a good target for therapeutic intervention in adult tumour-induced neoangiogenesis. Furthermore, its expression is greatly increased in the vasculature of xenografted as well as endogenous human tumours and was shown to be induced by hypoxia, a condition frequently present in the tumour milieu. Despite this wealth of data, the mechanistic basis of Dll4 function in general, and in the tumour angiogenesis context in particular, remains to be elucidated. *In vitro* work involving various endothelial cell lines have so far yielded contradictory results, that probably only *in vivo* experimental work is likely to clarify.

The embryonic lethal phenotype of *Dll4* null mutants makes it impossible to characterize its postnatal function in vivo using conventional gene targeting. We are therefore using inducible gene deletion and overexpression strategies to create loss- and gain-of-function mutants to address the various aspects of Dll4 function *in vivo* in newborn and adult mice. The genetically modified mouse lines already established in our laboratory enable us to carry out a number of *in vivo* experiments that will help dissect the role of Dll4/Notch signaling in the regulation of vascular branching, specification of tip cell identity and tumour angiogenesis.

Alternative splicing in developmental control and stress response in *Arabidopsis thaliana*.

MEMBERS: Paula Duque.

STUDENTS AND TECHNICIANS: Raquel Carvalho and Sofia Carvalho.

EXTERNAL COLLABORATORS: Nam-Hai Chua (The Rockefeller University, New York, USA).

Alternative splicing (AS) has recently emerged as an important mechanism for generating proteome diversity and regulating gene expression. Very few alternatively-spliced transcripts are known in plants, but recent bioinformatics studies indicate that AS plays a

far more significant role in plants than previously thought. Their unique developmental plasticity and stress tolerance, developed as a result of their sessile growth habit, suggests that plants offer exceptional opportunities to reveal AS mechanisms. We are investigating the biological relevance of AS in plant stress and development and the regulatory mechanisms of plant pre-mRNA splicing, which remain poorly understood. Reverse genetic approaches in *Arabidopsis* are being used to examine the functional significance of splice variants of development- and stress-associated genes and of individual serine/arginine-rich (SR) proteins, which are established key players in mammalian AS. Moreover, we will employ a computational approach in an attempt to identify plant splicing enhancer cis-acting elements (ESEs) recognized by SR proteins.

Alternative splicing of an *Arabidopsis* RING E3 ubiquitin ligase involved in plant stress and development.

MEMBERS: Paula Duque.

STUDENTS AND TECHNICIANS: Sofia Carvalho.

EXTERNAL COLLABORATORS: Nam-Hai Chua (The Rockefeller University, New York, USA).

As sessile organisms, plants developed adaptive developmental and physiological strategies to cope with environmental stress. These range from morphological modifications to physiological adaptation at the cellular level, but the basis of the capacity for adaptation lies ultimately at the level of the genome. Alternative splicing (AS) has recently emerged as an important mechanism for generating proteome diversity and regulating gene expression. Its prevalence in many genomes shows that it may play important roles in biological processes, and recent studies indicate that plant AS events occur in response to environmental changes or at particular developmental stages. Nevertheless, very few alternatively-spliced transcripts have been reported in plants so far. On the other hand, the ubiquitin/26S proteasome pathway, a tightly regulated and highly specific protein degradation system essential for organism survival, contributes significantly to plant development and stress tolerance by affecting a wide range of processes, and several plant E3 ubiquitin ligases have been implicated in the transduction of environmental signals.

In preliminary studies, we have identified an *Arabidopsis thaliana* gene encoding a RING E3 ligase involved in protein ubiquitination that undergoes AS. Exon skipping excludes the only nuclear localization signal in the predicted protein and determines the subcellular localization of two splice forms — the longer isoform is targeted to the nucleus, while the shorter localizes to the cytoplasm. Both alternatively-spliced transcripts are upregulated during senescence and upon application of the stress-response hormone abscisic acid (ABA), but the magnitude of this induction is more than two fold higher for the splice variant encoding the nuclear isoform. These results strongly suggest a role for AS of this ubiquitination gene in plant developmental control and stress responses.

This project proposes to extend these studies and conduct a detailed functional analysis of this poorly characterized RING E3 ligase. To that end, stress- and development-specific expression and splicing patterns will be investigated and reverse genetics approaches in

Arabidopsis used to examine the functional significance of both splice forms. Moreover, assessment of the ubiquitination capacity of each isoform and identification of the target protein(s) using *in vitro* ubiquitination and yeast two-hybrid assays hold much promise for unraveling the mode of action of this alternatively-spliced gene and its role in plant development and stress.

Herbicide resistance in *Arabidopsis thaliana*: role of plant multidrug resistance transporters.

MEMBERS: Paula Duque.

STUDENTS AND TECHNICIANS: Alexandra Duarte.

EXTERNAL COLLABORATORS: Isabel Sá-Correia and Miguel Cacho Teixeira (Insituto Superior Técnico, Lisbon, Portugal).

The widespread use of herbicides has led to an increasing number of resistant weed species and classes of herbicides to which resistance has evolved, with some biotypes currently showing multiple-resistance to various of these agrochemicals. Herbicide resistance has the potential to cause not only large economic losses in agriculture, but also deleterious effects on the environment and human health, as a result of rising herbicide application rates. The lack of a basic understanding of the molecular mechanisms underlying herbicide resistance remains the greatest obstacle to the use of biotechnology to deal with this problem. The Biological Sciences Research Group at Instituto Superior Técnico (IST) has recently shown that three *Saccharomyces cerevisiae* genes encoding plasma membrane multiple drug resistance (MDR) transporters determine yeast resistance to 2,4-dichlorophenoxyacetic acid (2,4-D), one of the selective herbicides most successfully used worldwide. Two of these genes, ScPDR5 and ScPDR18, belong to the pleiotropic drug resistance (PDR) subfamily of ABC drug efflux pumps, whereas the other MDR transporter (ScTPO1) is a member of the major facilitator superfamily (MFS). Based on these findings, we have been analyzing the effects of 2,4-D application on the expression of poorly characterized *Arabidopsis thaliana* MDR transporter genes showing high sequence similarity to the yeast resistance determinants. First results show a clear transcriptional activation of one MFS and two PDR genes in response to the herbicide. This strongly suggests an involvement of these putative plasma membrane drug efflux pumps in the plant's response to herbicidal concentrations of 2,4-D and pinpoints excellent candidates for herbicide resistance determinants in *A. thaliana*. The IGC and IST groups are collaborating to investigate the role of genes encoding MDR transporters of the ABC and MF Superfamilies in plant herbicide resistance. The functional analysis of these novel or poorly characterized plant genes involve gene expression analysis and reverse genetic approaches in *Arabidopsis*, heterologous expression and complementation experiments in yeast, and membrane transport assays in both systems. These studies, using the plant model *A. thaliana* and the fundamental model eukaryote *S. cerevisiae*, should accelerate understanding of the molecular mechanisms governing herbicide resistance, currently one of the most unexplored and exciting topics in plant biology, with important implications for agriculture, the environment, and human health.

Ionic basis of apical cell growth and morphogenesis in pollen tubes.

MEMBERS: José A. Feijó, Erwan Michard, Filipa Alves.

STUDENTS AND TECHNICIANS: Ana Bárbara Santos, Pedro Dias, Catarina Silva.

EXTERNAL COLLABORATIONS: Ana Bicho (Requimte, UTL-UNL), Gerhrad Obermeyer (Univ. Salzburg, Austria), Matthew Gilliam (Univ. Adelaide, Australia), Uwe Ludwige (Univ. Tuebingen, Germany).

Genetic control of pollen tube growth and sperm function.

MEMBERS: José A. Feijó, Rui Martinho, Jorg Becker.

STUDENTS AND TECHNICIANS: Filipe Borges, Catarina Silva.

EXTERNAL COLABORATIONS: Liam Dolan (John Innes Res.Ctr., Norwich, UK), Sheila McCormick (U. Berkeley, USA), Carlos Plancha (IMM), Rob Martienssen (Cold Spring Harbor Lab, NY, USA).

Ionic basis of signalling during mycorrhizal symbiosis.

MEMBERS: José A. Feijó, Alessandro Ramos.

STUDENTS AND TECHNICIANS: Pedro Dias, Catarina Silva.

EXTERNAL COLABORATIONS: Arnold Façanha (UENF, Brazil), Miguel Teixeira (ITQB, Oeiras, Portugal).

Neurogenesis: from embryos to ES cells.

MEMBERS: Domingos Henrique.

The main objective of the group is to investigate the molecular mechanisms that regulate the genesis of neurons in vertebrate embryos. Our aim is to characterize the molecular events that control the generation of neural stem cells in the embryo, how these cells are maintained and how they give rise to the multitude of neurons that compose the adult CNS. A better knowledge about these fundamental mechanisms is a pre-requisite for the development of cellular replacement therapies to treat neurodegenerative diseases, with a significant impact on human health.

Genetic screen for epithelial repair genes.

MEMBERS: António Jacinto, Ana Catarina Santos, Isabel Campos, Dulce Azevedo.

STUDENTS AND TECHNICIANS: Jennifer Geiger, Vanessa Carlos.

Wound healing is essential to organisms throughout the animal kingdom to restore tissue integrity after injury both during embryonic and adult life. To get more insight into this poorly understood process we have established a simple assay in *Drosophila* that will allow us to start dissecting the mechanisms of wound repair in simple epithelia. We are currently undertaking a large-scale genetic screen with the aim of finding new genes involved in *Drosophila* embryonic epithelial repair. For that purpose we have optimised a wounding assay consisting in laser wounding 50 *Drosophila* embryos and screening for

unclosed wounds ~16 hours after. We have successfully tested and calibrated our wounding assay in a pilot screen using mutations known to affect embryonic or larval wound closure. We confirmed that in our assay, *rho1* mutant embryos displayed a wound closure phenotype, as previously demonstrated, and we found that mutants for Jun-related antigen (Jra), a member of the JNK signalling pathway show wound closure defects in our assay. We have started the screen and so far we have tested almost 700 transposon insertions, all mapped and expected to affect the function a gene. We have identified 30 mutants that affect wound closure significantly, including some that display very dramatic phenotypes. The genes that are affected by these mutations will be studied in more detail using the full range of genetic and molecular tools available in *Drosophila*, and we will characterize their precise roles in the process of wound healing in the embryo and other epithelial tissues.

Integrating the genetics, mechanics and phenomenology of embryonic wound healing.

MEMBERS: António Jacinto.

STUDENTS AND TECHNICIANS: Marco Antunes.

EXTERNAL COLLABORATORS: Wayne Brodland (Univ. Waterloo, Canada), Shane Hutson (Vanderbilt Univ., Nashville, USA).

Embryonic wound healing is both a mechanical process, driven by intra- and intercellular forces, and a genetic process, regulated by an array of signalling molecules. Nature has adeptly integrated the mechanics and genetics; and if we scientists are to understand the multiple, hierarchical interactions involved, we must take a similarly integrated view. Here, we propose a collaborative approach involving biological physicists, developmental biologists and mechanical engineers. Our goal is to understand the fundamental biology and dynamics of embryonic wound healing – to connect the phenomenology of wound-margin shortening to the underlying cytoskeletal mechanics and genetic regulatory networks. We see embryonic wound healing as a process of epithelial re-sealing and an extension of normal morphogenesis – one where we can use variations in the tissue/wound geometry to investigate the full range of capabilities of the systems that generate and regulate morphogenetic/wound-healing forces. Our primary model system will be the epidermal flank of fruit fly embryos (*Drosophila melanogaster*) at stage 15, just after dorsal closure. This model system is well-suited to integrated genetic and mechanical studies because we can generate arbitrarily-shaped wounds with laser-microsurgery, and follow the cellular details of wound closure in living, GFP-labeled embryos via confocal microscopy. In addition, previous screens have identified many *Drosophila* mutants and transgenic constructs with defects in wound healing that can be used to manipulate the system genetically and will help us to establish correlations between the genetics and the cell mechanics.

Hemocyte chemotaxis in *Drosophila*.

MEMBERS: António Jacinto, Soren Prag.

STUDENTS AND TECHNICIANS: Marco Antunes.

EXTERNAL COLLABORATORS: Will Wood (Univ. Bristol, UK).

Tissue repair involves not only the damaged cells and neighbours that close the hole, but also inflammatory cells that are recruited to the wound site to clear the debris. In *Drosophila* this role is played by hemocytes, macrophage-like cells that are the main cell type of the *Drosophila* immune system. In this project we propose to establish a robust and quantitative *in vivo* hemocyte migration assay during pupa development and during three phases of hemocyte migration in response to wounding that is established; namely 1) initial sensing, 2) sustained chemotactic migration towards the wound, and 3) final cease in migration when reaching the wound. This assay will be used to analyse the migratory behaviour of haemocytes after genetic alteration of integrin expression and activity status. Furthermore, we will characterise haemocyte migration phenotypes upon mutation and alteration in expression levels of extracellular matrix proteins (outside-in) and intracellular integrin-associated proteins (inside-out). Finally we will identify genetic interaction between integrins and intracellular signalling proteins responsible for actin rearrangements during haemocyte migration.

The origin of blastemal cells during zebrafish caudal fin regeneration.

MEMBERS: António Jacinto, Nuno Afonso.

STUDENTS AND TECHNICIANS: Mariana Simões, Sara Mauricio de Sousa.

EXTERNAL COLLABORATORS: Joaquin Rodrigues Leon (IGC and CMRB, Barcelona, Spain).

The caudal fin of adult zebrafish has recently become a popular model for regeneration studies due to some advantages it presents as compared to other systems. The zebrafish is amenable to tissue and genetic manipulation and the animals are relatively easy to rear and maintain. The caudal fin of this teleost is constituted by multiple mineralised bony fin rays, which have a dermal origin. Each of the rays is composed of segmented concave facing hemirays, each of which surrounded by scleroblasts, osteoblast-like cells secreting the extracellular matrix of the bony fin rays. In the interior of these bony rays, a heterogeneous population of cells includes connective tissue and blood vessels. In the interray compartment, venous capillaries, nerves and connective tissue are also present. The regenerative process of the caudal fin takes two weeks and goes through three steps: wound healing, blastema formation and regenerative outgrowth. The aim of this project is to investigate the origin of blastemal cells during fin regeneration. We will take advantage of Cre/loxP system and tissue specific promoters to mark the different fin cell types with GFP and fate map the regenerating tissue. We will determine whether the blastemal cells result from dedifferentiation of fin tissue or from the activation of stem cells that reside in the tissues that constitute the fin. The relative contribution of each cell type to the regenerated tissues will be also evaluated.

The contribution of nerves for zebrafish caudal fin regeneration.

MEMBERS: António Jacinto, Nuno Afonso.

STUDENTS AND TECHNICIANS: Mariana Simões, Sara Mauricio de Sousa.

Limb (amphibians) and fin (zebrafish) regeneration are known to be dependent on the concomitant regeneration of peripheral axons into the blastema whose function is to stimulate division of the dedifferentiated blastemal cells in the early regenerate. Studies of nerve dependence in zebrafish fins offer new genetic and live imaging tools to investigate this process at the cellular and molecular level. We will take advantage of the zebrafish lines that express GFP in the nervous system to identify and manipulate axons in live animals. The GFP expressing lines will allow us to identify the position of axons in the organism, to follow the re-ennervation in live regenerating fins, and to correlate precisely the re-ennervation events with the behaviour of the regenerate. Sections of axons that innervate the caudal fin will be transected at selected time points after amputation to investigate the requirement of nerves at each phase of regeneration. We will determine if the nerves are required for wound healing, blastema formation, blastema proliferation or patterning. After establishing the fish tail as a model to study re-innervation in caudal fin regeneration we will be able to test the function of neural mitogenic factors that have been proposed to have a role in the communication between the nerve terminals and the blastema, based in studies from other systems.

The role of telomerase in zebrafish regeneration.

MEMBERS: António Jacinto, Miguel Godinho Ferreira.

STUDENTS AND TECHNICIANS: Rita Mateus.

EXTERNAL COLLABORATORS: Leonor Santos Ruiz (Univ, Malaga, Spain).

Zebrafish have constitutively telomerase activity in somatic cells throughout their lives, from embryos to adults. This is apparently different from mammalian telomerase regulation since in most mammalian somatic tissues telomerase activity is dramatically decreased in early stages of development except for germ cells and stem cells. Our collaborators have shown that telomerase is present in adult fins and its expression activated upon amputation. We aim to elucidate its function during regeneration using antisense morpholinos to downregulated the enzymatic activity of both the telomerase catalytic subunit (TERT) and the RNA template (TR). The effects of downregulating telomerase will be analyzed in detail in regenerating fins.

Hox genes in the development of the axial skeleton.

MEMBERS: Moisés Mallo.

STUDENTS AND TECHNICIANS: Tânia Vinagre, Ana Nóvoa, Joana Bom.

Hox genes have been known for a long time to play a central role in defining the 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 its length. Recent work has shown that, in addition to their classical role in the control of the identity of specific segments of the vertebral

column, Hox genes might also define global vertebral domains. For example, we have shown that Hox group 6 is able to provide thoracic characteristics (i. e. induce rib formation) to the vertebrae and that Hox group 10 genes are responsible for the genesis of the lumbar area by blocking rib formation. In this morphogenetic process Hox groups 10 and 6 work by modulating the same developmental process (formation of ribs) in opposite directions. We are exploiting this experimental system to understand the molecular mechanisms of Hox gene activity. We have found that what Hox group 6 and 10 genes do is to modulate the cellular responses of a part of the somite, its lateral border (known as the hypaxial lip), to signals provided by surrounding tissues, most particularly the lateral plate mesoderm and the surface ectoderm. We have shown that when the Hox group 6 genes are operating, an interlimb kind of response is triggered in this hypaxial lip, resulting in rib development. Conversely, the Hox group 10 effects a limb-type of response in the lateral somite, resulting in a ribless phenotype. Interestingly, the effect of both these genes is codified even before the somite is physically formed. Our current work focuses on the molecular mechanisms of all these Hox-mediated activities.

Hoxb4 in the proliferation of hematopoietic stem cells.

MEMBERS: Moisés Mallo, Leonor Parreira, Natalia Moncaut.

EXTERNAL COLLABORATORS: Bernd Schiedlmeier and Hannes Klump (Hannover Medical School, Hannover, Germany).

It has been shown that Hoxb4 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 an 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 the response of the progenitor cells to Fgf signalling. In addition, and quite surprisingly, the Fgf signalling has a negative effect on the expansion of hematopoietic progenitors, as evidenced by the strong increase in the number of hematopoietic colonies formed when Fgf signalling is blocked (with SU5402) concomitant with the induction of Hoxb4. This effect was confirmed in vivo in experiments aimed at the repopulation of the hematopoietic system of lethally irradiated mice. We have also performed an Affymetrix GeneChip screen for genes under the modulation of Hoxb4 in our ESC system. We have compared our data with that obtained by our collaborators Bernd Schiedlmeier and Hannes Klump using a complementary system based on the controlled expression of HoxB4 introduced into bone marrow-derived hematopoietic stem cells using retroviral vectors. Our comparison gave an overlapping set of Hoxb4 targets that are mostly engaged in the modulation of signalling pathways. This confirmed and expanded our data involving the functional association of Hoxb4 with Fgf signalling. These experiments also uncovered an important association of the Hoxb4 gene with TNF signalling, which has functional relevance according to the results from hematopoietic repopulation experiments.

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

MEMBERS: Moisés Mallo, Victoria Gallego.

STUDENTS AND TECHNICIANS: 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 have investigated whether the Hoxb3 antisense transcript could work in trans. For this, we produced transgenic embryos overexpressing this transcript in areas where Hoxb3S is normally expressed and assessed its effects on the expression of the Hoxb3S transcript. So far, it seems that this Hoxb3AS transcript is not able to modulate expression of the sense version in trans. In addition, we have shown that a miRNA might be produced from an intron within the Hoxb3AS immature transcript. We have produced miRNA libraries from rhombomere 4 of mouse embryos to try to identify such a miRNA. Moreover, we have performed experiments based on the production of BAC transgenics to identify the promoter/enhancer elements controlling the expression of Hoxb3AS for their ulterior inactivation in vivo.

Development of the heart outflow tract.

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. During this process, the first and second aortic arches disappear between days 9 and 11 of embryonic age. We are investigating how this process is controlled at the cellular and molecular levels. We have found that the first two aortic arches fail to become associated with smooth muscle cells, which in this area of the embryo are derived from the neural crest. We hypothesized that the presence or absence of these neural crest-derived smooth muscle cells play a role in the remodeling of the aortic arches. We are testing this hypothesis by creating transgenic mice which overexpress, in the first two arches, genes able to induce differentiation into smooth muscle cells (like myocardin). Some of our preliminary data using double immunofluorescence and 3D reconstruction of the optical sections obtained with the confocal microscope, indicate that the presence of smooth muscle cells might indeed prevent aortic arch vessels from degenerate. We are further analyzing this issue and performing experiments to determine the molecular basis for this process.

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

MEMBERS: Moisés Mallo.

STUDENTS AND TECHNICIANS: Filipa Moraes, Ana Nóvoa, Joana Bom.

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 various derivatives. We have previously shown that Bmp2 is essential for the early steps of neural crest formation. In the absence of this gene no migratory neural crest cells can be detected. We now wanted to understand if Bmp2 was required for the induction of NCCs or for the migration of induced NCCs. Using a variety of neural crest markers we showed that neural crest progenitors are formed in the absence of Bmp2, indicating that Bmp2 activity might be required for migration of induced NCCs. A similar conclusion was reached from the analysis of a transgenic line that expressed RFP in the neural crest cells, when this line was introduced in the Bmp2 mutant background. While RFP-positive cells could be seen, thus demonstrating the production of NCCs, they remained in the neural tube. We could also show that the absence of migratory NCCs in Bmp2 mutant embryos is not due to their elimination by cell death. The neuroectoderm of these embryos fail to close and create abnormal folds both along the anterior-posterior and medio-lateral axes, which are associated with an apparent medio-lateral expansion of the neural tube. Despite this abnormal morphology, no major changes in the anterior-posterior patterning of the neural tube occurred in the absence of Bmp2 activity, as determined by the analysis of a variety of molecular markers. Perhaps the most interesting finding resulting from our experiments is that, while BMP signalling seems to be essential for early development of NCCs both in mouse and chicken embryos, the molecular cascade downstream of the BMP signal may be different in both vertebrates.

Zygotic transcriptional activation after egg fertilization in *Drosophila melanogaster*.

MEMBERS: Rui Martinho.

STUDENTS AND TECHNICIANS: Rui Tostões, André Rosa.

EXTERNAL COLABORATIONS: Jordi Casanova (IBMB-CSIC, Barcelona, Spain).

The embryonic developmental program of most multicellular organisms relies on maternally encoded gene products until the zygotic genome becomes transcriptionally active during midblastula transition (MBT). Although the processes that control transcriptional activation of the zygote are remarkably important, the molecular mechanisms controlling it are poorly understood.

In *Xenopus* and in *Drosophila* transcriptional activation of the zygotic genome occurs after a series of rapid nuclear divisions (without an increase of the total mass of the embryo). It has been proposed that the induction of MBT only occurs once a hypothetical nucleocytoplasmic ratio threshold is obtained. However, since in mammalian embryos there is a minor activation of the zygotic genome during late 1-cell stage and a major

activation during 2-cell stage, the nucleocytoplasmic ratio (N/C ratio) hypothesis is not likely to apply to mammals.

Drosophila melanogaster early embryonic development relies on maternally encoded gene products. In *Drosophila*, such maternal proteins and RNAs are loaded into the egg during oogenesis and they regulate multiple events during early embryonic development. After fertilization, as the number of nuclei rapidly increases, there is a maternal to zygotic transition in which the soma suddenly becomes transcriptionally active and many of the maternally encoded products are rapidly degraded. Since *Drosophila*'s maternal to zygotic transition (MZT) is known in other organisms as midblastula transition (MBT) we will hereby follow this denomination.

Although the bulk of zygotic transcriptional activation only occurs during interphase 14, a few genes (known as "early genes") are transcribed as early as nuclear division 9. The "early genes" known functions include sexual determination, embryonic patterning and cellularization. We hypothesize that the precocious expression of these genes results from the need of having their basic developmental roles in place before the embryo becomes competent for widespread transcriptional activation. Such sequential activation of transcription suggests that MBT is a highly regulated embryonic transition.

Transcriptional activation of the zygotic genome during MBT correlates with major changes in the chromosome and nuclear organization of the syncytial blastoderm nuclei. In *Xenopus* some transcriptional transactivators are bound to the chromatin even before the induction of MBT, which suggests that the repressed chromatin is transcriptionally committed even before the induction MBT. Yet, since in *Drosophila* the gap genes expression (e.g., *krüppel* and *giant*) are successively refined during blastoderm and since the pair rule genes expression is initiated in broad domains and only co-evolves with the expression of the gap genes during interphase 14, we hypothesize that during syncytial blastoderm the nuclei are initially transcriptional "naive" and lack some of the mechanisms required for the fine-tuning of gene expression.

We speculate that compaction of the oocyte nucleus during oogenesis and absence of transcriptional activity during the later stages of oogenesis and early syncytial blastoderm is likely to have a major impact in the RNA interference and epigenetic processes responsible for transcriptional regulation fine-tuning. Consistent with this possibility, we observed that syncytial blastoderm nuclei have reduced levels of chromatin modifications known to be important for transcriptional regulation (Rui Martinho, unpublished data). We propose that the transcriptional activation of the "early genes" during mid-syncytial blastoderm will facilitate the accumulation of the necessary epigenetic and RNA interference regulatory mechanisms that will correctly regulate gene expression during later stages of embryonic development.

Work done in the laboratory of Ruth Lehmann (NYU-Medical Center, USA), and further developed in our laboratory, allowed the identification of a large collection of maternal mutants defective for blastoderm cellularization. The isolated mutations affect the function of genes putatively required for nuclei division and/ or migration, transcriptional regulation, and membrane invagination and/ or establishment of cell polarity. Since transcriptional activation of the zygotic genome is required for blastoderm cellularization, we expect that some of the isolated mutants are likely to be defective for the induction of MBT.

The main objective of this research proposal is to study the molecular and cellular mechanisms responsible for the induction and accurate transcriptional activation of the quiescent zygotic genome after oocyte fertilization. We expect that a better understanding of these processes will shed light not only on a remarkably important developmental transition, but will also allow a better understanding of how are global transcriptional changes induced and coordinated.

Formation and morphogenesis of epithelial cells in *Drosophila melanogaster*.

MEMBERS: Rui Martinho.

STUDENTS AND TECHNICIANS: Ana Rita, Tânia Ferreira.

EXTERNAL COLABORATIONS: António Jacinto (IMM, Lisbon, Portugal).

During development a cell may be faced with the prospect of carrying out diverse cell biological processes simultaneously. If such processes are interdependent or potentially incompatible these events are likely to be tightly coordinated. For example, the cell division and cell migration machinery utilize much of the same cytoskeleton components. In *Drosophila melanogaster*, cell division is delayed until the prospective mesoderm invagination is completed. *tribbles* mutants fail to delay mitosis, resulting in the abortion of prospective mesoderm invagination.

In *Drosophila*, early embryonic development is syncytial and a polarized epithelium forms *de novo* through a process known as blastoderm cellularization. *Drosophila* embryonic development starts with thirteen nuclear divisions without cytokinesis. Until nuclear division 7 all nuclei are located in the interior of the embryo. The nuclei migrate outward to the egg periphery during nuclear division 8 and 9, with most nuclei arriving at the surface of the embryo during interphase 10. After four additional nuclear divisions, the cortical nuclei arrest mitosis during interphase 14. Once mitosis is arrested, the cortical nuclei become synchronously encased by polarized invaginations of the plasma membrane (furrow canals). During cellularization the furrow canals expand as the result of polarized insertion of newly synthesized plasma membrane, and adjacent cells begin to form polarized cell-cell contacts.

Induction of blastoderm cellularization only occurs once the nuclei have migrated to the cortical region of the embryo and arrested division. If they fail to reach the cortical region or fail to arrest division, this results in the abortion of cellularization. Since the development of *Drosophila* blastoderm is the result of several interdependent cell biological processes that are occurring simultaneously, or sequentially in an extremely short period of time, we hypothesize that active coordination of these processes is of particular importance for the viability of the embryo.

Work done in the laboratory of Ruth Lehmann (NYU-Medical Center, USA), and further developed in our laboratory, allowed the identification of a large collection of maternal mutants defective for blastoderm cellularization. Analysis of these mutants allowed the identification of at least 9 complementation groups. The isolated mutations affect the function of genes putatively required for nuclei division and/ or migration, transcriptional regulation, and membrane invagination and/ or establishment of cell polarity. One of the isolated complementation groups is allelic to *scraps*, which encodes Anillin. Anillin is a

conserved furrow component, required for cytokinesis in *Drosophila* and vertebrate tissue culture cells. Anillin is likely to play an important role in the coordination between the actin cytoskeleton and the blastoderm invaginating membranes.

Our main goal is to further elucidate the molecular and cellular mechanisms required for blastoderm cellularization. Achieving this goal will give an important contribution in the understanding of the processes controlling the cell cycle and the establishment of a polarized epithelium. We are particularly interested in the mechanisms responsible for the coordination of these two biological processes since they are likely to give an insight into the processes controlling epithelial-mesenchymal transition and carcinoma progression. We propose that given the unique developmental characteristics of *Drosophila* early embryogenesis these processes will be particularly amenable for genetic and biochemical dissection.

Notch in embryonic hematopoiesis.

MEMBERS: Leonor Parreira, Isabel Alcobia, Andrea Gomes.

STUDENTS: Pedro Saavedra.

While the importance of the Notch-pathway in post-natal hematopoiesis has been unequivocally demonstrated, its role in embryonic hematopoiesis remains largely unknown. During embryogenesis, hematopoiesis proceeds through sequential waves which take place in distinct tissue microenvironments (or niches). The first, appears in the blood islands of the extra-embryonic yolk sac (YS), is mostly made of primitive nucleated erythrocytes and apparently devoid of HSC capacity (long-term reconstitution of all myeloid and lymphoid lineages in adult recipients). Shortly after, pluripotent hematopoietic progenitors arise within the embryo in the aorta-gonad-mesonephros region (AGM). AGM hematopoietic progenitors are pluripotent, originating lymphoid, myeloid and definitive erythrocytes and have HSC potential. After a short period of AGM hematopoiesis, definitive and fully-functional HSC appear in the fetal liver, which will remain the major hematopoietic organ until birth, when hematopoiesis shifts permanently to the bone marrow. Loss of function studies indicate that the two earliest stages of hematopoiesis are differently affected by Notch-signaling. Absence of canonical RBP-Jk pathway does not prevent the onset of primitive erythropoiesis in the YS (rather increasing the number of erythroid colonies) while severely blocks definitive AGM hematopoiesis. The reasons for this difference are unknown.

In order to address this issue, we have made use of Embryoid-Bodies (EB) derived from Embryonic Stem Cells (ESC), an *in vitro* model known to faithfully reproduce *in vivo* embryonic hematopoiesis. The data show that Notch-signaling-related genes are dynamically and differentially transcribed in EB undergoing commitment into a hematopoietic program. A transient and abrupt up-regulation of Delta4 occurs at the transition of primitive to multi-lineage hematopoiesis (day 4), followed by up-regulation of Jagged2 and Jagged1 at day 6, when “definitive” hematopoiesis is already fully established. 3D analysis of developing embryoid bodies revealed that changes in gene transcription were accompanied by a progressive spatial segregation of EB-cells into regions of distinct cellular densities and molecular composition. These changes

culminated, at day 6, in the emergence of a discrete dense-cellular-region enriched in Notch 1, Notch 4, Jagged 1 and Jagged 2 and, also, in ESC-markers, as well as in “niche” molecules, such as osteopontin, heparan sulphates and beta-catenin. Early hematopoietic precursors were predominantly located in this region. Together, the data suggest that Notch-signaling might have a role in these early stages of hematopoiesis.

Accordingly, inhibition of γ -secretase activity or overexpression of a dominant-negative form of MAML1 (a co-activator of all Notch-receptors-mediated signaling) in developing EBS, was found to markedly increase the hemangioblast-potential at day 3 while severely decreasing multilineage hematopoiesis at day 6. However, the examination of functional read-outs of Notch-activity showed that conventional Notch-target genes are not up-regulated in the EB until days 5/6 and that biochemical evidence for canonical S3-cleavage of Notch receptors was barely detectable only at day 4, the transition from primitive to multilineage hematopoiesis. Furthermore, Notch receptors and ligands were rarely found at the cell membrane, rather being located in the cytoplasm in an endoplasmic reticulum and/or late-endosomal pattern. This, together with the observation that non-S3-cleaved forms of Notch were consistently present in the nucleus of EB-cells, makes it likely that Notch receptor processing in the early stages of embryonic development is distinct, yet functional, from that occurring at later stages. As a whole, the data suggest that Notch-signaling is active and regulates the earliest ontogenic step of hematopoiesis, the generation of the hemangioblast.

Manuscript in preparation: “Portrayal of the Notch system in an established *in vitro* model of yolk sac hematopoiesis: evidence for a functional role of Notch in the generation of the hemangioblast”. Alcobia I, Gomes AC, Saavedra P, Santos AP, Oliveira S, Laranjeiro R, Zilhão R, Parreira L and Cidadão A.

II – Embryoid Bodies derived from Delta 4 ^{+/-} Embryonic Stem Cells have reduced hemangioblast and hematopoietic potential (ongoing).

The observation that Delta4 expression was markedly and transiently up-regulated at day 4 of EB development (see above) makes this Notch-ligand a likely candidate for a functional role on the regulation of the transition from primitive to multi-lineage hematopoiesis.

To investigate this possibility, EBs were generated from ESC derived from mice heterozygous for a null (and haplo-insufficient) mutation of Delta4 (Duarte et al, *Genes & Dev.* 2004 18: 2474-2478) and, time-course clonogenic assays performed to assess their hemangioblast and hematopoietic potential.

The data show that Delta4^{+/-} EBs differ from their normal counterparts by a severe reduction (6-10 fold) in the number of hemangioblast colonies (BL-CFC) at day 3, followed by a less severe reduction in hematopoiesis from days 6 to 10 (2.2 for BFU-E; 1.7 for CFU-GEM; 1.6-fold reduction for CFU-G), though no morphological abnormalities were observed both in hemangioblast and hematopoietic colonies (in any cell-lineage). Moreover, the number of secondary EBs was also reduced, indicating that DI4^{+/-} EB have reduced stem cell capacity.

Since the hemangioblast gives rise to endothelial as well as to primitive hematopoietic cells the role of Delta 4 in the generation of endothelial cells was further assessed by 1)

replating individual wild-type and $DI4^{+/-}$ BL-CFCs in methyl-cellulose under endothelial-growth conditions, to determine their capacity to originate secondary colonies with adherent cells (endothelial) and, 2) by culturing $DI4^{+/-}$ (or wild-type) Flk-1⁺ cells sorted at day 3 (hemangioblast precursors) on matrigel in the presence of VEGF and bFGF (conditions that favor endothelial differentiation), followed by analysis of CD31 expression over time. In both experiments, the potential of $DI4^{+/-}$ cells to originate endothelial cells was similar to that of wild-type cells. Therefore, the data show that Delta4 is not essential for the specification of the hemangioblast from immature Flk-1⁺ cells, nor for its subsequent differentiation into endothelial and hematopoietic cells but is required to regulate the pool size of these cell lineages.

Experiments to address the mechanisms underlying these observations are in progress.

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

MEMBERS: Joaquín Rodríguez-León.

STUDENTS AND TECHNICIANS: Ana Raquel Tomás.

EXTERNAL COLLABORATORS: Juan Carlos Izpisua Belmonte (The Salk Institute for Biological Studies, La Jolla, CA, USA).

A signalling 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 signalling center need to be tightly regulated, both at the spatial and temporal levels. The vertebrate limb is paradigmatic in the study of signalling centers. Limb outgrowth requires first, the formation and maintenance of different signalling 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.

The role of ion dynamics during vertebrate limb development.

MEMBERS: Joaquín Rodríguez-Léon, Ana Catarina Certal Afonso.

STUDENTS AND TECHNICIANS: Joana Freire Monteiro.

EXTERNAL COLLABORATORS: José Feijó (IGC, FCUL), Alan Shipley (Applicable Electronics, USA), Joseph Kunkel (University of Massachusetts, USA).

Endogenous electric fields (EFs) are steady voltage gradients known to be present in living organisms for more than two centuries, but neglected since. The asymmetric nature of the ion distribution across membranes is the basis of membrane electrical potentials and specific ionic mechanisms have been recently shown to underlie a number of processes such as wound healing, tissue regeneration, early development, and cell proliferation. These mechanisms involve significant cellular activities of ion transport through the plasma membrane, where different ion transporters control ion dynamics in a tight and spatially-restricted manner, generating localized stable currents and interfering with downstream genetic cascades specifying developmental pathways.

Vertebrate limbs have for long been used as developmental model systems to study organ formation. During limb bud initiation, a cross-talk between the mesoderm and the ectoderm in the lateral side of the embryo has been described as critical. One suggested epigenetic mechanism for limb bud initiation involves endogenous electric current loops in the limb field. A steady current was shown to precede the formation of limb buds in mouse and chick embryos, and its artificial disruption results in abnormal limbs, which argues for an early physiological control in limb bud formation. It has been suggested that sodium could be involved in the generation and maintenance of this current but other ions may also prove to be critical and thus there are yet no conclusive studies at this point.

Endogenous currents are also associated with limb regeneration, which shares some features with normal limb development. Despite the numerous physiological studies, the underlying ions driving those currents remain unknown. The elucidation on the nature and functional significance of these currents will for sure open avenues for new therapeutic strategies and drug screenings.

With this project we want to unveil the physiological and molecular basis of endogenous ionic currents and cellular ion dynamics during vertebrate limb development and regeneration. For this purpose we will use mainly two developmental models: the chicken embryo as a general model due to its feasibility for manipulation, gene expression studies and imaging, and adult zebrafish as a regeneration model due to its genetic tools, namely the availability of mutants. We will describe in detail the ion dynamics in the flank of the chicken embryo just prior and during limb bud initiation, and during the regeneration of amputated caudal fins in fish. By using a unique combination of specific techniques, such as the ion-specific vibrating probe coupled with advanced ion imaging to measure ion fluxes and ion concentrations, respectively, we will generate a spatial and temporal map of the dynamics of certain ions like protons, calcium, potassium, chloride or sodium in both

the lateral side of the embryo and in regenerating stumps. Transgenic mice and fish overexpressing constitutively genetic-encoded ion probes will also be generated for ion imaging in deeper tissues. A major goal of this project is to find a link between the genes involved in limb bud initiation and the respective ion dynamics. After interfering with ion transporter activities or expression, in situ hybridisations will be performed at different time points for key genes. Fish mutants for these same transporters will also be analysed. As background for this project we have a set of preliminary data supporting the idea that, as very recently discovered in other developmental mechanisms, ions play a fundamental role in both organ formation and regeneration.

Evaluation of the ability of cardiac, bone marrow and embryonic pluripotent cells to functionally integrate into the myocardium.

MEMBERS: Luís Rosário.

STUDENTS AND TECHNICIANS: Isa Matos.

EXTERNAL COLLABORATORS: Doris Bachtrog (University of San Diego, California, USA).

The identification of cells, from the bone marrow and from the heart, that can divide and differentiate into cardiomyocytes has changed the paradigm of the heart as a post mitotic organ. This paradigm shift raises new questions concerning heart biology and physiology, as well as the physiopathology of cardiovascular diseases. This project focuses on detecting how newly formed cardiomyocytes integrate the myocardium, looking for the mechanic and electric coupling of cardiomyocytes in three ways: 1) identification of the cellular organelles essential for electromechanical coupling; 2) detecting if and when they become operative; 3) correlate invasive and destructive methods applied in the animal model, with non-invasive and non-destructive methods to detect the functional integration of newly formed cardiomyocytes.

As this paradigm shift starts to have therapeutic implications this project seeks to observe the interplay between stem cells and a disease processes in which they may be applied: myocardial infarction

Functional study of the molecular clock during vertebrate limb development.

MEMBERS: Leonor Saúde and Susana Pascoal.

STUDENTS AND TECHNICIANS: Andreia Pinto and Paulo Raimundo.

EXTERNAL COLLABORATORS: Isabel Palmeirim (Universidade Minho, Braga, Portugal).

A molecular clock that controls periodic somite formation from presomitic mesoderm is operating in vertebrate organisms, such as chick, mouse and zebrafish. Several lines of evidence gathered from cell culture experiments suggest that this molecular clock may not be an exclusive property of presomitic cells, but it might rather be a general mechanism providing cellular temporal information. In fact, there is now evidence that the molecular clock operates in the chick forelimb bud strongly suggesting a role during limb formation. The tetrapod forelimb bud is considered the vertebrate homologue of the zebrafish pectoral fin bud, since they share structural organization and gene expression repertoires. We

propose to take advantage of the zebrafish as a genetic tractable system to dissect the functional role of the molecular clock during vertebrate limb development.

Determination of the expression patterns of the clock-related genes her7, deltaC and notch1a during stages of pectoral zebrafish fin bud development.

Fin bud development in zebrafish occurs from 24 to 72 hours-post-fertilization (hpf) at the standard temperature of 28.5°C. Embryos were fixed at several time points within the 24-72 hpf interval in 4% paraformaldehyde (PFA), dehydrated in methanol and stored at -20°C. These embryos were analyzed by whole-mount mRNA *in situ* hybridization using specific probes for the *her7*, *deltaC*, and *notch1a* genes, according to the protocol described by Thisse and Thisse (1998). The early pectoral fins arise at 24 hpf as a small aggregate of mesenchymal cells at the level of the third somite. At this level a weak expression of *her7*, *deltaC* and *notch1a* can be observed in the entire fin bud. At 31 hpf the apical epidermal cells lining the anterior-posterior axis (A-P) of the bud thicken to form a transient ridge which is similar to the tetrapod apical ectodermal ridge (AER), one of the signaling centers of the limb bud. Another signaling centre of the tetrapod limb bud is the zone of polarizing activity (ZPA) also present at this stage in the posterior part of the zebrafish fin bud. By 34 hpf, the apical epidermal cells undergo a morphological change, detach from the underlying mesenchyme and progressively form an epidermal fold separated by a sub-epidermal space. As the fin buds grow, the first skeletal elements start to condensate by 37 hpf in the centre of the fin bud, and will give rise few hours later to the cartilaginous endochondral disk. At 48 hpf, the epidermal fold starts to elongate and mesenchymal cells start to invade the structure. *her7*, *deltaC* and *notch1a* have a strong expression pattern at 34 hpf to 50 hpf. *her7* and *deltaC* are expressed at these stages in the ZPA, AER, mesenchymal cells, endochondral disk and fin fold. *notch1a* at 34 hpf is expressed in all fin bud, but at 46 hpf this gene is specifically expressed in two stripes of cells at the proximal part of the fin. This expression pattern mimics the expression of *myoD*, a marker of the muscle cells of the fin. The expression of these genes is no longer detected at later stages (72 hpf).

Evaluation of the dynamic behavior of the clock-related genes her7, notch1a and deltaC in the pectoral fin bud.

The whole-mount mRNA *in situ* hybridization with the clock-related genes *her7*, *deltaC* and *notch1a* allow us to determine if the expression patterns obtained in embryos at the exactly same stage of development show differences at the level of the pectoral fins. The expression patterns observed for *her7* and *deltaC* are indeed different for the same stage of development; we can found two types of expression pattern for fin buds hybridized under precisely the same conditions. In some embryos the entire fin is labelled, while in others, *her7* and *deltaC* expression can only be observed at the level of the proximal domain. These differences in embryos at the same stage can be observed within the 36 hpf to 50 hpf interval. Thus, we can conclude that these genes have a dynamic expression at the level of the fins. However, the expression pattern observed for *notch1a* does not

have this dynamic behavior. The next step is to find the cycle periodicity of the *her7* and *deltaC* dynamic expression.

Analyze the fin phenotype of the deadly seven (notch1a^{-/-}), beamter (deltaC^{-/-}) and mind bomb (E3 ubiquitin ligase^{-/-}) zebrafish mutants.

To characterize the fin cartilaginous pattern in these mutants we performed a skeletal staining of fins beginning at developmental age of 72 hpf to 6 days of zebrafish development. Embryos were staged, fixed, dehydrated and stained for 5 hours to overnight with Alcian Blue. After removal of the pigment with H₂O₂ in KOH, the muscles were rendered transparent by digestion with trypsin. Fish were stained overnight with Alizarin Red and stored after transferring to an ascending series of KOH/glycerol. The mutants *deadly seven (notch1a^{-/-})* and *beamer (deltaC^{-/-})* have normal fins compared with the wild type (WT). The *mind bomb (E3 ubiquitin ligase^{-/-})* mutant has fins smaller than the WT and the cells of the endochondral disk and fin fold seem to be mixed.

The role of Notch signalling in the determination of left-right identity during zebrafish development.

MEMBERS: Leonor Saúde and Susana Lopes.

STUDENTS AND TECHNICIANS: Luís Pacheco, Andreia Pinto and Paulo Raimundo.

Zebrafish mutants of the Notch signalling pathway show defects in the expression of typical left-sided markers such as *nodal*, *lefty2* and *pitx2*. In order to clarify these abnormal expression patterns we have made a detailed study of the morphogenesis/ciliogenesis of the Kupffer's vesicle (KV). This transient organ has been identified as having an equivalent role to the mouse node in the fish left-right determination as it is also formed by ciliated cells that generate a leftward flow. Some of these morphogenesis/ciliogenesis phenotypes point to early problems in the fate specification of the precursors of the KV

Analysis of the degree of conservation of Terra function and its direct targets.

MEMBERS: Leonor Saúde.

STUDENTS AND TECHNICIANS: Raquel Lourenço and Sara Pereira.

It has already been described that *terra* knockout in the mouse leads to defects in late somite differentiation. Nevertheless, nothing is known about the effect on LR patterning and segmentation clock synchronization.

We have obtained from David Zarkawer's laboratory *terra* heterozygous mice, which are being crossed in order to collect the *terra* homozygous mutant embryos. Once we have them, we will check for the axial rotation, heart looping and laterality of the lungs, liver and stomach. Probes for genes known to be involved in the LR patterning such as *nodal*, *cerl2*, *lefty1*, *lefty2* and *pitx2* are being synthesised so that their expression can be addressed in the mutants.

We will also determine if, like in zebrafish embryos injected with *terra*-morpholino, in *terra^{-/-}* mice embryos there are problems in early somite formation. *In-situ* hybridizations will be performed with the somite marker *uncx4.1*, to address the number of somites between the

left and right sides, and also with the cycling genes *hes1*, *hes7*, *lunatic fringe*, *axin2*, *nkd1* and *snail*, to address for a possible desynchronization of their expression between both sides of the PSM.

Define and compare cell migration patterns between the left and the right side of Hensen's node.

MEMBERS: Leonor Saúde.

STUDENTS AND TECHNICIANS: Raquel Mendes, Gabriel Martins.

We wanted to determine if the morphological and molecular asymmetries of a stage 5(HH) Hensen's node influence the pathways and velocity of ingressing cells. We compared the migratory pathways of small populations of PSM and somites precursors located on the left vs. on the right side after labelling with vital dyes. Our results show that at 6 hours-after-labelling the precursor cells on the left reached a more anterior position when compared with the precursor cells located on the right side of the node. This suggests that the morphological and molecular asymmetries within the node are translated into asymmetries in migration.

Transcriptional regulation and function of chick Cerberus during embryonic development.

MEMBERS: Ana Teresa Tavares, José António Belo.

STUDENTS AND TECHNICIANS: Ana Cristina Silva, Natacha Marreiros.

The apparent symmetry of the vertebrate body disguises profound asymmetries in the development and placement of internal organs. Remarkably, the laterality of organs within the body cavity is conserved in all vertebrate species studied to date, which suggests that the determination of the left-right axis is controlled by a highly conserved pathway. The TGF-beta-related molecule Nodal plays an essential in left-right patterning of all vertebrate embryos. Previous reports have shown that zebrafish and mouse Cerberus-related proteins Charon and Cerberus-like-2 (Cerl-2), respectively, act in the node region to prevent Nodal signal from crossing to the right side, whereas chick Cerberus (cCer) has an unclear function in the left side mesoderm. In this study, we investigated the transcriptional regulation and function of chick Cer in left-right development. By analyzing the enhancer activity of *cCer* 5' genomic sequences in electroporated chick embryos, we identified a *cCer* left-side enhancer that contains two FoxH1 and one SMAD binding site. We have shown that these Nodal-responsive elements are necessary and sufficient for the activation of transcription in the left side mesoderm. In transgenic mouse embryos, *cCer* regulatory sequences behave as in chick embryos, suggesting that the *cis*-regulatory sequences of *Cerberus-related* genes have diverged during vertebrate evolution. Moreover, our findings from *cCer* overexpression and knockdown experiments indicated that cCer is a negative feedback regulator of Nodal asymmetric signaling. We propose that chick Cer and mouse Cerl-2 have evolved distinct regulatory mechanisms but retained a conserved function in left-right development, which is to restrict Nodal activity to the left side of the embryo.

Characterisation and applications of a novel hemangioblast-specific transcriptional enhancer.

MEMBERS: Ana Teresa Tavares, Vera Teixeira, Gabriel G. Martins.

STUDENTS AND TECHNICIANS: Natacha Marreiros.

During early embryogenesis, both hematopoietic and endothelial lineages derive from aggregates of mesodermal cells that form the blood islands in the extraembryonic yolk sac. This observation has led to the hypothesis that both lineages originate from a common precursor known as the hemangioblast. During the study of *cCer* transcriptional regulation in chick embryos, we isolated short *cis*-regulatory regions that are able to drive the expression of the *eGFP* reporter gene specifically in hemangioblast precursors and blood islands. The sequence analysis of those DNA fragments identified several binding sites for transcription factors implicated in vasculogenesis and hematopoiesis, including GATA and ETS. Based on these observations, we are analysing the regulation of this hemangioblast-specific enhancer and using it in lineage tracing and embryonic stem cell differentiation studies of vascular and blood cell development. In the future, the hemangioblast-specific reporter may become a valuable tool for the screening of agents or genetic mutations that perturb vascular and blood cell development as well as for hemangioblast-targeted gene therapies.

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

MEMBERS: Sólveig Thorsteinsdóttir, Gabriela Rodrigues, Gabriel G. Martins.

STUDENTS AND TECHNICIANS: Pedro Rifes, Catarina Lopes, Rita Amândio.

EXTERNAL COLLABORATORS: Isabel Palmeirim and Raquel Andrade (Escola das Ciências de Saúde, Universidade do Minho, Braga, Portugal); Leonor Saúde (Instituto Gulbenkian de Ciência, Oeiras, Portugal), Anne-Gaelle Borycki (University of Sheffield, Sheffield, UK).

Somites are transient epithelial segments laid down early in vertebrate development. 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. During this transition, the composition of the ECM surrounding somitic cells changes. Very little is known about what factors control this ECM turnover and even less is known about how this ever-changing ECM influences the differentiation state, the shape and the locomotive behaviour of the cell it interacts with. Moreover, although knowledge of how paracrine signalling and transcription factors control the segmentation and formation of somites is now advanced, almost nothing is known about how they regulate, and are regulated by, the ECM and ECM receptor mediated signalling.

We are using high resolution 3D imaging/analysis techniques to characterise the adhesive properties of cells and the organisation of their surrounding ECM as they progress from the PSM towards being incorporated into epithelial somites. We also focus on how different cell types cooperate to produce the ECM, how cells respond to contact with different types of ECMs, and what receptors of the integrin family are involved. Our results show that the PSM expresses the fibronectin matrix assembly receptor, integrin $\alpha 5 \beta 1$, whereas

ectoderm is practically the only tissue to express fibronectin mRNA. We performed experiments to demonstrate that the “ectoderm factor”, known for many years to promote somitogenesis in PSM explants, is not a diffusible factor, but is most likely fibronectin protein, necessary to build the fibronectin matrix of the PSM (Rifes et al., 2007). Further ongoing research focuses on producing 4D films of somitogenesis and identifying what cellular movements and cell shape changes are involved in the transformation of the anterior PSM into somites. Furthermore, we are performing experiments to address what signalling pathways are activated upon ECM engagement by different integrins in the PSM.

More than putting cells into place during development: cell-extracellular matrix interactions in myogenesis.

MEMBERS: Sólveig Thorsteinsdóttir, Gabriela Rodrigues, Gabriel G. Martins

STUDENTS AND TECHNICIANS: Luís Marques, Ana Gaspar

EXTERNAL COLLABORATORS: Fernanda Bajanca (ICVS, University of Minho, Portugal); Anne-Gaelle Borycki (University of Sheffield, Sheffield, UK); Margaret Buckingham, (Pasteur Institute, Paris, France).

In the last decade, a strong research effort has been put forth to unveil the genetic control of muscle development. This research is not only important for the understanding of the origin and progression of muscle dystrophies but is essential for the design of successful therapies to correct these crippling and sometimes fatal diseases. The building of muscles from myogenic precursor cells is a complex process and understanding how these cells arise in the embryo and how they interact to produce functional muscle is of outmost importance. A lot of progress has been made in the last decade and the genetic hierarchies involved in muscle determination and differentiation are now well known. The next step is to understand how these genetic hierarchies translate into alterations in cell behaviour, such as migration, elongation and fusion; all essential processes in muscle formation. However, practically nothing is known about how the genes activated upon myogenic determination (the Myogenic Regulatory Factors, MRFs) activate downstream targets involved in modulating cell behaviour.

The obvious candidates to support changes in cell shape and motility are molecules of the extracellular matrix (ECM). Cells interact with the ECM through transmembrane receptors, called integrins. Integrins are not only involved in ECM recognition and binding, but also trigger a variety of intracellular signalling pathways which affect cell behaviour. Although integrins and their ligands have been shown to play a role in advanced phases of muscle development, the knowledge about their functions in early myogenic events is still relatively scarce. In addition, a bridge between the paracrine or genetic control of myogenesis and cell behaviour through integrin or ECM modulation is now starting to be established.

In recent years, our group has focused on the study of cell-ECM interactions during myogenesis in the embryo. In this project, we have established three major aims:

1. determine to what extent integrin expression patterns can be used as markers for the different populations involved in producing skeletal muscle;
2. extend our knowledge on what signalling pathway is activated by the $\alpha 6 \beta 1$ integrin

during epaxial myotome formation and how this pathway relates to better known pathways (Wnt and Shh) linked to epaxial myogenesis;

3. determine what cell-ECM interactions are involved in the much less known hypaxial (ventral and limb) myogenesis.

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 comodity of the reader. A good example of this contention is the fact that this sector of the IGC’s activities could well be “dissolved” in several others, or else, include various projects listed under other headings (Stress and Inflammation, Developmental Biology, etc.). Yet, this grouping aims at underlining that several apparently diverse interests converge in cytoskeleton structure, dynamics and functions.

Integrative study on centrosome biogenesis and function.

MEMBERS: Mónica Bettencourt-Dias and José Pereira-Leal.

STUDENTS AND TECHNICIANS: Zita Santos.

Centrosomes play a very important role in cell physiology, being involved in a multiplicity of disorders. However, their biogenesis and function are poorly understood. We are using integrated approach to this problem, combining computational data mining with experimental studies in the versatile model organism *Drosophila melanogaster* and human tissue culture cells. We are taking advantage of the recent outburst of genomic and proteomic data to build a comprehensive annotated list of centriole-associated genes, predict novel components and regulators. These structures are present in every branch of eukaryotes suggesting a “core” conserved molecular mechanism for centriole biogenesis. We used comparative genomics and phylogenetic analysis to study the evolution of proteins known to be involved in centriole assembly. In *C.elegans*, SPD-2 is the first protein to be recruited to the centrosome and is responsible for ZYG-1 recruitment. This protein in turn recruits a complex of SAS-6 and SAS-5, necessary for the formation and elongation of a central tube. Posterior assembly of the microtubules onto the central tube is dependent on SAS-4. We mapped new putative orthologs of these proteins throughout the tree of life, including flagellated fungi, whose genome is currently being sequenced. We used sensitive search methods, developed classification criteria for each protein, and combined these with detailed phylogenetic analysis for orthology assignments. Our results show that the presence of proteins directly involved in structural aspects of centriole assembly, such as SAS-6 and SAS-4, correlates with the existence of centrioles. Strikingly, “triggers”/“regulators” of centriole duplication, such as SAK/PLK4, appeared late in evolution, in flagellated fungi and animals. This suggests that SAK/PLK4 role was previously fulfilled by another kinase, perhaps the founder of its family, Polo. Furthermore, it suggests that the appearance of SAK/PLK4 may be associated with linking centriole duplication to other aspects of centriole and cellular physiology. Other proteins such as

SPD-2 or CP110 on the other hand seem to be defining other functions of these structures on specific branches of the tree of life.

Dissecting the role of SAK kinase in *denovo* and templated centriole biogenesis.

MEMBERS: Mónica Bettencourt-Dias.

STUDENTS AND TECHNICIANS: Ana Rodrigues-Martins and Inês Bento.

EXTERNAL COLLABORATORS: David M Glover (University of Cambridge, UK), Giuliano Callaini (University of Siena, Italy).

SAK/PLK4 is a distinct member of the polo-like kinase family. We have recently reported that *Drosophila* cells progressively lose centrioles, the core structure of centrosomes, following downregulation of SAK by mutation or RNAi. SAK mutants lose their centrioles during the mitotic divisions preceding male meiosis but still produce cysts of 16 primary spermatocytes as in wild-type. Mathematical modelling of the stereotyped cell divisions of spermatogenesis can account for such loss by defective centriole duplication. Depletion of SAK in human cells prevents centriole duplication and gives rise to mitotic abnormalities, pointing to a conserved role of this protein in centrosome formation. To further dissect the function of SAK in centriole biogenesis we have overexpressed this kinase in both S2 cells and in *Drosophila* embryos. Overexpression of SAK leads to centrosome amplification. SAK overexpression during oogenesis leads to amplification of centrosomes in the embryo with developmental arrest. Both oogenesis and early embryogenesis are normal with centrosome amplification occurring at the end of first mitosis. Interestingly, fertilization is not needed for centrosome amplification to occur; indicating that overexpression of SAK can lead to *de novo* centrosome formation. We have explored this result to further understand the role of SAK in templated and *de novo* centrosome formation. Differences in the pattern and timing of centrosome appearance in embryos and unfertilized eggs are consistent with their different origins. Hence, we have shown that SAK is a master regulator of centrosome biogenesis, being involved in both templated and *de novo* centriole formation. We are now further investigating the regulation of SAK activity in canonical and *de novo* biogenesis.

Regulation of SAK/PLK4 protein levels throughout cell cycle.

MEMBERS: Mónica Bettencourt-Dias.

STUDENTS AND TECHNICIANS: Inês Cunha-Ferreira.

EXTERNAL COLLABORATORS: David M Glover (University of Cambridge, UK).

Centrosomes are the major microtubule organising center (MTOC) of animal cells, contributing to accurate chromosome segregation, amongst other processes. Centrosomes duplicate once per cell cycle in coordination with DNA replication. Duplication of this structure is triggered and dependent on the activity of a conserved kinase, SAK/PLK4. We have demonstrated that regulation of SAK/PLK4 protein levels is critical to restrict its function, preventing centrosome amplification. We have shown that SAK/PLK4 is degraded by the proteasome, via the SCF/Slimb ubiquitin ligase. This complex physically interacts with SAK/PLK4, and in its absence, SAK/PLK4 levels accumulate, leading to a SAK/PLK4-

dependent increase in centrosome number. Our results provide a mechanism for the control of centrosome copy number, opening new avenues for understanding centrosome amplification observed in human diseases. We are further investigating how SAK levels are controlled throughout the cell cycle, in particular whether other enzymes cooperate with the SCF complex to target SAK for degradation.

The Role of D-SAS-6 in the regulation of centriole biogenesis.

MEMBERS: Mónica Bettencourt-Dias.

STUDENTS AND TECHNICIANS: Ana Rodrigues-Martins and Inês Bento.

EXTERNAL COLLABORATORS: David M Glover (University of Cambridge, UK), Inês Ferreira, (University of Cambridge, UK), Giuliano Callaini (University of Siena, Italy).

It is clear from recent studies, that a group of proteins discovered in *C.elegans*, which includes ZYG-1, SAS-4, SAS-5 and SAS-6, is part of a centriole-assembly protein module. SAS-6 is a major player in this process; its absence leads to a lack of centriole duplication in worms and humans. It is crucial to identify how SAS-6 is regulated and what its function is in order to understand centriole biogenesis. Our previous studies have shown that SAK kinase, the *Drosophila* and human orthologue of ZYG-1, is also an essential player in centriole duplication. Our data suggests that SAS-6 functions downstream of SAK. We are using a variety of cell biology, genetic and biochemical approaches to study the function and regulation of SAS-6.

G2/M regulation by the polarity-related kinase Par-1.

MEMBERS: Mónica Bettencourt-Dias.

STUDENTS AND TECHNICIANS: Joana Lamego.

EXTERNAL COLLABORATORS: David M Glover, University of Cambridge, UK, Daniel St Johnston, University of Cambridge, UK and Helene Doerflinger, University of Cambridge, UK.

Par-1 is a conserved serine-threonine kinase that mediates the mechanism of cell polarity in a variety of animal species. Interestingly, mutations of Par proteins in *C.elegans* not only affect the polarity of the embryo but also lead to loss of cell cycle asynchrony in the first mitotic divisions. However it is not clear how the polarity and cell cycle progression phenotypes are connected. In an attempt to identify protein kinases required for cell cycle progression we found that depletion of the Par-1 protein in *Drosophila* tissue culture cells leads to a delay in the G2/M transition of the cell cycle. Our results show that depletion of Par-1 leads to a 30% increase in the population of cells with 4N DNA content. We have used a variety of cell cycle markers to characterise this phenotype showing that these cells are diploid, being delayed in the G2/M transition. There are several isoforms of this kinase, being only one of them responsible for establishing cell polarity in *Drosophila*. We investigated which isoforms are involved in cell cycle progression, using gene silencing through RNAi, specific for each one of the isoforms. Curiously, the isoform responsible for the G2 delay is the same involved in cell polarity. We are now further investigating how Par-1 regulates cell cycle progression, in particular whether it plays a role upstream of

negative G2/M regulators, such as Wee1 and Myt1. An understanding of the role of Par-1 in cell cycle progression and how it relates to its function in cell polarity may shed light on the relationship between those two processes and how they contribute to tumorigenesis.

Intracellular cross talk between mitosis and apoptosis.

MEMBERS: Sukalyan Chatterjee.

STUDENTS AND TECHNICIANS: Ana Girio, Ana Lucia Mena.

This project has identified Erk5 as a survival factor in mitosis. In mitosis Erk5 is activated by phosphorylation and possibly not via the known cognate residues. Ablation of activation of Erk5 in mitosis causes cell death in a caspase dependent manner. The project is continuing with the aims to identify the upstream signaling cascade(s) leading to the phosphorylation of Erk5 and the downstream targets conferring survival in mitosis

Intercellular Cross-talk between Neurons and microglia.

MEMBERS: Sukalyan Chatterjee, Teresa Faria Pais

STUDENTS AND TECHNICIANS: Catarina Figueiredo, Carlos Araujo.

Glial cells and neurons are in constant reciprocal signalling both under physiologic and neuropathologic conditions. Microglia are resident macrophage-like cells that mediate innate and adaptive immune responses within the central nervous system (CNS) following stress and injury. Microglial activation is often associated with neuronal death during inflammation of the central nervous system, although microglia is also known to exert a neuroprotective role. This project, has investigated the interplay between neurons and microglia in the perspective of neuronal survival. The microglial conditioned medium (MCM) from stimulated microglia show increased neurotoxicity mediated through N-methyl-D-aspartate receptor (NMDAR). Neurons can reduce this toxicity by diffusible factor(s) which modulate the transcription of glutaminase, an enzyme that produces glutamate, an agonist of the NMDAR. This enzyme also plays a role in necrotic neuron mediated activation and neurotoxicity of microglia. These findings may have implications to our understanding of microglial neurotoxicity during injury associated with neuroinflammation.

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

MEMBERS: Lisete Fernandes.

STUDENTS AND TECHNICIANS: Joana Monteiro, João Coelho.

The Yap family of bZIP transcriptional factors in *Saccharomyces cerevisiae* contains members that are central players in cellular responses to stress challenges such as: Yap1 in oxidative and cold signals. From this point of view, Yaps constitute an excellent tool to address the cross-talk of signaling pathways, in particular, the cross-talk of cold and oxidative signals. In addition to its involvement in nuclear-cytoplasmic trafficking of transcription factors, the cytoskeleton is a well-known network target of oxidative and cold signals. Exploiting whether the cross-talk of cold and oxidative signaling pathways occurs

at the level of such network led to insights on the role of specific cytoskeleton components on transcriptional regulatory events upon oxidative signals.

Molecular mechanisms bridging protein folding and transcription activation.

MEMBERS: Lisete Fernandes.

STUDENTS AND TECHNICIANS: João Coelho; Susana Broa.

GimC, also designated prefoldin, is a cytoplasmatic complex composed of 6 distinct subunits (Gim1-Gim6 and Pfd1-Pfd6 in *Saccharomyces cerevisiae* and mammals, respectively) that promotes the formation of functional actin and tubulin, essential components of the cytoskeleton. GimC binds to nascent chains of its substrates, delivering them, after completion, to the chaperonine TRiC/CCT for efficient folding. However, the Gim proteins are not essential and data in the literature suggest alternative functions for several of the GimC subunits, e.g., archaeobacteria contains a complex homologue of prefoldin and inherent substrates do not include actin/tubulin. Results obtained with *gim*-mutants indicate different activities of each Gim subunit within the cell. The cell's ability to regulate global gene transcription was found to be functional. However, a deficiency in regulation of specific gene expression, in null mutants of γ -like subunits, prompts for a particular importance of these subunits, namely under stress conditions. Analysis of molecular targets for this deficiency is under study.

End-protection and DNA repair at *S. pombe* telomeres.

MEMBERS: Miguel Godinho Ferreira, Tiago Carneiro.

STUDENTS AND TECHNICIANS: Hugo Almeida, Miguel Teixeira.

Telomeres, the natural chromosome ends of eukaryotes, have unique properties that distinguish them from damage-induced DNA ends. Most human somatic cells lack telomerase, the enzyme responsible for generating telomeres. Proliferation of cells lacking telomerase leads to telomere depletion and ultimately to telomere loss. When telomere function is lost, chromosome ends are treated as DNA breaks that, in most cases, leads to cell death. If the cell survives, chromosomes will be joined by their ends yielding dicentric entities that can break upon mitosis. This, in turn, causes unequal distribution of genetic information to daughter cells as well as to the formation of new unprotected ends. The ensuing genomic instability is thought to be involved in the development of cancer. The proposed work uses the fission yeast (*Schizosaccharomyces pombe*) as a model system. Studies in fission yeast have proven fruitful in identifying fundamental principals in several biological processes, particularly those concerned with cell cycle regulation. Taz1 protects chromosome-ends from being recognized as deleterious breaks and used as substrates of DNA repair. The outcome of these processes at telomeres varies greatly through the cell cycle, leading to chromosome-end fusions and lethality in G1 and chromosomal rearrangements in G2. Our aims will be to (1) find new participants and further characterize the known components responsible for chromosome-end protection and (2) understand the principles governing dysfunctional telomeres as substrates for DSB repair.

Inhibition of genomic instability by telomere protection.

MEMBERS: Miguel Godinho Ferreira.

STUDENTS AND TECHNICIANS: Hugo Almeida.

We plan to identify and characterize new components responsible for chromosome-end protection in a screen based on a novel assay. Previous attempts to discover proteins involved in telomere protection have been based on telomere functions unrelated to end-protection (such as subtelomere gene silencing). In contrast, the strategy proposed in this study is designed to directly capture telomere-telomere fusions. Novel proteins and regulatory principals uncovered in this work are likely to be conserved. Understanding how telomeres are protected from DNA repair and, in its failure, how the cells respond to unprotected telomeres will provide insights to the initial stages of tumourogenesis. Identification of the key players in these events will novel targets for cancer therapy and early detection.

Identification of anti-checkpoint proteins at fission yeast telomeres.

MEMBERS: Miguel Godinho Ferreira, Tiago Carneiro.

STUDENTS AND TECHNICIANS: Vanessa Borges.

Telomeres, the natural chromosome ends of eukaryotes, have unique properties that distinguish them from damage-induced DNA double strand breaks (DSBs). Whereas a single DSB can halt cell cycle progression and activates repair processes, telomeres prohibit checkpoint activation and DNA repair, which leads to chromosome-end fusions - a source of genomic instability and a step in tumourigenesis. Recent work has revealed an apparent contradiction: although telomeres prohibit checkpoints and DNA repair, several components involved in these processes are, not only present at telomeres, but also critical for normal telomere function. Thus, similar to deleterious DNA ends, telomeres recruit potentially dangerous proteins to chromosome-ends. The mechanism whereby telomeres prevent checkpoints and DNA repair at chromosome-ends remains to be elucidated. I propose to identify and characterize the mechanism that protects telomeres from being recognized as damaged DNA. I have generated two novel assays that will allow me to test directly whether a protein or a process is required for telomere protection. The first will allow me to screen for components that are involved in preventing chromosome-end fusions or DNA damage recognition. The second assay will test whether these components are sufficient to confer telomere-like protection to a newly formed DSB. Understanding how telomeres protect chromosome-ends from DNA damage checkpoints and repair and, in its failure, how the cells respond to unprotected telomeres will provide insights to the initial stages of tumourogenesis. Identification of the key players in these events will provide specific candidate targets for therapeutic intervention and possible tools for early diagnostic.

Using *Drosophila* to understand the molecular control of actin dynamics in aberrant active cell migration.

MEMBERS: Florence Janody and Beatriz García Fernández.

STUDENTS AND TECHNICIANS: Sofia Raquel Rebelo.

EXTERNAL COLLABORATORS: Helena E. Richardson, Peter MacCallum (Cancer Institute, Melbourne, Australia).

Most solid tumors arise within the confine of normal epithelia. Aggressive cancer is a multistage process, which involves disruption of polarised epithelial architecture, loss of proliferation control, resistance to cell death and invasion/metastasis. These transitions can be trigger when neoplastic Tumor suppressor genes (nTSG) are mutated or when the dynamics of the actin microfilament system is affected. Our goal is to get insights on how the actin cytoskeleton is regulated by Actin Binding proteins (ABP) during tumor progression. We are taking advantage of *Drosophila* genetics, which permits to relatively easily manipulate cancer-related genes, such as nTSG, in the context of intact epithelia, combine to a large scale genomics approach, to:

Task 1: Determine how the filamentous actin network is organized and the activity of ABP regulated at different steps of the tumoral proceess, induced by the loss of nTSG.

Task2: Identify new ABP that prevent or promote abnormal cell migration of *lgl* mutant cells.

Task 3: Characterize the transcriptome dynamics of this tumoral process, at different steps of the invasive process.

Cytoskeleton organization is differentially regulated in genetically distinct epithelia: The case of the Capping Protein heterodimer.

MEMBERS: Florence Janody.

STUDENTS AND TECHNICIANS: Sofia Raquel Rebelo, Mauricia Vinha, Pedro Miguel Gaspar, Nuno Miguel Palha.

Abstract: Actin is one of the most abundant and highly conserved proteins in Eukaryotes. Actin monomers can polymerize into filaments, forming the actin cytoskeleton. In addition to control numerous processes, such as the generation and maintenance of cell morphology and polarity, endocytosis, intracellular trafficking, contractility and cell division, the actin microfilament system is considered to be the engine of cellular migration. Actin filaments growth, stability, disassembly and also their organization into functional higher-order networks are controlled by a plethora of actin-binding proteins (ABP), strongly conserved between species. Most studies to address how the actin microfilament system is regulated used in vitro assays or cells in culture. However, in multicellular organisms, the role of a particular actin isoform and its regulation within a tissue remain obscure.

Several lines of evidence strongly suggest that the different actin isoforms, in addition to the ABP that regulate their dynamics, have specialized functions depending of the tissue context. Indeed, we found that the Capping Protein heterodimer (CP), which inhibits the addition and loss of actin monomers to the filament (+) end, prevents epithelium to mesenchymal-like transition and cell extrusion only in a subset of epithelia. This suggests that cytoskeleton organization is tightly regulated in genetically distinct epithelia. Extrusion of *cpa* mutant cells is dependant of the Vestigial (Vg) transcription factor, which specify the wing blade epithelium and enhances transcription of *cpa* in this region. Thus, one function of Vg is to specify the cytoskeletal changes that lead to morphogenesis of the adult wing.

In order to get some insight on how cytoskeleton organization is regulated in genetically distinct epithelia to achieve distinct morphological outcomes and to promote a framework of sufficient strength to withstand forces that place tension on a single cell within epithelia, we are investigating:

Task 1: What are the primary consequences of CP loss in genetically distinct wing disc epithelia.

Task 2: Identify the Vestigial target genes that specify cytoskeleton changes by oligonucleotide microarrays.

Task 3: Identify which domains of CP mediate CP functions in diverse epithelia by performing a structure function analysis of the CP heterodimer in transgenic flies.

Task 4: Identify molecules that regulate the activity or the sub-cellular localization of CP.

Study of the function and the regulation of tubulin cofactor A in mammalian cell lines and during mouse development.

MEMBERS: Helena Soares.

STUDENTS AND TECHNICIANS: Sofia Nolasco, João Gonçalves.

EXTERNAL COLLABORATORS: Juan Zabala (Facultad de Medicina, Departamento de Biología Molecular, Universidad de Cantabria, Spain) and Jesus Del Mazo (Centro de Investigaciones Biológicas-C.S.I.C., Madrid, Spain).

Tubulin cofactors (TBCA-E) are proteins involved in the process of tubulin folding and the production of α/β -tubulin heterodimers, the building blocks of microtubules (MTs). The function of these proteins was established mainly by tubulin *in vitro* folding assays, and their precise role *in vivo* has remained elusive. However, there are increasing evidences indicating that the tubulin cofactors may play a role in the regulation of MT cytoskeleton dynamics.

In mammals TBCA is encoded by one gene with introns as described in the data bases. In mouse the *Tbca* gene is localized in the chromosome 13 (TBCA13). However, we identified a second *Tbca* transcribed gene in chromosome 16 (TBCA16) which structure suggests a retro transposition origin.

TBCA13 is more abundant in the mouse testis than in other tissues. Interestingly, we observed that *Tbca13* and *Tbca16* genes show differential expression patterns during the process of testis maturation. Until now, we are only able to detect the TBCA13 protein *in vivo* (by tandem electrospray mass spectrometry). However, we can not exclude that TBCA16 protein could be expressed at a precise moment and/or at a precise stage of development. Alternatively, and taking into account our previous RNAi results, it is possible that the TBCA16 mRNA could have a regulatory role in the *Tbca13* gene expression.

To better understand the function of these two proteins we performed several overexpression experiments of *Tbca13* and/or *Tbca16* genes in mammalian cell lines. Curiously, we detected significant amounts of both TBCAs in the protein insoluble fraction (pellet). We are investigating why TBCA is present in the pellet, if it is aggregated due to overexpression, if it is associated with other proteins, or if it is confined to a specific sub-cellular compartment.

In parallel, to better study the function of the mouse *tbca*, we will start the creation of a function model *in vivo*. In this context, we will produce knockout (ko) mice for *tbca* gene (in chromosomes

13) that will constitute the biological model to study the role during development, specifically of the testis. we are about to finish the targeting construct.

Recently, we also started the characterization of a gene encoding a protein containing a TBCC domain. This protein contains also a domain that is present in proteins that are able to interact with actin. Experiments are in progress to evaluate the role of this protein using RNAi and over-expression experiments as well as biochemical approaches.

The role of the cytosolic chaperonin CCT and tubulin Cofactor E of the ciliate *Tetrahymena* in the assembly and dynamics of functional specialized microtubule structures.

MEMBERS: Helena Soares.

STUDENTS: Miguel Coelho, Pedro Sanches, Teresa Cruto and João Gonçalves.

EXTERNAL COLLABORATORS: Jacek Gaertig (Department of Cellular Biology, University of Georgia, Athens, USA), Luis Viseu Melo (Departamento de Física, Instituto Superior Técnico, Lisbon, Portugal).

Cofactor E is one of five tubulin-specific chaperones known as Cofactors A–E that participate in the pathway leading to the assembly of the α/β -tubulin heterodimer, the subunit that polymerizes to form microtubules (Mts). TBCE is also able to rescue α -tubulin from the native dimer, inducing depolymerisation of the Mts. Several neurodegenerative diseases have been ascribed to TBCE mutations, for example the Kenny-Caffey syndrome. *Tetrahymena thermophila* assembles 17 types of distinct functional microtubular structures, being an ideal model to investigate the role of TBCE in MT assembly and dynamics, or even in signal transduction mechanisms. We identified the *Tetrahymena* TBCE gene by comparing the human TBCE aminoacid sequence with TIGR *Tetrahymena* gene predictions database. The *Tetrahymena* Cofactor-E gene encodes a protein that presents the conserved domains of the human protein.

To characterize the TBCE function we created two *Tetrahymena* CU522 strains carrying exogenous versions of TBCE with distinct C-terminal tags, -HA and -GFP. Studies using confocal immunofluorescence microscopy show that TBCE localizes mainly in the cytoplasm. Both TBCE-HA and TBCE-GFP overexpression result in destabilization of the most dynamical Mts. At the cortex level the transversal Mts are the more affected which results in altered cell shapes (e.g. twisted form) probably resulting from depolymerization of transversal Mts bands. To go further in these studies we also created somatic knockouts using Neo2 cassette (Pm-r). These cells have cytokinesis impairment with absence of amitosis in the macronucleus which became unstable. Dramatic alterations at the cortex and basal bodies number are also observed. Undivided masses of cells (monsters) remain viable and able to assemble basal bodies which conflicts with extensive depolymerisation of cytoplasmic Mts.

On the other hand we observed that the TBCE gene mRNA levels are up-regulated during ciliary regeneration and also increase upon mechanical stress, a process probably resulting in cytoskeletal remodelling. Moreover, *T. thermophila* cells with abnormal cytoskeleton had increased levels of TBCE. All the data taken together led to the hypothesis that mechanical deformations in the microtubular cytoskeleton induce TBCE

gene expression. To test this hypothesis we developed an experimental setup, in which ferromagnetic particles are mechanically excited acting on cellular structures. In practice, *Tetrahymena* cells were fed with ferromagnetic particles. The ingested elements are inside vacuoles in the cell's cytoplasm, which are connected with the microtubular system. An alternating magnetic field (AMF) is then applied to a volume of cells over a period of time. The internalized particles align with the field producing a vibrating movement that mechanically stresses the cells. By varying the oscillation frequency of the AMF it should be possible to mimic an internal heat shock (at high frequencies). This would allow for a study on different stress responses in the cell. Preliminary data seem to indicate that TBCE gene expression is indeed induced at low frequencies whereas at higher frequencies this induction is smaller. Therefore, the gene response might be dependent on the vibration frequency applied.

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

MEMBERS: Helena Soares.

INTERNAL COLLABORATORS: 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).

Species of the genus *Besnoitia*, which are classified in the subfamily *Toxoplasmatinae* of the phylum Apicomplexa, are widely distributed intracellular protozoan parasites of vertebrates that have recently received increasing attention due to their, real or apparent, increased prevalence. In this scenario, besnoitiosis in domestic animals should act as an alert and draw our attention to the wild life. Although the first descriptions of bovine besnoitiosis, caused by *B. besnoiti*, are from France and Portugal at the end of the XIX century and beginning of the XX century, its occurrence in Europe received little or no attention until the last decade of the XX century. Since then, numerous cases have been reported in Portugal, Spain and France. The life cycle of *B. besnoiti* is poorly understood and the definitive host, although its existence has been postulated, has never been identified. The capacity of bloodsucking insects for transmitting mechanically the parasite from infected to uninfected bovine has been demonstrated, and the existence of other intermediate hosts can't be ruled out. For developing our studies there was a clear need for the isolation of naturally occurring *B. besnoiti*. We succeeded to isolate the parasites directly by inoculation of bradizotes, obtained by mechanical disruption of bovine skin cysts, into cell cultures. By this process we obtained isolates from south of Portugal, north of Spain and southeast of Spain. Our recent studies also showed that there is a cross-talk between the host and parasite microtubule cytoskeleton and that such event plays an important role during the first steps of cell host invasion.

In order to address the diversity among *B. besnoiti* we determined the 18S, ITS1, 5.8S, ITS2 and 28S rDNA nucleotide sequence from these isolates, as well as from an isolate from Israel (kindly provided by Varda Shkap, Kimron Veterinary Institute). These

sequences, together with rDNA sequences from other species of *Besnoitia* and related genus available in the GenBank database, were aligned according to their primary structure similarity using ClustalW. Phylogenetic analysis was inferred by genetic distance using Kimura two parameter, maximum parsimony and maximum likelihood. The extremely high level of identity found among our *B. besnoiti* sequences (99-100%) argues in favor of a clonal population, and for all rDNA regions it was possible to infer an independent cluster for the *Besnoitia* members which is formed by two distinct groups: *B. akodonii*, *B. jelissoni*, *B. darlingi* and *B. oryctofellisi*, in one group, and *B. besnoiti*, *B. bennetti* and *B. tarandi* in another, more homogeneous, group. This latter group would include *B. caprae* that has been claimed to be indistinguishable from *B. besnoiti*. However, host species barriers for parasites within this group have to be further investigated. Presently, we are validating in house developed diagnostic methods to test samples from different animal species.

Study of physical properties of microtubules by AFM techniques. Use of microstructured actuators for directional control of microtubules.

MEMBERS: Helena Soares.

STUDENTS: Ruben Ramalho and Pedro Sanches.

EXTERNAL COLLABORATORS: Luis Viseu Melo (Departamento de Física, Instituto Superior Técnico, Lisbon, Portugal); Susana Freitas (INESC-NM).

Microtubules are powerful spatial organizers in the cell which interact with multiple proteins and organelles and are involved in a variety of functions from cell division, compartmentalization, signal transducing, polarity to morphogenesis. The possibility of using them to direct the position and movement of other molecules makes these polymers attractive candidates for bionanotechnological applications, such as biomimetic actuators, sensors, etc. Thus, the control and manipulation of microtubules is an important part of the ongoing effort to produce new MEMS (microelectromechanical systems) that integrate biological components for use in medical and biological applications. We propose to use static electric fields to control the direction of microtubules adsorbed to a substrate and have so far demonstrated this method applied to a bulk sample of microtubules adsorbed to functionalized mica. To achieve micron-level control of microtubule structures, we have, during this year, designed and constructed a microstructured substrate containing multiple capacitor arrays to achieve locally controlled adsorption of microtubules. The device is currently undergoing its first testing and optimization cycle.

Characterization of the Mob1-like proteins in higher eucariotes.

MEMBERS: Álvaro Tavares.

STUDENTS: Claudia Florindo, Catarina Samora.

EXTERNAL 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).

The *Drosophila melanogaster* genome has four genes with homology to *S. cerevisiae* MOB1, a gene required to promote mitotic exit and cytokinesis. Conservation of the

homologous genes in *Drosophila* (Dmobs) suggested they may participate in late mitotic events in higher eukaryotes. To test this possibility we have analysed the effects of loss of function and over-expression of each of the *Drosophila* dmob genes, in addition to the sub-cellular localisation of their products. Phenotypic characterisation of the dmob mutant fly lines generated, suggests that the *Drosophila* MOB proteins may not be involved in mitotic exit or cytokinesis. Despite this the Mob genes are essential for the viability of the organism, and have non-overlapping functions. For example, Dmob1 null mutants are male sterile but show no cell division abnormalities during spermatogenesis. On the other hand, Dmob2 null mutants die at late larval stage with severe mitotic defects. Dmob3-YFP and Dmob4-YFP show similar localisation patterns on the centrosomes and kinetochores during mitosis. Most interestingly we have shown that clonal mutants of the Mob4 gene in *Drosophila* results in the formation of tumors. In addition to the *Drosophila* studies we are also analysing the function of the Mob-like genes in humans, *Tetrahymena* (in collaboration with Helena Soares) and *Arabidopsis* (in collaboration with Paula Duque).

Study of mitotic kinases in *Drosophila melanogaster*.

MEMBERS: Álvaro Tavares.

STUDENTS: Lucia Mentelova, Ramakrishna Prabhu.

EXTERNAL COLLABORATORS: David Glover (CRC Cambridge, UK), Ariane Abrieu (France) and Rui Gomes (FCUL, Lisbon, Portugal).

kinases peak during M phase and this activation has been attributed to phosphorylation. Polo is a pleiotropic kinase which has multiple roles during mitosis, such as centrosome maturation, G2/M transition, spindle formation, metaphase to anaphase transition, chromosome segregation and cytokinesis. We have been conducting a search for POLO substrates. Our focus is on the kinetochore substrates. In addition to polo we are also studying the function of the mitotic kinase Mps1. It is suspected that Mps1 has an important role for centrosome function in addition to its role on the kinetochores. Using *Drosophila* and chemical genetics we are trying to clarify if Mps1 has also a centrosomal function. Simultaneously, we have been trying to identify Mps1 substrates on the kinetochore complex.

Defining the mitotic kinetochore structure in a higher eukaryote *Drosophila melanogaster*.

MEMBERS: Álvaro Tavares.

STUDENTS: Gonçalo Costa, Fátima Dias, Lucia Mentelova.

EXTERNAL COLLABORATORS: Ana Coelho (ITQB, Oeiras, Portugal), Peter Roepstorff (Univ Southern Denmark, Odense, Denmark).

The molecular mechanisms ensuring accurate chromosome segregation during meiosis and mitosis are critical to the conservation of euploidy (normal chromosome number) in eukaryotic cells. A dysfunctional kinetochore represents one possible source for chromosome instability and the generation of aneuploidy and cancer development. Kinetochores are large complex protein structures that assemble at the centromeric regions of each sister chromatid and perform three key functions: attach chromosomes to

the spindle, co-ordinate microtubule dynamics to allow chromosomes movement along the spindle, and generate the 'wait' signal that prevents anaphase onset until all the chromosomes are correctly aligned on the spindle. Recent proteomic studies suggest that kinetochores of *Saccharomyces cerevisiae* are comprised of at least 60 proteins. Although some of these proteins are conserved from yeast to humans, the majority of proteins in high eukaryote kinetochores are still to be identified. In order to identify and characterize kinetochore proteins in *Drosophila* we have developed method to obtain enriched mitotic kinetochore protein fractions. From these fractions we have identified 89 proteins of which 26 are encoded by uncharacterized genes. We have now studied the function of seven of these new genes in detail and we found that, by tagging with GFP followed by expression in S2 cells, they localize with the mitotic kinetochore. Functional studies, using mutants and RNAi assays, show that lack of these proteins result in typical kinetochore phenotypes, with cells presenting chromosomes misalignment and missegregation during mitosis. Taken together these results show that the approach to isolate kinetochore proteins was successful. In addition to the *Drosophila* studies, we have analysed the function of the human homologues for two of these proteins and found similar mitotic defects in chromosomes segregation, and result in polyploid cells. These results indicate that the function of the kinetochore genes is conserved across species. We believe that continued identification of conserved kinetochore components in model systems as *Drosophila melanogaster* will provide a rich resource of candidate genes that may be mutated or misregulated in human cancers.

Inter-species cell-cell signalling in bacteria.

MEMBERS: Karina Xavier and Michal Bejerano Sagie.

This project focuses on the molecular mechanisms bacteria use for inter-cellular communication. This process, called quorum sensing, involves the production, release, and response to signal molecules termed autoinducers. Quorum sensing enables a bacterial population to regulate activities as a multi-cellular group. Behaviours regulated by quorum sensing are often crucial for successful bacterial-host relationships; both symbiotic and pathogenic. Most autoinducers are species-specific, however one autoinducer called autoinducer-2 (AI-2), is produced and detected by a wide variety of bacteria allowing inter-species communication.

This project relies on a multi-disciplinary approach to study AI-2 systems promoting bacterial inter-species communication. By studying quorum sensing in *Escherichia coli*, we have characterized one of the first AI-2 systems. We will pursue the characterization of the *E. coli* AI-2 system, and will also investigate novel AI-2 signalling systems in other bacteria to understand the network architecture controlling AI-2 signalling at the species level. We have developed the first laboratory system to study inter-species AI-2 signalling in consortia, so once we identify novel AI-2 circuits, we will use this set up to study inter-species cell-cell communication in complex bacterial communities.

Quorum sensing in *Escherichia coli*.

MEMBERS: Karina Xavier.

STUDENTS AND TECHNICIANS: João C. Marques and António Santos.

EXTERNAL COLLABORATORS: Stephan Miller (Swarthmore College, Pennsylvania, USA).

We have shown that in the enteric bacterium *Escherichia coli* AI-2-regulates a system that internalizes and degrades the AI-2 signal. Specifically, at high population densities, *E. coli* uses this system to remove AI-2 produced by itself and also AI-2 produced by other species present in the same co-culture. AI-2 internalization by *E. coli* has the consequence of interfering with other species' ability to use AI-2 to regulate their group behaviours by quorum sensing. We predict that this mechanism of interference with AI-2 signalling has important consequences in natural niches colonized by *E. coli*, such as the human gut, where many different species of bacterial species co-exist and depend on quorum sensing for efficient colonization.

The aim of this project is to characterize the molecular mechanisms of AI-2 internalization in *E. coli*. In this project we will use a combined biochemical, genetic and chemical approach to:

- (i) Identify and characterize the mechanisms involved in regulating AI-2 secretion and internalization in *E. coli*.
- (ii) Characterize the transcriptional regulator of the *E. coli* quorum sensing system and to identify all the genes and functions regulated by this system.
- (iii) Characterize the enzymes and metabolites involved in the AI-2 degradation pathway.

This work will allow us to characterize the mechanism involved in accumulation and perception of the signalling molecule, the metabolic pathway involved in turning off this signal, and finally to identify the bacterial behaviours regulated by this system. Since the *E. coli* system for AI-2 internalization represents the first example of interference with AI-2-mediated quorum sensing, characterization of this system will be valuable in the design of clinical and biotechnological strategies intended to manipulate quorum sensing dependent behaviours such as virulence.

Identification and characterization of quorum sensing systems involved in bacterial inter-species communication.

MEMBERS: Karina Xavier.

STUDENTS AND TECHNICIANS: Michal Bejerano-Sagie and Catarina S. Pereira.

EXTERNAL COLLABORATORS: Stephan Miller (Swarthmore College, Pennsylvania, USA) and Michko Taga (MIT, Boston, USA).

The broad use of AI-2 as a signal controlling group behaviors in bacteria makes it an especially interesting system to study bacterial inter-species communication but also for the development of new therapeutic interventions to control processes regulated by quorum sensing. Toward these goals, it is essential to identify mechanisms used for AI-2 detection and relay. In this project a newly identified AI-2 internalization system in the plant symbiont *Sinorhizobium meliloti* will be characterized in order to evaluate the function of this system during the symbiosis this organism establishes with its host, the alfalfa plant. To assess the diversity of these systems, AI-2 detection systems from additional bacterial

species will be identified and characterized, and the structures of their active AI-2 molecules and their inter-conversions characterized. Mutants impaired in these AI-2 signalling pathways will be constructed, and used to study bacterial-bacterial, and bacterial-host interactions.

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.

Optical-genetic identification and control of serotonin neurons in behaving animals.

MEMBERS: Zach Mainen and Guillaume Dugue.

COLLABORATORS: Susana Lima (IGC, Oeiras, Portugal).

Serotonin is an important neurotransmitter implicated in a wide variety of physiological functions and pathophysologies but whose function is not well understood. Critically, very little is known about the activity of serotonin-releasing neurons in the brain. This problem is greatly exacerbated by the difficulty in identifying serotonin-containing neurons. To address these problems we are using a combination of behavioral analysis, electrophysiological recording and optical-genetic probes targeted through specific promoters to this class of cells. By selectively activating serotonin neurons with light delivered through implanted fiber optics, we will be able to positively identify them during recordings and to specifically activate them, allowing us to test specific hypotheses concerning the role of serotonin in brain function and behavior.

The role of the auditory cortex in associative learning.

MEMBERS: Marta Moita.

STUDENTS AND TECHNICIANS: Raquel Antunes.

In this project we are studying the neural mechanisms underlying fear generalization. We are using 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. How each pathway contributes to the acquisition of auditory fear conditioning remains unclear. It has been proposed that the direct thalamic route is faster (fewer synapses), but less accurate than the indirect cortical route. Thus, it might be expected that the cortical route to the amygdala prevents generalization of the fear response to sounds that were not paired with footshock. To test this possibility, we lesioned specifically the indirect route (by lesioning MGv, the only auditory thalamic nucleus that provides the major source of input to primary auditory cortex, and does not project to the amygdala). We then tested the fear response of rats to a tone that was paired with shock (tone+) and one that explicitly unpaired (tone-). We found that control rats fear more the tone+ than the tone-, whereas lesioned rats showed equivalent levels of fear to both stimuli. We are now starting a series of experiments where instead of a permanent lesion to MGv we will temporarily inactivate through targeted drug infusions. Finally, we intend to characterize the responses of amygdala neurons to sounds (both in naive and trained rats) and test the effect of MGv lesions on these response profiles.

The role of the amygdala in trace fear conditioning.

MEMBERS: Marta Moita.

STUDENTS AND TECHNICIANS: Marta Guimarães.

This project aims at understanding the mechanisms underlying learning of an association between stimuli separate in time. To this end we use auditory fear conditioning. In this paradigm animals learn to fear a previously neutral tone after it is paired with an aversive stimulus, usually a footshock. In delay fear conditioning (dFC) the two stimuli co-terminate and in trace fear conditioning (tFC) the tone and the shock are separated by a brief interval of time. During auditory fear conditioning (both delay and trace) rats learn to fear the tone and the training environment in which conditioning took place (contextual fear conditioning). The mechanisms underlying the association between a tone and shock, that occur separate in time, remain elusive. One possibility is that the association between tone and shock is mediated by what is common between the two stimuli, i.e. the physical context. In particular, it has been proposed that tFC is a form of second order conditioning, where learning the context-shock association would correspond to a first order conditioning and the tone-shock association would correspond to a second order association mediated by the context. To test this hypothesis we took advantage of a behavioral protocol that prevents the formation of an association between the training context and shock. If fear of the tone during tFC depends on the association between the training environment and shock, then preventing the ability to fear the training context should also prevent the acquisition of fear of the tone. This should only be true in the case of tFC, where tone and shock are separate, but not in the case of dFC where tone and shock co-terminate. It is known that overexposure to the training environment significantly decreases the ability of the training environment to become associated with shock, a process called latent inhibition. Thus, we overexposed rats to the conditioning chamber, after which we trained rats with a single tone followed after several seconds by shock in that same chamber. A second group of rats was trained with a single tone-shock pairing (where tone and shock co-terminate). As expected, rats in both groups failed to acquire fear of the training

environment confirming that our latent inhibition protocol worked. However, rats in both groups learned to fear the tone. These results suggest that acquisition of a fear of a tone in a tFC paradigm is independent of context fear memory formation. We are now testing whether learning to fear the tone in our trace conditioning protocol still depends on the hippocampus, even though it is independent of context fear. To do so we are performing temporary inactivation of the hippocampus through bilateral infusion of muscimol, a drug that effectively shuts down activity of excitatory neurons.

Prisoner's dilemma in rats.

MEMBERS: Marta Moita, Elio Sucena, Isabel Gordo.

STUDENTS AND TECHNICIANS: Duarte Viana.

In the Prisoner's Dilemma, two individuals simultaneously choose one of two strategies: cooperate or defect. The resulting payoff depends on both players' choices (Table I). To qualify as a Prisoner's Dilemma, the payoffs must conform to the following set of inequalities: $T > R > P > S$.

		Against	
		C	D
Payoff to:	C	R	S
	D	T	P

Mutual cooperation results in a moderate reward (R), but mutual defection leads to low payoffs for both players (P). When one cooperates and the other defects, the defector receives the largest possible reward (T) and the cooperator receives the smallest possible reward (S). This implies that mutual cooperation is better than mutual defection, but for an individual player, there is a sizable temptation to defect. Therefore, the evolutionarily stable strategy in a one-shot game is defection. Nevertheless, stable cooperation can emerge if the cooperative interactions occur repeatedly, the opening move is cooperative, and from that point on, each player copies the other's moves. This winning strategy is a version of reciprocity called Tit-For-Tat (TFT). So far, sustained cooperation in a Prisoner's Dilemma game has only been observed in humans. However, it has been recently shown that female rats show reciprocal cooperative behavior. Thus, we are testing whether in the context of the prisoner's dilemma, rats also sustain cooperation. We have shown that rat's can learn the rules of the task and the payoff matrix, since at the beginning of the game they start at chance but gradually increase the defection rate when playing against a pseudo-random (PR) strategy. We found that when playing against a TFT strategy that should lead to sustained cooperation by the other player, defection rates raise more slowly and are lower than when playing against PR. We have tested rats in a number of prisoner's dilemma matrices: homogeneous matrices (all rewards), heterogeneous matrices (qualitatively different punishments and rewards). These yielded similar results. We have also tested both females and males, since in natural populations females seem more prone to cooperate, which could be the result of a sexual dimorphism. We found similar behavior although females' behavior was more variable.

Winners and losers: social modulation of hormones, brain and behaviour.

MEMBERS: Rui Oliveira.

STUDENTS AND TECHNICIANS: Miguel Simões.

EXTERNAL COLLABORATORS: Adelino Canário (Univ. Algarve, Portugal), David Gonçalves (ISPA, Portugal), Günther Zupanc (Jacobs University, Bremen, Germany).

The nature vs nurture debate has been ubiquitous in the history of the Behavioral Sciences, and the approaches of the social and the biological sciences to the study of behaviour have been seen as almost mutually exclusive. However, more recently, a growing body of literature has documented social influences on genetic constitution and gene expression, functioning of the endocrine and nervous systems, and immune activity. These results suggest that the effects of social factors on the expression of behavior must have underlying biological processes. Therefore, a major challenge in current psychobiology is to understand how psychosocial factors can modulate biological mechanisms. In social species individuals must fine-tune the expression of their social behavior according to the social environment in which they live. Theoretically, the cellular, molecular and physiological basis of motivational changes underlying this behavioural plasticity can be explained either by structural reorganization or by biochemical switching of the neural networks underlying social behaviour, depending on the effects of social context on behaviour being long-lasting and slow or rapid and reversible, respectively. These potential neural mechanisms underlying phenotypic plasticity have a parallel in hormonal mechanisms: structural organization of neural circuits can be influenced by organizational effects of hormones, while biochemical switches can be driven by activational effects of hormones on central pathways underlying behaviour. The major endocrine candidates to play a mediator role in the control of behavioural plasticity are androgens and glucocorticoids, since both classes of steroid hormones respond to social interactions and have effects on the expression of social behavior. Therefore, the social context in which an individual lives influences their circulating steroid levels which in turn may influence the behavior that the individual expresses in that particular context. Thus, steroid hormones may be viewed as moderators of agonistic and sexual motivation according to the social environment that they are facing, helping the individual to fine-tune its behavior according to social context. This rationale implies 3 causal steps: a) social modulation of steroid levels; b) steroid hormones acting on the neural mechanisms underlying social behavior, at an activational (functional) or an organizational (structural) level; and (c) changes in the activity/structure of these neural mechanisms adjust the expression of subsequent behavior according to the social context. So far in our lab we have been focusing mainly on the social influences on circulating androgen levels (see references). With the current project we aim to test the hypothesis that the endocrine responsiveness to the social environment mediates behavioral plasticity, by acting on an activational or organizational fashion, depending on the duration of the social experience. Thus, we predict that short-term social interactions will activate neuromodulatory mechanisms, while long-term experiences will have structural reorganization effects. Moreover, we also hypothesize that structural changes induced by long-term social experiences might be flexible according to social context. Neurogenesis assessed by cell proliferation markers (BrdU) together with neuronal markers (HuD and NeuN), will be used

as an indicator of structural reorganization. Changes in the gene/protein expression of neuromodulatory systems (arginine-vasotocin and isotocin) and in steroid receptors (AR, ER, GR) and steroid metabolizing enzymes (aromatase and 11-beta-HSD) will be used as indicators of biochemical switching. The proposed study requires a model species with a complex social system that can be easily manipulated in the lab, and whose individuals could be analyzed at the physiological, cellular and molecular level. The cichlid fish, *Oreochromis mossambicus* provides one such model system. The following questions will be addressed: 1) Which brain areas are activated by social interactions, and if winning and losing experiences activate different brain areas (using the expression of the immediate early genes)? 2) How animals integrate multiple social experiences (winning/losing sequential social interactions), happening at different time-scales, and how it affects steroid circulating levels, neurogenesis and neuromodulation, and the subsequent expression of behavior? 3) If long-term induced changes in structural reorganization and biochemical switching mechanisms can be manipulated by induced reversals on social status? Together, these three questions will allow a better understanding of how the social environment affects the adult brain and modulates behavioral flexibility.

THEORETICAL AND COMPUTATIONAL BIOLOGY

Given the scientific interests of the IGC in “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 has had evident 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, Kalet Leon.

STUDENTS AND TECHNICIANS: Tiago Paixão, Iris Vilares, Nuno Sepúlveda.

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 (Carneiro et al. Immunol, Rev. 2007). Further studies of regulatory T cells and their function proceed along three 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. Methodologically, this is leading us to advance several experimental and computational tools to deal with the three color imaging, and to develop algorithms for cell simulation as well as for automated feature detection and quantification of 2D and 3D images.

Second, we study 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. The "eco-evolutionary" dynamics of these diverse cell populations predicts a spontaneous partitioning of the T cell repertoire into two pools of clones in a stationary immune system: the first pool constitutes the vast majority of T cells and is made of small clones that are poorly stimulated by normal presentation of body antigens and are devoid of regulatory T cells, the second pool is made of also of small clones, which albeit being strongly stimulated by normal body antigen presentation contain regulatory T cells that prevent clonal expansion. The former pool contains the precursors of immune responses to invading pathogens, while the latter pool contains the clones that are involved in tolerance or autoimmunity. Aiming to assess the predictions concerning the repertoire of T cells, we are employing statistical methods to infer and quantify the diversity and clonal size distribution in T cell subpopulations.

Third, we study how the regulatory T cells dynamics and regulation is coupled with growing antigens, such as microorganisms and tumors. These theoretical studies suggest a role for the T cell dynamics promoting the diversity of intestinal flora, as an essential part of vertebrate physiology. Regarding tumors we identified two modes of tumor progression that differ in the engagement of regulatory T cells, and thus in the potential for therapeutic intervention. The results of these studies provide guidelines for the rational design of immunotherapies for cancer subtypes based on vaccination, immunosuppression and surgery.

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).

Lymphocyte antigen-receptor diversity is the hallmark of the vertebrate immune system. Diversity is thought to be critical for host defense, and also to prevent immunopathologies arising when diversity is truncated. However, there are no straightforward methods to

measure diversity in a pool of lymphocytes, and the ones that exist pose yet unresolved statistical problems. Oggle et al. (2003) have put forward a promising methodology to estimate diversity of antigen-receptor repertoire genes based on microarray technology. 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. In the group we developed a full quantitative analysis of this technique aiming to better assess the precision and accuracy of the estimates it leads to. This model was calibrated with data obtained with standard oligos of known diversity, allowing to establish confidence intervals for estimates, and to detect the presence of and quantify systematic errors. This methodology, as well as other that lead to average diversity, will lead to estimates when cell populations are very heterogeneous in terms of the representation of individual antigen receptor variants. We therefore set out to develop new statistical tools that allow to infer the distribution of clonal sizes based on sequencing and enumeration of antigen receptor variants in a cell population. We redeployed statistical methods used in ecology to estimate biodiversity, and demonstrated, by reanalysing published data on enumeration of TCR variants in T cell subpopulations, that these methods have enough power to infer not only the average diversity but to characterize the clonal size distribution.

Monoclonal anti-CD8 therapy induces disease amelioration in the K/BxN mouse model of spontaneous chronic arthritis.

MEMBERS: Margarida Carneiro, Bruno Raposo, Isabel Belo, Maria João Lagareiro

EXTERNAL COLLABORATORS: Luis Graça (IMM, Lisbon, Portugal), Ana Água-Doce (IMM, Lisbon, Portugal), José António Pereira da Silva (Hospitais da Universidade Coimbra, Coimbra, Portugal), Steffen Gay (Medical School Univ. Zurich, Switzerland).

In the rheumatoid synovium CD8⁺ T cells comprise up to 40% of all infiltrating T cells. Moreover, CD8⁺ T cells have been linked to the formation of synovial ectopic germinal centers in RA, and they produce pro-inflammatory cytokines. Hence, CD8⁺ T cells appear to be intimately involved in initiating and maintaining the chronic inflammation in the RA synovium. Nevertheless, studies on animal models of arthritis, have not been able to clearly identify the role of CD8⁺ in inflammatory synovitis.

Using the K/BxN mouse model of spontaneous chronic arthritis, which shares many similarities with human RA, we have studied the potential of CD8⁺ T cell depletion with monoclonal antibodies in stopping and reversing experimental arthritis progression.

K/BxN mice with established chronic arthritis received intraperitoneally (IP) 200µg of depleting anti-mouse CD8 monoclonal antibody (mAb, clone YTS169) or isotype control. Another group of K/BxN mice with established disease underwent total thymectomy followed by IP injection of 300µg of YTS169. Disease progression was scored twice weekly for 4 months, and blood collected to determine serum levels of TNF-α, and changes in frequency of CD8 and CD4 T cells, and B cell. Joints were collected for histology. Mice treated with anti-CD8 had an improvement in the arthritis score already 5 days after the mAb injection. Disease signs improved on average 60%-70% when compared to the control group. Even though recovery of the CD8⁺ T cell population was

associated with an increase of the arthritis score, when compared to the control group the anti-CD8 treated animals maintained a lower arthritis score for over 100 days. When anti-CD8 was administered up to 5 days before the presence of clinical signs of disease, initiation of arthritis could be postponed for 3 weeks (recovery of CD8 T cells). Mice subject to total thymectomy kept permanently a very low arthritis score. Histological analysis showed an absence of inflammatory infiltrate in the anti-CD8 treated animals, and signs of cartilage synthesis. One month after anti-CD8 injection the serological levels of TNF-alpha dropped significantly ($p<0.05$) from $11.6\pm 2.1\mu\text{g/ml}$ to $7.3\pm 0.9\mu\text{g/ml}$. No changes were observed in the serum levels of the disease related anti-glucose-6-phosphate-isomerase antibodies. Interestingly, after 30 days the peripheral blood frequency of IgD-CD24hi memory B cells in the anti-CD8 treated group was significantly higher ($p<0.05$) than in the control group ($23.4\pm 3.7\%$ vs $14.1\pm 2.3\%$, respectively). Additionally, arthritic K/BxN mice presented a significantly lower ($p<0.05$) frequency of peripheral blood CXCR4+CD8+ T cells, than non-arthritic control litter-mates ($28.9\pm 7.2\%$ vs $50.4\pm 2.3\%$, respectively). The present study presents evidence that CD8+ T cells play a pivotal role in initiating and maintaining chronic synovitis in a mouse model of experimental chronic arthritis. In arthritic mice the lower frequency of circulating CD8+ T cells expressing the CXCR4 chemokine receptor suggests, that CD8+ T cells might accumulate in the synovium where there is an over-expression of the chemokine CXCL12. By reducing the levels of TNF-alpha and blocking the homing of memory B cells and inflammatory macrophages into the synovium, treatment with depleting anti-CD8 mAb improves arthritis signs and cartilage synthesis.

Anti-TNF therapy induces the recovery of peripheral blood memory B cells in patients with Rheumatoid Arthritis.

MEMBERS: Margarida Carneiro.

EXTERNAL COLLABORATORS: João Eurico da Fonseca (IMM/Hospital de Santa Maria, Lisbon, Portugal), Rita Moura (IMM, Lisbon, Portugal); Pamela Weinmann (IMM, Lisbon, Portugal); Ruth Fritsch (Medical School University of Utrecht, The Netherlands); Peter Lipsky (NIAMS-NIH, USA).

In order to study the changes in peripheral memory B cell subpopulations in RA patients, and to understand the possible role of TNF in regulating changes in specific memory, we analysed the frequency and distribution of B cell subsets in the peripheral blood of very early untreated RA patients, as well as in the peripheral blood and synovial membrane of active RA patients with long-standing disease. Subsequently, we assessed whether treatment with a specific TNF-blocker normalized the distribution of these peripheral B cell subsets. Our results show, for the first time, that RA patients, independent of disease duration, have a much lower frequency of peripheral blood pre-switch IgD⁺CD27⁺ memory B cells than healthy individuals, whereas post-switch IgD⁻CD27⁺ accumulate with increased disease duration. Additionally, we present evidence that pre-switch IgD⁺CD27⁺ memory B cells accumulate in the synovial membrane of RA patients under the influence of TNF, since anti-TNF therapy increased the frequency of pre-switch IgD⁺CD27⁺ peripheral memory B cells. These results document disease-related and TNF-dependent

abnormalities in memory B cell sub-sets in RA and suggest that part of the success of TNF neutralizing therapy could relate to normalization of memory B cell abnormalities.

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Analysis of T-cell diversity and repertoire overlap of naïve and regulatory CD4⁺ T cells in mice and humans.

MEMBERS: Jose Faro.

STUDENTS AND TECHNICIANS: Nuno Sepúlveda.

EXTERNAL COLLABORATORS: António Bandeira (Institut Pasteur, Paris, France), Guy Gorochov (Hôpital Pitié Salpêtrière, Paris, France).

The estimation of T-cell receptor diversity and overlap of different T-cell populations requires the estimation of the number of how many different TcR- or TcR- chains expressed by those cell populations. Different approaches have been followed until now in mice. All of them involved analyzing the diversity of a given VJ combination in a single cell sample from 1 or 2 mice. Recently we have developed new methods to estimate both the diversity and overlap of TcR- chains in T cell populations. The diversity method requires analyzing the number of different chains of a given VJ combination for each of two independent cell samples of a T-cell population, as well as the number of common sequences between them. The overlap method is based on the observed diversity in the cell samples of each of two T-cell populations and the number of common sequences between those two samples, as well as the estimated total diversity within each T-cell population bearing the studied VJ combination. We have applied those methods to two distinct mouse VJ combinations (V 3-J 1.6 and V 11-J 2.2) varying more than 5-fold in cell numbers. Preliminary results suggest that cell subsets expressing distinct VJ combination may display different relative TcR- chain diversities. That is, in mice the diversity found for a given VJ combination apparently cannot be extrapolated to other VJ combinations.

We have also started to analyze human TcR- chain sequence data for PBL CD4⁺ naïve and Foxp3⁺ T-cell samples expressing V 5 from both healthy and patient donors. In spite

of the relatively high degree of diversity estimated for both T-cell populations a surprising high degree of overlap was found between Treg cells from different individuals or the same individual at different times. In contrast, no overlap was found between naïve T cells from different individuals or between naïve and Tregs within each individual.

Do CD4⁺ Treg cells influence memory B-cell generation and affinity distribution of selected B cells in germinal centers?

MEMBERS: Jose Faro, Margarida Carneiro, Luis Graça.

STUDENTS AND TECHNICIANS: Ana Água-Doce.

EXTERNAL COLLABORATORS: África González (University of Vigo, Vigo, Spain).

In a humoral thymus-dependent immune response a fraction of activated B and T cells (including regulatory T cells) migrate into primary follicles forming germinal centers (GC). These are dynamically organized, short lived microenvironments where intense proliferation, apoptosis, and hypermutation of immunoglobulin V(D)J genes of B cells takes place. They are essentially involved in generation of memory B cells and in the affinity maturation of antibodies. Failures in controlling the survival or differentiation of GC B cells may lead to autoimmune processes or to depression of B-cell memory generation. Yet, there is little knowledge on how the molecules and cells involved operate at a cell population level to drive and regulate the GC reaction. We are starting a project that aims at analyzing the potential impact of regulatory T cells in the dynamics of GCs, and their role in controlling antigen-specific or self-reactive B cell survival and differentiation in GCs from healthy and autoimmune mice.

Studies on the effect of organic and metallic nanoparticles on the physiology of different cell types.

MEMBERS: Jose Faro.

STUDENTS AND TECHNICIANS: Belén Díaz, Christian Sánchez.

EXTERNAL COLLABORATORS: África González (University of Vigo, Vigo, Spain), Manuel Arruebo (University of Zaragoza, Zaragoza, Spain).

Inorganic nanoparticles (NP) show great potential for medical therapy. However, biocompatibility studies are essential to determine their safety. We have compared five different inorganic NPs for their cytotoxicity, internalization or reactive oxygen species (ROS) production using tumoral and normal human blood cells as well as murine macrophages. We find striking differences depending on the cell type and no direct correlation between ROS production and cell toxicity. These results warn against the current lack of standardization of toxicity procedures for NPs and how easily this may mislead to wrong conclusions about their safety.

BioGrid - Parallel algorithms for gene annotation.

MEMBERS: Pedro Fernandes, Arlindo Manuel Limede de Oliveira, Ana Teresa Correia de Freitas, José Carlos Alves Pereira Monteiro, Luis Miguel Teixeira D Avila Pinto da

Silveira, Paulo Ferreira Godinho Flores, Alexandra Sofia Martins de Carvalho, Alexandre Paulo Lourenço Francisco, Ana Paula Proença Ramalho, Orlando Miguel Cruz da Anunciação, Pedro Tiago Gonçalves Monteiro.

STUDENTS AND TECHNICIANS: Sérgio Mendes Costa (IST, Lisbon, Portugal).

Finishing the implementation of several programs for motif discovery in sequences using suffix tree algorithms. The development of the MUSA, PSMILE and RISO programs, started at INESC, was completed. The programs were adapted to run on the interactive portal of HERMES, the High Performance Computer available at the IGC. The work was extended using the same methodology, to encompass bi-clustering algorithms: the classical Cheng & Church and the newly developed versions using suffix tree as well. This second set of programs is useful in microarray data analysis for example. All the programs have been correctly documented in a user's manual that is made available online. The software has been extensively tested.

Criticality in epidemiology.

MEMBERS: Gabriela Gomes, Nico Stollenwerk, Natalia Mantilla-Beniers.

STUDENTS AND TECHNICIANS: Paula Rodrigues, Ricardo Águas, Maíra Aguiar.

EXTERNAL COLLABORATORS: Lisa White (Wellcome Trust South-East Asia Programme, Thailand), Robert Snow (KEMRI-University of Oxford-Wellcome Trust Collaborative Programme, Kenya), Alberto Pinto (Universidade do Minho), Eduardo Massad (Universidade de São Paulo, Brazil).

Mathematical models have become standard tools in epidemiology and public health. Particularly useful to intervention design are situations where small changes in controllable factors lead to major transitions in system behaviour. In tuberculosis, we are investigating the concept of a reinfection threshold in transmission, above which we describe a scenario of high prevalence of disease associated with low vaccine effectiveness. In malaria, we have identified thresholds for eradication and resurgence in regions of mesoendemic transmission in sub-Saharan Africa. Although the two thresholds coincide initially, the threshold for resurgence increases with time leading to optimistic prospects for the sustainability of eradication efforts. In spatial epidemiological systems, we analyse reinfection models with pair approximation methods. In dengue fever outbreaks in Singapore, the breeding place structure of aedes mosquitos leads to scale-free distributions of weekly dengue cases.

Multi-Strain dynamics and chaos.

MEMBERS: Nico Stollenwerk.

STUDENTS AND TECHNICIANS: Maíra Aguiar.

EXTERNAL COLLABORATORS: Bob Kooi (Vrije Universiteit Amsterdam), Francisco Lemos, Sonia Diniz (Public Health Service Minas Gerais, Brazil), Bernard Cazelles (Centre National de la Recherche Scientifique, Paris, France).

An epidemic multi-strain model with temporary cross-immunity shows chaos, even in a previously unexpected parameter region. Motivated by experimental findings of antibody-

dependent-enhancement (ADE), dengue fever models with enhanced infectivity have previously shown deterministic chaos. We challenge the assumption of enhanced infectivity on the grounds that secondary infections are likely to lead to serious hemorrhagic dengue fever (HDF) requiring hospitalization, reduced exposure to mosquitoes and, therefore, reduced contribution to transmission. Including temporary cross-immunity in such models we find a deterministic chaotic attractor in a more realistic parameter region of reduced infectivity on secondary infection. This opens new ways for the analysis of existing data sets. Beyond dengue, our findings apply to any multi-strain epidemiological system with altered infectivity upon secondary infection.

Pathogen diversity and disease epidemiology.

MEMBERS: Gabriela Gomes, Gareth Weedall, Isabel Gordo.

STUDENTS AND TECHNICIANS: Sander van Noort.

EXTERNAL COLLABORATORS: Julia Gog (Cambridge University, USA).

Mathematical models are developed to link the genetic and antigenic diversity of viruses, bacteria and eukaryotic pathogens with the epidemiology of the associated diseases. This is especially interesting for pathogens that cause repeated infections throughout life and disease severity is partly determined by the history of infection. For influenza A, we are addressing the patterns of punctuated drift evolution. For falciparum malaria, we are investigating the distributions of genetic diversity observed in different sub-families of the large multi-gene family of erythrocyte membrane protein 1 (PfEMP1) genes and the associated malaria syndromes. Models are also developed to assess the effects of pathogen-specific population structures and selective pressures upon genetic diversity. Where appropriate, we explore the epidemiological and evolutionary consequences of existing and potential vaccines and investigate the practical implications of this research for vaccine design.

Geographical heterogeneity of tuberculosis incidence in Portugal.

MEMBERS: Gabriela Gomes, Ana Cristina Paulo.

Tuberculosis incidence in Portugal is one of the highest in Europe and has the particularity of having a marked heterogeneous distribution among different geographical areas. At National level the principal indicators estimated by the WHO are very optimistic and we should expected, according to WHO criteria, a decrease of at least 7% a year in tuberculosis incidence. We estimated the average decrease between 2000 and 2004 to be of 0.3% a year, which is modest compared to what is expected. The reasons for this slow decrease are directly linked to the geographical pattern of tuberculosis in the country. The trend in tuberculosis incidence is very heterogeneous at the Freguesia level and curiously we did not find any Freguesia with a consistent upward or downward trend. The trend was correlated with the trend on the number of individuals accessing DOTS, pulmonary or extra-pulmonary forms and to a lesser extent to the number of months (in average) for the duration of treatment. The impact of HIV was also addressed and we develop a neural network to classified cases of HIV based on subsidiary information already taken at

individual level in the tuberculosis database. This has two important implications; first, it allows to correct the estimate on the number of HIV-TB coinfections since at present for about 60% of the individuals in the SVIG, HIV status is not known and, second, it will allow to properly estimate the very optimistic detection rates, at national and regional levels, since this is based on the prevalence of HIV-TB coinfection in the population.

Modelling biological control of mammal pest species in island ecosystems.

STUDENTS AND TECHNICIANS: Nuno Oliveira.

EXTERNAL COLLABORATORS: Frank Hilker (University of Alberta, Canada), Manuel Carmo Gomes (FCUL, Lisbon, Portugal).

Invasive species are viewed as one of the most significant causes of biodiversity loss. Introduced feral cat populations, in particular, are an important threat to many island vertebrate populations, namely seabirds. Release of a pathogenic parasite, like the Feline Immunodeficiency Virus (FIV), has been suggested to be an efficient control measure of these mostly immune-naïve invasive populations. Such approach was theoretically investigated here, using mathematical models that describe the potential effects of introducing FIV into a host cat population that acts as a superpredator embedded in a simple island food web. Results show that FIV cannot fully eradicate cat populations in sub-Antarctic islands, but can be an efficient agent for their long-term control, allowing for the recovery of their endangered prey. However, this study emphasises that the control of superpredators by pathogenic virus can also cause harmful effects, such as community destabilization or the so-called mesopredator release effect, a burst of mesopredators following superpredators' control. Despite its potential as a tool in conservation biology, virus introduction should be preceded by preliminary empirical and theoretical studies on the ecological systems one wants to intervene.

Internet-based surveillance of influenza-like illness.

MEMBERS: Gabriela Gomes, Marion Muehlen, Nico Stollenwerk, Vitor Faustino.

STUDENTS AND TECHNICIANS: Sander van Noort, José Lourenço.

Gripenet is an internet-based system to monitor the influenza-like illness in real-time with the direct participation of volunteers recruited from the general population. The system is based on a website – www.gripenet.pt – specially created to: inform and educate the population about the disease; gather information on the population's health through online surveys. The system permits the graphic representation, processing and analysis of data on the development of the disease in Portugal in real time. The work is combined with the development of mathematical models that simulate the development of the influenza epidemic spread in Portugal and evaluate intervention scenarios. Geographic and demographic information is included with various levels of detail. A major component of our activity has a European dimension, aiming at extending the system implementation to other countries to facilitate the characterization influenza spread and increase awareness.

Evolution of protein trafficking pathways.

MEMBERS: Jose B. Pereira-Leal.

The evolution of the eukaryotic endomembrane system and the transport pathways of their vesicular intermediates are poorly understood. A common set of organelles and pathways seems to be present in all free-living eukaryotes, but different branches of the tree of life have a variety of diverse, specialized organelles. We are developing algorithms to reconstruct trafficking pathways from complete genome sequences, and are now focusing on Rab/Ypt proteins, small guanosine triphosphatases with tissue-specific and organelle-specific localization that emerged as markers for organelle diversity. We developed an automated Rab identification and classification system and used it to study the evolutionary dynamics of protein trafficking pathways in the kingdom Fungi, a sister kingdom of Animals. We identify and annotate these proteins in 26 genomes representing near one billion years of evolution, multiple lifestyles and cellular types. We found that surprisingly, the minimal set of Rab/Ypt present in fungi is similar to, perhaps smaller than, the predicted eukaryotic ancestral set. This suggests that the saprophytic fungal lifestyle, multicellularity as well as the highly polarized secretion associated with hyphal growth did not require any major innovation in the molecular machinery that regulates protein trafficking. The Rab/Ypt and other protein traffic-related families are kept small, not paralleling increases in genome size, in contrast to the expansion of such components observed in other branches of the tree of life, such as the animal and plant kingdoms. Our analysis suggests that multicellularity and cellular diversity in fungi followed different routes from those followed by plants and metazoa.

The evolution of biomolecular networks.

MEMBERS: Jose B. Pereira-Leal.

STUDENTS AND TECHNICIANS: Rita Rasteiro.

EXTERNAL COLLABORATORS: Emmanuel Levy (MRC Laboratory of Molecular Biology, UK), Sarah Teichmann (MRC Laboratory of Molecular Biology, UK).

A network is a representation suitable for describing complex systems with many interconnected components, for example the totality of the known protein-protein interactions in a given cell can be represented as a "protein interaction network". The use of such representations immediately makes available a range of analytical tools that were developed in mathematics, physics and sociology, among others, permitting a more quantitative description of biological phenomena and uncovering the principles of biological organization. We are interested in understanding the balance of evolutionary forces that generates the large-scale features of cellular networks, and to what extent these are universal, *i.e.* if they act on different levels of cellular organization. We are focusing on the modular character of protein interaction networks. Functional modularity is a concept from engineering, but that in biology represents spatially, temporally or chemically isolated sets of biological components, accomplishing a discrete biological function. We have been focusing on the role of gene duplication in the evolution of modularity and showed that duplication of self- interactions is a major evolutionary pathway for the generation of modularity in cellular networks, and in the evolution of protein complexes, an important

class of functional modules. We are now investigating whether domain gains and losses can occur within protein complexes, and found in a test case that we analyzed in detail that this is the case. Analysis of the global effect of this mechanism of protein evolution will follow.

Mechanisms of gene loss in parasitic genomes.

MEMBERS: Jose B. Pereira-Leal.

STUDENTS AND TECHNICIANS: André Mendonça.

Intracellular parasitism is well known to be accompanied by genome compaction and reduction. The genomes of these organisms reduce the size of their intergenic sequences, shorten their introns and even the termini of protein coding genes. Furthermore, there is extensive gene loss resulting in genomes with unusually low numbers of genes. Gene loss is one of several mechanisms of genome evolution. It is frequently the fate of duplicate genes, either because they are incompletely duplicated, or because redundancy with the original gene is sufficient to make the cost of having an extra copy too high and the gene is quickly pseudogenized. In the case of intracellular parasites, gene loss is believed to be neutral, as a consequence of host compensation lifting the selective pressure on many genes of the parasite genome. However, this scenario has never been tested. We are investigating the extent of neutrality of gene loss in parasitic genomes in comparison to their free-living counterparts, using a combined data analysis and modeling approach.

The evolutionary origins of the bacterial spore coat.

MEMBERS: Jose B. Pereira-Leal.

STUDENTS AND TECHNICIANS: Renato Alves.

EXTERNAL COLLABORATORS: Adriano Henriques (ITQB – UNL, Portugal).

Bacteria like those belonging to the genus *Bacillus* make endospores with a thick coat that has proven to be amongst the most resistant structures in nature. The bacterial spore can withstand pressures in excess of 40 GPa, temperature extremes of up to 500°C, the extreme cold of complete vacuum, and latency periods of hundreds of millions of years and still be able to germinate. We are investigating the composition of the bacterial coat, the structure believed to be partly responsible for such resilience. We are using bioinformatics methods to trace the known components of this structure throughout the tree of life, and are using this and other approaches to predict novel components of the bacterial spore coat, that we validate in the laboratory. One outcome of our study is the construction of a data integration system for bacterial coat components.

Integrative study on centrosome biogenesis and function.

MEMBERS: Jose B. Pereira-Leal, Monica Bettencourt-Dias.

STUDENTS AND TECHNICIANS: Zita Santos.

Centrosomes play a very important role in cell physiology, being involved in a multiplicity of disorders. However, their biogenesis and function are poorly understood. We are using

integrated approach to this problem, combining computational data mining with experimental studies in the versatile model organism *Drosophila melanogaster* and human tissue culture cells. We are taking advantage of the recent outburst of genomic and proteomic data to build a comprehensive annotated list of centriole-associated genes, predict novel components and regulators. These structures are present in every branch of eukaryotes suggesting a “core” conserved molecular mechanism for centriole biogenesis. We used comparative genomics and phylogenetic analysis to study the evolution of proteins known to be involved in centriole assembly. In *C.elegans*, SPD-2 is the first protein to be recruited to the centrosome and is responsible for ZYG-1 recruitment. This protein in turn recruits a complex of SAS-6 and SAS-5, necessary for the formation and elongation of a central tube. Posterior assembly of the microtubules onto the central tube is dependent on SAS-4. We mapped new putative orthologs of these proteins throughout the tree of life, including flagellated fungi, whose genome is currently being sequenced. We used sensitive search methods, developed classification criteria for each protein, and combined these with detailed phylogenetic analysis for orthology assignments. Our results show that the presence of proteins directly involved in structural aspects of centriole assembly, such as SAS-6 and SAS-4, correlates with the existence of centrioles. Strikingly, “triggers”/“regulators” of centriole duplication, such as SAK/PLK4, appeared late in evolution, in flagellated fungi and animals. This suggests that SAK/PLK4 role was previously fulfilled by another kinase, perhaps the founder of its family, Polo. Furthermore, it suggests that the appearance of SAK/PLK4 may be associated with linking centriole duplication to other aspects of centriole and cellular physiology. Other proteins such as SPD-2 or CP110 on the other hand seem to be defining other functions of these structures on specific branches of the tree of life.

FLAD (Fundação Luso Americana para o Desenvolvimento) COMPUTATIONAL BIOLOGY COLLABORATORIUM

The chief aim of the FLAD CBC is to establish, enable, and foster an international, collaborative network of associated institutions and scientists. It is an open host organization designed to enable intense cooperation amongst researchers from national and international institutions: the center hub of a collaborative network of research institutions. Its chief aims are to provide suitable facilities for visiting scientists, and hosting informatics technology to enable continuing off-site collaboration and research in Mathematical and Computational Biology.

In our second year of operation, most funded visitors result from submitted collaborative proposals. In September 2007 we launched various publicity efforts in the community at large. Interviews were given on the radio (e.g. on Rádio Clube Português and RDP 3), TV (RTP) and various print media, especially around the time of the European Conference on Artificial Life which the collaboratorium organized in September.

During 2007, we hosted the following scientists: Alaa Abi Haidar (Indiana University, USA), Pierre Baldi (University of California, Irvine, USA), Nuno Bandeira (University of California San Diego, USA), Randy Beer (Indiana University, USA), Luis Bettencourt (Los Alamos National Laboratory, USA), Peter Cariani (The Templeton Foundation and Harvard Medical School, USA), Melvin Cohn (The Salk Institute, USA), Edward Flach (Oxford/Indiana University, USA), Zvi Grossman (National Institutes of Health), Reinhard Laubenbacher (Virginia Tech, USA), Manuel Marques-Pita (University of Edinburgh, UK), Kiran Patil (BioCentrum-DTU at the Technical University of Denmark, Denmark), Jose Principe (University of Florida, USA), Luis Rocha (Indiana University, USA), Miguel Rocha (Universidade do Minho, Portugal), Isabel Rocha (Universidade do Minho, Portugal), Peter Todd (Indiana University, USA), Alessandro Vespignani (Indiana University/Turin), and Janet Wiles (The University of Queensland, Australia).

We can also report that 9 publications were produced from work developed in the visits sponsored by the collaboratorium in such journals as Genome Biology and Conservation Genetics. The FLAD collaboratorium also supported the process of grant submission by various visiting groups (e.g. by the groups of Lounes Chikhi and Gabriela Gomes), as well as various academic activities (e.g. support for the MIT|Portugal Program, Bio-Engineering Systems program) and conferences (e.g. hosting the 2007 European Conference on Artificial Life).

ECTOPIA

Ectopia is a laboratory hosting artists from different backgrounds interested in exploring the intersection of art and science. It fosters the development of collaborative projects involving artists and researchers. Ectopia provides resident artists access to the research being conducted at the Instituto Gulbenkian de Ciência. During the residency, the artists are exposed to the research through seminars and informal discussions with the scientists, being encouraged to develop collaborative projects. In addition, the researchers are also exposed to the artists and invited to take advantage of those collaborations in their scientific projects.

During 2007 the experimental art laboratory Ectopia hosted its first research projects, participated in several actions to divulge to artists the possibility of developing artistic residencies at the IGC, and started international collaborations with the objective of facilitating the hosting of foreign artists as well as giving a wider international exposure to projects developed at Ectopia.

MEMBERS: Marta de Menezes.

STUDENTS AND TECHNICIANS: Maria Manuela Lopes.

EXTERNAL COLLABORATORS: Nicola Triscot and Derek Hales (Arts Catalyst and University of Huddersfield, UK), Belinda Quirke and Noel Kelly (The Solstice Art Center and The Art Project, Ireland), Anne Kienhuis (The Art and Genomic Centre, The Netherlands), Jurij Krpan (Kapelica Gallery, Slovenia), Suncica Ostoic (The Kontejner, Croatia), Denisa Kera, Ciant (Check Republic), Monica Bello (Capsula, Spain), Antonio Franco (Museu Extremeno de Arte Contemporaneo, Spain), Laura Beloff (La Laboral, Spain), Jose Librero (Centro Andaluz de Arte Contemporaneo), Oron Catts, SymbioticA, Australia).

EXHIBITIONS

Decon, Instituto Gulbenkian de Ciênciam, Oeiras, Portugal; **Artists in Labs**, Enter3 Festival, Prague, Czech Republic. Curator: Denisa Kera.; **Decon**, Petit Cabanon, Porto, Portugal. Curator: Inês Moreira.; **Proteic Portrait**, Museo Extremeño e Iberoamericano de Arte Contemporáneo (MEIAC), Badajoz, Spain. Curator: Inês Moreira.; **Bios4**, Centro Andaluz de Arte Contemporáneo (CAAC), Sevilla, Spain. Curator: António Cerveira Pinto.; **Genesis**, Centraal Museum, Utrecht, The Netherlands. Curator: Emilie Berg Gomar.; **Depósito**, Reitoria da Universidade do Porto, Porto, Portugal. Curator: Paulo Cunha e Silva.

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 2007 or later are listed below. FCT derives from several programs, and are all awarded on the basis of competitive applications: (1) institutional support, as positions for scientists and technicians, in the framework of the Laboratório Associado ITQB/IBET/IGC; (2) institutional support, in the framework 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 Grants

POCI/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/BCI/47972/2002

Juan Carlos Belmonte

Mechanisms of regulation of nodal during left-right axis development.

PTDC/SAU-OBD/69928/2006

José A. Belo and Jorge Carneiro

Genetic and biochemical control of Cerl-2 in during early development.

PTDC/BIA-BCM/69912/2006

José A. Belo

Study of the role of vertebrate ADTK1, a novel Ser/Thr/Tyr kinase gene family.

POCTI/SAU-MNO /58192/2004

Jocelyne Demengeot

Inflammatory components in the biology of regulatory T cells and the prevention of auto-immune disease.

PTDC/SAU-MII/71402/2006

Jocelyne Demengeot

Quantitative and Qualitative T cell receptor repertoire requirements for immune tolerance establishment and maintenance.

PTDC/BIA-BCM/73195/2006

Mónica Bettencourt-Dias

Regulation of centriole biogenesis and function: an integrative approach.

PTDC/SAU OBD/73194/2006

Mónica Bettencourt-Dias

Regulation of the tumorigenesis-related kinase SAK/PLK4.

POCI/SAU-MMO/60333-2004

Jorge Carneiro

T cell production in HIV immunodeficiency.

PTDC/SAU-BEB/65992/2006

Margarida Carneiro

Effects of inflammation on bone and cartilage: collagen pattern and biomechanical properties.

POCTI/CBO/4765/2002

Sukalyan Chatterjee

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

POCI/BIA-BDE/55758/2004 and PPCDT/BIA-BDE/55758/2004

Francisco Dionisio and Isabel Gordo

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

PTDC/BIA-BDE/66180/2006

Francisco Dionisio

The role of commensal bacteria in the spread of antibiotic resistance genes.

PTDC/MAT/66426/2006

Francisco Dionisio

Mathematical models of evolutionary processes.

PTDC/CVT/71084/2006

António Duarte

Investigation of Notch signalling function in physiological and tumour angiogenesis.

POCTI P/CVT/56015/2004

António Duarte

Arteriogenesis: identification of new members of the Notch pathway involved in arterial cell fate determination.

PTDC/AGR-AAM/67858/2006

Paula Duque

Herbicide resistance in *Arabidopsis thaliana*: role of plant multidrug resistance transporters.

POCI/BIA-BCM/60046/2004

José A. Feijó

Oscillatory behavior in pollen tubes: fundamental biochemical and biophysical mechanisms for the regulation and of cell growth and morphogenesis.

POCI/AGR/58320/2004

José A. Feijó/Helena Pereira

Large scale screen for genes important in production associated phenotypes in *Vitis*.

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.

POCI/SAU-MMO/59913/2004

C. Fesel

Autoantibody repertoires and regulatory T-cells in human and murine lupus.

PTDC/SAU-OB/66438/2006

Miguel Godinho Ferreira

Inhibition of genomic instability by telomere protection.

PTDC/BIA-BCM/67261/2006

Miguel Godinho Ferreira

Identification of anti-checkpoint proteins at fission yeast telomeres.

POCTI/MAT/58528/2004

Gabriela Gomes

Reinfection thresholds and the management of recurrent infections.

POCTI/BSE/46856/2002

Isabel Gordo and Francisco Dionisio

Population genetics of adaptation in *E. coli*.

PTDC/BIA-BDE/73163/2006

Isabel Gordo, Helena Soares

Rates of mutations in the protozoa *Tetrahymena thermophila*.

PTDC/BIA-BDE/65276/2006

Isabel Gordo

Diversity and molecular evolution of pathogen populations.

POCI/SAU-MMO/55974/2004

Luís Graça

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

PTDC/SAU-MII/64279/2006

Luís Graça

Co-receptor and co-stimulation blockade for the induction of regulatory T cells in allergic airways disease.

PTDC/SAU-NEU/71310

Domingos Henrique

Stem cell based therapy for inner ear hair cell regeneration.

PTDC/SAU-OBG/75106

Domingos Henrique

Notch signaling and regulatory mechanisms during mammalian neurogenesis.

POCTI/BCI/48577/2002

António Jacinto

Cell-cell recognition and sorting during *Drosophila* morphogenesis.

POCTI/BIA-BCM/58389/2004

António Jacinto

Inflammation and chemotaxis in *Drosophila* embryos.

PTDC/SAU-OBG/73112/2006

António Jacinto

Molecular mechanisms of bone formation during caudal fin regeneration in zebrafish.

PTDC/SAU-OBG/7391/2006

Florence Janody

Using *Drosophila* to understand the molecular control of actin dynamics in aberrant active cell migration.

PTDC/SAU-MII/65029/2006

Cristina João

IMMUNE RECOVERY AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION -
MODULATION BY IG AND POTENTIAL CLINICAL APPLICATION.

PTDC/BIO/70815/2006

Rasmus Larsen

In vivo delivery of anti-inflammatory chimeric proteins: assessment of therapeutic
application.

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.

POCI/DG/BIA/82013/2006

Rui Martinho

Analysis of early transcriptional activation and germ-line segregation in *Drosophila
melanogaster*.

PTDC/BIA-BCM/69256/2006

Rui Martinho

Characterization of the transcriptional activation of the quiescent zygotic genome after
oocyte fertilization.

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

Role of auditory cortex in associative learning.

PTDC/SAU-NEU/71647/2006

Marta Moita

How do we learn the association of events separate in time? A Study on the
mechanisms underlying auditory trace fear conditioning.

POCTI/SAU-IMI/57946/2004

Maria Mota

The role of Heme oxygenase-1 and its products in the course and pathology of malaria infection.

POCI/SAU-MMO/58413/2004

Luisa Mota-Vieira

Study of genetic diversity of the Azorean population.

PTDC/SAU-GMG/64426/2006

Sofia A. Oliveira

Genetic epidemiology of stroke in the post-genomic era.

PTDC/SAU-GMG/64428/2006

Sofia A. Oliveira

microRnomics and proteomics of Parkinson's disease.

PTDC/PSI/71811/2006

Rui Oliveira

Winners and losers: social modulation of hormones, brain and behaviour.

PTDC/SAU-MII/69796/2006

Elisabetta Padovan

The role of Toll-like receptors in the control of intercellular communication during T lymphocytes activation.

POCTI/SAU-NEU/56986/2004

Teresa Pais/Sukalyan Chatterjee

Role of activated brain macrophages in animal models of neurological diseases.

PTDC/SAU-MII/69290/2006

R.M.E. Parkhouse.

Mechanism of cell cycle arrest induced by an HCMV viral gene.

POCTI/BCI/37953/2001

Leonor Parreira

Notch-signaling in normal hematopoietic differentiation.

POCI/IMI/61057/2004

Carlos Penha-Gonçalves

Determinantes genéticos de resistência à infecção hepática em modelos murinos de malária.

POCTI/SAU-MMO/57995/2004

Carlos Penha-Gonçalves

Diabetes tipo 1: Imunopatologia e factores de susceptibilidade genética.

POCI/SAU-MMO/62964/2004

Carlos Penha-Gonçalves

Genética da resposta imune inata na diabetes tipo 1 murina.

POCI/SAU-MMO/63284/2004

Joaquín Rodríguez-Léon

The cell biology and molecular basis of neural tube closure in the chick embryo.

PTDC/SAU-OBD/64628/2006

Leonor Saúde

Functional study of the molecular clock during vertebrate limb development.

PTDC/SAU-OBD/71596/2006

Leonor Saúde

Identification of the direct target genes of Terra using a ChIP-on-chip approach.

PTDC/SAU-MII/72027/2006

Miguel Seabra

Mechanismos moleculares de formação do vacúolo parasitário na infecção por malária.

PTDC/BIA-BCM/73146/2006

Miguel Seabra

Mecanismos moleculares de motilidade de organelos.

POCTI/BIA-BCM/60670/2004

Pedro Simas

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

POCTI/SAU-IMI/57365/2004

Pedro Simas

Herpesvirus modulation of B-lymphocyte function.

PTDC/SAU-MII/65017/2006

Pedro Simas

Herpesvirus-encoded microRNAs as novel regulators of host gene expression: molecular function during latency in B-lymphocytes.

POCI/CTM/61622/2004

Helena Soares

MTube - Study of physical properties of microtubules by AFM techniques.

POCTI/SAU-MNO/56066/2004

Miguel P. Soares

Modulation of atherosclerosis by the protective gene heme oxygenase-1: molecular mechanisms and therapeutic applications.

POCTI/BIA-BCM/56829/2004

Miguel P. Soares

Molecular mechanisms underlying the protective effect of Heme Oxygenase- 1: interaction with the NF-kappaB signal transduction pathway.

POCTI/BSE/48402/2002

Élio Sucena

Adaptation in outbred populations of *D. melanogaster*: a molecular genetics, developmental and behavioral analysis

POCTI/BIA-BDE/60950/2004

Élio Sucena

VERTICAL TRANSMISSION OF *WOLBACHIA* BACTERIA IN *DROSOPHILA*.

POCTI/BIA-PRO/60337/2004

Álvaro Tavares

Characterisation of the mitotic checkpoint in *Drosophila*: function of the proteins Mps1 and CENP-ana.

POCTI/BME/46257/2002

Ana Teresa Tavares

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

POCI/SAU-MMO/59725/2004

Ana Teresa Tavares

Characterisation and applications of a novel hemangioblast-specific transcriptional enhancer.

POCTI/BSE/48228/2002

Henrique Teotónio

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

POCI/BIA-BDE/61127/2004

Henrique Teotónio

Experimental evolution of outcrossing in *Caenorhabditis elegans*.

POCTI/BCI/42040/2001

Sólveig Thorsteinsdóttir/ Isabel Palmeirim

New aspects on coordinating limb bud development.

POCI/BIA-BCM/59201/2004

Sólveig Thorsteinsdóttir

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

PTDC/BIA-BCM/67437/2006

Sólveig Thorsteinsdóttir

More than putting cells into place during development: cell-extracellular matrix interactions in myogenesis.

PTDC/SAU-GMG/64519/2006

Astrid Moura Vicente

Molecular genetics and functional genomics of autism spectrum disorders.

PTDC/SAU-GMG/64426/2006

Astrid Moura Vicente

Genetic epidemiology of stroke in the Post-genomic era.

POCI/DG/BIA/82010/2006

Karina B. Xavier

Quorum Sensing in *Escherichia coli*.

PTDC/BIA-BCM/73676/2006

Karina B. Xavier

Identification and characterization of quorum sensing systems involved in bacterial inter-species communication.

European Union Grants

Marie Curie Intra-European Fellowship LIF-025885

Magarida Carneiro

Cellular and molecular dynamics of ectopic germinal center formation in rheumatoid arthritis.

INFRASTRUCTURE EUROPEAN MOUSE MUTANT ARCHIVE: EMMAINF

Jocelyne Demengeot

BIOCONTRACT - Cooperation in mutualisms: contracts, markets, space, and dispersal (European Science Foundation - EUROCORES - TECT).

Francisco Dionisio

Marie Curie International Reintegration Grant 029143

Paula Duque

Alternative Splicing in Arabidopsis.

MEXT-CT-2004-14338

Gabriela Gomes

Reinfection thresholds and the management of recurrent infections.

CT.06.EPI.205.1.0

Gabriela Gomes, Nico Stollenwerk (and other European partners)

Working group to develop mathematical and statistical models and analysis of protective factors for HIV infection among injecting drug users.

Coordinator: Mirjam Kretzschmar (University of Bielefeld).

LSHG-CT-2003-503494

Domingos Henrique

FunGenES- Functional genomics in engineered ES cells.

LSHM-CT-2003-504468

António Jacinto, Joaquin Rodriguez-Leon, Isabel Palmeirim, Solveig Thosteinsdottir

Cells into Organs: Functional genomics for development and disease of mesodermal organ systems.

Marie-Curie RTD REG/D.5(2006)D/506117

Sara Magalhães

Sex and the maintenance of diversity in heterogeneous habitats.

Marie Curie International Reintegration Grant 029165

Rui Martinho

Analysis of early transcriptional activation and germ-line segregation in *Drosophila melanogaster*.

Marie Curie Intra-European Fellowship 024563

Sofia A. Oliveira.

Stroke Genetics and Genomics.

LSH-2005-1.2.5-1

Miguel P. Soares

Engineering of the porcine genome for xenotransplantation studies in primates: a step towards clinical application.

MRTN-CT-2004-512348

Álvaro Tavares

Research and Training Network "Spindle Dynamics"

Marie Curie International Reintegration Grant 031108

Karina B. Xavier

Inter-species cell-cell signalling in bacteria

Other Grants

Shering-Plough Inc. Research Grant

Margarida Carneiro

Impairment of oxidative burst and tolerance breakdown: consequences for rheumatoid Arthritis.

Octapharma Portugal

Jocelyne Demengeot

Ivlg therapy in SLE, regulating the regulators.

Royal Society International Joint Projects 2006/R2

Mónica Bettencourt-Dias.

Convénio Grices

Mónica Bettencourt-Dias.

APCL (Portuguese Association against Leukemia)

Sérgio Dias and Ana Cachaço

Mechanisms involved in bone marrow recovery following radio or chemotherapy: a role for the vascular compartment.

GlaxoSmithkline

Sérgio Dias

Characterization of bone marrow niches that impede or favor leukemia engraftment and expansion.

Liga Portuguesa Contra o Cancro and Crioestaminal

Sérgio Dias

Mechanisms of endothelial differentiation from endothelial progenitors.

Crioestaminal, Sociedade Portuguesa de Oftalmologia, Smart Matrix SDR LINK Project (MRC, UK)

Sérgio Dias

A role for endothelial progenitors in health and in disease.

FLAD

Sérgio Dias

Importância do metabolismo do butirato, resultante da fermentação bacteriana das fibras no cólon, no aparecimento e progressão do carcinoma colorrectal.

Liga Portuguesa Contra o Cancro and Novartis

Sérgio Dias

Angiogenesis in leukemias and myelodysplastic syndromes.

Ministry of Education and Science, Spain

Jose Faro

Studies on the role of regulatory T lymphocytes in the germinal centre reaction: a bio-mathematical approach.

Consellería de Innovación e Industria (Xunta de Galicia) Spain.

Jose Faro.

Nanoparticles in biological systems: immune response and diagnostic. Evaluation of the immune response to metallic nanoparticles and their application in diagnostic.

Association for International Cancer Research, UK

Miguel Godinho Ferreira

End-protection and DNA repair at *S. pombe* telomeres.

Fundação Calouste Gulbenkian

Gabriela Gomes, Sander van Noort

Gripenet.pt.

POSC 651/4.2/C/REG

Gabriela Gomes, Vítor Faustino, Paula Macedo, José Lourenço

Gripenet.pt.

AHFMR (Altera Heritage Foundation for Medical Research, Canada)

Gabriela Gomes

Meningitis vaccine design and implementation including modeling.

Coordinators: Anthony Schryvers (University of Calgary) and Rolando Pajon Feyt (Finlay Institute, La Habana, Cuba).

Human Frontiers Science Program Research Grant RGP21/2007

António Jacinto, Shane Hutson, Wayne Brodland,

Integrating the genetics, mechanics and phenomenology of embryonic wound healing.

Centro de Biologia do Desenvolvimento.

Florence Janody.

Understand how cytoskeleton organization is regulated in genetically distinct epithelia:
The case of the Capping Protein heterodimer.

APCL (Portuguese Association against Leukemia)

Cristina João

Immune Reconstitution after stem cell transplantation.

APCL (Portuguese Association against Leukemia)

Cristina João

Recuperação imunológica em doentes com linfoma não Hodgkin tratados com Rituximab, associado ou não a quimioterapia.

Bolsa de Investigação Pfizer Professor João Cid dos Santos

Moisés Mallo

Real time in vivo studies of heart outflow tract morphogenesis and ther role of the *Tbx1* gene.

Fundação Bial

Marta Moita

How do we learn to associate events separate in time: a study using trace auditory fear conditioning.

GEMI Fund AgaLinde Healthcare.

Maria Manuel Mota.

Mechanism of HO-1/CO and profilaxis/therapeutical aplication of CO in the pathogenesis of severe malaria.

M2.1.2/I/013/2007

Luisa Mota-Vieira

The study of inbreeding, recessive mutations and genomic homozygosity in the Azorean population.

Center for Human Genetics, Duke University

Sofia A. Oliveira

Parkinson's disease proteomics.

3R Research Foundation Switzerland.

Elisabetta Padovan

Adjuvanticity of microbial-derived particles and synthetic analogs *in vitro*.

Wellcome Foundation

R.M.E. Parkhouse

African swine fever virus: development of vaccines and epidemiological investigations.

Medinfar

Carlos Penha-Gonçalves

Influence of natural polyphenolic compounds on the pathogenesis course of Type 1 Diabetes.

Fundação Calouste Gulbenkian

Luis Rosario

Evaluation of the ability of cardiac, bone marrow and embryonic pluripotent cells to functionally integrate into the myocardium.

Phillip Morris External Research Program

Miguel P. Soares

Anti-atherogenic effect of inhaled carbon monoxide: assessment of mechanism of action and potential therapeutic applications.

POCI/DIV/2005/00236

ÉLIO SUCENA

O MILHO E A CIÊNCIA: A HISTÓRIA DO MILHO E DO HOMEM.

Autism Genome Project (AGP) – International Consortium for the Genetics of Autism

Astrid Vicente

Autism Speaks, British Medical Research Council (MRC), Health Research Board of Ireland (HRB), Genome Canada, Canadian Institutes for Health Research (CIHR), Southwest Autism Research and Resource Center (SARRC) and Hilibrand Foundation.

The Autism Simplex Collection (TASC) - International Consortium for the Genetics of Autism

Astrid Vicente

Autism speaks.

Comissão de Fomento da Investigação em Cuidados de Saúde

Astrid Vicente

Estudo de factores imunológicos e de neuroprotecção em pacientes com autismo.

Comissão de Fomento da Investigação em Cuidados de Saúde

Astrid Vicente

Estudo de factores genéticos envolvidos na susceptibilidade aos acidentes vasculares Cerebrais e evolução dos doentes aos 3 e 12 meses.

Fundação Astra-Zeneca

Astrid Vicente

Biomarkers in Alzheimer's disease: the lipid homeostasis/oxidative stress connection.

Programa de Bolsas FLAD/NSF

Karina B. Xavier

Portugal-EUA: parcerias e redes para investigação.

ECTOPIA Research Projects

Decon – Deconstruction, Decontamination, Decomposition.

Artistic Project developed by Marta de Menezes in collaboration with Lígia Martins at the ITQB. In 2007 was the development and production of the project, as well as its first exhibitions. The project had the support of the European Consortium SOPHIED, of the Instituto da Artes and Ministerio da Cultura, and from IBET. This year it was exhibited in Sevilla, Prague, Porto and at the IGC.

Ethology. Artistic Project developed by Maria Manuela Lopes in collaboration with a variety of groups from the IGC and IBMC. Supported by the Instituto das Artes/Ministério da Cultura and the Ciência Viva. Now in its developing stage.

In the beginning there was the Word. Artistic project developed by Marta de Menezes specially for an exhibition at Faulconer Gallery in Grinnell, USA. Developed with the help and collaboration of Paula Duque in IGC.

Arte e Ciência. International Conference organized by Ectopia in collaboration with IBMC/INEB at the Museu Nacional Soares dos Reis in Porto. The conference was supported by IBMC/INEB and the Ciência Viva.

Evolutionary Cultures. Ectopia is now the Coordinating partner of the European Network Evolutionary Cultures of institutions connected with art and science projects. ArtsActive. In November Ectopia became a member of this international network, with the objective of promoting the hosting of international artists for Ectopia. www.artsactive.net.

PUBLICATIONS

1. Ali F., Hamdulay S.S., Kinderlerer A.R., Boyle J.J., Lidington E.A., Yamagushi T., Soares M.P., Haskard D.O., Randi A.M. and Mason J.C. Statin-mediated cytoprotection of human vascular endothelial cells: a role for Kruppel-like factor 2-dependent induction of heme oxygenase-1. *J Thrombosis Haemostasis* (2007) 5:2537-2546.
2. André J.-B. and Day T. Perfect reciprocity is the only evolutionary stable strategy in the continuous iterated prisoner's dilemma. *J Theor Biol* (2007) 247:11-22.
3. Arenkiel B.R., Peca J., Davison I.G., Feliciano C., Deisseroth K., Augustine G.J., Ehlers M.D. and Feng G. In vivo light-induced activation of neural circuitry in transgenic mice expression channelrhodopsin-2. *Neuron* (2007) 54:205-218.
4. Becker J.D. and Feijó J.A. How many genes are needed to make a pollen tube? Lessons from transcriptomics. *Ann Bot* (2007) 100:1117-1123.
5. Belo J.A., Bento M.C. and Tavares A.T. Functional analysis of novel genes differentially expressed genes in heart/hemangioblast precursor cells (H/HPC). *Dev Biol* (2007) 306:406-407.
6. Bejerano-Sagie M. and Xavier K.B. The role of small RNAs in quorum sensing. *Curr Opin Microbiol* (2007) 10:189-198.
7. Bettencourt-Dias M. and Glover D.M. Centrosome biogenesis and function: centrosomics brings new understanding. *Nat Rev Mol Cell Biol* (2007) 8: 451-463.
8. Carneiro J., Leon K., Caramalho I., van den Dool C., Gardner R., Oliveira V., Bergman M.-L., Sepúlveda N., Paixão T., Faro, J., and Demengeot J. When three is not a crowd: A crossregulation model of the dynamics and repertoire selection of regulatory CD4 T cells. *Immunol Rev* (2007) 216:48-68.
9. Carneiro-Sampaio M., and Coutinho A. Immunity to microbes: Lessons from primary immunodeficiencies, *Infection Immun* (2007) 75:1545-1555.
10. Carneiro-Sampaio M., and Coutinho A. Tolerance and autoimmunity: lessons at the bed-side of primary immunodeficiencies. *Advances Immunol* (2007) 75:1545-1555.
11. Casalou C., Fragoso R., Moura Nunes J.F. and Dias S. VEGF/PLGF induces leukemia cell migration via P38/ERK1/2 kinase pathway, resulting in Rho GTPases activation and caveolae formation. *Leukemia* (2007) 21: 1590-1594.

12. Chora A.A., Fontoura P., Cunha A., Pais T.F., Cardoso S., Ho P.P., Lee L.Y., Sobel R.A., Steinman L. and Soares M.P. Heme oxygenase-1 and carbon monoxide suppress autoimmune neuroinflammation. *J Clin Invest* (2007) 117:438-447.
13. Cohn M. On a key postulate of T-cell receptor restrictive function: the V-gene as a single pool encoding recognition of the polymorphic alleles of the species major histocompatibility complex. *Immunology* (2007) 120:140-142.
14. Combadão J., Campos P.R.A., Dionisio F. and Gordo I. Small-world networks decrease the speed of Muller's ratchet. *Genet Res* (2007) 89:7-18.
15. Correia A.C., Costa M., Moraes F., Bom J., Nóvoa A. and Mallo M. *Bmp2* is required for migration but not for induction of neural crest cells in the mouse. *Dev Dyn* (2007) 236:2493-2501.
16. Correia C. and Vicente A.M. Pharmacogenetics of risperdone response and induced side effects. *Personalized Med* (2007) 4:271-293.
17. Correia S.P.C., Dickinson A. And Clayton N.S. Western scrub-jays anticipate future needs independently of their current motivational state. *Curr Biol* (2007) 17:856-861.
18. Costa M.J.L., Pedro L., de Matos A.P.A., Aires-Barros M.R., Belo J.A., Gonçalves J. and Ferreira G.N.M. Molecular construction of bionanoparticles:chimaeric SIV p17-HIV 1 p6 nanoparticles with minimal viral protein content. *Biotechnol Appl Biochem* (2007) 48:35-43.
19. Coutinho A.M., Oliveira G., Katz C., Feng J., Yan J., Yang C., Marques C., Ataíde A.S., Miguel T., Borges L., Almeida J., Currais A., Bento C., Mota-Vieira L., Temudo T., Santos M., Maciel P., Sommer S.S. and Vicente A.M. *MECP2* coding sequence and 3'UTR variation in 172 unrelated autistic patients. *Am J Med Genet Neuropsychiatric Genet* (2007) 144:475-483.
20. Coutinho A.M., Sousa I., Martins M., Correia C., Morgadinho T., Bento C., Marques C., Ataíde A.S., Miguel T., Moore J.H., Oliveira G. and Vicente A.M. Evidence for epistasis in the determination of platelet serotonin levels and in autism etiology. *Hum Genet* (2007) 121:243-256.
21. Cunha-Rodrigues M., Portugal S., Febbraio M. and Mota M.M. Bone marrow chimeric mice reveal a dual role for CD36 in *Plasmodium berguei* ANKA infection. *Malaria J* (2007) 6:Art. Nº 32.
22. Dionisio F. Selfish and spiteful behaviour through parasites and pathogens. *Evol Ecol Res* (2007) 9:1199-1210.

23. Dionisio F. and Gordo I. Controlling excludability in the evolution of cooperation. *Evol Ecol Res* (2007) 9:365-373.
24. Douradinha B., van Dijk M.R., Ataíde R., van Gemert G.J., Thompson J., Franetich J.F., Mazier D., Luty A.J., Sauerwein R., Janse C.J., Waters A.P. and Mota M.M. *Int J Parasitol* (2007) 37:1511-1519.
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26. Fernández B.G., Arias A.M. and Jacinto A. Dpp signalling orchestrates dorsal closure by regulating cell shape changes both in the amnioserosa and in the epidermis. *Mech Dev* (2007) 124:884-897.
27. Ferreira, M.G. Telomeres on the Cdk roller coaster ride, *Nat Cell Biol* (2007) 9:22-23.
28. Ferrer E., Bonay P., Foster-Cuevas M., González L.M., Dávila I., Cortéz M.M., Harrison L.J.S., Parkhouse R.M.E. and Gárate T. Molecular cloning and characterisation of Ts8B1, Ts8B2 and Ts8B3, three new members of the *Taenia solium* metacestode 8 kDa diagnostic antigen family. *Mol Biochem Parasitol* (2007) 152:90-100.
29. Ferrer E., Gonzalez L.M., Martinez-Escribano J.A., Gonzalez-Barderas M.E., Cortez M.M., Davila I., Harrison L.J.S., Parkhouse R.M.E. and Garate T. Evaluation of recombinant HP6-Tsag, an 18kDa *Taenia saginata* oncospherical adhesion protein, for the diagnosis of systicercosis. *Parasitol Res* (2007) 101:517-525.
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SEMINARS AT THE IGC

The IGC runs regular seminar series, of internal and external seminars, with international speakers. Below is a listing of all seminars held in 2007.

JANUARY

Rossana Henriques

The 40S ribosomal protein S6 kinase represses cell proliferation through the RBR1 E2F pathway in Arabidopsis.

The Rockefeller University, USA.

John F. Kearney

Marginal Zone B cells: players in innate and adaptive immunity.

University of Alabama, USA.

Zsuzsanna Izsvak

Regulation and application of transposable elements in vertebrates.

Max-Delbrück-Centrum für Molekulare Medizin, Berlin, Germany.

Eric Westhof

RNA structural biology

Architecture et Réactivité de l'ARN.

Inst. Biologie moléculaire et cellulaire, CNRS, Strasbourg, France.

Judith L. Campbell

A genetic network controlling genome stability in yeast.

California Institute of Technology, USA.

Bill Newsome

Value-based decision-making: from behavior to neural circuits.

Howard Hughes Medical Institute and Stanford University, USA.

António Coutinho

The IGC.

IGC, Oeiras, Portugal.

Luis Ferreira Moita

Genetic dissection of immune response signaling pathways.

IMM, Lisbon, Portugal

Nicole Gorfinkiel

Cellular junctions during *Drosophila* dorsal closure.

University of Cambridge, UK.

Francisco Dionísio
The evolution of antibiotic resistance: costly advantageous mutations.
IGC, Oeiras, Portugal.

Tim Hunt
Getting in and out of mitosis.
Imperial Cancer Research Fund, UK.

Hélder Maiato
Ensuring mitotic fidelity in mammals.
IBMC, Porto, Portugal.

Rute Nascimento
Identification and utility of novel, "unassigned" virus host "evasion" genes.
IGC, Oeiras, Portugal.

Mark Dessing
Beyond four colors.
Becton-Dickinson Biosciences Europe, Belgium.

FEBRUARY

Jörg Kirberg
Beyond competition for the APC - hierarchical clues in homeostatic proliferation.
Max Planck of Immunobiology; Freiburg, Germany.

Katia Koelle
The role of antigenic neutrality in the evolutionary dynamics of influenza.
Center for Infectious Disease Dynamics, Penn State University, USA.

Teresa Pais
Microglia and T cell activation in cerebral malaria.
IGC, Oeiras, Portugal.

Miguel Soares
Targeting Heme Oxygenase-1 to overcome the pathogenesis of inflammatory diseases.
IGC, Oeiras, Portugal.

Anthony P. Monaco
Genetics of neurodevelopmental disorders.
Director, Wellcome Trust Centre for Human Genetics, Univ. of Oxford, UK.

Ricardo Ribeiro dos Santos

Cellular therapy for chronic chagasic cardiomyopathy and hepatopathies: the Brazilian experience.

Centro de Pesquisas Gonçalo Moniz, Fiocruz and Hospital São Rafael, Salvador, Brazil

Mark Feitelson

New tools to study the pathogenesis of chronic hepatitis B and C virus infections.

Thomas Jefferson University, USA.

Santiago Zelenay

Prevention and cure of spontaneous and severe encephalomyelitis by inducing de novo generation of specific regulatory T cells in vivo.

IGC, Oeiras, Portugal.

MARCH

Robin Weiss

Blocking the docking of HIV.

University College London, UK.

Robin Weiss

Parasitic cancer cells.

University College London, UK.

J. A. Cole

How to use micro-arrays: sense, nonsense, lists and new insights into microbial physiology.

University of Birmingham, UK.

José Pereira-Leal

What decides the functional fate of gene duplicates?

IGC, Oeiras, Portugal.

Stacey Efsthathiou

The molecular basis of herpes simplex virus latency and reactivation.

Cambridge University, UK.

Joaquín León

Asymmetric gonad development in birds, a matter of size?

IGC, Oeiras, Portugal.

Sebastian Amigorena

Antigen presentation and T cell activation by dendritic cells.

U365 INSERM, Institut Curie, Section Recherche, Paris, France.

Jorge Vieira

What is the rate at which adaptation happens at the molecular and phenotypic level?

IBMC, Porto, Portugal.

Arcadi Navarro

Associations between chromosomal rearrangements and genic evolution in mammals.

University Pompeu Fabra, Barcelona, Spain.

Leonor Saúde

The establishment of the left-right axis in vertebrates.

IGC, Oeiras, Portugal.

Jim Watson

Rules for Science.

Cold Spring Harbor Laboratory, USA

Danny Reinberg

Chromatin and its impact on gene expression and cellular memory.

Howard Hughes Medical Inst., NYU School of Medicine-Smilow Res. Center, USA.

APRIL

Sérgio Dias

Endothelial progenitors in tumor angiogenesis: what we now know.

IGC, Oeiras, Portugal and IPO, Lisbon, Portugal.

Moisés Mallo

All you always wanted to know about Hox and you never dared to ask.

IGC, Oeiras, Portugal.

Matt Rockman

The genetic basis of heritable phenotypic variation in *C. elegans*.

Princeton University, USA.

Sofia Oliveira

Human Genetics @ IGC: past, present and future.

IGC, Oeiras, Portugal.

Benedikt Kost

Maintenance of polarized Rac/Rop signalling and cell expansion at the pollen tube tip.
Heidelberg Institute of Plant Sciences, Univ. of Heidelberg, Germany.

Alice Cheung

Functional contribution of formins, a family of actin nucleation proteins, the polarized cell growth process in pollen tubes.
University of Massachusetts, Amherst, USA.

Lorena Riol Blanco

What is the function of the immunological synapse of the dendritic cells?
Centro de Investigaciones Biológicas (CSIC), Madrid, Spain.

Álvaro Tavares

Tension will tear us apart: kinetochores and chromosome segregation.
IGC, Oeiras, Portugal.

Alessandro Vespignani

Computational approaches to the worldwide spread of emerging.
Indiana University School of Informatics, Indiana, USA.

David Sherratt

Bacterial chromosome dynamics.
Iveagh Professor of Microbiology, University of Oxford, UK.

Graham Wilkie

People Programme in FP7.
DG Research, European Commission.

Reinhard Laubenbacher

Biochemical network inference.
Virginia Bioinformatics Institute, VirginiaTech, USA.

MAY

Ginès Morata

Apoptosis in tumor formation in drosophila.
Centro de Biología Molecular del CSIC, Madrid, Spain.

Juan Rivera

Greasing of the allergic and inflammatory responses; lipids as intrinsic and extrinsic regulators.
Chief, Molecular Inflammation Section, Director, Office of Science and Technology, NIH, USA.

Vincent Archambault

Starting with a protein interaction to tackle cellular function - case story on *Drosophila Polo Kinase*.

University of Cambridge, UK.

Guillaume Dugue

Golgi cells: a network underlying oscillations in the cerebellar cortex?

Ecole Normale Superior, Paris, France.

Miguel Seabra

Membrane traffic and disease.

IGC, Oeiras, Portugal.

Elizabeth Jones

The role of hemodynamic forces during mammalian development.

Inserm Unit 833, Dep. of Experimental Medicine, Collège de France, Paris, France.

Steven Suchting

The Notch ligand Dll4 negatively regulates VEGF-induced angiogenic sprouting.

Inserm Unit 833, Dep. of Experimental Medicine, Collège de France, Paris, France.

Francesco Colucci

The many ways of activating innate immunity lymphocytes.

Babraham Institute, UK.

Joe Paton

Stimulus and action values in amygdala and basal ganglia circuits: from systems to synapse.

Columbia University, New York, NY.

Domingos Henrique

Making Neurons: from the embryo to ES cells.

IGC, Oeiras, Portugal and FMUL, Lisbon, Portugal.

Valérie Nicaise

Host factors recruited by plant viruses during the infection process.

INRA, Bordeaux, France.

Patrick Phillips

The emerging synthesis between evolutionary genetics and molecular biology.

University of Oregon, Eugene, USA.

Hideki Garren

DNA vaccines for autoimmune disease: preclinical and clinical results in MS and type I diabetes.

Co-founder and VP of Research Bayhill Therapeutics, Adjunct Clinical Faculty, Stanford University, USA,

Jeroen Dobbelaere

A genome-wide Visual RNAi screen to identify New centrosome components.

The Wellcome Trust/Cancer Research UK, The Gurdon Institute, University of Cambridge, UK.

Lisete Fernandes

OS-signaling in transcription regulation: Yap1 activity under altered actin dynamics.

IGC, Oeiras, Portugal.

Robin Allshire

CENP-A chromatin at fission yeast centromeres.

Wellcome Trust Centre Cell Bio, Institute of Cell Biology, School Biological Sciences, University of Edinburgh, UK.

Francois Balloux

The long March of human genes.

University of Cambridge, UK.

Helena Soares

Identification of a novel pathway for CD4+ T cell priming.

IGC, Oeiras, Portugal.

Miguel Godinho-Ferreira

The hunt for the anti-checkpoint activity at telomeres.

IGC, Oeiras, Portugal.

JUNE

Peter Fraser

Transcription factories and nuclear organization of the genome.

Head, Lab. Chromatin and Gene Expression, Babraham Research Campus, Cambridge, UK.

Karina Xavier

Termination of the quorum sensing signalling cycle by enteric bacteria.

IGC, Oeiras, Portugal.

Dan Holmberg
Dissecting the pathogenesis of Type I diabetes in an animal model.
Umea University, Sweden.

Marta Alarcón-Riquelme.
The Genetics of SLE: an update.
Associate Professor in Medical Genetics, Rudbeck Lab., Uppsala, Sweden.

João Pedro Simas
Gammaherpesvirus suppression of NF-kB transcriptional activity via a EC5S ubiquitin complex that targets nuclear p65/RelA.
IGC, Oeiras, Portugal and IMM, Lisbon, Portugal.

Christian Conrad
Automatic primary and secondary screening in genome wide RNAi live cell-based assays.
Imaging Facility, EMBL, Germany.

Marta Moita
Of dilemmas and fear.
IGC, Oeiras, Portugal.

European Patent Office
Patenting in Biotechnology and Pharmacy.
European Patent Office, Munich, Germany.

Juan J. Lafaille
Antigen induced Foxp3+ regulatory T cells.
Skirball Inst. of Biomolecular Medicine, New York University School of Medicine, USA.

Paula Duque
Herbicide resistance in arabidopsis: the role of plant MDR transporters.
IGC, Oeiras, Portugal.

Luis Bettencourt
Growth, innovation, scaling and the pace of life in cities.
Santa Fe Institute, USA.

José Principe
Brain machine interfaces.
University of Florida, USA.

Josh Brickman

Conflict between self-renewal & lineage commitment waged in the battle field of the early embryonic mesendoderm.

Institute for Stem Cell Research, University of Edinburgh, UK.

JULY

Pedro Rifes

Redefining the role of ectoderm in somitogenesis: a player in the formation of the fibronectin matrix of presomitic mesoderm

IGC, Oeiras, Portugal.

Leonor Santos Ruiz

Fishing in Costa del Sol. Research on teleostean fin regeneration at the University of Málaga, Spain.

Facultad de Ciencias, Campus de Teatinos, Málaga, Spain.

Astrid Moura Vicente

Finding genes for complex brain pathologies: autism, stroke, Alzheimer's disease.

IGC, Oeiras, Portugal.

Amadeu Soares

New ecotoxicological tools and more – biodiversity and other challenges.

Universidade de Aveiro, Portugal.

Leandro Nunes de Castro

LVCOn - Virtual laboratory in natural computation.

LSIn, UniSantos, Brazil.

Hinrich Schulenburg

Evolution of *C. elegans*-parasite interactions.

Zoological Institute, University of Tuebingen, Germany.

Rui Martinho

De novo formation of 6000 polarized epithelial cells in less than 4 hours: a forward genetics approach

IGC, Oeiras, Portugal.

Luís Rocha

A Computational Model of RNA Editing and the Evolution of Regulation and Memory in Dynamic Environments.

IGC, Oeiras, Portugal and Indiana University, USA.

Thiago Carvalho

Development and function of marginal Zone B cells.

IGC, Oeiras, Portugal.

Ken Harris

How do neurons work together? Insights from auditory cortex.

Rutgers University, USA.

Allen Braun

Using multimodal neuroimaging methods to study recovery in post-stroke aphasia.

NIH, USA.

Mónica Bettencourt Dias

Right time, right place and only once: the control of centriole birth.

IGC, Oeiras, Portugal.

John Driver

Strain Dependant Maturation of Tolerogenic and Immunogenic Mouse Dendritic Cells Induced by Activated NKT Cells.

The Jackson Laboratory, USA.

Kiran R. Patil

Systems biology of metabolic networks: Looking beyond the genome.

Center for Microbial Biotechnology, Biocentrum-DTU, Technical University of Denmark, Denmark.

Manuel Marques-Pita

Representational Redescription: From collective computation in cellular automata, to understanding the conceptual properties of gene regulatory network.

University of Edinburgh, UK.

Leonor David

Mucin glycosylation in cancer - a short version of long story of translational research.

IPATIMUP, Porto, Portugal.

António Jacinto

Reconstructing the epithelium.

IGC, Oeiras, Portugal.

SEPTEMBER

Lounes Chikhi

Population crash in Borneo orang-utans: insights from genetic data.

IGC, Oeiras, Portugal and CNRS, Paris, France.

Isabelle Olivieiri

Specialization versus speciation in nitrogen-fixing bacteria.

Université Montpellier, France.

Peter Cariani

Temporal codes and timing nets for neural information processing.

Templeton Foundation, USA.

Raquel Sá-Leão

Epidemiology of *pneumococci* in Portugal: unique features and unexplored opportunities.

ITQB/UNL, Oeiras, Portugal.

Sérgio Cunha

Exposure to infections in early life as risk factor for asthma phenotypes in later childhood.

Instituto de Saúde Coletiva, Universidade Federal da Bahia, Brasil.

Matti Hakama

Cancer: genetic, communicable or something else.

Tampere School of Public Health, Finland.

Volodymyr Dvornyk

Genetics of menopause and menarche: why should we study it?

Kent State University, UK.

Lília Perfeito

Evolution at warp speed.

IGC, Oeiras, Portugal.

Joachim Lingner

Regulation of telomerase at chromosome ends.

Inst. Technology Lausanne (EPFL), Swiss Inst. Experimental Cancer Research (ISREC), Switzerland.

Juan Mata

A genome-wide view of posttranscriptional regulation.

Department of Biochemistry, University of Cambridge, UK.

Ligia Deus/ Rita Neres

Mouse malaria: liver genetics and pregnancy-associated pathology.

IGC, Oeiras, Portugal.

Edward Louis

An overview of the Madagascar biodiversity & biogeography project at Omaha's Henry Doorly Zoo.

Henry Doorly Zoo, Omaha, Nebraska, USA.

Sérgio Dias

Endothelial cells and signalling pathways in normal and malignant bone marrow.

IGC, Oeiras, Portugal and IPO, Lisbon, Portugal.

Gines Morata

Cell competition, apoptosis, and tumor progression.

Centro Biología Molecular Severo Ochoa, Universidad Autónoma Madrid, Spain.

OCTOBER

Jorg Becker

A whole-genome transcriptomics approach to sexual reproduction and apical cell growth in *arabidopsis thaliana*.

IGC, Oeiras, Portugal.

Stephen Suomi

Serotonin, aggression and gene X environment interactions in rhesus monkeys and other primates.

National Institutes of Health, Bethesda, Maryland, USA.

Sidonia Fagarasan

Dynamic interactions between bacteria and B cells in GALT.

Research Center for Allergy and Immunology, RIKEN Yokohama, Japan.

Osami Kanagawa

Imaging at RIKEN, Is seeing believing?

Riken Center for Allergy and Immunology, RIKEN Yokohama, Japan.

Tiago Paixão

The stochastic basis of somatic variation.

IGC, Oeiras, Portugal.

Richard Vaughan-Jones

Spatial regulation of intracellular pH and Ca²⁺ in heart: role of gap junctions and sarcolemmal transporters.

Burdon Sanderson Cardiac Science Centre, Oxford, UK.

Florence Janody

Cell-type specific regulation of the actin cytoskeleton during epithelial morphogenesis in *Drosophila*.

IGC, Oeiras, Portugal.

Richard Borowsky

What happens in the dark ?

New York University, USA.

Simon Tavaré

Arrays are dead: long live resequencing!

University of Cambridge, UK

Marcos Antezana

Highly conserved regimes of neighbor-base-dependent mutation.

The University of Chicago, USA.

Henrique Teotonio

The genetics of adaptation in sexual organisms.

IGC, Oeiras, Portugal.

Nuno Bandeira

A new approach to the identification of proteins and post-translational modifications.

UC San Diego, USA.

Claude Desplan

Detection and processing of color information in *Drosophila*.

New York University, USA.

Thomas Lenormand.

An alternative theory for the dominance of mutations.

CNRS, Montpellier, France.

Helfrid Hochegger

Cdk1 independent activation of aurora kinase A at the G2/M transition.

Genome Damage and Stability Centre, University of Sussex, UK.

NOVEMBER

Anthony Dean

Revealing an ancient adaptation.

University of Minnesota, St. Paul, USA.

Jocelyne Demengeot

Tuning and specifying mature lymphocyte differentiation upon cellular activation.
IGC, Oeiras, Portugal.

Linda Ottoboni

Molecular mechanism controlling lymphocyte recruitment in inflamed vessels and outlook on EAE.
Deutsches Krebsforschungszentrum, Heidelberg, Germany.

Carlos Tadokoro

Regulatory T cells in action.
Skirball Inst. of biomolecular medicine, New York Univ. School of Medicine, NY, USA.

Gabriel Martins

Imaging cells into organs.
IGC, Oeiras, Portugal.

Vittoria Colizza

Understanding epidemic control strategies.
Institute for Scientific Interchange Foundation, Turin, Italy.

Phong T. Tran

Microfluidics, microtubules, and cell shape.
University of Pennsylvania, Philadelphia, USA and Institut Curie, Paris, France.

Jose Belo

Handedness and hearts: new endeavors in the study of the vertebrate embryo.
IGC/IBB/CBME/Univ. Algarve, Portugal.

Bonnie Bassler

Cell-Cell communication and virulence in *vibrio cholerae*: a new quorum-sensing pathway and a new autoinducer.
Princeton University, New Jersey, USA.

Maria Mota

Approaching malaria from the host side.
IGC, Oeiras, Portugal/IMM, Lisbon, Portugal.

Ute Saunders

Dendritic cell mediated activation of a Type 2 T-Independent immune response.
University of Alabama at Birmingham, USA.

Hugo Almeida

Revealing the mechanism of DNA-end protection using a direct assay for testing telomeric integrity.

IGC, Oeiras, Portugal.

Vanessa Borges

Identification of the anti-checkpoint activity at telomeres.

IGC, Oeiras, Portugal.

Paul Frankland

Neural mechanisms of memory consolidation.

Program in Neurosciences & Mental Health, Hospital for Sick Children, Toronto, Canada.

Brian Charlesworth

Mutation, selection and genome evolution.

University of Edinburgh, UK.

Rob Martienssen

Slicing, spreading and copying silent heterochromatin with RNA interference.

Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, USA.

Rosalina Fonseca

Rules of heterosynaptic plasticity in excitatory synapses.

IGC, Oeiras, Portugal.

DECEMBER

Sara Magalhães

Adaptation to novel host plants in herbivorous mites: the role of genetic variance and phenotypic plasticity.

IGC, Oeiras, Portugal.

Minus va Baalen

The evolution of cooperation and communication.

Université Pierre et Marie Curie, Paris, France.

Élio Sucena

Development and evolution: in and around *Drosophila* oogenesis.

IGC, Oeiras, Portugal.

Paula Alexandra Videira

Revealing the role of sialylation in the immunobiology of dendritic cells.

FMUNL, Lisboa, Portugal

Nuno Gomes

Evolution of mammalian replicative aging.

University of Lisbon, University of Southwestern Medical Center at Dallas, USA.

José Faro

Approaches to analyse TcR diversity and overlap between T-cell populations in mice and humans.

IGC, Oeiras, Portugal.

Adriano Henriques

Development in an undomesticated *Bacillus subtilis*.

ITQB/UNL, Oeiras, Portugal.

Education and Training

GRADUATE EDUCATION

Graduate education has always been a strong commitment of the IGC. 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 in Biomedicine, which ran until 2005. In 2005, a new PhD Programme in Computational Biology was launched, in collaboration with the Fundação para a Ciência e a Tecnologia and Siemens Portugal.

PHD PROGRAMME IN COMPUTATIONAL BIOLOGY

The Fundação para a Ciência e a Tecnologia (FCT), SIEMENS SA Portugal, and the Fundação Calouste Gulbenkian decided to join efforts to promote a pilot PhD Programme in Computational Biology, in collaboration with PT foundation and FCCN.

The PhD Programme in Computational Biology (PDBC) aims to ensure the training of a limited number of PhDs in this area at an internationally competitive level, thus fulfilling the urgent need to educate young students and scientists in this nascent interdisciplinary research field. This programme 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.

DIRECTION

Jorge Carneiro, IGC, Oeiras (Programme Director) – since September 2007

Luis Rocha, Indiana University, USA (Collaboratorium Director)

Marie-France Sagot, INRIA, France (Programme Director) – left August 2007

BOARD OF TRUSTEES

FCT Representative (Chair Person)

FCG Representative

SIEMENS SA Portugal Representative

Maria do Carmo Fonseca, IMM, Lisbon

Fernando Lopes da Silva, UVA, NL

Alexandre Quintanilha, IBMC, Porto

Amílcar Sernadas, IST, Lisboa

PROGRAMME COMMITTEE

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

The PDBC was designed as a four-year programme divided into one year of full-time courses, workshops and projects covering 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 internationally competitive laboratory. The PDBC has so far recruited three classes of 12 students per year, selected on highly competitive national (and international) calls for applications. The first and second classes are already carrying out research training in recognised labs in Europe and USA. The third class is currently undergoing the first phase of courses. The first two editions relied on over 100 faculty members, half of which from overseas institutions, and the other half from institutions in Portugal. After these two years of activity, the worldwide visibility of the PDBC is patent in the fact that its website <http://bc.igc.gulbenkian.pt> has been ranking as number one in the Google search for “Ph.D. Computational Biology”.

In 2007 Marie-France Sagot (INRIA, France), stepped down as PDBC Director, to be replaced by Jorge Carneiro (IGC), with Claudio Soares (ITQB) as Deputy-Director.

STUDENTS OF THE COMPUTATIONAL BIOLOGY PHD PROGRAMME IN 2007

Ana Vieira dos Santos Cruz	José Alexandre Teles
Daniel Gil Gonçalves Ferreira	Nuno Miguel Martins Tenazinha
Hélder António Martins Pedro	Patrícia M. Simões
Hélio Ernesto Coronel Machado Pais	Pedro Pintado Jorge Gonçalves
Inês de Santiago Domingos de Jesus	Rita Martins Rocha
João André Coutinho Viana Alpedrinha	Sandra Cristina Milheiro Cabral Botelho
João Filipe da Custódia Dias	

Computational Biology PhD Programme for 2006/2007

JANUARY 8TH – FEBRUARY 23RD 2007

Molecular Sequence and Structures. Molecular Evolution and Sequences

RNA Structure and Regulation

François Michel, Eric Westhof, Pascal Romby

Protein Structure

Claudio Soares and António Baptista

Science Communication Workshop

Jorge Carneiro, Mónica Dias, Clare Sansom, Ana Moutinho

Databases

Luis Torgo

Machine Learning I: Bayesian decision theory, Naïve Bayes, Bayesian Classifiers, Bayesian Networks; Linear and Logistic Regression, Feature Selection, Kernel Regression, Mutual Information

João Gama, Mario Figueiredo

FEBRUARY 12TH – FEBRUARY 15TH

Workshop: Genome Dynamics and Introduction to host-parasite systems

Gene Ontologies

Sofia Pinto

Information Retrieval

Francisco Couto

Machine Learning II: Boosting, Trees, Biclustering; Neural Networks, PCA, Multidimensional Scaling Clustering

Arlindo Oliveira, Luis Borges de Almeida, Ana Fred

FEBRUARY 26TH – MARCH 23TH

Evolutionary and Functional Genomics. Genome structures

DNA Structure and Regulation

Richard Lavery

Introduction to Dynamic Systems

João Miranda Lemos

Statistics for Microarray Data

Stephane Robin

Bifurcation Theory

Frank Hilker, Petri Nets, Ina Koch

MARCH 26TH – MARCH 30TH 2007

Biological Networks. Protein Interaction Networks

Neural Organization; Models of Network Evolution; Evolution of Modularity in Complex Networks; Detecting Modularity; Experimental Methods to identify protein-protein interactions; Prediction of protein-protein interactions

José Pereira Leal, Chris Wiggins, Adriano Henriques

APRIL 16TH – MAY 11TH 2007

Modeling of Metabolic Networks

Stephan Schuster and Michael Savageau

Complex Networks

Prediction and predictability in complex systems; Understanding the structure and function of complex networks; Computational approaches to the worldwide spread of emerging infectious diseases

Alessandro Vespignani

Discrete Models

Reinhard Laubenbacher

Python Programming II

Philippe Veber

MAY 14TH – MAY 18TH

Workshop: Exploring some potential new paths for computational biology

MAY 21ST – MAY 25TH

Systems Biology. Population Biology: From host-parasite dynamics to immune system development

Epidemiology

Host-Parasite Co-evolution

Within host dynamics

Immune System

Jorge Carneiro, Gabriela Gomes, Paula Macedo, Natalia Mantilla Beniers, Ricardo Aguas, Isabel Gordo, Ruy Ribeiro

MAY 28TH – JUNE 8TH

Biological Networks. Genetic Regulatory Networks

Introduction to the modeling and simulation of genetic regulatory networks

Hidde de Jong, Delphine Ropers, Pedro Monteiro

Logical modelling of genetic networks

Denis Thieffry, Claudia Chaouiya

JUNE 11TH – JUNE 15TH

Systems Biology

Image processing and applications to Microarray analysis

Gene expression measurements

Pattern recognition

Gene network identification and analysis

Junior Barrera, Roberto Cesar

JUNE 18TH

Workshop: Career Development

PDBC and career construction; An Inside-Out Approach to Launching a Great Science Career; The Changing Worldwide Biotechnology Industry - How Your Career May Be Affected; Case-studies.

Jorge Carneiro, Dave Jensen, Antonio Coutinho, Gabriela Gomes, Rui Alves, Nuno Palma

JUNE 25TH – JUNE 26TH

Workshop: Cell and Tissue Morphodynamics: From models to imaging and back

Jorge Carneiro, Stan Maree, Tilo Beyer, Filipa Alves, Veronica Grieneisen, Waine Brodland, Koto Miura, Jake Hofman, Gabriel Martins, Isabel Palmeirim, Leonor Saude, António Jacinto

Computational Biology PhD Programme for 2007/2008

SEPTEMBER 17TH – SEPTEMBER 21ST

Welcome week

SEPTEMBER 24TH-SEPTEMBER 29TH

Introductory Module

Hypothesis Driven Research Course/A Primer in Algorithms and Data Structures

Some key experiments in Drosophila development: a personal view; Cell Cycle; Cell Biology; Molecular biology and genetics: an historic perspective; Computational biology; Use of in vitro model systems

Rui Martinho, Gines Morata, Alvaro Tavares, Jose Feijo, Moises Malo, Jose Pereira-Leal, Monica Dias

OCTOBER 1ST – OCTOBER 10TH

Statistics/Introduction to R/Programming with Python

Statistics

Introduction to R

Programming in Python

Dinis Pestana, Nuno Sepulveda, Andreias Bohn, Aurelien Naldi, Cristina Paulo

OCTOBER 15TH – OCTOBER 20TH

Genetics

Mendelian genetics; Drosophila genetics; Drosophila genetics tools; Quantitative genetics; Mouse transgenesis; Immune system genetics; Epigenetics; Non-model organism genetics

Elio Sucena, Fernando Roch, Rui Martinho, Henrique Teotonio, Moises Mallo, Jocelyne Demengeot, Richard Borowsky

Statistics/Programming with Python

Statistics; Python Practice

Dinis Pestana, Aurelien Naldi, Jose Lourenco

OCTOBER 22ND – NOVEMBER 2ND

Evolution

Introduction to Evolutionary Biology; The Comparative Method; Molecular genetics; Phenotypic evolution; Introduction to development and evolution; The evolution of

anterior-posterior patterns in insects The evolution of eye spots in butterflies; Evolution of recombination; Genetic Basis of Adaptation; The evolutionary history of humans; Biochemical basis of selection

Henrique Teotonio, Isabel Gordo, Elio Sucena, Claude Desplan, Patricia Beldade, Thomas Lenormand, Lounes Chikhi, Anthony Dean

Statistics

Dinis Pestana

NOVEMBER 5TH - NOVEMBER 9TH

Within Cell Biology

Cell Division; Regulation of gene expression; Signal transduction; Membrane trafficking; Cytoskeletal architecture and cellular morphogenesis

Alvaro Tavares, Monica Bettencourt Dias, Miguel Godinho Ferreira, Rui Martinho; Ana Costa-Pereira, Silvia Magalhaes dos Santos, Miguel Seabra, Phong Tran

Statistics

Dinis Pestana

NOVEMBER 12TH – NOVEMBER 16TH

Between Cell Biology

Cell-junction, cell-adhesion; Cell-adhesion remodeling; Extracellular matrix; Cell movement: 3D reconstructions; Control of cell sorting during development; Cell synchronization during development; Immunological Synapses; Neuronal Synapses; Communication among bacteria

Vasso Kostourou, Solveig Thorstendottir, Gabriel Martins; Moises Mallo; Leonor Saude; Jocelyne Demengeot; Rosalina Fonseca; Bonnie Bassler, Carina Xavier

Logics

Manfred Kerber

NOVEMBER 19TH

Workshops: Making the most of your work

Carol Featherstone, Daphne Goodfellow

NOVEMBER 20TH – NOVEMBER 23RD

Cell integration

From molecules to disease; Mechanisms of disease: cancer; Mechanisms of disease: inflammation; Cellular and Molecular Mechanisms of Regeneration

Miguel Seabra, Sergio Dias, Florence Janody, Miguel Soares; Elly Tanaka

Linear algebra, PCA and LDA

Recalling linear algebra; Principal component analysis; Linear discriminant analysis

Jorge Cadima

NOVEMBER 24TH

Student scientific meeting

NOVEMBER 26TH – NOVEMBER 30TH

Theory of evolution and population genetics/SVM

DNA sequence divergence between species; DNA sequence variability within species; Basic theory of selection, maintenance of variation by selection; Basic theory of the evolutionary effects of finite population size (genetic drift); Mutation and selection, directional selection; Molecular variation and evolution; Multiple loci and linkage; Detecting selection from DNA sequence data disequilibrium

Brian Charlesworth

Support Vector Machine

Thomas Stibor

DECEMBER 3RD –DECEMBER 7TH

Sequence Alignment

Burkhard Morgenstern, Colin Dewey

DECEMBER 10TH - DECEMBER 13TH

Sequence Alignment |Phylogenetics

Sequence alignment

Colin Dewey

Phylogenetics

Andreas Spillner

GULBENKIAN PHD PROGRAMME 2007/2008

DIRECTOR

Henrique Teotónio

The Gulbenkian PhD Programme 2007 is aimed at providing a selected set of students with top quality courses and workshops during three months followed by four years of a thesis project to be carried out at the Institute, in any of the areas that are currently part of our research. The ten students admitted in 2007 were selected from a pool of close to 150 applicants from several countries by a selection committee, which then also evaluated the PhD proposals.

The PhD Gulbenkian Programme has as its main objective to provide excellence education to students and form the next generation of researchers in Biology.

STUDENTS OF THE GULBENKIAN PHD PROGRAMME 2007

Ana Inês da Cunha Ferreira
Ana Teresa dos Santos Avelar
Barbara Jezowska
Barbara Vreede
Clara de Fátima Alves Pereira
Ivo Marguti
Mariana Coelho Correia da Silva
Migla Miskinyte
Patrícia Inácio
Ricardo de Sousa e Paiva

Gulbenkian PhD Programme for 2007

SEPTEMBER 17TH – SEPTEMBER 21ST

Welcome week

SEPTEMBER 24TH-SEPTEMBER 29TH

Introductory Module, Hypothesis Driven Research Course

Some key experiments in Drosophila development: a personal view; Cell Cycle; Cell Biology; Molecular biology and genetics: an historic perspective; Computational biology; Use of in vitro model systems

Rui Martinho, Gines Morata, Alvaro Tavares, Jose Feijo, Moises Malo, Jose Pereira-Leal, Monica Dias

OCTOBER 8TH – OCTOBER 11TH

Biostatistics

Isabel Gordo, Francisco Dionísio, Costantin Fesel, Ana Cristina Paulo

OCTOBER 15TH – OCTOBER 20TH

Genetics

Mendelian genetics; Drosophila genetics; Drosophila genetics tools; Quantitative genetics; Mouse transgenesis; Immune system genetics; Epigenetics; Non-model organism genetics

Elio Sucena, Fernando Roch, Rui Martinho, Henrique Teotonio Moises Mallo Jocelyne Demengeot, Richard Borowsky

OCTOBER 22ND – NOVEMBER 2ND

Evolution

Introduction to Evolutionary Biology; The Comparative Method; Molecular genetics; Phenotypic evolution; Introduction to development and evolution; The evolution of anterior-posterior patterns in insects The evolution of eye spots in butterflies; Evolution of recombination; Genetic Basis of Adaptation; The evolutionary history of humans; Biochemical basis of selection

Henrique Teotonio, Isabel Gordo, Elio Sucena, Claude Desplan, Patricia Beldade, Thomas Lenormand, Lounes Chikhi, Anthony Dean

NOVEMBER 5TH - NOVEMBER 9TH

Within Cell Biology

Cell Division; Regulation of gene expression; Signal transduction; Membrane trafficking; Cytoskeletal architecture and cellular morphogenesis

Alvaro Tavares, Monica Bettencourt Dias, Miguel Godinho Ferreira, Rui Martinho; Ana Costa-Pereira, Silvia Magalhaes dos Santos, Miguel Seabra, Phong Tran

NOVEMBER 12TH – NOVEMBER 16TH

Between Cell Biology

Cell-junction, cell-adhesion; Cell-adhesion remodeling; Extracellular matrix; Cell movement: 3D reconstructions; Control of cell sorting during development; Cell synchronization during development; Immunological Synapses; Neuronal Synapses; Communication among bacteria

Vasso Kostourou, Solveig Thorstendottir, Gabriel Martins; Moises Mallo; Leonor Saude; Jocelyne Demengeot; Rosalina Fonseca; Bonnie Bassler, Carina Xavier

NOVEMBER 19TH

Workshops: Making the most of your work

Carol Featherstone, Daphne Goodfellow

NOVEMBER 20TH – NOVEMBER 23RD

Cell integration

From molecules to disease; Mechanisms of disease: cancer; Mechanisms of disease: inflammation; Cellular and Molecular Mechanisms of Regeneration

Miguel Seabra, Sergio Dias, Florence Janody, Miguel Soares; Elly Tanaka

NOVEMBER 24TH

Student scientific meeting

NOVEMBER 26TH – NOVEMBER 30TH

Systems Biology I

Theories of antibodies; Tuning and specification of immune response; Evolution of the immune system; Memory formation and consolidation; Synaptic plasticity; Hippocampus and cortex interactions; Neurons and memory formation; Nervous and immune systems; Cognition, memory

In-lab sessions

Vasco Barreto, Jocelyne Demengeot, Louis DuPasquier, Marta Moita, Rosalina Fonseca, Paul Frankland

DECEMBER 3RD –DECEMBER 7TH

Systems Biology II

**Networks in Biology; Interactions individual-environment; Adaptative dynamics;
Interaction among individuals**

Isabel Gordo, Sara Magalhaes, Pierrick Labbé, Minus van Baalen

GULBENKIAN / CHAMPALIMAUD PHD PROGRAMME IN NEUROSCIENCE 2007/2008

DIRECTOR

Zachary Mainen

In 2007 the first edition of the Gulbenkian-Champalimaud PhD Programme in Neuroscience was launched. This doctoral programme in neuroscience aims to train students to perform innovative and integrative research into the biological bases of behavior. The programme is sponsored by a collaboration between the FCT, the Champalimaud Foundation and the Gulbenkian Foundation and works in close collaboration with the Champalimaud Foundation Neuroscience Programme (CNP) at the IGC.

During the first year, students attend courses organized and taught by a combination of internal faculty and invited international researchers. This initial training phase aims at providing students with a broad background and common language in biology. The curriculum is varied in format and emphasizes active participation and discussion and practical exercises. Advanced workshops and symposia are also part of the curriculum. The goal is to develop critical and creative thought and to gain exposure to a variety of perspectives on the biology of the nervous system.

Autumn semestre courses focus on core concepts in biological systems including genetics, evolution, within-cell biology, between-cell biology, and ecology. These courses are co-organized with the two other PhD programmes at the IGC. The spring semester is devoted to in depth work in neuroscience, organized in a series of 12-15 courses of intense short courses one or two weeks. The semester begins with a two week general introduction and includes physiology, development, sensory systems, motor systems, learning and cognition. Each course aims to span from basic introduction to current research topics. Perspectives and approaches are designed toward maximizing diversity and include engineering, biomedicine, ethology, evolutionary biology and computational neuroscience. There is a strong quantitative component to the curriculum, and students are instructed in Matlab and data analysis, models and other projects.

The first year of the curriculum takes place at IGC. During this spring of the first year, students choose a laboratory to conduct their thesis research project, guided by the programme director and neuroscience core faculty. Portuguese students are eligible to work in any laboratory in the world. Foreign students can choose laboratories at the IGC or elsewhere in Portugal. A yearly retreat is planned in conjunction with CNP laboratories.

STUDENTS OF THE GULBENKIAN/CHAMPALIMAUD PHD PROGRAMME IN NEUROSCIENCE 2007/2008

Ana Margarida Lago Agrochão
Iris Margarida Donga Vilares
José Joaquim Fernandes
Maria Inês Alves Vicente
Maria Isabel Santos Lestro Henriques
Mariana Marcelino Belchior Cardoso
Patrícia Marçal Alves Correia
Patrício Manuel Vieira Simões
Pedro Nuno Galvão Ferreira
Rodrigo Manuel Abril de Abreu

GULBENKIAN/CHAMPALIMAUD PhD Programme in Neuroscience for 2007

SEPTEMBER 17TH – SEPTEMBER 21ST

Welcome week -Introduction

SEPTEMBER 24TH -SEPTEMBER 29TH

Hypothesis Driven Research Course

Some key experiments in Drosophila development: a personal view; Cell Cycle; Cell Biology; Molecular biology and genetics: an historic perspective; Computational biology; Use of in vitro model systems

Rui Martinho, Gines Morata, Alvaro Tavares, Jose Feijo, Moises Malo, Jose Pereira-Leal, Monica Dias

OCTOBER 4TH – OCTOBER 12TH

Statistics/Introduction to R

Statistics

Introduction to R

Dinis Pestana, Nuno Sepulveda, Andreias Bohn, Ana Cristina Paulo

OCTOBER 15TH – OCTOBER 20TH

Genetics

Mendelian genetics; Drosophila genetics; Drosophila genetics tools; Quantitative genetics; Mouse transgenesis; Immune system genetics; Epigenetics; Non-model organism genetics

Elio Sucena, Fernando Roch, Rui Martinho, Henrique Teotonio Moises Mallo Jocelyne Demengeot, Richard Borowsky

OCTOBER 22ND – NOVEMBER 2ND

Evolution

Introduction to Evolutionary Biology; The Comparative Method; Molecular genetics; Phenotypic evolution; Introduction to development and evolution; The evolution of anterior-posterior patterns in insects The evolution of eye spots in butterflies; Evolution of recombination; Genetic Basis of Adaptation; The evolutionary history of humans; Biochemical basis of selection

Henrique Teotonio, Isabel Gordo, Elio Sucena, Claude Desplan, Patricia Beldade, Thomas Lenormand, Lounes Chikhi, Anthony Dean

Statistics

Dinis Pestana

NOVEMBER 5TH - NOVEMBER 9TH

Within Cell Biology

Cell Division; Regulation of gene expression; Signal transduction; Membrane trafficking; Cytoskeletal architecture and cellular morphogenesis

Alvaro Tavares, Monica Bettencourt Dias, Miguel Godinho Ferreira, Rui Martinho; Ana Costa-Pereira, Silvia Magalhaes dos Santos, Miguel Seabra, Phong Tran

Statistics

Dinis Pestana

NOVEMBER 12TH – NOVEMBER 16TH

Between Cell Biology

Cell-junction, cell-adhesion; Cell-adhesion remodeling; Extracellular matrix; Cell movement: 3D reconstructions; Control of cell sorting during development; Cell synchronization during development; Immunological Synapses; Neuronal Synapses; Communication among bacteria

Vasso Kostourou, Solveig Thorstendottir, Gabriel Martins; Moises Mallo; Leonor Saude; Jocelyne Demengeot; Rosalina Fonseca; Bonnie Bassler, Carina Xavier

Matlab

Susana Vinga

NOVEMBER 19TH

Workshops: Making the most of your work

Carol Featherstone, Daphne Goodfellow

NOVEMBER 20TH – NOVEMBER 23RD

Cell integration

From molecules to disease; Mechanisms of disease: cancer; Mechanisms of disease: inflammation; Cellular and Molecular Mechanisms of Regeneration

Miguel Seabra, Sergio Dias, Florence Janody, Miguel Soares; Elly Tanaka

Linear algebra, PCA and LDA

Recalling linear algebra; Principal component analysis; Linear discriminant analysis

Jorge Cadima

NOVEMBER 24TH

Student scientific meeting

NOVEMBER 26TH – NOVEMBER 30TH

Systems Biology I

Theories of antibodies; Tuning and specification of immune response; Evolution of the immune system; Memory formation and consolidation; Synaptic plasticity; Hippocampus and cortex interactions; Neurons and memory formation; Nervous and immune systems; Cognition, memory

In-lab sessions

Vasco Barreto, Jocelyne Demengeot, Louis DuPasquier, Marta Moita, Rosalina Fonseca, Paul Frankland

DECEMBER 3RD –DECEMBER 7TH

Systems Biology II

Networks in Biology; Interactions individual-environment; Adaptative dynamics; Interaction among individuals

Isabel Gordo, Sara Magalhaes, Pierrick Labbé, Minus van Baalen

THE GULBENKIAN TRAINING PROGRAMME IN BIOINFORMATICS (GTPB)


The Gulbenkian Training Programme in Bioinformatics provides hands-on education in Bioinformatics, either to service-oriented personnel, or to scientists and students, particularly from the IGC, with specific interests in a given topic. In 2007, the Programme followed its activity plan that was previously prepared, aiming at providing a set of four thematic courses, in addition to the two introductory level courses.


This Programme is now a reference for many similar initiatives in Europe. Sharing of students and teachers with other programmes such as the one at the University of Cambridge – Dep Genetics and the one at the European Bioinformatics Institute already takes place. A group of Spanish research institutes (CIPF, IMIM, CNIO, etc.) are organizing a way of joining this initiative. Agreements are under way to formalize these cooperative efforts. The success of this programme is due to its enhanced design and standardized methodology. All the courses are fully documented in CDROM.


Courses in 2007

PB07 - Perl & BioPerl for Biomedical Researchers
May 7th - 11th

EBS07 - Elementary Bioinformatics Skills
May 21st - 26th

ENSEMBL-A  User Level Ensembl - genome exploration
June 19th

ENSEMBL-B  User Level Ensembl - mining genomes for SNPs
June 20th

ENSEMBL-C  Ensembl - Programming workshop
June 21st - 22nd

MDA07 - Microarray Data Analysis
June 25th - 27th

PSFP07 - Protein Structure and Function Prediction
September 24th-28th

ICMDA07 - Introductory Course on Microarray Data Analysis
October 15th-19th

MEPA07 - Molecular Evolution Phylogenetics and Adaptation
November 19th-23rd

IMMUNO07 - Immunoinformatics
December 17th-21st

Attendees

PORTUGUESE ATTENDEES: 116
FOREIGN ATTENDEES : 18
TOTAL OF ATTENDEES : 134

Instructors in 2007

PB07 - Francisco Couto (FCUL, Lisbon, Portugal).

EBS07- David P Judge (University of Cambridge, Cambridge, UK).

ENSEMBL-A - Xose Fernandez (EBI, Hinxton, UK).

ENSEMBL-B - Xose Fernandez (EBI, Hinxton, UK).

ENSEMBL-C - Javier Herrero (EBI, Hinxton, UK).

MDA07 - Joaquin Dopazo (CIPF, Valencia, Spain), Fatima Al-Sharour (CIPF, Valencia, Spain), Hernan Dopazo, CIPF (Valencia, Spain).

PSFP07 - Michael Tress (CNIO, Madrid, Spain).

ICMDA07 - Nuno Barbosa Morais (University of Cambridge, Cambridge, UK), Matt Ritchie (University of Cambridge, Cambridge, UK), Simon Tavaré (University of Cambridge, Cambridge, UK).

MEPA07 - Hernan Dopazo (CIPF, Valencia, Spain)
Leonardo Arbiza (CIPF, Valencia, Spain)

IMMUNO07 - Pedro Reche (Universidad Complutense, Madrid, Spain), Jan Andersson (University of Basel, Basel, CH), Michael Tress (CNIO, Madrid, Spain).

PORTUGUESE CENTRE FOR BIOINFORMATICS (CENTRO PORTUGUÊS DE BIOINFORMÁTICA – RECURSOS DE ALTA PRESTAÇÃO: CPB-RAP)

The services delivered by this resource have been granted to academic users on request, and have helped in developing new skills in university students, mainly from Instituto Superior Técnico in Lisboa.

The initial set of Bioinformatics tools has been increased by addition of public domain packages. Plans to do this with many more have been laid-out, based on the refinement of the techniques of integrating new software on the existing platform in a standardized way, delivering not only an interactive service through the web but also webservices that allow for remote access in a programmatic way. Addition of resources is mainly limited by the availability of manpower to do it according to the established methodology.

The resource has served about 115 users in interactive mode via a web portal. So far, every user is able to get full performance from individual machines. Average load throughout the year is estimated at 38% and peak performance has been reached only in 4% of the running time. The system is therefore able to supply full performance when needed.

In simulation jobs (mainly in Population Genetics and Evolution) the system has delivered service to local users. In this type of usage, the ability to run long term jobs for many days is preferred over the possibility of reaching peak performance. The system has served a minimum of 20 simultaneous long duration jobs of this type in every single day.

Science and Society

Over the last few years, the IGC has developed a Science Communication Programme to promote public engagement in science through direct, bilateral communication, i.e. dialogue between scientists and the public. The year 2007 witnessed the start of important new activities engaging different actors, such as the edition of a science fiction book written in close collaboration with scientists, science communicators and teachers and the beginning of the development of a philanthropy programme aiming at establishing alternative funding opportunities for scientific research, at the same time as further involving the Portuguese society in science and scientific culture.

In 2007, the following activities, detailed by target audience, were organized by the Science Communication Office of the IGC:

MEDIA

Press Office

Press office duties include preparing and sending out press releases, organizing press conferences and a permanent dialogue and support of the science journalists' work.

IGC activities were covered in

national newspapers: 24 Horas, Correio da Manhã, Destak, Diabo, Diário Económico, Diário de Notícias, Expresso, Jornal de Letras, Jornal de Negócios, Jornal de Notícias, Metro, Notícias da Manhã, OJE, Página 1, Primeiro de Janeiro, Público, Semanário Económico, SOL, Tal & Qual

magazines: Boa Forma, Caras, Channel Partner, Notícias Magazine (Diário de Notícias/Jornal de Notícias), Exame, Única (Expresso), flash!, Focus, Visão, Ingenium, Se7e, Nova Gente, Pais & Filhos, Sábado, Tabu (SOL), Visão

specialized publications for medical professionals: Anamnesis, Gestão Hospitalar

local press: Açoriano Oriental, As Beiras, Correio do Minho, Correio do Ribatejo, Diário Açores, Diário Coimbra, Diário de Aveiro, Diário do Minho, Diário de Leiria, Diário Sul, Jornal da Madeira, Jornal de Leiria, Povo da Beira

online press: Ciência.pt, Ciência Hoje, Diário Digital, Dinheiro Digital, Farmácia.com.pt, TSF online, netfarma.pt, Orelhas.pt, Portugal Diário, Público Última

Hora, Rádio Renascença Online, RTP Online, Sapo, Saúde na Internet, Sercultura online, SIC Online, SOL Online, Tek.sapo.pt, TSF Online, TVI Online

television stations: RTP1, RTP2, RTPN, SIC Notícias, TVI

radio stations: Antena 1, Rádio Renascença, TSF

A total of 242 direct references to the IGC or IGC scientists were made in these publications.

COORDINATOR: Sofia Cordeiro (IGC)

Media kits

Media kits about each research group at the IGC were prepared, including a brief description of the group's research interest and activities, relevant pictures illustrating the group's recent discoveries and pictures of group members. These kits are a fundamental instrument not only to provide journalists with background information for their reports but also when rapid and easy access to information about a group or scientist is needed, such as in the event of an award. These kits were specifically conceived for media, but will also be available to prospective candidates to the IGC research teams, school teachers, students of all teaching levels and the general public, through the IGC website and eventually in a small copy number publication available for specific purposes.

COORDINATION: Sofia Cordeiro (IGC), Mónica Bettencourt-Dias (IGC, Comunicar Ciência), Sheila Vidal (IGC)

SCIENCE WRITING: Susana Lamas (Comunicar Ciência)

STUDENTS AND SCIENCE TEACHERS

School Visits to the IGC

A total of 397 students visited the IGC in 2007, originating from 13 schools of the whole country, from pre-schoolers to university students. A total of 38 IGC scientists were involved in these visits.

ORGANIZATION: Sofia Cordeiro (IGC); Carla Rodrigues (IGC), Ana Rita Marques (IGC)

Book Presentation “Os Factos da Vida”, by Ana Saldanha, Editorial Caminho

Within the scope of the IGC Science Communication project (ref. POCTI/DIV/2005/00045) supported by Ciência Viva — Agência Nacional para a Cultura Científica e Tecnológica, the well known Portuguese children's writer Ana Saldanha, wrote a new novel entitled “Os

Factos da Vida” (The Facts of Life), for children and youngsters ages 8 through 14. The goal was the production of fiction book to break down stereotypes about science and the day-to-day life of a scientist in the field of Life Sciences. This project and this book were built on a partnership¹ between science communicators, scientists, teachers, students and the writer.

In order to promote the project and the book “Os Factos da Vida”, by Ana Saldanha, the IGC together with some partners of this project organized three book public presentations, all integrated in the Science and Technology Week events:

DATE AND PLACE: 19th November, Instituto de Biologia Molecular e Celular, Porto

ORGANIZATION: Sónia Martins (IBM), Júlio Bórlido (IBMC), Sheila Vidal (IGC)

COLLABORATION: Editorial Caminho, Mónica Sousa (IBMC)

PARTICIPANTS: 60 (30 students, teachers, scientist, project partners and journalists)

DATE AND PLACE: 22nd November, Esc. Secundária Luís de Freitas Branco de Paço de Arcos

ORGANIZATION: School’s library, Sheila Vidal (IGC)

COLLABORATION: Susana Pascoal (IGC)

PARTICIPANTS: 85 (75 students and teachers)

DATE AND PLACE: 23rd November, Instituto Gulbenkian de Ciência, Oeiras

ORGANIZATION: Sheila Vidal (IGC)

COLLABORATION: Editorial Caminho, Susana Pascoal (IGC)

PARTICIPANTS: 100 (75 students, 10 teachers, scientists and partners)

¹**Team:** Ana Godinho, Head, Science Communication and Outreach, IGC; Ana Saldanha, writer, Porto; António Amorim, Biology secondary school teacher, Escola Secundária Luís de Freitas Branco, Paço de Arcos; Frederico Jesus, student, Escola Secundária Luís de Freitas Branco, Paço de Arcos; Margarida Afonso, Lecturer in Education Studies, Escola Superior de Educação de Castelo Branco; Sheila Vidal, Science Manager, IGC; Sónia Martins, Science Communicator, Instituto de Biologia Molecular e Celular, Porto.

Book Impact Evaluation “Os Factos da Vida”, by Ana Saldanha, Editorial Caminho

Within the scope of the mentioned IGC Science Communication project (ref. POCTI/DIV/2005/00045) and for the evaluation of the impact of the book on youngsters ages 12 through 14 we designed a workshop entitled “Os Factos da Vida” for 8th grade students in which participants were asked to fill in a small questionnaire and make a drawing of a scientist. The workshop was carried out in 27 schools groups/657 students around the country.

COORDINATION: Sónia Martins (IBMC) and Sheila Vidal (IGC)

WORKSHOP DESIGN: Sónia Martins (IBMC)

PARTICIPANTS/COLLABORATION: 657 students / 35 teachers

SCIENTISTS

2007 Crioestaminal Award Presentation Ceremony

DATE AND PLACE: 21st November, IGC, integrated in the Science and Technology Week events

DESCRIPTION: This year awarded to an IGC scientist, Mónica Bettencourt-Dias, the Crioestaminal Award distinguishes outstanding work in biomedical research in Portugal and is decided by a renowned international jury. As the institution where the distinguished scientist develops her research, the IGC hosted the award presentation ceremony.

SPECIAL GUEST: Deborah Ward, Breakthrough Breast Cancer Institute, London.

ORGANIZATION: Associação Viver a Ciência, Crioestaminal, Sheila Vidal (IGC), Greta Martins (IGC)

PARTICIPANTS: 60

PHILANTROPY

Fundraising for Science - New approaches for research funding in Portugal (Workshop)

DATE AND PLACE: 22nd November, IGC

DESCRIPTION: A workshop directed at providing information on how alternative funding strategies for research in Portugal can be designed. Based on the UK experience and on what is already being done in Portugal to raise funds for scientific research and medicine, strategies were proposed on how the scientific and clinical communities can interact with the general public to raise money for science.

ORGANIZATION: Maria João Leão (IGC), Margarida Trindade (Associação Viver a Ciência), Sheila Vidal (IGC) e Greta Martins (IGC)

SPEAKERS: António Parreira (Associação Portuguesa Contra a Leucemia, Portugal), Deborah Ward (Breakthrough Breast Cancer, UK), Frances Austin (Associação Laço, Portugal), Margarida Trindade (Associação Viver a Ciência, Portugal), Maria João Leão (Instituto Gulbenkian de Ciência, Portugal), Vera Lopes (Liga Portuguesa Contra o Cancro, Portugal)

PARTICIPANTS: 50 (Scientists, medical doctors, journalists, etc)

PROMOTING IGC ACTIVITY

“Breaking down stereotypes in the life sciences through fiction”, Project (ref. POCTI/DIV/2005/00045) presentation, Ana Saldanha, Sónia Martins, Margarida Afonso, António Amorim, Frederico Jesus, Sheila Vidal, Ana Coutinho. Oral communication at the II Communicating Science Meeting – National meeting of science communicators, 2nd Edition, Porto, July 2007

OTHERS

In 2007 the **Bioinformatics Unit** teamed up with secondary schools to develop web-based bioinformatics practicals to be used by secondary school teachers, complementing the national curriculum. The goal of this project is to make use of the fact that bioinformatics requires few resources when compared to experimental biosciences, and thus can be used as a basis for enquiry-based learning.

We are working in partnership with the schools:

Escola Secundária da Quinta do Marquês em Oeiras Oeiras
Escola Secundária Miguel Torga em Queluz

Developing web-based practicals for students (12th grade/“12º ano”), testing these in the classroom and having feedback from 130 students, and testing them with 7 biology teachers. Simultaneously we are training them to teach these practicals, while developing

teacher training sets to accompany the on-line practicals. These practicals can be found at the url: www.bioinformatica-na-escola.org

In parallel we participated in the following high-school events:

“Workshop Genoma Humano”, Semana Cultural da Escola Secundária José Afonso, Loures, 29 Janeiro. (Isabel Marques).

“Identificação de genes envolvidos em cancro mama, prostata e pulmão, recorrendo a recursos Bioinformáticos” - Projecto Área Escola , Escola Secundária Jorge Peixinho, Montijo. (Isabel Marques).

Symposia, Conferences and Meetings Organised by the IGC

Workshop: Behaviour pathologies: biological approaches 14-16 February 2007

Instituto Gulbenkian de Ciência

ORGANISERS: Post-doctoral fellows (IGC, Oeiras, Portugal)

Behaviour pathologies comprise several chronic disorders that in most cases represent a permanent and complete disability, with patients requiring social, medical, and economic support all their lives. The understanding of the complex etiology of these diseases is fundamental for the development of effective therapies and prevention. Emerging technologies in fields such as those of molecular biology, animal models, functional studies, and brain imaging, strongly contribute towards understanding behaviour pathologies. Interactions between these different areas of research provide an integrative view of how genetics, physiology, structure, and function come together to originate these disorders. The main goal of this workshop is to gather scientists developing cutting edge research in these several areas, stimulating creative thinking to achieve progresses in the study of behavior pathologies, such as autism and schizophrenia, among others.

This Workshop is integrated in a workshop series organized by the post-doctoral fellows of the Instituto Gulbenkian de Ciência (IGC). Throughout the years, the IGC has established as a major priority the promotion of the highest educational level in biological and biomedical sciences. With the aim of reinforcing their contribution to the scientific events held at the Institute, the post-doctoral fellows of the IGC have joined to organize a series of workshops in different fields of excellence.

Análise evolutiva da religião Fundação Calouste Gulbenkian 12-13 March 2007

ORGANISER: Henrique Teotónio (IGC, Oeiras, Portugal)

The phenomenon of Religion was the subject of this workshop, especially at its institutional level, the churches. Evolutionary theory was discussed as a conceptual framework to explain the group behavior of individuals, that set themselves as institutions, to resolve materialistic problems such as lack of political unity, social unrest and economic differences. Individual attempts to explain religions were not discussed at depth. Several international researchers from evolutionary biology, anthropology and theology were invited to give lectures on their views. Group selection may explain why institutions such as the church can provide a material basis for their evolution, and correlated with that for the belief in metaphysics by individuals. We attempted to promote a scientific not teleological discussion of this theme. The workshop took place at the Fundação Calouste Gulbenkian during two days and was a success based on the number of attendees and the interest it gathered from all participants.

Introduction to light microscopy workshop

Instituto Gulbenkian de Ciência

4 April 2007

ORGANISERS: Nuno Moreno (IGC, Oeiras, Portugal) and Gabriel G. Martins (FCUL, Lisbon, Portugal/IGC, Oeiras, Portugal).

This course combined a series of introductory lectures on optics, microscopy and image acquisition and analysis. The course also included practical sessions where attendants were training in using the IGC microscopy equipment.

Symposium reconstruction of the past: from big bang to language

Fundação Calouste Gulbenkian

4-5 May 2007

ORGANISER: Sydney Brenner

From Big Bang to Language, the evolutive path of planet earth and of the human being was the object of analysis in a symposium organised on May 4-5 at the Fundação Calouste Gulbenkian. The goal of science is to rationally derive the laws of nature that explain the existence of the world and of the human being. The cosmology, physics, chemistry and biology all allow today to "reconstruct the past" of the universe and of the earth, as well as the evolution of mankind, of language and of society. This conference series brought together world specialists who described the evolutive pathway, under the scientific perspectives of the beginning of this 21st century.

The conference was organised by Sydney Brenner, 2002 Nobel Prize winner in Medicine or Physiology, who conducted the opening and closing sessions of the conference. Gerry Gilmore, Professor of Experimental Philosophy from the University of Cambridge, UK, talked about the Big Bang, and Leslie Orgel, Professor of Prebiotic Chemistry at the Salk Institute, USA, discussed the "Origin of the Darwinian Evolution". A path into the "Origin of Cellular Life" was the proposal of Jack Szostak, Researcher at the Howard Hughes Medical Institute, followed by a conference by Simon Conway Morris, Professor of Evolutionary Paleobiology, on "Early Metazoans". Robert Foley, Director of the Leverhulme Centre for Human Evolutionary Studies at the University of Cambridge, UK and Svante Pääbo, Director of the Max-Planck Institute for Evolutionary Anthropology in Leipzig, Germany, approached the theme of Human Origin from two perspectives: the study of "Bones and Stones" and "The Genomic Perspective on Human Origin", respectively. The final speaker was Guy Deutscher, Professor at the Department of Languages and Cultures of Ancient Mesopotamia in the University of Leiden, Holland, who talked about the "Evolution of Language". Sydney Brenner closed the meeting with a conference on "Reconstructing the Past" that effectively summarised these two fantastic days.

Workshop microarrays in clinical oncology – promises and pitfalls

Sociedade das Ciências Médicas, Lisbon

30 June 2007

ORGANISERS: José Pereira Leal (IGC, Oeiras, Portugal), Carlos Caldas and Nuno Barbosa-Morais (Dept. of Oncology, University of Cambridge, UK)

and

Introduction to microarray data analysis

15th-19th October (GTPB courses)

ORGANISERS: José Pereira Leal (IGC, Oeiras, Portugal), Simon Tavaré, Nuno Barbosa-Morais and Matt Ritchie (Dept. of Oncology, University of Cambridge, UK).

The Bioinformatics unit elected as one of its missions to help develop the interface between bioinformatics and medical research and application. In collaboration with the “Sociedade de Ciências Médicas” we test ran a new format that combined a workshop at the Sociedade de Ciências Médicas aimed at raising awareness in the biomedical community for a problem or application, and then follow it by an intensive hands on course to provide specific training in that problem or application.

Mechanisms of early development: cell fate determination, morphogenesis and patterning

Instituto de Medicina Molecular

31 August – 2 September 2007

ORGANISERS: António Jacinto (IMM, Lisbon, Portugal/ IGC, Oeiras, Portugal) and Claudio Stern (UCL, London, UK)

The symposium Mechanisms of early development: cell fate determination, morphogenesis and patterning was mainly focused on the early stages of embryonic development. It aimed to integrate the research activities of the European Network of Excellence Cells into Organs, whose main objective is to bring together several research groups to elucidate molecular and cellular processes underlying specification and differentiation of mesodermally derived organ systems.

This meeting, with less than 200 participants, allowed intense discussion about recent progress in this busy area.

Neural basis of reward and decision making

4-7 September 2007

Instituto Gulbenkian de Ciência

ORGANISER: Rui Costa (IGC, Oeiras, Portugal/NIH, USA)

In September 2007 a very productive and successful 3 day symposium on the “Neural bases of reward and decision making” was held at the Institute Gulbenkian de Ciência. This symposium took place between the 4th and 7th of September and inaugurated the series of Champalimaud Neuroscience Workshops at the Institute Gulbenkian de Ciência (www.igc.gulbenkian.pt).

The workshop was organized by Rui Costa and Greta Martins from the Institute Gulbenkian de Ciência with the collaboration of Bernard Balleine, John O'Doherty and Masamichi Sakagami.

The participants consisted of twenty four speakers from key groups around the world engaged in experimental, computational, and theoretical approaches to investigate the neural bases of reward and decision making and students and postdoctoral fellows

selected based on their curriculum vitae and a letter of intention. The workshop was opened by the representatives of the Fundação Calouste Gulbenkian and the Fundação Champalimaud. During the workshop, the speakers presented their work and participated in interactive discussions on the main issues and controversies in the field. The meeting encompassed joint breaks and meals, as well as social activities, in order to foster further discussions. The format of the workshop helped to generate substantial integration between the different and sometimes disparate approaches and started to foster future collaborations.

The impression of all the participants was extremely positive and the results of a workshop evaluation survey completed after the meeting revealed that the participants thought that overall the meeting was excellent with an average rating of 4.6 from 1 (poor) to 5 (excellent).

Besides the extremely positive feedback at the scientific level this meeting served as a catalyzer for the incipient neuroscience community at the Institute and facilitated the introduction of the Champalimaud Neuroscience Program at the Instituto Gulbenkian de Ciência to the international Neuroscience Community.

Lisbon epidemiology consortium – inaugural meeting
Instituto Gulbenkian de Ciência / Universidade de Lisboa
10-11 September 2007

ORGANISER: Gabriela Gomes (IGC, Oeiras, Portugal)

This meeting included a series of scientific presentations by candidate members of the consortium, public lectures by two members of the Scientific Advisory Board and a strategy meeting of the consortium with the advisory board.

Workshop on Population genetics modelling and habitat fragmentation: separating recent and ancient events for efficient conservation
Instituto Gulbenkian de Ciência
19-21 September 2007.

ORGANISER: Lounès Chikhi (IGC, Oeiras, Portugal/CNRS, Paris, France)

Genetic data are increasingly used to describe patterns of diversity within and between populations of endangered species living in increasingly fragmented habitats. This workshop aimed at bringing together conservation biologists and population geneticists to discuss

(i) the current needs of conservation biologists, (ii) the current limitations and possibilities of population genetics methods to separate natural from anthropogenic processes, including simulation tools (iii) the future of genomic data and computer simulation in conservation genetics.

This Workshop was funded by The European Science Foundation within the ConGen Programme.

Workshop theoretical and experimental perspectives on serotonin function

Hotel Vila Galé, Ericeira/Instituto Gulbenkian de Ciência

4-6 October 2007

ORGANISER: Zachary Mainen (Fundação Champalimaud/IGC, Oeiras, Portugal)

The function of serotonin has long been regarded as an enigma due to the great diversity of phenomena in which it has been implicated, ranging from disorders including depression, schizophrenia, pain, and anxiety to hallucinations, rhythmogenesis, temporal discounting, social dominance, metabolism, behavioral inhibition and reversal learning. Yet serotonin is produced by a relatively small set of neurons—the raphe nuclei—and broadcast widely up and down the neuraxis, suggesting a unified aspect to its function.

The purpose of this workshop was to bring together researchers from a wide variety of perspectives to exchange data and ideas on this most mysterious of neurotransmitters and to contemplate a unifying theory of serotonin function. The meeting explored new conceptual links between the diverse functions of serotonin and new avenues for addressing pathologies related to its dysfunction.

LA PI training course

Hotel Ericeira

1st course 18-20 November 2007

2nd Course 22-24 November 2007

ORGANISERS: Mónica Bettencourt-Dias (IGC, Oeiras, Portugal), Rita Abranches (ITQB, Oeiras, Portugal), Mariana Pinho (ITQB, Oeiras, Portugal), Greta Martins (IGC, Oeiras, Portugal), Cristina Lopes (ITQB, Oeiras, Portugal).

Leading a research group in the life sciences requires management skills: group leaders are expected to hire, manage and teach staff, coordinate and devise the theme(s) of the lab, acquire and manage the appropriate funding and possibly perform teaching duties. Young group leaders are often not adequately prepared for these challenges during their training as scientists although their success and the continuation of their careers depend not only on their scientific talent but also on the individual's skills as the manager of a research team. The EMBO Lab Management courses address basic issues in the areas of staff selection, leadership, effective problem solving and communication.

Trainers: Management consultant team (CJ Fitzwilliams) that gives the course at EMBO and has given course to IMM (Portugal). The courses have been offered on a regular basis since 2005 and have continuously received extremely positive feedback by the over 100 participants.

Fundraising for science. New approaches for research funding in Portugal

Instituto Gulbenkian de Ciência

22 November 2007

ORGANISERS: IGC Science Administration and Associação Viver a Ciência

With this workshop we have tried to understand how fundraising for science is applied in the UK, how much investment fundraising represents to a research institute and the

benefits obtained from that investment, what is being done in Portugal to raise funds for scientific research and medicine and learn how scientific and clinical communities can interact with the general public to raise money for science.

III Encontro nacional de biologia evolutiva

Instituto Gulbenkian de Ciência

21 December 2007

ORGANISERS: Sara Magalhães, Isabel Gordo, Francisco Dionisio (IGC, Oeiras, Portugal),

This is an annual meeting of Portuguese researchers in Evolutionary Biology. In this meeting Portuguese scientists doing research in this field in Portugal and all over the world get together to present and discuss their results. It is a time to get acquainted with the type of problems that are dealt with by the Portuguese evolution community and to try to establish connections between Portugal and other countries in this field.

3rd Annual Gulbenkian Alumni Meeting

Instituto Gulbenkian de Ciência

27 December 2007

ORGANISERS: Bruno Silva-Santos (IGC, Oeiras, Portugal/ IMM, Lisbon, Portugal), Mário Gomes-Pereira (Inserm, Paris, France), Greta Martins (IGC, Oeiras, Portugal).

This meeting had around 100 participants and comprised five scientific seminars (on Immunology, Neurosciences, Developmental Biology and Evolution) by former Gulbenkian PhD students, and a debate on “Biomedical Research in Portugal: Now and Soon”. This debate involved representatives of the Portuguese Government (Secretary of State Manuel Heitor), the Gulbenkian and Champalimaud Foundations, and of the best biomedical research institutes in the country.

Theses

The students listed below prepared and successfully defended their theses in 2007. The research projects were developed either fully or partially at the IGC.

PhD THESES

Rute Conceição do Nascimento Veríssimo Afonso “An unsigned herpesvirus gene inducing cell cycle and apoptosis”, ITQB, Universidade Nova de Lisboa, Oeiras, Portugal, March 2007.

Sílvia Cristina de Paiva e Almeida “Manipulating of T and B cell biology through transgenic expression of a virus gene inhibiting transcription via NF-kB and NFAT”, ITQB, Universidade Nova de Lisboa, Oeiras, Portugal, November 2007.

Margarida Almeida-Santos “The role of Notch ligands delta-like1 and Jagged1 in the function of mature B cells”, Faculdade de Medicina da Universidade de Lisboa, Lisbon, Portugal, January 2007.

Marta Alexandra Garcia Campos “The role of VEGF during a malária infection”, ITQB, Universidade Nova de Lisboa, Oeiras, Portugal, July 2007.

Marta Carapuço. “The role of Hox genes in the development of the branchial arches and axial skeleton”. Universidade Nova de Lisboa, Lisbon, Portugal, February 2007.

Sílvia Costa “Interactions between the cytoskeleton and the ionic fluxes in the regulation of cell polarity in pollen tubes”, Faculdade de Ciências da Universidade de Lisboa, Lisbon, Portugal, April 2007.

Catarina Figueiredo “ Role of neuron-microglia interactions in NMDA receptor mediated cell death” ITQB, Universidade Nova de Lisboa, Oeiras, Portugal, December 2007.

Rita Leonor Alves Cabral Figueiredo Fior, “The role of *hes* genes in the controlled production of neurons” Faculdade de Medicina da Universidade de Lisboa, Lisbon, Portugal, April 2007.

Beatriz Fernandez Garcia “Analysis of the role of Dpp signalling during the morphogenetic process of dorsal closure in *Drosophila melanogaster*” ITQB, Universidade Nova de Lisboa, Lisbon, Portugal, January 2007.

Cátia Igreja “Molecular characterization of endothelial progenitor cells differentiation in physiological and pathological conditions”. Faculdade de Ciências da Universidade de Lisboa, Lisbon, Portugal. October 2007.

Cláudia Istrate “Host and immune responses to natural and experimental rotavírus infection” ITQB, Universidade Nova de Lisboa, Oeiras, Portugal, April 2007.

Joana Paes de Faria Monteiro “Oxidative stress-signaling and Yap1 transactivation in *Saccharomyces cerevisiae*”, Faculdade de Ciências da Universidade de Lisboa, Lisbon, Portugal, March 2007.

Joana Moreira “Mathematical modelling of interacting cell populations: germinal center dynamics and spatio-temporal pattern formation in the Zebrafish, *Danio rerio*”, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal, January 2007.

Tiago Paixão “The stochastic basis of somatic variation”. Universidade do Porto, Porto, Portugal. October 2007. [Short listed for the Reinhart Heinrich Doctoral Thesis Award, of the European Society for Mathematical and Theoretical Biology, for the best thesis in 2007].

Cristina Rodrigues “Revealing host factors important for hepatocyte infection by *Plasmodium*”, Faculdade de Ciências da Universidade de Lisboa, Lisbon, Portugal, November, 2007.

Lénia Rodrigues “M2 protein of MHV-68 modulates B-cell Function”, Faculdade de Medicina da Universidade de Lisboa, Lisbon, Portugal, March 2007.

Sérgio Simões “The posterior spiracles of the *Drosophila* embryo: a model system to study cytoskeleton and adhesion regulation” Faculdade de Ciências da Universidade de Lisboa, Lisbon, Portugal, April 2007.

Ana Sofia Gírio Veloso “Regulation of the pro-apoptotic protein Bim by the kinase Erk5 in mitosis”, ITQB, Universidade Nova de Lisboa, Oeiras, Portugal, July 2007.

Santiago Pablo Zelenay “Origin and function of regulatory T cells” Faculdade de Ciência da Universidade de Lisboa, Lisbon, Portugal, June 2007.

MSc THESES

Vanessa Borges, “Identificação da actividade de anti-checkpoint nos telómeros”, Faculdade de Ciências da Universidade de Lisboa, Lisbon, Portugal, 2007.

Marta Caridade “Geração de células T reguladoras específicas para alérgeno”, Faculdade de Ciências da Universidade de Lisboa, Lisbon, Portugal, November 2007.

João Nuno Santos Coelho “Caracterização de mutantes *gim* em condições de stresse”, Faculdade de Ciências da Universidade de Lisboa, Lisbon, Portugal, 2007.

Joana Louçã “*Wolbachia* infection of *D. melanogaster*: an ecological study of infection and transmission” University of Amsterdam, Amsterdam, Holland, August 2007.

João C. Marques “Termination of the AI-2 signalling cycle in enteric bacteria” Universidade de Lisboa, Lisbon, Portugal, December 2007.

Catarina Osório. “Angiogenic profile in MDS and genetic characterization of the endothelial compartment- biologic and clinical relevance”. Faculdade de Medicina, da Universidade do Porto, Porto, Portugal, July 2007.

Luís Pacheco “Vesícula de Küpffer – Estudos funcionais de mutantes da via Delta/Notch e do gene *terra*”, Faculdade de Ciências da Universidade de Lisboa, Lisbon, Portugal, 2007.

Pedro Sanches, “Magnetic micro- and nano-particles as a tool for cell biology” Instituto Superior Técnico, Universidade Técnica de Lisboa, Portugal, November, 2007

BSc THESES

Rui Buzaco “The effect of the depletion of CD25+ cells in disease progression of serum induced arthritis”, Universidade de Évora, Évora, Portugal, October 2007.

Pedro Miguel Gaspar “Determine whether the capping protein heterodimer promote vesicles trafficking”, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal, September 2007.

Nuno Morgado “Pregnancy in disease development in the K/BxN mouse model of spontaneous chronic arthritis”, Universidade de Évora, Évora, Portugal, October 2007.

Nuno Miguel Ribeiro Palha “Investigate the role of capping proteins in proximal-distal patterning of the *Drosophila* wing disc epithelium”, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal, September 2007.

Participation of IGC Staff in Conferences, Seminars and Scientific Meetings

JANUARY

Carneiro J.

Quantitative insights into monoallelic expression of cytokine genes: the double life of a gene locus.

Invited seminar, Instituto de Medicina Molecular, Lisbon, Portugal.

Coutinho A.

Escola Superior de Tecnologias da Saúde, Parque das Nações, Lisbon, Portugal.

Mallo M.

Hox genes in development: what is new?

CABIMER, Seville, Spain.

Mallo M.

Some news about Hox genes.

Guy's Hospital, King's College London, London, UK.

Mota MM.

Malaria Infection:approaching the host.

Keystone Symposium Drugs Against Protozoan Parasites, Tahoe City, California, USA.

Rodríguez-León J.

The mouse as a model in developmental biology

Lusofona University, Developmental Biology Course, Lisbon, Portugal.

Trindade A.

Sobre-expressão de dll4 provoca hipertrofia arterial e defeitos vasculares através do bloqueio de vias pró-angiogénicas.

Jornadas do ensino pós-graduado da FMV, Faculdade de Medicina Veterinária, Lisbon, Portugal.

FEBRUARY

Costa G.

New insights on *Drosophila* kinetochore.

Meeting Proteomics and Pathology: from both sides of the Atlantic Ocean, Joint Congress of Spanish Proteomics Society (SEProt) and European Proteomics Association (EuPA), Valencia, Spain.

Coutinho A.

Challenge for biomedical research.

Centro de Pneumologia, Universidade de Coimbra, Coimbra, Portugal.

Mallo M.

Designing the body with Hox genes.

Centro Nacional de Biotecnologia, Madrid, Spain.

Muehlen M, van Noort S.

Internet-based surveillance of influenza-like illness (ILI) more uniform across european countries.

International Meeting of Emerging Diseases and Surveillance, Vienna, Austria.

Nóvoa A.

7th Transgenic Technology Meeting TT2007, Brisbane, Australia.

Oliveira S.

Identification of stroke susceptibility genes using the genomic convergence approach.

1^o Congresso Português do AVC, Porto, Portugal.

Parkhouse R.M.E.

Diagnosis and control of cystercercosis.

Conference Zoonoses e Migrações Humanas, Évora, Portugal.

Rodríguez-León J.

Role of FLRT3 in the caudal fin regeneration of adult zebrafish.

Seminar at Instituto de Medicina Molecular, Lisbon, Portugal.

Stollenwerk N.

Pair approximations in models with reinfection.

Royal Holloway University London (RHUL), UK.

Tavares A.

Mechanism of cell division.

2007 Oeiras Lab Associated Symposium, Vimeiro, Portugal.

Trindade A., Kumar S.R., Schemet J.S., Lopes-da-Costa L, Becker J., Jiang W., Liu R., Gill P.S. and Duarte A.

Overexpression of Delta-like 4 induces arterialization and attenuates vessel formation in developing mouse embryos.

Poster at the Gordon Research Conference on Vascular Cell Biology, Ventura, California, USA.

MARCH

Bergman M.L.

Identifying T cell subset phenotype and function: alpha/beta, gamma/delta, regulator, helper, NK & cytotoxic T cells.

Hertfordshire, London, UK.

Carneiro J.

Doenças autoimunes e o paradoxo da esterilização.

Tertúlias Científicas de Viana. Viana do Castelo, Portugal.

Carneiro M.

Artrite reumatóide: uma doença, infelizmente, muito feminina.

Encontro da Associação Portuguesa de Mulheres na Ciência, Universidade Técnica de Lisboa, Lisbon, Portugal.

Demengeot J.

Immune tolerance and T cell repertoire selection.

Immunology course. Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal.

Dias S.

Angiogenesis signaling pathways and the bone marrow microenvironment.

Fourth International Conference on Tumor Microenvironment. Florence, Italy.

Dias S.

Endothelial differentiation.

Stem Cells as a Tool in Toxicology, Coimbra, Portugal.

Dias S.

Aberrant signaling pathways in bone marrow disease.

First meeting of the Portuguese Signaling Network (SINAL), IMM, Lisbon, Portugal.

Gomes A.C., Alcobia I., Parreira L., Cidadão A.

Notch in embryonic hematopoiesis.

Poster at the Keystone Symposia on Molecular and Cellular Biology: Stem Cell Interactions with their Microenvironmental Niche, Keystone, USA.

Janody F. and Rebelo S.

Actin capping proteins maintain epithelium integrity of vestigial-expressing cells in the wing blade epithelium.

47rd Annual *Drosophila* Research Conference, Philadelphia, USA.

Marques A.R., Tostões R., Marty T., Martinho R.

Isolation and characterization of new maternal mutants defective for blastoderm cellularization.

Poster at the 48th Annual *Drosophila* Conference, Philadelphia, USA.

Marques I.

Análise de genomas.

Workshop Conferências de Biologia Molecular, Instituto de Superior de Ciências da Saúde-Sul (ISCSS), Monte da Caparica, Portugal.

Mentelova L.

Isolation and characterization of *Drosophila Melanogaster* kinetochore proteins.

48th Annual *Drosophila* Research Conference, Philadelphia, USA.

Pereira-Leal J.

Redes de interação biológicas - da bancada, para o computador, para a vida real.

XIENEB, Universidade de Aveiro, Aveiro, Portugal

Ramalho R.R., Soares H. and Melo L. V.

Microtubule behavior under strong electric fields: an atomic force microscopy study.

Poster at the 4th NanoSpain Workshop, Sevilla, Spain.

Sanches P., Soares H. and Melo L. V.

Detection of magnetically marked structures in cilia by magnetic force microscopy.

4th Poster at the 4th NanoSpain Workshop, Sevilla, Spain.

Seabra M.

Rab GTPases, organelle motility and disease.

Inherited Disorders of Cellular Trafficking Meeting, London, UK.

Soares M.

Targeting heme oxygenase-1 to overcome the pathogenesis of inflammatory diseases.

Invited lecture. Max Planck Institute for Infection Biology, Berlin, Germany.

Vilas-Boas F., Fior R., Henrique D.

Expression of a new chick hes gene, hes6-1 during embryonic development.

Poster presented at BSDB Spring Meeting, Edinburgh, UK.

APRIL

Abranches E., Bekman E., Henrique D.

Microarray analysis of embryonic stem cell-derived neural precursors.

2nd Annual International Meeting of the Portuguese Society for Stem Cells and Cellular Therapy – From Biology to Therapy, Coimbra, Portugal, April, 2007.

Águas R, Fernandes H, Gomes G, Gordo I, Mantilla-Beniers N, Rodrigues P, van Noort S.

One week meeting under the auspices of the royal society joint project grant strain dynamics: theoretical framework for antigenic diversity. Cambridge University, UK.

Alcobia I, Gomes A.C., Saavedra P., Cidadão A., Duarte A., Parreira L.

Effects of Delta4 haplo-insufficiency in the emergence and development of mouse hemangioblast and multilineage hematopoietic development.

2nd International Meeting of the Portuguese Society for Stem Cells and Cell Therapies (SPCE-TC), Coimbra, Portugal.

Bekman E., Henrique D.

Neuronal production in vitro from embryonic stem cells.

2nd Annual International Meeting of the Portuguese Society for Stem Cells and Cellular Therapy – From Biology to Therapy, Coimbra, Portugal.

Bergman M.L. and Demengeot J.

Cellular subsets involved in thymic selection of regulatory T cells

WIR: World Immune Regulation Meeting, Davos, Switzerland.

Cachaço A.S., Santos A.C. and S. Dias.

Extracellular matrix molecules undergo cell- and type-specific turnover during bone marrow recovery following irradiation: relevance for organ homeostasis and in malignancy.

Poster at the AACR Annual Meeting, Los Angeles, USA.

Carneiro J. and Paixão T.

Noise in biochemical processes.

Kinetic modelling course of the PhD Program in Experimental Biology and Biomedicine. University of Coimbra. Coimbra, Portugal.

Carneiro M.

When B cells loose their memory: a study of chronic granulomatous disease patients.

University of Lund, Sweden.

Certal A.C., Santos M.R., Rodríguez-León J.
The potassium channel ERG1 is involved in the regulation of cell proliferation and apoptosis during limb development.
International Chick Meeting, Barcelona, Spain.

Coutinho A.
Biologia, biotecnologia, bioquímica.
Ciência em Portugal – Ciência 2007. Associate Laboratories Meeting. Fundação Calouste Gulbenkian, Lisbon, Portugal.

Demengeot J.
Synergies between V-regions and innate receptors on lymphocytes.
5th Annual Meeting of the Association for Immunotherapy of Cancer - CIMT/ 3rd International Conference Strategies for Immune Therapy 2007, Würzburg, Germany.

Duarte J, Água-Doce A, Caridade M, Oliveira V, Sakaguchi S, Graca L.
Reprogramming the immune system with tolerogenic antibodies in autoimmune arthritis.
World Immune Regulation Meeting, Davos, Switzerland.

Gomes A.C., Alcobia I., Parreira L., Cidadão A.
Notch signaling in embryonic hematopoiesis: a view from the microenvironment.
2nd International Meeting of the Portuguese Society for Stem Cells and Cell Therapies (SPCE-TC), Coimbra, Portugal.

Graça L
Reprogramar o sistema imunitário em alergia e autoimunidade: lições de modelos animais.
Clinical Session, Hospital de Santa Maria, Lisbon, Portugal.

Mallo M.
Manipulating gene expression in the mouse.
1st International Course of Immunology FIOCRUZ/YAKULT, Salvador de Bahia, Brazil.

Martins G.G., Amândio R., Rifes P., Palmeirim I. and Thorsteinsdóttir S.
Cell-Matrix transformations associated with somite boundary formation in chick embryos.
Poster at the International Chick Meeting, Barcelona, Spain.

Mota-Vieira L.
Contributo da genética para a história do povoamento dos Açores.
Conference on Escola Básica e Secundária das Lajes do Pico, Azores, Portugal.

Mota-Vieira L.

A biologia da vida humana: conhecer para melhor prevenir a gravidez na adolescência.
Conference on Escola Básica e Secundária das Lajes do Pico, Azores, Portugal.

Oliveira S.

Caso de sucesso no 6º PQ.

Sessão de Informação sobre o Programa Pessoas –7º Programa-Quadro de Investigação da UE, Universidade de Évora, Portugal.

Pereira-Leal J.

The role of gene duplication in the evolution of modularity in protein interaction networks.

Universidade de Coimbra, Coimbra, Portugal.

Pereira-Leal J.

Bioinformática - da investigação à aplicação.

II Conference in Biotechnology, Universidade Lusófona, Lisbon, Portugal.

Rifes P., Carvalho L., Lopes C. Andrade R., Rodrigues G., Palmeirim I. and Thorsteinsdóttir S.

Redefining the role of ectoderm in somitogenesis: a player in the formation of the fibronectin matrix of presomitic mesoderm.

International Chick Meeting, Barcelona, Spain.

Saavedra P., Alcobia I., Gomes A.C., Oliveira S., Duarte A., Parreira L. Cidadão A.

Haplo-insufficiency of the Notch-ligand Delta 4 in ESC-derived embryoid bodies is associated with epithelial-mesenchymal transition and decreased hematopoietic potential in a gamma-secretase independent manner.

Poster at the 2nd International Meeting of the Portuguese Society for Stem Cells and Cell

Therapies (SPCE-TC), Coimbra, Portugal.

Soares M.

Heme Oxygenase-1: “Master-Switch” in the resolution of inflammatory reactions.

Invited lecture. First international course of Immunology, Fiocruz-Yacult, Salvador da Bahia, Brazil.

Soares M.

Regulation of inflammatory reactions.

Invited lecture. First international course of Immunology, Fiocruz-Yacult, Salvador da Bahia, Brazil.

Soares M.

Regulation of inflammatory reactions.

Invited series of lectures at the PhD Program of the Universidade Federal de São Paulo (USP), São Paulo, Brazil.

Stollenwerk N.

Modelling reinfection in hepatitis C.

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), Lisbon, Portugal.

Stollenwerk N.

Multi-strain models show critical fluctuations in *Neisseria meningitidis* epidemiology.

Neisseria Vaccine Conference, Varadero, Cuba.

Zelenay S.

De novo differentiation of protective and curative antigen specific Foxp3⁺ regulatory T cells upon inflammatory immunization.

Immunology course, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal.

MAY

Bekman E., Abranches E., Henrique D.

Neuronal production in vitro from ES cells.

Poster at Annual Meeting Sociedade Portuguesa de Neurociências, Ofir, Portugal.

Benedito R., Trindade A., Hirashima M., Henrique D., Lopes da Costa L., Rossant J., Gill P.S. and Duarte A.

Loss of Notch signalling induced by Dll4 cause arterial calibre reduction by increasing endothelial cell response to angiogenic stimuli.

Poster at the 7th European School of Haematology Conference on Angiogenesis, Albufeira, Portugal

Bettencourt-Dias M.

Biogénese de centróssomas no ciclo celular e no cancro.

FCUL, Lisbon, Portugal.

Branco CC.

Assessment of linkage disequilibrium in the Azores population.

Oral communication in the 3rd International Meeting on Genetics of Complex Diseases and Isolated Populations, Turin, Italy.

Carneiro J.

A brief history of bioinformatics and systems biology.

Postgraduate Course on Bioinformatics and Systems Biology. Escola de Ciências da Saúde. Braga, Portugal.

Carneiro J.

Bioquímica e biologia teórica.

Jornada comemorativa. Bioquímica 25 anos. Dialogos entre a Ciência e a Cultura. FCUP and ICBAS, Universidade do Porto, Porto, Portugal.

Carneiro J.

Complexity in the lymphocyte interaction networks regulating autoimmunity.

Advanced Course on Complexity of Biological Networks. Epigenomics Project. Évry, France.

Carneiro J.

O sistema imunitário como fonte de inspiração para cognição artificial.

Invited Seminar at Instituto Politécnico de Leiria. Leiria, Portugal.

Carneiro M.

B cell biology.

A Day with B cells and joints Symposium, Institute for Molecular Medicine, University of Lisbon, Lisbon, Portugal.

Carneiro T., Borges V. and Godinho Ferreira M.

DNA repair and DNA damage checkpoint inhibition are separable functions of telomeres. Telomeres and Telomerase. Cold Spring Harbour, New York, USA.

Chora A.

Heme oxygenase-1 and carbon monoxide down-regulate self-reactive T cell activation and inhibit neuroinflammation.

Invited lecture. Instituto de Medicina Molecular, Lisbon, Portugal.

Coutinho A.

O papel de Portugal no mundo.

Conference Portugal em EXAME 2007: campeões no Mundo, Centro Cultural de Belém, Lisbon, Portugal.

Coutinho A.

A Biotecnologia e a Informática.

Pequenos-Almoços no Parque, Taguspark, Oeiras, Portugal.

Coutinho A.

Lost in translation: immunology, as a case-study in molecular medicine.

Opening conference, 6th Congress European Federation of Internal Medicine, Lisbon, Portugal.

Duarte A.

Use of mouse embryonic stem cells in biomedical research.

1st International Consensus Meeting: New horizons in cell and tissue banking, Estação Zootécnica Nacional, Vale de Santarém, Portugal.

Duque P.
From basic genomics to systems biology.
EMBO Conference Series, Ghent, Belgium.

Gomes G.
Trends in theoretical epidemiology.
CIM (Centro Internacional de Matemática) Annual Meeting, Coimbra, Portugal.

Gomes G.
A European infrastructure for epidemic forecast.
Workshop on Modelling of Control Strategies for Health Threats, Luxemburg.

Gomes G.
Gripenet.
12th Annual EISS (European Influenza Surveillance Scheme) Meeting, Malaga, Spain.

João C.
Reconstituição imune após transplante autólogo de precursores hematopoiéticos.
Centro de Investigação Patobiológica Médica (CIPM), Instituto Português de Oncologia de Francisco Gentil, Lisbon, Portugal.

Mallo M.
Building up a skeleton with Hox genes.
Netherlands Institute for Developmental Biology, Hubrecht Laboratorium, Utrecht, Holland.

Marques J.
Termination of the AI-2 signalling cycle in enteric bacteria.
ASM 107th General Meeting. Toronto, Canada.

Moraes F.
The role of neural crest-derived smooth muscle cells in remodeling embryonic arteries.
7th ESH Euroconference on Angiogenesis, Albufeira, Portugal.

Mota-Vieira L.
O genoma humano e sua hereditariedade.
Conference at Escola Secundaria Domingos Rebelo, Ponta Delgada, Azores, Portugal.

Parkhouse R.M.E.
Principles of vaccine design.
Lecture at the Universidade de Lisboa, Lisbon, Portugal.

Pereira C. S.
Universal AI-2 Signalling in *Sinorhizobium meliloti*.
ASM 107th General Meeting. Toronto, Canada.

Pereira-Leal J.
Compartimentalização em biologia celular - uma abordagem bioinformática.
IV Fórum Inova, Universidade Nova de Lisboa, Lisbon, Portugal.

Rodrigues P.
Reinfection - embedding immunology into epidemiology.
Advanced Course on TB: The recrudescence of an infectious disease: tuberculosis.
Scuola Superiore d'Immunologia Ruggero Ceppellini, Napoli, Italy.

Rodríguez-León J.
FLRT3 modulates FGF signaling in adult zebrafish fin regeneration.
III Annual Meeting of the European Network of Excellence Cells Into Organs, Les Embiez, France.

Saúde L.
4th Plenary Meeting of the Cells into Organs Network of Excellence.
Les Embiez conference centre, Les Embiez, France.

Trindade J.R., Ferguson J., Carvalho M.B., Martins I., Rodrigues C., Garcia H., Leitão C., Petkovic M., Becker J.D., Afonso C., Branco L., Esperança J.M.S.S., Seddon K.R., Rebelo L.P.N. and Silva Pereira C.
Neoteric white-biocatalysis for drug synthesis.
BIT's 5th Congress of International Drug Discovery Science and Technology, Shanghai, China.

Trindade J.R., Ferguson J., Carvalho M.B., Martins I., Rodrigues C., Garcia H., Leitão C., Petkovic M., Becker J.D., Afonso C., Branco L., Esperança J.M.S.S., Seddon K.R., Rebelo L.P.N. and Silva Pereira C.
Bio-Ionic Liquid Laboratories.
BIT's 5th Congress of International Drug Discovery Science and Technology. Shanghai, China.

Zelenay S.
Dominant tolerance mediated by Foxp3⁺ regulatory T cells.
Immunology course. Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal.

JUNE

Alves F. Feijó J.A.

Theoretical models for ion dynamics and cell polarization in pollen tubes. XIV International Workshop on Plant Membrane Biology, Universidad Politecnica de Valencia, Valencia, Spain.

Alves F. Feijó J.A:

Theoretical models for ion dynamics and cell polarization in pollen tubes. Gordon Research Conference on Cellular Osmoregulation: Sensors, Transducers & Regulators, Centre Paul Langevin, Aussois, France.

Batista S., Reis C. and Godinho Ferreira M.

Rad32(Mre11)/Rad50/Nbs1 complex is required for NHEJ repair in early G1 cells. Poster at the IV international fission yeast meeting. Copenhagen, Denmark.

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

Functional analysis of novel genes differentially expressed in chick heart/hemangioblast precursor cells (H/HPC).

Poster at the 1st Pan American Congress of Developmental Biology/SDB 66th Ann Meeting, Cancun, Mexico.

Bettencourt-Dias M.

Self-assembly and modularity in centriole assembly.

Poster at the Chromo2007 meeting, Torku, Finland.

Bettencourt-Dias M.

Self-assembly and modularity in centriole assembly.

CRMB, Montpellier, France.

Cachaço A.S., Santos A.C. and Dias S.

Involvement of TNF α in leukaemia onset and bone marrow turnover.

Poster at the 14th Congress of European Hematological Association, Vienna, Austria.

Carvalho-Santos Z.

What can evolution tell us about centriole duplication.

Poster at the Brinkley Mitosis meeting, California, USA.

Casalou C., Fragoso R., Moura Nunes J.F. and Dias S.

FLT-1 Activation by VEGF/PLGF induces Leukemia cell migration via p38/Erk1/2 kinase pathway, resulting in Rho GTPases activation and caveolae formation.

Poster at the EHA Annual Meeting, June 2007 Viena, Austria.

Corte-Real J.

Genetic control of the homeostatic levels of serum IgM in the mouse.
37th Scandinavian Society for Immunology Meeting, Turku, Finland.

Corte-Real J.

Genetic control of B cell autoreactivity in type 1 diabetes.
Jornadas de Ensino Pós-Graduado da Faculdade de Medicina Veterinária/Simpósio do CIISA, Lisbon, Portugal.

Feijó J.A.

Ions-in, ions-out, the way pollen tubes use water flow to underlie growth and morphogenesis regulation.
Gordon Research Conference on Cellular Osmoregulation: Sensors, Transducers & Regulators, Aussois, France.

Feijó J.A.

Plants, sex and pollen tubes: how evolution shapes special cells for special purposes.
I Encontro Científico Multidisciplinar, Vigo University, Spain.

Feijó J.A.

Member of the international scientific committee, Chairman, International Congress on Plant Membranes, Valencia, Spain.

Fernandes P.

Using Web Services to deploy Bioinformatics applications straightforwardly.
EMBRACE Network of Excellence, A European Model for Bioinformatics Research and Community Education, Swiss Institute of Bioinformatics, Deploying Web Services for Biological Sequence Annotation, Geneva, Switzerland.

Fragoso R., Igreja C. and Dias S.

A putative role for VEGFR-1 (FLT-1) in B cell commitment and differentiation.
EHA Annual Meeting, Viena, Austria.

Gallego-Díaz V.

Role of an antisense transcript of Hoxb3 in mouse development.
Pan-American SDB Congress, Cancun, Mexico.

Gonçalves J., Nolasco S., Bellido J., Zabala J.C. and Soares H.

Pursuing the in vivo tubulin Cofactor A role.
Poster at the EMBO-FEBS Workshop on Chaperones in Normal & Aberrant Protein Folding, Aging & Cancer. Tomar, Portugal.

Graça L.

T-regulatory cells and tolerance: mechanisms of suppressive function.

9th Banff Conference on Allograft Pathology, Coruna, Spain.

Graça L.

Reprogramming the immune system in allergy.

Institut de Pharmacologie Moléculaire et Cellulaire, Nantes, France.

Mallo M.

Hox genes are still alive and in business.

Institute for Stem Cell Research, University of Edinburgh, Edinburgh, UK.

Michard E., Feijó J.A.

Glutamate receptors as a key element in the H⁺ and Ca²⁺ signaling pathways cross-talk in pollen tube growth.

14th International workshop on plant membrane biology, Valencia, Spain.

Mira N.P., Lourenço A.B., Fernandes A.R., Becker J.D., Sá-Correia I.

Adaptive response and resistance to acetic and propionic acids: Involvement of Haa1p- and Rim101p- dependent regulons.

3rd European Federation of Biotechnology Conference: Physiology of Yeasts and Filamentous Fungi, Helsinki, Finland.

Mira N.P., Becker J.D., Sá-Correia I.

Saccharomyces cerevisiae adaptation to acetic acid stress involves the Haa1p-dependent response regulon.

3rd European Federation of Biotechnology Conference: Physiology of Yeasts and Filamentous Fungi, Helsinki, Finland.

Moncaut N.

Mediators of Hoxb4 hematopoietic-promoting activity.

Pan-American SDB Congress, Cancun, Mexico.

Mota-Vieira L.

O papel do biólogo em saúde: um testemunho das actividades realizadas em contexto hospitalar.

Conference at Universidade dos Açores, Ponta Delgada, Azores Portugal.

Nolasco S., Bellido J., Gonçalves J., Cruto T., Zabala J.C. and Soares H.

TBCA16, a putative mouse pseudogene with a biological function?

Poster at the EMBO-FEBS Workshop on "Chaperones in Normal & Aberrant Protein Folding, Aging & Cancer". Tomar, Portugal.

Nóvoa A.

10th Felasa-iclas Joint Meeting 2007, Cernobbio, Italy.

Oliveira S.

Genetic epidemiology.

Mestrado de Neurociências, Faculdade de Medicina de Lisboa, Lisbon, Portugal.

Parkhouse R.M.E.

Invited Discussant at the Christophe Mérieux Conference, Trends in Virology, Annecy, France.

Penha-Gonçalves C.

Immunogenetics of Type 1 diabetes: a genetic paradigm of autoimmunity.

Lição de provas de agregação., Reitoria da Universidade de Lisboa, Lisbon, Portugal.

Penha-Gonçalves C.

Malaria in Príncipe Island: a search for genetic factors controlling the carrier status.

New York Academy of Science International Conference, Severe Malaria: Pathogenesis and Intervention Strategies, Stockholm. Sweden.

Pereira-Leal J.

The evolution of modularity in biomolecular networks.

Faculty of Life Sciences, University of Manchester, Manchester, UK.

Rifes P., Carvalho L., Lopes C. Andrade R., Rodrigues G., Palmeirim I. and Thorsteinsdóttir S.

Redefining the role of ectoderm in somitogenesis: a player in the formation of the fibronectin matrix of presomitic mesoderm.

Poster at the Cells into Organs Workshop: Designing the Bodyplan: Developmental Mechanisms, Leiden, The Netherlands.

Rodrigues-Martins A.

Self-assembly and modularity in centriole assembly.

Poster at the Brinkley Mitosis Meeting, California, USA.

Rodríguez-León J.

FLRT3 modulates FGF signalling in adult zebrafish fin regeneration.

Seminar at IBILI, Universidade de Coimbra, Coimbra, Portugal.

Seabra M.

Rab GTPases, membrane traffic and retinal disease.

FASEB Summer Conference, Snowmass CO, USA.

Silva A. C., Vitorino M., Filipe M., Marques S., Becker J., Steinbeisser H. and Belo J.A.
Study of *Xenopus* orthologues of novel genes expressed in the mouse AVE.
Poster 1st Pan American Congress of Developmental Biology/SDB 66th Ann Meeting,
Cancun, Mexico.

Silva-Santos B.
Innate mechanisms of tumour immunosurveillance.
Annual Meeting of the European Molecular Biology Organization (EMBO) Young
Investigator Programme, Heidelberg, Germany.

Soares M.
Heme oxygenase-1 is a protective gene that counters the pathogenesis of cerebral
malaria.
Invited lecture, Rockefeller University, New York, USA.

Soares M.
Heme oxygenase-1 is a protective gene that counters the pathogenesis of cerebral
malaria.
Invited lecture, Brigham and Women Hospital, Harvard, Medical School, Boston, USA.

Soares M.
Heme oxygenase-1 is a protective gene that counters the pathogenesis of cerebral
malaria.
Invited lecture, University of Massachusetts Medical School, USA.

Soares M.
Heme oxygenase-1 is a protective gene that counters the pathogenesis of cerebral
malaria.
Invited lecture. National Institute of Health, Washington DC, USA.

Soares M.
Heme oxygenase-1: a protective gene that counters the pathogenesis of inflammatory
diseases.
Invited lecture, University of Alabama at Birmingham, USA.

Tavares A.
Molecular characterization of *Drosophila* kinetochore.
Seminar on the 3rd Spindle Dynamics Workshop, Sitges, Spain

Vinagre T.
Mechanisms of Hox functional specificity: The role of Hoxa10 in rib formation.
Pan-American SDB Congress, Cancun, Mexico.

JULY

Afonso N. , Simões M.

FLRT3 modulates FGF signalling during regeneration of the adult zebrafish caudal fin.
The 5th European Zebrafish Genetics and Development Meeting, Amsterdam, The Netherlands.

Aguiar M.

Deterministic chaotic dynamics in a multi-strain model with temporary cross-immunity.
II Conference on Computational and Mathematical Population Dynamics, Campinas, Brazil.

Aguiar M.

New chaotic attractor in dengue: Deterministic chaotic dynamics in a multi-strain model with temporary cross-immunity.
X International Dengue Course and Symposium, La Habana, Cuba.

Alves F.

Out of the flatland: towards three-dimensional modeling in *Drosophila* development and pollen germination.
Dynamics of Regulatory Networks II (Scientific Gathering), Centro Internacional de Ciências A.C., Cuernavaca, Mexico.

Becker J.D., Takeda S., Dolan M., Feijó J.A.

Transcriptional profiling of *arabidopsis* root hairs and pollen defines an apical growth signature.
Plant Biology and Botany 2007 Joint Congress, Chicago, USA.

Carneiro J.

Know thy self. The immune system as a cognitive system.
Caminhos da Complexidade: Ciências da Vida, Encontros da Arrábida. Arrábida, Portugal.

Carvalho S.

A novel alternatively-spliced RING E3 ubiquitin ligase regulates ABA-mediated germination responses in *Arabidopsis*.
2nd ISSS Workshop on Molecular Aspects of Seed Dormancy and Germination
Salamanca, Spain.

Certal A.C., Santos M.R., Rodríguez-León J.

Erg1 regulates cell proliferation and apoptosis during normal vertebrate limb development
6th European Biophysics Congress, London, UK.

Coelho M. and Soares H.

CER is a *Tetrahymena thermophila* tubulin cofactor E (TBCE) homolog involved in microtubule dynamics and remodeling.

FASEB Summer Research conferences on Ciliate Molecular Biology, Tucson, Arizona, USA.

Coutinho A.

Inflection point strategies for global public goods.

UBS Philantropy Forum, Penha-Longa Hotel, Sintra, Portugal.

Leon K.

Modulation of the immune system-tumor relationship.

Caminhos da Complexidade: Ciencias da Vida, Encontros da Arrábida. Arrábida, Portugal.

Lopes S.

Functional study of the molecular clock during pectoral fin development.

Poster at the 5th European Zebrafish Genetics and Development Meeting, Amsterdam, Netherlands.

Lourenço R.

Functional study of the molecular clock during pectoral fin development.

Poster at the 5th European Zebrafish Genetics and Development Meeting, Amsterdam, Netherlands.

Mantilla-Beniers N.B.

Epidemiological signature of alternative cross-immune responses to BCG.

II Conference on Computational and Mathematical Population Dynamics, Campinas, Brazil.

Pascoal S.

Functional study of the molecular clock during pectoral fin development.

Poster at the 5th European Zebrafish Genetics and Development Meeting. Amsterdam, Netherlands.

Pereira-Leal J.

Evolution of protein-protein interaction networks.

Gordon Research Conference, Structural, Functional & Evolutionary Genomics, Cambridge, UK.

Rodrigues L., Filipe J., Seldon M., Anrather J., Soares M.P. and Simas J.P.
Gammaherpesvirus suppression of NF- κ B transcriptional activity via a EC₅S ubiquitin complex that targets nuclear p65/RelA.

32th International Herpesvirus Workshop, Asheville, USA.

Rodríguez-León J.

FLRT3 modulates FGF signalling during regeneration of the adult zebrafish caudal fin.
Poster at the 5th European Zebrafish Genetics and Development Meeting. Amsterdam, Holand.

Saúde L.

Functional study of the molecular clock during pectoral fin development.
Poster at the 5th European Zebrafish Genetics and Development Meeting.
Amsterdam, Netherlands.

Seabra M.

Organelle motility.
Chairman. 6th European Biophysics Society Congress, London, UK.

Silva-Santos B.

57th Lindau Meeting for Nobel Laureates in Physiology and Medicine, Lindau, Germany.

Simões B., Vitorino M., Silva A.C., Conceição N., Belo J.A., Cancela M.L.

Phenotypic observation of *Xenopus laevis* MGP loss of function.
Poster at the 32nd FEBS Congress, FEBS2007, Vienna, Austria.

Stollenwerk N.

Rich dynamics in multi-strain epidemiological models.
II Conference on Computational and Mathematical Population Dynamics, Campinas, Brazil.

Stollenwerk N.

New chaotic attractor in dengue: positive lyapunov exponent in a multi-strain model with temporary cross-immunity.
X International Dengue Course and Symposium, La Habana, Cuba.

Xavier K.B.

Termination of the AI-2 signalling cycle in enteric bacteria.
Gordon Research Conference. Microbial Adhesion & Signal Transduction, Salve Regina University, USA.

Zelenay S.

RIKEN Research Center for Allergy and Immunology (RCAI) International Summer Program. Yokohama, Japan.

AUGUST

Alves F.

Modeling ion dynamics and cell polarization in pollen tubes.

Departamento de Genética del Desarrollo y Fisiología Molecular, Instituto de Biotecnología da Universidade Nacional Autónoma de México, Cuernavaca, México.

Alves F.

A model for the spatial regulation of gene expression in *Drosophila* early development.

Mechanisms of Early Development, Cell Fate Determination, Morphogenesis and Patterning, Instituto Medicina Molecular, Lisbon, Portugal.

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

Functional analysis of novel genes differentially expressed in chick heart/hemangioblast precursor cells (H/HPC).

Poster at the Symposium Mechanisms of early development: cell fate determination, morphogenesis and patterning, Instituto de Medicina Molecular, Lisbon, Portugal.

Bettencourt-Dias M.

Self-assembly and modularity in centriole assembly.

Harvard Medical School, Boston, USA

Bettencourt-Dias M.

Self-assembly and modularity in centriole assembly.

FASEB Summer Research Conferences, Biology of Cilia and Flagella, Vermont, USA

Bettencourt-Dias M.

Right time, right place and only once: the control of centriole birth.

Samuel Lunenfeld Research Institute, Toronto, Canada.

Borges A. C., Trindade A., Becker J., Costa L. L. and Duarte A.

Identification of novel downstream targets of Dll4 in the endothelium of the mouse embryo.

Poster at the Symposium of Mechanisms of Early Development, Cell fate determination, morphogenesis and patterning, Instituto de Medicina Molecular, Lisbon, Portugal.

Campos I., Geiger J., Santos A., Carlos V., Jacinto A.

The search for new genes involved in wound healing in *Drosophila*.

Cells into Organs Symposium, Mechanisms of early development: cell fate determination, morphogenesis and Patterning, Instituto de Medicina Molecular, Lisbon, Portugal.

Carneiro J.

From natural to artificial immune systems and back.

ICARIS2007, 6th International Conference in Artificial Immune Systems, Santos, Brazil.

Correia C., Sepulveda N. and Paulino C.

Mapeamento Bayesiano de fenotipos binários complexos.

15th Annual Congress of the Portuguese Society for Statistics, Lisbon, Portugal.

Coutinho A.

Hot topics in immunology: immunoregulation.

ImmunoRio2007, 13th International Congresso f Immunology, Rio de Janeiro, Brazil

Cunha A.

Marine Biological Laboratory summer course, Pathogenesis of neuroimmunologic diseases, Woods Hole, USA.

Ferrari L., Gardner R., Sousa AE, Ogle B., Platt J.F., Cascalho M, and Carneiro J.

Precision and accuracy of antigen-receptor genetic diversity estimates using microarray technology.

Poster at the IMMUNORIO 2007, 13th International Conference in Immunology, Rio de Janeiro, Brazil.

Gallego-Díaz V

Role of an antisense transcript of Hoxb3 in mouse development.

Mechanisms of early development: cell fate determination, morphogenesis and patterning, Lisbon, Portugal.

García Fernández B. and Janody F.

Mechanisms of early development: cell fate determination, morphogenesis and patterning.

Instituto de Medicina Molecular, Lisbon, Portugal.

Gonçalves L., Filipe M., Bento M., Silva A.C., Steinbeisser H. and Belo J.A.

Analysis of the role of the novel gene ADTK1 during mouse development.

Poster at the Symposium Mechanisms of early development: cell fate determination, morphogenesis and patterning. Instituto de Medicina Molecular, Lisbon, Portugal.

Gordo I.

Patterns of genetic variation in pathogen populations.

11th Congress of The European Society for Evolutionary Biology, Upssala, Sweden.

Lamego J.

G2/M regulation by the polarity-related kinase Par-1

Poster at the Mechanisms of early development: cell fate determination, morphogenesis and patterning, Lisbon, Portugal.

Liguori G.L., Borges A.C., D'Andrea D., Liguoro A., Gonçalves L., Salgueiro A.M., Pêrsico M. G. and Belo J.A.

Cripto-independent nodal signalling promotes positioning of the a-p axis in the early mouse embryo.

Poster at the Mechanisms of early development: cell fate determination, morphogenesis and patterning, Instituto de Medicina Molecular, Lisbon, Portugal.

Lopes P, Teotónio H, Sucena E, Magalhães S.

Evolution of pesticide resistance in *C. elegans*.

Poster at the Congress of the European Society for Evolutionary Biology, Uppsala, Sweden.

Mallo M.

Patterning the axial skeleton by Hox genes.

Mechanisms of early development: cell fate determination, morphogenesis and patterning, Lisbon, Portugal.

Marinho C.

A mouse model exhibiting pathological features of human pregnancy-associated malaria.

Poster at the 13th International Congress of Immunology, Rio de Janeiro, Brazil.

Marques S., Salgueiro A.M., Silva A.C. and Belo J.A.

Functional activity studies on the role of *cerl-2* in the mouse node.

Poster at the Symposium Mechanisms of early development: cell fate determination, morphogenesis and patterning, Instituto de Medicina Molecular, Lisbon, Portugal.

Monteiro J.F., Shipley A.M., Rodríguez-León J. and Certal A.C.

Dynamic extracellular ion fluxes are involved in fin regeneration in zebrafish.

Poster at the Symposium on Mechanisms of Early Development, Lisbon, Portugal

Moraes F.

The role of neural crest-derived smooth muscle cells in remodeling embryonic arteries.

Mechanisms of early development: cell fate determination, morphogenesis and patterning, Lisbon, Portugal.

Parkhouse R.M.E.

Interacción patógeno-huesped: una arma de doble filo.

UNAM, Mexico DF, México.

Parkhouse R.M.E

Interaccion patógeno-huesped: hecha la ley hecha la trampa.

CINVESTAV, Instituto Politecnico Nacional, Mexico DF, Mexico.

Perfeito L., Fernandes L., Mota C. and Gordo I.

Adaptive mutations in bacteria: high rate and small effects.

Poster at the 11th Congress of The European Society for Evolutionary Biology, August 20-25, Upssala, Sweden.

Rebelo M., Fontes M.F., Caramalho I., Zelenay S., Duarte J., Martins C., and demengeot J.

Role of IL-7 in the homeostasis of CD4 T cells.

13th International Congress of Immunology. Rio de Janeiro, Brazil.

Salgueiro A.M., Filipe M. and Belo J.A.

Functional study of Mad4, a novel gene expressed in the AVE during early mouse embryonic development.

Poster at the Symposium Mechanisms of early development: cell fate determination, morphogenesis and patterning, Instituto de Medicina Molecular, Lisbon, Portugal.

Sepulveda N. and Carneiro J.

How broad is the regulatory T cell repertoire.

IMMUNORIO 2007- 13th International Conference in Immunology. Rio de Janeiro, Brazil.

Sepulveda N., Paulino C. D. and Carneiro J.

Estimação da diversidade dos receptores de linfocitos T.

15th Annual Congress of the Portuguese Society for Statistics, Lisbon, Portugal.

Sepúlveda N., Paulino C. D. and Carneiro J.

How diverse are regulatory T cells that protect an organism against autoimmunity?

Joint Annual Meetings of The Society for Mathematical Biology and The Japanese Society for Mathematical Biology. San Jose, Ca, USA.

Sepulveda N., Paulino C. D. and Carneiro J.

Estimation of T-cell receptor diversity.

56th Session of the International Statistical Institute, Lisbon, Portugal.

Silva A.C., Filipe M., Vitorino M., Steinbeisser H. and Belo J.A.

Role of XADTK1 during *Xenopus laevis* development.

Poster at the Symposium Mechanisms of early development: cell fate determination, morphogenesis and patterning, Instituto de Medicina Molecular, Lisbon, Portugal.

Silva Pereira C., Trindade J.R., Ferguson J., Petkovic M., Martins I., Leitão C., Carvalho M.B., Rodrigues C., Garcia H., Becker J.D., Esperança J.M.S.S., Seddon K.R. and Rebelo L.P.N.

Functional ionic liquids application to whole cell biocatalysis for drug production.

COIL meeting. Yokohama, Japan.

Silva-Santos B.

CD27 and Bcl2 A1 provide survival and differentiation signals to developing thymocytes.

13th International Congress of Immunology, Rio de Janeiro, Brazil.

Soares M.

Regulation of inflammatory and immune responses by the stress responsive gene Heme Oxygenase-1 (HO-1) and its end-product Carbon Monoxide (CO).

Chairing session and invited lecture. Mini-symposium, Control of inflammation, 13th International Congress of Immunology, Rio de Janeiro, Brazil.

Vitorino M., Silva A. C., Steinbeisser H. and Belo J. A.

Functional study of the novel gene XADTK1-L2 during *Xenopus laevis* embryogenesis.

Poster at the Symposium Mechanisms of early development: cell fate determination, morphogenesis and patterning, Instituto de Medicina Molecular, Lisbon, Portugal.

Zelenay S., Bergman M.L. and Demengeot J.

Prevention and cure of spontaneous and severe encephalomyelitis by inducing de novo generation of specific regulatory T cells in vivo.

13th International Congress of Immunology, Rio de Janeiro, Brazil.

SEPTEMBER

Belo J.A.

Generating asymmetries in the early vertebrate embryo: the role of the Cerberus-like family.

Eddy De Robertis Symposium, EMBL, Heidelberg, Germany.

Caiado F., Real C., Dias S. Inhibition of the Notch-Delta pathway at early steps of endothelial progenitor differentiation impairs adhesion and spreading to the ECM via integrin modulation.

Poster at the ELSO Meeting, Dresden, Germany.

Carvalho R.

SR45, a plant-specific splicing factor, regulates development and stress response in *Arabidopsis thaliana*.

Poster at the X Congresso Luso-Espanhol de Fisiologia Vegetal, Alcalá de Henares, Spain..

Chora A., Fontoura P., Cunha A., Pais T., Cardoso S., Ho P.P., Y Lee L., Sobel R.A., Steinman L. and Soares M.:

Heme Oxygenase-1 and carbon monoxide suppress autoimmune neuroinflammation.
Poster at the 5th International Congress Heme Oxygenases 2007, Krakow, Poland.

Coelho M., Cruto T. and Soares H.

CER is a *Tetrahymena thermophila* tubulin cofactor E (TBCE) homolog involved in microtubule dynamics and remodeling.

Poster at the Sixth international congress ELSO 2007, Dresden, Germany.

Coutinho A.

Recrutamentos e promoções de docentes e de investigadores: experiência institucional extra-universitária com ligações às universidades.

Novas Conversas da Nova. Campus de Campolide, Lisbon, Portugal.

Dias S.

The importance of the vascular compartment in bone marrow function.

Vascular Wall and Endothelium Meeting, Faculdade de Medicina de Lisboa/IMM, Lisbon, Portugal.

Duarte, A.

Notch signalling in the regulation of arteriogenesis and angiogenesis (oral presentation).

Advanced Course on Vascular Wall and Endothelium, Faculdade de Medicina de Lisboa, Lisbon, Portugal.

Duarte J., Zelenay S., Bergman M.L., Martins A.C., Demengeot J.

Foxp3 expression by regulatory T cells is labile and conditions their function.

JFEBS 14th International Summer School on Immunology, Immune System: Genes, Receptors and Regulation, Hvar, Croatia.

Epiphany S.

Induction of HO-1 during *Plasmodium* liver infection protects infected hepatocytes by controlling the inflammatory response.

Poster at the Gordon Research Conference on Malaria, Oxford, UK:

Gaspar A., Bajanca F. and Thorsteinsdóttir, S.

Expression patterns of extracellular matrix molecules laminin, collagen type IV fibronectin and their integrin receptors in the early phases of hypaxial myogenesis.

Poster at the Symposium on Mechanism of early development: cell fate determination, morphogenesis and patterning, Lisbon, Portugal.

Gomes G.

The SIRI model in epidemiology.

University of Utrecht, The Netherlands.

Graça L.

Tolerance induction with monoclonal antibodies: from transplantation to allergy.
Nuffield Department of Surgery, University of Oxford. Oxford, UK.

Jacinto A.

Wound healing in *Drosophila*.

The Healing Foundation Centre Opening Symposium, 2007, The Healing Foundation Centre, Manchester, UK.

Lopes C., Rifes P., Andrade R.P., Thorsteinsdóttir, S. and Rodrigues G.

Expression pattern of fibronectin and its integrin receptors (Itga5 and Itga4) during early stages of chick embryo development.

Poster at the Symposium on Mechanism of early development: cell fate determination, morphogenesis and patterning, Lisbon, Portugal.

Lopes S.

The role of zebrafish gene *terra* during left-right development.

Poster at the Mechanisms of early development: cell fate determination, morphogenesis and patterning. Instituto de Medicina Molecular, Lisbon, Portugal.

Lourenço R.

Identification of the *terra* target genes in zebrafish.

Poster at the Mechanisms of early development: cell fate determination, morphogenesis and patterning.

Instituto de Medicina Molecular, Lisbon, Portugal.

Marques A.R., Tostões R., Marty T., Martinho R.

Differential requirements for mitotic sister-chromatid cohesion in the soma and in the germ-line.

European *Drosophila* Research Conference, Vienna, Austria.

Marques L., Bajanca F., Rodrigues G. and Thorsteinsdóttir, S.

Is the PI3K/Akt pathway involved in mouse skeletal myogenesis?

Poster at the Symposium on Mechanism of early development: cell fate determination, morphogenesis and patterning, Lisbon, Portugal.

Martins G.G., Amândio R., Rifes P., Palmeirim I. and Thorsteinsdóttir S.

Cell-Matrix transformations associated with somite boundary formation in chick embryos.

Poster at the Symposium on Mechanism of early development: cell fate determination, morphogenesis and patterning, Lisbon, Portugal.

Mendes R.

Do cells from the left and right side of Hensen's node have different migration pathways?

Poster at the Mechanisms of early development: cell fate determination, morphogenesis and patterning. Instituto de Medicina Molecular, Lisbon, Portugal.

Mentelova L

Characterisation of the *Drosophila Melanogaster* Mob Homologues.

20th European *Drosophila* Research Conference, Vienna, Austria.

Mota MM.

Malaria Infection:approaching the host.

ELSO 6th Annual Meeting, Frontiers of Cellular, Developmental and Molecular Biology, Dresden, Germany.

Muehlen M.

Yearly meeting of the European TropNet network on imported tropical diseases.

IHMT, Lisbon, Portugal.

Muehlen M.

Poster presentation of GripeNet: real-time internet-based ILI surveillance

ESEI Conference, Lisbon, Portugal.

Neres R.

A mouse model exhibiting pathological features of human pregnancy-associated malaria.

Poster at the Gordon Research Conferences Malaria, Magdalen College, Oxford, UK.

Pacheco L.

The role of zebrafish gene terra during left-right development.

Mechanisms of early development: cell fate determination, morphogenesis and patterning

Instituto de Medicina Molecular, Lisbon, Portugal

Pamplona A., Rodrigues C., Chora A., Epiphany S., Cunha-Rodrigues M., Soares M.P. and Mota M.M.

Suppression of cerebral malaria by heme oxygenase-1 and carbon monoxide.

Gordon Research Conferences,Malaria; Magdalen College, Oxford, UK.

Pamplona A., Ferreira A., Balla J., Jeney V., Balla G., Epiphany S., Chora A., Rodrigues C., Pombo-Gregoire I., Cunha-Rodrigues M., Portugal S., Soares M.P. and Mota M.M. Heme oxygenase-1 and carbon monoxide suppress the pathogenesis of experimental cerebral malaria.

Poster at the 5th International Congress Heme Oxygenases 2007, Krakow, Poland.

Pascoal S.

Functional study of the molecular clock during pectoral fin development.

Poster at the Mechanisms of early development: cell fate determination, morphogenesis and patterning. Instituto de Medicina Molecular, Lisbon, Portugal.

Pereira F.

Identification and characterization of new *Drosophila* kinetochore proteins.

ELSO meeting, Dresden, Germany.

Pereira-Leal J.

The bioinformatics of modular biological systems.

Centro de Informática e Tecnologias de Informação, Universidade Nova de Lisboa, Lisbon, Portugal.

Pombo-Grégoire I., Cardoso S., Noordeloos A.M., Correia M., Sanz-González S., Chora A., Larsen R., Duckers E. and Soares M.

Heme oxygenase-1 derived carbon monoxide inhibits atherosclerosis.

5th International Congress Heme Oxygenases 2007, Krakow, Poland.

Raposo B.

Combined anti-CD8 and anti-TNF therapy induce disease remission in the K/BxN model of spontaneous chronic arthritis.

2007 Baltic Immunology Summer School, Lund University, Sweden.

Rifes P., Carvalho L., Lopes C. Andrade R., Rodrigues G., Palmeirim I. and Thorsteinsdóttir S.

Redefining the role of ectoderm in somitogenesis: a player in the formation of the fibronectin matrix of presomitic mesoderm.

Poster at the Symposium on Mechanism of early development: cell fate determination, morphogenesis and patterning, Lisbon, Portugal.

Sambo R.

Genetic susceptibility to cerebral malaria in Angolan children.

Poster at the Gordon Research Conferences Malaria, Magdalen College, Oxford, UK.

Saúde L.

Mechanisms of early development: cell fate determination, morphogenesis and patterning. Instituto de Medicina Molecular, Lisbon, Portugal.

Seabra M.

Molecular mechanisms of platelet secretion.

UK-Netherlands Platelet Meeting, London, UK.

Seldon M., Silva G., Pejanovic N., Larsen R., Pombo-Gregoire I., Filipe J., Anrather J., Soares M.

Heme oxygenase-1 inhibits the expression of adhesion molecules associated with endothelial cell activation via a mechanism that targets serine in nuclear factor kappa B (NF- κ B) RelA.

Poster at the 5th International Congress Heme Oxygenases 2007, Krakow, Poland.

Serpa J., Dias S.

Butyric acid treatment of resistant colon cancer cells promotes the acquisition of a more angiogenic phenotype.

Poster at the ELSO Meeting, Dresden, Germany.

Silva G., Cunha A., Pombo-Grégoire I., Seldon M. and Soares M.

The anti-apoptotic effect of heme oxygenase-1 in endothelial cells involves the degradation of p38 mitogen activated protein kinase (MAPK) isoform.

Poster at the 5th International Congress Heme Oxygenases 2007, Krakow, Poland.

Stollenwerk N.

Rich dynamics in multi-strain epidemiological models: from evolution towards criticality to reinfection threshold and new deterministic chaotic attractors.

Lisbon Epidemiology Consortium Inaugural Meeting, Universidade de Lisboa, Lisbon, Portugal.

Soares M.

Heme oxygenase-1: a protective gene that counteracts the pathogenesis of inflammatory diseases.

Invited lecture, 5th International Congress Heme Oxygenases 2007, Krakow, Poland.

Soares M.

Heme oxygenase-1: in organ transplantation.

Invited lecture: State of the art, 5th International Congress Heme Oxygenases 2007, Krakow, Poland.

Tavares A.

Identification and characterization of *Drosophila* kinetocore proteins.

20th European *Drosophila* Research Conference, Vienna, Austria.

Tokaji L., Bonaparte D., Marinho-Cavalcante M., Chora A., Cardoso S., Silva G., Soares M.

Heme Oxygenase-1 prevents the development of severe sepsis: mechanism of action.

Poster at the 5th International Congress Heme Oxygenases 2007, Krakow, Poland.

OCTOBER

Aguiar M.
Chaos in a multi-strain dengue model and data.
CNRS, Paris, France.

Bettencourt-Dias M.
Making centrioles with and without a template.
IBMC, Porto; Portugal.

Carneiro J.
Modelling T cell activation in autoimmunity.
Workshop on Systems Biology in Medicine. Long Beach CA, USA.

Carneiro J.
Biologia Computacional na busca de um sensor de imunodiversidade.
Workshop Sensores. ISEL, Lisboa. Portugal.

Cortes H, Marcelino E, Waap H, Reis Y, Hemphill A, Müller N, Gottstein B, Soares H, Marques I, Leitão A.
Bovine besnoitiosis: an emerging disease in Europe?
Poster at the Second Symposium of the Belgian Wildlife Disease Society (BWDS), Wildlife diseases, environment and man. Queen Astrid Military Hospital, Brussels, Belgium.

Coutinho A.
Nature-CNIO (Spanish National Cancer Research Centre) Conference on Oncogenes and Human Cancer: The next 25 years, Madrid, Spain.

Coutinho A.
Que progressos nas ciências da vida?
Conference A Ciência terá Limites? Fundação Calouste Gulbenkian, Lisbon, Portugal.

Feijó J.A.
Live cell imaging methods: new tools and old tricks.
Advanced. Live Cell Microscopy Workshop, CNIO, Madrid, Spain

Florindo C.
Human Mob1 is required for cytokinesis by destabilizing microtubules at the midbody
EMBO-Workshop, Molecular Mechanisms of Cell Cycle Control in Normal and Malignant Cells, Spetses, Greece.

Gomes G.
The reinfection threshold in epidemiology.

IX Jornadas de Biologia Aplicada, Universidade do Minho, Portugal.

Mallo M.

Designing the vertebrate skeleton with the help of Hox genes.

Colloquium on Hox genes in Development and Evolution, Les Treilles, France.

Muehlen M.

Presentation of GripeNet: advantages and potential of an internet-based ILI surveillance system.

ESCAIDE, Stockholm, Sweden.

Pereira C. S.

Interference with AI-2-mediated signalling in *Sinorhizobium meliloti*.

3rd ASM Conference on Cell-Cell Communication in Bacteria. Austin, Texas.

Rodríguez-León J.

FLRT3 modulates FGF signalling in adult zebrafish fin regeneration.

Seminar at Universidade Nova de Lisboa, MSc Developmental Biology course, Monte da Caparica, Portugal.

Seabra M.

Molecular mechanisms of melanosome biogenetics and motility in pigmented cells.

European Society for Pigment Cell Research, Bari, Italy.

Soares M.

Heme oxygenase-1 and carbon monoxide suppress autoimmune neuroinflammation.

Invited lecture, Collaborative Research Center SFB 548, Workshop Mechanisms of Autoimmunity, Clinic of the Johannes Gutenberg-University Mainz, Germany.

Soares M.

Heme Oxygenase-1: Controlling the pro-oxidant effects of free heme.

Invited lecture, SFRR-Europe 2007 Meeting, Vilamoura, Portugal.

Tavares A.

Identification and characterization of new kinetochore proteins.

Seminar on EMBO-Workshop, Molecular Mechanisms of Cell Cycle Control in Normal and Malignant Cells, Spetses, Greece.

Teotónio H.

The genetic basis of adaptation in sexual species.

Microsoft Frontiers in Biology, Trento, Italy.

Vitorino M., Silva A. C., Steinbeisser H. and Belo J. A.

Functional study of the novel gene XADTK1-L2 during *Xenopus laevis* embryogenesis.
Poster at the V Italian-German Xenopus Meeting, Lovenno di Menaggio, Italy.

Xavier K.B.

AI-2 Processing in enteric bacteria.

3rd ASM Conference on Cell-Cell Communication in Bacteria. Austin, Texas.

NOVEMBER

Abranches E., Bekman E., Henrique D.

Microarray Analysis of ES cell derived neural precursors.

Poster at EMBL Conference on Functional Genomics with Embryonic Stem Cells,
Heidelberg, Germany.

Agua-Doce A, Wollenberg I, Caridade M, Graca L.

Reprogramming the immune system in allergy with a non-depleting anti-CD4 antibody.

XXXIII Annual Meeting of the Sociedade Portuguesa de Imunologia, Instituto de
Medicina Molecular, Lisbon, Portugal.

Aguiar M.

Bifurcation analysis of routes to chaos in a multi-strain dengue model.

Department of Theoretical Biology, Faculty of Earth and Life Sciences, Free University
of Amsterdam, The Netherlands.

Almeida S., Oliveira V., Parkhouse R.M.E.

Impact of B/T cell restricted transgenic expression of A238L, a viral host evasion gene
of the african swine fever virus (ASFV).

Poster and oral presentation, XXXIII Annual Meeting of the Sociedade Portuguesa de
Imunologia, Instituto de Medicina Molecular, Lisbon, Portugal.

Antunes R.

Neuroscience 2007, SFN 37th Annual Meeting, San Diego, USA.

Becker J.D., Borges F., Gomes G., Gardner R., Moreno M., McCormick S, Takeda S,
Dolan L. and Feijó J.A.

A whole-genome transcriptomics approach to sexual reproduction and apical cell
growth in *Arabidopsis thaliana*.

RNA 2007, Vimeiro, Portugal.

Bekman E., Abranches E., Henrique D.

Neuronal production in vitro from ES cells.

Poster at EMBL Conference on Functional Genomics with Embryonic Stem Cells,
Heidelberg, Germany.

Bettencourt-Dias M.

Right time, right place, control of centriole number.

Wadsworth Center, Albany, USA.

Borges A. C.

Identification of downstream targets of Dll4 in the endothelium of the mouse embryo.

VII International Symposium on Experimental Techniques, Universidade do Algarve, Faro, Portugal.

Borges F., Gomes G., Gardner R., Moreno N., McCormick S., Feijó J.A., and Becker J.D.

Comparative transcriptomics of *Arabidopsis* sperm cells to decipher the role of male gametes in double fertilization.

RNA 2007 Vimeiro, Portugal.

Caramalho I.

Regulatory T cells maintain effector cells at check in NOD mice protected from Diabetes by LPS treatment.

XXXIII Annual Meeting of the Sociedade Portuguesa de Imunologia, Instituto de Medicina Molecular, Lisbon, Portugal.

Caridade M, Oliveira V, Agua-Doce A, Graca L.

In vitro conversion of naive T cells into Foxp3+ regulatory T cells by suboptimal activation.

Poster at the XXXIII Annual Meeting of the Sociedade Portuguesa de Imunologia, Instituto de Medicina Molecular, Lisbon, Portugal.

Carneiro J.

Regulation robustness in the immune system.

X Meeting 2007, 3rd International Conference of the Brazilian Association for Bioinformatics and Computational Biology. São Paulo, SP, Brazil.

Carneiro J.

Round table on Bioinformatics Education together with Jorge Guimarães, Helaine Carrer, Glória Franco, Paulo Mazzoncini.

X Meeting 2007, 3rd International Conference of the Brazilian Association for Bioinformatics and Computational Biology. São Paulo, SP, Brazil.

Carneiro J.

Regulation robustness in the immune system.

Invited Plenary Lecture at Centre for Computational and Systems Biology(CosBi), Microsoft Research, University of Trento, Trento, Italy.

Carneiro M.

Chair, I Convenção Danone de Probióticos, Centro de Congressos de Lisboa, Lisbon, Portugal.

Carvalho R.

A plant-specific SR protein regulates plant stress responses.

RNA 2007 Vimeiro, Portugal.

Chora A.

Heme oxygenase-1 and carbon monoxide down-regulate self-reactive T cell activation and inhibit neuroinflammation.

Invited lecture for the CytoMed Award 2007. Congress of the Portuguese Society of Immunology, Instituto de Medicina Molecular, Lisbon, Portugal.

Correia S., Reis A.L., Ferreira P., Santos L., Goodburn S., Leitão A., Parkhouse R.M.E. Identification of non-assigned, non-homologous virus genes inhibiting interferon responses.

Poster, XXXIII Annual Meeting of the Sociedade Portuguesa de Imunologia, Instituto de Medicina Molecular, Lisbon, Portugal.

Cortes H, Waap H, Reis Y, Marcelino E., Soares H, Marques I, V. Caeiro, Shkap, A Hemphill, Leitão A.

Isolation and in vitro culture of *Besnoitia besnoiti* from infected animals without any use of laboratory animals.

Poster at the VIII International Symposium on Experimental Techniques, Universidade do Algarve, Faro, Portugal.

Coutinho A.

Lost in translation.

Annual Meeting Sociedade Portuguesa de Hematologia, Albufeira, Portugal.

Coutinho A.

Lessons from primary immunodeficiencies.

XXXIII Reunião Annual da SPI, Manipulating the immune response: a therapeutic tool, Instituto de Medicina Molecular, Lisbon, Portugal.

Coutinho A.

Fé, Agnosticismo e Ciência.

Ciência na Almedina. Livraria Almedina, Lisbon, Portugal.

Coutinho A.

Teaching genetics in Portugal.

11ª Reunião da Sociedade Portuguesa de Genética, Centro de Cultura e Congressos da Ordem dos Médicos, Porto, Portugal.

Demengeot J.

Inducing immune activities to lower auto-immunity.

XXXIII Annual Meeting of the Sociedade Portuguesa de Imunologia, Instituto de Medicina Molecular, Lisbon, Portugal.

Dias S.

The importance of angiogenesis in bone marrow diseases.

Annual Meeting of the Portuguese Society of Hematology, Albufeira, Portugal.

Duarte J, Agua-Doce A, Sakaguchi S, Fonseca JE, Graca L.

Reprogramming the immune system with tolerogenic monoclonal antibodies in autoimmune arthritis.

Poster at the XXXIII Annual Meeting of the Sociedade Portuguesa de Imunologia, Instituto de Medicina Molecular, Lisbon, Portugal.

Duarte J., Zelenay S., Bergamn M.L., Martins A.C., Demengeot J.

Foxp3 expression by regulatory T cells is labile and conditions their function. João H. XXXIII Annual Meeting of the Sociedade Portuguesa de Imunologia, Instituto de Medicina Molecular, Lisbon, Portugal.

Faro J.

Nanotechnology in medicine: diagnosis and therapy.

International Symposium, Centro Social Caixanova, Vigo, Spain.

Feijó J.A.

The control of apical cell growth and morphogenesis by ion dynamics.

Génoscope/Génopole, Évry, Paris, France.

Ferrari L., Gardner R., Sousa AE, Ogle B., Platt J.F., Cascalho M, and Carneiro J.

Precision and accuracy of antigen-receptor genetic diversity estimates using microarray technology.

Poster at the X Meeting 2007, 3rd International Conference of the Brazilian Association for Bioinformatics and Computational Biology. São Paulo, SP, Brazil.

Gabriel A.M., Pereira C., Goulart L.F., Leal B., Fontes M. F., Vasconcelos C., Ferreira C. M., Martins J., Matos-Costa J., Martins B., Lima M., Demengeot J., Fesel C.

Treatment of Systemic Lupus Erythematosus with intravenous immunoglobulin (ivlg) and its effect on T-cell subsets and immunoregulation.

Poster at the XXXIII Annual Meeting of the Sociedade Portuguesa de Imunologia, Instituto de Medicina Molecular, Lisbon, Portugal.

Graça L.

O sistema imunitário e sua relação com o corpo.

Congresso Português de Psiquiatria. Estoril, Portugal.

Guimarães M.

Neuroscience 2007, SFN 37th Annual Meeting, San Diego, USA

João C.

Scientific Coordinator of the 1^o Curso de Investigação Clínica em Oncologia Médica, Instituto Português de Oncologia de Francisco Gentil, Lisbon, Portugal. (Duration of 3 months).

João C., Guimarães A., Antão I., Ferreira P., Ferreira I., Teixeira G., Miranda N., Abecassis M.

Condicionamento para transplante de precursores hematopoiéticos com Irradiação Corporal Total – A experiência da UTM e do Serviço de Radioterapia do IPOFG de Lisboa.

Annual Meeting of the Portuguese Society of Hematology, Albufeira, Portugal.

Mallo M.

The role of Hox genes in skeletal development.

Meeting of the Portuguese Society for Human Genetics, Porto, Portugal.

Mira N.P., Lourenço A.B., Fernandes A.R., Becker J.D., Sá-Correia I.

The RIM101 pathway is involved in *Saccharomyces cerevisiae* resistance to propionic acid.

MICRO'07-BIOTEC'07-XXXIII JPG, Lisbon, Portugal.

Mira N.P., Becker J.D., Sá-Correia I.

Saccharomyces cerevisiae adaptation to acetic acid stress involves the Haa1p-dependent response regulon.

MICRO'07-BIOTEC'07-XXXIII JPG, Lisbon, Portugal.

Mota-Vieira L

Co-chair of the session “Population genetics of common diseases”

11th meeting of the “Sociedade Portuguesa de Genética Humana”, Porto, Portugal

Muehlen M.

Organized a training module for GIS applications in epidemiology.

University of Zaragoza, Spain.

Muehlen M.

Opportunity and added value of field epidemiology training in Portugal.

Portuguese Epidemiology Congress, Lisbon, Portugal.

Muehlen M.

Importance of adherence to GCP guidelines in conducting field trials.

I Encounter of the Iberian Platform for Malaria, Lisbon, Portugal.

Neres R.

A mouse model that can contribute to study malaria in pregnancy.

Poster at the XXXIII Annual Meeting of the Sociedade Portuguesa de Imunologia, Instituto de Medicina Molecular, Lisbon, Portugal.

Oliveira T, Caridade M, Agua-Doce A, Graca L.

Prevention of allergic airways disease in allergen-specific TCR-transgenic mice with a non-depleting CD4 monoclonal antibody.

Poster at the XXXIII Annual Meeting of the Sociedade Portuguesa de Imunologia, Instituto de Medicina Molecular, Lisbon, Portugal.

Oliveira V., Soares H., Almeida S., Parkhouse R.M.E.

The impact of B cell restricted transgenic expression of a mouse herpes virus (MHV-68) host evasion gene.

Poster, XXXIII Annual Meeting of the Sociedade Portuguesa de Imunologia, Instituto de Medicina Molecular, Lisbon, Portugal.

Padovan E.

Distinct molecular signatures in human monocytes and dendritic cells predict adjuvant activity and pyrogenicity of toll-like receptor agonists.

XXXIII Annual Meeting of the Sociedade Portuguesa de Imunologia, Instituto de Medicina Molecular, Lisbon, Portugal.

Parkhouse R.M.E.

Chairman, XXXIII Annual Meeting of the Sociedade Portuguesa de Imunologia, Instituto de Medicina Molecular, Lisbon, Portugal.

Pierdomenico F., João C., Carvalho S., Chagas M., Vieira M.R., Gomes da Silva M., Salta M.G.

Estadiamento e avaliação de resposta à terapêutica em doentes com Linfoma de Hodgkin utilizando TAC e PET.

Annual Meeting of the Portuguese Society of Hematology, Albufeira, Portugal.

Pires A.E., Cabral S., Esteves S., Gomes da Silva M., Porrata L., Markovic S., João C.

Efeito da infusão de imunoglobulina ou seus derivados sobre a reconstituição imunológica após transplante autólogo de precursores hematopoiéticos (TAPH) num modelo animal (ratinho).

Annual Meeting of the Portuguese Society of Hematology, Albufeira, Portugal.

Queirós A., Pires A.E., Cabral S., Esteves S., Gomes da Silva M., Porrata L., Markovic S., João C.

Treatment with polyclonal immunoglobulin and its fragments optimize recovery of IgM serum levels after autologous stem cells transplantation (ASCT).

XXXIII Annual Meeting of the Sociedade Portuguesa de Imunologia, Instituto de Medicina Molecular, Lisbon, Portugal.

Ribot J.

The CD27 co-receptor provides differentiation and activation signals to Treg and gd T cells.

XXXIII Annual Meeting of the Sociedade Portuguesa de Imunologia, Instituto de Medicina Molecular, Lisbon, Portugal.

Tenreiro S., Vargas R.C., Becker J.D., García-Salcedo R., Ramos J., Sá-Correia I.

Mechanisms of resistance to quinidine and quinidine targets: post-genomic approaches using the eukaryotic model *Saccharomyces cerevisiae*. MICRO'07-BIOTEC'07-XXXIII JPG, Lisbon, Portugal.

Teotonio H.

Workshop on Evolutionary topics for Ecologists.

Instituto Nacional de Pesquisa da Amazônia, Manaus, Brasil.

Xavier K.B.

The regulation of quorum sensing by small RNAs.

RNA 2007. Vimeiro, Portugal.

Zelenay S., Bergman M.L., Fontes M.F., Demengeot J.

Pro-inflammatory immunization promotes differentiation of Foxp3⁺ regulatory T cells that prevent and revert autoimmunity.

XXXIII Annual Meeting of the Sociedade Portuguesa de Imunologia, Instituto de Medicina Molecular, Lisbon, Portugal.

DECEMBER

Águas R, Gomes G.

Prospects for sustainable malaria control in sub-Saharan Africa.

Centre for International Health, University of Barcelona, Spain.

Bettencourt-Dias M.

Right time, right place, control of centriole number.

Poster at the American Society for Cell Biology, Washington DC, USA

Caiado F., Real C., Dias S.

Inhibition of the notch-delta pathway at early steps of endothelial progenitor differentiation impairs adhesion and spreading to the ecm via integrin modulation.

Poster at the American Society of Hematology 49th ASH annual meeting and exposition, Atlanta, USA.

Garcia-Fernández B.

Drosophila Melanogaster como modelo animal para estudos de Biologia do Desenvolvimento.

Aula de Biologia do Desenvolvimento da Universidade Lusófona, Lisbon, Portugal.

Geiger J.

Searching *Drosophila* for new genes involved in wound healing.

The 47th Annual Meeting of the American Society for Cell Biology, Washington Convention Center, Washington DC, USA

Muehlen M.

Assessment of field conditions for the establishment of a demographic surveillance system in Angola.

Provincia do Bengo, Angola.

Pereira F.

Molecular characterization of *Drosophila* kinetochore.

ASCB 47th Annual Meeting, Washington DC, USA

Pereira-Leal J.

What does evolution tell us about centriole duplication ?

Poster at the Annual meeting of the American Society for Cell Biology, Washington, USA.

Pires A.E., Cabral S., Esteves S., Gomes da Silva M., Porrata L., Markovic S., João C.

The effect of infusion of polyclonal immunoglobulin or its fragments in the improvement of the immune reconstitution after autologous stem cells transplantation (ASCT) in a murine model.

Poster at the American Society of Hematology meeting 2007, Atlanta, GA, USA.

Rodrigues-Martins A.

Self organization and modularity in centriole assembly.

American Society for Cell Biology, Washington DC, USA.

Saúde L.

Como é controlado o posicionamento dos órgãos internos em vertebrados ?

Congresso Nacional da Sociedade Portuguesa de Cirurgia Pediátrica. Escola de Ciências da Saúde, Braga, Portugal.

Awards, Prizes and Nominations

The following awards, prizes and committee nominations for IGC research staff were obtained in 2007.

Mónica Bettencourt-Dias

Crioestaminal award

Eppendorf Young European Investigator

2007 Pfizer Prize for Basic Research (Ana Rodrigues Martins and Mónica Bettencourt-Dias)

Bolsa Professor Doutor António Xavier- Installation Grants for Research Group Leaders. Oeiras City Council.

Radio Clube Português/Jornal Metro 2007: Ciência e Pensamento

“Seeds of Science/Sementes de Ciência 2007” Ciência Hoje (www.cienciahoje.pt)

Margarida Carneiro

General Secretary of the Portuguese Society for Immunology

Angelo Chora

Citomed Prize in Immunology 2007 “Heme oxygenase-1 and carbon monoxide suppress autoimmune neuroinflammation”.

António Duarte

Menção Honrosa Prémio Científico UTL/Santander Totta

Joana Duarte

Best poster award. Annual Meeting of the Sociedade Portuguesa de Imunologia, Lisbon, Portugal. November 14-16 2007.

Reprogramming the immune system with tolerogenic monoclonal antibodies in autoimmune arthritis.

Authors: Duarte J, Agua-Doce A, Sakaguchi S, Fonseca JE, Graca L.

Lisete Fernandes

Director of Department, DCNE, Escola Superior de Tecnologia da Saúde de Lisboa, Lisbon, Portugal.

Miguel Godinho-Ferreira

Career Development Fellowship

Fundação para a Ciência e Tecnologia, Portugal
(PTDC/SAU-OB/66438/2006).

Constantin Fesel

NEDAI prize for autoimmunity research.

Gabriela Gomes

Scientific Coordinator of the Lisbon Epidemiology Consortium.

Isabel Gordo

Council member of the European Society for Evolutionary Biology Career Development Grant from Fundação para a Ciência e Tecnologia (declined)

Luís Graça

Secretary of the Sociedade de Ciências Médicas de Lisboa.

Scientific secretary of the Sociedade Portuguesa de Imunologia.

Moisés Mallo

Editorial Board, Developmental Dynamics

Madalena Martins

Biomarkers in Alzheimer's Disease: The Lipid Homeostasis/Oxidative Stress Connection.

Prize Fundação Astrazeneca (Programa de Apoio à Investigação).

Luisa Mota-Vieira

Secretary of the "Sociedade Portuguesa de Genética Humana", from November 2007

Member of scientific commission of the "Sociedade Portuguesa de Genética Humana", from November 2006 until October 2008

Member of the Community Genetics Network, since September 2007.

Marion Muehlen

Vice President of the EPIET Alumni Network (EAN) board

Ana Nóvoa

Travel Award, for students and technicians, to attend the TT2007 Meeting in, Brisbane, Australia.

Rui Oliveira

President of the Sociedade Portuguesa de Etologia.

R.M.E.Parkhouse

President of Sociedade Portuguesa de Imunologia until 2009

Member of evaluation pannel of Redes y Centros de Investigación Corporativa, Spain

Member of evaluation pannel of Neglected Tropical Diseases Initiative, Nuffield, Gulbenkian and Volkswagen Foundations

Member of organizing committee, International Herpesvirus Workshop

Member of organizing committee, International Meeting, “Cellular and Cytokine Interactions in Health and Disease”.

José Pereira-Leal

Bolsa Professor Doutor António Xavier- Installation Grants for Research Group Leaders. Oeiras City Council.

Miguel Seabra

Vice-President of Scientific Council of Faculdade de Ciências Médicas, Universidade Nova de Lisboa

Member of the External Scientific Board of IBILI, Universidade de Coimbra

Member of Editorial Board of J. Biol. Chem.

Member of Editorial Board of Biochem. J.

Member of Editorial Board of Pigment Cell Res.

Member of MRC College of Experts

Bruno Silva-Santos

European Molecular Biology Organization (EMBO) Young Investigator Programme – Installation Grant, 2007-2012.

Treasurer of the Sociedade Portuguesa de Imunologia from November 2007.

Margarida Santos

Prémio Pulido Valente Ciência 2007 – Organização Funcional da Célula

Helena Soares

Scientific Council President of the Escola Superior de Tecnologia da Saúde de Lisboa, Lisbon, Portugal.