



# **INSTITUTO GULBENKIAN DE CIÊNCIA**

## **ANNUAL REPORT 2004**

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# **FUNDAÇÃO CALOUSTE GULBENKIAN**

## **BOARD OF ADMINISTRATION**

The Fundação Calouste Gulbenkian, established by Calouste Sarkis Gulbenkian by his Will dated June 18, 1953, is a private Institution of general public utility, endowed with legal personality. The aims of the Foundation are charity, art, education and science. The members of the Board of Administration in 2004 were:

### **President**

Emílio Rui Vilar

### **Honorary President**

Mikhael Essayan

### **Executive Trustees**

José Blanco (retired in September 2004)

Diogo de Lucena

Isabel Mota

Eduardo Marçal Grilo

Teresa Gouveia (since November 2004)

### **Non-Executive Trustees**

André Gonçalves Pereira

Eduardo Lourenço

Artur Santos Silva

## **INSTITUTO GULBENKIAN DE CIÊNCIA**

### **BOARD OF DIRECTORS**

The Board of Directors for the Instituto Gulbenkian de Ciência (IGC) ensures that the activities at the Institute follow the guidelines and objectives defined by the Board of Administration of the Fundação Calouste Gulbenkian. The members of the Board of Directors for 2004 were:

### **Board of Directors**

Diogo de Lucena (Chairman)

João Caraça

Manuel Rodrigues Gomes

Manuel Carmelo Rosa

Horácio Menano

António Coutinho

## **SCIENTIFIC ADVISORY BOARD**

The Scientific Advisory Board of the IGC scrutinises the scientific progress and teaching programmes, as well as the recruitment and activity of personnel and research groups. The Scientific Advisory Board also advises the Board of Administration of the Fundação Calouste Gulbenkian on all matters relevant to the mission of the Institute. The members of the Scientific Advisory Board for 2004 were:

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

The Scientific Advisory Board met at the IGC on 21-23 July 2004.

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

## **STAFF**

### **DIRECTOR**

António Coutinho

### **DEPUTY-DIRECTORS**

Sérgio Gulbenkian  
José Mário Leite

### **CHIEF TECHNOLOGICAL OFFICER**

Matthias Haury

## RESEARCH MEMBERS

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

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

Dulce Azevedo (FCT)  
Jörg Becker (FCT)  
José António Belo (UALG)  
Mostafa Bendahmane (FCT)  
Marie-Louise Bergman (Marie-Curie-EU)  
Jorge Carneiro (Lab. Associado)  
Margarida Carneiro (IGC)  
Cristina Casalou (IPO/FCT)  
Pierre-André Cazenave (Univ. Paris VI/Institut Pasteur/CNRS/FCT)  
Ana Catarina Certal (FCT)  
Sukalyan Chatterjee (FCG)  
Melvin Cohn (Salk Institute/FCT)  
Susana Constantino (IPO/FCT)  
Ana Paula Coutinho (FCT)  
António Coutinho (CNRS/FCG)  
Pedro Coutinho (FCT)  
Ana Crespo (UE)  
Jocelyne Demengeot (FCG)  
Sérgio Dias (IPOFG/FCT)  
Francisco Dionísio (FCT)  
Sabrina Epiphany (FCT)  
José Faro (Univ. Salamanca)  
José Feijó (FCUL)  
Lisete Fernandes (ESTSL)  
Carlos Alberto Ferreira (HUSM)  
Constantin Fesel (FCT)  
Carlos Penha Gonçalves (Lab. Associado)  
Simone Gines (FCT)  
Gabriela Gomes (FCT)  
Guilherme Gonçalves (Ministério da saúde)  
Isabel Gordo (FCT)  
Christophe Gregoire (FCT)  
Isabel Pombo Gregoire (FCT)  
Sérgio Gulbenkian (FCG)  
Werner Haas (FCG)  
Matthias Haury (FCG)

Domingos Henrique (FMUL)  
António Jacinto (FCT)  
Gregory King (Univ. Warwick)  
Abdelkader Lakmeche (FCT)  
Joaquin Rodriguez León (FCT)  
Giovanna Liguori (FCT)  
Moises Mallo (Lab. Associado)  
Moises Marinho (UE)  
Gabriela Martins (FCT)  
Marta Miranda (FCT)  
Kalet Leon Monzon (Cent. Immunol. Mol. Cuba/IGC)  
Maria Mota (FCT)  
Sofia Oliveira (Marie-Curie/EU)  
Maria Teresa Faria Pais (FCT)  
Isabel Palmeirim (ECSUM)  
Ana Maria Pamplona (FCT)  
Michael Parkhouse (FCG)  
Paula Parra (FCT)  
Leonor Parreira (FMUL/IMM)  
Cristina Paulo (FCT)  
Sylviane Pied (INSERM/Inst. Pasteur)  
Ricardo Pimenta-Araújo (FMUL)  
Ana Rita Ponce (IGC)  
Miguel Prudêncio (FCT)  
Gabriela Rodrigues (FCUL)  
Ana Paula Santos (FCT)  
Ana Catarina Moreira dos Santos Sarzedas (FCT/EMBO)  
Leonor Tavares Saúde (FCT)  
Elsa Seixas (FCT)  
Gabriela Silva (FCT)  
João Pedro Simas (FMUL)  
Helena Soares (ESTSL)  
Miguel Che Parreira Soares (Lab. Associado)  
John Stewart (Univ. Tech. Compiègne/CNRS)  
Élio Sucena (FCT)  
Álvaro Augusto Tavares (ISTUL)  
Ana Teresa Tavares (FCT)  
Vera Lucas Teixeira (FCT)  
Henrique Teotónio (FCT)  
Solveig Thorsteinsdottir (FCUL)  
Maria de Jesus Trovoada (FCG)  
Johann Truccolo (FCT)  
Filipa Vala (FCT)  
Tatiana Vassilevskaia (Astrazeneca/FCT)  
Miguel Vaz Afonso (IGC)  
Astrid Vicente (INSA-RJ)

Luisa Mota Vieira (HDES)  
Sheila Vidal (IGC)  
Ana Margarida Vigário (FCT)  
Andrew Waters (Univ. Leiden/FCG)  
William Wood (FCT)

## **STUDENTS**

### **Ph.D. Students**

Sónia Albuquerque (ICBAS/FCT)  
Isabel Alcobia (FMUL/FCT)  
Sílvia Almeida (ITQB/UNL/FCT)  
Paulo Alves (FMUL/FCT)  
Fernanda Bajanca (FCUL/FCT)  
Marta Barreto (FCUL/FCT)  
Leonor Boavida (FCUL/FCT)  
Ana Cristina Borges (UALG/FCT)  
Ana Sofia Cachaço (FCUL/FCT)  
Dinis Calado (FMUL/FCT)  
Marta Campos (FMUL/FCT)  
Iris Caramalho (ICBAS/FCT) - Postdoc since October 2004  
Daniel Carapau (FCUL/FCT)  
Marta Carapuço (ITQBUNL/FCT)  
Ângelo António Chora (FMUL/FCT)  
Jaime Combadão (ITQB/UNL/FCT)  
Sofia Cordeiro (FCUL/FCT)  
Catarina Correia (FCTUL/FCT)  
Sílvia Correia (ITQB/UNL/FCT)  
Vasco Correia (ICBAS/FCT)  
Sofia Côrte-Real (FMUL/FCT)  
Sílvia Costa (FCUL/FCT)  
Ana Margarida Coutinho (FCUL/FCT)  
Margarida Cunha (ICBAS/FCT)  
Célia Domingues (FCUL/FCT)  
Ana Paula Elias (FCUL/FCT)  
Mariana Faria (FCUL/FCT)  
Elisabete Fernandes (FCT)  
Beatriz Fernandez (ITQB/UNL/FCT)  
Manuela Ferreira (FCUL/FCT)  
Catarina Figueiredo (ITQB/UNL/FCT)  
Mário Rui Filipe (FCT/UNL/FCT)  
Cláudia Florindo (FCT/UNL/FCT)  
Francisca Fontes (FMUL/Hospital Egas Moniz/ Ministério da Saúde)  
Rita Fragoso (FCUL/FCT)  
Ana Rita França (FMUL/FCT)

Catarina Freitas (FCUL/FCT)  
Rui Freitas (ITQB/UNL/FCT)  
Rui Gardner (ICBAS/FCT)  
Susana Godinho (IST/FCT)  
Mário Grãos (FCUL/FCT)  
Vincent Guiyedi (Inst. Pasteur)  
Anja Hagemann (FCUL/FCT)  
Cátia Igreja (FCUL/FCT)  
Pedro Lares (FCUL/FCT)  
Patrícia Leirião (IHMTUNL/FCT)  
Ana Sofia Lopes (FCUL/FCT)  
Sofia Marques (FMUL/FCT)  
Maria Hortense Matos (ITQB/UNL/FCT)  
Ana Lúcia Mena (ITQB/UNL/FCT)  
Joana Monteiro (FCUL/FCT)  
Filipa Moraes (ITQB/UNL/FCT)  
Joana Moreira (FCUL/FCT)  
Nuno Moreno (IGC/FCUL)  
Inês Mota (Univ. Évora)  
Rute Nascimento (FMUL/FCT)  
Hélia Neves (FMUL/CEBIP/FCT)  
Sofia Nolasco (FCUL/FCT)  
Helena Nunes (ITQB/UNL/FCT) - left December 2004  
Vivian Oliveira (ITQB/UNL/FCT)  
Tiago Paixão (ICBAS/FCT)  
Lília Perfeito (ITQB/UNL/FCT)  
Diogo Pimentel (PGDB/FCT)  
Ana Margarida Prado (FCUL/FCT)  
Ana Sofia Quina (FMUL/FCT)  
Manuel Rebelo (FCUL/FCT)  
Ana Luisa Reis (FMVUTL/FCT)  
Cristina Rodrigues (FCUL/FCT)  
Lénia Rodrigues (FMUL/FCT)  
Paula Rodrigues (UNL)  
Sofia de Albuquerque Rodrigues (ECSUM/FCT)  
Maria do Rosário Sambo (FMUL/Hospital Pediátrico Luanda, Angola)  
Margarida Santos (FMUL/FCT)  
Ana Cecília Seixas (FCUL/FCT)  
Mark Seldon (ICBAS/FCT)  
Ana Cristina Silva (FCUL/FCT)  
Susana Silva (FCUL/FCT)  
Sérgio Simões (FCUL/FCT)  
Laszlo Tokaji (ITQB/UNL/FCT)  
Ana Sofia Veloso (ITQB/UNL/FCT)  
Ana Maria Vieira (FCUL)  
Santiago Zelenay (FCUL/FCT)



**M.Sc. Students**

Bruno Mateus (IST)

**B.Sc. Students**

Ricardo Águas (Univ. Évora)

Ricardo Ataíde (Univ. Lusófona)

Catarina Barroca (FCUL)

Pedro Campinho (FCUL)

Catarina Correia (Univ. Lusófona)

Vanessa Cristão (FCUL/PRODEP) - left September 2004

Tânia Cruz (Univ. Lusófona)

Joana Duarte (FCUL)

João Duarte (Univ. Coimbra)

Lurdes Duarte (Univ. Évora)

Valter Duarte (Univ. Lusófona)

Diogo Fonseca (Univ. Algarve) - left July 2004

Joana Duarte (FCUL)

Andreia Cunha (FCUL)

Célia Faria (Univ. Lusófona)

Dinis Gokaydin (FCUL)

João Gonçalves (FCUL)

Lara Lourenho (FCUL)

Raquel Lourenço (Univ. Lusófona)

Ramiro Magno (FCUL)

Diogo Manoel (Univ. Évora)

Ana Paula Martins (IST)

Rui Martins (FCUL)

Susana Matias (FCT/UNL)

Inês Matos (Univ. Évora)

João Melo (Univ. Coimbra)

Carla Milagre (Univ. Lusófona)

Ester Morgado (École Sup. Techniques de Biol. Apl., Paris, France)

Ana Margarida Nunes (FCUL)

Isabel Peixeiro (FCTUL)

Ana Catarina Ribeiro (FCUL)

Pedro Rifes (FCUL/PRODEP)

Yara Reis (Univ. Beira Interior)

Joana Rodo (Univ. Évora)

Pedro Santos (FCUL)

Luís Saraiva (Univ. Évora)

Ana Filipa Simões (FCUL) - left July 2004

Inês Sousa (FCUL)

Pedro Vale (Univ. Évora)

Duarte Viana (Univ. Évora)

Tânia Vinagre (Univ. Lusófona)

Marta Vitorino (Univ. Algarve)

**Laboratory Technical Support**

Ana Água-Doce (BIC/FCT)  
Tânia Aires (BTI/FCT)  
Ana Alexandra Almeida (BI/FCT)  
Sofia Andrade (BI/FCT)  
Paulo Almeida (Lab. Associado)  
Gilberto Bento (BI/FCT)  
Margaret Bento (IEFP)  
Paulo Bettencourt (BTI/FCG)  
Cláudia Bicho (BI/FCT)  
Dolores Bonaparte (BTI/FCG)  
Daniela Brites (BTI/FCT)  
Marisa Cabrita (BTI/FCG)  
Lara Carvalho (BTI/FCG)  
Maria do Céu Conceição (BTI/FCG)  
Ana Neves Costa (BI/FCT)  
Sofia Couceiro (IEFP)  
Judite Dias (IEFP)  
Lígia Deus (IEFP)  
Sara Fidalgo (Marie-Curie/EU)  
Carla Fernandes (BTI/FCG)  
Ricardo Ferreira (IEFP)  
Lídia Fonseca (BTI/UE)  
Ana Cristina Gaspar (BTI/FCG)  
Alexandre Gonçalves (BIC/FCT)  
Lisa Gonçalves (BI/FCT)  
Alexis Gonzalez (FCG)  
Susana Magrito (BTI/FCG) - left March 2004  
Sara Marques (BI/FCT)  
Ester Morgado (IEFP)  
Ana Nóvoa (Lab. Associado)  
Dominique Ostler (BTI/FCG) - left June 2004  
Filipe Pinto (IEFP)  
Rui Rodrigues (BIC/FCT) - left April 2004  
Nuno Sepúlveda (BIC/FCT)  
Catarina Silva (BTI/FCT)  
Susana Silva (IEFP) - left September 2004  
Sofia Simões (BTI/UE) - left December 2004  
João Tiago Sousa (FCG)  
Ana Teles (IEFP)  
Sónia Ventura (BI/FEDER)

## **ADMINISTRATIVE, SECRETARIAL AND TECHNICAL STAFF**

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

### **Administrative and Secretarial Staff**

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

### **Laboratory Technical Staff**

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

### **Technical Support Staff**

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

## UNITS AND SERVICES

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

**Animal Facility:** Jocelyne Demengeot

**Bioinformatics:** Pedro Fernandes

**Cell Imaging:** Sérgio Gulbenkian/Matthias Haury

**Histology and Histopathology:** Miguel Soares/Sérgio Gulbenkian

**Informatics:** Matthias Haury

**Library and Scientific Information:** Sérgio Gulbenkian

**Science and Society:** Ana Paula Godinho Coutinho

**Sequencing and Genotyping:** Carlos Penha-Gonçalves

**Theoretical and Computational Biology:** Jorge Carneiro

**Transgenic Unit:** Moises Mallo

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

## INTRODUCTION

This is the 6<sup>th</sup> Annual Report of the Instituto Gulbenkian de Ciência (IGC) after the last reform. The Institute continues to count on the quality and devotion of all its scientists, students and support personnel, on the attention and excellence of its Scientific Advisory Board, as well as on the understanding, guidance, and support from the Board of Administration of the Fundação Calouste Gulbenkian (FCG). If the project has developed satisfactorily, those are the main reasons, together with the supportive cooperation of many institutions, in Portugal and abroad, not the least the National Research Council (Fundação para a Ciência e a Tecnologia, FCT), and the two partner Institutes in the Oeiras Campus (ITQB and IBET) with whom the IGC composes an Associated Laboratory at the FCT. Along these six years, the strategic choices and the operational model adopted by the Institute have proven appropriate for pursuing its missions. With a budget that has been kept roughly constant by the FCG, the Institute has renewed six of its eight wings, equipped several units in its technological platform and a number of laboratories, has installed some 35 autonomous groups, has produced excellent science in a variety of fields, and has promoted the PhD education of some 250 new scientists. The responsibilities for nearly all of the groups at the IGC has been given to scientists who were attracted to Portugal from abroad, together with an even larger number of post-docs, bringing about a significant reinforcement of the national scientific community in life sciences. Most of the PIs at the IGC are very young, but have already proven their ability to find financial support to conduct independent, internationally competitive research in full autonomy. A considerable number of these have now been recruited in institutions across the country, representing as many seeds of a certain way of doing science that is the essence of the project itself. The IGC is, thus, only a platform in the overall project, which aims at progressively becoming an Institute with no walls, where we all share the same spirit and, perhaps, pride and nostalgia for what we did together in Oeiras. The Board of Administration of the Gulbenkian Foundation has now approved a 5-years plan aimed at consolidating the Institute's structure and activity, giving us all the reasons to believe that, as long as the project remains coherent, valuable and centrifugous, the efforts and devotion of all will be matched by the necessary support.

Clearly, all those scientists, Portuguese and foreigners alike, were attracted to Oeiras by the project itself, for the IGC offers no perspective of career or tenure-track positions, but a solid intellectual and technological environment, as well as an exclusive preoccupation with science and its practice in an open, cooperative manner. While this generous attitude of so many is most rewarding and encouraging, it is also clear that the very foundations of the project remain fragile. This is the first thing to note in the present Annual Report, seven years after the IGC's reform was enacted and many young scientists were supported to find their way: the bottle-neck in the Portuguese science system is, today, at the stage of career development. The public investments in doctoral and post-doctoral fellowships along the last 20 years, together with several doctoral programs and the opening of new research institutions, have given outstanding results and brought into science a large population of young women and men, decided to dedicate their lives to scientific research, to the promotion of science in society, or to

various entrepreneurial aspects in a technology-driven society. In turn, this resulted in a healthy increase in the competition for fellowships and research contracts. Thus, the rates of success among applicants for PhD and post-doctoral fellowships have decreased to levels that are comparable to those of European countries with stronger scientific traditions. Furthermore, an increasing number of Portuguese post-docs bring external fellowships (Marie Curie, EMBO, etc.) into the national system, which now hosts reasonable numbers of European and other foreign post-docs, even students. While these are all very positive signs of development, this dynamics of growth brought the community to a serious bottleneck, for there is essentially no research career in the country. Once the young scientist to-be completes the conventional cycle of education (PhD and post-doc) on public money, there are few possibilities ... other than re-applying for another post-doctoral fellowship. A quick screen of Portuguese young scientists reveals that many of them are now, after consecutive post-docs, in their last years of support, with little perspectives other than, perhaps, emigrating again for more developed countries. As there is no research career in Portugal, the most obvious future for a scientist is to compete for a teaching position at the University. Given the embarrassingly low number of PhD-holders with teaching duties (and jobs) at the Universities, this would seem to provide the best conditions for University research to recover. Yet, the extreme inbreeding of Portuguese Universities, the rules that regulate admission to such positions, and in some cases, the poor attractiveness of many a University department, all together result in today's situation of many excellent post-docs without support, being "held out" by University departments that keep their positions for non-PhD holders. National Laboratories offer a similar picture: they harbor hundreds of research positions, but continue to await the structural reform that everyone considers necessary and would open up those positions to external competition. There are very few other alternatives.

The excellent governmental initiative that launched the Associated Laboratories 5 years ago is certainly a step in the right direction, particularly as it encouraged the establishment of new autonomous research groups with fresh leaderships. Yet, the 5-years positions that have been created at the Associated Laboratories are far too few to contemplate even the best 10% of all young scientists who are "produced" in active institutions. In addition, given their strategic importance for the evolution of the whole science and technology system, Associated Laboratories, rather than indiscriminately contemplating all different scientific areas, should be criteriously distributed according to the expected (or desired) differential growth in each area. The overall distribution of public investments in science and technology, however, continues to reflect historical biases that neither correspond to current scientific production, nor to the expectations of the public. Health sciences, for example, receives today less than 10% of the total public investments! Finally, rather than "democratically" distributed by the various Centers as traditionally done in this country, Associated Laboratory positions should be reserved to the very top institutions, selected with extreme rigor and focused on excellence, while continuously evaluating the institution's ability to attract excellent candidates as well as the performance of the recruited scientists. Incidentally, specific support for Associated Laboratories should be strictly reserved to create such research positions, and they should not be diverged for consolidating institutional infrastructures, whatever necessary these might be. Above all, we need to attract and retain excellent young people.

With University and National Laboratory careers essentially closed, with too few possibilities to compete for a public research career, young scientists are left with no alternatives, all the more so as private research in the industry and “start-ups” remains extremely weak, not for the lack of investigators but for the absence of the necessary structural conditions for its development. The paucity of science-financing “charities” in Portugal adds to the problem, particularly if they concentrate their support where it is least necessary, i.e., in PhD students and post-docs, rather than innovating with career-development programmes. Some of our young scientists, after some years of enthusiastic work in the country, are already returning abroad, favorably competing for excellent positions where they can do precisely what we had expected from them when investing in their education: good science. In summary, all efforts in the education of new scientists along the last two decades may be jeopardised, if the public and private institutions do not succeed in reversing this trend. The consequences would be dramatic: this would deprive the country of the best, leaving it inhabited by mediocrity alone, which would have in hands the education of the new generations.

This is certainly the major structural problem in the national science and technology system, to which the IGC’s mission is particularly sensitive. Thus, as the Institute aims at identifying, educating, “incubating” and “exporting” new leaders in biomedical research, its operation requires openness in a market driven by excellence only. Other structural problems, while disturbing, are less critical: the irregularity and unpredictability of competitive calls for research projects is more of a problem than the paucity of funds, but we all hope it will be corrected, and it will become less critical once the European Research Council is eventually established. These considerations are quite relevant at a time when the IGC reached the stage to initiate the turnover of its scientists, and organize their departure to other institutions. One of the most rewarding achievements of last year was, precisely, the agreement between APIFARMA (the national association of pharmaceutical drug companies) and the FCG to establish a fund to support the setting-up of laboratories for those emigrating from the IGC to other institutions in Portugal. While the IGC will not profit from those moneys, they contribute at the heart of the project itself.

The scientific strategy of the IGC has been adapted to its mission of generating new leaderships, but also to the size of the Institute, to the stage of maturity in biomedical science and to the needs of the country. It is based on sharing the values of science and on the notion of cooperativity, amongst people, ideas, approaches and technologies, research projects and programmes, all concerned with “a project” that goes well beyond all of us. This was discussed in the 2003 Annual Report and I will not deal with it again. Enough to recall that that the institution is small enough to entertain centralised technological facilities and a high level of “connectivity” with frequent interactions amongst all its groups, and, yet, a large enough size to diversify its scientific content, provided all programmes are coherent in the institutional profile and objectives. The “maturity” of modern biology makes this possible, as we all use the same basic principles, the same language and the same technologies, and may thus claim “unity” in the whole field: there are no longer valid reasons to segregate evolutionists from developmental biologists, geneticists from immunologists, microbiologists from cell biologists, plants from animals, flies from mice and men. In addition, biology is finally

providing for the scientific basis of medicine, such that “disease-oriented” research may well be “basic” and *vice versa*. Scientific research is either good or bad, irrespective of being basic or applied. This has allowed for frequent productive interactions between scientists and groups working on apparently distinct problems, and I continue to think that the IGC’s competitive edge, if there is one, is to be found at the interfaces between research topics that are usually not in the same “department” or small institution. The statutory chart of the IGC reflects this strategy, defining our research on “the genetic and molecular basis of the evolution and development of complex biological systems”. Not surprisingly, therefore, the other distinctive characteristic of much that is done at the Institute is the concern with “organism-centered” questions and approaches, the notion that “systems” have properties, often the most interesting even from the “translational” point of view, that cannot be reduced to its components or processes. “System’s Biology” has recently come to the limelight by the back door, I am afraid, as an approach to deal with the massive volumes of data that are produced in “omics”-type science. Research at the IGC, however, did not have to wait for this fashion, and much of the Institute’s investment and contributions, not the least in the setting-up of a solid theoretical biology core, has this specific concern. It was obvious, therefore, the necessity of launching educational initiatives in Bioinformatics and to put together a novel PhD programme in Computational Biology. This programme will operate on the basis of a “Co-laboratorium”, a platform where active research is conducted by resident and visiting scientists on subjects of concern to the empiricists, perhaps the best way of introducing mathematicians to biology and biologists to the wonders of dynamic systems and networks. This was another achievement of the past year that shall now be announced to start operating in 2005. As other PhD programmes previously conducted by the IGC, this is an experiment that will extend over a 5 years period, which being innovative, certainly involves risks. Conversely, if successful, it will survive, perhaps elsewhere and under the responsibility of others, for as long as its value will justify it.

Within this general framework, one area of modern biology was particularly fit and, yet, entirely absent from the IGC’s agenda. This was the large field of neurosciences, which has gained a remarkable progress over the last few years, to the point that some speak of its new foundation. A variety of novel molecular and cellular approaches, together with new technologies for *in vivo* recordings of multiple neuronal groups in rodents going about their business, as well as the development of new paradigms for behavioral studies, all have brought unsuspected horizons to the field. It is not too risky to predict that, along the next decades, neurosciences will embody many of the most basic scientific questions in biology, and will concern translational research of the highest social relevance. As a significant part of the IGC space remains empty and awaiting renewal, it seemed appropriate to extend the Institute’s potential in education and research while giving it a higher scientific coherence, by bringing in new neurosciences. The IGC’s Scientific Advisory Board defended this evolution, defining the strategies on how to make the project into a relevant contribution from the Gulbenkian Foundation. The Board of Administration has then approved the proposal, such that we could start recruiting and setting-up groups with specific programmes in this field. The year of 2004 marks the beginning of this endeavor by the installation of the first group on behavioral neurosciences.



The development of neurosciences at the IGC will follow a few strategic principles. First, as in other fields, the viability of this project will also depend upon sharing of ideas and approaches, let alone facilities, by all the scientists involved, amongst themselves and with all other groups in the Institute. Science is what scientists do, and those who join this project must be excellent, generous, and engaged in the wider project. Second, it is only worth starting neurosciences at the IGC's if the specific projects involved would be better done here than elsewhere. In other words, neurosciences at the IGC must profit from the structure of the Institute and of its current scientific content in other areas. This means that neurosciences must be conducted on a strong basis of cell and molecular biology, "take" from the Institute's environment in evolution and development, and profit from the organism-centered biology done here in genetics, immunology, infection and inflammation. The next strategic choice concerned the area in the neurosciences field that shall be developed at the Institute. In as much as the IGC is concerned with organism-centered biology, "systems neurosciences" should be our area, with a clear preference for rodent (mouse) models, arguably the best to obtain molecular and cellular correlates of behavior, because of both the methods for genetic manipulation, electro physiology and imaging, as well as the type of behaviors that are available to analyses. The fact that this particular area is relatively less developed in Europe, as compared to the USA, poses some problems but may also represent a unique opportunity for international relevance. Obviously, the current developments of Computational Biology and Systems Neurosciences at the IGC bring many points in common, such that their simultaneity offers opportunities that we shall attempt to exploit. Launching neurosciences at the IGC is a project that evolves risks for both the scientists who will engage and carry the project, and for the institutional consolidation in its diversity. Yet, it is our conviction that the road is open, after the "proof of principle" and the vitality of the Institute's activity in other fields. We all have great expectations.

By opening, a few years ago, a Science Communication Office the IGC had innovated in Portugal, and its example has now been followed by other institutions, resulting in a stronger presence of science in schools and in its higher visibility at the media and with the public at large. While the exuberance of activities in this area owes much to the competence and dedication of its responsible, I must thank all at the Institute for their never-failing enthusiasm and availability for promoting science in the most various contexts. This last year, the IGC has launched a new "Grant's office", for informing scientists of current possibilities for grants and awards, and helping them in preparing applications or negotiating contracts. The results of this experiment have been remarkable, far exceeding the expected positive impact, most of us having difficulty today of imagining life without it. Speaking of awards, 2004 was an exceptional year for the IGC. Internationally, scientists at the Institute were distinguished with two very prestigious awards: a Marie Curie Excellence Grant and a EURYI, among various other contracts and grants. In Portugal, IGC's scientists were awarded some of the most prestigious prizes, notably the Pfizer Prizes for Medical Research (both the main and the junior prizes), the Pfizer Research Fellowship, three Terry Fox Fellowships for cancer research, the AMI Prize for Infectious and Parasitic Diseases, the CESPU International Prize, and the Pulido Valente Science Prize. I hope that the Gulbenkian Foundation and the other agencies that continue to support our activity will find in such public

recognition of IGC's science and of its impact in Portugal further arguments for their decision. At the Institute, because we believe in what we are doing, we are all very grateful for the opportunity to enact our commitment to this project.

## **RESEARCH**

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

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

### **Experimental evolution**

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

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

In the next few years, we new groups in this area shall be installed, re-inforcing evolutionary thinking in other programs at the Institute, and contributing to promote the study and public knowledge of evolution in Portugal.

## **Evolution of Virulence: the case of plasmids**

Members: Francisco Dionisio

Students and Technicians: Pedro Vale and Rui P. Martins

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

## **Population Genetics of adaptation in *E. coli***

Members: Isabel Gordo, Francisco Dionísio and Lisete Fernandes

Students and Technicians: Lilia Perfeito and Catarina Barroca

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

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

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

## **Evolutionary Dynamics of mutator cells**

Members: Isabel Gordo, Francisco Dionsio and Lisete Fernandes

Students and Technicians: Lilia Perfeito

External Collaborators: Ivan Matic (INSERM, Faculté de Médecine Necker-Enfants Malades, Université Paris V, France)

Certain populations of viruses, bacteria and cancer cells have abnormally high mutation rates. These are known as mutators. The mutator phenotype is a two-edged sword: on one hand it confers the potential for a faster adaptation but on the other hand a mutator carries the cost of producing a higher number of deleterious mutations. In this project we try to analyze the cost and benefits of cells with a high mutation rate.

## **Adaptive evolution in spatially structured asexual populations**

Members: Isabel Gordo

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

All natural populations are structured to some extent. In this project we perform theoretical studies of the process of adaptation in a spatially structured asexual population. The model assumes a local competition for replication, where each organism interacts with its nearest neighbors for reproduction. Our preliminary results show that the substitution rate of beneficial mutations is smaller for a spatially structured population than that seen for homogeneous populations. Furthermore, the substitution rate decreases as the number of neighbors for local competition is reduced. Besides, we also observe that the limit value of the rate of substitutions is now a decreasing function of the rate of deleterious mutation, which is contrary to the behavior of homogeneous populations.

## **Patterns of genetic diversity in chromosomes with restricted recombination**

Members: Isabel Gordo

External Collaborators: Peter Andolfatto (University of San Diego, California)

In this project we are analyzing the levels and pattern of genetic diversity in chromosomal regions with low levels of recombination. The model assumes that a gene is composed of neutral and selected sites and that the selected sites are under weak purifying selection. We study several statistics commonly used to describe genetic diversity and several standard statistical tests in population genetics and molecular evolution. Finally we compare the theoretical results with the data of natural populations of *Drosophila melanogaster*.

## **Patterns of variation in subdivided asexual populations**

Members: Isabel Gordo

Students and Technicians: Jaime Combadão

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

## **Recombination Activating Genes 1 and 2 and Vertebrate Genome Stability**

Member: Leonor Sarmento, Jocelyne Demengeot, Carlos Penha Gonzalves, Antonio Jacinto, Moises Mallo.

Students and Technicians: Paulo Almeida, Ana Novóa, Paulo Bettencourt

We have previously reported the generation of transgenic mice expressing the Rag1 and 2 genes both continuously throughout lymphocyte development and constitutively in most non-lymphoid tissues. We showed that ectopic expression of the Rag genes is lethal, both to lymphocytes, and to the organism as a whole. These animals display growth retardation and early death reminiscent of mice deficient in double strand break repair molecules. We developed a novel transgenic system, where Rag1 and 2 are independently expressed. Analyses of these mice suggest that over-expression of only one of the Rag genes is sufficient to induce severe pathological lymphopenia, a result that prompted us to design and new inducible transgenic animals, currently under elaboration. We are also investigating the impact of Rag-1 and 2 on the vertebrate genome evolution by introducing these genes in invertebrate organisms and by a bio-informatic approaches.

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

Members Elio Sucena and Filipa Vala

Endosymbiosis (the symbiotic interaction between a host and an intracellular symbiont) is a powerful evolutionary force because endosymbiosis is a source of novel metabolic functions. Wolbachia are cytoplasmic, vertically transmitted alpha-proteobacteria which require an eukaryotic cell to survive. The fact that closely related Wolbachia strains can establish both positive and negative interactions with their hosts makes it the ideal symbiont to study transition from (endo-)parasitism to (endo-)mutualism. Specifically, associations with these bacteria provide a mean to study the mechanisms by which a host becomes dependent on its symbiont. To this goal, it is important to characterize the

genetics underlying host-symbiont interactions. The transition from parasitism to mutualism involves a switch of selection forces acting on the host: while selection acting on Wolbachia always favors efficient vertical transmission (this is the only route by which it can colonize new hosts); selection on the host acts to increase vertical transmission of the endosymbiont once the latter has become important for survival and/or reproduction. Our project aims at characterizing how Wolbachia finds its way into the reproductive tissues of a developing host. Presently, one single study describes the patterns of distribution of Wolbachia during oogenesis/early development. With regard to the distribution of bacteria in oocyte/syncytial embryo of *Drosophila*, that study shows these patterns fall into three categories: along a posterior to anterior axis, along an anterior to posterior axis, and a uniform distribution along this axis. The former two resemble the distribution of *Drosophila* oocyte polarity determinants like *oskar* and *bicoid*, respectively. Also, three cytological studies suggest that Wolbachia associate to cell cytoskeleton components to migrate.

With this project we propose to identify the host developmental cues that the endosymbiont uses to reach gonad tissues, using a combination of immunostaining techniques for confocal microscopy analysis, ectopic expression of genes and mutant lines of candidate genes of *Drosophila melanogaster*. The present proposal asks three questions: 1. Does the distribution of Wolbachia in early embryos rely on oocyte polarity determinants or their regulators; 2. Do Wolbachia associate with cytoskeleton components to create these patterns; 3. Do Wolbachia reach gonadal tissues by actively following the host endogenous pole cell migration cues or do they reach the gonads by hitchhiking in association with these cells?

### **The genetic and molecular basis of evolutionary novelty**

Members: Elio Sucena

Students and Technicians: Filipe Pinto Teixeira

The basic concept of evolutionary novelty is that of a new trait, usually an anatomical or morphological one, that opens up the possibility of a wide adaptive radiation into new niches. Development is both a good source of evolutionary hypothesis and an operative element for evolutionary change as it is during Development that morphology is established. Gene recruitment (Co-option) events that precede and are essential for morphological novelties are key innovations at the genetic level which may underlie differences in cellular growth and morphogenetic processes between related organisms. The model system *Drosophila*, provides us with an excellent handle to tackle the question of Evolutionary novelties and for the dissection of the molecular mechanisms that underlie the process of Co-option.

During Oogenesis in *Drosophilids* the sheet of epithelial cells covering the oocyte (follicle cells, FCs), undergo a genetic programmed morphogenetic movement that defines, on the anterior-dorsal portion of the egg, two symmetrically tubular projections that shape the dorsal appendages (DAs) of the egg. Such structures are absent in other dipteran families. The Developmental Genetics of this process has been described in great detail permitting a comparative molecular study between *Drosophilidae* and its sister taxa.

In brief, the development of DAs is driven by the combined effect of two morphogens on a subset of the FC epithelium: Gurken (Grk) and decapentaplegic (dpp). Integration of these two signals are necessary and sufficient to determine the correct position, number and shape of DAs. Grk emanating from the oocyte, and dpp secreted by an anterior population of FCs, trigger the EGFR pathway in the anterior-dorsal FCs. The EGFR pathway is subsequently activated and spatially refined through an autoregulatory cascade involving Rhomboid, Spitz and Argos. This process defines two bilateral groups of competent cells expressing the gene Broad-complex that will undergo morphogenesis and express other markers such as pipe, Bullwinkle, shark and JUN kinase.

These pathways are highly conserved in evolution and play many roles through animal development. Nevertheless, they can evolve new roles, such as eliciting the differentiation of DAs in drosophilids, without deleterious pleiotropic effects.

This project aims at identifying the step(s) in the cascade that triggered the co-option of the cascade to shape the eggshell of Drosophilids in a novel manner. For this we will characterize the transcriptional profile of the genes involved in this process in the dipteran *Anopheles*, using in situ hybridization and immunostaining and compare it with the well-characterized *Drosophila* process. The candidate genes identified in this manner will, subsequently, be tested functionally using transgenesis in both dipteran species.

This experimental and conceptual set-up constitutes the first attempt at describing in molecular detail the developmental genetic mechanism for evolutionary novelty.

### **The molecular genetics of adaptation to octanoic acid in outbred populations of *D. melanogaster***

Members: Elio Sucena

Students and Technicians: Gilberto Bento

Combining experimental evolution with a candidate gene approach we aim at determining the historical dynamics of the adaptative process.

Adaptation to the toxin octanoic acid is ecologically and historically relevant for such a process has been undertaken by the *D. sechellia* lineage, a closely related species of *D. melanogaster*. Previous work, namely QTL analysis by Corbin Jones, has identified the genomic regions involved in *D. sechellia* resistance to octanoic acid. What will be the response of a sexual outbred population of *D. melanogaster* to selection for this toxin? Does experimental evolution recapitulate natural evolution? What is the distribution of effects observed, at the genome level, through the process of adaptation to a new environmental pressure?

### **Experimental Reverse Evolution in *Drosophila Melanogaster***

Members: Henrique Teotonio

Students and Technicians: Daniela Brites, Tânia Aires, Sérgio Santos

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



The irreversibility of evolution is extreme form of evolutionary constraint. It is the impossibility of return to evolutionary states that were once possible. Irreversibility can occur at several biological levels and the study of the genetic mechanisms underlying this process has had renewed theoretical interest and intense empirical efforts. We described the reverse evolution in phenotypic traits of several differentiated populations all descendent from a common ancestor when the ancestral environment was re-imposed upon them. It was found that despite the occurrence of adaptation to the ancestral environment reverse evolution is highly contingent on previous evolutionary history: the life-history dynamics between populations are dissimilar, some respond rapidly while others do not, some converge to ancestral phenotypic states while others do not. Neither exhaustion of genetic variability during previous differentiation nor the presence of gene interactions explain this contingency. The answer appears to lie in the diverse ways populations can evolve to the same level of ancestral fitness. Are these patterns of reversibility at the phenotypic level mirrored with genetic reversibility? How parallel are phenotypic and molecular trajectories during reverse evolution? These are the questions we are currently pursuing with studies of the population genetics at candidate genes.

### **Evolution of Outcrossing in *Caenorhabditis elegans***

Members: Henrique Teotonio

Students and Technicians: Diogo Manoel

External Collaborators: Patrick Phillips (University Oregon Eugene, USA).

Androdioecy is a rarely occurring mixed mating system where males co-exist with hermaphrodites in the same population. Why do males persist if selfing has many associated evolutionary benefits? Current research is addressing the hypothesis that males are evolutionarily maintained due to their role in promoting outcrossing. Mating system theory is being directly tested by manipulating levels of outcrossing and mutational load while studying the adaptation of variable populations to novel environments. We hope to understand if the expression of deleterious mutations through inbreeding depression, the sorting of beneficial mutations through recombination, or their interaction are the primary factors responsible for the evolution of male function.

### **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.

### **Genetics of familial stroke (STROKENETICS study)**

Members: Sofia Oliveira

Students and Technicians: Sara Fidalgo

External Collaborators: José Ferro (Hospital de Santa Maria, Lisbon, Portugal); Isabel Henriques (Hospital Espírito Santo, Évora, Portugal); Miguel Viana-Baptista (Hospital Egas Moniz, Lisbon, Portugal) Ana Amélia Pinto (Hospital Fernando Fonseca, Amadora Sintra, Portugal); Manuel Correia (Hospital Geral de Santo António, Porto, Portugal); Assunção Tuna (Hospital Geral de Santo António, Porto, Portugal); João Ramalho Fontes, Hospital São Marcos, Braga, Portugal).

The goal of this project is to create a stroke biobank to identify genes that influence the risk for developing stroke, also called susceptibility genes. Stroke is the third leading cause of death in the developed world. It is even more disabling than lethal, and the persistent neurological impairment and physical disability caused by stroke have a substantial socioeconomic cost. Stroke is a complex disease resulting from the interplay of environmental and genetic factors. Major known risk factors include family history, age, hypertension, hypercholesterolemia, diabetes, cardiovascular disease, smoking and alcohol consumption. Identification of genes increasing susceptibility to stroke would have far-reaching public health impact, from enhancing motivation to make behavioral and lifestyle changes in susceptible individuals to providing basic biological and clinical information about the development, prevention and treatment of stroke.

The genetic component has been demonstrated in twin and family studies, in animal model studies, and mutations have been found in several genes in rare classical Mendelian forms of stroke. However, very few susceptibility genes specific for the common forms of stroke have been identified and association studies have mostly reported conflicting results. We will use a novel genomic convergence approach that combines genomic screening, expression analysis, and association studies to identify

susceptibility genes.

The first and key step in this endeavor is the creation of a high quality stroke biobank. During the two years of this project we will collect the biological samples and information from 800 individuals originating from 200 Portuguese multiplex families with ischemic stroke.

The unified, comprehensive, and multidisciplinary approach that we outline has not yet been implemented in other studies of stroke but the availability of new genetic, molecular and statistical tools makes such an approach both timely and essential.

### **Genetics of sporadic stroke (GENOPORT study)**

Members: Sofia Oliveira

Students and Technicians: Sara Fidalgo

External Collaborators: José Ferro (Hospital de Santa Maria, Lisbon, Portugal); Isabel Henriques, (Hospital Espírito Santo, Évora, Portugal); Miguel Viana-Baptista (Hospital Egas Moniz, Lisbon, Portugal); Ana Amélia Pinto (Hospital Fernando Fonseca, Amadora Sintra, Portugal); Manuel Correia (Hospital Geral de Santo António, Porto, Portugal); Assunção Tuna (Hospital Geral de Santo António, Porto, Portugal); João Ramalho Fontes (Hospital São Marcos, Braga, Portugal).

Since the genetic basis of ischemic stroke may be different in families with a history of stroke (multiplex families) and in families without a history of stroke (sporadic cases), and sporadic cases are the most common, we propose to collect a second dataset of 500 sporadic stroke patients, 500 unaffected family members and 500 controls. This dataset will be used to test our findings in the familial dataset, to test findings from other research groups, and to test novel candidate genes for association.

### **Genetics of malaria in mouse models**

Members: Carlos Penha Gonçalves

Students and Technicians: Lígia Deus

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

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

The work-plan comprises (1) the genetic mapping of resistance loci using genetic crosses of mouse strains that are resistant to malaria and (2) the isolation of the underlying genetic factors by an approach that combines candidate gene analysis and positional cloning.

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

### **Genetics of lymphocyte homeostasis**

Members: Carlos Penha Gonçalves  
Students and Technicians: Joana Rodo

The goal of this project is to characterize the genetics of the homeostasis of the number of lymphocytes within the lymphoid organs. The workplan includes:  
to study the establishment of lymphocyte homeostasis during the ontogenesis of the lymphoid organs in different laboratory mouse strains and **(2)** to genetically map and to identify the genetic factors involved in the homeostatic mechanisms that control the number of lymphocytes in the lymphoid organs of the mouse.

### **Genetic Epidemiology of Autism**

Members: Astrid Vicente and Constantin Fesl  
Students: Ana Margarida Coutinho, Catarina Correia, Marta Barreto, Ricardo Ferreira, Isabel Peixeiro, Pedro Santos, António Currais, Inês Sousa  
Colaboradores: Guiomar Oliveira (Hospital Pediátrico de Coimbra, Coimbra, Portugal), Luisa Diogo (Hospital Pediátrico de Coimbra, Coimbra, Portugal), Patrícia Maciel (Universidade de Braga, Braga, Portugal), Steve Sommer (City of Hope National Medical Centre, CA, USA).

*Genetic Epidemiology of Autism in Portugal:* Our present database and sample collection now includes 259 nuclear families with one or more autistic patients and extensive clinical, behavioral, genealogical and biochemical information on patients and relatives. In this sample we have been analysing the association of candidate genes of the serotonergic system, and others, with autism and with associated quantitative endophenotypes, as well as exploring the hypothesis of the involvement of autoimmune mechanisms in the disease pathogenesis. We have demonstrated the role of serotonin transporter gene variants in the determination of hyperserotonemia, which is present in a proportion of autistic patients (Coutinho AM *et al*, 2004). We have also found that other genes encoding serotonin receptors and enzymes involved in serotonin metabolism, and in some cases the interaction between variants of such genes, influence the levels of serotonin in autism (Coutinho AM *et al*, *in preparation*). The role of autoimmune factors, namely anti-brain autoantibodies has been assessed in our population sample, and we reported the occurrence of a particular brain antigen, as yet unidentified, to which antibodies are produced in a significant fraction of autistic patients. This trait was not heritable, and we hypothesize that such autoantibodies maybe produced as a response of the immune system to a brain insult, arguably occurred during CNS development (Silva S

*et al*, 2004). Other observations in our patients suggest that neuroprotective factors are challenged in autism, namely the increased levels of serum BDNF, which in our sample we found not to be genetically determined by variation at the *BDNF* gene. We are further investigating key molecules involved in neuroprotective mechanisms. The *Autism Genome Project*, a consortium led by the National Alliance for Autism Research (NAAR) with the objective of identifying genes for autism, has entered the preparation of Phase II. This consortium is conducting a genome wide association scan for autism in a sample population of unprecedented size. This collaborative effort will undoubtedly yield crucial information for the understanding of autism etiology, while opening opportunities for follow up research by the associate groups on the pathological mechanisms associated with autism symptoms and deficits.

*Autism and Mitochondrial Disorder – microarray analysis of the expression profile of nuclear genes for mitochondrial enzymes in autism.* In the epidemiological survey of autism and known etiologies in Portugal, we have found an unexpectedly high rate of biochemical markers of mitochondrial dysfunction, and confirmed the occurrence of mitochondrial disease associated with autism in 7.2% of the patients. We have now confirmed mitochondrial dysfunction markers in 17.2% of 210 patients with autism, leading to mitochondrial disease in 7 of fully evaluated 20 patients. No mtDNA mutations were detected in this sample. We are therefore testing nuclear genes encoding proteins involved in mitochondrial function for association with autism. One such gene, *SLC25A12*, encoding the mitochondrial aspartate/glutamate carrier, has been reported to be associated with autism. However, we did not confirm this association in our sample of 241 nuclear families, nor an involvement of variants of this gene in the determination of lactate or pyruvate levels (Correia C *et al*, submitted).

*Clinical, epidemiological and genetic study of Rett Syndrome in Portugal.* In the context of a collaborative project with Patricia Maciel at Universidade de Braga and Steve Sommer at the City of Hope National Medical Center, we screened our autism patients for mutations in the *MECP2* gene, located on the X chromosome, which causes mental retardation and Rett syndrome. This disorder often presents with autistic symptoms, and our hypothesis is that, while known *MECP2* mutations in Rett syndrome are likely non-viable in males, mutations that have a less severe deleterious effect on protein expression/function may lead to the autistic phenotype in males or females. The whole coding sequence, including a recently described exon and the 3'UTR have been screened. A number of polymorphic variants, present in patients and controls were found in the coding region, and high sequence variability was found in the 3'UTR. We have also found a novel missense change in exon 3 in a male patient with autism and mental retardation that was not present in control individuals. The missense change is located in an interdomain leading to a change in aminoacid properties that alters protein conformation and likely affects the function of the neighbour transactivation domain. The patient had an aunt who suffered from mental retardation, with likely regression as accounted by the mother, and who died of uncontrolled seizures at age seven. The description of this patient's symptoms is compatible with Rett syndrome. The mutation is present in the heterozygous and non-affected mother and maternal grandmother of the proband, and X-inactivation assays, although not fully conclusive, are compatible with a skewed inactivation of the X chromosome bearing the mutation in the mother. We therefore conclude that an autistic phenotype may be caused by mutations in *MECP2*

with a less severe deleterious effect on protein function, in a small number of cases (Coutinho AM *et al*, *submitted*).

### **Pharmacogenetics of risperidone therapy in autism spectrum disorders**

Member: Astrid Vicente

Students: Ana Margarida Coutinho, Catarina Correia, Pedro Santos

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

We are investigating the role of selected candidate genes in the variability of response, in efficacy/side effects, to specific medication for autism, aiming at the prediction of individual response based on specific genotypic and phenotypic information, with a major impact on therapeutic decisions in clinical settings. Clinical and psychological evaluation procedures have been validated, as well as genetic and biochemical characterization techniques. Patient recruitment has been initiated, and sample collection at multiple time points is ongoing.

### **Genetics of Human Systemic Lupus Erythematosus (SLE)**

Members: Astrid Vicente, Constantin Fesel and Jocelyne Demengeot

Students: Marta Barreto, Ricardo Ferreira, Lara Lourenço

Colaboradores: Carlos Ferreira (Hospital de Santa Maria, Lisbon, Portugal), Berta Martins (ICBAS, Porto, Portugal), Carlos Vasconcelos (Hospital de São João, Porto, Portugal), Assoc. Doentes com Lúpus, (Lisbon, Portugal).

SLE is a multifactorial disorder with heterogeneous presentation, in which genetic susceptibility plays a major role. The main objective of this study is the identification and characterization of genetic susceptibility factors for SLE. The strategy used is the identification of lupus-associated traits that are genetically less complex, and therefore more amenable to genetic mapping. The collection of SLE patients and family members has progressed throughout 2004, in collaboration with the Associação de Doentes com Lupus, Hospital de Santa Maria, Hospital do Divino Espírito Santo, Hospital de Sto. António and ICBAS. Presently, 74 multigenerational families have already been collected, including 140 patients and 210 unaffected relatives. Identification and collection of familial cases is progressing in the Azorian islands. A database has been established, gathering clinical and serological information as well as disease-associated phenotype and genetic data. Given that antinuclear antibody (ANA) production is a main characteristic of SLE, we have been analysing autoantibody reactivities in patients and relatives, and determining heritability of these traits in multiplex families. The epitope specificities of the antinuclear antibodies in patients and unaffected relatives and controls are also being analyzed, and preliminary results indicate that specific ANA are inherited among affected and unaffected family members (Ferreira R *et al*, *submitted*). We have also been investigating the role of regulatory cells (Treg) in SLE. We have established that there is a significant difference in Treg cell numbers between patients and controls,

which are decreased in patients and particularly in women. Treg cell numbers were found to be decreased in two patients after the onset of SLE and after an acute episode (Barreto M *et al*, *in preparation*). We also found that Treg cell numbers are highly heritable in families affected with SLE. We therefore have been analyzing genetic factors that might be involved in the determination of Treg cell numbers and consequently might be susceptibility factors for SLE. Specific genes involved in regulatory cell function have been tested as candidates for disease susceptibility. We have found that variants of the *CTLA-4* gene are associated with SLE (Barreto M *et al*, 2004), and are involved in the determination of the numbers of regulatory T cells. The *TGF  $\beta$*  gene was also found to be associated with Treg cell numbers. Measurement of the expression levels for these genes suggests that the variability of Treg cell numbers is not influenced by expression levels but by a deleterious functioning of either molecule (Barreto M *et al*, *in preparation*). Interaction of the two genes is currently being assessed. Another gene involved in Treg function, *FOXP3*, has also been shown to be associated with SLE. We demonstrate that in patients, that have lower numbers of regulatory T cells, expression of this gene is increased (Barreto M *et al*, *in preparation*).

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

Members: Luisa Mota-Vieira

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

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

This project aims to build a DNA bank of 1,000 healthy unrelated individuals (about 0.8% of the current population) living in the Azorean island of São Miguel (131,609 inhabitants). Resulting of a collaboration with the Hematology Department of the Hospital of Divino Espirito Santo, the only hospital located in S. Miguel island, the DNA bank follows the international ethical guidelines and has been approved by the Hospital's ethical committee. Blood samples (7.5 mL) were collected with appropriate Informed Consent, which includes (1) the distribution of free informative leaflet explaining the goals of proposed studies, the confidentiality of the personal data, and the methods of identification and storage of DNA samples; and (2) an interview with a health professional for additional information. All samples in the repository are anonymous and have self-reported data concerning sex, age, birth and current living places (locality and municipality in the island), and parental birthplaces. The samples are representative of all the island's municipalities ( $r=0.995$ ,  $p<0.01$ ). The majority (87%) of the participants are male, with mean age of 36.3 y (18-64y). Birthplace analysis reveals that 902 (90%) have both parents born in São Miguel. Moreover, 477 (54%) have their parents born in the same locality, confirming high rate of endogamy in rural area. To date, this DNA bank was used to assess the Y-chromosome phylogeny and diversity in Azorean population (Pacheco PR *et al*. *Ann Hum Genet*, 2005, *in press*). [This work is concluded and was funded by DRCT, Azores].

## **The Y chromosomal heritage of the Azores population.**

Members: Luisa Mota-Vieira

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

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

The Azores, a Portuguese archipelago located in the North Atlantic Ocean, had no native population when the Portuguese first arrived in the 15th century. The islands were populated mainly by Portuguese, but Jews, Moorish prisoners, African slaves, Flemish, French and Spaniards also contributed to the initial settlement. To understand the paternal origins and diversity of extant Azorean population, we typed genomic DNA samples from 172 individuals, using a combination of 10 Y-biallelic markers (YAP, SRY-1532, SRY-2627, 92R7, M9, sY81, Tat, SRY-8299, 12f2 and LLY22g) and the following Y-chromosomal STR systems: DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393 and DYS385. We identified nine different haplogroups, most of which are frequent in Europe. Haplogroup J\* is the second most frequent in Azores (13.4%), but it is modestly represented in mainland Portugal (6.8%). The other non-European haplogroups, N3 and E3a, which are prevalent in Asia and sub-Saharan Africa, respectively, have been found in Azores (0.6% and 1.2%, respectively) but not in mainland Portugal. Microsatellite data indicate that mean gene diversity (D) value for all the loci analysed in our sample set is 0.590, while haplotype diversity is 0.9994. Taken together, our analysis suggests that the current paternal pool of the Azorean population is, to a great extent, of Portuguese descent with significant contribution from people with other genetic backgrounds (Pacheco PR et al. *Ann Hum Genet*, 2005, in press). [This work is concluded and was funded by DRCT, Azores].

## **Genetic diversity of the Azorean population revealed by microsatellite loci**

Members: Luisa Mota Vieira

Students and Technicians: Claudia C. Branco

This project aims to characterize the genetic variability of the Azorean population. It will be carried out in two cohorts: The São Miguel's population and the whole Azorean population. For this purpose, we selected 15 short tandem repeats (STRs) markers, highly polymorphic for the European population. This selection was based on their location on different chromosomes, thereby avoiding, the presence of linkage between markers. STRs were typed by Polymerase Chain Reaction (PCR) with fluorescently labelled primers. An aliquot of 1 µl of each PCR product was combined with 0.5 µl CEQ™DNA size standard kit 400, 29 µl formamide deionized (Qbiogene), and run on a CEQ™8000 Genetic Analysis System (Beckman Coulter). At moment, 15 STR markers are genotyped for 250 individuals from the anonymous DNA bank of São Miguel population. Our preliminary analysis indicates that allele frequencies obtained for São Miguel's population do not differ from those obtained for mainland Portugal and other European



populations. Despite this similarity, we identified for some markers rare alleles that are not described for mainland Portugal. The heterozygosity values calculated for each STR typed vary from 65.3% for TPOX to 93% for D18S51, although the majority of markers show values superior to 80%. These data suggest that the selected markers are very informative for this population. [Ongoing project; CCB is a fellowship of FCT (SFRH/BD/ 12254/ 2003); this work was funded by DRCT, Azores].

### **Study of the genetic diversity of São Miguel population through the analysis of HLA loci.**

Members: Luisa Mota-Vieira

Students and Technicians: Paula R. Pacheco and Ann Lismond

This project aims to improve our knowledge of São Miguel population genetic diversity, through the polymorphism analysis of HLA-A, HLA-B and HLA-DRB1 loci. Blood samples were taken, after informed consent, from 106 unrelated blood donors, whose parents were born in São Miguel island. HLA typing was carried out using polymerase chain reaction with sequence specific primers (Olerup SSP HLA-A-B-DR SSP Combi Tray kit). Statistical analysis was performed with Arlequin v2.0. At the HLA-A locus, we identified 16 HLA-A alleles, of which A\*02 (GF=0.2500), A\*01 (GF=0.1509) and A\*24 (GF=0.1368) are the most frequent. Of the 24 HLA-B alleles found, B\*44 (GF=0.1557), B\*08 (GF=0.1368) and B\*14 (GF=0.0708) are the most prevalent. At HLA-DRB1 locus we found 13 alleles, of which the most prevalent are DRB1\*07 and DRB1\*03 (GF=0.1698 and GF=0.1651, respectively) followed by DRB1\*13 (GF=0.1462). All genotype frequencies are in Hardy-Weinberg equilibrium. The most frequent haplotype in São Miguel is HLA-A\*01-B\*08-DRB1\*03 (HF=0.0802) which is of West European origin. Albeit at lower frequency, we also found the Iberian North African haplotype HLA-A\*30-B\*18-DRB1\*03 (HF=0.0047), the Iberian Berbers haplotype HLA-A\*02-B\*51-DRB1\*13 (HF=0.0047) and two Mongol haplotypes: HLA-A\*02-B\*50-DRB1\*07 (HF=0.0047) and HLA-A\*02-B\*44-DRB1\*04 (HF=0.0142). Moreover, the preliminary interpopulation analysis highlights a strong relatedness with other European populations and input of people from different origins. These findings agree with our previous results on the Y chromosomal heritage of São Miguel population, and will be useful for Azorean studies of autoimmune diseases related to HLA genotypes [Ongoing project; this work is funded by DRCT, Azores].

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

Members: Luisa Mota-Vieira

Students and Technicians: Laura de Fez

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

The knowledge of the type, frequency and distribution of recessive mutations is very important in populations living in small geographic areas. This study aims to characterize the hereditary hemochromatosis (HH, OMIM 235200), a recessive and iron overload disease mainly caused by mutations in the *HFE* gene, in the population of São Miguel island, the biggest (746.79 km<sup>2</sup>) and the most populated (131,609 inhabitants) island of the Azores. Our approach is based on a screening by a PCR-RFLP of the 3 principal *HFE* mutations (C282Y, H63D and S65C) in a control population of 203 unrelated blood donors, representing the 6 municipalities of the island, and in 14 HH patients with elevated transferrin saturation levels (TS>40%). In the control group, we observed allele frequencies similar to those found in Central Europe: 4.93% for C282Y, 21.67% for H63D, and 1.97% for S65C. Moreover, nine genotypes were identified: wild/wild (50.74%), wild/H63D (31.03%), wild/C282Y (7.88%), H63D/H63D (4.93%), wild/S65C (2.96%), C282Y/H63D (0.99%), and C282Y/C282Y, H63D/S65C, H63D-S65C/H63D all with a frequency of 0.49%. We also investigated the geographic distribution of the mutant alleles within the island. Only for C282Y, the more severe mutation, we observed difference between East (9.3%) and West (3.6%). The molecular analysis of the 14 HH patients revealed no more than 3 subjects with the C282Y mutation: 2 were C282Y homozygotes and 1 was C282Y heterozygote. Of the remaining 11 patients, 1 was H63D/S65C compound heterozygote, 3 were H63D heterozygotes, and 7 have no characteristic *HFE* mutations. In the non-C282Y patient's group, we excluded the presence of a new mutation in this codon by DNA sequencing. In order to investigate the cause of iron overloading in these patients, other genes involved in iron homeostasis will be analyzed. In conclusion, our data indicate that the molecular basis of hereditary hemochromatosis in São Miguel's population is more complex than expected [Ongoing project; this work is funded by DRCT, Azores].

### **Genetic and consanguinity of congenital heart disease in Azores**

Members: Luisa Mota-Vieira and Teresa Cymbron

Students and Technicians: Rita Cabral

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

Congenital heart disease (CHD) is the clinical manifestation of anomalies in embryonic cardiac development, being the most frequent of all clinically significant birth defects. In 2004, we carried out the epidemiological characterization of CHD in São Miguel island, during a 10 years-period (January 1992 to December 2001). The children were closely followed up by the only hospital in this island. A total of 189 patients with CHD were diagnosed in São Miguel population, indicating an average incidence of 9.20 per 1000 live-birth. During this period the incidence of CHD ranged from 4.77 to 12.75. The most frequent cardiac alterations found were ventricular septal defect (38.1%), atrial septal defect (12.2%), and persistent ductus arteriosus (11.6%). These three lesions amount to 61.9% of all CHD cases. Other lesions present were aortic stenosis (6.9%), atrioventricular septal defect (5.3%), pulmonary stenosis (4.8%), tetralogy of Fallot and pulmonary atresia with VSD (3.7%), coarctation of the aorta and transposition of the

great arteries, both with 2.7%. The remaining cardiac malformations showed a frequency below 2%. These data agree in general with other recent studies. The main differences are a higher frequency of persistent ductus arteriosus (11.6%) and atrial septal defect (12.2%), and a lower frequency of transposition of the great arteries. Death occurred in 10.6% (n=20) of the children enrolled in this study. The cardiac lesions with higher mortality were pulmonary atresia with VSD and transposition of the great arteries. Until now, three familial clusters were detected, representing a total of 9 patients, most of which affected with VSD. The data described here represents the first characterization of congenital heart disease in the Azorean population. [Ongoing project; this work is funded by FCT (POCTI/ESP/49236/2002) and DRCT, Azores].

### **Molecular classification of breast cancer using *cDNA-Microarray* Hybridization**

Members: Bernardo R. Peixoto

Students and Technicians: Laura de Fez Sayas and Maria João Vasconcelos

External Collaborators: Sérgio Verjovski-Almeida and Eduardo M. Reis, Instituto de Química - Universidade de São Paulo, Brasil.

Two-color spotted-glass cDNA microarray analysis is a powerful tool for gene expression analysis of human pathological conditions, including cancer. cDNA hybridizations generate fluorescence intensity values from two different labeled RNA samples, which are generally used to calculate gene expression ratios for subsequent analysis. Ratiometric data analysis minimizes various sources of variation related to the construction or hybridization of the microarrays, thus facilitating comparison of gene expression across different arrays. While direct comparison of two RNA samples in the same microarray provides the highest level of precision, for practical reasons indirect comparisons through a common RNA reference are the most used experimental design in comparative gene expression profiling of human tumors. A major drawback associated to the use of a common reference RNA in large-scale tumor profiling studies is the requirement of a large amount of high-quality reference sample to allow the comparison of gene expression across different datasets. During 2004, we evaluated an indirect method to generate gene expression ratios derived from the co-hybridization of human RNA samples and a 27-mer reference oligonucleotide (RefOligo) that is complementary to every feature on the microarray. This method was originally proposed by Church and co-workers (*PNAS-USA* 99:7554-7559, 2002) to control intensity ratios in yeast gene expression experiments, a system with much lower gene expression complexity. We performed hybridizations using a fixed amount of Cy3-labeled RefOligo against distinct Cy5-labeled targets derived from prostate, breast and kidney tumor samples. Reconstructed ratios between all tissue pairs derived from RefOligo hybridizations were compared to ratios obtained from direct hybridizations, and reconstructed ratios derived from hybridization of each tissue against a reference RNA pool. The results, which have been described in detail in a manuscript recently submitted for publication (Eduardo M. et al. Indirect measurement of differential gene expression using an oligonucleotide reference sample), show that the RefOligo method produce more accurate measurement, with reduced variability and higher correlation of replicates compared the reference RNA

pool. The unlimited availability of an inexpensive, chemically synthesized reference oligonucleotide makes its use very convenient in large-scale projects where the availability of an RNA pool is usually restrictive. We will use this method to continue with the project of assaying the breast tumor samples collected in Azores. Currently, the tumor bank contains 58 samples, all have been treated to isolate and amplify RNA for hybridization assay, which will start within a month (April 2005). Finally, the establishment of a coordinated collection, annotation and storage of tumor samples, with centralized online database, in a bigger scale, involving many Institutions would represent an important starting point for future collaborative cancer genomics projects in Portugal. [Ongoing project; this work is funded by FLAD (Proj. L-V-383/2002) and by DRCT, Azores].

## **Virology & Immunity**

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

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

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

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

Students and Technicians: Sílvia Almeida, Sílvia Correia, Rute Nascimento, Vivian Oliveira and Sónia Ventura

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

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

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

## **Control of African swine fever (ASF) through improved diagnosis**

Members: R.M.E. Parkhouse

Students and Technicians: Ana Luísa Reis

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

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

Our previous work has identified the 12 principle serological determinants of ASFV and in this project recombinant forms of these proteins have been produced as potential serological diagnostic probes. Their utility is currently being assessed using sera from infected pigs.

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

Members: Pedro Simas and Marta Miranda

Students and Technicians: Patricia Madureira, Sofia Marques and Lenia Rodrigues

External Collaborators : Stacey Efstathiou (University of Cambridge, Cambridge, UK)

Studies into the molecular basis of gammaherpesvirus latency have been hindered by the lack of amenable animal model systems and the lack of fully permissive cell lines, which are required for the genetic manipulation of these viruses. This project centres on the utilisation of a gammaherpesvirus, designated murine herpesvirus 68 (MHV-68), whose pathogenesis can be readily investigated in the laboratory mouse (for recent reviews see Simas & Efstathiou, 1998; Virgin VI & Speck, 1999). MHV-68 is genetically related to Epstein-Barr virus and Kaposi's sarcoma associated herpesvirus, which are important Human pathogens. Experimental infection of inbred strains of mice with MHV-68 results in acute productive infection of the lung followed by latent infection of B-lymphocytes and macrophages. Comparison of the genomic organisation of MHV-68 with other gammaherpesviruses shows that they have large blocks of co-linearly arranged conserved genes, interspersed with virus specific ORFs and a number of cellular homologues, which are predicted to determine the particular biological properties of these viruses, e.g. host range, immune evasion, latency and disease. MHV-68 has 14 unique such genes, designated M1 to M14, and a number of cellular homologues, including a complement control protein, a D-type cyclin and an IL8 receptor. In addition to these cellular homologues two of the 'M' genes, M1 and M11, show low level similarity to serpins and bcl2 cellular proteins, respectively.

Our research interests are focused in trying to understand how these cellular homologues and unique ORFs coordinate their functions with those of a B-lymphocyte and result in immune evasion and persistent infection. Recent studies have identified selective transcription of a number of viral genes during latent infection in spleen, including M2, M3 and M9 (Simas et al., 1999; Virgin et al., 1999; Hussain et al., 1999). We have recently shown that the M3 gene, encodes a secreted chemokine binding protein (Parry et al., 2000). As yet no function has been allocated to either M2 or M9 and both of these ORFs do not show significant homology to any known proteins.

The proposed project aims to elucidate the function of these two ORFs, M2 and M9, by determining their respective cellular molecular targets and analysing the biology of deletion mutant viruses. The procedure chosen will use, in parallel, the Yeast Two-Hybrid System and MALDI peptide mass mapping combined with sequence database searching, to identify cellular molecular targets for these proteins. Once interacting cellular proteins have been identified it may be possible to predict and test for function, in vivo, by constructing MHV-68 recombinants with either deleted or modified M2 or M9

genes. This approach should give valuable insights into general mechanisms by which gammaherpesviruses evade immune responses and establish persistent/latent infections in the host.

### **Antiviral peptides blocking herpes simplex virus type 1 entry into cells**

Members: Pedro Simas and Marta Miranda

Students and Technicians: Lidia Fonseca

External Collaborators: Massimiliano Galdiero (University of Napoli, Napoli, Italy)

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

This project will develop a new class of anti-HSV-1 drugs – peptides that act at the cell surface blocking virus entry. Peptides will be designed and tested to several HSV glycoproteins, namely gB, gD, gH and gL, which are all involved in virus attachment and penetration. Because such peptides will function via an extracellular novel mechanism they should prove complementary, not competitive, with current antiviral chemotherapies; predictably combined therapy, as with anti-HIV therapies, may prove the most common use of antiviral peptides that block virus entry.

### **Transcriptome analysis of germinal centre B cells during gammaherpesvirus latent infection**

Members : Pedro Simas

Students and Technicians: Sofia Marques and Rui Freitas

External Collaborators: Paul Lyons and Sytacey Efstathiou (University of Cambridge, Cambridge, UK).

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

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

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

This project investigates the effect that MHV68 latent infection has upon GC B cell physiology by analyzing their transcription profile. We propose to use a strategy involving transgenic mice with a floxed EGFP allele that only becomes functional upon Cre mediated excision. In this model, Cre will be provided by a recombinant MHV68 resulting in the fluorescent tagging of latently infected cells. This makes possible the purification of pure populations of latently infected GC B cells, a 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 determining the molecular basis for gammaherpesvirus infection but also provide clues on what gene products (either cellular or viral) may have therapeutic uses themselves or may be targets for therapeutic intervention.

### **Improved Strategies for a Retroviral Gene Therapy Vector Development: an Enveloped Virus**

Members: Matthias Haury

External Collaborators: Ana Sofia Valente Coroadinha and Manuel Carrondo (IBET, Oeiras, Portugal).

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

### **Inflammation & Immunity**

Inflammation is a stress reaction causing ‘*rubor, calor, dolor, tumor*’ (redness, heat, pain and swelling) but it represents the body’s defense to a variety of injuries. Inflammatory reactions often occur as a result of microbial infections, involving both the immediate activation of the “innate immune system”, as well as the adaptive response of lymphocytes, cooperating in the clearance of pathogens. Inflammatory reactions should thus be perceived as a beneficial response that allows the immune system to deal with invading microbes. If uncontrolled, however, “innate” responses might be lethal, as in septic shock, while chronic inflammation often leads to tissue damage, at the origin of degenerative diseases (e.g., atherosclerosis, rheumatism, multiple sclerosis), many of which are autoimmune and continue to represent a serious therapeutic challenge. To be



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

### **Dynamic and function of regulatory T cells during inflammatory responses**

Members: Alexis Perez, Jorge Carneiro, Matthias Haury, Marie Louise Bergman, Jose Feijo, Antonio Coutinho, Jocelyne Demengeot

Students and Technicians: Iris Caramalho, Miguel Vaz-Afonso, Santiago Zelenay, Francisca Fontes, Manuel Rebelo, José Martins, Lurdes Duarte, Nuno Moreno.

Acute inflammatory immune responses to normally innocuous microbes can be lethal and are a frequent cause of death in immuno-suppressed patients. In several experimental systems of infection or colonization by commensals, CD4<sup>+</sup> CD25<sup>+</sup> regulatory T cells (Treg) prevents these deleterious inflammatory responses and limit also protective immune responses. We set out to understand the nature of the triggering signal necessary for Treg function during inflammatory responses. We evidenced that regulatory T cells are activated, expanded, and acquire higher effector efficiency upon inflammatory stimuli. This activation is in part mediated by Toll Like Receptors selectively expressed by this subpopulation of T cells but other pro-inflammatory signals such as IL-15 participate in this dynamic. Moreover, we generated evidence indicating that Treg control the innate and adaptive responses to inflammatory stimuli by a mechanism independent of foreign protein. Finally we revealed that a large reservoir of differentiated FoxP3<sup>+</sup> regulatory T cells is encompassed in the CD25<sup>+</sup>-CD45RB<sup>low</sup> subset of CD4 cells, and rapidly recruited to the CD25 expressing cell pool upon homeostatic activation. This subset is overrepresented in IL-7 deficient mice, which contrarily to many lymphopenic models, does not develop lymphoproliferative autoimmune disease, a finding we explored to reveal that in addition to TCR specificities, Treg differentiation follows a developmental program distinct from the bulk of other conventional T cells.

Taken together these findings points to a scenario whereby Treg are activated and expanded by the very same immune responses they are controlling, most likely ensuring a limit in the amplification mechanism involved in an immune response. Ongoing work aims at determining whether the finding that inflammatory reactions enhance Treg numbers and function can serve to define preventive strategies to strengthen immune tolerance in mouse models of spontaneous Type I Diabetes, Lupus Erythematosus and multiple sclerosis. However, acute inflammation may also correlate with the onset of

autoimmune disease. To further explore this double edged effect, we used the anti-myelin basic protein (MBP) TCR transgenic mice, a murine model for human Multiple Sclerosis developed by J.Lafaille (Cell 1994, 78: 399). Our findings indicate that reduced commensal colonization as well as administration of immunosuppressors and anti-inflammatory compounds, such as Hydrocortisone, increases mice susceptibility to induced encephalomyelitis. Moreover, alteration in Tregs in this system leads to autoimmunity when it is combined with pertussis treatment, while pertussis administration before induction appears to be protective.

To further clarify the dynamic of Tregs during inflammatory reactions, we invested in setting the conditions to visualize in vivo the cellular interactions that take place during these reactions. Recent advance in the use of multi-photon laser scanning microscopy enable live intravital detection of individual cells, the monitoring of their trafficking and the visualization of their interactions within lymphoid tissue. In an attempt to further understand the cellular mechanism and the kinetic of immune regulation in situ, we merged expertise in i) cellular and molecular immunology, ii) mathematical modeling of cellular interactions and iii) live multi-photon intravital microscopy. This year we concentrated our efforts to the set up of experimental conditions allowing the simultaneous monitoring of three cellular subsets in lymphnodes, spleen and skin of lived mice. We established the conditions adapted to chronic and acute recording of regulatory T cells, conventional lymphocytes and APC interactions and trafficking at a minimal depth of 150µm during at least 3 hours. As a complement to these in situ analyses, we developed novel in vitro assays where the same three cellular subsets are seeded together in semi-solid cultures and their individual responses monitored using classical read out and 4D live microscopy.

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

Members: Miguel Soares and Gabriela Silva

Students: Mark Pena Seldon

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

The pro-inflammatory phenotype associated with endothelial cell (EC) activation must be tightly controlled so that inflammation does not lead to disease. One of the mechanisms by which this occurs relies on the expression of “protective genes”. These control not only the pro-inflammatory phenotype associated with EC activation but in addition protect EC from undergoing apoptosis. We found that the stress responsive enzyme heme oxygenase-1 (HO-1) is a prototypical protective gene.

Expression of HO-1 in EC controls the extent of E-selectin and VCAM-1 expression, two pro-inflammatory genes expressed upon exposure of EC to pro-inflammatory stimuli such as TNF-α or IL-1β. Expression of HO-1 in EC blocks the activation of nuclear factor kappa B (NF-κB), a transcription factor essential to support the expression a these pro-inflammatory genes. It is Fe chelation, afforded by HO-1 expression in EC, that inhibits NF-κB activity. Fe chelation does not interfere with phosphorylation/degradation of the NF-κB inhibitor IκBα or with NF-κB nuclear translocation but instead targets the

RHD domain of p65/RelA to suppress its transcriptional activity. The mechanism by which this occurs is being addressed but presumably involves the phosphorylation status of p65/RelA.

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

Members: Miguel Soares and Gabriela

Students: Mark Pena Seldon

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

Heme oxygenase-1 (HO-1) controls inflammatory reactions preventing the pathogenesis of inflammatory diseases. This protective effect is associated with inhibition of endothelial cells (EC) apoptosis, an effect mediated via the ability of HO-1 to degrade heme into the gas carbon monoxide (CO). The anti-apoptotic effect of HO-1-derived CO is abrogated by the pyridinyl imidazol SB203580, a specific inhibitor of p38 $\alpha$  and p38 $\beta$  mitogen activated protein kinases (MAPK). How HO-1-derived CO interacts with p38 $\alpha$  and/or p38 $\beta$  to prevent EC apoptosis remained to be established. We found that expression of p38 $\alpha$  in EC is pro-apoptotic while that of p38 $\beta$  is cytoprotective, as assessed by specific siRNA-mediated p38 $\alpha$  and p38 $\beta$  inhibition. Expression of HO-1 in EC targets the active form of p38 $\alpha$  but not that of p38 $\beta$  for degradation by the 26S proteasome. This effect is impaired when HO-1 enzymatic activity is inhibited by tin protoporphyrin and can be mimicked by exogenous CO, suggesting that HO-1 inhibits p38 $\alpha$  expression via the generation of CO. The anti-apoptotic effect of HO-1 is reverted when p38 $\alpha$  but not when p38 $\beta$  are ectopically expressed suggesting that the ability of HO-1 to act as “molecular switch” decreasing the expression of p38 $\alpha$  while promoting signaling via the anti-apoptotic p38 $\beta$  MAPK isoform is required for its anti-apoptotic activity in EC.

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

Members: Miguel Soares, Moises Calvacante and Tatiana Vassilevskaia

Students and Technicians: László Tokaji

External Collaborators: AstraZeneca, Manchester, UK

We have assessed the feasibility of a “target” delivery system in terms of preventing septic shock. This technology is based on the use of small peptides, referred to as protein transduction domains (PTD). These can enter cells “spontaneously” with very high efficiency. The best-described among these is a eleven amino acid (YGRKKRRQRRPPQ) peptide, derived from the human immunodeficiency virus (HIV). It has been shown to be “spontaneously” incorporated in cultured cells at around 100% efficiency. When linked/fused to other molecules such as proteins these are “spontaneously” incorporated into cultured cells as well. Perhaps, more importantly this

TAT peptide can be used *in vivo* to achieve expression of fused/linked proteins in virtually all cell types. We have generated a chimeric protein containing TAT and the full-length heme oxygenase-1 (HO-1) sequences. Based on the potent cytoprotective and anti-inflammatory of HO-1 we hypothesized that this TAT-HO-1 protein may be used therapeutically to prevent the deleterious effects of acute inflammatory reactions such as septic shock. We show hereby that TAT-HO-1 prevents endotoxic as well as septic shock in mice. We also provide mechanistic evidence suggesting that this protective effect relies on the ability of TAT-HO-1 to suppress the release of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and HMGB-1 by activated monocyte/macrophages (M $\phi$ ), two central effectors in the pathogenesis of endotoxic and septic shock. The protective effect of TAT-HO-1 was observed when this protein was administered several hours after the onset on these acute inflammatory reactions, suggesting that it can be used therapeutically.

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

Member: Miguel Soares and Jocelyne Demengeot

Student: Angelo Chora

Collaborators: Abelhadi Saoudi (Inserm - Unité de Recherche U563 Centre de Physiopathologie Toulouse-Purpan Pavillion Lefèvre, CHU Purpan, France).

Induction of experimental autoimmune encephalomyelitis (EAE) in rodents is well-established prototypic T helper (Th) cell-mediated autoimmune disorder that recapitulates the human demyelinating disease multiple sclerosis. Clinical symptoms are all directly attributed to injuries to the central nervous system and manifest over relatively short periods (relapse episodes) followed by longer ones during which the intensity of the clinical signs decreases significantly (remission episodes). We have hypothesized that the remission and resistance phases of this disease are regulated through the expression of specific genes that protect target cells in the brain (i.e. oligodendrocytes) from T cell and monocyte/macrophage (M $\phi$ ) mediated injury. In addition these genes may also down-modulate the immune response that is directed against the target cells. EAE was induced in C57Bl/6 mice using a standard myelin oligodendrocyte glycoprotein-derived peptide (MOG35-55) in incomplete Freud's adjuvant supplemented with heat-inactivated *Mycobacterium tuberculosis*. Mice received the HO-1 inducer Cobalt Protoporphyrin IX (CoPPIX) or Zinc Protoporphyrin IX (ZnPPIX), a protoporphyrin that is structurally identical to CoPPIX but that does not induce the expression of HO-1. An additional group received equivalent volumes of phosphate buffered saline (PBS), the vehicle solution used for protoporphyrin administration. Induction of HO-1 expression after the appearance of the clinical signs of the disease afforded a net suppression in terms of disease progression in all animals analyzed. Perhaps more important is the observation that induction of HO-1 expression fully reverted the clinical signs of disease about 50% of the animals analyzed. Control animals treated with PBS or ZnPPIX showed normal disease progression with severe paralysis during the active phase of disease and partial remission thereafter. Our preliminary data suggests that exposure to CO during the active phases of EAE yields similar results to those of HO-1 induction. The proliferation of anti-

MOG35-55 CD4 T cells was evaluated *in vitro* following *in vivo* immunization with MOG35-55. Induction of HO-1 expression suppressed the ability of anti-MOG35-55 CD4 T cells to proliferate when re-challenged *in vitro* with this peptide, as compared to ZnPPiX or PBS treated controls. Administration of ZnPPiX had no significant effect in terms of modulating T cell proliferation, as compared to control mice that received PBS. Using adoptive transfer experiments we found that the ability of HO-1 to suppress the proliferation of anti-MOG35-55 CD4 T cells was mediated via a small (<2%) population of antigen presenting dendritic cells.

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

Member: Miguel Soares, Isabel Pombo Gregoire and Moises Mallo

Student: László Tokaji

Heme oxygenase-1 (HO-1) is a stress responsive enzyme that catabolyzes heme into three products: the gas carbon monoxide (CO), biliverdin and iron. The hypothesis tested in this proposal is that inhaled CO prevents the pathogenesis of atherosclerosis via a mechanism that involves the modulation of monocyte/macrophage activation as well as the inhibition of smooth muscle cell (SMC) proliferation. When maintained under a “high cholesterol” diet, ApoE<sup>-/-</sup> mice have plasma cholesterol levels that are up to 4 to 5 times the normal levels and develop atherosclerotic lesions. These are identical to those observed in humans, in terms of their anatomic sites, cell constitution and histomorphology. We have set up a colony of ApoE<sup>-/-</sup> mice at the Instituto Gulbenkian de Ciência and established the kinetics of atherosclerotic lesion formation in these mice when maintained under normal chow (3% fat) versus “high cholesterol diet” (15.8% fat, 1.25% cholesterol, 0.5% sodium cholate). As expected ApoE<sup>-/-</sup> mice developed atherosclerotic lesions under normal chow with the extent of these lesions being significantly increased when these mice are fed with high fat diet. The lesions appeared mainly in the aortic arch and at branch points. This was correlated with cholesterol levels that reached 2000mg/dl after one week of treatment with high fat diet as opposed to the 200mg/dl observed in animals under normal diet. We then set-up experimental conditions allowing the exposure of these mice to exogenous CO and obtained preliminary data suggesting that CO does suppresses the development of atherosclerosis in these mice. We will now confirm this data and will cross ApoE<sup>-/-</sup> mice with mice that are deficient for genes involved in the signal transduction pathway triggered by CO in smooth muscle cells and monocyte/macrophages so that we can assess whether these genes are involved in the anti-atherogenic effects of CO.

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

Member: Matthias Haury

Students and Technicians: Dinis Calado, Ana Teles

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

We have generated a new mouse transgenic mouse strain to study the expression of IL-10 in vivo, and we are currently characterizing this strain to study expression patterns of IL-10 in various cell types. We are now also analyzing the allelic expression of IL-10 in various situations ex-vivo and in-vitro. These studies are carried out in collaboration with the laboratory of Dr. Dan Holmberg, Umea University, Sweden.

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

Members: R.M.E. Parkhouse

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

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

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

Finally, we have developed potential vaccines currently being tested for bovine and porcine cysticercosis, based on a recombinant oncospherical surface and secreted molecule. Interestingly this molecule is a functional adhesion molecule, possibly facilitating tissue

invasion by the parasite in the intermediate host, and so constitutes a rational basis for a vaccine.

### **Malaria & immunity**

Malaria remains the most devastating parasitic disease worldwide. In any given year, nearly ten per cent of the global population will suffer from malaria — 500 million clinical cases — and more than 1 million will die. In Africa, the disease kills one child in twenty before 5 years of age, representing nearly 10% of over 10 million children who die at these ages. In addition, malaria has a major negative impact in economic development and stability of many developing countries. Various attempts at eradicating malaria have thus far failed.

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

At the IGC, 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. Thus, *Plasmodium* can invade many types of cells but only fully develops inside hepatocytes, indicating a critical role of the host cell in sustaining parasite growth and development. As seen above, inflammatory reactions can be pathogenic, and cerebral malaria provides one such example. Hence, regulation of the acute responses to infection is investigated, also because absence of effective immunity can be thought to result from excessive

regulation. The expansion of a particular population of T cells and its accumulation in the brain of infected mice, have been correlated with cerebral malaria. Current work aims at the control of such pathogenic lymphocytes by Regulatory T cells, and at dissecting the effects of pathogenic lymphocytes on endothelial, astro and microglial cells of the brain, using genomics and proteomics and hoping to identify new predictive markers for the infection outcome. The risk for malaria infection and disease varies wildly across Tropical Africa, and the overall results of therapeutic or environmental interventions also very widely, suggesting unexpected thresholds in transmission. Furthermore, to be effective, interventions in malaria need not be radical, as they might bring prevailing conditions across those thresholds. By developing mathematical models, we aim at a better understanding of malaria epidemiology and control. We have recently shown that variations in the “reinfection threshold” that is intrinsic to the population dynamics of recurrent infections may explain those discrepancies.

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

Member: António Coutinho and Elsa Seixas

Student: Dominique Ostler

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

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

### **The role of Toll-like receptors in cerebral malaria**

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

Student: Vasco Correia

Despite intensive research, the pathogenic mechanisms of cerebral malaria – the major cause of death in *P. falciparum* infection - are not fully characterised. Known central features are the T cell-dependence of the process in murine malaria, a key role of TNF-alpha, and the sequestration of mature forms of parasitized erythrocytes and ring stages



within the microvasculature of the major body organs, following interactions between surface molecules on parasitized red blood cells and host receptors.

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

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

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

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

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

### **In search of malaria mitogens**

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

Using three inbred mouse strains and *P. berghei* as well as *P. chabaudi chabaudi*, we have now analysed in detail the alterations in cellular composition and state of activation of lymphocytes in representative secondary lymphoid organs, bone marrow and thymus. The results confirm that malaria infection is accompanied by a marked polyclonal activation of T and B lymphocytes and serum hypergammaglobulinemia. We initiated, therefore, the search for “malaria mitogens”, following two complementary approaches: on the one hand, we screen plasmodium products for activating lymphocytes and/or dendritic cells; on the other hand, we use the information contained in the recently

completed plasmodium genome sequence in order to identify “candidate” molecules with this ability.

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

Member: Elsa Seixas

Student: Dominique Ostler

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

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

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

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

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

Students: Ana Rita França

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

A greater appreciation of the mechanisms of innate immunity during the infection should provide critical clues on how manipulation of the immune system may best be achieved. Thus we propose to characterize the cellular and molecular mechanisms underlying innate immune processes induced by *Plasmodium* sporozoites and to clarify whether these innate mechanisms are beneficial or not to the parasite and/or host. The ultimate

goal is to elucidate the role and the significance of TLRs in hepatocytes, and to clarify the pathways initiated by recognition of sporozoites in both the initial interaction and during the development of an immune response.

### **Plasmodium-Host interaction during the liver stages of infection.**

Members: Maria M. Mota and Miguel Prudêncio

Since it is becoming evident that intracellular parasite are masters at manipulating the host cell pathways for their own benefit to create a more hospitable environment, we proposed to characterize the hepatocyte response to *Plasmodium* sporozoite infection. In addition, we also proposed to take a careful examination on the host apoptosis pathways altered by infection as well as to identify *Plasmodium* genes responsible for apoptosis inhibition. During the first year of this project we have already started the preliminary analysis of microarray experiments based on a single hybridisation. In addition, we also came across a *Plasmodium* molecule candidate for the role of host cell apoptosis inhibition by the parasite.

### **The role of HGF during the liver stages of a malaria infection.**

Members: Maria M. Mota and Sabrina Epiphanyo

Students and Technicians: Susana Silva, Sonia Albuquerque

External Collaborators: Silvia Giodano (IRCC, Turino, Italy). Ana Rodríguez (NYU, New York, USA).

*Plasmodium*, the causative agent of malaria, migrates through several hepatocytes before initiating a malaria infection. We have previously shown that this process induces the secretion of hepatocyte growth factor (HGF) by traversed cells, which renders neighbor hepatocytes susceptible to infection. The signaling initiated by HGF through its receptor MET has multifunctional effects on various cell types. Our results reveal a major role for apoptosis protection of host cells by HGF/MET signaling on the host susceptibility to infection. Inhibition of HGF/MET signaling induces a specific increase in apoptosis of infected cells leading to a great reduction on infection. Since HGF/MET signaling is capable of protecting cells from apoptosis by using both PI3-kinase/Akt and, to a lesser extent, MAPK pathways, we determined the impact of these pathways on *Plasmodium* sporozoite infection. Although inhibition of either of these pathways leads to a reduction in infection, inhibition of PI3-kinase/Akt pathway caused a stronger effect, which correlated with a higher level of apoptosis in infected host cells. Altogether, the results show that the HGF/MET signaling requirement for infection is mediated by its anti-apoptotic signal effects. These results demonstrate for the first time that active inhibition of apoptosis in host cell during infection by *Plasmodium* is required for a successful infection.

## **T- Cell response in pathogenesis of malaria**

Members: Sylviane Pied, Margarida Vigario.

Students: Tania Cruz.

Collaborators: Virgílio do Rosario, (CMDT Lisbon, Portugal), Danièle Voegtle and Pierre-André Cazenave, (Institut Pasteur Paris, France).

Cerebral malaria (CM) is one of the most severe complications of *Plasmodium falciparum* infection. Several observations in malaria patients, and experimental models suggest that T cells are implicated in cerebral malaria (CM) pathogenesis. Using the experimental model of cerebral malaria induced in C57BL/6 mice by *Plasmodium berghei* ANKA, we found that the development of the neuropathology is associated with an expansion of CD8<sup>+</sup> T cells bearing Vβ8<sup>+</sup> T cell receptor (TCR) chains in the blood and an accumulation of CD8<sup>+</sup> T cells in the brain. The exact mechanism by which these cells are involved is at present unknown. We hypothesized that in CM susceptible mice the neuropathology could be, at least in part, the result of an inefficient control of pathogenic effector T cells by CD4<sup>+</sup>CD25<sup>+</sup> regulatory T. Surprisingly, in an experimental model of CM induced by *P. berghei* ANKA (PbA) the number of CD4<sup>+</sup>CD25<sup>+</sup> T cells expressing Foxp3 increased during the infection. These CD4<sup>+</sup>CD25<sup>+</sup> Treg cells displayed an activated phenotype and a biased CDR3 TCRVβ repertoire. Consistent with their activated status, CD4<sup>+</sup>CD25<sup>+</sup> Treg cells isolated from PbA-infected mice showed an enhanced regulatory activity *in vitro*. CM was not exacerbated in mice infected with PbA one month after treatment with anti-CD25. However, splenic CD8<sup>+</sup> T cells expressing CD69 were increased in these mice. Taken together, these results show that *Plasmodium* infection leads to an increase in the number of CD4<sup>+</sup>CD25<sup>+</sup> Treg cells exhibiting *in vitro* suppressive function. However, they are not efficient to protect mice from CM.

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

Members: Sylviane Pied, Johann Truccolo

Students: Rui Rodrigues (left in April 2004)

Collaborators: Béatrice Régnault (Affymetrix platform Institut Pasteur Paris, Paris, France).

Both host and parasite factors play a role in mechanisms leading to the development of Cerebral Malaria (CM) during *Plasmodium falciparum* infection. However, the cascade of events resulting from the interactions between malaria parasite and brain cells and their involvement in the neuropathogenesis remains totally unknown.

The aim of this project is to analyse parasite and parasite-specific T-cells interactions with cells of the brain-blood-barrier (BBB). For this, we have developed an *in vitro* model of BBB to analyse changes induced in astrocytes, endothelial and microglial cells following exposure to *Plasmodium berghei* ANKA, a parasite strain able to induce CM in susceptible mice, and CD8<sup>+</sup> T cells recruited in the brain of *P. berghei* ANKA infected mice developing CM. Using parasite stained with a fluorescent vital dye, we analyzed by microscopy morphological and physiological changes induced by *P.berghei* blood stages

when interacting with either endothelial or microglial, astroglial cells. We also investigated modifications in genes transcription using the Affymetrix using GeneChip murine genome U74v2 microarray.

We found that stimulation of primary cultures of microglial cells and astrocytes from C57BL/6 mice with *P. berghei* ANKA clone 1.4 blood stages leads to up or down-regulation of 249 genes. Among them 19 were belong to genes involved in the immune response.

### **Analysis of the repertoires of Immunoglobulins E (IgE) self-reactivity to brain antigens in *Plasmodium falciparum* infected patients manifesting different clinical forms of malaria.**

Members: Sylviane Pied, Constantin Fesel

Students and Technicians: Joana Duarte

External Collaborators: Vincent Guiyedi (Institut Pasteur, Paris, France)

Despite much clinical and scientific effort, the physiopathology of pernicious malaria in humans infected with *Plasmodium falciparum* remains obscure. Most studies done up to now, aiming to analyse immunoglobulin E response during malaria have suggested a pathogenic role for this class of Ig as it increased in patients developing severe disease. In order to elucidate these phenomena, we studied in *P. falciparum*-infected children from different cohorts: non-severe clinical manifestations, severe forms without cerebral affection and cerebral malaria total and parasite specific IgE response by Elisa and IgE autoreactivity using a quantitative immunoblot coupled with principal component analyses.

We observed a decrease in total IgE levels in cerebral malaria groups when compared to asymptomatics and patients manifesting acute or severe non cerebral malaria. We also found more self reactive IgE to brain antigens in the asymptomatic group. Moreover, the IgE self- reactivity to brain gradually decreases with the severity of the disease particularly in cerebral malaria patients. These results favour a protective role for IgE in malaria patients.

The originality of this project in an integrated approach toward the role of the immune system in the development of *P. falciparum* infection, since different components of the immune reaction (taken into account in their globality, due to the originality of the utilized methods) as well as the parasite genotype and the association with cerebral manifestation, are studied in the same patients. This could allow to identify new markers with predictive value for the expected development of the infection.

### **Role of microglia activation in cerebral malaria associated neuropathogenesis**

Member: Sukalyan Chatterjee, Teresa Faria Pais

Students and Technicians: Catarina Figueiredo, M<sup>a</sup> Hortense Matos

External Collaborators: Laura Santambrogio, (Harvard Medical School Boston, Boston, USA).

Numerous studies both in humans and in animal models have shown that neurodegenerative disorders and infections of the CNS are concomitant with the activation of brain macrophages, historically known as microglial cells. Although, like other glial cells, brain macrophages may have a neuroprotective role their uncontrolled activation enhances the neuropathology associated with the different disease. Activated microglia have up-regulated surface receptors and produce cytokines which initiate a local immune response. Cerebral malaria (CM), the most serious clinical complication of infection with the parasite *Plasmodium falciparum* causes millions of deaths each year and neurological sequelae in the survivors. There are evidence that microglia are activated very early on after infection. Whether activation of microglia is a key event in causing neuronal damage culminating in CM is still a matter of debate.

Although the activation of brain macrophages is associated with both human and mouse cerebral malaria (CM) the relative contributions of the heterogeneous populations of brain macrophages to the disease are unknown, We have dissociated for the first time inflammatory monocytes from resident brain macrophages in mice developing CM when infected with *Plasmodium berghei*. Our results suggest that parenchymal brain macrophages are most likely contribute to the subsequent phases of infection that culminate in death of mice with CM syndrome, rather than just being activated as a consequence of overwhelming brain inflammation. It is unclear whether apoptosis sets in to cause neuronal damage and what are the determinants of the cell death cascade. One hypothesis is that the parasite mediates neuronal cell death and then signals from dying neurons activate microglia. It is also likely that infection can activate microglia which causes cytotoxic damage to neurons. The ongoing project is addressing these issues and will also investigate the signaling mechanisms in activation of microglia. Moreover, the project will investigate the role of quinolinic acid secreted by activated microglia in the neuropathogenesis of CM.

## **Developmental Biology in animals and plants**

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

to interactions among groups working in apparently distinct areas, which may result in very fruitful collaborations.

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

### **Polar growth, orientation and morphogenesis in pollen tubes**

Members: Jorge Carneiro and Jose Feijó

Students and Technicians: Ramiro Magno

The pollen tube is a cell with polar growth that delivers the male gametocyte to the female oocyte, navigating through the female tissues in the flower. Aiming to better understand the mechanism by which the pollen tube navigates through the female tissues we modeled its polar growth, orientation and morphogenesis. We have shown that the growth and orientation of the pollen tube in three dimensions can be described by the spatio-temporal orchestration of the rates of growth of the rigid cell wall surface and of the incompressible cytoplasm volume. The simulation of these processes is computationally efficient making it possible to simulate thousands of pollen tubes simultaneously and also to assess different hypotheses about the mechanisms controlling of pollen navigation. Future research along these lines will be to identify the molecular control mechanisms.

### **Isolation and study of novel head-inducing genes expressed in the Anterior Visceral Endoderm**

Members: José A. Belo

Students and Technicians: Mário Filipe, Sara Marques, Lisa Gonçalves, Ana C. Silva

External Collaborators: Herbert Steinbeisser (Dep. Human Genetics, Medical School University of Heidelberg, Germany).

Several reports point to an involvement of the mouse Anterior Visceral Endoderm (AVE) in early anterior neuroectoderm induction. The inhibition of BMP4, Xnr1 and Xwnt8 signalling was shown to be necessary for the correct induction of forebrain in *Xenopus laevis*. Several secreted antagonists of BMP4, Nodal and Wnt8 pathways, like Cer-1, Lefty1 and Dkk1 are expressed in the AVE, in a region underlying the prospective anterior neuroectoderm.

In order to further characterise the molecular mechanisms that play a role in the early forebrain induction, a transgenic mouse line was generated in which EGFP is expressed in the AVE, under the control of the promoter region of the Cer-1 gene. In this transgenic line the A-P axis reorientation could be followed, by the fluorescently labelled AVE cells, even before gastrulation. This allowed us to microdissect the anterior-distal (Ad)

region and the diametrically opposed proximo-posterior (pP) region of the E5.5 mouse embryo. Gene expression profiling of both Ad and pP regions using GeneChips ® (Affymetrix ®) identified several new transcripts expressed at the very early stages of A-P axis establishment.

Preliminary analysis of the temporal and spatial expression patterns of the newly identified genes are being conducted. Several new genes have been identified so far to be asymmetrically expressed in the AVE.

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

Members: José A. Belo

Students and Technicians: Ana C. Borges

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

In the present work we generated *cerberus-like;cripto* double mutants in order to uncover a genetic interaction between these two factors. According to our hypothesis, in the context of the *cripto* null mutation, the loss of *cer-1* would increase the level of free Nodal protein in the extracellular space. By disrupting *cripto* (and consequently, the nodal canonical pathway) and increasing nodal levels, we may redirect nodal molecules to a Cripto-independent receptor complex and rescue the *cripto* null mutant phenotype. This strategy may allow us to uncouple *nodal* and *cripto* functions during embryonic development.

Preliminary data provided insight into an alternative Nodal signalling pathway that is Cripto-independent and accounts for a basal level of Nodal activity capable to overcome gastrulation in the mouse embryo. This work also highlights the importance of double mutant studies to uncover complex regulation networks occurring during mouse development, and the importance of antagonist and co-receptors to modulate Nodal signalling, in order to achieve optimal levels for embryonic development.

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

Members: José A. Belo

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

External Collaborators: Michelangelo Cordenonsi (Department of Medical Biotechnologies, University of Pádova, School of Medicine, Padua. Italy).

Correct establishment of the L/R body asymmetry in the mouse embryo requires asymmetric activation of the evolutionary conserved *Nodal* signaling cascade in the L-LPM. Furthermore, the presence of Nodal in the node is essential for its own expression in the L-LPM. By sequence homology analysis, we have identified a novel mouse gene of the *Cerberus-like* family, that we designated *cerberus-like 2* (*cerl-2*). Here, we have characterized the function of *cerl-2*, a novel Nodal antagonist, which displays a unique



asymmetric expression on the right side of the mouse node. *cerl-2* knock-out mice display multiple laterality defects including randomization of L/R axis. Strikingly, these defects can be partially rescued by removing one nodal allele. Our results demonstrate that Cerl-2 plays a key role restricting the Nodal signaling pathway towards the left side of the mouse embryo by preventing its activity in the right side.

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

Members : José A. Belo and Vera Lucas-Teixeira

Students and Technicians: Sara Marques

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

Recognizable relevance has been given to the intracellular pathways by which *Fgf8* is regulated and modulated. Recently, it has been demonstrated that the dual mitogen activated protein kinase phosphatase-3 (*Mkp3*), plays a role as a negative feedback modulator of the MAPK/ERK FGF8 signaling in chick limb bud development.

We have investigated the role of the mouse *Mkp3* and its functional relationship with the *Fgf8* signaling pathway in the mouse IsO using gene transfer micro-electroporation assays and protein-soaked-bead experiments. Here we demonstrate that *Mkp3*, beyond any other known modulators, has a fast, direct and strong negative action on the MAPK/ERK-mediated FGF8 signaling in the mouse neuroepithelium.

We have also started to generate the necessary tools to inactivate MKP3 in mouse ES cells by homologous recombination.

### **Transcriptional Regulation of *Caronte* during Embryonic Development**

Members: José A. Belo and Ana T. Tavares

Students and Technicians: Sofia Andrade

*Xenopus Xcer*, mouse *Cer-1* and chick *Car* are expressed in equivalent embryonic structures such as the anterior endomesoderm, anterior visceral endoderm, and hypoblast, respectively. In mouse and chick embryos, these genes are also expressed in the anterior definitive mesendoderm. However, at later stages, *Xcer* transcripts are no longer detected, mouse *Cer-1* RNA is found in the rostral domain of nascent somites and presomitic mesoderm, and chick *Car* is expressed in the left lateral plate and paraxial mesoderm. The general aim of this project is to dissect the transcriptional regulatory mechanisms that establish these similarities and differences in the expression patterns of the *Cerberus-like* gene family.

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

(1) to perform a detailed analysis of *Car* expression pattern in the developing chick embryo;

(2) to optimise the transfection technique used to introduce *Car* reporter constructs into developing chick embryos (electroporation in New culture);

(3) to identify the transcriptional *cis*-regulatory elements that drive *Car* expression, and the transcription factors that bind those elements and enhance (or repress) *Car* transcription;

(4) to investigate the regulation of *Car* reporter constructs by signalling molecules that may repress or activate *Car* asymmetric expression (*i.e.*, BMP4, SHH and Nodal); and

(5) to determine if the upstream regulators of *Car* expression are conserved in mouse embryos.

### **Mechanisms of plant cell growth and morphogenesis**

Member: José Feijó

Students and Technicians: Ana Catarina Certal, Sofia Cordeiro, Ana Margarida Prado, and Leonor Boavida

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

### **Pollen stigma interaction and sexual plant reproduction**

Member: José Feijó

Students and Technicians: Ana Catarina Certal, Sofia Cordeiro, Ana Margarida Prado, and Leonor Boavida.

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

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

## **Epithelial dynamics and adhesion during *Drosophila* dorsal closure**

Members: Antonio Jacinto

Students and Technicians: Beatriz Garcia Fernandez, Sérgio Manuel de Matos Simões, Anja Hagemann, Pedro Almeida Laires

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

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

## **Wound repair using *Drosophila* as a model system**

Members: Antonio Jacinto, William Jonathan Wood

External Collaborators: Paul Martin (Depts Physiology and Biochemistry, School of Medical Sciences, University of Bristol, University Walk, Bristol, UK).

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

are involved in dorsal closure are also functionally required to initiate or drive the wound closure. We are using our wounding system to test mutants defective for morphogenetic genes that may be required during wound healing.

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

Members: Antonio Jacinto, William Jonathan Wood

External Collaborators: Paul Martin (Depts Physiology and Biochemistry, School of Medical Sciences, University of Bristol, University Walk, Bristol, UK).

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

### **The role of Tbx1 in embryonic development**

Members: Moisés Mallo

Students and Technicians: Filipa Moraes, Ana Nóvoa

External Collaborators: Virginia Papaioannou (Columbia University, New York, USA)

The Tbx1 gene belongs to the T-box-containing family of transcription factors. A variety of genetic analyses, both in mice and humans, indicate that Tbx1 is the major responsible for the pathological manifestations observed in DiGeorge syndrome patients.

We are studying the role of Tbx1 during development using mice carrying a null mutation in this gene. These mutant mice contain a variety of malformations including abnormal branching of the heart outflow tract, deficiencies in the branchial arch derivatives, agenesis of pharyngeal glands and abnormal development of the auditory system. We have analyzed in detail the middle and inner ear phenotypes of the *Tbx1* null mice. The middle ear is strongly affected. Its skeletal components are malformed to varying degrees, some being slightly hypoplastic and others completely absent. However, a seemingly normal-looking tympanic membrane can still be recognized. Middle ear anomalies are associated with other skeletal deficiencies in the branchial arch-derived skeleton. These phenotypes derive from a combination of the failure of the posterior branchial arches to develop and the misrouting of neural crest cells. The inner ears of *Tbx1*<sup>-/-</sup> animals are hypoplastic. No vestibular or cochlear structures are detectable, but

the endolymphatic duct, the cochleovestibular ganglia and residual sensory patches are still identifiable. Molecular analyses indicate that *Tbx1* is not required for the establishment of spatial patterns in the otocyst, but rather for their maintenance. The inability of the *Tbx1*<sup>-/-</sup> embryos to keep properly segregated functional domains in the otocyst is likely the cause of the strong inner ear phenotypes observed in these mutants. We have started to analyze role of *Tbx1* in the development of the heart outflow tract, which is going to be the main focus of the future work.

### **The role of Hox genes in the patterning of the axial skeleton of the mouse**

Members: Moisés Mallo

Students and Technicians: Marta Carapuço, Ana Nóvoa.

Hox genes are key regulators of the specification of the morphological identities of the vertebrae, the basic unit of the axial skeleton. Embryologically, the axial skeleton derives from the somites, segmental units organized in pairs on both sides of the developing neural tube. Somites are formed in a rostro-caudal sequence by the epithelialization of mesenchymal cells at the rostral end of the presomitic mesoderm. We have analyzed when, during development, are the Hox providing specific differentiation programmes to the somites. Using transgenic experiments, we have found that, at least for some of these genes (most particularly the Hox 10 group), the activity of these genes seems to be required in the presomitic mesoderm, during somitic formation and not when the somite is differentiating into their skeletal derivatives. For other Hox genes, it seems that the activity is required at different levels for specific processes. Thus, the Hox11 group, defines the development of the sacrum when the corresponding somites are still in the presomitic mesoderm, but are required in the differentiating somite to generate caudal vertebrae. These functions are indeed in keeping with the patterns of Hox gene expression, which seem to fit better with their functional domains when the relevant somites are still under formation in the presomitic mesoderm than in the resulting somites. These results are very important for the proper design of experiments that will allow the further analysis of the mechanisms of Hox gene activity during the specification of the regional identities of the axial skeleton.

### **The role of Gbx2 in the patterning of the neural tube and of the inner ear**

Members: Moisés Mallo

Students and Technicians: Marta Carapuço, Ana Nóvoa, Tânia Vinagre.

We have initially identified *Gbx2* in a molecular screen for downstream effectors of *Hoxa2* activity. When we analyzed mice carrying a null mutation for *Gbx2*, we found that the inner ear of these mutants is variably affected. Additional analyses showed that the neural tube of *Gbx2* mutant embryos develop abnormally, not only at the level of the isthmus (as previously described) but that they have a more generalized mispatterning of the forebrain, midbrain and hindbrain. Among these defects were the presence of

anomalies in the post-otic hindbrain, a phenotype that could explain the inner ear phenotype in terms of abnormal placode induction rather than of differentiation problems of the otic vesicle. We have started a complete characterization of the neural phenotype which will be complemented with the analysis of the differentiation of the otic vesicle. When the molecular characterization is ready and we have a hypothesis for the generation of the abnormalities in the inner ear of Gbx2 embryos. Accordingly, we will design functional studies, mostly using transgenic and gene modification technologies in the mouse, to test these hypotheses.

### **The role of Bmp2 in the early development of the mouse neural crest cells**

Members: Moisés Mallo

Students and Technicians: Catarina Correia

We have previously shown that Bmp2 is required for the formation of migratory neural crest cells in mouse embryos. However, it is still unclear whether this factor is involved in the production of these cells, in their migration or both. To address this issue, we have started to establish a interspecific grafting system, which will allow to evaluate the neural crest-inducing capabilities of the dorsal neural tube of the mouse in chicken embryos. This assay is based on the observation that neural tube corresponding to an area that is normally not fated to produce neural crest cells is transplanted into the prospective surface ectoderm, neural crest cells are induced at the interface between these two tissues. In our experimental design, we graft the dorsal neural tube of E8.0 mouse embryos into the prospective surface or neural ectoderm of stage 4 chicken embryos. The production of chicken-derived neural crest cells using specific molecular markers, is the method to evaluate the inducing capabilities of the mouse tissues. We will compare the activities of the above-mentioned mouse tissues from wild type and Bmp2 mutant embryos. We expect these results will give the first clues to understand the role of Bmp2 in early neural crest development and that they will help to design further experiments to fully understand how Bmp2 control this embryonic process.

### **The role of Hoxb4 in hematopoiesis**

Members: Moisés Mallo, Leonor Parreira

Students and Technicians: Ana Cristina Santos, Ana Catarina Ribeiro

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

We are interested in understanding the molecular mechanisms mediating Hoxb4 activity in the formation of hematopoietic stem cells. We have found that the FGF and Notch signalling pathways play essential roles in this process, as determined by the effects produced by specific inhibitors of these pathways. HoxB4 seems to potentiate the response of ES cells to FGF signals, which seem to be involved in the fine tuning of the HoxB4-stimulated production hematopoietic stem cells. Our data shows that, while inhibition of FGF signalling blocks formation of HSCs in normal ES cells, when this inhibition is performed on HoxB4-induced cells the production of immature hematopoietic progenitors seems to be stimulated. This effect correlates with variations in Fgf signalling activities in these cells. We are working to determine the molecular basis for the HoxB4-dependent stimulation the sensitivity of ES cells to Fgf signals and on the physiological relevance of this process. In addition, we are undertaking a systematic approach to understand the mediators of HoxB4- induced generation of HS cells by analyzing the transcripts of ES cells in the presence and absence of Hoxb4 using the Affymetrix chip technology. Functional analyses with the obtained candidate genes will follow to gain insights into how Hoxb4 controls the formation of hematopoietic stem cells.

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

Member: Leonor Parreira

Students: Isabel Alcobia, Ana Sofia Quina and Hélia Neves

The choice between alternative cell-differentiation pathways is regulated by direct intercellular contacts mediated by trans-membrane proteins expressed by adjacent and apparently equivalent stem cells. Two protein families involved in this process are the Notch receptors and their ligands, the Delta and Jagged proteins. Both protein families are phylogenetically conserved and involved in several developmental scenarios including the decision processes underlying the functional divergence of CD4/CD8 T lymphocytes and the choice between  $\alpha\beta$  and  $\gamma\delta$  T-cell receptors in mouse thymocytes. Using a cell coculture assay we have recently observed that the Notch ligand Delta-1 completely inhibits the differentiation of human hematopoietic progenitors into the B-cell lineage while promoting the emergence of cells with a phenotype of T-cell/natural killer (NK) precursors. In contrast, Jagged-1 did not disturb either B- or T-cell/NK development. Furthermore, cells cultured in the presence of either Delta-1 or Jagged-1 can acquire a phenotype of NK cells, and Delta-1, but not Jagged-1, permits the emergence of a de novo cell population coexpressing CD4 and CD8 (*Jaleco et al, J Exp Med, 2001, 194:991-1001*).

Now, the effects of these Notch ligands on myeloid development are under investigation. To do this, a long-term culture assay is used, where bone marrow stromal cells transduced with human Delta-1 or Jagged-1 cDNAs, are co-cultured with normal human progenitors, followed by the analysis of their differentiation potential in methylcellulose clonogenic assays. Preliminary results indicate that Delta-1 and Jagged-1 differ also have differential effects in myelopoiesis, namely on the proliferation, clonogenic and differentiation properties of pluripotent myeloid precursors.

## **Two closely related buttonhead-like transcription factors, *Sp8* and *Sp9* regulates *Fgf8* expression and limb outgrowth in vertebrate embryos**

Members: Joaquín Rodríguez León

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

Initiation and maintenance of signaling center is a key issue during embryonic development. The apical ectodermal ridge, a specialized epithelial structure is a pivotal signaling center for limb outgrowth as a source of FGF8. Here we show that two closely related buttonhead-like zinc finger transcription factors, Sp8 and Sp9 are expressed in the AER, and regulate Fgf8 expression and limb outgrowth. Embryological and genetic analyses revealed that Sp8 and Sp9 are ectodermal targets of FGF10 signaling, and Wnt/b-catenin signaling differentially regulates Sp8 and Sp9. Functional analyses unveiled their role as positive regulator of Fgf8 expression. Moreover, dominant negative approach and morpholino-based knockdown analysis indicate cooperative action of Sp8 and Sp9 in the expression of Fgf8 and limb outgrowth. Our study revealed that two closely related factors, Sp8 and Sp9 act in the expression of Fgf8 and vertebrate limb outgrowth.

## **A new role for BMP5 during limb development acting through the synergic activation of Smad and MAPK pathways**

Members: Joaquín Rodríguez León

External Collaborators: Vanessa Zuzarte Luís, Juan Antonio Montero Simón, Ramón Merino, Juan Hurlé González (Dept. Anatomy and Cell Biology. University of Cantabria, Santander, Spain).

We have characterized both Smad proteins and MAPK p38 as intracellular effectors for the action of BMPs in the developing limb autopod. Activation of Smad signalling involves the receptor-regulated genes Smad1 and -8, and the inhibitory Smad6, and results in both the upregulation of gene transcription and protein phosphorylation with subsequent nuclear translocation. MAPK p38 is also quickly phosphorylated after BMP stimulation in the limb mesoderm. Treatment with the inhibitor of p38, SB203580, showed that this kinase has a major function in the chondrogenic effect mediated by BMPs but is also the effector for the activation of several genes in the interdigital mesoderm associated with the onset of apoptosis. Our results strongly suggest that Smad and MAPK pathways act synergistically in the BMP pathway controlling limb development.



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

Members: Joaquín Rodríguez León

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

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

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

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

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

### **Developmental Genetics of Digit Patterning in *Chamaeleo chamaeleon***

Members: Joaquin Leon and Élio Sucena

External Collaborators: Juan Carlos Izpisua Belmonte and Yasuhiko Kawakami (The Salk Institute for Biological Studies, San Diego, California, USA.)

Variability in digit length and shape constitutes a paradigm for the study of functional evolution of Vertebrate features. For example, the digit structure of arboreal *Chamaeleo* is radically different from its terrestrial ancestral relatives and stems from profound changes in the developmental programs that shape the autopod.

Differences along the Anterio-Posterior (AP) axis are determined by the interplay of two cellular and developmental processes, AP Patterning and Apoptosis. These processes have been shown to depend upon differential changes of the spatial and temporal regulation of a conserved set of genes. This differential regulation determines differences between species, as well as between forelimb and hindlimb.

During limb development, a group of cells located in the posterior mesenchyme, the Zone of Polarizing Activity (ZPA) acts as the organizer of AP polarity. Such organizing activity depends on the action of a network of conserved genes namely, *shh*, *gli3*, *ptc*, several *hox* genes, *gremlin*, *bmp2* and *dhand*.

The detailed pattern of digit structure is shaped by the concerted action of apoptosis-induction genes in finely delimited areas. Amongst the genes involved in this process, several *bmps*, *gremlin*, *fgfr3*, *msx-2*, *bambi* and *dkk1*, are pivotal for the onset of programmed cell death.

This project aims at characterizing and comparing the expression pattern of such genes in *Chamaeleo chamaeleon*. For this purpose we will construct an embryonic cDNA library that will be used in the isolation of the above mentioned candidate genes. Through *in situ* hybridization we will describe the genetic basis for the AP and apoptotic patterns of the autopod in *C. chamaeleon*.

Such characterization will be the basis for a comparative study of digit formation between *C. chamaeleon* and other vertebrates, and will clarify the genetic basis of forelimb versus hindlimb patterning.

This study will contribute to the understanding of the mechanistic basis for morphological evolution.

### **Molecular and Cellular Characterization of Segmentation in the Chick Embryo**

Members: Isabel Palmeirim and Leonor Saúde

Students and Technicians: Alexandre Gonçalves

The discovery that segmentation and somite formation is a process guided by Medial-PSM raises the question: What are the genes responsible for the segmentation autonomy of Medial-PSM? A subtraction screening of cDNA libraries from Medial *versus* Lateral chick PSM was performed in order to determine the genes differentially expressed in Medial-PSM. From the first round screening one new gene (clone 43) was selected for study based upon its differential pattern of expression in the Medial-PSM using whole-mount/cross section *in situ* hybridisation techniques. The role of clone 43 in establishing the segmentation autonomy is being analysed. Additionally, from the first round screening, two new genes (clone 28 and clone 45) were selected based on their specific pattern of expression in anterior PSM and their absolute complementary expression to the secreted factor *fgf8*. These genes are therefore good candidates for the establishment of the maturation territory in anterior PSM and their relation with Fgf and Retinoic Acid pathways is under study.

## **The role of *terra* during chick early development**

Members: Isabel Palmeirim and Leonor Saúde

Students and Technicians: Raquel Lourenço

The zebrafish gene *terra* encodes a putative zinc-finger protein that is specifically expressed in the Lateral PSM. Therefore, it could be involved in establishing the lateral *versus* medial identity within the PSM. The chick homologue of *terra* was identified in a public EST database search using the amino acid sequence of zebrafish, mouse and human. Two chick ESTs with 80% identity with the human *terra* were purchased from MRC gene service. Whole-mount RNA *in situ* hybridisation experiments were performed to reveal the temporal and spatial expression pattern of *terra*. The pattern of expression of *terra* suggests a role in somitogenesis. To gain some insight into the signals that regulate the expression pattern of *terra*, a series of embryological experiments are being performed. In order to understand the role that *terra* might play in somitogenesis it is absolutely necessary to obtain its full-length sequence. Once the full-length *terra* clone is obtained, gain-of-function experiments using electroporation and loss-of-function experiments using RNAi will be performed.

## **Time control during vertebrate embryonic development**

Members: Isabel Palmeirim

Students and Technicians: Catarina Freitas, Sofia Rodrigues

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

The temporal and spatial regulation of somitogenesis requires an intrinsic molecular oscillator, which is translated into cyclic gene expression of the denominated cycling genes. In this study, we demonstrated that the expression of several cyclic genes is already dynamic at the level of the prospective  $\square$ omatic territory, and that medial and lateral prospective  $\square$ omatic cells, located in distinct territories, express these genes in an asynchronous way. These observations led us to conclude that the segmentation clock is providing cellular positional information not only in the anterior/posterior but also in the medial/lateral PSM axis. Moreover we showed that medial and lateral PSM cells are differently committed to the segmentation programme: in contrast to medial cells, lateral PSM cells isolated from their medial counterpart are not able to form morphological segments nor to express segmentation genes, including the cycling genes. In the anterior third of the PSM, immediately before somites are formed, the information for somite formation is restricted to the medial cells. We are currently performing microsurgical assays using quail-chick grafting as well as *in ovo* insertion of barriers at different levels in the chick embryo to uncover when, during development, the information for segmentation is conferred to the medial PSM cells. Unpublished data from our group

raises the possibility that Hensen's node might be responsible for conferring segmentation autonomy to prospective medial PSM cells.

### **The molecular clock is operating during limb bud development**

Members: Isabel Palmeirim and Joaquín Rodríguez-Léon

Students and Technicians: Susana Pascoal (Univ Minho, Portugal), Mónica Ferreira (Univ. Minho, Portugal).

Previous results suggested that the molecular clock described for somitogenesis could also be providing positional information to limb cells, since *hairy2* presented a very dynamic expression pattern in the limb bud. In order to establish that this dynamic pattern presents a cyclic behaviour, work was performed to determine the cycle time period. This was done using *in ovo* ablation of one limb and further incubation of the embryo for varied periods of time, after which *in situ* hybridization was performed on both limbs and *hairy2* expression patterns compared. The results obtained indicate that our goals have been achieved. To determine the functional relevance of *hairy2* cyclic expression in limb bud development, and its possible involvement in limb segmentation, work is being performed to temporally characterize limb structure formation. *Hairy2* expression pattern was further studied in later stages of chick embryo development, namely during digit formation.

In order to clarify the functional role of both *hairy1* and *hairy2* in limb development, misexpression of these genes in the limb is desirable. To do so, the gene must be cloned in an appropriate vector for retroviral construction. *Hairy2* nucleotide sequence is not available and the probe used in our lab for *in situ* hybridization comprises only the 3' end of the gene. The portion of the chicken genome that has been sequenced only includes this same fragment of the gene, lacking the sequence coding for the first 96 aminoacids of Hairy2 protein. Several experimental approaches were used in an attempt to clone the entire *hairy2* gene, including RACE (Rapid Amplification of cDNA Ends), and amplification of genomic DNA by PCR, using different primers and amplification conditions, as well as colony hybridization of a cDNA library, all of which were unsuccessful. Given the difficulty in characterizing this particular region of the gene, we have established contact with the curators of the chicken genome sequencing project in order to determine what may be done to overcome it. *Hairy1* is presently being cloned in an appropriate viral vector.

### **Extracellular matrix and somitogenesis: causes and consequences**

Members: Sólveig Thorsteinsdóttir, Isabel Palmeirim, Gabriela Rodrigues

Students and Technicians: Pedro Rifes

It has previously been shown that cultured explants of chick presomitic mesoderm (PSM) only form somites in the presence of the overlying ectoderm. However, it is not clear if the ectoderm factor is a soluble factor or whether it is the ectoderm or PSM extracellular

matrix. We are culturing isolated PSM with or without ectoderm and manipulate the availability of the ECM to determine the relative importance of each in somitogenesis.

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

Members: Sólveig Thorsteinsdóttir and Gabriel G. Martins

Students and Technicians: Fernanda Bajanca, Ana Sofia Cachaço, Ana Sofia Lopes

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

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

### **The Role of VEGF and its Receptors in Tumour Growth and Angiogenesis**

Members: Sérgio Dias, Susana Constantino, Cristina Casalou

Students and Technicians: Rita Fragoso, Ana Paula Elias

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

In this Project we have addressed several aspects of tumor angiogenesis, in the context of solid and liquid (hematologic) neoplasms. Our work focuses on the role and regulation of Vascular Endothelial Growth Factor (VEGF) and its receptor tyrosine kinases, working both as paracrine signaling partners as well as autocrine stimulators of neoplastic growth. As paracrine signals, we have found out that VEGF and its receptors are actively regulated within endothelial cells, and are now exploiting the mechanisms of VEGF splicing by neoplastic cells, under the influence of distinct environmental signals (ie, conferring some organ specificity to VEGF production). In addition, we have also described for the first time the existence of internal and external VEGF/VEGF receptor autocrine loops on malignant cells (leukemias). We described further that the activation of internal or external VEGF/VEGF receptor loops results in turn in the activation of

distinct signaling pathways and as such different cellular functions. Finally, still within the aims of the Project, we have recently described a previously unrecognized role for VEGF receptor-1 in regulating the sub-organ localization of neoplastic cells (subsets of acute leukemias). VEGFR-1 stimulation (or blockade) results in the modulation of the localization of the leukemia cells within the bone marrow, which may have serious consequences for therapeutic efficacy in treating such diseases. We are now exploiting the molecular mechanisms that regulate leukemia cell movement within the bone marrow microenvironment. In parallel, we will evaluate the regulation of VEGF splicing, in response to different microenvironment signals, within the bone marrow microenvironment.

### **Angiogenesis and Prostate Cancer**

Members: Sérgio Dias

Students and Technicians: Cátia Igreja, Margarida Courinha

External Collaborators: Shahin Rafii (Cornell University) Manuel Coelho (H. Curry Cabral, Lisbon, Portugal)

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

In the present project we investigate the relative contribution of angiogenesis (classical pathway) and EPC towards prostate cancer growth and metastasis formation. The obtained results will be studied in the context of tumor development and eventual resistance to conventional therapies.

### **Notch signaling and lymphocyte regulation**

Members : Jocelyne Demengeot and Leonor Perreira

Students: Manuel Rebelo, Margarida Santos

It is well established that Notch signalling condition B-lymphocyte fate at early developmental stages, however little is known on the involvement of such signal during mature B cell differentiation to effectors stages. We investigated the effects of the Notch ligands Delta-like-1 (Dll1) and Jagged-1 (Jg1) on mouse B cell activation. Purified B cell subsets were co-cultured with S17 stromal cells expressing either Dll1 or Jg1, and exposed to the activation stimuli LPS, anti-CD40 and/or anti- $\mu$  mAbs. Monitoring cell proliferation, survival, activation, Ig secretion and transcription of specific genes revealed that Dll1 and Jg1 differentially affect the latest stages of B cell activation. The ligand Dll1 increases the frequency of activated B cells that differentiate to Immunoglobulin (Ig)

secreting cells, while Jg1 promotes the survival of differentiated plasma cells but not their Ig secretion. Finally, immuno-histochemistry analysis establishes that Dll1 and Jg1 are differentially expressed in restricted areas of the spleen where B cell terminal differentiation is tightly controlled. These findings indicate that specific Notch ligand-receptor interactions locally facilitate or limit B cell engagement to terminal differentiation. Ongoing work aims at defining the consequence of this regulation on the extend and nature of antibody mediated immune responses.

### **Apoptosis vs. Differentiation – the Cell-fate of Cajal Retzius cells**

Members: Matthias Haury, Paula Parra Bueno

Students and Technicians: Helena Cabaço, Duarte Viana

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

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

### **Theoretical and Computational Biology**

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

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

to signal transduction in lymphocytes, maturation of immune responses and lymphocyte population dynamics, notably, in diseases of the immune system. In evolutionary biology, mathematical modelling and experiments were combined to address, in simple systems using bacteria and plasmids, host-parasite co-evolution, emergence of antibiotic resistance, and the evolutionary forces responsible for the generation and maintenance of diversity in populations.

Functional genomics also combines computational and experimental biology, as it makes use of computation to extract novel information from the very extensive genomic and proteomic databases now available in a variety of living organisms. The IGC's agenda is to analyse genomes in search of unnoticed structural signatures of how biological systems operate, how diseases emerge, or how hosts and parasites co-evolve. Genome-scale technology creates statistical and computational problems on its own, as novel sources of biological information accumulate, notably with high-throughput screening methods, such as gene-chip technologies.

Mathematical biology is also increasingly relevant to epidemiology, particularly to accurately represent the natural dynamics of recurrent and persistent infections, and to predict the impact of interventions. While attempting to derive quantitative frameworks, researchers at the IGC developed a strong, novel concept: reinfection threshold is a notion that was introduced in the quantification of pathogen transmission, and is gaining an increasing number of applications.

### **Nature, origin and dynamics of regulatory T cells**

Members: Jorge Carneiro, Jocelyne Demengeot, Marie-Louse Bergman

Students and Technicians: Nuno Sepúlveda and Tiago Paixão

Regulatory CD4<sup>+</sup>CD25<sup>+</sup> T cells prevent autoimmune pathologies by suppressing other cells. We have shown, by first principle modeling, that the regulatory function of these cells cannot be explained by competition alone, and requires that regulatory cells actively inhibit disease causing cells and use the later as a growth factor (Leon et al. J Theor Biol, 2000; Leon et al. J Immunol 2001). Together with Demengeot's group (IGC, Oeiras), we gave experimental support to this crosstalk mechanism (Leon, PhD Dissertation 2002). On theoretical grounds, we have shown that the same crosstalk mechanism allows for an efficient self-nonself discrimination (Leon et al. J Theor Biol 2003) and can explain the epidemiological observation that autoimmune disease incidence is inversely correlated with the overall prevalence of infections (Leon et al. J. Autoimmunity 2004). Currently we are assessing the details of the interactions between antigen presenting cells, regulatory T cells and their targets by mathematical modeling and by experimental analyses of the spatial redistribution of the three cell types *in vitro* and *in vivo* by confocal microscopy. Recent developments of our theoretical models led us to predict how the repertoire of regulatory T cells is selected and maintained homeostatically. Future work along these lines involves the development of new techniques to assess repertoire diversity experimentally in order to test our predictions.



## **Heterogeneity in cell populations**

Members: Jorge Carneiro

Students and Technicians: Tiago Paixão and Nuno Sepúlveda

External Collaborators: Dejan Milutinovic (Instituto Superior Técnico, Lisbon, Portugal).

The quantity of membrane receptors per cell is distributed in cell populations that are otherwise qualitatively homogeneous. Hitherto, modelers and experimentalists have neglected the origin and functional consequences of this heterogeneity. Typically, experimental measurements as well as models deal with average amount of receptors per cell neglecting the higher moments of the distribution. We asked whether we could extract more information from the shape and dynamics of the distribution of the antigen receptor (TCR) per cell in a T lymphocyte population. We have shown that the variance of the distribution of TCR molecules per lymphocyte and the way it changes in time provides a straightforward method to assess TCR-triggering and downmodulation models that would be indistinguishable when tested by comparison to mean TCRs per cell. We further demonstrate that whether or not the predictions of mean field models can be used to study a mechanism depends on the shape of the distribution of TCRs per cell and on the sources of variance. Trying to tackle this issue we provide two non-mutually exclusive hypotheses for the mechanism generating the distribution of receptors per cell: a chain of stochastic amplification events underlying gene expression pathways and protein level regulation; and the serial conjugation of the T cell and APC presenting agonistic peptides. We proposed new experimental procedures to estimate the relative contributions of these cell autonomous and cell heteronomous processes. Future work involves the realization of these experiments and the validation of the approach.

## **ZF-Human Disease: A Database for Zebrafish Models of Human Diseases**

Members: Pedro Coutinho

Students and Technicians: João Melo

The zebrafish due to its genetic and embryological properties is an optimal animal model to study human diseases at a cellular and molecular level. To approach this systematically, we have defined the zebrafish proteins that are the best candidates for the generation of animal models that could mimic human genetic diseases and susceptibilities.

We gathered the human sequences associated to at least one disease or susceptibility to disease, at the Online Mendelian Inheritance in Man (OMIM) database, to perform a FASTA analysis of the zebrafish proteins. The resulting information was compiled and integrated with zebrafish *in-situ* and mutant allele data to generate the ZF Human Disease Database (ZF-HDD).

For the 2452 human conditions with an associated human sequence, 908 were matched to a zebrafish protein. In total, 2670 zebrafish proteins were matched and from these, the

associated mRNA expression and mutant alleles have been characterised for 109 and 33, respectively.

The zebrafish mutant allele data was used to validate the approach by showing that this systematic identification of candidate proteins/genes to model Human diseases can be used to select zebrafish proteins that can be used for the generation of animal model of human disease. The ZF-Human Disease database will be available online.

### **Genome Analysis of *Plasmodium* Identifies a Novel Mechanism for Creating Diversity**

Members: Pedro Coutinho, Isabel Gordo and Maria Mota

External Collaborators: Peter Preiser (Nanyang Technological University, Singapore).

The methodology to characterise selection pressure on proteins is usually based on comparison of nucleotide alleles, or orthologues, or alternatively in the calculation of volatility, a measurement that requires good knowledge of the specific transcriptome. These cause difficulties when analysing species that are evolutionary distant and for which there is limited information. To solve this problem we have developed a new gene property, the potential mutability (*pmut*) that measures locally a gene's potential to undergo mutations that will change the amino acid sequence that it encodes for. This method can be applied to any coding sequence from any species to identify regions that are under positive/negative pressure to vary. On species with a relatively well described transcriptome, it can be used to identify the genes under relatively higher positive/negative selection pressure. Using *pmut*, we confirm strong selective pressures on previously characterised antigens and on the *var* gene family in the human malaria parasite *Plasmodium falciparum*. Importantly this approach shows that *var* genes are under other selective pressures besides antibody recognition. This second mechanism of selection is applied uniformly across the coding sequence of PfEMP1 proteins' structural domains as well as across the parasite's life cycle. Furthermore, we show that this selection is conserved across *Plasmodium* species. The application of our method to transcriptomes is highly sensitive, flexible, fast and it is an ideal tool for identification of genes relevant for pathogen-host relationships that can be applied to many distinct pathogens and hosts.

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

Members: Jose Faro, Margarida Carneiro

Students and Technicians: Ana Agua-Doce, Joana Moreira

External Collaborators: Africa Gonzalez (Univ. Vigo, Vigo, Spain); Michal Or-Guil (Humboldt Univ., Berlin, Germany); Santiago Velasco (Univ. Salamanca, Salamanca, Spain).

The present study aims to shed some light on controversial aspects of the GC reaction, namely the mechanism(s) involved in their peculiar burst-like dynamics. Four different mechanisms with a common core will be investigated (decrease in available Ag on FDCs, decrease of Ag-specific Th cells, decrease of probability of getting favourable mutations after a maximum is reached, phenotype changes in FDCs). A mathematical modeling strategy will be developed in order to derive experimentally testable predictions that can discriminate between the different postulated mechanisms. One of the models will include a stochastic component to account for accumulation of favourable and unfavourable mutations. The variables of those models are experimental measurable quantities, namely: amount of antigen trapped in complexes on FDCs, number of centrocytes and centroblasts, FDCs, and Th cells in GCs. The models describe the population dynamics of those variables as ordinary differential equations (ODEs). This mathematical approach will be complemented by an experimental analysis of different key parameters entering the models. In particular, it will be assessed: a) the decline of available Ag-antibody complexes on FDCs; b) the kinetics of hypermutations increasing antibody affinity (data from the literature will be used to test the corresponding model); c) the kinetics of GC Th cells responsible to Ag-specific centrocytes; d) phenotypic changes in FDCs and Th cells influencing centrocyte differentiation. Disclosing the force(s) driving the GC reaction will contribute to a better understanding of T-dependent humoral responses, including the kinetics of memory B cell generation and antibody affinity maturation and their role in different autoimmune pathologies.

### **The reinfection threshold and epidemiological puzzles**

Members: Gabriela Gomes and Isabel Gordo

Students and Technicians: Inês Mota, Madalena Patricio, José Nuno Martins, Ricardo Águas

External Collaborators: Fonseca Antunes (Direcção Geral de Saúde, Portugal).

The *reinfection threshold* is a concept that we have recently introduced in the quantification of pathogen transmission, which is gaining an increasing number of applications. First, populations that exceed this threshold sustain high levels of infection and tend to be insensitive to interventions, leading to our recently proposed mechanism for the controversial variable efficacy of bacille Calmette-Guérin (BCG) vaccine against pulmonary tuberculosis. Second, a reinfection threshold appears to have a regulatory role in pathogen diversity, leading to our recently proposed mechanism for the unusual patterns of influenza evolution. Third, in diseases where reinfection is essential to the maintenance of acquired clinical immunity (such as pertussis and malaria) interventions that reduce transmission below a reinfection threshold are expected to increase disease prevalence. Reinfection thresholds appear responsible for many unresolved inconsistencies registered in recurrent infections, but perhaps the most exciting is that reinfection thresholds can be manipulated by suitable interventions and used in control planning. This practical implication has never been explored.

## **Dose-response in infection and epidemiological patterns**

Members: Gabriela Gomes, Mostafa Bendahmane

Students and Technicians: Paula Rodrigues

External Collaborators: Alessandro Margheri, Carlota Rebelo (FCUL, Lisbon, Portugal), Graham Medley (University of Warwick, Warwick, UK).

Transmission of infection requires that susceptible individuals encounter the infectious agent, and this is typically through contact with infectious individuals. There is good documentation on dose-response relationships in terms of infection, disease, and immunity. The majority of such empirical studies conclude that there is a minimal dose required to induce a response, and above this threshold the probability of response increases nonlinearly with the dose of exposure. We incorporate these observations into models of transmission, and describe epidemiological patterns not captured by the simpler mass-action transmission models.

## **Population dynamics of varicella-zoster virus: the influence of zoster on the control of varicella by mass vaccination**

Members: Gabriela Gomes, Cristina Paulo

External Collaborators: Divisão de Doenças Transmissíveis (Direcção Geral de Saúde, Portugal).

The National Advisory Group for Vaccination (Comissão Técnica de Vacinação) is considering the approval for the use of varicella (chickenpox) vaccine as already happens in other European countries. As in the case of measles and other infectious diseases, predictions of mathematical models of varicella transmission dynamics can play an important role in the decision to license the vaccine and in the design of a specific vaccination program for the Portuguese population. Initial models were based on a somewhat simplified view of the natural history of varicella infection and did not incorporate shingles. In fact, varicella is a disease caused by the primary infection of the varicella-zoster virus (VZV). After the first infection the VZV becomes dormant in the dorsal root ganglia from where it can reactivate some years later causing herpes-zoster (shingles). This latter disease can have a crucial role in the maintenance of chickenpox in small populations as individuals with zoster can spread the VZV and infect susceptible individuals. Nonetheless very little is known about zoster epidemiology and of its relative infectiousness compared to varicella. Recent mathematical models have incorporated zoster to test some assumptions about its relative importance on the dynamics of VZV. The first specific objective of this project is to compare models already proposed. A second specific objective is to propose a new modeling approach based on old arguments about the relative importance of reinfection *versus* reactivation of zoster on the dynamic of this virus. This is of particular interest since it can give some insight into whether the

dynamics can be influenced by the *reinfection threshold* a very new concept that seems to have a crucial importance to explain for instances observed discrepancies about vaccine efficacy against TB in different populations.

### **Vaccination against diphtheria, tetanus and rubella in Portugal: Seroepidemiological study to assess strategies and support evidence based decisions**

Members: Gabriela Gomes, Guilherme Gonçalves

External Collaborators: Maria Augusta Santos (INSA, Porto, Portugal), Maria de São José Nascimento (FFUP, Porto, Portugal).

Project Description: The aim of the project is to produce evidence to support decisions related with the Portuguese National Vaccination Program (evidence based Public Health); vaccination is a very efficient way to prevent disease and its consequences (morbidity and mortality); vaccination has been safe and cost effective preventing diphtheria, tetanus and rubella. Three specific objectives were established. First, to identify and measure the decrease of protective antibodies (against diphtheria, tetanus and rubella), dependent on the number of doses received and time since last vaccination. Second, to assess vaccine efficacy measured as the increase in specific protective (against diphtheria and tetanus) antibody concentration, and dependent on the previous concentration of antibody and age at vaccination. Third, to assess the efficiency of transplacental transport of antibodies against rubella.

### **Cell Biology: Mitosis, Cytoskeleton, and Stress**

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

#### **Cell cycle dependent regulation of apoptosis and survival**

Member: Sukalyan Chatterjee

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

The regulated coordination of cell division and cell-death is a key determinant for normal physiological functions and cell number homeostasis. Apoptosis (programmed cell death) is a mechanism by which organisms eliminate unwanted or damaged cell. Cells have developed surveillance mechanisms that control the progression through the cell cycle by

assuring that the initiation of one event only occurs after successful completion of the previous one. The evidence pointing to the cross talk between the cell cycle and apoptosis are sparse but not lacking, although the details are still largely unknown. Caspases and pro-apoptotic Bcl-2 family of proteins have been shown to be upregulated in S phase after G<sub>0</sub> arrest. In addition, Bcl-2 and Mcl-1 have been reported to influence and be influenced by the cell-cycle. Moreover, activation of several cell cycle related proteins has been implicated in apoptosis in post-mitotic neurons. This communication between cell cycle regulators and the apoptotic machinery led us to the hypothesis that competence for self-destruction of cells might change along the cycle. The regulation of survival and cell death is a key determinant of cell fate. Recent evidence shows that survival and death machineries are regulated along the cell cycle. We show here that BimEL (a BH3-only member of the Bcl-2 family of proteins) is phosphorylated in mitosis. This post-translational modification is dependent on MEK and growth factor signalling. Interestingly, FGF signalling seems to play an essential role for this process, since in presence of serum, inhibition of FGF receptors abrogated phosphorylation of Bim in mitosis. Moreover, we have shown bFGF to be sufficient to induce phosphorylation of Bim in serum-free conditions in any phase of the cell cycle and also to significantly rescue cells from serum-deprivation induced apoptosis. Our results show that, in mitosis, Bim is phosphorylated downstream of growth factor signalling in a MEK-dependent manner with FGF signalling playing an important role. We suggest that phosphorylation of Bim is a decisive step for survival of proliferating cells.

### **Regulation of proteolysis during mitosis**

Member: Álvaro Tavares

Student: Mariana Faria

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

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

proteins during this stage of the cell cycle. We wish to determine the nature of these proteins, and for such we will use a combined approach of RNAi and MALDI mass spectrometry. In addition, the detailed characterization of the mutant phenotype, using classical genetics and confocal microscopy, will help to clarify the role of those proteins during mitosis (or better say, what happens if they're not degraded) and of the proteosome itself.

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

Members: Álvaro Tavares and José António Belo

Student: Claudia Florindo

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

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

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

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

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

## **Mitotic roles for the Plkk and Mps1 kinases**

Member: Álvaro Tavares

Students: Paulo Alves, Mariana Faria, Dinis Gokaydin.

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

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

Member: Álvaro Tavares

Students: Susana Godinho, Célia Domingues.

Collaborators: David Glover (Cambridge, UK)



The accurate segregation of chromosomes at mitosis is essential for the provision of genetic material to ensure cell viability. Defects in any stage of this process can lead to cell death or, in higher organisms, the development of cancer. Multipolar spindles have often been observed in human cancers in situ as well as an abnormal number of centrosomes. Identification of the molecular targets of centrosome kinases and elucidation of the pathways that regulate centrosome duplication, separation and function provide novel opportunities for therapeutic intervention. We previously showed that the protein kinase polo is a centrosomal kinase, and that is required for the formation of a bipolar spindle and for the proper execution of cytokinesis. We wish to understand how the activity of the polo protein kinase is regulated and how it functions at the level of the centrosomes. We previously found that polo proteins, either from *Drosophila* embryo extracts or from *Xenopus* egg extracts, bind to several proteins forming different stable complexes. We are now on the process of identifying the complexes' components in total embryo extracts and in centrosome preparations. We want to characterize these proteins, sorting which are polo substrates and which are activators. Taking advantage of *Drosophila* genetics we have also searched and isolated new genes required for spindle assembly and centrosome function, some of which coding for proteins with high degree of homology with the *Saccharomyces cerevisiae* proteins. We are now on the process of characterising these genes

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

Members: Lisete Fernandes

Students and Technicians: Alexandra Almeida, Cláudia Bicho, Joana Monteiro

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

In this context, the involvement of Yaps as the target regulatory proteins in both phenomena has been ascertained by addressing (a) the specific role of Yap1 under cold signals as well as (b) the specific role of YAP4 under equivalent conditions.

So far, our data does not support a link between Yap1 (as a transcriptional activator) and processes described in literature as the basis for cold-phenotypes such as defects in transcription, decrease mRNA stability, etc. The study of the specific role of YAP4, under same conditions, was approached by determining the type of transcriptional activity of this regulator as well as by developing a genetic screening to identify genes essential to the cold-phenotype of *yap4* cells. This loss-of-function genetic screening has generated mutants that revert *yap4* cold-tolerance. The identification of such mutations by

complementation assays was performed. In order to determine the functional link between Yap4 and the mutant selected, the later is being functionally characterized.

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

Members: Lisete Fernandes

Students and Technicians: Diogo Fonseca, Joana Monteiro, Marcos Pinho

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

In order to understand the specific signaling downstream of Yap1, we are addressing the study of GTFs as specific targets of oxidative signals generated by hydrogen peroxide. In this context, we have selected GTFs which should be primary targets of Yap1 and thus targets of oxidative stress. Contrary to our predictions, we have found that its gene expression is altered in the absence of Yap1. To further explore the relevance of the GTF on Yap1-mediated transcription, *gtf* mutants with impaired oxidative stress response (but with normal physiological behaviour) have been generated and the mutations were identified by DNA sequencing. Characterization of Yap1 activity in *gtf* mutants' background is being analysed.

### **Study the function and the regulation of tubulin cofactor a expression in mammalian cells submitted to microtubule depolymerising agents**

Members: Helena Soares

Students and Technicians: Sofia Nolasco

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

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

putative targets of microtubules dynamic regulation by controlling the synthesis, flux and transport of tubulin. In this context we are studying the cofactor A functions by RNAi in HeLa and MCF-7 cells. In mammalian cells cofactor A is an essential gene opposite to that observed in yeast cells. Moreover, the cofactor A knockdown induces subtle depolymerisation of microtubules (Mts) as revealed by indirect immunofluorescence; FACS analysis shows that cofactor A RNAi promotes cellular proliferation inhibition by G1 cell cycle arrest and induces apoptosis. In MCF-7 cells the cofactor A knockdown triggers a dramatic cell shape modification resembling differentiation. Additionally, HeLa cells present a blebbing phenotype that is probably associated with early apoptotic events dependent on caspase-3 activity. Interestingly, there is no “marginal” blebbing in MCF-7 cells – defective caspase-3 cell line. We are also investigating if cofactor A is able to respond to an increase of native  $\alpha/\beta$ -tubulin heterodimers pool inside of the cell. The steady-state levels of cofactor A mRNA (studied by real time PCR) and TBCA- $\beta$ -tubulin protein dimer (studied by PAGE analysis) decrease in HeLa cells submitted to different Mt depolymerising agents (cold-shock, colchicine and nocodazol), probably affected by the amount of free native tubulin heterodimers. Interestingly, the steady-state amount of cofactor A protein did not change during the referred treatments. This last observation is probably related to the half-life of the cofactor A protein (>24h under physiological conditions). We also established that the decrease of the amount of the TBCA- $\beta$ -tubulin dimer is a response to the specific increase of the native  $\alpha\beta$ -tubulin heterodimers and not to alterations in the translation machinery provoked by Mt cytoskeleton disorganization. This indicates that tubulin folding pathway is able to respond to an increase of native  $\alpha/\beta$ -tubulin heterodimers pool.

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

Members: Helena Soares

Students and Technicians: Cecília Seixas

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

Cilia and flagella are complex motile and sensory organelles that extend from the cell surface. They generally present a highly ordered basic structure of nine peripheral microtubule (Mt) doublets arranged around of two central Mts forming the axoneme. This structure is nucleated by the basal body anchoring it into the cell body. An external membrane continuous with the cell plasma membrane surrounds the axoneme. The distal end of the central pair and doublet Mts are attached to the membrane via a cap. There are increasing evidences that defects in cilia components are associated with human disorders, therefore understanding how they are assembled and which mechanisms underlie this complex event is crucial.

In ciliate *Tetrahymena* exponentially growing cells we observed by indirect immunofluorescence that the cytosolic chaperonin CCT-subunits, TpCCT $\alpha$ ,  $\delta$ ,  $\epsilon$  and  $\eta$ -are associated with mature cilia. CCT is a hetero-oligomeric complex of about 900 kDa composed of two back-to-back rings, each containing eight different, although related,

gene products. This chaperonin mediates the folding of actin and tubulin. Therefore we hypothesize that CCT-subunits could be involved in transport/protection of tubulin heterodimers that are going to be assembled into the axoneme structure. Alternatively tubulin is transported in a non-native structure and the chaperonin would be necessary to allow *in situ* incorporation of the new heterodimer in the growing axoneme.

To test these hypotheses we investigated in which component of the cilium CCT-subunits were present. Cilia suspension was fractionated into an axonemal fraction, a ciliary membrane fraction (containing cytoplasmic matrix) and a fraction containing the caps. Our preliminary results suggest that CCT- $\alpha$ ,  $\eta$ ,  $\xi$  and  $\delta$  subunits are present in the axonemal and membranar fractions. Using Atomic Force Microscopy (AFM), it was possible to observe the presence of a gradient of antibodies against CCT-subunits along the axoneme. Moreover, in the early stages of cilia assembly, a crater-like structure with 280 nm of diameter and 60 nm of height, composed of 9 units (each containing a Mt doublet) was observed. As cilia grow (90 nm of length, still without cap), 3-fold structures are found on the axoneme wall. Each of the 3 units making up these structures shows the same dimensions of each of the 9 units at the top. Our hypothesis is that the growing axoneme is assembled from these pre-mounted structures, each containing 3 Mt doublet sections, instead of being assembled from individual components (*e.g.* tubulin heterodimers). Probably these structures are mounted in the cell body and then migrate over the outer axoneme wall to their assembly site. These results support the idea that tubulin chaperonin CCT should be involved in this process.

To better understand the role of CCT in cilia biogenesis we decided to perform heterokaryon knockout of CCT- $\alpha$  and CCT- $\delta$  subunits in *T. Termophila*. For this, constructs containing a *neo* cassette and two targeting fragment of 1,5 kb of the target gene were built. Germline shooting experiments were achieved with this cassette, in order to substitute one of the copies of the gene in the micronucleus of the cells. The functionality of this cassette has already being confirmed, by shooting cells in their somatic macronucleus. The somatic transformants obtained are being used to test if the gene is essential and also they will be useful, after rescue with an inducible plasmid, in determining the consequence of the reduction of CCT levels in ciliogenesis.

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

Members : Helena Soares

Students and Technicians: Yara Reis

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

The coccidium *Besnoitia besnoiti* (Apicomplexa: Sarcocystidae) is a cyst-forming parasite responsible for bovine besnoitiosis that causes high losses in the bovine production. The disease is encountered mainly in a chronic form with scleroderma,

seborrhoea and alopecia as predominant clinical signs, accompanied by poor body condition and infertility in males and abortion in females.

A 4 years old cow presenting severe skin lesions characteristic of besnoitiosis was culled. Cysts in subcutaneous tissues were disrupted and merozoites were collected and inoculated in Vero cell cultures. This corresponds to the 3<sup>th</sup> isolation of *Besnoitia* at world level. Differently from the two first ones (Israel and South Africa) propagation of *B. besnoiti* was achieved by this method demonstrating that the use of laboratory animals is not necessary.

The 18S-5.8S-28S rDNA unit of the isolated *Besnoitia* was amplified by PCR and sequenced. The obtained sequences and those available in databases were used to perform a phylogenetic analysis by parsimony and maximum-likelihood methods. This analysis showed that the Portuguese isolated strain is distinguishable from *Besnoitia besnoiti* and other *Besnoitia* species sequences available in databases.

We have also investigated the role of protozoa and host cell microtubule (Mt) cytoskeleton in the first steps of invasion. Using indirect immunofluorescence we observed that the Mt cytoskeleton of *B. besnoiti*, similarly to other known Apicomplexa protozoa, possesses a set of subpellicular Mts spirally arranged that extend from the conoid in the apical end to 2/3 of the cell body. Strikingly, when *B. besnoiti* interact with the host cell we observed a dramatic re-organization of the protozoa Mt cytoskeleton. This is characterized by the loss of the subpellicular Mts giving raise to tubulin globular-like structures. These observations are in agreement with the remarkable morphological changes observed in the surface of the protozoa by atomic force microscopy. Preliminary results show that the treatment of the free protozoa cells with the antimitotic agent Nocodazol does not affect the subpellicular Mts of the parasite maintaining its capacity to invade the host cells. On the other hand, the depolymerization of host cell Mts by the same anti-Mt agent does not inhibit the invasion by the protozoa. Studies are in progress to clarify the relationship between the observed *B. besnoiti* Mt modifications and the initial steps of host cell invasion.

### **Expression and cellular localization of eRF3 in human cells: studies under oxidative stress conditions**

Members: Helena Soares

Students and Technicians: João Gonçalves

External Collaborators : Miguel Brito (Departamento das Ciências Naturais e Exactas, ESTeSL, Lisbon, Portugal); Fernando Antunes (Departamento Química e Bioquímica, FCUL, Lisbon, Portugal).

The eukaryotic release factor 3 (eRF3) is a GTP-binding protein that stimulates the action of the eukaryotic release factor 1 (eRF1) during translation termination. Furthermore, eRF3 has also been implicated in: translation initiation control; polyadenylated mRNA decay; cell cycle progression; cytoskeleton organization, and apoptosis.

In this work, the effect of steady-state H<sub>2</sub>O<sub>2</sub> concentrations on the mRNA and protein levels of eRF3 was studied in HeLa and MCF-7 cell lines. The effect on mRNA steady-state levels was evaluated by the quantification of mRNA by real-time quantitative RT-

PCR with Taqman<sup>®</sup> probes. Alterations in protein steady-state levels were determined by western blotting using polyclonal antisera against eRF3. Additionally, the intracellular localization of eRF3 was studied by cell fractionation followed by western blotting and also by indirect immunofluorescence.

The two cell lines presented different responses to the oxidative stress both in terms of mRNA and protein levels. The amount of eRF3 mRNAs was unaffected by the treatments in MCF-7 while in HeLa it was markedly increased. Besides from being very abundant in the cytoplasm, eRF3 was also observed in the nucleus. In fact, in western blots, several bands corresponding to eRF3 (possible isotypes/isoforms) were observed in the nuclear fraction. In both cell lines the treatment with H<sub>2</sub>O<sub>2</sub> caused no alterations in the cytoplasmatic eRF3 protein levels. In contrary, in nuclear fraction, changes in the protein levels of the different putative isotypes/isoforms were observed in response to the oxidative stress. These modifications were different in the two cell lines studied.

The possibility of eRF3 co-localize with elements of the cytoskeleton was also investigated by indirect immunofluorescence. Interestingly, eRF3 do not co-localize with microtubules, but co-localization was observed with actin microfilaments especially in filopodia.

### **The functional organization of chromatin in the nucleus**

Member: Leonor Parreira

Students: Isabel Alcobia, Ana Sofia Quina and Hélia Neves

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

the variation in the composition of these heterochromatic structures may contribute to the dynamic relocation of genes in different nuclear compartments during cell differentiation, what might have functional implications for cell-stage-specific gene expression (Alcobia et al, submitted Nov. 2002).

### **cDNA Microarray Technology in Diagnosis and Monitoring for Oncology patients**

Members: Sérgio Dias, Jorge Becker

Students and Technicians: Cátia Igreja, Margarida Courinha

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

The initial work aimed at establishing the necessary “circuits” to allow regular collection of tumor samples to be classified and studied further, and also the safe and detailed storage of such samples (the building of a tumour biobank). The first sets of samples analyzed by cDNA array technology were Thyroid, Salivary gland tumours and lymphomas.

## **RESEARCH CONTRACTS**

The research activities at the IGC are supported to a significant level by the National Research Council (Fundação para a Ciência e a Tecnologia, FCT), but also by the European Union and by a few private corporations. All research contracts signed by the IGC that pertained, at least in part, to 2004 or later are listed below. The support from the FCT comes through various mechanisms, all awarded on the basis of competitive applications: (1) institutional support, as positions for scientists and technicians, in the frame of the Laboratório Associado ITQB/IBET/IGC; (2) institutional support, in the frame of the Unidade de Investigação FCT on “Tolerância Natural”; (3) individual support, as fellowships to visiting scientists, post-doctoral fellows, PhD students and technicians (these are indicated in the lists of people at the Institute); (4) group support, as research contracts (listed below); (5) sporadic support for the organization of scientific meetings, as indicated. We are pleased to acknowledge this support as it has become absolutely essential to the activities of the Institute.

### **FCT PROJECTS**

POCTI/BCI/41725/2001

Jörg Becker

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

POCTI/MGI/46477/2002

Jorge Carneiro

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

POCTI 36312/1999

Jorge Carneiro

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

POCTI/BCI/42249/2001

Sukalyan Chatterjee

Molecular mechanisms of microtubule mediated signalling under oxidative stress.

POCTI/ CBO/47565/2002

Sukalyan Chatterjee

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



POCI/43063/MGI/2001

Jocelyne Demengeot

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

POCTI/43411/ BCI/ 2001

Jocelyne Demengeot

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

POCTI/CBO/38391/2001

Sérgio Dias

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

POCTI/MGI/36413/1999

José Faro

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

POCTI/39906/FCB/2001

José Feijó/Dora Brites

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

POCTI/BCI/46453/2002

José Feijó

Uma abordagem genética e molecular à biofísica da comunicação célula-célula durante a reprodução sexuada em plantas.

POCTI/BCI/37862/2001

Lisete Fernandes

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

POSI/SRI/47778/2002

Pedro Fernandes

BioGrid - Parallel Algorithms for Gene Annotation

POCTI/MGI/40478/2001

Pedro Fernandes

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

POCTI/MAT/47510/2002

Gabriela Gomes

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

POCTI/BSE/46856/2002

Isabel Gordo

Population genetics of adaptation in *Escherichia coli*.

POCTI/P/BIO/10091/1998

Matthias Haury

IL-10 GFP Transgenic mice.

POCTI/BCI/41909/2001

António Jacinto

Epithelial dynamics and adhesion during *Drosophila* dorsal closure.

POCTI/BCI/48577/2002

António Jacinto

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

POCTI/MGI/43466/2001

Moisés Mallo

Molecular mediators of *Hoxa2* function during mouse development.

POCTI/MGI/46337/2002

Moisés Mallo

Reversible gene inactivation in the mouse.

POCTI/MGI/385632/2001

Maria Mota

*Plasmodium*-Host interaction during the liver stages of infection.

POCTI/MGI/44517/2002

Maria Mota

The role of HGF during the liver stages of a malaria infection.

POCTI/ SAU-NEU/56986/2004

Teresa Pais/Sukalyan Chatterjee

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

POCTI/BCI/42040/2001

Isabel Palmeirim

New aspects on coordinating limb bud development.

POCTI/MGI/45100/2002

Michael Parkhouse/Ana Crespo

Viral modulation of cell division, apoptosis and interferon responses.

POCTI/NSE/39166/2001

Paula Parra-Bueno

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

POCTI/32576/BCI/2000

Leonor Parreira

The functional organization of the chromatin in the nucleus.

PRAXIS SAU/14000/1998

Leonor Parreira

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

POCTI 36392/1999

Carlos Penha Gonçalves

Genetics of Malaria in wild mouse models.

POCTI/MGI//36369/1999

Sylviane Pied

T- Cell response in pathogenesis of malaria

POCTI/MGI/46719/2002

Sylviane Pied

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

POCTI/45914/BCI/2002

Leonor Saúde

Molecular and Cellular Characterization of Segmentation in the Chick Embryo.

POCTI/34240/1999

Pedro Simas

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

POCTI/ESP/46378/2002

Pedro Simas

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

POCTI/BIA 10097/1998

Helena Soares

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

POCTI/MGI/37296/2001

Miguel Soares

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

POCTI/BIA-BCM/56829/2004

Miguel 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

The molecular genetics of adaptation to octanoic acid in outbred populations of *D. melanogaster*

POCTI/BME/33221/1999

Álvaro Tavares

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

POCTI/BCI/41735/2001

Álvaro Tavares

Functional characterisation of the mitotic kinases DPlkk and DMps1.

POCTI/CBO/39099/2001

Álvaro Tavares

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

POCTI/BIA-PRO/60337/2004

Álvaro Tavares

Characterisation of the mitotic checkpoint in *Drosophila*: function of the proteins Mps 1 and CENP-ana.

POCTI/BSE/48228/2002

Henrique Teotónio

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

POCTI/BCI/40754/2001

Sólveig Thorsteinsdóttir

Extracellular matrix and somitogenesis: causes and consequences.

POCTI/BCI/47681/2002

Sólveig Thorsteinsdóttir

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

POCTI/ESP/39636/2001  
Astrid Vicente  
Genetic Epidemiology of autism.

POCTI/FCB/44706/2002  
Astrid Vicente  
Pharmacogenetics of risperidone therapy in autism spectrum disorders.

## **EUROPEAN UNION PROJECTS**

Marie Curie Fellowship FP6-2002-M-5  
Marie Louise Bergman  
Treg\_invivo\_imaging.

EVR1-CT-2001-40017  
Pedro Fernandes  
BIOCASE – A Biological Collection Access for Europe

IST 1999-20469  
Pedro Fernandes  
EMBER - Design of a European Biocomputing Educational Resource

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

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

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.

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

MIRG-CT-2004-513760  
Sofia Oliveira

Stroke genetics.

QLK2-CT-2001-02216

Michael Parkhouse

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

MGI/36403/99

Michael Parkhouse

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

QLK3-CT-2001-00422

Miguel Soares

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

QLK2-CT-2002-00810

Pedro Simas

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

## **OTHER PROJECTS**

cDNA Microarray Technology in Diagnosis and Monitoring for Oncology patients.

Sérgio Dias

Fundação Calouste Gulbenkian.

Angiogenesis in Prostate Cancer.

Sérgio Dias

Portuguese Society of Urology and from Abbott Pharmaceuticals.

Mechanisms of Endothelial Differentiation from Endothelial Progenitors.

Sérgio Dias

Liga Portuguesa Contra o Cancro.

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

António Jacinto

069880 Wellcome Trust, UK.

AstraZeneca

Miguel Soares

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

NIH RO1: HL67040-01

Miguel Soares

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

GEMI Fund AgaLinde Healthcare

Miguel Soares

Inhaled CO and Multiple Sclerosis.

Research and Training Network

Álvaro Tavares/Thomas Surray (Heidelberg)

Spindle Dynamics

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## SEMINARS AT THE IGC

### January

Michael McMurray (Fred Hutchinson Cancer Research Center, Seattle, USA)  
*Age-induced genomic instability in budding yeast.*

Tânia Reis (Fred Hutchinson Cancer Research Center, Seattle, USA)  
*Negative Regulation of *DE2F1* by CDKs controls cell cycle timing.*

Heinrich Hoerber (Wayne State University, School of Medicine, USA)  
*Nano-Bio-Science, new tools and old structures.*

Moises Mallo (IGC)  
*To build or not to build, that (could be) the question.*

Antonio Alcami (Centro Nacional de Madrid, Universidad Autónoma de Madrid, Madrid, Spain)  
*Modulation of chemokine activity: lessons from viruses.*

Neeta Roy (Cornell University, NY, USA)  
*Progenitors of the adult human white matter - the present and future prospects.*

Jenefer M. Blackwell (Cambridge Institute for Medical Research, Wellcome Trust, UK)  
*From genomes to vaccine in leishmaniasis.*

Gord Fishell (Skirball Institute, NYU School of Medicine, USA)  
*Making up your mind: the assignment of cell fate in the developing telencephalon.*

### February

Guilherme Neves (Whitehead Institute for Biomedical Research, MIT, USA)  
*Individual neurons express distinctive combinations of alternatively spliced *Dscam* isoforms.*

Oscar Marín (Instituto de Neurociencias, Universidad Miguel Hernández & CSIC, San Joan d'Alacant, Spain)  
*Cellular and molecular mechanisms of neuronal migration in the cerebral cortex.*

Pedro Simas (IGC)  
*Viral latency in B cells.*



Justin Crowley (Center for the Neural Basis of Cognition, Carnegie Mellon University, USA)

*Ocular dominance column development revisited: a motivation for time-lapse, in vivo, two-photon imaging.*

Élio Sucena (IGC)

*Evolution of Development: problems and approaches.*

João Barata (IMM/FMUL, Lisbon, Portugal)

*Interleukin-7-mediated signaling in the biology of T-cell acute lymphoblastic leukemia.*

Gonçalo Abecassis (University of Michigan, Michigan, USA)

*The future of association studies in complex diseases.*

## **March**

Ruth Lehmann (Skirball Institute, New York University, USA)

*Navigating the embryo: germ cell migration in Drosophila.*

Fiona Watt (Institute for Cancer Research, UK)

*Regulation of Stem Cell fate in mammalian epidermis.*

Chris Marshall (Institute for Cancer Research, UK/Center of Cell and Molecular Biology, London, UK)

*GTPase signaling pathways in tumor biology.*

Rui Alves (Dept. Ciències Mèdiques, Univ. Lleida, Spain)

*Investigating design at different levels in Molecular Biology.*

Maria João Saraiva (IBMC, Porto, Portugal)

*Towards therapies for protein misfolding disorders: lessons from transthyretin amyloidosis.*

Henrique Teotónio (IGC)

*Studying adaptation with experimental evolution.*

Kevin Marsh (Wellcome Trust Research Labs, Kilifi, Kenya)

*Why don't we know what makes you immune to malaria?*

Florence Janody (Skirball Institute of Biomolecular Medicine, New York University Medical Center, USA)

*A eye specific screen identifies new components involved in transcriptional regulation and cell architecture in Drosophila*

José António Belo (IGC)

*Establishing the embryonic body axes: new players, new concepts.*

Stan Maree (Theoretical Biology and Bioinformatics. Utrecht University, The Netherlands)

*Cell motility driven by actin polymerization: a multiscale modeling.*

Ana Paula Godinho Coutinho (IGC)

*Taking science beyond the IGC gates: an 18 month review.*

## **April**

Juha Partanen (Institute of Biotechnology, University of Helsinki, Finland)

*Intercellular communication between developing mid- and hindbrain.*

Greg King (Univ. Warwick, Warwick, UK)

*Why is there so LITTLE carbon dioxide in the atmosphere?*

Nelson Saibo (Gent University, Belgique/ITQB/UNL)

*Hormonal effects on Arabidopsis thaliana hypocotyl growth.*

Krista Rombouts (Universita degli Studi Firenze, Firenze, Italy)

*Liver Fibrosis - from the bench to clinical targets.*

Momtchilo Russo (Universidade S. Paulo, S. Paulo, Brazil)

*Immunomodulation of experimental asthma.*

Dinis Calado (IGC)

*Regulation of IL-10 Gene Expression at the Allele Level.*

Pedro Coutinho (IGC)

*P. falciparum multigene families and selection pressures.*

## **May**

Matthias Haury (IGC)

*High Speed Cell Sorting*

Paul McNeil (Medical College of Georgia, USA)

*How cells reseal and what happens when they cannot.*

Gabriela Gomes (IGC)

*Reinfection thresholds and epidemiological puzzles.*

Herbert Steinbeisser (Institute for Human Genetics, Medical School, University of Heidelberg, Germany)

*Shaping the vertebrate body: regulation of morphogenesis in the Xenopus embryo*

Ana Catarina Moreira dos Santos (IGC)

*Isoprenylation and cell attraction: From Yeast to Zebrafish.*

Paula Almeida Coelho (IBMC, Porto, Portugal)

*Mitotic Chromosomes: behind the structure.*

Christiana Ruhrberg (University College London, UK)

*Shared signals control neuronal migration and vascular patterning in the developing brain.*

## **June**

Isabel Gordo (IGC)

*To be or not to be imperfect? Dynamics of mutators in bacterial populations.*

Fernanda Bajanca (FCUL, Lisbon, Portugal/IGC)

*Myotome formation in the mouse: Does the extracellular matrix play a role?*

Mario Delgado (Instituto de Parasitologia y Biomedicina, Granada, Spain)

*VIP: a "very important peptide" in immunology. Potential therapeutic applications.*

Marilia Cascalho (Mayo Clinic College of Medicine, USA)

*B cells and immunoglobulin promote T cell receptor diversification.*

Suzanne Bourgeois (The Salk Institute, California, USA/IGC)

*Nutrition and Cancer.*

Stuart Marshall-Clarke (Univ. Liverpool, Liverpool, UK)

*Flu takes its Toll(s).*

Ana Catarina Certal (IGC)

*Proton Homeostasis and Regulation of Growth in Pollen Tubes.*

Edgar Cruz e Silva (Universidade de Aveiro, Aveiro, Portugal)

*Characterization of the PPI interactome in human testis.*

Iris Caramalho (IGC)

*Regulatory CD4 T cell function and dynamics in inflammation.*

## July

Alfonso Martinez Arias (University of Cambridge, UK)  
*Modulation of Wnt signalling by Notch: rhyme and reason.*

Sérgio Dias (CIPM-IPO, Lisbon, Portugal/IGC)  
*Endothelial Differentiation.*

Samuel Aparício (University of Cambridge, Cambridge, UK)  
*A reverse genetics screen in mice for new mammalian physiology.*

Gregory Jefferis (University Stanford, USA)  
*How the Fly brain connects to the Fly Nose.*

Jody Rosenblatt (MRC Laboratory for Molecular Cell Biology, London, UK)  
*Cell death and division: new roles for myosin II.*

Mike Reid (Cancer Research UK, Lincoln's Inn Field, London, UK)  
*Live imaging of the inflammatory response in Zebrafish.*

## September

Filipa Moraes (IGC)  
*The role of Tbx1 in mouse ear development.*

José Maria Alvarez Mosig (Instituto de Ciências Biomédicas da Universidade de São Paulo, Brazil)  
*The murine infection by Sylvio X10/4 clone of T. cruzi: a model of human chronic Chagas disease.*

Erwan Michard (Max-Planck Institute for Mol.Plant Physiol., Germany)  
*Functional characterization of a plant K<sup>+</sup> channel: the Arabidopsis AKT2 channel and its unique phospho-regulation.*

Jorge Vieira (IBMC, Porto, Portugal)  
*Drosophila americana: adaption at the nucleotide, chromosomal and genome level.*

Clemens Utzny (INSERM, Institut Claude de Preval, Toulouse, France)  
*Receptor triggering and specificity encoding: tales of T cell activation?*

Fredrik Ivars (Lund University, Sweden)  
*Regulatory T cells and Tolerance.*

Alan Perelson (Los Alamos National Laboratory, USA)  
*HIV Infection: Where do we stand with respect to therapy and vaccination.*

Rik Korswagen (Univ. Medical Center, Utrecht, The Netherlands)

*Wnt signaling in C.elegans: regulation of neuroblast migration and a novel function of beta-catenin in FoxO signaling.*

## **October**

Takeshi Watanabe (Riken Research Center for Allergy and Immunology, Japan)

*News from the Far East.*

Salvatore Valitutti (INSERM, Institut Claude de Prével, Toulouse, France)

*Immunological synapse: a manifestation of inter-cellular information transfer.*

Guilherme Gonçalves (Min. Saúde, Portugal/IGC)

*Epidemiology, Field Epidemiology and Public Health.*

Alexandre Akoulitchev (Exeter College, Sir William Dunn School of Pathology, University of Oxford, Oxford, UK)

*Non-coding RNA in transcription: from initiation to termination.*

Ana Cristina Borges (IGC)

*The role of cerberus-like genes in embryonic axes formation.*

Henry Tuckwell (IGC)

*Some properties of a discrete time stochastic epidemic model of SIR type*

*University of California, San Diego, USA.*

## **November**

Santiago Zelenay (IGC)

*Revealing cryptic regulatory T cells.*

Adam Chippindale (Queen's University, Canada)

*Gender Load: The cost of genetic conflict between the sexes.*

Georgios Pyrowolakis (Biozentrum, Basel University, Switzerland)

*Nuclear interpretation of BMP/Dpp signaling in Drosophila.*

Alcino Silva (Brain Research Institute, UCLA, USA)

*Molecular and Cellular mechanisms of information acquisition and storage.*

Miguel Seabra (Imperial College, London, UK/IGC)

*Rab GTPases and intracellular membrane organisation: players, mechanisms and mouse models of human disease.*

Luisa Mota Vieira (Hospital do Divino Espírito Santo, Ponta Delgada, Azores/IGC)

*Population genetics in Azores: What can we learn from surnames and Y-chromosomes.*

Iain Hagan (University of Manchester, Manchester, UK)

*Coupling cell division with the environment: p38 stress pathways regulate MPF via Polo at the spindle pol.*

Margarida Vigário (IGC)

*Regulatory T cells in experimental cerebral malaria.*

## **December**

Vicente Andrés (Instituto de Biomedicina de Valencia, Valencia, Spain)

*Cell cycle control, microarray analysis and atherosclerosis: Lessons learnt from induced murine models.*

Marta Barreto (IGC)

*Identification and characterization of genetic susceptibility factors for systemic lupus (SLE).*

Stephen Proulx (Center for Ecology and Evolutionary Biology, University of Oregon, USA)

*The evolution of Genome Complexity: The Rise and Proliferation of Genetic Interactions.*

Edward Feil (University of Bath, Bath, UK)

*Reconstructing micro-evolutionary events in bacteria.*

Jerry Turnbull (University of Liverpool, Liverpool, UK)

*Heparan Sulfate - Decoding the Functions of a Dynamic Cell Regulator in Development and Disease.*

Maria Manuel Mota (Presidente da Associação Viver a Ciência/IGC)

*A Associação Viver a Ciência*

Luis V. Rizzo (Instituto de Ciências Biomédicas, Universidade de S. Paulo, Brazil)

*Cytokines in autoimmunity.*

Vasco Barreto (Rockefeller University, USA)

*Activation-induced cytidine deaminase (AID): learning from the C terminus.*

Luis Rocha (Indiana University, USA/IGC)  
*The foundation of a co-Laboratorium*

**Conference Series “Este Mundo e o Outro”**  
**Organisation: Leonor Parreira**

**April**

Diogo de Lucena (FCG, Lisbon, Portugal)  
*O "Homo Oeconomicus".*

Luis Sobrinho (IPO, Lisbon, Portugal)  
*Prolactin and maternal behaviour-adaptation and mal-adaptation-a tale of two cultures.*

**May**

Manuel Castro Caldas (AR.CO, Lisbon, Portugal)  
*Ineffable Monuments.*

Vasco Graça Moura (Member of the European Parliament, Novelist, Literary Translator and Poet)  
*Poetry and Painting in Portuguese Literature.*

**June**

Máximo Ferreira (Museu da Ciência, Lisbon, Portugal)  
*De Ptolomeu ao Mundo de Hoje.*

**July**

Jorge Dias de Deus (IST, Lisbon, Portugal)  
*O melhor dos Mundos.*

Ana Sousa Dias (RTP, Lisbon, Portugal)  
*Última Hora.*

## TEACHING

### **POST-GRADUATE EDUCATION**

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

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Ricardo Miguel Neto da Silva  
Daniel Vieira Noro e Silva Sobral  
Bernardo Figueiredo Luís Miranda de Távora

**Gulbenkian PhD Programme in Biomedicine for 2004**

**5-16 Jan: Immunology**

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

Jorge Carneiro (IGC, Oeiras, Portugal)

**19-29 Jan: Host Pathogen Interaction**

Organiser: Mike Parkhouse (IGC, Oeiras, Portugal)

John Skehel (National Institute for Medical Research, London, UK)

Tony Minson (University of Cambridge, Cambridge, UK)

John McCauley (Institute for Animal Health, Berkshire, UK)

Linda Dixon (Institute Animal Health Pirbright Lab, Surrey, UK)

Covadonga Alonso (INIA, Madrid, Spain)

Antonio Alcami (University of Cambridge, Cambridge, UK)

Pedro Simas (IGC, Oeiras, Portugal)

Murray Selkirk (Imperial College of Science, Technology & Medicine, London, UK)

Jennefer Blackwell (Wellcome Trust Centre, Cambridge, UK)

Sylviane Pied (Institut Pasteur, Paris, France/IGC, Oeiras, Portugal)

Margarida Vigário (IGC, Oeiras, Portugal)

Maria Mota (IGC, Oeiras, Portugal)

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

Organiser: Gordon Fishell (Skirball Institute, NYU Medical Center, NY, USA)

Oscar Marín (Instituto de Neurociencias, Univ. Miguel Hernández, Alicante, Spain)

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

Organiser: Miguel Vaz Afonso (Max-Planck Institute, Martinsried, Germany)

Rosalina Fonseca (Max-Planck Institute, Martinsried, Germany)

Guoping Feng (Duke University, Durham, USA)

Justin Crowley (Carnegie Mellon Univ., Pittsburg, USA)

Karim Nader (McGill University, Montreal, Canada)

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

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

Allen Braun (NIH, Bethesda, USA)

Alex Martin (NIH, Bethesda, USA)

William Tecumseh Fitch (Univ. of St. Andrews, Fife, UK)

Miguel Castelo-Branco (IBILI, Faculdade de Medicina, Coimbra, Portugal)

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

Organiser: Carlos Penha Gonçalves (IGC, Oeiras Portugal)

Gonçalo Abecasis (Center for Statistical Genetics, Ann Arbor, USA)

Susana Campino (IGC, Oeiras, Portugal)

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

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

Chris Marshall (Institute of Cancer Research, London, UK)

Fionna Watt (Keratinocyte Lab., London Research Institute, London, UK)

Paula Gameiro (IPOFG, Lisboa, Portugal)

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

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

**15-17 Mar: Evolution**

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

**18-19 Mar: Epidemiology**

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

**22-26 Mar: Theoretical Biology**

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

**29 Mar-2 Apr: Genomics & Bioinformatics**

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

**6-7 April: Science Communication**

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

**14-16 April: Chemical Genetics**

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

**1-2 Sept: Introduction to Biology**

Organiser: Jorge Carneiro (IGC, Oeiras, Portugal)

**3-8 Sept: Evolution**

Organiser: Francisco Dionísio (IGC, Oeiras, Portugal)  
Faculty: Gregory Velicer (Univ. Tuebingen, Germany)  
Arcadi Navarro (Unitat de Biologia Evolutiva, Univ. Pompeu Fabra, Barcelona, Spain)

**Tools and Basics: Techniques**

Organiser: Sukalyan Chatterjee (IGC, Oeiras, Portugal)

**8 Sept: Introduction to Macromolecules**

Faculty: Lisete Fernandes (IGC, Oeiras, Portugal)

**9 Sept: Protein purification & analysis, Enzyme activity**

Faculty: Christophe Gregoire (IGC, Oeiras, Portugal)

**10 Sept: Spectroscopy**

Faculty: Ricardo Franco (ITQB, Oeiras, Portugal)

**Recombinant DNA & Nucleic acid analysis**

Faculty: Lisete Fernandes (IGC, Oeiras, Portugal)

**13 Sept: Structure**

Faculty: Vítor Paixão (ITQB, Oeiras, Portugal)

Margarida Archer (ITQB, Oeiras, Portugal)

**14 Sept: Imaging**

Faculty: Peter Clark (Imperial College, London, UK)

**15-17 Sept: Cell biology**

Faculty: Susana Godinho (IGC, Oeiras, Portugal)

Célia Domingues (IGC, Oeiras, Portugal)

Paulo Alves (IGC, Oeiras, Portugal)

Mark Seldon (IGC, Oeiras, Portugal)

**20-24 Sept: Fly, Fish, Worms, Mouse & Human genetics**

Organiser: António Jacinto (IGC, Oeiras, Portugal)

Faculty: Hendrik Korswagen (Hubrecht Laboratory, Utrecht, The Netherlands)

Astride Vicente (IGC, Oeiras, Portugal)

Jorge Vieira (IBMC, Porto, Portugal)

Jörg Becker (IGC, Oeiras, Portugal)

José António Belo (IGC, Oeiras, Portugal)

Leonor Saúde (IGC, Oeiras, Portugal)

Marta Barreto (IGC, Oeiras, Portugal)

Sérgio Simões (IGC, Oeiras, Portugal)

Ana Catarina Santos (IGC, Oeiras, Portugal)

**28-30 Sept-: The dynamic Nucleus**

Organiser: Sukalyan Chatterjee

Faculty: Ana Sofia Quina (IGC, Oeiras, Portugal)

José Braga (IMM, Lisboa, Portugal)

Dinis Calado (IGC, Oeiras, Portugal)

**1-8 Oct: Transcription**

Organiser: Sukalyan Chatterjee (IGC, Oeiras, Portugal)

Faculty: Patrick Cramer (Gene Centre, Univ. of Munich, Munich, Germany)

Andreas Ladurner (EMBL, Heidelberg, Germany)

Maria Pia Cosma (TIGEM, Naples, Italy)

Richard Festenstein (Imperial College, London, UK)

Lisete Fernandes (IGC, Oeiras, Portugal)

**10-12 Oct: Replication, Recombinant and Telomere Biology**

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

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

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

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

**13-15 + 18-19 Oct: mRNA Biogenesis**

Organiser: Margarida Gama-Carvalho (IMM, Lisboa, Portugal)

Faculty: Alexandre Akoulitchev (Sir William Dunn School of Pathology, Oxford Univ. Oxford, UK)

Alexandra Moreira do Carmo (IBMC, Porto, Portugal)

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

Noélia Custódio (IMM, Lisboa, Portugal)

José Rino (IMM, Lisboa, Portugal)

Anita Gomes (IMM, Lisboa, Portugal)

**20-22 + 25-26 Oct: Advanced Protein Chemistry**

Organiser: Joerg Hoehfeld (Institute for Cell Biology, Bonn, Germany)

Faculty: Joerg Martin (Max-Planck-Institute, Tuebingen, Germany)

Thomas Langer (Institute of Genetics, Cologne, Germany)

**8-12 Nov: Apoptosis**

Organiser: Yuri Lazebnik (Cold Spring Harbor Laboratory, Cold Spring Harbor, USA)

Faculty: Michael Hengartner (IMB, University of Zurich, Switzerland)

David Vaux (The Walter and Eliza Hall Institute, Victoria, Australia)

Scott Kaufmann (Mayo Clinic, Rochester, USA)

**15-19 Nov: Cell fate decisions & stem cell biology**

Organiser: Tariq Enver (John Radcliffe Hospital, Oxford, UK)

Faculty: William A. Harris (Dept. of Anatomy, Univ. of Cambridge, Cambridge, UK)

Matthew Loose (The Institute of Genetics, Nottingham Univ., Nottingham, UK)

Roger Patient (John Radcliffe Hospital, Oxford, UK)

**22-26 Nov: Cell cycle**

Organiser: Álvaro Tavares (IGC, Oeiras, Portugal)

Faculty: Iain Hagan (Univ. of Manchester, UK)

Jonathon Pines (Wellcome/CRC Cambridge, UK)

Cláudio Sunkel (IBMC, Porto, Portugal)  
Susana Godinho (IGC, Oeiras, Portugal)  
Mariana Faria (IGC, Oeiras, Portugal)

**1-3 Dec: Integrated Cell Biology I - Motility & Cell Adhesion**

Organiser: Vânia Braga (Imperial College, London, UK)  
Faculty: Anne Ridley (Ludwig Institute, London, UK)

**8-10 Dec: Integrated Cell Biology II - Organelles**

Organiser: Graça Raposo (Institut Curie, Paris, France)  
Faculty: Evelyne Coudrier (Institut Curie, Paris, France)

**13 Dec: Integrated Cell Biology III – Cell Signalling**

Organiser: Marcus Thelen (Institute for Research in Biomedicine, Bellinzona, Switzerland))

**Annual Meeting of PGDB in Porto  
16-20 December 2004**

Tradition was broken this year. For the first time the Annual Meeting of the PGDB was not held in Curia in September but in Porto in December. And it was a joint meeting of the three doctoral programmes on areas of Biomedical Sciences running in Portugal. From the 16<sup>th</sup> to the 20<sup>th</sup> December students from GABBA (Graduate Program in Areas of Basic and Applied Biology, University of Porto), PDBEB (Doctoral Programme on Experimental Biology and Medicine, University of Coimbra) and PGDB (Gulbenkian PhD Programme in Biomedicine, gathered together to present their work, exchange their experiences and discuss science.

Oral presentations (50 from PGDB students in a total of 93) were held in plenary and simultaneous sessions and PGDB-4 students presented posters (15 in a total of 39) as part of their requirements for passing the first year of the graduate programme. The posters were kept for the whole duration of the meeting, enabling frequent daily discussions around them.

Members of the PGDB research review committee (António Jacinto, Vasco Barreto, Miguel Castelo-Branco, Miguel Godinho Ferreira and Isabel Palmeirim) followed closely all the presentations and provided feedback to the PhD programme Executive.

There were two keynote conferences by invited speakers: Professor Matthias Hentze, EMBL, Heidelberg, on “Internationality, Interdisciplinarity and Independence: Advanced training at the EMBL”, and Professor Mary Ritter, Imperial College, London, UK, on “Creating a Graduate school within a University”, both addressing the very important issue of Graduate Program Models, leading to fruitful discussion within that special session.

Many important visitors kindly participated in the Meeting: the President of FCT (Foundation for Science and Technology), Professor Ramôa Ribeiro, Professor Diogo de Lucena (FCG board member); Dr<sup>a</sup> Idalina Salgueiro (FLAD) and various senior academic representatives of Portuguese Universities.

### **INTERNAL PHD PROGRAMME (PDIGC)**

#### **Director**

Sérgio Gulbenkian

#### **Staff**

Maria Matoso

The aim of the PDIGC is to provide a research training environment at the IGC so that PhD students can develop the skill and knowledge to contribute to research as professionals. The Programme encourages creativity, critical reflection, conceptual development and professional competence, judgement and confidence.

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

### **Annual Meeting of PDIGC 27-29 October 2004**

The second annual PDIGC meeting took place at the IGC from 27<sup>th</sup> to 29<sup>th</sup> October 2004. The scientific sessions included presentations from 56 students and plenary lectures given by Prof. Martin Raff (UCL, London, UK) who talked about his scientific life and Prof. Leonor Parreira (IGC/IMM, Lisbon, Portugal) who talked about Stem cells.

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

The intensity of the PGBIOINF (see below) absorbed most of current resources and time. Yet, the IGC organized one introductory course on bioinformatics, under the topic “Biological Sequence Analysis”. The course took place from December 13<sup>th</sup> to 17<sup>th</sup>, for an attendance of 20 students, 14 of which from the IGC. Faculty: David P. Judge, Department of Genetics, University of Cambridge, Cambridge, UK and Lisa Mullan, European Bioinformatics Institute, Hinxton, UK. Isabel Marques assisted on the practicals.

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

The third edition of the programme started on the 8<sup>th</sup> of September. There are now 15 students in PGBIOINF. The PGBIOINF course that was taught at the IGC was Introduction to Bioinformatics.

Faculty: Pedro Fernandes and Mário G. Silva .

Earlier in 2004, Part B of the second edition took place in the IGC. 12 advanced seminars took place and the 8 students of that edition completed the course in due time.

### **Courses:**

March 22-25

Functional and Comparative Genomics

Faculty: Luciano Milanesi, ITB, Milano, Italy

March 29 - April 2

Population Dynamics and Epidemiology

Faculty: Gabriela Gomes, IGC, Oeiras, Portugal

Manuel Carmo Gomes, FCUL, Lisboa, Portugal

April 5-8

Gene Identification and Prediction

Faculty: Enrique Blanco Garcia, IMIM, Barcelona, Spain

Charles Chapple, IMIM, Barcelona, Spain

April 19-23

Protein Structure and Function Prediction

Faculty: Michael Tress, CNB, Madrid, Spain

Manuel Gomez, Madrid, Spain



April 26-30

Genetic Expression and Microarrays

Faculty: Joaquin Dopazo, CNIO, Madrid, Spain

Juan M. Vaquerizas, CNIO, Madrid, Spain

May 3 - May 7

Population Genetics

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

Octavio Paulo, FCUL, Lisbon, Portugal

May 10-14

Gene Ontology

Faculty: Robert Stevens, Univ. Manchester, Manchester, UK

Amelia Ireland and Helen Parkinson, European Bioinformatics Institute, Hinxton, UK

May 24-28

Data Mining and Data Warehousing

Faculty: Christian Blaschke and José M. Fernandez, CNB, Madrid, Spain

June 1-4

Phylogenetics and Molecular Evolution

Faculty: Hernan Dopazo, CNIO, Madrid, Spain

June 7-9

Proteomics, Transcriptomics and Metabolomics

Faculty: Luciano Milanesi, ITB, Milan, Italy

Alexander Kel, Biobase, Hannover, Germany

June 14-18

Limits and Expectations in Bioinformatics

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

Pedro Fernandes and Ana Sofia Figueiredo, IGC, Oeiras, Portugal.

June 21-24

Genetic Analysis

Faculty: Astrid Vicente, C. Fesel, M. Barreto, A. Coutinho, C. Correia, R. Ferreira (IGC, Oeiras, Portugal)

## SCIENCE AND SOCIETY

The science communication programme at the IGC targets four different audiences: science journalists, schools (teachers and pupils), scientists and the public. Although specific activities are developed, tailored to each audience, the underlying rationale to the entire programme is the promotion of *engagement* in science, through direct, two-way communication.

### The Media

#### *Scientists-Journalists Club*

The aims of these informal meetings between science journalists and research scientists are:

- To discuss, within context, the background to the most recent findings in the life sciences;
- To discuss the social, ethical, political and economic implications of life science research;
- To establish a network of journalists and scientists, thus fostering communication links and better understanding between the two groups (Peters, 1999).

Table I summarizes the meetings held in 2004.

**Table 1.** Scientists-journalists Club meetings held in 2004.

Date	Topic	Scientists	Journalists
8 <sup>th</sup> January	Inflammation: infections, arteriosclerosis and auto-immune disease	Miguel Soares (IGC)	JN, Grande Reportagem, Público, Visão
4 <sup>th</sup> February	Gene chips: making the most of the mountain of available genetic information	Jorg Becker (IGC)	JN, Grande reportagem, Público, 2010 (2x) Focus
3 <sup>rd</sup> March	The evolution of Bacteria, antibiotic resistance and multi-resistance	Francisco Dionísio, Isabel Gordo (IGC)	Radio
25 <sup>th</sup> March	Genetically modified plants	Margarida Oliveira (ITQB)	Freelancer

#### *Press Office duties*

These include liaison with the media, preparing and sending out press releases and organising press conferences. The aim is to provide journalists with media subsidies, in

the form of press releases, to aid in their task of reporting the research undertaken at the IGC. Selected press releases have been placed online at AlphaGalileo, the European science news agency.

### **Science Teachers**

#### *Biology in Modern Times Seminars (Ciclo de Conferências Biologia dos Tempos Modernos)*

These seminars, held every other month during the school year, were targeted at secondary school teachers. The main aims are:

- Updating teachers on the latest developments in life science research both in terms of concepts and methods;
- Fostering the teachers' interest and enthusiasm in scientific research and in science in general;
- Building a network of scientists (from graduate students to senior scientists) and school teachers, looking to future collaborations.

**Table 2** summarizes the sessions held during the 2003/04 school year. Each session was made up of three seminars, by three different scientists, grouped by general themes. Between 40 to 60 teachers were present in each session.

**Table 2.** Biology in Modern Times seminars during the 2003/04 school year.

Date	Theme	Titles
21st Nov 03	Immunology	The cells that make up their DNA (António Coutinho, IGC) Natural and pathological auto-immunity (Jorge Carneiro, IGC) Inflammation and the immune response (Miguel Soares, IGC)
16th Jan 04	Evolution	Studying adaptation through experimental evolution methods (Henrique Teotónio, IGC) Evolution and Development: a case of morphological convergence (Élio Sucena, IGC) The evolution of the Y chromosome (Isabel Gordo, IGC) Epidemiology and evolution of the flu virus (Gabriela Gomes, IGC)
19 <sup>th</sup> Mar 04	Biotechnology	Therapeutical applications of animal cell technology (Helder Cruz, ECBio) The 'omic' revolution (Miguel Silva Santos, STAB Vida) Portuguese solutions to applied biological projects (Luís Amado, APBio)
21 <sup>st</sup> May 04	Developmental	A step towards the next generation in the fruit fly,

	Biology	Drosophila melanogaster (Ana Catarina Santos, IGC) The segmentation clock in vertebrates (Leonor Saúde, IGC) Animal models of human genetic disease (José António Belo, IGC)
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### *'Inspirar Ciência' Workshop for teachers*

In order to better contribute to the social, economic and cultural well-being of a nation, the public's engagement in science should begin at school, involving both students and their teachers. Students should be encouraged and enthused to learn more about the scientific and technological developments that play such an important role in modern societies.

Along these lines, the Instituto Gulbenkian de Ciência (Portugal), the Instituto de Medicina Molecular (Portugal) and the European Molecular Biology Laboratory (Germany) organised the *Inspirar Ciência* workshop on Developmental Biology, with the aim of bringing scientists and school teachers together. A total of 12 school teachers, from all over Portugal, took part in the workshop, held at the IGC, from 13-15<sup>th</sup> of April 2004. Several topics and the most recent techniques related to Developmental Biology were covered, including a debate on the social implications of animal research. The workshop had a strong practical emphasis, and care was taken to design and perform experiments which are easily transferable to the classroom.

The topics covered during the workshop, and the participating scientists, are listed in Table 3.

**Table 3.** Topics and scientists of the Inspirar Ciência workshop for Biology teachers.

Topic	Scientists
Introduction to Developmental Biology	Ana Cristina Borges (IGC)
Reproduction in plants	Leonor Boavida (IGC)
The cell cycle	Álvaro Tavares with Célia Domingues, Cláudia Florindo, Mariana Faria e Susana Godinho (IGC)
Drosophila melanogaster: genes and phenotypes	António Jacinto with Sérgio Simões, William Wood, Ana Catarina Santos e Beatriz Fernandez (IGC)
Teaching resources on the Web	António Jacinto (IGC) and Francisca Menezes Ferreira (IMM)
Vertebrate embryonic development	Alexandra Manaia (EMBL) with Sofia Rodrigues, Leonor Saúde, Joaquín León (IGC)

Round Table discussion: The use of animals in life science research

Ana Godinho Coutinho (Chairperson, IGC)  
Participants: António Coutinho (IGC), Maria do Carmo Fonseca (IMM), Andrew Moore (EMBO), Selene Veiga (Direcção Geral Veterinária), Dolores Bonaparte (IGC)

**Organisers:**

Ana Godinho Coutinho (Instituto Gulbenkian de Ciência, Portugal)

Ana Cristina Borges (Instituto Gulbenkian de Ciência, Portugal)

Sofia Rodrigues (Instituto Gulbenkian de Ciência, Portugal)

Francisca Menezes Ferreira (Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Portugal)

Alexandra Manaia (European Learning Laboratory for the Life Sciences, European Molecular Biology Laboratory, Alemanha).

*Communications at local schools*

By invitation from the schools, the following communications were made, at schools in the Lisbon area:

‘O cientista neutro: não mais?’, Seminar, Núcleo de Filosofia, 12th March 2004, Escola Secundária Artística António Arroio, Lisbon, Portugal.

‘Why learn science? A scientist’s point of view’. Round-table discussion, 1º Encontro Nacional de Núcleos de Estágios de Física e Química, 23rd April 2004, Escola Secundária, Queluz, Lisbon, Portugal.

**The Public**

*‘Dialogar Ciência’ debate*

As a direct outcome of the weekend conference with IGC scientists and Oeiras residents, a public debate with IGC researchers was organised and held, by invitation from the Oeiras council. The debate took place in the auditorium of the Oeiras Public Library on 24<sup>th</sup> January 2004.

The panellists were selected from IGC researchers so as to represent scientists at different stages of their careers, from undergraduate to director of the institute.

**Participants:**

Ana Filipa Simões (BSc student; Haematopoiesis Lab)

Daniel Carapau (PhD Student; Malaria Cell Biology Lab)

Isabel Gordo (Post-doc; Population Genetics Lab)

José Feijó (Group leader; Plant Development Lab)

José Mário Leite (Deputy-Director)

Ana Coutinho (Science Communications Manager)

### *Science writing*

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

- ‘A gas, Viagra and reproduction in plants’, *Fundação Calouste Gulbenkian Newsletter*, Issue 57, October 2004, Fundação Calouste Gulbenkian, Lisbon
- ‘Inflammation and protective genes: the common denominators in cardiovascular and auto-immune disease’, *Fundação Calouste Gulbenkian Newsletter*, Issue 56, September 2004, Fundação Calouste Gulbenkian, Lisbon
- ‘Science communication in a culturally diverse world’, *Fundação Calouste Gulbenkian Newsletter*, Issue 55, July-August 2004, Fundação Calouste Gulbenkian, Lisbon
- ‘Manipulating genes: switching them off, then on, and then off again, at will’, *Fundação Calouste Gulbenkian Newsletter*, Issue 54, June 2004, Fundação Calouste Gulbenkian, Lisbon
- ‘Mathematics and epidemiology in the battle against tuberculosis’, *Fundação Calouste Gulbenkian Newsletter*, Issue 53, May 2004, Fundação Calouste Gulbenkian, Lisbon
- ‘Cell division: making it just right’, *Fundação Calouste Gulbenkian Newsletter*, Issue 52, April 2004, Fundação Calouste Gulbenkian, Lisbon
- ‘On the heels of the herpesvirus’, *Fundação Calouste Gulbenkian Newsletter*, Issue 51, March 2004, Fundação Calouste Gulbenkian, Lisbon

### **School Visits at the IGC (2004)**

Colégio São Martinho (Coimbra)	40
Esc.Sec. Bocage (Setúbal)	36
Esc. Sec. Amadora	20
Esc.Sec. Bocage (Setúbal)	36
Colégio Sagrado Coração Maria (Lx)	21
Esc. Sec. Caminha	19
Colégio Sagrado Coração Maria	24
Esc.Sec. Sobral Monte Agraço	22
Colégio Académico (Lisboa)	15
Esc.Sec. Júlio Dantas (LAGOS)	65
Esc.Ant.SenaFaria Cast.-Branco	30
Esc Sec. de Odivelas	27
Esc.Sec.P. Ant. Macedo - Stº André	50
Saint Dominic's	14
Colégio Sagrado Coração Maria (Lx)	28
Colégio Sagrado Coração Maria (Lx)	14
Saint Dominic's	15
Esc.Sec. Francisco Simões (Almada)	35
<b>TOTAL 18 visits; 12 schools</b>	<b>511 students</b>

## **SYMPOSIA, CONFERENCES AND MEETINGS ORGANISED BY THE IGC**

**EMBO/GBC Plant Development  
Instituto Gulbenkian de Ciência  
22 March-7 April 2004**

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

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

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

**EMMA Meeting  
Instituto Gulbenkian de Ciência  
3-19 June 2004**

**Organisers: Moisés Mallo (IGC, Oeiras, Portugal) and Dolores Bonaparte (IGC, Oeiras, Portugal).**

The European Mouse Mutant Archive (EMMA) is a European Community funded consortium aimed at the preservation and distribution of mutant mouse strains created in the countries of the European Union. The member of this consortium, which include the “Consiglio Nazionale delle Ricerche” (Monterotondo, Italy), the “Centre National de la Recherche Scientifique” (Orleans, France), The “Medical Research Council” (Harwell, UK), the “Karolinska Institutet” (Stockholm, Sweden), the “National Research Centre for Environment and Health” (Munich, Germany), the “European Bioinformatics Institute” (Hinxton, UK) and the “Instituto Gulbenkian de Ciência” (Oeiras, Portugal), meet four times per year to evaluate the progress of the enterprise, discuss technical problems, design new strategies and standardize operating procedures and quality controls among the members. The meeting held in June was one of the four annual meetings and included the Technical Working Group and the Database Group.

**EMBO/FEBS Workshop on AFM Applications in Biology**

**Instituto Gulbenkian de Ciência**

**6-9 de July 2004**

**Organisers: Helena Soares (IGC/ESTeSL, Lisbon, Portugal), Luis Melo (IST, Lisbon, Portugal), Pedro Brogueira (IST, Lisbon, Portugal) and Heinrich Höerber (Wayne Medical School, Wayne State Univ., Detroit, USA).**

AFM is a powerful technique with a wide range of applications in biology, allowing not only for imaging but also for different types of probing and manipulation at nanometric scale. New outstanding applications are reported almost everyday. AFM systems are also becoming widely available. It is therefore timely to bring together scientists engaged in different aspects of AFM applications in biology to present the latest advances and promote cross-fertilisation in the field so that the potential of this technique can be fully explored.

The purpose of this conference was to create a platform for the dissemination and exchange of new results and ideas in the field of Atomic Force Microscopy (AFM) applications in biological sciences.

**Summer School in “Mathematics in Biology and Medicine”**

**Instituto Gulbenkian de Ciência**

**20-24 September 2004**

**Organisers: Jorge Carneiro (IGC, Oeiras, Portugal), Francisco Dionisio (IGC, Oeiras, Portugal), Jose Faro (IGC, Oeiras, Portugal), Gabriela Gomes (IGC, Oeiras, Portugal), Isabel Gordo (IGC, Oeiras, Portugal).**

This course is aimed at graduate students and early postdocs interested in research at the interface between mathematics, biology and medicine. The role of mathematical formalisms in providing insight into biological and medical processes became apparent at the beginning of the 20th century. The approach has since increased in popularity, especially during the past 10-20 years. This new phase of expansion is, to a large degree, stimulated by new developments in molecular biology and computation. Appropriate mathematical models are in great demand in many areas of biology. Topics covered: population dynamics and genetics, ecology, epidemiology, immunology and developmental biology.



**Mathematics in Biology and Medicine 2004**  
**Instituto Gulbenkian de Ciência**  
**20-24 September 2004**

**Organisers:** Gabriela Gomes (IGC, Oeiras, Portugal), Jorge Carneiro (IGC, Oeiras, Portugal), Pedro Coutinho (IGC, Oeiras, Portugal), Francisco Dionísio (IGC, Oeiras, Portugal), José Faro (IGC, Oeiras, Portugal), Isabel Gordo (IGC, Oeiras, Portugal).

Summer school consisted of 6 courses taught by international experts in the mathematical modelling of biological and medical systems. The school accommodated around 70 participants from various countries worldwide.

**Antigenic Diversity and Malaria Modelling**  
**Instituto Gulbenkian de Ciência**  
**26-29 September 2004**

**Organisers:** Gabriela Gomes (IGC, Oeiras, Portugal)

Workshop to organize a Research Training Network on “Exploring Pathogen Diversity in Disease Epidemiology and Vaccine Research – EXPAND”.

**Lymphocytes: Biology and Medicine”. XXX Annual Meeting of the Portuguese Society of Immunology**  
**Instituto Gulbenkian de Ciência**  
**30 September-2 October 2004**

**Organisers:** Jocelyne Demengeot, Carlos Penha Gonçalves and Maria Francisca Moraes-Fontes (IGC, Oeiras, Portugal)

This meeting pioneered the association of the Portuguese Society of Immunology with national Clinical Medical Societies, namely the Portuguese Societies of Haematology, Rheumatology and Internal Medicine (NEDAI) bringing together scientists and clinicians, on the subjects of transplantation, autoimmune disease and haematological malignancies.

## THESES

### PhD Theses

**Leonor Boavida** “Uma abordagem molecular e biofísica aos mecanismos de interacção célula-a-célula durante o processo reprodutivo em plantas”, University of Lisbon, Lisbon, Portugal.

**Ana Cristina Borges** “Study of genetic and biochemical interactions with mouse cerberus like genes”, Faculdade de Engenharia dos Recursos Naturais, University of Algarve, Faro, Portugal.

**Íris Maria Ferreira Caramalho** “Regulatory CD4 T cell function and dynamics in inflammation” Institute of Biomedical Sciences Abel Salazar, University of Porto, Porto, Portugal.

**Ana Catarina Certal** “Expressão e caracterização molecular de transportadores iónicos durante a germinação do grão de pólen e crescimento do tubo polínico”, University of Lisbon, Lisbon, Portugal.

**Elizabete Barata Fernandes** “Métodos estatísticos no mapeamento de QTLs”, University of Lisbon, Lisbon, Portugal.

**Claudia Susana Reste Florindo** “Cloning and characterization of human Mob1-like proteins”, FCT/UNL, Lisbon, Portugal.

**Rui Gardner.** “Towards understanding the prooxidative effects of superoxide dismutase: a mathematical approach”. ICBAS, Porto, Portugal.

### MSc Theses

**Núno Sepúlveda.** “Modelos estatísticos para a acção conjunta de dois loci em fenótipos binários complexos”. Instituto Superior Tecnico, Lisbon, Portugal.

### BSc Theses

**Ricardo Jorge Alexandre Águas** “Modelação da epidemiologia da tosse convulsa: A dicotomia imunidade parcial / imunidade temporária”, University of Évora, Évora, Portugal.

**Claúdia do Céu Afonso Bicho** “Identification of genes involved with YAP4 function in cold response”, University of Lisbon, Lisbon, Portugal.

**Tânia Cruz** “Efeito dos globulos vermelhos sobre a proliferação das células T em resposta à infecção por malária”, Lusofona University, Lisbon, Portugal.

**Joana Duarte** “Does IgE play a role in the pathogenesis of human cerebral malaria?”, University of Lisbon, Lisbon, Portugal.

**João Duarte** “An integrated approach to transcription regulation in arabidopsis thaliana”, University of Coimbra, Coimbra, Portugal.

**Lurdes Duarte** “Análise crítica de modelos teóricos representativos da dinâmica de Centros Germinais”, University of Évora, Évora, Portugal.

**Diogo Nuno Freiria Cardoso Barros da Fonseca** “Construction and selection of sua7 mutants sensitive to oxidative stress”, FERN, University of Algarve, Faro, Portugal.

**João António Lourenço Gonçalves**, “Estudo da localização celular e do efeito de condições de stress na expressão de eRF3 em células humanas”, University of Lisbon, Lisbon Portugal.

**Raquel Lourenço** “Terra, a new player in early chick development”, Lusófona University, Lisbon, Portugal.

**Ramiro Magno**. “Geometria e dinâmica do crescimento do tubo polínico”. Instituto Superior Tecnico, Lisbon, Portugal.

**Bruno Gonçalo Douradinha Mateus** “The role of Pb36p protein during the hepatic stage of malaria infection”, Instituto Superior Tecnico, Lisbon, Portugal.

**Ana Paula Rodrigues Martins** “Estudo da cinase Mps1 de Drosophila melanogaster”, Instituto Superior Técnico, Lisbon, Portugal.

**Lilia Perfeito**, “Dynamics of mutators in E. coli populations”, University of Lisbon, Lisbon, Portugal.

**Yara Reis**, "Estudos sobre a infecção por Besnoitia besnoiti", Beira Interior University, Covilhã, Portugal.

**Pedro Gonçalo Reis Rifes** “Possible role of the extracellular matrix in chick somitogenesis”, Faculty of Sciences, University of Lisbon, Lisbon, Portugal,

**Ana Filipa da Costa Simões** “A via de sinalização notch na organogénese do timo” University of Lisbon, Lisbon, Portugal.

## **PARTICIPATION IN ACADEMIC COMMITTEES**

### **José António Belo**

Member of the Jury of the MSc. Thesis, Catarina Figueiredo da Mota, University of Aveiro, Aveiro, Portugal, January 2004.

Member of the Jury of the PhD Thesis, Luísa Vasconcelos, UNL/ITQB, Oeiras, Portugal, Julho 2004.

Member of the Jury of the PhD Thesis, Ana Cristina Borges, University of Algarve, Faro, Portugal, December 2004.

### **Jorge Carneiro**

Member of the Jury of the Ph.D Thesis, Dejan Milutinovic, Instituto Superior Técnico, Lisbon, Portugal, Setembro 2004.

### **António Coutinho**

Member of the Jury of the PhD Thesis, Margarida Lima, ICBAS, University of Porto, Porto, Portugal, February 2004.

Member of the Jury of the PhD Thesis, Sandra Sousa, Université Paris 7-Denis Diderot, Institut Pasteur, Paris, France, November 2004.

Evaluation panel, Apoio à inserção de doutorados e mestres, Agência de Inovação, Lisbon, Portugal, December 2004.

Institutional Evaluation at São Paulo University, São Paulo, Brazil, December 2004.

### **Jose Faro**

Member of the Jury of the BSc Thesis of Lurdes Duarte, University of Evora, Evora, Portugal, October 2004.

### **José Feijó**

Member of the Jury of the Ph.D Thesis, Ana Catarina Certal, University of Lisbon, Lisbon, March 2004.

Member of the Jury of the Ph.D Thesis, Leonor Boavida, University of Lisbon, Lisbon, Portugal, November 2004.

Arguing member Ana Catarina Santos, University of Lisbon, Lisbon, Portugal, January 2004.

Arguing member Rita Teodoro, ICBAS, Porto, Portugal, February 2004.

**Lisete Fernandes**

Member of the Jury of the BSc Thesis, Cláudia do Céu Afonso Bicho, University of Lisbon, Lisbon, Portugal, July 2004.

Member of the Jury of the BSc Thesis, Diogo Nuno Freiria Cardoso Barros da Fonseca, University of Algarve, Faro, Portugal, July 2004.

**Gabriela Gomes**

Member of the Jury of the PhD Thesis, Ana Cristina de Almeida Santos Paulo, University of Lisbon, Lisbon, Portugal, May 2004.

Member of the Jury of the BSc Thesis, Ricardo Jorge Alexandre Águas, University of Évora, Évora, Portugal, September 2004.

**Isabel Gordo**

Member of the Jury of the BSc Thesis, Lilia Perfeito, University of Lisbon, Lisbon, Portugal, July 2004.

**Moisés Mallo**

Member of the Jury of the PhD Thesis, Luis Filipe Costa de Castro, University of Porto, Porto, Portugal, October 2004.

**R.M.E. Parkhouse**

Member of External Review Board de la Red de Investigación de Centros de Enfermedades Tropicales (RICET), Madrid, Spain, July 2004.

**Leonor Saúde**

Member of the Jury of the BSc Thesis, Raquel Lourenço, Lusófona University, Lisbon, Portugal, September 2004.

**Helena Soares**

Member of the Jury of the PhD Thesis, José Eduardo Ferreira de Oliveira Gomes, University of Lisbon, Lisbon, Portugal, June 2004.

Member of the Jury for “Provas Públicas para Professor Coordenador”, Área Científica de Farmácia, Escola Superior de Tecnologia da Saúde de Lisboa, Lisbon, Portugal, April 2004.

Member of the Jury for “Provas Públicas para Professor Adjunto”, Área Científica de Anatomia Patológica, Citológica e Tanatológica, Escola Superior de Tecnologia da Saúde de Lisboa, Lisbon, Portugal, June 2004.

**Álvaro Tavares**

Member of the Jury of the PhD Thesis of José Eduardo Gomes, University of Lisbon, Lisbon, Portugal, September 2004.

Member of the Jury of the PhD Thesis of Maria Luísa Caramalho Abrunhosa Vasconcelos, Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Lisbon, Portugal, July 2004.

**Sólveig Thorsteinsdóttir**

Member of the Jury of the BSc Thesis of: Bernardo Távora, Filipe Teixeira and Catarina Leitão, University of Lisbon, Lisbon, Portugal, July 2004.

Member of the Jury of the BSc Thesis, Raquel Lourenço, Faculty of Sciences, Lusófona University, Lisbon, Portugal, September 2004.

Member of the Jury of the BSc Thesis of: Marta Costa, Tatiana Resende and Pedro Rifés, University of Lisbon, Lisbon, Portugal, October 2004.

**Gabriela Rodrigues**

Member of the Jury of the BSc Thesis of: Bernardo Távora, Catarina Leitão, Andreia Bernardo, Marta Carvalho and Mafalda Rato, University of Lisbon, Lisbon, Portugal, July 2004.

Member of the Jury of the BSc Thesis of: Marta Costa, Tatiana Resende and Mafalda Nascimento, University of Lisbon, Lisbon, Portugal, October 2004.

## HONOURS AND AWARDS

**Íris Caramalho, Thiago Lopes-Carvalho, Santiago Zelenay, Manuel Rebelo, Vanessa Oliveira, Gustavo Rosa, Matthias Haury, Jorge Carneiro and Jocelyne Demengeot**

- Prémio Pfizer de Investigação  
1st prize ex-equos

**Margarida Carrolo, Silvia Gordiano, Laura Cabrita-Santos, Simona Corso, Ana M. Viagário, Susana Silva, Patricia Letrião, Daniel Carapau, Rosario Armas-Portela, Paolo M Cornoglio, Ana Rodrigues and Maria M Mota**

- Prémio AMI Saúde - "Doenças infecciosas e parasitárias"  
1st Prize

**Susana Constantino Rosa Santos (IPO/IGC)**

- Prémio Pulido Valente – Ciência: Cancro – Ciência Básica e Clínica  
1st prize

**Susana Constantino Rosa Santos e Sérgio Dias (IPO/IGC)**

- Prémio Pfizer de Investigação  
1st prize ex-equos

**António Coutinho**

- Appointed member of the “core” group of the Advisory Council of the Riken Research Center for Allergy and Immunology, Japan
- Appointed member of “Conselho Consultivo das Ciências da Saúde” of Fundação para a Ciência e a Tecnologia
- “Estímulo à Excelência” Award from Fundação para a Ciência e a Tecnologia

**Margarida Cunha**

- Prémio Pfizer para Jovens Investigadores  
“Menção Honrosa”

**Margarida Cunha, Graça Coelho and Maria M. Mota**

- Prémio Internacional de Investigação CESPU 2004 - Um contributo para o desenvolvimento das Ciências e Tecnologias da Saúde.  
“Menção Honrosa”

**Manuela Cristina Fernandes Ferreira (IPO/IGC)**

- Bolsa NRS/LPCC – Terry Fox - Oncologia – Jovens investigadores

**Moises Mallo**

- Bolsa de Investigação Pfizer - Professor João Cid dos Santos

**Andreia Mendonça (IPO/IGC)**

- Bolsa NRS/LPCC – Terry Fox - Oncologia – Jovens investigadores

**Maria M Mota**

- European Young Investigator Award

**Rute Conceição do Nascimento**

- Bolsa NRS/LPCC – Terry Fox - Oncologia – Jovens investigadores

**R.M.E. Parkhouse**

- “Estímulo à Excelência “ Award from Fundação para a Ciência e a Tecnologia

**Miguel Soares**

- Prémio Internacional de Investigação CESPUI 2004 - Um contributo para o desenvolvimento das Ciências e Tecnologias da Saúde.  
1st prize

**Làszlò Tokaji**

- Prémio Pfizer para Jovens Investigadores  
1st prize



## **PARTICIPATION OF IGC PERSONNEL IN CONFERENCES, SEMINARS AND SCIENTIFIC MEETINGS**

### **January**

Coutinho A.  
Innate and adaptive Immunity.  
Immunology Course 2003-2004, Pasteur Institute, Paris, France.

Coutinho A.  
Ciência e Cultura.  
Curso Mestrado. Depto. Biologia Univ. Aveiro, Aveiro, Portugal.

### **February**

Becker J.  
Unveiling the transcriptome of *Arabidopsis thaliana* pollen with full-genome oligonucleotide arrays.  
XXXI Jornadas de Genética. ITQB, Oeiras, Portugal.

Boavida L.  
New insights into cell-cell interactions during the reproductive process in *Arabidopsis thaliana*.  
Poster .XXXI Jornadas de Genética. ITQB, Oeiras, Portugal.

Certal A.C.  
The Molecular Basis of Proton Homeostasis in Growing Pollen Tubes.  
Poster. XXXI Jornadas de Genética. ITQB, Oeiras, Portugal.

Coutinho A.  
Ora então, vamos à vida!  
Conferences, Despertar para a Ciência, Auditório Reitoria Universidade do Porto, Porto, Portugal.

Coutinho A.  
The PGDBM experience.  
ESRM2004 (Euroscience, Marie Curie Fellowship Association, Eurodoc, Postgraduates International Meeting), Fundação Calouste Gulbenkian, Lisbon, Portugal.

Feijó J.  
Quantos genes são precisos para fazer uma célula e uma planta?  
Invited Conference, FCUL, DNA50, Lisbon, Portugal.

Parkhouse, R.M.E.  
Host-Pathogen Interactions.  
Tropical Animal Science Programme of the Veterinary School, Universidade Técnica de Lisboa, Lisbon, Portugal.

Soares M..  
Protective genes suppress atherosclerosis.  
Jornadas de Doença Cardiovasculares. Lisbon, Portugal.

## **March**

Coutinho A.  
Ora então, vamos à vida!  
Conferences, Despertar para a Ciência, Auditorio Reitoria Universidade de Coimbra, Coimbra, Portugal.

Coutinho A.  
EMBO Council, Heildelberg, Germany.

Cymbron T, Cabral R, Anjos R, Macedo C, Duarte CP and Mota-Vieira L.  
Incidence and familial aggregation of CHD in Azores islands.  
Poster. Keystone Symposia on Cardiac Development and Congenital Heart Disease, Colorado, USA.

Demengeot J.  
Function and dynamic of regulatory T cells during inflammatory responses.  
Keystone Symposia: “Regulatory/Suppressor T cells”, Banff/Alberta, Canada.

Filipe M., Marques S., Becker J., Belo J.A.  
Isolation and study of novel head-inducing genes expressed in the anterior visceral endoderm.  
Developmental Biology Annual Symposium and Genetics 2004, University of Warwick, UK.

Soares M.  
Heme oxygenase-1 controls the pro-inflammatory phenotype of activated endothelial cells.  
Medical University Vienna, Vienna, Austria.

Zelenay S., Fontes M. F., Água Doce A., Oliveira P., Coutinho A. and Demengeot J.  
Transient depletion of CD4<sup>+</sup> CD25<sup>+</sup> regulatory T cells results in a dramatic and persistent increase in certain auto-antibodies.  
Poster. Keystone Symposia: “Regulatory/Suppressor T cells”, Banff/Alberta, Canada.

## **April**

Coutinho A.

Os dados pessoais e a genética, elementos essenciais à análise de risco.

Jornadas em Direito dos Seguros “Segredo profissional, confidencialidade, privacidade e protecção de dados – A genética e os seguros”, Faculdade de Direito da Universidade de Lisboa, Lisbon, Portugal.

Coutinho A.

O Admirável mundo novo da biologia.

Ciência na Almedina, Livraria Almedina, Lisbon, Portugal.

Coutinho A.

1<sup>st</sup> meeting of the Research Center for Allergy and Immunology Advisory Council  
Riken, Institute of Physical and Chemical Research, Kanagawa, Japan.

Coutinho P.

From zebrafish development to computational parasitology.

Departamento de Ciências Médicas, Universidade da Beira Interior, Portugal.

Parkhouse R.M.E.

Control of cisticercosis.

Instituto de Biomédicas, Universidad Carabobo, Venezuela.

Silva S, Marques S., Haury M., Rodriguez J. and Parra P.

Expressing GFP in the cortical subplate and hippocampal pyramidal cells at different time points.

Poster. New Insights on Developmental Neurobiology Symposium, Cadiz, Spain.

Thorsteinsdóttir S.

Kick-off” meeting of the Network of Excellence “Cells into Organs” (EU/FP6).

Utrecht, The Netherlands.

## **May**

Barata E.

Métodos estatísticos no mapeamento de QTLs.

Reunião Anual da Sociedade Portuguesa de Estatística, Evora, Portugal.

Cortes, H., Leitão, A., Vidal, R., Soares, H., Marques, I., Reis, Y., Waap, H., Pereira Fonseca, I., Fazendeiro, I., Ferreira, ML and Caeiro, V.

Isolation of *Besnoitia besnoiti* from naturally infected cow in Portugal, phylogenetic analysis of its 18S rRNA.

1st Annual Workshop, “Apicomplexan biology in the post-genomic era”, Lisbon, Portugal.

Coutinho A.

Ora então, vamos à vida!

Escola Secundária de Castelo Branco, Castelo de Branco, Portugal.

Coutinho A.

A nova medicina preditiva.

Investigação Biomédica e Administração de Saúde, Escola Nacional de Saúde Pública, Lisbon, Portugal.

Coutinho A.

Doenças inflamatórias imunomediadas.

1ª Reunião de Doenças inflamatórias imunomediadas, Schering-Plough Farma, Lda., Hotel Pestana Palace, Lisbon, Portugal.

Coutinho A.

Investigação clínica ao serviço dos doentes.

78º Aniversário da Associação Protectora dos Diabéticos de Portugal, APD, Lisbon, Portugal.

Cunha-Rodrigues M., Febbraio M., Mota MM:

Malaria in CD36 knockout mice.

Poster. Apicomplexan biology in post-genomic era, COST Action 857, 1st Annual Workshop, Lisbon, Portugal.

Epiphany S, Garcia CR, Rodrigues-Cunha M, Rodrigues C, Leirião P, VanGemert GJ, Sauerwein R, Mota MM.

The role of melatonin during the hepatic stages of a malaria infection.

Poster. Apicomplexan biology in post-genomic era, COST Action 857, 1st Annual Workshop, Lisbon, Portugal.

Feijó J.

Ion dynamics and the regulation of apical growth.

1st. GABBA workshop”

IBMC, Porto, Portugal.

Feijó J.

Ion dynamics and the regulation of apical growth.

Reunião Anual da Soc. Bioquímica Brasileira, Caxambu, Brazil.

Fragoso R. and Dias S.

A role for FLT-1 in acute lymphoblastic leukemia.

Poster. 4th European Conference on Angiogenesis, Helsinki , Finland.

Leirião P., Albuquerque S., Mota MM. and Rodriguez A.

Hepatocyte apoptosis during malaria infection and phagocytosis by dendritic cells.

Poster. Apicomplexan biology in post-genomic era, COST Action 857, 1st Annual Workshop, Lisbon, Portugal.

Martins S., Douradinha B. and Mota MM.

Role of cytoskeleton during hepatocyte infection by plasmodium sporozoites.

Poster. Apicomplexan biology in post-genomic era, COST Action 857, 1st Annual Workshop, Lisbon, Portugal.

Santos SC and Dias S.

Mechanisms of KDR/VEGF internalization in endothelial cells.

Poster (selected out of 250 as the Best Poster). International Cell Biology Meeting; Dubrovnik, Croatia

Sepulveda N.

Modelos estatísticos para a acção conjunta de dois loci em fenótipos binários complexos.

Reunião Anual da Sociedade Portuguesa de Estatística, Évora, Portugal.

Tavares A.

Mob1-like proteins Mob4A and Mob4B are required for cytokinesis in human cells

The Cell Cycle Meeting, Cold Spring Harbour Laboratories, USA.

## **June**

Bettencourt-Dias M, Coutinho AG and Araújo SA.

Training scientists to communicate to lay audiences: successes and limitations of a science communication workshop

PCST8 Meeting, Barcelona, Spain.

Cabral R, Branco CC, Costa S, Caravello GU, Tasso M and Mota-Vieira L.

Geography of surnames in Azores: specificity and spatial distribution analysis.

Poster. Annual Meeting of the European Society of Human Genetics, Munich, Germany.

Carneiro, J.

Regulation of the immune system.

Regulation. Historical and Current Themes in Theoretical Biology. Humboldt University, Berlin, Germany.

Coutinho A.

A review of the major immunological concepts implicated in the understanding of tolerance to self.

11<sup>th</sup> N.A.T. Meeting “Inducing and detecting allograft tolerance in humans”

Cité des Congrès, L’Atlantique, Nantes, France.

Coutinho A.

Second generation immune networks.

From autoopoiesis to neuroPhenomenology: a tribute to Francisco Varela.

Amphithéâtre Richelieu, University Paris-Sorbonne, Paris, France.

Coutinho AG, Araújo SA and Bettencourt-Dias M.

An experiment in two-way, direct communication between scientists and the public in Portugal.

PCST8 meeting, Barcelona, Spain.

Fernandes P.

PGBIONF: the FCUL/IGC Post Graduate Programme in Bioinformatics

Special meeting on european strategies in bioinformatics post-graduate education, organized by the EU Project BioSapiens, at the European Bioinformatics Institute, Hinxton, UK.

Mota-Vieira L, Pacheco PR, Almeida ML, Cabral R, Carvalho J, Costa S, Rego P, Branco CC, Loura M, Peixoto BR, Araújo AL and Mendonça P.

Human DNA bank in São Miguel Island, Azores: Assembly and analysis.

Poster. Annual Meeting of the European Society of Human Genetics, Munich, Germany.

Nunes Cabaço H., Haury M., Fairen A. and Parra P.

A transgenic mouse expressing GFP in the cortical subplate and hippocampal pyramidal cells at different time points.

Poster. FENS, Lisbon, Portugal.

Pacheco PR, Branco CC, Costa S, Cabral R, Cymbron T, Peixoto BR and Mota-Vieira L.

The Y chromosomal heritage of São Miguel’s population (Azores).

Poster. Annual Meeting of the European Society of Human Genetics, Munich, Germany.

Santos A. C.

HoxB4 in embryonic hematopoiesis.

Poster. 2<sup>nd</sup> Annual Meeting of the Society for Stem Cell Research, Boston, USA.

Soares M.

Heme oxygenase-1: a protective response in organ transplantation.

2004 FASEB Summer Research Conferences. Snowmass, Denver Colorado, USA.

Trindade M, Coutinho AG and Bettencourt-Dias M.  
Promoting science in developing countries – a young scientists’ initiative in Mozambique and Angola.  
PCST8 Meeting, Barcelona, Spain.

## **July**

Borges A.C., Liguori G., Graziella Persico M. and Belo J.A.  
The role of mouse cerberus-like and cripto in early mouse development.  
63<sup>rd</sup> Annual Meeting of the Society for Developmental Biology”, University of Calgary, Canada.

Calado D., Holmberg D. and Haury M.  
Regulation of IL-10 expression at the allele level.  
Poster. 12th International Congress of Immunology and 4th Annual Conference of FOCIS 2004 Montreal Canada.

Carvalho C.R., Rodriguez-Léon J., Delphini M.C., Pascoal S., Vieira C., Thorsteinsdóttir S., Duprez D., Izpisua Belmonte J.C. and Palmeirim I.  
Dynamic expression pattern of hairy2 during limb bud development: is hairy2 counting time?  
Poster. 8th International Conference Limb Development & Regeneration”, Dundee, Scotland, UK.

Certal, A.C.  
Imaging pollen tubes with two-photon microscopy.  
13th Workshop on Plant Membrane Biology. Montpellier, France.

Certal, A.C.  
Proton Homeostasis and Regulation of Growth in Pollen Tubes.  
Poster. 13th Workshop on Plant Membrane Biology. Montpellier, France.

Cordeiro S.  
Polarized growth and membrane domains: ion dynamics in pollen tube growth.  
13th International Workshop on Plant Membrane Biology Montpellier, France.  
Poster.

Coutinho A.  
Cooperação científica entre Países Lusófonos.  
XIV Encontro da Associação das Universidades de Língua Portuguesa, Escola Politécnica da Universidade de São Paulo, São Paulo, Brazil.

Demengeot J.

Function and dynamic of regulatory T cells during inflammatory responses.

12<sup>th</sup> International Congress of Immunology and 4<sup>th</sup> Annual Conference of FOCIS,  
Montreal, Canada.

Feijó J.

Ion dynamics and the regulation of apical growth.

Plenary lecture and Chairman of the session “Ions and development”

13th International Workshop on Plant Membrane Biology Montpellier, France.

Figueiredo C., Pais T.F. and Chatterjee S.

Differential response of microglia and neurons to quinolinic acid mediated cell death.

FENS 2004, Lisbon, Portugal.

Fontes M.F., Zelenay S, Perez A, Rebelo M, Caramalho I and Demengeot J

Drug induced immunosuppression as a cause of autoimmunity.

12<sup>th</sup> International Congress of Immunology and 4<sup>th</sup> Annual Conference of FOCIS

Montreal, Canada.

Poster.

Gomes G.

Reinfection thresholds and epidemiological puzzles.

VII International Meeting on Molecular Epidemiology of Infectious Diseases

Valencia, Spain.

Monteiro J., Fernandes L.

Possible role of TFIIB under non-physiological conditions.

Poster presented at the “Yeast Genetics & Molecular Biology 2004 Meeting”

Washington, Seattle, USA

Moraes F.

Tbx1 is required for proper neural crest migration and to stabilize spatial patterns during middle and inner ear development.

Poster. 63<sup>rd</sup> Annual Meeting of the Society for Developmental Biology, University of Calgary, Alberta, Canada.

Rebelo M, Fontes M.F., Perez A, Oliveira P. and Demengeot J.

IL-7 is dispensable for regulatory T cell generation.

Poster. 12<sup>th</sup> International Congress of Immunology and 4<sup>th</sup> Annual Conference of FOCIS  
Montreal, Canada.

Seixas, C., Melo, L.V., Brogueira, P. and Soares, H.

Cilia assembly during tetrahymena reciliation studied by AFM

EMBO/FEBS Workshop on AFM Applications in Biology, IGC, Oeiras Portugal.



## August

Carvalho C.R., Rodriguez-Léon J., Delphini M.C., Pascoal S., Vieira C., Thorsteinsdóttir S., Duprez D., Izpisua Belmonte J.C. and Palmeirim I.

Temporal Control of limb bud development: evidences of a molecular clock  
Poster. Santa Cruz Conference on Developmental Biology, USA.

Coutinho A.

Who controls science?

ESOF 2004 (Euroscience Open Forum), Stockholm, Sweden.

Monteiro J., Fernandes L.

Possible role of TFIIB under non-physiological conditions.

Poster presented at the “6th EMBL Transcription Meeting”.

Heidelberg, Germany.

Prado A.M.

Nitric oxide participates in growth regulation and re-orientation of pollen tubes

18th International Congress on Sexual Plant Reproduction in Beijing, China.

## September

Almeida, S., Oliveira, V., Parkhouse, R.M.E.

The construction of transgenic and gene knockout mice is a powerful strategy for elucidating and manipulating mechanisms of immunity in health and disease.

Poster. XXX Annual Meeting of the Portuguese Society of Immunology. IGC, Oeiras, Portugal.

Barreto M., Ferreira R., Fesel C., Fontes M.F., Santos E., Alves M., Cortez-Dias N., Pereira C., Martins B., Andreia R., Mota-Vieira L., Vasconcelos C., Ferreira C., Demengeot J., Vicente A.

Gene and lymphocyte analysis in systemic lupus erythematosus

XXX Annual Meeting of the Portuguese Society of Immunology. IGC, Oeiras, Portugal.

Bettencourt P., Barreto V., Lopes Carvalho T., Demengeot J.

Characterization of transgenic mice ubiquitously expressing the recombination activating genes-1 and -2 independently.

Poster. XXX Annual Meeting of the Portuguese Society of Immunology. IGC, Oeiras, Portugal.

Bergman M. L., Martins J. and Demengeot J.

Live imaging of lymphocyte motility by intravital microscopy

Poster. XXX Annual Meeting of the Portuguese Society of Immunology. IGC, Oeiras, Portugal.

Calado D.

Regulation of IL-10 expression at the allele level.

XXX Annual Meeting of the Portuguese Society of Immunology. IGC, Oeiras, Portugal.

Caramalho Í., Zelenay S., Bergman M. L., Perez A., Oliveira P. and Demengeot J.

Lipopolysaccharide and regulatory and regulatory T cells: partners in the protection of NOD mice from diabetes

Poster .XXX Annual Meeting of the Portuguese Society of Immunology. IGC, Oeiras, Portugal.

Carvalho, T.J., T. Paixão, J.Carneiro.

Is cytokine expression really monoallelic or simply stochastic? .

Poster. XXX Annual Meeting of the Portuguese Society of Immunology. IGC, Oeiras, Portugal.

Correia, S., Crespo, A., Parkhouse, R.M.E.

Modulation of cell division and interferon responses.

Poster. Workshop Biology of Virus Infection, EMBO, Heidelberg, Germany.

Coutinho A.

Why are we all becoming allergic?

Opening lecture, 26º Congresso Anual da Sociedade Europeia de Nutrição Clínica e Metabolismo, ESPEN 2004, Centro de Congressos Lisboa, Lisbon, Portugal.

Coutinho A.

EMBO Council

Heidelberg, Germany

Cunha-Rodrigues M, Febbraio M and Mota MM.

Malaria in CD36 knockout mice

International Molecular Parasitology Meeting XV, Woods Hole, USA.

Deus L.G., Almeida P., d'Avila J., Vigário A.M., Mota M.M., Penha-Gonçalves C.

A locus controlling malaria liver stage infection maps to a 17 cM region on mouse chromosome 17

Poster. XXX Annual Meeting of the Portuguese Society of Immunology. IGC, Oeiras, Portugal.

Figueiredo C., Pais TF. and Chatterjee S.

Mechanism of resistance of microglia to quinolinic acid mediated cell death.

ISNI 2004, Venice, Italy.

Filipe M., Marques S., Silva A. and Belo J.A.

The AVE and the cerberus-like gene family: searching for new players establishing asymmetries in the early mouse embryo.

Mouse Molecular Genetics” Cold Spring Harbor Laboratory, NY, USA.

Fontes M.F., Zelenay S, Perez A, Rebelo M, Caramalho I and Demengeot J.  
Drug induced immunosuppression as a cause of autoimmunity.  
Poster. XXX Annual Meeting of the Portuguese Society of Immunology. IGC, Oeiras, Portugal.

Leirião P., Albuquerque S., Corso S., van Gemert G-J. Sauerwein R.W, Rodriguez A., Giordano S. and Mota MM.  
HGF/MET signaling protects Plasmodium infected host cells from apoptosis.  
International Molecular Parasitology Meeting XV, Woods Hole, USA.

Mallo M.  
Transgenics and knock outs: general principles.  
Course on Laboratory Animal Science, Universidade do Minho, Braga, Portugal.

Mallo M.  
Hox genes and the regulation of skeletal identity.  
EMBO Practical Course, Anatomy and Embryology of the Mouse.  
Zagreb University School of Medicine. Zagreb, Croatia.

Moraes F.  
Tbx1 is required for proper neural crest migration and to stabilize spatial patterns during middle and inner ear development.  
Poster. EMBO Practical Course, Anatomy and Embryology of the Mouse.  
Zagreb University School of Medicine. Zagreb, Croatia.

Matos, MH  
Classical and alternative activation of microglia: a split role for GM-CSF.  
ISNI 2004, Venice, Italy.

Nascimento, R., Crespo, A., Parkhouse, R.M.E.  
VGAP a novel viral protein inducing cell cycle arrest and apoptosis.  
Poster. European Life Science Organisation Meeting (ELSO), Nice, France.

Oliveira V.L., Mar Albà. M, Crespo, A., Parkhouse R.M.E  
Inhibition of toll-like receptor signaling by african swine fever virus.  
Poster. XXX Annual Meeting of the Portuguese Society of Immunology. IGC, Oeiras, Portugal.

Pais T.F. and Chatterjee, S.  
Characterization of macrophage brain cell population in experimental cerebral malaria.  
ISNI 2004, Venice, Italy.

Paixão, T, J. Carneiro.

How normal is the lognormal? Stochastic protein synthesis and the ubiquity of the lognormal.

Poster. Summer School on mathematics in Biology and Medicine. IGC, Oeiras, Portugal.

Palmeirim, I.

The segmentation clock and the A-P patterning.

European Network of Excellence, Geneve, Switzerland.

Pamplona A.; Rodrigues C.D. and Mota MM.

Heme oxygenase-1 protects mice from cerebral malaria.

International Molecular Parasitology Meeting XV, Woods Hole, USA.

Poster.

Parkhouse, R.M.E.

Estrategias racionales para controlar los patógenos

Universidad Nacional Autonoma de Mexico, Mexico.

Parkhouse, R.M.E.

Host-pathogen Interaction: A reciprocal two-edged sword.

XXX Annual Meeting of the Portuguese Society of Immunology. IGC, Oeiras, Portugal.

Rebelo M, Fontes M.F., Perez A, Oliveira P. and Demengeot J.

IL-7 is dispensable for regulatory T cell generation.

XXX Annual Meeting of the Portuguese Society of Immunology. IGC, Oeiras, Portugal.

Poster.

Reis, A., Correia, S., Leitão, A., Parkhouse, R.M.E.

Cell biology function and diagnostic potential of the immunodominant serological epitopes of African swine fever virus Infection.

Poster. Workshop Biology of Virus EMBO, Heidelberg, Germany.

Rodo J.

A role for MHC class II molecules in B cell activation by LPS.

XXX Annual Meeting of the Portuguese Society of Immunology. IGC, Oeiras, Portugal.

Santos M., Rebelo M., Água-Doce A., Neves H., Parreira L and Demengeot J.

Distinct effects of notch ligands delta-1 and jagged-1 on mature B cell differentiation to Ig secreting cells

Poster. XXX Annual Meeting of the Portuguese Society of Immunology. IGC, Oeiras, Portugal.

Sepúlveda, N., P. Pereira, J.Carneiro.

Stochastic modeling of gamma gene rearrangement of the gammadelta T lymphocyte receptor.

Poster. XXX Annual Meeting of the Portuguese Society of Immunology. IGC, Oeiras, Portugal.

Soares M.

Protective responses in ischemia reperfusion injury.  
Annual meeting of FUJISAWA, Lisbon, Portugal.

Soares M.

Heme oxygenase-1 expression: A fine balance between immunity and disease.  
Institute of Parasitology and Biomedicine, CSIC, Granada, Spain.

Soares M.

Heme oxygenase-1: a stress responsive gene that controls inflammatory reactions.  
XX International Congress of the Transplantation Society. Austria Center Vienna, Austria.

Tavares A.

Human Mob1-like proteins Mob4A and Mob4B are required for cytokinesis  
The Cell Biology of Cancer, Oxford, UK.

Veloso A., Grãos M. and Chatterjee S.

Role of the apoptotic protein .Bim in determining cell fate during cell cycle.  
ELSO 2004, Nice, France.

Vigário A.M., Gorgette O., Dujardim H., Cazenave P.-A., Six A., Bandeira A. and Pied S.

Regulatory CD4<sup>+</sup>CD25<sup>+</sup> T cells expand during Plasmodium infection but do not prevent cerebral malaria.

XXX Annual Meeting of the Portuguese Society of Immunology. IGC, Oeiras, Portugal.

Zelenay S., Lopes Carvalho T., Caramalho I., Rebelo M. and Demengeot J.

CD25 and Foxp3: Labile but reliable markers of functional regulatory T cells.

Poster. FEBS International Summer School on Immunology. The Immune System: Genes, Receptors and Regulation. Ionian Village, Western Ploponese, Greece.

Zelenay S., Lopes Carvalho T., Caramalho I., Rebelo M. and Demengeot J.

CD25 and Foxp3: Labile but reliable markers of functional regulatory T cells

Poster. XXX Annual Meeting of the Portuguese Society of Immunology. IGC, Oeiras, Portugal.

## **October**

Almeida, S., Oliveira, V., Parkhouse, R.M.E.

The construction of transgenic and gene knockout mice is a powerful strategy for elucidating and manipulating mechanisms of immunity in health and disease.

Poster. Sociedade Brasileira de Imunologia, RJ, Brasil.

Barreto M., Ferreira R., Fesel C., Fontes M.F., Santos E., Alves M., Cortez-Dias N., Malheiro F, Pereira C., Martins B., Andreia R, Mota-Vieira L, Vasconcelos C, Ferreira C, Demengeot J, Vicente A.

Systemic lupus erythematosus (SLE) patients show low levels of CD4+CD25+CD45RO+ regulatory T Cells, which are highly heritable and correlated with autoimmune disease manifestations within affected families.

Lupus International Meeting, New York, USA.

Becker J.

A genomic view of the commitment to cell growth, cell division control and regulation of gene expression in arabidopsis pollen.

Symposium on Frontiers in Sexual Plant Reproduction II. Albany, USA.

Boavida L.

Spatial and temporal resolution of cell-to-cell interactions during the progamic phase in *Arabidopsis thaliana*.

Symposium on Frontiers in Sexual Plant Reproduction II. Albany, USA.

Carneiro, J.

Como o sistema imunitário conta as suas células? Um périplo pela modelação matemática do sistema imunitário.

CMAF, University of Lisbon, Lisbon, Portugal.

Correia C., Coutinho A.M., Bento C., Marques C., Ataíde A., Miguel T., Borges L., Oliveira G. and Vicente A.M.

Association of the 5-HTT1A gene region with autism spectrum disorders.

Annual Meeting of the American Society for Human Genetics, Toronto, Canada.

Coutinho A

“Self and nonsense”. Contribuições de Nelson Monteiro Vaz.

SBI Meeting – Homage Prof. Nelson Vaz, Ouro Preto, Brazil.

Coutinho A.

Ora então, vamos à vida!

Conferences Despertar para a Ciência, Auditório da Biblioteca Municipal de Faro, Faro, Portugal.

Coutinho A.

From innate to adaptive immunity.

Cours d’Immunologie Approfondie 2004-2005.

Institut Pasteur, Paris, France.

Coutinho A.

A Genética na imunologia: da violação do dogma central à resolução das doenças auto-imunes.

Opening Lecture, Sociedade Portuguesa de Genética, Auditório da Estação Agronómica Nacional, Oeiras, Portugal.

Coutinho A.M., Oliveira G., Glanzmann C., Feng J., Yan J., Yang C, Marques C., Ataíde A., Miguel TS., Temudo T., Maciel P., Sommer SS. and Vicente A.M.

MECP2 variation in a sample of portuguese autistic patients.

Annual Meeting of the American Society for Human Genetics, Toronto, Canada.

Coutinho P.

Potential mutability and ZF-HDD models.

Departamento de Electrónica e Telecomunicações, Aveiro University, Aveiro, Portugal.

Cymbron T, Anjos R, Macedo C, Duarte CP and Mota-Vieira L.

Epidemiological characterization of Congenital Heart Disease in Sao Miguel island, Azores (Portugal).

Poster. 54<sup>th</sup> Annual Meeting of the American Society of Human Genetics, Toronto, Canada.

de Fez L, Cabral R, Branco CC, Pacheco PR and Mota-Vieira L.

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

Poster. 54<sup>th</sup> Annual Meeting of the American Society of Human Genetics, Toronto, Canada.

Feijó J.

Ion dynamics and the regulation of apical growth.

Plenary lecture. Symposium on Frontiers in Sexual Plant Reproduction II. Albany, USA.

Feijó J.

Ion dynamics and the regulation of apical growth.

Plenary lecture. FEBS course on microspectromics, Wageningen, Holland.

Feijó J.

Ion dynamics and the regulation of apical growth.

Reunião da Sociedade Portuguesa de Bioquímica, Vilamoura, Portugal.

Haury M.

A inovação tecnológica em Portugal através de uma carreira internacional.

Altran Business Meeting, Pav. Conhecimento, Lisbon, Portugal.

Pacheco PR, Lismond A. de Fez L, Cabral R, Branco CC and Mota-Vieira L.

Genetic history of Sao Miguel island (Azores): A HLA perspective.

Poster. 54<sup>th</sup> Annual Meeting of the American Society of Human Genetics, Toronto, Canada.

Parkhouse, R.M.E.

Immune responses against viral infection.

International Postgraduate Programme “Life and Health Sciences”, Minho University, Braga.

Parkhouse, R.M.E.

Control de la cisticercosis humana por una vacuna recombinante y un diagnóstico molecular: Perspectiva de la investigación nacional e internacional.

V Jornadas de Divulgación Científica “Dr Witremundo Torrealba”, Universidad Carabobo, Venezuela.

Rodriguez-Leon J.

Role of calcium signaling in the establishment of left-right axis.

2<sup>nd</sup> Chemistry Forum. Universidade Nova de Lisboa, Portugal.

Tavares A.T., Andrade S. and Belo J.A.

Transcriptional regulation of caronte asymmetric expression.

The Cell in Development (III CRG Annual Symposium), Barcelona, Spain.

Teotónio H.

Phenotypic plasticity and evolvability.

Experimental Evolution Workshop.

University of Fribourg, Fribourg, Switzerland.

Vicente A.M., Correia C., Coutinho A., Diogo L., Grazina M., Oliveira C., Oliveira G.

Lack of association of the mitochondrial aspartate/glutamate carrier SLC25A12 gene with autism or with markers of mitochondrial dysfunction in autistic patients.

Annual Meeting of the American Society for Human Genetics, Toronto, Canada.

Vieira A.

Pectin dynamics and hydrogel behavior during early germination of *E. globulus* pollen grains.

Poster. Symposium on Frontiers in Sexual Plant Reproduction II. Albany, USA.

## **November**

Carneiro, J.

Como o sistema imunitário conta as suas células ? Uma breve introdução à modelação matemática do sistema imunitário.

CIMA, Évora University, Portugal.



Costa S.

Influência da dinâmica do citoesqueleto de actina na regulação dos fluxos protónicos em tubos polínicos.

XXXIX Reunião Anual da Sociedade Portuguesa de Microscopia Electrónica e Biologia Celular, Aveiro, Portugal.

Coutinho A.

EMBO/EMBL Anniversary, Maunhein, Germany.

Coutinho A.

EMBO fellows meeting, The Rockefeller University, New York, USA.

Coutinho A.

A condição Pós-Humana, Técnica, Ciência e Cultura no Século XXI.

“Tendências da Cultura das Redes em Portugal”, Instituto Franco-Português, Lisbon, Portugal.

Coutinho A.

A Célula como património da vida.

8º Seminário Nacional do Conselho Nacional de Ética “Da célula ao embrião”, Fundação Calouste Gulbenkian, Lisbon, Portugal.

de Fez L, Cabral R, Branco CC, Pacheco PR and Mota Vieira L.

Estudo das mutações do gene HFE na população da ilha de São Miguel (Açores).

Poster. 8ª Reunião da Sociedade Portuguesa de Genética Humana, Porto, Portugal.

Fernandes P.

Bioinformática: compreender a Biologia usando informação.

Escola Superior de Biotecnologia, Porto, Portugal.

Fernandes, P.

Inserting the activity of procura in IGC's bioinformatics services.

2<sup>nd</sup> Annual Meeting of the Portuguese Proteomics Network: “New frontiers in Proteomics Technology”, Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisbon, Portugal.

Moreno N.

Membrane turnover and regulation in pollen tubes.

XXXIX Reunião Anual da Sociedade Portuguesa de Microscopia Electrónica e Biologia Celular, Aveiro, Portugal.

Pacheco PR, Branco CC, Cabral R, de Fez L, Araújo AL, Peixoto BR, Mendonça P and Mota Vieira L.

O cromossoma Y dos Açorianos: Filogenia e Diversidade.

Poster. 8ª Reunião da Sociedade Portuguesa de Genética Humana, Porto, Portugal.

Soares M.  
Protective responses in ischemia reperfusion injury.  
Advanced training in organ transplantation. ESOT course, Rotterdam, The Netherlands.

Vieira A.  
Pectin dynamics and hydrogel behavior during early germination of *E. globulus* pollen grains.  
XXXIX Reunião Anual da Sociedade Portuguesa de Microscopia Electrónica e Biologia Celular, Aveiro, Portugal.

## **December**

Becker J.  
A genomic view of the commitment to cell growth, cell division control and regulation of gene expression in arabidopsis pollen.  
XIV Congresso Nacional de Bioquímica. Vilamoura, Portugal.

Becker J.  
A genomic view of the commitment to cell growth, cell division control and regulation of gene expression in arabidopsis pollen.  
Plant Genomes: From Sequence to Phenome, Cold Spring Harbor, NY, USA.

Bento M., Tavares A.T. and Belo J.A.  
Subtractive cloning of differentially expressed genes in chick heart/hemangioblast precursor cells (H/HPC).  
XIV Congresso Nacional de Bioquímica. Vilamoura, Portugal.

Borges A.C., Liguori G., Graziella M. and Belo J.A.  
Cerberus-like and cripto genetically interact during mouse early development.  
XIV Congresso Nacional de Bioquímica. Vilamoura, Portugal.

Certal A.C.  
Proton homeostasis and regulation of growth in pollen tubes.  
XIV Congresso Nacional de Bioquímica. Vilamoura, Portugal.

Costa G, Tavares A., Roesptorff P., and Coelho AV  
Drosophila kinetochore protein profile  
XIV Congresso Nacional de Bioquímica, Vilamoura, Algarve, Portugal.

Coutinho A.  
Avaliação Institucional da Universidade de São Paulo, Universidade de São Paulo, São Paulo, Brazil.

Domingues C, Wainman A, Glover D and Tavares A.  
DMob4, an essencial gene in Drosophila, is a potential NDR/tricornered kinase regulator

XIV Congresso Nacional de Bioquímica, Vilamoura, Algarve, Portugal.

Echevarria D., Martinez S., Marques S., Teixeira V. and Belo J.A.  
Mkp3 is a negative feedback modulator of Fgf8 signaling in the mammalian Isthmic organizer.

XIV Congresso Nacional de Bioquímica. Vilamoura, Portugal.

Faria M., Alves P., Pimentel S., Godinho S., Domingues C., Florindo C., Martins A., Gomes R. and Tavares A

Drosophila Mps1 protein kinase is essential for spindle checkpoint establishment

XIV Congresso Nacional de Bioquímica, Vilamoura, Algarve, Portugal.

Feijó J.

Ion dynamics and the regulation of apical growth.

XIV Congresso Nacional de Bioquímica. Vilamoura, Portugal.

Filipe M., Marques S., Becker J. and Belo J.A.

Isolation and study of novel genes expressed in the prospective anterior and posterior regions of E5.5 embryo.

XIV Congresso Nacional de Bioquímica. Vilamoura, Portugal.

Filipe M., Silva A.C., Vitorino M., Steinbeisser H. and Belo J.A.

Expression pattern of Xenopus orthologs of novel genes expressed in the mouse AVE.

XIV Congresso Nacional de Bioquímica. Vilamoura, Portugal.

Florindo C., Perdigão J., Fesquet D., Pines J. and Tavares A.

Mob1-like proteins Mob4A and Mob4B are required for cytokinesis in human cells

XIV Congresso Nacional de Bioquímica, Vilamoura, Algarve, Portugal.

Godinho S., Glover D and Tavares A.

Depletion of Drosophila Polo kinase reveals an important role in chromosome resolution and segregation during mitosis

XIV Congresso Nacional de Bioquímica, Vilamoura, Algarve, Portugal.

Godinho S., Glover D and Tavares A.

Depletion of Drosophila Polo kinase reveals an important role in chromosome resolution and segregation during mitosis

44<sup>nd</sup> Annual Meeting of the ASCB, Washington, USA.

Igreja C., Courinha M., Silva MG. and Dias S.

Novel AC133 isoforms distinguish circulating from incorporated AC133 cells during tumor growth.

Poster 46<sup>th</sup> Meeting of the American Society of Hematology. San Diego, California. USA.

Marques S., Borges A.C., Silva A.C., Cordenonsi M. and Belo J.A.  
The activity of the Nodal antagonist Cerl-2 in the mouse node is required for correct L/R  
body axis.  
XIV Congresso Nacional de Bioquímica. Vilamoura, Portugal.

Prado A.M.  
Nitric oxide a new molecule in growth regulation and re-orientation of pollen tubes.  
XIV Congresso Nacional de Bioquímica. Vilamoura, Portugal.