



# **INSTITUTO GULBENKIAN DE CIÊNCIA**

## **ANNUAL REPORT 2001**

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**This Report can be consulted at the IGC website: <http://www.igc.gulbenkian.pt>**

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**BOARD OF ADMINISTRATION  
OF THE  
FUNDAÇÃO CALOUSTE GULBENKIAN**

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 2001 were:

Doutor Victor de Sá Machado (Chairman)

Dr. José Blanco

Dr. Mikhael Essayan

Dr. Emílio Rui Vilar

Prof. Doutor Diogo de Lucena

Dra. Isabel Mota

Prof. Doutor Eduardo Marçal Grilo

## **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 2001 were:

Prof. Doutor Eduardo Marçal Grilo (Chairman)

Prof. Doutor António Coutinho

Prof. Doutor João Caraça

Prof. Doutor Manuel Rodrigues Gomes

Dr. Horácio Menano

Dr. Manuel Carmelo Rosa

The Board of Directors met at the IGC on 18 September 2001.

### **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 2001 were:

Prof. Sydney Brenner (Chairman)

Prof. Philippe Kourilsky

Prof. Nicole Le Douarin

Prof. Martin Raff

Prof. Kai Simons

Prof. Susumu Tonegawa

Prof. Hans Wigzell

Prof. Lewis Wolpert

The Scientific Advisory Board met at the IGC on 19-21 March 2001.

## **STAFF**

### **DIRECTOR**

António Coutinho

### **DEPUTY-DIRECTORS**

Sérgio Gulbenkian

José Mário Leite

## **RESEARCH MEMBERS**

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

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

Jan Andersson (Univ. Stockholm/FCG)

Jorg Dieter Becker (Univ. Bielefeld/FCT)

José António Belo (FCG)

Juan Carlos Izpisua Belmonte (Salk Inst./FCG)

Sergiy Bobrovnyk (Palladin Inst. Biochemistry, Kiev/OTAN)

Paula Parra Bueno (FCT)

Jorge Carneiro (FCT)

Cláudia Rocha Carvalho (Univ. Fed. Minas Gerais/FCG)

António Gil Pereira de Castro (ICBAS/FCT)

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

Sukalyan Chatterjee (FCG/FCT)

Melvin Cohn (Salk Inst./FCT)

Suzanne Bourgeois Cohn (Salk Inst.)

António Coutinho (CNRS/FCG)

Ana Crespo (UE)

Jocelyne Demengeot (FCG/FCT)

Francisco Dionísio (FCG/FCT)

José Faro (Univ. Salamanca/FCT)

José Feijó (FCUL/FCT)

Lisete Fernandes (Esc. Sup. Tec. Saúde Lisboa)

Carlos Alberto Ferreira (HUSM)

Constantin Fesel (Weizmann Institute/FCT)

Carlos Penha Gonçalves (FCG)

Zvi Grossman (Tel Aviv Univ/FCT)

Simone Gines (FCT)

Sérgio Gulbenkian (FCG)

Werner Haas (Linden Technologies, Boston, USA)  
Matthias Haury (FCG)  
Dan Holmberg (Umea Univ./FCG)  
Shohei Hori (Tokyo Univ./FCG)  
Rodney Langman (Salk Inst./FCT)  
Moises Mallo (Lab. Associado)  
Maria Marone (Universita Cattolica Del Sacro Cuore)  
Jane Megid (FAPESP)  
Javier Morcillo (FCG)  
Maria Teresa Faria Pais (FCT)  
Isabel Palmeirim (FCG/Univ. Minho)  
Michael Parkhouse (FCT)  
Leonor Parreira (IHEFMUL/CEBIP)  
Joana Perdigão (FCT)  
Sylviane Pied (INSERM/FCT)  
Dominic Poccia (Amherst College Senior Sabbatical award)  
Joaquin Rodriguez (FCT)  
Susana Santos (FCT)  
Leonor Tavares Saúde (FCT)  
Helena Soares (Esc. Sup. Tec. Saúde Lisboa/FCT)  
Miguel Parreira Soares (Lab. Associado/FCT)  
João Pedro Simas (FMUL/FCT)  
John Stewart (Univ. Tech. Compiègne/CNRS)  
Ana Teresa Tavares (FCT)  
Alvaro Augusto Tavares (IST/FCT)  
Alexandra Teixeira (FCT)  
Vera Lucas Teixeira (FCT)  
Solveig Thorsteinsdottir (FCUL)  
Ramon Trelles (FCT)  
Tatiana Vassilevskaia (FCG/IGC)  
Astrid Moura Vicente (FCG/IGC)  
Luisa Mota Vieira (Hospital do Dívino Espírito Santo, Ponta Delgada)  
Ana Margarida Vigário (FCT)  
Ari Waisman (Cologne Univ/FCT)  
Andrew Waters (Leiden Univ./FCG)

## **STUDENTS**

The following students worked at the IGC for all or part of the year.

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

Sílvia Almeida (FCUL/FCT)  
Paulo Alves (FMVUTL/FCT)  
Sebastien Bagot (Inst. Pasteur Paris, Hosp. Pitié-Salpêtrière)

Marta Barreto (FCUL/FCT)  
Dinis Pedro Calado (FMUL/FCT))  
Fernanda Maria Bajanca (FCUL/FCT)  
Leonor Boavida (FCUL/FCT)  
Ana Cristina Borges (FCUL/FCT)  
Déborah Braun (Univ. Paris VI/École Normale Supérieure)  
Ana Sofia Cachação (FCUL/FCT)  
Susana Gomes Campino (FCUL/FCT)  
Iris Caramalho (ICBAS/FCG)  
Thiago Lopes Carvalho (Univ. Estadual Campinas/FCG/FCT)  
Cristina Casalou (FCUL/FCT)  
Ana Catarina Certal (FCUL/FCT)  
Sofia Cordeiro (FCUL/FCT)  
Ana Margarida Coutinho (FCUL/FCT)  
Margarida Cunha (FCG/IGC)  
Célia Domingues (FCUL/FCT)  
Nádia Silva Duarte (FCUL/FCT)  
Mariana Faria (FCUL/FCT)  
Mário Rui Filipe (FCUL/FCT)  
Cláudia Florindo (UNL/FCT)  
Francisca Fontes (H.Egas Moniz)  
Catarina Freitas (FCUL/FCT)  
Sandra Penélope Freitas (FERNUA/FCT)  
Susana Godinho (FCUL/FCT)  
Mário Grãos (FCUL/FCT)  
Patrícia Madureira (ICBAS/FCT)  
Sofia Pinto Guia Marques (ICBAS/FCT)  
Joana Monteiro (FCUL/FCT)  
Kalet León Monzón (Univ. Havana/FCG)  
Rute do Nascimento (FCUL/FCG)  
Hélia Neves (FMUL/CEBIP/FCT)  
Sofia Nolasco (FCUL/FCT)  
Leonor Orge (LNIV)  
M<sup>a</sup> Gabriela Rodrigues (FCUL)  
Sofia de Albuquerque Rodrigues (FMUM/FCT)  
Ana Cecília Seixas (FCUL/FCT)  
Mark Jan Seldon (ICBAS/FCT)  
Valerie Soulard (Inst. Pasteur Paris, Hosp. Pitié-Salpêtrière)  
João Sousa (FCUL/Inst. Rocha Cabral/FCT)

### **M.Sc. Students**

Cláudia Dias (FCUL)

### **B.Sc. Students**

Nuno Duarte Afonso (FCUL)

Inês Martins Alves (FCUL)



José Antão (FCUL)  
Ricardo Bandarrinha (FCUL)  
Ana Bartolomeu (ICBAS)  
Lara Carvalho (FCUL)  
Inês Conceição (FCUL)  
Ana Costa (FCUL)  
Lara Costa (FCUL)  
Andreia Feijão (IST)  
Pedro Geraldes (FCUL)  
Nuno Geraldo (Univ. Évora)  
Alexandre Gonçalves (FCUL)  
Andreia Lino (FCUL)  
Sara Marques (Univ. Évora)  
Vânia Parelho (FCUL)  
Susana Pascoal (Instituto Piaget)  
Gustavo Rosa (FCUL)  
Joana Santos (FCUL)  
Ana Catarina Silva (FCUL)  
Susana Silva (FCUL)  
Sofia Simões (FCUL)  
Ana Maria Vieira (FCUL)  
Cláudia Vieira (FCTUNL)  
Joana Vital (FCUL)

#### **Technical Support Students**

Maria da Luz Alvim (BIC/FCT)  
Sílvia António (BIC/ICBAS)  
Sílvia Correia (BIC/FCT)  
Vasco Correia (BTI/FCG)  
Carla Ferreirinho (BIC/FCT)  
Lídia Fonseca (BIC/FCT)  
Sara Lopes Marques (BTI/FCT)  
Vanessa Oliveira (BIC/FCT)  
Dominique Osteler (BTI/FCG)  
Nuno Sepúlveda (BIC/FCT)  
Ana Maria Vieira (BTI/CELBI)

#### **Others**

Catarina Figueiredo  
Joana Martins  
Marta Martins  
Maria Hortense Matos  
Carla Narciso  
Ana Margarida Prado  
Olívia Rodrigues

Dora Sabino  
Catarina Serrano  
Rui Soares  
Marta Vitorino

### **SHORT-TERM VISITORS**

The IGC benefits from a large number of visitors each year. Most come to follow up collaborations with colleagues at the IGC. Persons listed here did laboratory or theoretical work at the IGC during 2001.

José Maria Alvarez (Univ. São Paulo, São Paulo, Brazil)  
Irun Cohen (The Weizmann Institute of Science, Rehovot, Israel)  
Gianni Garotta (Serono International, SA, Geneva, Switzerland)  
Vincent Guiyedi (Inst. Pasteur Paris, Hosp. Pitié-Salpêtrière)  
Maria Isabel Campos dos Reis (Hospital Egas Moniz, Lisbon, Portugal)  
Luís Rocha (Los Alamos National Laboratory, Los Alamos, USA)  
Renato Rodrigues-Pousada (Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione, Rome, Italy)

### **Abbreviations**

CNRS	Centre National Recherche Scientifique (France)
DGV	Direcção Geral de Veterinária
EAN	Estação Agronómica Nacional
FCG	Fundação Calouste Gulbenkian
FCUL	Faculdade de Ciências da Universidade de Lisboa
FCUP	Faculdade de Ciências da Universidade do Porto
FMUL	Faculdade de Medicina da Universidade de Lisboa
FCMUNL	Faculdade de Ciências Médicas da Universidade Nova de Lisboa
FCT	Fundação para a Ciência e Tecnologia
FCTUNL	Faculdade de Ciência e Tecnologia da Universidade Nova de Lisboa
FERNUA	Faculdade de Engenharia de Recursos Naturais da Univ. do Algarve
FMVUTL	Faculdade de Medicina Veterinária da Universidade Técnica de Lisboa
FFUL	Faculdade de Farmácia da Universidade de Lisboa
IBMC	Instituto de Biologia Molecular e Celular da Universidade do Porto
ICBAS	Instituto de Ciências Biomédicas Abel Salazar
IEFP	Instituto do Emprego e Formação Profissional
INSA	Instituto Nacional de Saúde Dr. Ricardo Jorge
ISA	Instituto Superior de Agronomia da Universidade Técnica de Lisboa
INSERM	Institut National de la Santé et de la Recherche Médicale
ISCE	Instituto Superior de Ciências Educativas
ITQB	Instituto de Tecnologia Química e Biológica
LNIVL	Laboratório Nacional de Investigação Veterinária de Lisboa
UCSC	Università Cattolica del Sacro Cuore, Rome, Italy

## **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 2001.

### **Administrative and Secretarial Staff**

Ana Paiva Brandão  
Manuel Carvalho  
Manuela Cordeiro  
Jorge Costa  
Cristina Gonçalves (from April to September 2001)  
Fátima Mateus  
Maria Matoso  
Greta Martins  
João Nunes  
Ana Lícia Pires  
Ana Maria Santos  
Vítor Santos (from June 2001)  
Abílio Simões  
Teresa M<sup>a</sup> Sousa  
Lurdes Torres  
Maria Vasconcelos (left October 2001)

### **Laboratory Technical Staff**

M<sup>a</sup> Ressurreição Alpiarça  
Magda Carlos (left June 2001)  
Dolores Ferreira (from October 2001)  
Ana Cristina Gaspar (from June 2001)  
Ana C. Homem  
Bruce Lenhart  
Júlia Lobato  
Susana Magrito (from April 2001)  
Isabel Marques  
Rute Marques (left October 2001)  
Nuno Moreno  
Dolores Oliveira  
Patricia Rodrigues (left December 2001)  
Rosa M<sup>a</sup> Santos

### **Technical Support Staff**

António Gomes  
António C. Ligeiro  
João Carlos Lopes  
Paulo J. L. Martinho  
Carlos Nunes

João António B. Pires (left December 2001)  
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.

### **Cell Imaging**

Sérgio Gulbenkian/Matthias Haury

### **Genetic Manipulation of Mice and Rats**

Bruce Lenhart (until October 2001) Moises Mallo (from October 2001)

### **Animal Facility**

Bruce Lenhart

### **DNA Sequencing and Genotyping**

Júlia Lobato

### **Bioinformatics**

Pedro Fernandes

### **Library and Scientific Information**

Pedro Fernandes

## **CELL IMAGING UNIT**

A new Leica DIC DMR RA Microscope was installed. Together with a ultrasensitive cooled CCD camera and Metamorph Acquisition software it enables users to carry out combined DIC + Fluorescent imaging using high performance image processing algorithms.

During the year 2001 the histology service processed more than 3100 microscope slides.

The Unit has also produced some monoclonal antibodies in high quantities, using a new in vitro system. Custom labelling of monoclonal antibodies is available (FITC, Biotin, PE, APC, PE-Cy5, PE-Cy7), and additional colour combinations were tested for future production.

## **GENETIC MANIPULATION OF MICE AND RATS**

During 2001 the UMTG has undergone a transition phase with renovation of its staff and equipment. Although the operation of the UMTG has been limited by the constraints imposed by the reconstruction of the new animal house, it provided technical support for several ongoing projects at the Institute.

## **ANIMAL FACILITY**

Although being greatly limited because of the reconstruction work, the new animal facility began operations in its production capacities in 2001. At year's end, the facility was producing most of its stock strains. There were 3.540 animals produced (1.250 for experimental purposes at the IGC, 1.195 sold to other institutions and 136 given to secondary schools).

## **GENOTYPING AND SEQUENCING UNIT**

In the year 2001 the Genotyping and Sequencing Unit worked at full capacity of its 3 sequencing machines and has sequenced samples for IGC users and for the following institutions: ITQB – Instituto de Tecnologia Química e Biológica da Universidade Nova de Lisboa; Centro de Genética e Biologia Molecular da Universidade de Lisboa; Faculdade de Ciências Médicas da Universidade de Lisboa; Faculdade de Farmácia da Universidade de Lisboa; Faculdade de Medicina Veterinária da Universidade de Lisboa; IBET – Instituto de Biologia Experimental e Tecnológica, Oeiras; Instituto do Coração, Lisboa; Instituto de Histologia e Embriologia da Faculdade de Medicina de Lisboa; Instituto Superior de Psicologia Aplicada, Lisboa; Instituto Superior Técnico da Universidade Técnica de Lisboa; Universidade do Algarve and Laboratório Nacional de Investigação Veterinária de Lisboa.

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

## **BIOINFORMATICS UNIT**

The Bioinformatics service delivered consultancy and accompaniment services to more than 400 users. Users in all sectors of the IGC have gained familiarity with the local resources, namely updated databases and software. The Bioinformatics Unit has organized six courses, five in the IGC premises and one at the University of Minho. A total of over 100 people received training under the Gulbenkian Training Programme in Bioinformatics (2nd edition). The EMBER project members started producing teaching materials. A first meeting was held at the IGC and significant progress towards the definition of the content and layout of the book, CDROM and website was achieved. The BioCASE project was approved and a new contract has been signed, thus permitting the continuation of the efforts for establishing an European Collection Data System within the next 3 years.

## **LIBRARY AND SCIENTIFIC INFORMATION**

The number of titles in the IGC Library was maintained, but access to online reading doubled in 2001. Although it is restricted to the IGC premises, the use of this access has increased more than tenfold. The IGC Library was visited by about 7500 readers.

## **INTERNATIONAL RESEARCH STRUCTURES AT THE IGC**

- ?? European Mouse Mutant Archive (EMMA)
- ?? Laboratoire Européen Associé CNRS “Génétique et développement de la tolérance naturelle “

## **NATIONAL RESEARCH STRUCTURES AT THE IGC**

- ?? Unidade de Investigação da Fundação para a Ciência e a Tecnologia “Tolerância natural”
- ?? Laboratório Associado do Ministério da Ciência e da Tecnologia with ITQB, IGC/FCG and IBET.



## INSTITUTIONAL AGREEMENTS

**Protocol of Scientific cooperation between the Hospital do Divino Espírito Santo, through its Molecular Genetics and Pathology Unit (UGPM) and the Fundação Calouste Gulbenkian, through the Health and Human Development Service (SSDH) and the Instituto Gulbenkian de Ciência (IGC).**

A protocol of scientific cooperation was signed on 18 June 2001 between the Hospital do Divino Espírito Santo, through its Molecular Genetics and Pathology Unit and the Fundação Calouste Gulbenkian, through the Health and Human Development Service and the Instituto Gulbenkian de Ciência.

The objective of this contract is to support research previously approved by the Scientific Board of the IGC, which is conducted by the UPGM in collaboration with the IGC. To this effect, the FCG offers, through the IGC, its equipment and services and the use of its laboratories, in Oeiras, on a non-permanent basis by members of the UGPM team, as well as financial support, through the SSDH, for students and technicians travelling to and from the IGC. The FCG/SSDH/IGC also supports applications for external financing of projects elaborated by the UGPM.

**Protocol of cooperation between the Secretaria Regional da Educação e Assuntos Sociais da Região Autónoma dos Açores and the Fundação Calouste Gulbenkian, through the Serviço de Saúde e Desenvolvimento Humano and the Instituto Gulbenkian de Ciência.**

Aiming at strengthening biomedical research on the genetic bases of susceptibility to “complex” diseases, particularly autoimmune diseases and autism, a protocol of collaboration was signed on 18 June 2001 by the Secretaria Regional da Educação e dos Assuntos Sociais da Região Autónoma do Açores with the Fundação Calouste Gulbenkian.

Given the relative isolation of the Azorian population with an increased level of inbreeding and the consequent increase in the frequency of hereditary diseases due to genetic defects, the Hospital do Divino Espírito Santo of Ponta Delgada, Açores, decided to create a Molecular Genetics and Pathology Unit.

Within this context, and as health support is one of the principal objectives of the Fundação Calouste Gulbenkian, the Serviço de Saúde e Desenvolvimento Humano of the Foundation decided to significantly contribute towards equipment for this unit.

In turn, as the study of human diseases with “complex genetics” is one of the research interests at the IGC, this Institute decided to offer technological and scientific support by launching some projects of collaboration in the area of “complex diseases”.

## INTRODUCTION

2001 was the fourth year of operation of the Gulbenkian Science Institute (Instituto Gulbenkian de Ciência - IGC) after its last reform. The IGC has now grown to a total population of some 150 people, distributed in 21 research groups, visiting scientists, education programs, and support units & services. The indicators of our activity have naturally increased to new levels: scientific production, as measured by the quality of journals publishing our contributions, is second to no other institution in Portugal; many scientists of the IGC have been lecturing or presenting their results at a large number of meetings and courses, in Portugal and abroad; essentially all groups at the Institute were very successful in obtaining national and/or international support for their research projects; the new PhD Program (PGDB) continues to attract a large number of excellent applications, and to successfully run the respective graduate-courses, now including foreign students; the Gulbenkian Biology Courses (our international advanced-course program) were launched with a 3-weeks laboratory and lecture course on plant development that already gained EMBO (European Molecular Biology Organisation) sponsorship; the IGC has organized several other international courses, notably in the area of bioinformatics, as well as two international workshops: one in the context of the CNRS European Associated Laboratory, the other as the IV Gulbenkian Autumn Meeting, this time on Bioinformatics; a total of 94 public research seminars were held at the Institute in 2001 (including 2 “Gulbenkian Lectures: Frontiers in Modern Biology”), in addition to all other “internal” seminars, discussions and “journal club” meetings; finally, we have received visits of some 500 secondary school biology students with the respective teachers, and actively participated in other initiatives on Science & Society.

The Institute has also kept its dynamics of growth: the Scientific Advisory Board has approved several new intra-muros and “external” groups that have already started operating; the first two scientific appointments at the “Laboratório Associado” founded by the Science & Technology Ministry were open to international applications and awarded to young scientists with curricula that justified their appointments at the “Associate” level. Last year, together with the Serviço de Saúde e Desenvolvimento Humano of the Gulbenkian Foundation, the IGC has signed agreements with the Azorian Health Authorities, in order to launch new programs on the genetics of human diseases, and gained a new “external” research group at the Hospital do Divino Espírito Santo in Ponta Delgada. The Institute continued to attract excellent applications for group leaders from young scientists, as well as many candidates for student, post-doctoral and technical positions. We were very honoured with the decision of a few “senior investigators” with impressive records in science to move to Oeiras, a fact which speaks highly of the Institute’s scientific environment and the quality of its life.

More importantly, perhaps, the Institute has become a meeting point for personal interactions and scientific exchange, a turning platform for many who like to be here and

regularly come back to find intellectual openness and excitement, new challenges and a positive attitude to science. We believe that the difficulty of establishing a strong “institutional spirit” with a population of predominantly young scientists and students who turn-over at a high rate is being overcome with the participation of all those, here and elsewhere, who consider the IGC to be their home. The “IGC spirit”, built upon the “PGDBM/PGDB spirit” but enriched by further diversity and institutional components, is now a reality that is fostered every day by the communal use of our resources and equipments, by our “horizontal structure”, and by the sharing of scientific problems and excitement across different areas of biology and medicine. We are happy that this feeling of cohesion of principles and goals in the diversity of individuals and specific scientific interests, has started to gain yet further perspectives by its extension to the IGC’s neighboring institutions that are also our partners in the “Laboratório Associado”. While we still need to develop this relationship further, it is clear that, in many ways, ITQB, IBET and IGC already function as a single large institution, and share the conviction that the interest of the campus passes before that of each institute, let alone that of individual groups or scientists. The recent work for the preparation of our proposals to the call from the Research Council on heavy equipment has been a rewarding demonstration of this attitude.

The Institute’s mission to serve as an incubator for new scientific leaderships, notably to be inserted in the Portuguese scientific community, is based on a threestep strategy: first, to identify the best students and give them optimal conditions of education; second, to attract the most original and promising young scientists and give them support and full autonomy for a few years, such that they can prove their quality and capability; third, to make sure that they leave the IGC to take up better positions elsewhere. This strategy of identifying and investing in the best people only to push them out, is obviously “suicidal” for a conventional research institution. On the other hand, the goal of the Gulbenkian Foundation is precisely to contribute to the development of science, and of science in Portugal, rather than to support one more conventional research institute. The Foundation’s spending in the IGC as a “direct activity” is thus fully justified, because we are here to serve the scientific community in ways that no other institution in Portugal can do. Thus, other institutions naturally keep the best scientists for as long as they can, usually until they retire. It is thus very rewarding to see that the IGC’s “suicidal” strategy has actually begun to work and is bringing the expected fruits: three of our current group leaders were recruited for positions at different Universities, and will be moving on to the new laboratories whenever completing their 5 years contracts in Oeiras. These groups, we hope, will seed the “IGC spirit” elsewhere and will continue to be “IGC groups” wherever they are. The turn-over of groups and scientists at the Institute is thus a sine qua non condition of its operational model. Accordingly, in 2001, the very first group at the “new IGC” has moved abroad. Furthermore, several of our technical personnel, educated at the Institute in highly differentiated technologies, have now been recruited to other institutions, giving thus proof that another of our missions is being fulfilled.

The renewal of the Institute’s infrastructures was continued at a reasonable pace. Thus, we have completed the reconstruction of the animal house, the seminar rooms, the

cafeteria, and the general storage, as well as a new wing of laboratories. In addition, we have built a green-house and a “fly room”, and have installed facilities for the maintenance and breeding of frogs and zebra-fish, which form an essential part of the infrastructure necessary for an encompassing programme in developmental biology. We have proceeded with the re-equipment of the animal house and laboratories, including the installation of seven new laboratories in the new wing. Furthermore, new informatic systems were introduced in the administrative and financial management, in order to facilitate and speed up the ordering, purchase and distribution of equipments and consumable supplies. The year 2001 was also marked by the decision of the Board of Administration of the Gulbenkian Foundation to build a “faculty residence” in the Oeiras campus and to install here, together with the local authorities, a “science garden” dedicated to the promotion of biology with the general public, particularly with secondary school teachers and students.

The only thing at the Institute that did not grow in 2001 was the budget that was ascribed to the IGC by the Gulbenkian Foundation. The Institute is a department of the Foundation, not part of a university or of a state laboratory, and it cannot engage in educational or entrepreneurial for-profit activities. Hence, the IGC has no “core” financing other than the Gulbenkian Foundation’s budget (and a handful of positions we obtained from the Ministry of Science in the context of the Associated Laboratory), making it particularly sensitive to variations in the Foundation’s budget. The development of the Institute could nevertheless be continued, through the efforts of our scientists, and the money they have obtained in contracts with the Portuguese Research Council (Fundação para a Ciência e a Tecnologia - FCT), with the European Union, or with private entities. This also allowed us to fully participate in the Foundation’s efforts to reduce permanent staff personnel. We strongly hope that the current worldwide financial difficulties will yield to better times, and that the Board of Administration of the Gulbenkian Foundation will be in a position to pursue its policy of increasing the share of science in its spending. Science is still markedly underrepresented at the Foundation, while many prominent politicians share the view that science is today a fundamental pillar of democracy, and the motor of all socio-economical progress. We do hope that the Board of Administration of the Gulbenkian Foundation will continue to provide the necessary support to bring to fruition a promising new model of a scientific education & research institution in Portugal that has rapidly reached internationally competitive standards.

In what concerns science, the major progress in 2001 was the rapid integration of several new groups that, with their specific concerns and questions, come to greatly enrich the IGC’s present collective competence. The area of infections and immunity was reinforced with three groups working on malaria, all concerned with various aspects of host-parasite interactions. A group on inflammation is now complementing our efforts on cell biology, as well as on the physiopathology and genetics of autoimmune diseases, and might also help the work on infection. The area of developmental biology has gained two new groups that simultaneously strengthened our competences in genetic manipulation of mice and in confocal microscopy. Cellular stress is receiving increasing attention, particularly in what concerns cytoskeleton dynamics, a federating topic for several groups

at the Institute. With the arrival of new groups working on evolution of complex traits that is expected for 2002, the thematic coherence of the IGC will be close to completion.

Significant scientific advances were made in essentially all areas of research at the IGC. Biology is living very exciting times, for we now know of an increasing number of components and processes (genes, molecules, cells, intra- and inter-cellular pathways of signaling and communication), such that global properties and generic rules for systems operation and controls can be derived. It is exciting, for example, to discover that some of the “big questions”, such as spacio-temporal coordination in living systems, can be mediated by processes as simple as oscillations in ion (protons and calcium) concentrations. This was shown here for the growth of pollen tubes and for adult cells, and also for egg fertilization, where two distinct calcium-dependent pathways were identified, with clear implications for the technology of in vitro plant fertilization. Another example: a simple gas (carbon monoxide, CO) turns out to have such a variety of fundamental physiological roles that it is provoking a widespread excitement, not unlike that stirred by nitric oxide (NO) some years ago. One of the IGC’s new groups centers its interests on CO’s anti-inflammatory activities, opening the way to increasing cooperation with the immunologists, but also with groups interested in the biology of endothelial cells. Novel insights were also obtained into other highly complex systems, such as the organization of shape and size in the embryo: on the one hand, the cyclic expression of some genes in a given embryonic region now appears to be used by cells as information about their position in both antero-posterior and medial/lateral coordinates; on the other hand, after years of attention to single genes and/or pathways, it has become clear that “independent” protein factors synergize in the regulation of fundamental complex processes, such as cell death and the construction of organ shapes. We also understand better how cells divide and how daughter cells separate to become independent, through work done here on the characterization of centrosomes. The study of viral genes and proteins that subvert cellular functions continues to give us insights into the biology of both the cell and the virus: one group at the IGC has now identified a protein from herpes virus that, through binding to a major component of a molecular pathway used by the cell to receive signals from its environment, “occupies” that pathway to its “advantage”. The immunologists, in turn, are excited with the perspective that complex, “distributed” properties such as natural immunological tolerance, involving many cell types and developmental “programs”, are now yielding to a better understanding through the identification and characterization of a class of cells designated as “regulatory” which appear as “master players” in that process. They have advanced the field by the characterization of receptor specificities of such T cells in a mouse model of multiple sclerosis, and are on the way to identify similar cells in diabetes. The dream of producing T cells under controlled conditions in vitro is also being pursued, in collaboration with an “external” IGC group that is analysing the role of a cell-to-cell communication system that is widely expressed in the embryo and may hold the key to another “big question”: differentiative commitment. This group has shown that distinct Notch-1-ligands mediate alternative commitment signals to the differentiating hematopoietic cell, thus opening new perspectives in this exciting field of “stem cell” differentiation. Yet higher levels of complexity are better approached at the present time by theoreticians, for they are not limited in the construction of models and hypotheses by the complexities of the

experimental systems. Their work is fundamental, nevertheless, as they try to “make sense” of many empirical observations and are prime consumers of “data”. For example, novel theories were derived here for the understanding of processes involving multiple hierarchical levels of life (genes, chromosomes, cells, organisms, societies of organisms, all viewed as different levels of social groups), and for questions as complex as the control of their resilience, according to the degree of relatedness of the respective individual members. While these theoretical models deal with biological evolution, the parallel with our human societies is striking.

On technical grounds, the increasing utilization of “gene- or oligo-chips”, of cellular imaging, and of bioinformatics by several groups was, perhaps, the most significant development. This has led to new requirements and necessary investments in equipment, notably in microscopy, computers and DNA arrays stations.

Finally, 2001 marked the launching of systematic and organised concerns with the intellectual property generated at the Institute. Profiting from the arrival of an outstanding scientist who also has solid entrepreneurial experience, the IGC has now submitted its first patent, and is determined to engage in sustained efforts in this direction. Along the next few years, we hope to “incubate”, together with the new scientific leaderships, young entrepreneur-scientists as well, thus contributing to re-inforce this component of an active community in modern biomedical research.

António Coutinho

## **RESEARCH**

The IGC's scientific interests are centered on the genetic basis of development and evolution of complex systems, privileging organism-centred 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.

## **PROJECT REPORTS**

### **Genetics, disease and biology**

Member: John Stewart

The major work carried out during the past year is the further development of a new method of genetic analysis for multifactorial genetic diseases. The basis of this method is a threshold model in which the probability of developing disease is an S-shaped function of the number of "disease alleles". The input data required consist of: population frequency of the disease (PF), monozygotic twin concordance rate, and frequency in sibs of affected probands. The frequency of disease alleles can be estimated from data in the literature; for a wide range of genetic diseases (PF varying from 0.04% in Autism to 33% in Allergy), the average value is surprisingly high, 24% (range 1%-76%). This represents a reversal of current perspectives, according to which "disease alleles" are supposed to be very rare. Confirmation of this new perspective will require the identification of physiologically significant "sub-phenotypes" specific for each of the gene loci involved.

### **Genetic Epidemiology of Autism**

Members: Astrid Moura Vicente and Luísa Mota Vieira

Students: Ana Margarida Coutinho and Susana Silva

Collaborator: Guiomar Oliveira, Hospital Pediátrico de Coimbra (HPC)

The epidemiological survey for autistic children conducted by this group in 2000, which covered 20% of school children aged 7-10 in mainland Portugal and the Azorian Islands, identified 252 children suspected of suffering from autism spectrum disorder (ASD). During 2001, the identified children were evaluated for diagnosis meeting Autism Diagnostic Interview-Revised (ADI-R) criteria, by the clinical team from the HPC. The children with a confirmed diagnosis of ASD will be recalled during the next year to the HPC, for a multidisciplinary research protocol that includes clinical and neurological examination, developmental and cognitive assessment, and cytogenetic and biochemical analysis, and for pedagogic orientation. Biological samples for a cell, serum and DNA bank will then be collected. At the same time, the family history of these children will be

studied, using a structured instrument for diagnosis of psychiatric illness in relatives of the proband, when these are not available for direct examination. We want to test the hypothesis that cognitive or psychiatric dysfunction is more common in families of autistic probands than in the general population, and that therefore the same genetic defects may be underlying common behavior alterations in a spectrum of cognitive or psychiatric diseases segregating in these families. In parallel, the collection of biological samples from autistic patients routinely followed at the HPC, and first-degree relatives, has progressed. This population sample presently comprises 129 nuclear families with 136 autistic patients, meeting the ADI-R cutoff for diagnosis of ASD. For these patients a large body of clinical, biochemical and behavioral data is available, gathered following the research protocol mentioned above.

Genetic analysis of selected candidate genes and chromosomal regions in this population is underway. The chromosome 7q31 region, where both linkage to autism and a gene mutation responsible for Specific Language Impairment have been found, is being evaluated. Several markers covering this region were tested, and an association with ASD was replicated in this sample. The same markers are being tested for association with a phenotype of severe language deficit within the ASD sample, which is defined by low scores for language developmental quotient and near normal global developmental quotient, as tested using a specific development evaluation procedure (Griffiths Mental Development Scale II). High serotonin levels in platelets of a proportion of ASD patients have been consistently reported, but the significance of this observation in autism is not known. We have measured serotonin levels in patients and relatives, and are testing genes involved in the serotonin pathway for association with this phenotype. Allelic association of the serotonin transporter was found both to ASD and to hyperserotonemia in our population, in agreement with a role for the transporter in the etiology of autism. The hypothesis of an autoimmune dysfunction in autism is being tested, analyzing the occurrence of autoimmune reactivities against human brain tissue in the serum of patients and family members and age-matched controls. The patterns of antibody reactivities are being evaluated using the Panama Immunoblot technique and multiparametric analysis. Preliminary data evidenced shared reactivities within the nuclear families, but no significant differences in autoantibody profiles between autistic patients and age-matched controls.

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

Members: Astrid Moura Vicente, Carlos Ferreira, Constantin Fesel, Francisca Fontes, Luísa Mota Vieira, Jorge Carneiro, John Stewart, Dan Holmberg, Jocelyne Demengeot, Carlos Penha Gonçalves and António Coutinho

Student: Marta Barreto

The collection of SLE patients and family members has progressed throughout 2001, in collaboration with the Associação de Doentes com Lupus. Presently, 82 familial cases have been identified, and from these 16 multigenerational families have already been collected. Identification 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. The first objective of this work is to define disease-associated immune phenotypes, which can subsequently be genetically mapped. Given that antinuclear antibody (ANA) production is a main characteristic of SLE, we are focusing on the analysis of autoantibody reactivities in patients and relatives. We find that antinuclear antibodies are often present in relatives not filling the criteria for diagnosis of SLE, but that may suffer from minor symptoms that occur associated with the disease. Pilot segregation studies for ANA in these families indicates that, while the transmission pattern of SLE is not defined, the inheritance of ANA follows a clear pattern in some pedigrees. The patterns of autoantibody reactivity against nuclear extracts are being further studied using a quantitative immunoblot technique designated Panama Blot. Multiparametric analysis of the multiple antibody reactivities in these blots will be performed with the aim to define autoimmune traits associated with the disease and/or segregating within the families. Preliminary data obtained with this technique shows some reactivity differences between patients and healthy controls, and shared reactivities within affected and unaffected members of the same families. The epitope specificities of the antinuclear antibodies in patients and unaffected, but ANA-positive, relatives are being further investigated by immunofluorescence in Hep-2 cell nuclei.

### **Genetics of murine IDDM**

Members: Dan Holmberg and Carlos Penha Gonçalves

Students: Marie Louise Bergman and Susana Campino

This project aims at identifying and genetically map subphenotypes associated with disease development in the Non-Obese Diabetic (NOD) mouse model for type 1 diabetes. Autoimmune diabetes in the NOD mouse is a multifactorial and polygenic disease, controlled by at least 19 susceptibility loci, named insulin dependent diabetes (*Idd*) loci 1-19. To date, no etiological mutations have been definitely identified as contributors to T1D pathogenesis. Recently, we replicated our previous finding that the apoptosis resistance induced by dexamethazone maps to the *Idd6* region. We also studied the genetic control of the apoptosis resistance phenotype in a cohort of F2(B6xNOD) females and have obtained evidence of linkage of this phenotype to a region overlapping the *Idd6* region. Further, we have established *Idd6* congenic NOD and B6 strains, containing a region of the *Idd6* locus that appears to control thymocyte apoptosis resistance as well as to contribute to the control of type 1 diabetes.

### **Molecular basis of the DiGeorge syndrome**

Member: Moisés Mallo

Collaborator: Virginia Papaioannou, Columbia University, New York, USA.

The DiGeorge syndrome is a dominant disorder characterized by cardiac outflow tract anomalies, hypoplasia of the thymus and parathyroid glands, cleft palate and facial dysmorphogenesis. In humans, this syndrome is frequently associated with

microdeletions in the 22q11 chromosomal region. It has recently been shown that one gene located within this region, *Tbx1*, is the major gene responsible for the manifestations associated with this syndrome. However, these manifestations are present with variable penetrance and severity depending on the genetic background. This variability can be accounted for by the presence of other genetic loci, called modifiers, that influence the phenotype. We have found that inhibition of BMP signaling in the premigratory neural crest phenocopies the DiGeorge phenotype. Therefore, genes within this pathway are prime candidates to act as modifiers. We are testing this possibility using a genetic approach. In addition, we are performing a molecular analysis of the origin of the various malformations associated with the DiGeorge syndrome both in the *Tbx1* mice and in the "antiBMP" transgenic mice.

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

Members: Dan Holmberg, Carlos Penha Gonçalves, Sylviane Pied and Pierre André Cazenave

Student: Susana Campino

To elucidate the genetic mechanism involved in the control of susceptibility/resistance to murine malaria we are taking advantage of the fact that some inbred wild-derived mouse strains display unique phenotypes of resistance. Crosses of these mice with susceptible laboratory strains C57BL/6 are used for genetic mapping and identification of genes controlling resistance/susceptibility. Recently, we have studied the WLA strain, which is highly resistant to cerebral malaria. In a genome wide screening of a F2 cohort two loci linked to cerebral malaria resistance and two loci linked to resistance to hyperparasitemia have been identified. Using a congenic mouse approach we are currently aiming to identify and characterize candidate genes in these chromosomal regions.

### **Tradeoffs in the evolution of virulence**

Member: Francisco Dionisio

Student: Olivia F. R. Rodrigues

Virulence, defined as the amount of damage that a parasite causes to its host, is influenced by several evolutionary processes. For example, parasites are favored to exploit their hosts prudently to prolong infection and avoid killing the host. Thus, there is a tradeoff between prudent exploitation and rapid reproduction. That is, there is a tradeoff between transmission and virulence. In order to study the evolution of virulence, we are using three different approaches: an experimental approach using conjugative plasmids and their bacterial hosts; a computational approach by modeling the interaction and evolution of parasites and their hosts, in order to better understand experimental results, as well as to plan experiments with plasmids and bacteria; a theoretical approach, through the analysis of theoretical models of the evolution of competition, cooperation, symbiosis, virulence and mutualism. We have been studying the diversity of transfer

ability of plasmids among different bacterial types and the relationship of that diversity with the evolution of virulence.

## **HIV2 as an attenuated model of aids virus infection**

Members: Jorge Carneiro and Zvi Grossman

Collaborators: A.E Sousa, A. Loureiro, R. Victorino, Unidade de Imunologia Celular /CEBIP/Faculdade de Medicina de Lisboa, Portugal

The aim of this project is to clarify the causal relationships among CD4 cell depletion, HIV replication and immune activation observed during AIDS pathogenesis. Our strategy consists in comparing the immunodeficiencies caused by HIV1 and HIV2, and in using mathematical modelling in the interpretation of results. We stratified and paired groups of HIV-1 and HIV-2 infected patients whose levels of CD4 depletion fell in the same range and found that the two infections exhibited a similar imbalance in the naïve/memory-effector population ratios with comparable up-regulation of CD4 and CD8 cell-activation markers (HLADR, CD38, CD69, Fas molecule). Moreover, there was a similar increase in the frequency of cycling CD4 T cells (Ki67<sup>+</sup>), which was in strong correlation with the expression of activation markers, and a similar level of anergy, as assessed by the in-vitro lymphoproliferative responses to CD3 stimulation in the presence or absence of CD28 co- stimulation and to a panel of microbial antigens. These findings are surprising considering that the two HIV-associated immunodeficiencies are characterized by markedly different plasma viral loads and are known to display different rates of CD4 T cell decline and clinical prognosis. They suggest a causal relationship between immune activation and CD4 T cell depletion in HIV disease and only an indirect relationship of these parameters to the virus rate of replication. A definitive clarification of these relationships in terms of cause and effect would be relevant for the delineation of immune based strategies in the treatment of HIV disease. Mathematical models are being developed to better understand these relationships in quantitative terms, that will eventually lead to testable predictions.

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

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

Student: Sílvia Almeida

The aim of this project is to identify and exploit viral modifiers of apoptosis and cytokine responses (particularly TNFRs) as a potential source of novel health care pharmaceuticals for manipulation of immune responses and treatment of certain diseases. Such virus genes will be identified by nucleotide sequence and functional analysis of cloned viral ORFs and cDNA libraries of three large DNA viruses (African swine fever (ASFV), Ectromelia virus (EV) and Mouse herpes virus (MHV 68)). As a direct approach towards identifying novel virus evasion genes which do not have homologies in the data base, the

genes of these three viruses will be systematically screened in functional assays for their impact on apoptosis and for binding to chemokines and cytokines.

Most of the year's work has been occupied with subcloning virus genes into expression and retrovirus transfer plasmids. As a necessary prelude we have had to modify existing plasmids for expression (pcDNA.3) and retroviral cloning (pLXIN-neo and PBABE-puro). These modifications include the design of appropriate restriction sites for facile subcloning of (1) Expression (cDNA) libraries of mouse herpes virus (MHV68) and Ectromelia, the mouse pox virus (EV) and (2) The 60-80 viral ORF's of unknown function and unrecognised homology produced by PCR cloning from African Swine Fever Virus (ASFV) and MHV68 genomes. In addition, the plasmids have been modified to include an influenza haemagglutinin tag to facilitate identification, tracking, visualization and purification.

To date, we have cloned the bifunctional inhibitor of NFkB and NFAT (ORF 238 L of ASFV), pro-apoptotic, LC dynein-binding protein (ORF E183L of ASFV), and 3 anti-apoptotic genes (ORF M11 of MHV 68, ORF 224L of ASFV and ORF A179 of ASFV). Systematic cloning of genes with no recognizable homology in the data base is underway, and the construction of virus cDNA libraries from MHV68 and EV has been accomplished. These will now be submitted to functional assays to search for viral gene products that bind TNF or modify the induction of apoptosis.

### **Molecular Pathogenesis of Murine Gammaherpesvirus-68 in Mice and Molecular Interactions in Murine Gammaherpesvirus 68 Latent Infection of B-Lymphocytes**

Members: Pedro Simas and Alexandra Teixeira

Students: Patricia Madureira, Sofia Marques and Lidia Fonseca

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.

The research theme in my laboratory 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 & Efsthathiou, 1998; Doherty and Christensen, 2000). MHV-68 is genetically related to Epstein-Barr virus and Kaposi's sarcoma associated herpesvirus, which are important human pathogens. Experimental infection of inbred strains of mice with MHV-68 results in acute productive infection of the lung followed by latent infection of B-lymphocytes. Comparison of the genomic organisation of MHV-68 with other gammaherpesviruses shows the presence of virus specific ORFs and a number of cellular homologues, which are predicted to determine the particular biological properties of these viruses, e.g. host range, immune evasion, latency and disease. In MHV-68 fourteen such genes are present, designated M1 to M14, and several cellular homologues, including a complement control protein, a D-type cyclin and an IL8 receptor. In addition to these cellular homologues two of the  $\text{CEM}^1$  genes, M1 and M11, show low level similarity to serpins and bcl2 cellular protein, respectively.

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

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

### **T- cell responses in the pathogenesis of malaria**

Members: Sylviane Pied, Sergio Gulbenkian and Pierre-André Cazenave

Students: Rui Manuel Trindade Oliveira, Sebastien Bagot, Vincent Guiyedi

The main objective of our project is to analyse the T-cell response involved in the pathogenesis of Cerebral Malaria (CM). Immune responses triggered by the malarial parasite play a major role in controlling parasite replication (protection) but are also associated with pathological changes that occur during malarial infections (pathogenesis). An understanding of the interaction of the parasite with the host immune system would be essential for the development of interventions to reduce malarial morbidity and mortality. We have previously shown that the occurrence of the neuropathology is linked to ?? T cells in mice infected with *Plasmodium berghei* ANKA, a parasite strain able to induce cerebral malaria in susceptible mouse strains. We now focus on the characterisation of these ?? T cell subpopulations and on their pathogenic function. We have observed a significant increase in the number of CD8<sup>+</sup> T cells in the brain of mice developing CM when compared with those that do not develop the disease. These CD8<sup>+</sup> T cells exhibit a phenotype of activated cells as demonstrated by the level of expression of CD25, CD69, CD44 and CD62L surface markers and are not undergoing T cell apoptosis. They secrete ?-IFN and TNF. To further characterise CD8 population from the brain of CM positive mice, we have studied the repertoire of TCRBV genes that is expressed in these cells, using the Immunoscope approach on a large scale and new informatic tools (ISEApeaks) to analyze the huge amount of data gathered. Data obtained showed a polyclonal CD8 T cell response and allowed the identification of putative pathogenic, recurrent clones expanded in CM<sup>+</sup> mice but not in infected mice that do not developed CM (CM<sup>-</sup>). Finally, functional studies using are *in vivo* and *in vitro* systems are in progress with the aim to better understand the role played by these CD8<sup>+</sup> T cells in the pathological process.

### **Study of Immunogenic Proteins of Mycoplasma mycoides subsp. mycoides Small Colony Type.**

Members: Pedro Simas Jose Regalla and Rosario Goncalves (LNIV)

Students: Lidia Fonseca and Paula Silva (LNIV)

*Mycoplasma mycoides* subsp. *mycoides* (Mmm SC) is the causative agent of contagious bovine pleuropneumonia (CBPP) in domestic cattle. Due to its economic impact for agriculture this disease has been included in List A by the Office International des Epizooties and has been eradicated from most European countries. Although, extensive control and eradication programs have been implemented in Portugal this disease is still endemic. Part of the difficulties of the control and eradication programs are due to the fact that the available diagnostic tests fail to detect asymptomatic carrier animals and lack sensitivity and specificity.

The proposed work is part of a formal collaboration between the Laboratorio Nacional de Investigacao Veterinaria (LNIV) and the Instituto Gulbenkian de Ciencia (IGC). Its objective is to develop a specific, sensitive and rapid serological diagnostic test by: i) identification and cloning of Mmm SC immunodominant protein encoding genes (IGC), ii) epitope mapping of Mmm SC immunodominant proteins (IGC), iii) analysis of intraspecific variability of Mmm SC (LNIV) and iv) to develop improved serological reagents and tests for CBPP (LNIV).

### **Strain typing of BSE in Portugal and genetic susceptibility to prion diseases.**

Members: Pedro Simas and Alexandre Galo (LNIV)

Student: Leonor Orge

This project is part of a collaboration between the Laboratorio Nacional de Investigacao Veterinaria (LNIV) and the IGC. The objectives of this project are: i) to type the current agent of bovine spongiform encephalopathy in Portugal and ii) to determine Prnp gene polymorphism in selected Portuguese sheep populations.

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

Member: R.M.E. Parkhouse

Collaborators: T. Garate (Instituto de Salud Carlos III, Centro Nacional de Microbiologia, Madrid, Spain), L. Harrison (University of Edinburgh, Department of Tropical Animal Health, Centre for Tropical Veterinary Medicine, Scotland), E. Sciutto (Universidad Nacional Autonoma de Mexico, Institute de Investigaciones Biomedicas, Mexico), M. Cortez (Universidad de Carabobo, Venezuela) and 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 in pig and man. The related parasite, *Taenia saginata*, similarly infects man as an intestinal tapeworm but passes its metacestode stage only in cattle. Rural transmission is mediated by poor sanitation and uncontrolled pig and cow management practices, and so the prevalence of these parasites is an objective indicator

of rural poverty. Recently, population movement linked to close human/pig and cow contact in the rural-urban interface has exacerbated the problem. Control through improved sanitation is a major, long-term and expensive goal. This project focuses on the shorter-term, more cost-effective strategies of improving pig and cow management, including village pig vaccination (transmission control) and the development of sensitive and specific diagnostic assays to detect parasites and anti-parasite antibodies, the latter based on synthetic peptides, recombinant reagents and PCR, not parasite material. New diagnostic assays will improve hospital patient monitoring/treatment and man/pig screening and hence epidemiological knowledge.

To date, we have succeeded in developing the following diagnostic tests: 1) PCR tests for the differential diagnosis of cestode parasites (*Taenia solium*, *Taenia saginata* and *Echinococcus*); 2) Synthetic peptide based assays to detect antibodies to *Taenia* parasites 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 a potential vaccine for bovine cysticercosis, based on a recombinant oncospherical surface and secreted molecule which, interestingly is functionally, an adhesion molecule, possibly facilitating tissue invasion by the parasite in the intermediate host.

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

Member: José Feijó

Students: Ana Catarina Certal, Sofia Cordeiro, Ricardo Bandarrinha Almeida, Ana Margarida Prado, Ana Maria Vieira, Sílvia Costa 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: Ana Catarina Certal, Sofia Cordeiro, Ricardo Bandarrinha Almeida, Ana Margarida Prado, Ana Maria Vieira, Sílvia Costa 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.

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

Member: Isabel Palmeirim

Student: Catarina Freitas, Sofia Rodrigues and Inês Ribeiro

In Human, Mouse, Chicken, Fly or any other organism the time of embryonic development is maintained and strictly controlled. Each step of embryonic development will only achieve the desired effect if it occurs at the right moment and in the right place. Somites are transient, segmented embryonic structures that constitute the basis of the segmental pattern of the adult vertebrate body. They give rise to structures such as the axial skeleton, the dermis of the back and all striated muscles of the adult body, except those of the head. In the Chick embryo, a pair of somites buds off, in a highly coordinated fashion (every 90 minutes) from the cranial end of the presomitic mesoderm (PSM) while new PSM cells enter the caudal end of this tissue as a consequence of gastrulation. Very little is known about the spatial and temporal coordination of this segmentation process in vertebrates. Recently, two avian homologues of the hairy *Drosophila* gene, “c-hairy1” and “c-hairy2”, were cloned, and the study of their expression pattern showed that these genes are dynamically and cyclically expressed in the PSM, in an autonomous manner and with a periodicity corresponding to the formation time of one somite (Palmeirim *et al.*, 1997; Jouve *et al.*, 2000). The dynamic expression pattern of these genes (“c-hairy1” and more recently the “c-hairy2”) provided the first molecular evidence for the existence of a developmental clock linked to somitogenesis.

“c-hairy” genes are expressed in prospective segmented tissues like the PSM but are completely absent in presumptive unsegmented tissues such as the lateral mesoderm (LM). Nevertheless, LM cells are able to form segmented structures (somites) if CHO-Noggin expressing cells are grafted in the prospective area of this tissue inhibiting the BMP4 (Bone Morphogenic Protein 4) action in the LM (Tonegawa *et al.*, 1997). In addition, if stage 4 (HH) Quail Hensen's node is grafted isochronically and heterotopically in the lateral or extraembryonic region of a Chick embryo it will induce the formation of a secondary axis with neural tube, notochord and ectopic somites (Hornbruch *et al.*, 1979). We intend to evaluate the expression of the genes implicated in the clock controlling somitogenesis (i.e. the c-hairy genes) during the formation of these ectopic somites. If the formation of these ectopic somites results in the activation of the “c-hairy” genes, we will use the Quail/Chick chimera model to determine which cell type(s) is (are) able to upregulate this expression (Quail/donor/Hensen's node cells or



Chick/receptor/lateral mesodermal cells). Experiments involving ectopic overexpression of Noggin or BMP4 will allow us to evaluate the relationship between these genes and the segmentation clock. Finally we intend to study the expression of these genes in other tissues where they are expressed in order to understand whether this time counting system is exclusive for PSM cells or whether it represents a more general mechanism for counting time during vertebrate embryonic development.

### **Evidence for medial/lateral specification and positional information within the presomitic mesoderm**

Member: Isabel Palmeirim

Students: Catarina Freitas and Sofia Rodrigues

In the vertebrate embryo, segmentation is built on repetitive structures, named somites, which are formed progressively from the most rostral part of presomitic mesoderm, every 90 minutes in the avian embryo. The discovery of the cyclic expression of several genes, occurring every 90 minutes in each presomitic cell, has shown that there is a molecular clock linked to somitogenesis. In this work we demonstrate that a dynamic expression pattern of the cycling genes is already evident at the level of the prospective presomitic territory. The analysis of this expression pattern, correlated with a quail/chick fate-map, identifies a “wave” of expression travelling along the future medial/lateral presomitic axis. Further analysis also revealed the existence of a medial/lateral asynchrony of expression at the level of presomitic mesoderm. This work suggests that the molecular clock is providing cellular positional information not only along the anterior/posterior but also along the medial/lateral presomitic axis. Finally, by using an in vitro culture system, we show that the information for morphological somite formation and molecular segmentation is segregated within the medial/lateral presomitic axis. Medial presomitic cells are able to form somites and express segmentation markers in the absence of lateral presomitic cells. In contrast and surprisingly, lateral presomitic cells, deprived of their medial counterparts, are not able to organise themselves into somites and lose the expression of genes known to be important for vertebrate segmentation, such as *C-Delta-1*, *C-Notch-1*, *paraxis*, *c-hairy1*, *c-hairy2* and *lunatic fringe*.

### **Analysis of epithelium to mesenchyme transitions in integrin $\beta 1D$ knock-in embryos**

Member: Sólveig Thorsteinsdóttir

Students: Ana Sofia Cachão and Fernanda Bajanca

Collaborators: Christine Mummery and Susana Chuva de Sousa Lopes, Hubrecht

Laboratory, Univ. Utrecht; Arnoud Sonnenberg, Netherlands Cancer Institute, Amsterdam, The Netherlands

The  $\beta 1$  integrin subunit undergoes alternative splicing in its cytoplasmic domain giving rise to two different splice variants in the mouse:  $\beta 1A$  and  $\beta 1D$ . In the adult,  $\beta 1D$  is present on both adult cardiac and skeletal muscle, while all other tissues express  $\beta 1A$

(van der Flier et al., 1995, FEBS Lett. 369:340; Zhidakova et al., 1995, BBRC 214:279; Belkin et al., 1998, JCB 132:211). The  $\beta$ 1D splice variant starts being expressed very late in embryogenesis: from E17.5 in skeletal muscle and from around birth in cardiac muscle (van der Flier et al., 1997, Dev. Dyn. 210:472). Exon-specific  $\beta$ 1D knock-in mouse embryos have been generated via homologous recombination in ES cells in which  $\beta$ 1A was replaced by  $\beta$ 1D; this was lethal at midgestation on the original genetic background (50% 129Ola:50% FVB) and embryonic fibroblasts isolated from embryos homozygous for the  $\beta$ 1D knock-in allele (ki/ki) showed an impaired migratory potential in vitro (Baudoin et al., 1998, Genes Dev. 12:1202).

In this study, we backcrossed heterozygous mice onto a FVB background for four generations and determined genotype frequencies at different stages of development. A total of 728 embryos were collected from heterozygous crossings between E8.0 and E18.5. We found expected ratios of all genotypes until E14.0 and after that two periods of lethality were evident: 60% of homozygous  $\beta$ 1D knock-in (ki/ki) embryos were lost around E14.0 and the remaining 40% of  $\beta$ 1D ki/ki embryos died at birth.

Since previous analyses had indicated a defect in cell migration which, if identified, could be responsible for the early period of lethality observed, we studied the behaviour of three migrating cell populations in vivo: cranial neural crest cell (revealed by *snail* expression), limb muscle precursor cells (revealed by *pax3* expression) and primordial germ cells (revealed by alkaline phosphatase staining). No difference was observed between  $\beta$ 1D ki/ki embryos and their littermates, thus suggesting that  $\beta$ 1D can support the migration of cell populations in vivo. We therefore conclude that the early lethality observed is most likely due to a malfunction not related to embryonic cell migration. Since many dying embryos recovered soon after E14.0 were distinctly white in appearance, we investigated the morphology of the placenta of these embryos. Based on the abnormal morphology of the labyrinth layer of the placenta, we suggest that placental insufficiency might be responsible for the early lethality of  $\beta$ 1D ki/ki embryos.

About 10% of  $\beta$ 1D ki/ki embryos develop remarkably normally until birth, but at E18.5 and P1 they are distinctly thinner and/or smaller than their littermates. Histological analysis showed that this phenotype was due to a dramatic (35%) reduction in muscle mass, particularly evident in the trunk muscles and diaphragm. Quantification of primary vs. secondary fibres in E18.5 embryos showed that there was a proportionally equal reduction in the number of both fibre types. We are currently investigating the molecular mechanism underlying this reduction in muscle fibre number in  $\beta$  1D ki/ki embryos.

### **What is the function of colloid during somite differentiation?**

Member: Isabel Palmeirim

Student: Vanessa Zuzarte

A member of the bone morphogenetic protein-1 (BMP-1) family which encodes for a metalloprotease was identified in different organisms and named Tolloid in fly, fish and mammals and Xolloid in frog. This metalloprotease acts by a double inhibition mechanism to establish the graded activity of BMPs, genes involved in the establishment of the vertebrate embryonic dorsoventral axis. Tolloid proteins specifically cleave

Chordin allowing the activity of BMPs. Liaubet et al.,(1998) identified the chick-tolloid-like gene (colloid) and described its role in the patterning of the neural tube.

In the vertebrate embryo, somites constitute the basis of the segmental pattern of the body and give rise to the axial skeleton, the dermis of the back and all striated muscles of the body, except those of the head.

During differentiation, somites become polarised according to a mediolateral axis. BMP4 is produced by the lateral plate mesoderm and has been proposed to play an important role in lateral somite specification (Pourquié et al, 1996). Determination of the dorsomedial mesodermal lineages requires the inhibition of BMP4 activity by proteins such as Noggin, Chordin or Follistatin.

We analysed the pattern of colloid expression during the somitogenesis, in chick embryos at different stages of development. This gene is expressed in the most anterior somites and its expression varies according to somite differentiation reflecting the mediolateral patterning. By using a tridimensional embryo tissue culture technique and in ovo operations we determined the role of surface ectoderm, neural tube, notochord and lateral plate in the induction and maintenance of colloid expression.

### **Formation of the myotome in the mouse**

Member: Sólveig Thorsteinsdóttir

Students: Fernanda Bajanca and Marta Luz

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

In vertebrates, the cells that will form the epaxial (dorsal) skeletal muscles, are derived from the dorso-medial part of the somites. Myocyte precursors undergo an epithelium-mesenchyme transition and organise themselves into a parallel array of mononucleated myocytes located in the dorsal part of the embryo, the epaxial myotome.

A recent model of myotomal formation in the mouse (Venters et al., Dev. Dyn. 216:219, 1999) suggests that myocyte precursors arise in the dorsomedial edge of the dermomyotome where they proliferate. These cells then undergo an epithelium to mesenchyme transition at the most medial part of the caudal and rostral edges of the dermomyotome and then migrate to a central area under the dermomyotome. After the cells reach the central portion of the dermomyotome, they start elongating in a rostral and caudal direction, forming the elongated morphology characteristic of myotomal myocytes. New cells are constantly added to the most medial portion of the myotome, while the older cells are displaced laterally with the continuous growth of the embryo.

In this project, we are characterising the expression pattern of extracellular matrix molecules and their integrin receptors in order to determine how cell migration and elongation is regulated in this system. We find that the expression pattern of integrins is dynamic and variable, suggesting that several migration patterns are involved in the formation of the mouse myotome along its different stages of development. Furthermore, we are performing an analysis of embryos homozygous for the *myf5* null allele (Tajbakhsh et al., Nature 384:266, 1996), where the epaxial myotome does not form

normally, in order to understand what cell-extracellular matrix interactions are essential for the morphogenesis of this tissue.

### **Specification of vertebrate limb bud.**

Members: Juan Carlos Izpisúa Belmonte, Ramón Díaz Trelles, Simone SanMartín Gines and Joaquín Rodríguez León

Students: Sofia Simões, Sara Marques and Alexandre Gonçalves

In the last years several groups have isolated some genes involved in the specification of limb identity. These genes are members of the T-box family. Only two of them are expressed in a restricted fashion in the forelimb (tbx5) and hindlimb (tbx4). We are interested in the study of genetic pathways that control the establishment of a specific limb. In order to find new genes that govern the morphogenesis of a forelimb or a hindlimb we have used a DNA microarray technology. We have compared RNA extracted separately from hindlimb and forelimb in a microarray that contains 4.608 genes obtained from a total limb library. The result will allow us to identify the genes that are expressed either in the hindlimb or in the forelimb. We will process these data and study the differential expression patterns of these genes during limb development. The next step will be the functional analysis of these genes and their interactions with other genes involved in growth, determination and specification of limbs.

### **New aspects on coordinating limb bud development**

Members: Isabel Palmeirim and Joaquín Rodríguez-Léon

Student: Cláudia Carvalho and Susana Pascoal;

The vertebrate limb originates from a dual contribution of lateral plate and somitic mesoderm (Chevallier et al., 1977). Through differential proliferation of the flank, specific regions of the lateral plate form buds at presumptive limb levels. Shortly thereafter, cells from the lateral edges of nearby somites migrate into the limb. All adult limb muscles derive from these migratory cells. The limb bud is enveloped by an overlying ectoderm, the distal tip, of which forms a specialized epithelial structure, the apical ectodermal ridge (AER) at the interface of dorsal and ventral territories (Saunders, 1948; Todt and Fallon, 1984). Cells directly under the AER remain undifferentiated in a region of distal mesenchyme in close proximity to the AER, denominated the “progress zone”, while condensation initiates in proximal limb regions, causing the humeral segment to form first, followed by the radius and ulna, and lastly by the digits. The first clues to how this complex shape and form is achieved came from the work of experimental embryologists who identified specific regions of the developing chick limb bud that are essential in directing growth and patterning. Of the three cardinal limb axes (D/V; A/P and P/D) the mechanisms that lead to cell fate specification along the P/D axis are the least understood. One paradigm, largely unmodified since its conception more than 20 years ago, is the progress zone model (Summerbell et al., 1973). According to this model, cell fate along the P/D axis is specified by the time spent in the progress zone.

Until now no evidence has been provided to explain how cells “know” the time they spend in the progress zone. Recently, in a completely different system – somitogenesis – Palmeirim et al., (1997) presented evidence for the existence of a molecular clock underlying the process of chick somitogenesis by showing that presomitic (PSM) cells undergo several cycles of *c-hairy1* gene expression, with a 90 minutes periodicity, corresponding to the time required to form one segment. These *c-hairy1* mRNA oscillations of expression occur in each PSM cell until it is incorporated into a somite. These results provide the first molecular evidence of a developmental clock linked to somitogenesis (Palmeirim et al, 1997). Later, this same type of behaviour was also described for a gene coding for a closely related transcription factor, *c-hairy2* (Jouve et al., 2000). Since “*c-hairy1*” and “*c-hairy2*” are also expressed in different limb bud regions such as the progress zone, with an apparent dynamic pattern of expression (preliminary results) we wondered whether these genes are implicated in the system of time counting that allows undifferentiated progress zone cells to “know” how much time they spend in the progress zone.

In addition, we intend to study the role of these genes in ZPA, AER and muscle development, since these genes are expressed in these tissues during limb bud development.

### **Mechanisms of head induction in vertebrates**

Members: José António Belo, Ana Teresa Tavares and Vera Teixeira

Students: Mário Rui Filipe, Ana Cristina Borges and Sara Marques

Mouse *cerberus-like*, a member of the *Cerberus/Dan* gene family is a secreted factor expressed in the Anterior Visceral Endoderm (AVE) of the pre-streak mouse embryo. This region has been implicated in anterior neural specification. The neural inducing and mesoderm inhibition activities of *Cer-1* result from specific inhibition of BMP4 and Nodal molecules, respectively. These activities are shared with the closely related *Xenopus cerberus* (a gene implicated in head induction in *Xenopus* experiments) and chick *Caronte*.

We have previously reported the targeted inactivation by homologous recombination in ES Cells of the mouse *cer-1* gene. Currently we are studying possible genetic interactions of *cer-1* with other genes involved in head formation to try to find the compensatory pathways involved in this process. A case of genetic compensation has been found between the BMP antagonists *chordin* and *noggin*. We have reported on the interaction of *cer-1* and *noggin* (Borges et al, 2001). Currently we are pursuing these studies using mouse mutants for the genes *goosecoid* (Borges et al, *submitted*) and *Otx2*, also known to be expressed in the AVE and to be involved in the process of head induction.

Using a differential screening approach, we are on the way to try to isolate novel genes expressed in the Anterior Visceral Endoderm at peri-gastrulation stages and with neural inducing properties. To this end we have generated the plasmid vector pPMcer1.EGFP, which contains the fluorescent marker EGFP under the control of the upstream promoter sequences of *cer-1*. By sorting EGFP positive cells we are able to generate a differential cDNA library which will allow us to identify novel genes expressed in the AVE. An

already identified novel gene related to *cer-l* has been inactivated by homologous recombination in ES cells. Chimeras have been obtained and are being crossed to generate a stable mutant mouse line that will be analyzed in order to access the role of this gene during embryonic development.

In pPMcerl.EGFP transgenic mouse lines, expression of EGFP can be observed in the AVE. When this plasmid is injected in dorsal blastomeres of *Xenopus* embryos, the *cer-l* promoter was able to drive the expression of EGFP in the deep endodermal cells of gastrulating embryos, overlapping with the domain of expression of *Xcer*. Electroporation of pPMcerl.EGFP in chick embryos also resulted into a *Caronte* like expression of EGFP.

We are now studying the regulation of *cerberus-like* promoter and use this promoter to drive the expression of Xwnt8, Xnr-1 or XBMP4 factors in the *Xenopus* embryo anterior endoderm. We hope to gain further insight into the mechanism by which *cerberus* is required for the induction of the head in the *Xenopus* embryo and the role of the controlled inactivation of specific signaling molecules in the anterior endoderm of the vertebrate embryo.

To investigate the extent of evolutionary conservation between the regulatory sequences of mouse, *Xenopus* and chick *cer-like* genes, intra and cross species promoter studies are being performed. To this end, a genomic clone containing the chick *caronte* gene was isolated by screening a chick genomic library with a probe against chick *caronte* cDNA. The analysis of the sequence of the chick *caronte* 5' genomic region and its comparison with the 5' regulatory regions of human, mouse and *Xenopus Cerberus* genes suggested the presence of several putative transcription factor-binding sites.

A 2.5 kb fragment of 5' genomic region of chick *caronte* was seen to drive the expression of a reporter gene into the hypoblast, anterior endoderm, heart precursor cells and left lateral plate.

The preliminary analysis of *Xenopus* embryos injected with *caronte*-promoter driven reporter constructs revealed that the reporter gene is expressed in the topological equivalent regions of the chick embryo, although not only on the left side lateral plate, but on both sides.

Interestingly, transgenic mice generated using this same construct show expression in the heart precursor cells and in the left lateral plate.

These results suggest that the upstream regulators of *caronte* expression are present in equivalent developing regions of the chick, *Xenopus* and mouse embryos.

## **Target genes for Hoxa2**

Member: Moisés Mallo

Collaborator: Nicoletta Bobola, Max-Planck Institute of Immunobiology, Freiburg, Germany

*Hox* genes are essential for different processes during embryonic development. In particular, *Hoxa2* is required for proper development of the hindbrain and the skeleton of the ear and neck. Because *Hox* genes are transcription factors, understanding how they work requires identification of the genes under their control. We have identified several potential targets for *Hoxa2* by a differential screening between wild type and mutant

tissues run on Affymetrix Gene Chips. Several of these candidates proved to be downstream of *Hoxa2* by in situ hybridization. We are now performing biochemical and genetic analyses on those candidates to determine whether they are functionally downstream of *Hoxa2* and whether they are direct or indirect targets of this gene.

## **Hematopoietic differentiation**

Member: Leonor Parreira

Students: Hélia Neves, Isabel Alcobia and Sofia Quina

A recently established research line is focused on the mechanisms underlying cell fate decision processes of normal hematopoietic stem cells. Specifically, the biological role of Notch receptors and their ligands, the Delta and Jagged proteins (coded by “cell-fate decision” genes), are under investigation. Making use of retroviral transducing systems, the expression of these genes is experimentally induced in in vitro assays specially designed for the study of hematopoietic differentiation. We have shown 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/NK precursors. In contrast, Jagged-1 did not disturb B nor T cell/NK development. Studies aimed at clarifying the biological reasons for these differential effects are now under way.

A second research line relates to the functional organization of chromatin in the nucleus of hematopoietic cells. Here, 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 an intrinsic gene spatial dynamics, established early in hematopoiesis, and perpetuated differentially in distinct cell lineages, may facilitate the collision of individual genes and their reciprocal recombination at subsequent stages of hematopoietic differentiation. This phenomenon may be mechanistically relevant for the occurrence of oncogenic gene rearrangements in human leukemia. A further ongoing study aims at extending our findings that chromocenters (associations of centromeres) in lymphoid and non-lymphoid cells represent cell-type-specific arrangements of centromeric heterochromatin. The dynamics of these spatial arrangements during sequential windows of hematopoietic differentiation is now under analysis. Our ultimate goal is to clarify the functional role of those heterochromatic compartments in the regulation of gene expression in hematopoietic cells.

## **Reversible gene inactivation in the mouse**

Member: Moisés Mallo

Student: Joana Martins

We have developed a genetic system that allows reversible inactivation of gene loci in the mouse. The system makes use of the regulatory elements of the bacterial tetracycline-

resistance operon, built into the gene locus of interest. In a first approach we have chosen the *Hoxa2* gene because the phenotype resulting from its inactivation has been well characterized in our and other labs. Using a knock-in strategy, we have introduced seven copies of the tetracycline operator (*tetO*) in the 5' area of the *Hoxa2* gene (we call this allele *Hoxa2<sup>tetO</sup>*). The transcriptional activity of the modified locus is then controlled by the tetracycline repressor (tetR) provided in *trans* as a transgene. We have generated lines expressing the tetR molecule in all tissues (using the human actin promoter) and in a tissue specific fashion (using one of the *Hoxa2* enhancers that drives expression in the hindbrain). In both cases, *Hoxa2* expression from the *Hoxa2<sup>tetO</sup>* locus was down-regulated by tetR and produced the corresponding phenotype. In addition, this repression can then be relieved by administration of doxycycline to the pregnant female. With this system, we are studying the temporal requirements of *Hoxa2* activity during development in the different embryonic areas where it is required. In addition, we are performing a variety of experiments to improve this technology, in order to make it available for its use in any other gene locus.

### **Generation structured models of cell proliferation and their usage to extract information from CFSE-profiles**

Member: Jorge Carneiro

Student: Kalet Leon

CFSE is a fluorescent dye that allows to quantify by FACS the frequency distribution of cells according to the number of rounds of division they underwent since labelling – the frequency distribution of cells in different generations. CFSE based techniques are frequently used to quantify cell proliferation and have been used to address issues of cell differentiation. Several models have been used to extract quantitative information from CFSE profiles. These models differ significantly in their set-up and in the meaning of their variables and parameters. This makes it very difficult to compare results and to assess the scope of conclusions they lead to. With the aim of overcoming this limitation in current theory for CFSE data analysis we developed two comprehensive models that describe the time evolution of the CFSE profile in a mixture of cells. According to the first model cells have division rates that are distributed, i.e. the cells are intrinsically heterogeneous. In the second model cells are homogeneous in their division rate but this is triggered by an interaction that is stochastic in nature. Models proposed by others can be derived from each of these two general models by particular parameter settings. Both general models, but none of their particular simplifications, can fit published data sets about the kinetics of CFSE profiles of in vitro stimulated T cells. The best fitting parameters reveal the following: 1) division time of T-cells is not Gaussian but follows an asymmetric distribution (e.g. gamma distribution); 2) the first round of division takes longer than subsequent rounds of division; 3) co-stimulation accelerates the kinetics of T cell division in every generation; 4) most of the variance in T-cell division can be explained by a single stochastic event leading to or involved in cell cycle.



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

Member: Jocelyne Demengeot

Students: Vasco Barreto, Thiago Carvalho

Collaborator: Miguel Godinho, UCSF

The vertebrate Immune System's hallmark is its ability to respond to a vast number of antigens specifically. This is achieved by antigen receptor gene assembly through somatic recombination of V, D and J segments. V(D)J recombination involves the activity of two lymphoid lineage specific proteins, the recombinase activating genes 1 and 2 (Rag1 and Rag2), and a set of ubiquitous DNA repair factors. Co-expression of Rag genes is lymphocyte specific and RAG protein levels are tightly regulated during lymphopoiesis and cell cycle. RAG1 and 2 target recombination signal sequences (RSSs) that flank antigen receptor coding segments. Rag genes were apparently transferred horizontally from a prokaryote or a virus into the vertebrate genome, 450 million years ago. While the V(D)J reaction is now well understood at the molecular level, its effects on cell cycle, genomic instability and genomic evolution remain largely unclear.

We generated 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 (Barreto *et al.*, Eur.J. Immunol., Dec. 2001). These animals display growth retardation and early death reminiscent of mice deficient in double strand break repair molecules.

We are now addressing the function the Rag genes *in vivo* and their relation to immune system development, oncogenesis and vertebrate genome evolution (review by Barreto *et al.*, submitted).

## **Modelling the activation and differentiation of lymphocytes**

Members: Jorge Carneiro, João Sousa, Zvi Grossman, Jose Faro

The aim of this project is to understand the activation and differentiation of mature lymphocytes as the consequence of the co-operation of signalling by receptors and the regulation of receptor expression. The project encompasses the study by mathematical modelling techniques of the early events of TCR signal transduction, of the cytokine receptor signalling, and of the regulation of the expression of both the TCR and cytokine receptors.

A mathematical model of TCR down-regulation and of the early signalling events of TCR triggering was already proposed [Sousa J. & Carneiro J. (2000) Eur. J. Immunol., 30, 3219]. The most unexpected result was that the kinetics of TCR down-modulation implies that the TCR triggering is ultrasensitive to changes in either ligand or TCR densities. This ultrasensitive response curve can be explained by either oligomerisation of TCRs or by the competition of two enzymes that respectively activate and deactivate ligand-bound TCRs (Sousa & Carneiro, in preparation). The later mechanism is efficient

even at very low ligand densities in contrast with the oligomerisation, suggesting a means by which different signalling cascades may be triggered depending on ligand densities (Sousa, Lino, & Carneiro, in preparation).

In collaboration with Zvi Grossman and Jose Faro we are elaborating further the concept of tuning of activation thresholds aiming to understand what is the contribution of tuning for lymphocyte homeostasis for peripheral self-tolerance. Indeed, it has been claimed that recurrent stimulation by self-MHC ligands leads to the persistence of peripheral T cell pools, and also to the adaptation of autoreactive T cells that persist in an anergic state and are thus unable to mount autoimmune responses. To better understand these processes we developed two models of tuning at the single cell level that share the same qualitative properties. One is the model of TCR down-regulation, which is complex compared to the second model, a minimalist model of tuning based in a Koshland-Golbeter hypersensitive molecular switch of T cell activation. In both models, a constant or slowly increasing stimulus will lead to adaptation and anergy but a sudden increase triggers T cell activation. A decrease in the stimulus will induce a transient state of strong anergy for a period of time. These models were used to understand the response of adaptable T cells for a stimulus delivered by APCs that results in proliferation and/or survival. Proliferative/survival responses that are tuneable by recurrent interactions with APCs show a dependence on T cell density (i.e. the higher the T cell density the higher the proliferative response), but this dependence is generically incompatible with homeostatic regulation. This result implies that if full T cell activation and homeostatic proliferation are both adaptable and dependent on recurrent interactions with stimulatory APCs then tuning of response thresholds cannot per se explain the persistence of anergic autoreactive T cells. Adaptation will rather lead to peripheral deletion of autoreactive T cells.

Cytokine receptor signalling pathways share a considerable number of components, from the receptors down to gene response elements. The aim of this work is to model cytokine signal transduction, exploring the consequences of these signalling pathways sharing receptors components. The first part of the project was modelling the interference between two members of the gc receptor family, namely IL-4R and IL-2R, as a prototype for sharing receptor chains. Using a mathematical model of receptor formation and cytokine binding, calibrated with experimental data, we demonstrate that under physiological conditions IL-2 may act as an inhibitor of IL-4 signalling in an activated T cell, that upregulated CD25. The model also predicts that IL-4 does not significantly inhibit IL-2 signalling. Using this model we predict the extent of interference between these two cytokines as cells transiently change the expression of cytokine receptor chains, namely CD25. Also, designing a cell population dynamics model based on autocrine IL-2 and IL-4 promotion of uncommitted Th precursors and committed Th2 cells, respectively, which takes into account interference between cytokines we suggest a simple mechanism for preventing commitment of an entire lymphocyte population to a particular differentiation pathway, and therefore to ensure functional pluripotentiality.

## **Mechanisms of lymphocyte co-operation involved in immune regulation**

Members: Jorge Carneiro, Jocelyne Demengeot and Shohei Hori

Students: Vanessa Oliveira, Kalet Leon and Iris Caramalho

The mechanisms by which the immune response is regulated are very important both for their fundamental interest and for their clinical applications in the modulation the immune response (either to increase it in case of vaccination; or to decrease it in case of transplantation and autoimmunity). In recent years, it became evident that regulatory CD4+CD25+ T cells play a fundamental role in preventing autoimmunity and pathogenic inflammation, and in controlling the size of the remaining CD4 T cell compartment. Inability to clone these cells and diversity of experimental settings are obstacles to the clarification of their mechanism of action and physiology.

The aim of this project was to get a better insight into the mechanism of action of regulatory cells following a novel approach. We identified all major classes of mechanism of interaction between regulatory and effector/target cells, and also their relevant subclasses. We built cell population dynamic models corresponding to each of these classes and performed phase space and bifurcation analysis. In this way we identified critical properties that allowed us to reject some hypotheses for their incompatibility with experimental data. The remaining candidate mechanisms were addressed experimentally using in vitro and in vivo assays.

We redesigned experimental approaches that allow to quantify cell numbers, and population dynamic parameters, namely proliferation rates measured by CFSE-labelling. We implemented a new experimental design that allowed us to follow both the population of regulatory and target cells in co-cultures and where we could quantify the result of cooperation between these cells. These approaches allowed us to positively identify the best candidate mechanism. To facilitate the quantitative analysis of the experimental data we developed software for analysis of FACS that is available at our site:<http://eaol.igc.gulbenkian.pt/eaol/ti/index.html>.

The overall results of this research program were twofold: methodological and immunological. On methodological grounds we have implemented and tested a novel approach in which mathematical models are instrumental in the analysis and comparison of different candidate mechanisms of lymphocyte cooperation. This approach has proven to allow the identification of critical, distinguishing properties that are experimentally testable, and in so doing be effective in narrowing down the list of alternative mechanisms. In immunological terms we could establish, following this approach, that regulatory CD4+CD25+ T cells interact with their targets/effector cells in multicellular conjugates with the APC, where they suppress their targets and where they receive signals from a growth factor. The regulatory cells are therefore dependent for survival and growth on the cells they suppress. This trait of their physiology has functional implications for the understanding and therapy of autoimmune diseases, that will now be addressed.

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

Members: José Faro, Jorge Carneiro, Sergio Gulbenkian, Matthias Haury and Jocelyne Demengeot

Germinal centers (GCs) are highly dynamic and short lived, specialized cellular structures, formed after primary lymphoid follicles during a humoral thymus dependent immune response. B cells in GCs undergo extensive proliferation, somatic hypermutation, massive cell death and antigen driven selection, and differentiation into memory B cells and plasma cells. GCs are, thus, essentially involved in memory B cell generation and affinity maturation of antibodies.

Previously, we have developed a minimal model of the somatic hypermutation process in immunoglobulin genes during a GC reaction (GCR), which seems suitable to analyse experimental data already available. We are now initiating a new project which aims to clarify the dynamics of the GCR by assessing four potential driving mechanisms: (1) the decline of Ag trapped on FDCs; (2) the kinetics of hypermutations increasing the affinity of antibodies; (3) the dynamics of GC T cell help; (4) phenotypic changes in FDCs influencing B cell differentiation. We will first explore mechanisms underlying hypermutation and affinity maturation by using our minimal model of somatic hypermutation.

## **The Biology of B cells**

Member: R.M.E. Parkhouse

Collaborator: Leopoldo Santos-Argumedo, Cininvestav, Instituto Politecnico Nacional, Mexico.

The essential focus here is to relate surface markers of B cells to B cell function and development:

## **CD45R, CD44 and MHC class II are signaling molecules for the cytoskeleton-dependent induction of dendrites and motility in activated B cells.**

The differentiation of lymphocytes can be studied through the analysis of surface "Markers" appearing or disappearing between discrete phases of the process. These molecules have been named differentiation antigens, and many of them have a Cluster of Differentiation (or CD) number. The precise function of some of these molecules is still obscure, but some of them have been identified as receptors for growth factors, adhesion molecules, growth inhibitory molecules, etc.

We recently showed that plate-bound anti-CD44 and anti-MHC Class II antibodies trigger filopodia formation in activated murine B cells. An increasing number of reports on B cell dendrite formation support the idea that such morphological transformation might be important in adhesion, cell motility and cell interactions essential to B cell

activity. In collaboration with the group of Santos-Argumedo, we now demonstrate a similar generation of dendrite and cell motility in activated B cells through interaction via CD45R. The dynamic formation of dendritic processes and associated induction of cell motility were characterized by a rapid, and multidirectional emission of dendrites with retractile behavior. The addition of cytochalasin B totally blocked dendrites formation and motility induced through either CD45R, CD44 or MHC II, suggesting that the necessary cytoskeletal rearrangements require active polymerization of actin. Preincubation of B cells with staurosporine (a PKC inhibitor) or BAPTA-AM (a calcium chelator) prevented these morphological changes, indicating additionally a requirement for a PKC-calcium-dependent activity. Dendrite formation and cellular motility, therefore, seem to be two manifestations of the same phenomenon, and CD44, CD45R and MHC II appear to be signaling molecules for the observed cytoskeleton-dependent morphological changes.

### **Ontogeny, distribution and function of CD38-expressing B lymphocytes in mice**

Another project is focused on the function of the lymphocyte ectoenzyme CD38. In this work we have analysed the expression of CD38, CD45R (B220), IgM and IgD on splenic B lymphocytes of different ages. Both immature (B220+ve, BCR-ve) and mature (B220+ve, BCR+ve) B lymphocytes expressed CD38. In spite of their expression of CD38 and IgM, splenic cells from neonatal mice failed to proliferate to either anti-CD38 or anti-IgM cross-linking even when IL-4 was present, but they did respond to LPS and anti-CD40. At two weeks of age splenic B cells began to respond to anti-CD38 and anti-IgM, with responses reaching adult levels by four weeks. Similarly, CD38 is expressed as early as B220 on the surface of progenitor B cells in the bone marrow. Although the distribution of CD38 on adult B cells from different lymphoid compartments was broadly similar, peritoneal B lymphocytes expressed significantly higher levels of CD38. Consistent with this observation, a detailed analysis, using IgM/IgD ratio and staining with anti-CD5 demonstrated that B1 lymphocytes have the highest expression of CD38. Interestingly, both immature B cells and peritoneal B1 lymphocytes were unresponsive to anti-CD38, however, they were activated by LPS or anti-CD40.

### **Role of Type I Interferon in the regulation of B cell activation and in the onset of autoimmune disease**

Member: Jocelyne Demengeot

Students: Deborah Braun and Iris Caramalho

Type I interferon (IFN-I) is constitutively produced in the bone marrow (BM) and induced at sites of inflammation and following infection by viruses or microorganisms. We have previously shown that IFN-I regulates the generation and selection of normal B cell populations in the BM. This year, we assessed the effects of IFN-I on mature B cell function by monitoring the responses of IFN- $\gamma$  treated murine splenic B cells to apoptotic, mitogenic and activating stimuli. A similar analysis was performed on BM mature B cells obtained from wild type or IFN-I Receptor deficient mice. IFN- $\gamma$  was

shown to induce B cells to a state of partial activation, an increased survival and resistance to Fas mediated apoptosis. We also found that IFN- $\gamma$  enhances B cell responses to BCR ligation. These results indicate that in addition to its inhibitory effect on viral replication and T cell apoptosis, IFN- $\gamma$  plays an essential role during an inflammatory response by lowering the threshold for B cell induction, thereby promoting fast and polyclonal antibody responses. (Braun et al #1, submitted).

These immuno-regulatory functions, together with a number of observations indicating that infection may alter the incidence of autoimmune diseases, prompted us to assess the effect of IFN-I on the onset and severity of the lupus-like disease developed by Fas-defective *lpr* mice. We show that sustained injection of poly I:C, a potent inducer of IFN-I, induced dramatic aggravation of the histological features, a ten fold increase in serum Ig and accumulation of activated B and T lymphocytes. In addition, introduction of a null mutation for the IFN- $\gamma$ -Receptor gene in the *lpr* background associates with a delay in the onset of glomerulonephritis, kidney depositions and lymphadenopathy. Our analyses highlight the role of the innate cytokine IFN-I on the exacerbation of inflammatory responses and demonstrate that IFN-I *per se* enhances autoimmunity in genetically predisposed individuals. We propose that the correlation between viral infection and emergence of autoimmune syndromes reported by others result from sustained production of IFN- $\gamma$ . (Braun et al #2, submitted).

### **Specificity requirements for selection and effector functions of CD25<sup>+</sup>4<sup>+</sup> regulatory T cells**

Members: Jocelyne Demengeot, Shohei Hori, Antonio Coutinho and Matthias Haury

Accumulating evidence has established that a subpopulation of CD4<sup>+</sup> T cells which constitutively express the CD25 molecule plays an indispensable role in preventing autoimmunity. However, little is known about the antigen specificities required for their development and effector functions. Previous studies have shown that anti-myelin basic protein (MBP) TCR transgenic mice spontaneously develop EAE when on a recombination-activating gene (RAG)-1-deficient genetic background (T/R<sup>-</sup>). In contrast, RAG-1 competent transgenic animals (T/R<sup>+</sup>) remain healthy, a result of the regulatory activities of T cells expressing endogenous  $\alpha\beta$  TCRs. We investigated the role of CD25<sup>+</sup>4<sup>+</sup> Treg cells in controlling EAE in this transgenic system. We show that T/R<sup>+</sup> animals contain MBP-specific anergic and suppressive CD25<sup>+</sup>4<sup>+</sup> cells, while T/R<sup>-</sup> mice do not. Adoptive transfer of WT or TG CD25<sup>+</sup>4<sup>+</sup> cells into T/R<sup>-</sup> mice prevented the development of EAE while non-TG cells from T/R<sup>+</sup> mice conferred only a limited protection due to their restricted repertoire diversity. The development of MBP-specific CD25<sup>+</sup>4<sup>+</sup> Treg was dependent on the co-expression of endogenous encoded TCR  $\alpha$  chains together with the TG-TCR. Moreover, we ruled out a role for infectious tolerance in the generation of MBP-specific regulatory T cells. Taken together, our analyses indicate that specificity to MBP is required for effector functions but not for selection of CD25<sup>+</sup>4<sup>+</sup> regulatory T cells (Hori et al #1 and 2, submitted).

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

Members: Jocelyne Demengeot, Shohei Hori and Matthias Haury

Students: Thiago Carvalho and Iris Caramalho

Acute inflammatory immune responses to normally innocuous microbes can be lethal and are a frequent cause of death in immuno-suppressed patients. Recent evidence demonstrated that a population of CD4<sup>+</sup> CD25<sup>+</sup> regulatory T cells (Treg) prevents *Helicobacter*-dependent inflammatory bowel disease (IBD) in immunodeficient mice. Likewise, we have shown that Treg prevent lethal inflammatory pneumonia (IPD) induced by the hyper-responsiveness of naïve CD4<sup>+</sup> cells triggered by *Pneumocystis Carinii* infection in mice (Hori S. et al #3, in press). The same population of Treg has also been shown to protect mice from fatal inflammatory autoimmune disease (AID).

Our recent data indicate that Treg dampen the expansion of naïve CD4<sup>+</sup> T cells and local inflammation induced by infectious agents, but also when it is sterilely induced (T. Lopes et al in preparation). Using this finding, we developed and explored an in vivo short-term assay where the cellular and molecular mechanisms underlying the operation of Treg, thus far unknown, can be readily analysed. In parallel we established a new ex vivo assay to monitor the survival and functional activation of limited number of cells (I. Caramalho et al, in preparation). This line of activity aims, therefore, at identifying the cellular and molecular mechanisms involved in such anti-inflammatory activity of regulatory T cells. As most AID involve sterile, inflammatory tissue damage, this work will help in defining potential therapeutic approaches for both immunopathology caused by hyper-responsiveness of the immune system, and autoimmune reactions (discussed in Coutinho et al, Immunological Reviews 2001. 182: 89-98).

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

Member: Matthias Haury

Student: Dinis Calado

We are in the process of generating a new transgenic mouse strain to study the expression of IL-10 ex-vivo in the regulatory T-cell subset, and to facilitate the isolation and characterisation of IL-10 expressing T regulatory cells. We are also characterising the subphenotype of regulatory T-cells and their localisation using multicolor (5-6 colors) flowcytometry and multiphoton confocal microscopy. These studies are carried out in collaboration with the laboratory of Dr. Dan Holmberg, Umea University, Sweden and Antonio Bandeira, Pasteur Institute Paris.

## **A practical alternative to limiting dilution analysis: application to the quantitative analyses of T cell receptor repertoire**

Members: Jocelyne Demengeot, John Stewart and Shohei Hori  
Collaborator: Alexis Colette, Institut Pasteur, Paris, France

In experimental immunology, a situation quite commonly arises in which there are a large number of potential events, but the probability of any individual event is small; and one wishes to measure the number of events which actually occur. In this type of situation, a standard procedure is limiting dilution analysis, a strategy to reduce the number of potential events to a point where, if a detectable signal is observed, indicates that only a single event has occurred. The frequency of occurrence may then be measured by simply counting the number of these events. However, this approach is relatively onerous, in that an extended series of preliminary experiments is necessary to determine the appropriate dilution; and a rather large number of repeated experiments at this dilution are then necessary to obtain an acceptable estimation of the frequency. The experimental load becomes particularly heavy if the data consist of several qualitative types of events with different frequencies, since different dilutions must be employed for each type of event.

We developed a new statistical method for estimating the number of events underlying a quantitative measurement and applied the method to the analyses of T cell receptors CDR3 length spectratypes (Hori et al #4 submitted). We show that this approach allows the direct and accurate quantification of lymphocytes expressing any antigen receptor with a given V, J and CDR3 length inside a diverse population of cells. This application provides an empirical validation of the method, firstly in terms of internal consistency, and secondly in terms of comparison with results obtained by more onerous classical methods. The potential applications of our method to the analysis of repertoire fluctuations are numerous: quantitative estimates of clonal expansion during an immune response, lymphocyte dynamics, principles of homeostatic regulation and control of tolerance versus autoimmunity.

Finally, the method is quite generally applicable to any situation where a quantitative signal arises from the summation of sub-signals from a discrete set of underlying events, and variation in the signal is due primarily to statistical fluctuation in the number of these events according to the Poisson distribution. Thus, an exemple beyond immunology, would arise from the classical procedure of counting cells in a histological section, if the manual count were replaced by an automated quantitative signal.

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

Member: Álvaro Tavares  
Students: Susana Godinho, Paulo Alves, Célia Domingues, Claudia Florindo.

We previously showed that the protein kinase polo is a centrosomal kinase, 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 have 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 in the process of identifying the complex's components in total embryo extracts and in centrosome preparations. We will characterise these proteins in order to determine 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. Mob1 is an essential gene in *S. cerevisiae*, and mutant alleles of this gene arrest in late mitosis, suggesting that Mob1p is essential for cytokinesis. It was shown that Mob1p binds to Mps1p, a protein kinase required for spindle-pole body duplication and for the mitotic checkpoint function, and to Dbf2p, a protein kinase required for progression through telophase. In order to gain a better insight into cell division mechanisms in higher eukaryotes, we have looked for possible homologues in *Drosophila melanogaster*. We have cloned four different Mob1-like genes in this organism (Dmob1-4), coding for proteins sharing very significant homology with the yeast protein. While all the four DMobs seem to be required for the first embryonic divisions, Dmob3 is the only one required at every stage of embryonic development. The intracellular localization of the Dmobs proteins was determined by immunofluorescence with specific polyclonal antibodies, and using GFP-tagged fusion proteins. Results indicate that the different proteins localize to the spindle poles during mitosis, with a specific accumulation at centrosomes, very much like described for the yeast Mob1p. In order to access the function of these proteins we have done dsRNA assays in S2 cells. Preliminary results indicate a requirement for these proteins during mitotic progression and centrosome duplication, as numerous cells are observed with monopolar spindles or with problems during cytokinesis. The function of these proteins seem to be highly conserved in higher eukaryotes as we have also cloned four human Mob1-like genes (with 60-90% identity with the *Drosophila* proteins).

### **Isolation and characterization of mitotic mutants**

Member: Álvaro Tavares

Students: Mariana Faria e Vânia Parelho

We have isolated two mutant *Drosophila* lines, created by insertion of a transposable P-element, resulting in a semi-lethal phenotype. One of these lines, line718/06, shows a high mitotic index in brain neuroblasts with a high proportion of cells in late anaphase with no apparent midbody formation being observed, suggesting that cells have problems exiting mitosis and executing cytokinesis. Mitotic neuroblasts show few abnormalities, such as anaphases with unfocused poles, which may indicate alterations in the centrosome integrity. Nevertheless, immunostaining of gamma-tubulin shows no alterations in the localization of this centrosomal component. The P-element was localized by in situ hybridization on polytene chromosomes at the region 95A, and it was

proved to cause the phenotype since the precise excision of this transposon reverts to the wild type. A stronger phenotype is exhibited in hemizygotes for the insertion of 718/06 over Df (3R) mbc-30, a deletion with breakpoints between 95A07 and 95C10. These individuals have about 15% polyploid cells. Usually, these polyploid cells have more than two centrosomes, which may suggest that after a failure in division at the end of mitosis, the cell continues the cycle in polyploidy. As in the mutant 718/06, these hemizygotes present a higher proportion of cells in telophase. Again, these observations suggest problems in the regulation of mitosis exit. New stronger alleles are now being generated by imprecise P-element excision, and the affected gene is being cloned.

### **The cytosolic chaperonin CCT and its substrate tubulin in *tetrahymena* cells**

Member: Helena Soares

Students: Cristina Casalou, Sofia Nolasco and Cecília Seixas

The main goal of the group is to understand the mechanism/s underlying microtubules (Mts) polymerizing/depolymerizing dynamics and Mts functional diversity in eukaryotic cells. We are also interested in how these mechanisms are related to signal transduction pathways triggered by stress conditions. Our immediate aim is to investigate: (i) How the tubulin folding machinery, the cytosolic chaperonin (CCT) and co-factors (A, B, C, D and E) control synthesis, flux and transport of tubulin; (ii) Which components of microtubule-organizing-centers (MTOCs) interact with the tubulin folding machinery to control Mt dynamics. Cellular responses to stress events require cytoskeleton rearrangements and changes in Mt dynamics. Therefore, our second aim is to investigate processes of signalling in the cell involving tubulin folding machinery and MTOCs components under stress. In these studies we are using two biological models, namely the ciliate *Tetrahymena* and mammalian cell lines. The ciliate *Tetrahymena* is being used as a biological model because this organism is a unicellular eukaryote that is easily deciliated and able to recover their cilia and also possesses, besides cilia, a great variety of functional distinct microtubular structures, offering good opportunities to study the mechanisms involved in tubulin and in the biogenesis of distinct functional classes of Mts. Cilia are complex Mt structures involved in generation of cell motility. Additionally, cilia may be polarized cell compartments that could be involved in transduction of signals from the external medium into the cell. The deciliation/reciliation event of *Tetrahymena* cells constitutes an attractive model system to study the induction and regulation of the set of genes involved in cilia assembly and function. Moreover, when cells divide the biogenesis of new cilia, are integrated in a complex program of differentiation and development. On the other hand the molecules that interact or make part of Mts in *Tetrahymena* cells are much more abundant in these cells than in mammalian cells. This facilitates the biochemical isolation and characterization of this/these molecule/s. Moreover, most of these molecules so far identified in *Tetrahymena* are quite similar to those of mammalian cells and are also integrated in similar structures. Interestingly, most mammalian cell types are capable of producing primary cilia that present simpler internal structures (9+0) compared to those of mobile cilia (9+2) in a way that seems to be cell cycle dependent. These structures, of unknown function, seem to be directly connected

with the extracellular matrix and involved in transducing signals from the external environment. For example, the artificial bending of these primary cilia causes a substantially increase in intracellular calcium. However, *Tetrahymena* is not readily amenable to genetic manipulations, which would facilitate the analysis of the function of the identified molecules. Moreover, one of ours aims is to understand if the molecules and the mechanisms underlying the biogenesis of specific classes of Mts and their dynamics are conserved among species or alternatively depend on the specialisation of the cell. These ideas led us to extend these studies to mammalian cells.

### **Isolation and characterization of the complexes-containing-tubulin during exponentially growing cells and cells submitted To hyperthermic stress**

Member: Helena Soares

Student: Cecília Seixas

Tubulin exists in *Tetrahymena* exponentially growing cells in different protein complexes with molecular masses ranging from 90-~500 kDa, as revealed by native gels. The distinct tubulin containing complexes have different abundances under hyperthermic stress. The amount of the ~500 kDa complex clearly increases in cells submitted to a hyperthermic stress. In order to characterize these tubulin complexes we have prepared post-mitochondrial extracts of *Tetrahymena* exponentially growing cells that were analysed in sucrose gradients from 10% to 40%. Subsequently, the fractions enriched in tubulin were pooled and applied into a FPLC gel filtration column (Superdex 200HR). The analysis of the obtained elution profile showed three main regions corresponding to 360-~500 kDa, ~200 kDa, 60-~120 kDa. The fractions corresponding to the region of 360-~500 kDa were further purified using ion-exchange chromatography (Q-Sepharose). The purified complex of ~500 kDa is composed at least by 12 distinct proteins with molecular masses ranging from 30-~150 kDa. Besides tubulin, two of these proteins were identified by immunoblot and immunoprecipitation techniques as CCT $\alpha$  and CCT $\beta$ -subunits. In cells submitted to a hyperthermic stress this complex seems to have the same composition but is much more abundant. These results suggest that under heat-shock, an event that probably requires cytoskeleton re-arrangements, tubulin seems to exist in specific protein complexes. Experiments are in progress in order to elucidate the identity of the unknown protein components and the role of some CCT subunits in this complex.

### **CCT chaperonin and CCT micro-complexes in cells recovering their cilia**

Member: Helena Soares

Student: Cristina Casalou

We have previously shown in *Tetrahymena* that the CCT-subunit gene family is up-regulated during cilia assembly suggesting its involvement in cilia biogenesis. In *Tetrahymena* exponentially growing cells CCT-subunits exist as part of a 900 kDa complex, as free subunits and/or in smaller oligomeric structures. Native PAGE analysis

combined with gel filtration chromatography and subsequent Western blot, showed that in the first minutes of reciliation the amount of the CCT micro-complexes is maintained contrasting with the decrease in levels of the 900 kDa complex. At 90 min of reciliation, the levels of the 900 kDa complex starts to recover. These studies show that during the process of cilia recovery there is a change in the ratio between the intact CCT chaperonin and the CCT micro-complexes species. This ratio is dramatic affected at 15 minutes of reciliation a moment characterized by the starting of axonema re-organization and cilia growing as observed by Atomic Force Microscopy. At 15 minutes of reciliation tubulin co-elutes in the same region of the elution profile with CCT micro-complexes suggesting an association between them. Strikingly, double-label immunofluorescence experiments show that CCT $\alpha$ , CCT $\beta$ , CCT $\delta$  and CCT $\epsilon$  co-localize with  $\alpha$ -tubulin within *Tetrahymena* cilia basal bodies, oral apparatus, cytoproct, structures where protein synthesis is not expected to occur. These results are in agreement with the observation that, after a post-mitochondrial fractionation, TpCCT $\alpha$ , TpCCT $\delta$ , TpCCT $\beta$  and TpCCT $\epsilon$  subunits are present in the insoluble fraction. Remarkably, during cilia recovery the levels of TpCCT $\beta$  in this fraction are increasing whereas the amount of TpCCT $\alpha$ , TpCCT $\delta$  and TpCCT $\epsilon$  are progressively decreasing. Moreover, in the insoluble fraction TpCCT $\epsilon$  seems to be part of a hetero-oligomeric complex of about 300 kDa. This work provides evidence that distinct CCT-subunits in smaller CCT complexes and/or as free subunits are present in cilia basal bodies and associated with complex Mt structures probably playing specific roles in cilia biogenesis. Additionally, there is a close relationship between the intact CCT and CCT micro-complexes indicating the existence of a complex regulatory mechanism between these species that can be modulated by alterations that affect Mt cytoskeleton and cilia structure.

### **Transcriptional regulation of the mammalian cofactor A. Implications in tubulin folding, microtubules biogenesis/dynamics and signalling pathways**

Member: Helena Soares

Student: Sofia Nolasco

It is our intention to investigate: i) if the cofactor A gene is able to respond to stress conditions (e.g. oxidative stress) that probably requires cytoskeleton re-arrangements; ii) if cofactor A exhibits a tissue-specific transcriptional regulation;

**a)** The project started with the identification of the number of genes encoding cofactor A (a molecular chaperone that accepts  $\alpha$ -tubulin after the folding assistance by CCT) in the mouse genome, as well as the characterization of their structure. Twelve positive phage plaques were isolated from a Sau3AI genomic mouse library using as probe a PCR product containing part of the coding region of cofactor A that was obtained by designing primers taking into account the cDNA sequence of cofactor A (Aloria and Zabala, unpublished results).

**b)** We determined sublethal oxidative and cold stress conditions in HeLa and hippocampal H19-7 cells and also sublethal conditions of antimetabolic agents that affect Mts i.e. taxol, nocodazole and colchicine. We have performed dose-response curves for different oxidant agents as for example hydrogen peroxide. Results of initial experiments

were not reproducible. Consequently, we started to submit a controlled number cells to a continuous flow of H<sub>2</sub>O<sub>2</sub> according to Antunes, F. and Cadenas, E. (2001) Free Radical. Biol. and Med., 30:1008-1018). After the definition of the oxidative conditions we started to study the expression of cofactor A by Northern blot analysis in control cells and cells treated with oxidants, submitted to cold-shock and antimitotic agents, using the gene specific probes. In contrast to the yeast homologue of cofactor A (Rbl2p) gene which appears to be up-regulated upon specific oxidative signals, the mammalian cofactor A expression was affected by the oxidative agents tested. Experiments are in progress to investigate the pattern of expression of the cofactor A gene in cells treated with different Mt perturbing agents. The expression of other genes of the tubulin folding pathway are also under investigation.

c) We started to dissect the regulatory region of the cofactor A gene through measurements of transcriptional activity of cofactor A gene/s using a reporter gene assay. A DNA fragment extending from the promoter region to the first third of the coding region of cofactor A gene was sequentially deleted resulting in DNA fragments of different sizes. These fragments were subcloned into a pGL3-basic firefly luciferase expression vector (Promega) and will be used to transfect mammalian cells lines. The constructs will be used to perform electrophoretic mobility shift assays (EMSA, bandshift) using nuclear protein extracts prepared from mammalian cell lines that are cultured under a variety of conditions. Further to experiments will be designed to identify regulatory cis-elements involved in tissue-specific regulation.

### **Cellular responses to stress challenges: - signal transduction and transcriptional regulation in yeast *Saccharomyces cerevisiae***

Member: Lisete Fernandes

Students: Joana Monteiro and Ana N. Costa

Cells cope with changes of the external and internal milieu like oxidative stress and temperature fluctuations. Eukaryotic cells respond to both stimuli by activating diverse signal cascades. How does the cell keep track of multiple cascades? What are the cross-talks between such cascades and how are they regulated? It has been demonstrated that cells undergoing stresses modulate gene expression and, transcriptional regulation has been strongly suggested as a key regulatory step in this event.

The Yap family of transcriptional factors in yeast *Saccharomyces cerevisiae* contains eight members respond to stress, including: Yap1 to oxidative and cold signals, Yap4 to compounds affecting cytoskeleton as well as in cold signals in yap1-deleted cells, Yap8 to arsenite. In addition Yap1, Yap2, and Yap8 are involved in resistance phenotypes to multiple drugs. Although it has been previously suggested that each Yap family member has a distinct biological function, the involvement of Yap proteins in different phenomena emphasizes putative overlaps in respective signalling cascades. Thus the Yap family is well suited to study the cross-talk of signalling pathways.

Our major aim has been focused in understanding the crossroad of cold and oxidative signalling pathways mediated by Yaps: (a) what is the specific role of Yap1 under cold signals? (b) how specific signals propagate to impinge on Yap proteins? and (c) what is the

mechanism of the activation by Yap1 and its relation to the basal machinery of RNA polymerase II?

### **Microtubules and Molecular Mechanisms of Signaling Under Stress**

Member: Sukalyan Chatterjee

Students: Mário Grãos and Maria Hortense Matos

This project focuses on the analysis of the mechanism of signal transduction in G<sub>2</sub>/M block mediated apoptosis by concentrating on the role of a number of proteins involved in the stress pathway and mechanism leading to apoptosis. Apoptosis is a mechanism of programmed cell death (PCD) that plays a decisive role both in normal development and disease. It is a highly conserved response to a signaling cascade whose initiating stimuli originate within the cell or from the external environment. It is involved in the deletion of autoreactive lymphocytes, the elimination of virally infected and malignant cells, and the development of complex multicellular organisms. Signaling cascades have been shown to be promiscuous. A single signaling protein or cascade is able to serve diverse physiological functions. The various signaling pathways are multifactorial, and specificity is hinged on 'which' and 'how' molecules interact in a given process. The emerging idea is that signals can be transduced through 'signalosomes', multimolecular entities of dynamic composition that assemble around scaffolding and adaptor molecules.

Microtubules (MTs) of the cytoskeleton can regulate the localisation of proteins in a signal dependent manner. We hypothesize that MTs act as spatiotemporal organizers of signaling and their dynamic properties may be part of the signal or the signaling mechanism itself. It is well established that microtubule perturbation and consequent apoptosis induced by Taxol, a widely used neoplastic agent, involve hyperphosphorylation of Bcl-2. Taxol stabilizes cytoskeletal microtubules by binding to  $\alpha$ -tubulin and preventing depolymerization and consequently blocking the cell cycle in the G<sub>2</sub>/M phase, causing mitotic arrest at metaphase. It has been shown that Taxol-mediated apoptosis is independent of MAP kinase signal transduction pathways in the concentration range in which Taxol is used in the clinic. A putative kinase downstream of Taxol-mediated bundling of microtubules, G<sub>2</sub>/M block and apoptosis may be involved. Using Taxol at clinically relevant doses which can be reached in vivo without toxic effects, it has been shown, in HeLa cells, that p38 and Ask are involved in Taxol-induced apoptosis, but not the stress kinase JNK.

Preliminary experiments performed with HeLa cells indicate that there is a postranslational modification, on microtubule polymerization, of Apoptosis signal-regulating kinase 1 (Ask1). Ask1 is a member of the MAPKKK family and can activate both the JNK and p38 signaling cascades. It profoundly influences the decision of cell fate, such as survival/differentiation or apoptosis, allowing cells to make appropriate responses to a variety of extracellular and intracellular stresses, by balancing and integrating several different signals. This project aims to understand the nature of the postranslational modification of Ask1 and its biological significance. It is likely that this modification is a phosphorylation event and if so, the kinase responsible for the phosphorylation will be identified and characterized.

We are currently interested in studying the levels, subcellular localization and putative interaction with MTs or MAPs of various pro and anti-apoptotic Bcl-2-family proteins, such as Bcl-2 and Bcl-xL and Bax, Bad, Bim, and Bid, respectively. We study these proteins in drug-challenged cells and during the cell cycle. Bim has been reported to be associated with the MTs through the motor protein dynein. Upon certain stimuli, it gets released and migrates to the mitochondria, where it interacts with Bcl-2 (an anti-apoptotic protein) and promotes the release of cytochrome c and subsequent caspase activation, leading to apoptosis. So far, we have shown that Bim is upregulated in cells undergoing apoptosis upon challenge with an oxidative stress inducer: menadione. In contrast, Bax does not seem to be upregulated under the same conditions.

Interestingly, we have also shown by various different techniques that Bim gets phosphorylated during or immediately prior to mitosis. Our results suggest that the pro-apoptotic protein Bim can be regulated at the level of transcription and at the level of phosphorylation. We will now study the subcellular localization of this protein during interphase and during mitosis, and identify the kinase(s) and phosphorylation site(s). Furthermore we will study the relevance of Bim's phosphorylation for its pro-apoptotic ability.

### **Neuron microglia cross talk and activation of microglia**

Members: Sukalyan Chatterjee and Teresa Pais

Student: Catarina Figueiredo

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. The mouse model of CM indicates that microglia, the resident macrophages of the brain, is activated very early after infection. Whether activation of microglia is a key event in causing neuronal damage culminating in CM is still a matter of debate. Using an *in vivo* mouse model for CM the microglial activation is being studied by immunocytochemistry using anti-CD11b and anti-CD40 antibodies and staining with a tomato lectin. Neuronal death will also be analysed in this model.

The cross talk between and the interdependence of activated microglia in neuronal damage is being investigated *in vitro* co-cultures of microglia, neurons or in co-cultures of neurons and microglia activated with extracts of parasitized erythrocytes. One hypothesis is that the parasite mediates neuronal cell death and signals from dying neurons activate microglia. In fact, our preliminary results show that necrotic HT22 (an immortalized mouse hippocampal cell line) and necrotic HeLa (tumor cell line) enhance the expression of CD40 in primary cultures of microglia. The effect of necrotic primary cerebellar neurons will be addressed and also the signaling mechanisms in activation of microglia by necrotic cells. It is likely that infection can activate microglia which causes cytotoxic damage to neurons. In order to answer this question primary microglia was stimulated with infected erythrocytes. The culture supernatants will be tested for cytokines as a read out of microglia activation.

In summary, we are addressing the signaling mechanisms in activated microglia by infection (extracts of parasitized erythrocytes) and dying neurons adopting a molecular

approach to identify secreted entities like cytokines, intracellular signaling cascades and mechanisms of transcriptional regulation. This may elucidate the crux of the balance between cytotoxic and protective role of microglia in CM.

### **Mechanism of apoptosis of Cajal-Retzius cells**

Members: Paula Parra, Sukalyan Chatterjee and Matthias Haury  
Technician: Maria Alvim

The major goal of this study is to investigate the ion channel properties and activity-dependent mechanisms in the induction of apoptosis in Cajal Retzius (CR) cells. These cells are fundamental for the guidance and emplacement of neurons of the cortex and the hippocampus and normally undergo apoptosis during the development of the central nervous system. Although the majority of the cells are lost, a subset of CR cells survive and differentiate into interneurons. Our objective is to examine the electrical differences of these two "subsets" of neurons, in order to determine if distinct ionic channels characterise two different cells populations or if there is a single population with differences in the regulation of channels that might be involved in the induction or prevention of apoptosis. The results of this study will form the basis to investigate in more detail the molecular mechanisms leading to the decision of CR cells to enter into either apoptosis or into the differentiating program.

Some hypotheses suggest a physiological role during synaptogenesis allowing for the correction of erroneous projections and the creation of pathways for axonal growth. Apoptosis is likely to be involved in controlling cell numbers in the CNS and could be the physiological fate of cells exhibiting transient functions like Cajal Retzius cells. Using transgenic mouse technology, we will address the following questions: What are the developmental cues that mediate cell fate decisions specially inducing differentiation/apoptosis in these cells? What neurotransmitters are involved and what is the signaling cascades that activates the apoptotic pathways?

### **Molecular mechanisms underlying 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**

Member: Miguel Che Parreira Soares  
Students: Mark Seldon, Rui Soares and Sofia Simoes

Endothelial cell (EC) apoptosis, such as it occurs during acute or chronic inflammation is a highly pro-inflammatory event. As such EC apoptosis is thought to contribute in a critical manner to the induction of irreversible tissue injury, organ failure and disease that can occur during acute and/or chronic inflammatory reactions. Understanding how EC become protected from undergoing apoptosis may be critical in the development of new therapeutic strategies aimed to suppress the deleterious effects associated with inflammatory reactions. One of the mechanisms by which EC can prevent apoptosis



relies on the expression of a series of cytoprotective genes. We are investigating the role of one such gene, the stress responsive gene heme oxygenase-1 (HO-1).

Under inflammatory conditions HO-1 is the rate-limiting enzyme in the catabolism of free heme, a pro-oxidant that accumulates during oxidative stress, into bilirubin, an anti-oxidant, and carbon monoxide (CO). In addition, HO-1 action on heme releases free iron that up-regulates the expression of ferritin, an anti-oxidant as well. In our previous studies, we have demonstrated that HO-1 protects EC from undergoing apoptosis, through a mechanism that is dependent on the generation of the gas CO. Contrary to other biological functions of CO, its ability to prevent EC apoptosis does not involve the activation of the enzyme guanylyl cyclase and/or the generation of cGMP. Instead, CO acts via a signaling transduction pathway that activates p38 mitogen activated protein kinases (MAPK). The mechanism(s) by which HO-1 and/or CO interact with this signaling transduction pathway and how this contributes to the overall anti-apoptotic effect of HO-1/CO remains to be established and is the focus of this proposal.

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

Member: Miguel Che Parreira Soares

Students: Mark Seldon, Rui Soares and Sofia Simoes

Microbial infections induce potent inflammatory reactions through a complex cascade of events involving the activation of resident macrophages (M $\phi$ ). Once activated, M $\phi$  secrete soluble pro-inflammatory molecules such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and IL-1 $\beta$ , which act on endothelial cells (EC) to change their normally anti-thrombotic and anti-adhesive phenotype into a pro-thrombotic and adhesive one, a phenomenon referred to as "EC activation". The phenotypic modifications associated with EC activation result in large measure from the expression of pro-inflammatory genes that encode cytokines/chemokines, adhesion and pro-thrombotic molecules. Expression of these pro-inflammatory genes is essential to initiate the recruitment and activation of leukocytes that will ultimately terminate microbial infections. However, their expression must be tightly regulated in order to avoid extensive immune stimulation that could induce EC apoptosis and tissue injury. In this project we are analyzing the mechanisms by which the expression of one anti-inflammatory gene, i.e. heme oxygenase-1 (HO-1), prevents the deleterious effects associated with inflammatory reactions. We have previously shown that this occurs through the ability of HO-1 to degrade heme and generate the gas carbon monoxide (CO), which acts as a signaling molecule to attenuate the expression of pro-inflammatory genes and to protect EC from undergoing apoptosis. The aim of this project is to further understand the molecular mechanisms underlying the anti-apoptotic effect of CO. Work from our laboratory has shown that the ability of CO to prevent EC apoptosis acts through a mechanism that depends on the activation of the transcription factor NF-Kb. The mechanism(s) by which HO-1 and/or CO interact with this signaling transduction pathway remain(s) to be established and are the focus of this project.

## 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 2001 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 Asssociado 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

PRAXIS/C/BIA/10094/1998

Jorge Carneiro

*Mechanisms of lymphocyte co-operation involved in immune regulation: an experimental and biomathematical approach.*

PRAXIS/BIO/10091/1998

Matthias Haury

*GFP tagged IL-10 knock in mice.*

PRAXIS/C/BIA/10097/1998

Helena Soares

*Study of the Tetrahymena tubulin complexes: An attempt to establish a functional relationship with microtubule assembly and dynamics.*

PRAXIS/P/BIO/14132/1998

Dan Holmberg

*Genetic Mapping of disease associated phenotypes in portuguese IDDM and SLE families and in mouse models*

PRAXIS/P/BIA/11034/1998

José Feijó

*As bases moleculares do crescimento in vitro e in vivo de tubos polínicos: uma abordagem multidisciplinar.*

PRAXIS/C/SAU/10265/1998

João Pedro Simas

*Molecular Pathogenesis of Murine Gammaherpesvirus-68 in Mice.*

MGI/36369/99-00

Sylviane Bernadette Pied

*T cell response in Pathogenesis of Malaria Infection*

MGI/36392/99-00

Carlos Augusto Gomes Barbosa da Penha Gonçalves

*Genetics of Malaria in Wild Mouse Models*

MGI/36403/99-00

Robert Michael Evans Parkhouse

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

MGI/36413/99-00

José Manuel Faro Rivas

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

ESP/34240/99-00

João Pedro Simas

*Molecular Interactions in Murine Gammaherpesvirus 68 latent infection of B-Lymphocytes*

BCI/34405/99-00

Álvaro Augusto Marques Tavares

*Isolation and characterisation of protein complexes containing the mitotic proteins polo and Dmob*

BCI/34599/99-00

Isabel Maria Palmeirim

*Time control during vertebrate embryonic development*

BCI/34772/99-00

José Alberto Feijó

*Genetic characterization of ion dynamics modulators in pollen tubes*

BME/36192/99-00

Juan Carlos Izpisua Belmonte

*Specification of vertebrate limb identity*

BSE/34794/99-00

Jorge Carneiro

*Dynamic invariance in biological systems*

CBO/36312/99-00

Jorge Carneiro

*HIV2 Infection as a Model for the Investigation of AIDS pathogenesis*

MGI/33570/99-00

Jocelyne Demengeot

*Immunobiological studies of microbial virulence immunomodulatory proteins that allow the survival of the secreting microorganism in the host*

MGI/32513/99-00

José António Belo

*Fungal and Mouse Models of human mitochondrial disease*

34350/99-00

José António Belo

*Genetic control of vertebrate development: the role of cerebrus-like family of secreted inhibitors and anterior visceral endoderm signaling*

36250/99-00

João Pedro Simas

*Study of Immunogenic Proteins of Mycoplasma mycoides subsp. mycoides small colony type*

33201/99-00

José Feijó

*The role of extensin peroxidases and extensin deposition in Plant development*

MGI/37296/2001

Miguel Soares

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

ESP/39636/2001

Astrid Moura Vicente

*Genetic Epidemiology of Autism*

BCI/42249/2001

Sukalyan Chatterjee

*Molecular mechanisms of microtubule mediated signaling under oxidative stress*

BCI/42040/2001

Isabel Maria Palmeirim

*New aspects on coordinating limb bud development*

MGI/38563/2001

Maria Manuel Mota

*Interacções Hospedeiro-Parasita durante os estadios hepaticos de infecções de Malaria*

BCI/41725/2001

Jörg Dieter Becker

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

BCI/41735/2001

Álvaro Augusto Tavares

*Functional characterization of the mitotic kinases DPIkk and DMps1*

BCI/37862/2001

Lisete Fernandes

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

BCI/419092001

António Jacinto

*Epithelial dynamics and adhesion during Drosophila dorsal closure*

BCI/43411/2001

Jocelyne Demengeot

*Effects of the recombination activating genes 1 and 2 on the vertebrate genome stability: onsequences and the cellular and at the organism level.*

BCI/43063/2001

Jocelyne Demengeot

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

MGI/43466/2001

Moises Mallo

*Molecular mediators of Hoxa2 function during mouse development*

FCB/39906/2001

José Feijó

*Inflammatory response and signaling between neurons and astrocytes during cell injury by sepsis, hypoxia-ischemia and hyperbilirubinemia.*

NSE/39166/2001

Paula Parra Bueno

*Apoptosis versus differentiation- The cell-fate of Cajal Retzius Cells*

MGI/40878/2001

Pedro Fernandes

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

## **OTHER PROJECTS**

### **European Union**

EU QLK3 – 2000 – 00362

Michael Parkhouse – Coordinator

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

EU QLK3-CT-2001-00422

Miguel Soares

*Targeting Heme-Oxygenase-1 or downstream processes: a new therapeutic approach for treatment of inflammation*

EU QLRI-CT-2001-00003

Sérgio Gulbenkian

*EMMA Works (European Mouse Mutant Archive non routine working programme)*

EU QLRI-CT-2001-01061

Sérgio Gulbenkian

*EMMANet (European Mouse Mutant Archive network)*

EU QLRI-CT-2001 – 01363

Pedro Fernandes

*EMBCORE*

EU EVR1 – CT-2001-400017

Pedro Fernandes

*BioCASE*

EU IST – 1999 – 20469

Pedro Fernandes

*EMBER*

EU QOL-2001

João Pedro Simas

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

## **Others**

Laboratoire Européen Associé CNRS

António Coutinho

*Genétique et développement de la tolerance naturelle*

ICCTI/413 CAPES, Brazil

Jocelyne Demengeot

*Tolerância Oral: Análise pelo Panamá-blotting e marcadores de activação linfocitária.*

Wellcome Trust Biomedical Research Collaborative Grant

054458/Z98/Z/MEP/LEC/CRD

João Pedro Simas

*Studies on the molecular pathogenesis of murine gammaherpesvirus infection of mice.*

Research contract with Astrazeneca

Miguel Soares

*Delivery of proteins in vivo*

Research Contract with STORA CELBI (Celulose Beira Industrial S.A.)

José Feijó

*Estudo da reprodução sexual em espécies florestais com interesse industrial para a indústria de celulose com vista à optimização dos processos de melhoramento genético e optimização da produtividade florestal.*

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Stewart J and Coutinho A. The biology of learning. In: O Futuro da Educação em Portugal. *Ministério da Educação. Coord. J. Caraça and R. Carneiro.* (In press).

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## SEMINARS AT THE IGC

### *January*

João Sousa (IGC, Oeiras, Portugal)

*Tunable activation thresholds, implications for tolerance and homeostasis of lymphocytes.*

Salvador Martinez (Univ. Murcia, Murcia, Spain)

*Developmental genes expression and cell fate in the nervous system.*

Chaya Kalcheim (Hebrew Univ. Jerusalem, Israel)

*Neural crest cell development.*

Stefano Piccolo (Univ. Padova, Padova, Italy)

*Biochemical properties of *Xenopus* cerberus as a model for head induction in vertebrates.*

Claudio Stern (Univ. Columbia, New York, USA)

*Chick gastrulation: establishing polarity in a regulative system.*

Luis Puelles (Univ. Murcia, Murcia, Spain)

*Topological fate-mapping of the early neural plate.*

Chaya Kalcheim (Hebrew Univ. Jerusalem, Israel)

*Myotome development.*

J.A. Belo (IGC, Oeiras, Portugal)

*Phenotypic analysis and genetic interactions of the chordin mouse KO.*

Stefano Piccolo (Univ. Padova, Padova, Italy)

*Establishing asymmetries in early Vertebrate development.*

Claudio Stern (Univ. Columbia, New York, USA)

*Molecular dissection of neural induction in the chick embryo.*

Guillermo Oliver (St.Jude Children's Res. Hosp., Memphis,USA)

*Early patterning of the visual system.*

Nicole Le Douarin (Acad. Des Sciences/Inst. d'Embryol. Cell. Mol., Nogent sur Marne, France)

*The organizer in the development of the amniote embryo.*

Luis Puelles (Univ. Murcia, Murcia, Spain)

*In vitro studies on planar induction effects upon the mouse locus coeruleus.*



Juan Lafaille (NYU Medical Center, Skirball Institute, New York, USA)  
*Ongoing experiments on regulatory T cells.*

## **February**

Alberto de Freitas Ribeiro (Univ.São Paulo, São Paulo, Brazil)  
*Immunocytochemistry of digestive enzymes in entereocytes of insects: mechanisms of secretion.*

António Freitas (Institut Pasteur, Paris, France)  
*B Lymphocyte Homeostasis.*

Steffi Koenen-Waisman (Univ. Cologne, Cologne, Germany)  
*IRF-1 Independent induction of MHC Class I by IFN?*

Lisa Marubio (Baylor College of Medicine, Houston, USA)  
*Molecular genetic approaches to studying the role of nicotinic acetylcholine receptors.*

Richard Miles (CNRS FRE 2199, Univ. Paris V, Paris, France)  
*From neuronal firing to pathological discharges in the hippocampus.*

## **March**

Thierry Bal (Institut de Neurobiologie A. Fessard, Paris, France)  
*Network oscillation, dynamic information processing and hybrid artificial-biological circuits in the thalamus.*

Alexandre Quintanilha (IBMC, Porto, Portugal)  
*All about IBMC.*

John McCauley (Institute for Animal Health, Surrey, UK)  
*Bovine Pestiviruses- RNA viruses with an unusual life-style and properties.*

Alan Rickinson, FRS (Univ. Birmingham, Birmingham, UK)  
*Epstein-Barr virus biology and the pathogenesis of EBV-associated tumours.*

Hans-Gerhart Burgert (Univ. of Munich, Munich, Germany)  
*Re-routing of cellular proteins by viral E3 proteins: An immune evasion strategy of adenoviruses.*

Tony Minson (Univ. of Cambridge, Cambridge, UK)  
*Herpes simplex virus: entry, exit and new approaches to vaccine construction.*

Sir John Skehel, FRS (National Institute for Medical Research, London, UK)  
*Influenza Virus Infection of Cells and its Inhibition.*

## ***April***

Sergiy Bobrovnyk (IGC, Oeiras, Portugal)  
*Ligand-receptor interaction: little tragedies of big science.*

Pedro Fernandes (IGC, Oeiras, Portugal)  
*Human Genome Bioinformatics.*

Anne-Frederique Antoine (École Normale Supérieure Lyon, Lyon, France)  
*Egg activation in maize : how to start a new plant.*

Shohei Hori (IGC, Oeiras, Portugal)  
*Regulatory CD4<sup>+</sup> T cells control immune responses both to self and to non-self.*

## ***May***

Joaquin Rodriguez León (IGC, Oeiras, Portugal)  
*Role of the BMP antagonist, Gremlin, during limb development.*

Cristina Costa (Hospital Fernando Fonseca, Sintra, Portugal)  
*New variant CJD and mad cow disease: We are what we eat.*

Manuel Carrondo (IBET, Oeiras, Portugal)  
*All about IBET.*

Dominic Poccia (IGC, Oeiras, Portugal/Amherst College, USA)  
*Restructuring the Nuclear Envelope at Fertilization.*

Jean David (CNRS UPR 9034, Gif-Sur-Yvette, France)  
*Multiple aspects of stress tolerance and adaptation in Drosophila.*

Manuel Santos (Univ. Aveiro, Aveiro, Portugal)  
*A functional genomics approach to the evolution of alternative genetic codes.*

Marta Barreto (IGC, Oeiras, Portugal)  
*Molecular and genetic studies of autoimmune disease: Type 1 Diabetes and Systemic Lupus Erythematosus.*

Cristina Casalou (IGC, Oeiras, Portugal)  
*Are the chaperonin containing-TCPI (CCT) subunits more than components of a folding machinery? Studies during Tetrahymena cilia biogenesis.*

## **June**

Sheila McCormick (Univ. Berkeley, Berkeley, USA)

*Cell signaling during pollen tube growth.*

Ana Crespo (IGC, Oeiras, Portugal)

*Expression of suppressors of cytokine signaling (SOCS) and macrophage function.*

Linda Dixon (Institute Animal Health, Surrey, UK)

*Evasion of host defences by African swine fever virus.*

Sofia Rodrigues (IGC, Oeiras, Portugal)

*The node clock is ticking.*

Esteban Domingo (Centro de Biología Molecular "Severo Ochoa", Univ. Autónoma de Madrid, Spain)

*New antiviral approaches studied with FMDV.*

Maria Mota (New York University Medical Center, New York, USA)

*Host-Parasite interactions during malaria liver infection.*

Lisete Fernandes (IGC, Oeiras, Portugal)

*Crossroad of cold and oxidative stress signaling pathways.*

Cristina Vieira (IBMC, Porto, Portugal)

*How recombination affects the way we think about plant gametophytic self-incompatibility systems.*

Jorge Vieira (IBMC, Porto, Portugal)

*How often are developmental genes the target of adaptive selection ? The fused gene story.*

## **July**

Irvin Cohen (The Weizmann Institute of Science, Rehovot, Israel)

*Patterns of Autoantibodies.*

Magdalena Zernicka-Goetz (Wellcome/CRC Institute/Univ. Cambridge, UK)

*Patterning of the Mouse Embryo: How early is it determined?*

David Glover (Univ. Cambridge, Cambridge, UK)

*Centrosomes: the Grand Central Stations of Mitosis.*

Ben Scheres (Univ. Utrecht, Utrecht, Netherlands)  
*Cell division and pattern formation in the Arabidopsis root.*

Bruce Alberts (President of the National Academy of Sciences, Washington DC, USA)  
*Spreading science throughout society.*

Juan Hurlé (Universidad de Cantabria, Santander, Spain)  
*Molecular control of digit morphogenesis.*

José Maria Álvarez Mosig (Univ. S. Paulo, S. Paulo, Brazil)  
*CD8+ T cells in experimental chronic Chagas disease.*

Melvin Cohn (IGC, Oeiras, Portugal/The Salk Institute, La Jolla, USA)  
*Contemporary models of the Self-Nonself discrimination: A general discussion.*

Stefan Grunert (Institute for Molecular Pathology, Wien, Austria)  
*Cooperation of TGF $\beta$  and ras signalling pathways during EMT and metastasis in mouse mammary epithelial cells.*

Francisco Dionísio (IGC, Oeiras, Portugal)  
*Selection of policing mechanisms in systems with division of labour.*

Manuel Nunes da Ponte (ITQB/UNL, Oeiras, Portugal)  
*All about ITQB.*

Thiago Carvalho (IGC, Oeiras, Portugal)  
*Regulatory T cells and Inflammation.*

Miguel Godinho Ferreira (Univ. Colorado Health Sciences Center, Denver, USA)  
*Decisions between life and death: What happens when telomeres are seen as DNA breaks.*

Claudia Rocha Carvalho (IGC, Oeiras, Portugal/Univ. Fed. Minas Gerais, Minas Gerais, Brazil)  
*Are cycling genes involved in limb patterning?*

Alvaro Tavares (IGC, Oeiras, Portugal)  
*The cellular MOB: dividing is a family business.*

Jorge Carneiro (IGC, Oeiras, Portugal)  
*A minimal model of chromatin competence for transcription.*

## **August**

Andrew Waters (Leiden University Medical Centre, Leiden, Netherlands)  
*Parasite molecules and their role the fertilisation and transmission of Plasmodium berghei.*

## **September**

Iris Caramalho (IGC, Oeiras, Portugal)  
*Immunoregulation and inflammation: lessons from in vitro assays.*

Sofia Cordeiro (IGC, Oeiras, Portugal)  
*Vibrating science: ions and cell polarity.*

Sandra Caldeira (ATV DKFZ, Heidelberg, Germany)  
*The potential role of HPV38 in the development of Non Melanoma Skin Cancers.*

## **October**

Deborah Braun (IGC, Oeiras, Portugal)  
*Type I Interferon, B lymphocyte activation and autoimmunity.*

Margarida Vigário (IGC, Oeiras, Portugal)  
*Cytokines and T cells in cerebral malaria.*

Alexander Poltorak (The Scripps Research Institute, La Jolla, USA)  
*Genetic analysis of innate immunity.*

Victor J. Small (Institute of Molecular Biology, Austrian Academy of Sciences, Salzburg, Austria)  
*Moving with the cytoskeleton: the network of life.*

Catarina Freitas (IGC, Oeiras, Portugal)  
*Information for segmentation is segregated within presomitic mesoderm.*

## **November**

João Pedro Simas (IGC, Oeiras, Portugal)  
*Gammaherpesvirus latency in B cells.*

Oreste Acuto (Institut Pasteur, Paris, France)  
*Molecular modifiers of TCR triggering threshold: cis and trans mechanisms.*

Olivier Lantz (Institut Curie, Paris, France)

*Role of MHC molecules and of Interleukin common gamma chain cytokines in the survival of CD4+ T cells.*

Ana Teresa Tavares (IGC, Oeiras, Portugal)

*Embryonic development of the vertebrate limb. The role of Rel / NF- KB transcription factors and co-factors.*

Daniel Cutler (MRC Lab for Molecular Cell Biology, UCL, London, UK)

*The Biogenesis of regulated Secretory Organelles.*

Genis Parra (Genome Informatics Research La, Instituto Municipal de Investigacion Medica, Barcelona, Spain)

*Gene prediction by comparative genomics: re-annotating human genome using mouse data.*

Sergi Castellano (Genome Informatics Research La, Instituto Municipal de Investigacion Medica, Barcelona, Spain)

*Prediction of non-canonical genes: the selenoproteins case.*

Vania Braga (Imperial College, London, UK)

*Cadherin adhesion and small GTPases.*

## **December**

Karim Labib (Paterson Institute for Cancer Research Christie Hospital NHS Trust, Manchester, UK)

*Regulation of the MCM2-7 protein complex.*

Ana Catarina Certal (IGC, Oeiras, Portugal)

*"Tell me how you pump, I'll tell you how you grow": a molecular approach to study pollen tube growth.*

Claudio Sunkel (IBMC, Porto, Portugal)

*The role of non-motor microtubule associated proteins in spindle function.*

Carl Smythe (Univ. Dundee, Dundee, Scotland)

*Analysis of checkpoint kinases in mammalian cells.*

Jonathon Pines (Univ. Cambridge, Cambridge, UK)

*Regulating mitosis by proteolysis*

Monica Sousa (IBMC, Porto, Portugal)

*Familial Amyloid Polyneuropathy: The tale of a growing fibril.*

Francesco Colucci (Institut Pasteur, Paris, France)

*Killing naturally: no longer Syk.*

Jan Andersson (Univ. Stockholm, Sweden)

*Immune responses in new born.*

Salvatore Valitutti (INSERM, Toulouse, France)

*Molecular dynamics of contact sites between T lymphocytes and antigen presenting cells.*

Leonor Tavares Saúde (IGC, Oeiras, Portugal)

*How to achieve a perfect body.*

Moises Mallo (IGC, Oeiras, Portugal)

*Reversible knock outs: a dream becoming true?*

Anita Gomes (Imperial College London, London, UK)

*Membrane Association and Targeting of Rab GTPases*

### **Gulbenkian Lectures : Frontiers of Modern Biology**

Host: Michael Parkhouse

In order to promote specially productive interactions of PhD students and young scientists with relevant figures in modern biology, a “Gulbenkian Lecture” series was launched in 2000. Selected invited speakers give seminars that are open to all interested scientists all over the country, but spend most of their time at the IGC in exclusive discussion with students.

### ***January***

Jim Smith, National Institute for Medical Research, UK

*Making Mesoderm: Upstream and downstream of Brachyury*

### ***May***

Carlos Martinez-A., National Center for Biotechnology, Madrid, Spain

*Chemokines: Key molecules in inflammation, cancer and HIV-1 infection*

## SYMPOSIA, CONFERENCES AND MEETINGS ORGANISED BY THE IGC

### *January*

#### **Gulbenkian Biology Courses**

##### **Plant Development: Molecular and Cellular Basis**

##### **Instituto Gulbenkian de Ciência**

**29 January – 16 February 2001**

Organiser: José Feijó (*IGC, Oeiras, Portugal and FCUL, Lisbon, Portugal*) and Margarida Oliveira (*IBET/UL, Lisbon, Portugal*)

With the description of several flowering plant genomes, molecular and genetic tools are becoming available for integrative organism-centred approaches. 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. Developmental biology has emerged as a most challenging area in which this is occurring, as it commands a full understanding of the spatial and temporal integration of processes that allow for growth, differentiation and morphogenesis. On the other hand, higher plants represent one of the major evolutionary trends and their genetic and experimental value lift them, specially in recent years, to a competitive and challenging field from where major fundamental discoveries have occurred.

This course aimed to provide the basis for this post-genomic work, with a solid theoretical background and practical training on the most utilised and versatile molecular tools and approaches, but creating the perception of need for interdisciplinary interfaces with cutting-edge biophysical and imaging techniques, and a strong sense of integrative biology. The faculty included some of the world-leading scientists on plant development. The course organization allowed for flexibility, free-discussion and aim at fostering productive interactions among all participants.

In 2001, it was attended by 16 international students, from 11 countries (1 Ass.Prof., 8 Post-Docs and 7 advanced PhD. Students), involving a Faculty of recognised international experts. Final program and details can be seen in [http://uic.igc.gulbenkian.pt/Plant\\_EMBO](http://uic.igc.gulbenkian.pt/Plant_EMBO) ).

Faculty: Richard Amasino, *Univ Wisconsin, Madison, USA*; Gerco Angenent, *Plant Research International B.V., Wageningen, The Netherlands*; Alice Cheung, *Univ. Massachusetts, Amherst, USA*; Enrico Coen, *John Innes Centre, Norwich, UK*; Nam-Hai Chua, *Univ Rockefeller, New York, USA*; Peter Hepler, *Univ Massachusetts, Amherst, USA*; Andrew J. Millar, *Univ Warwick, Coventry, UK*; William J. Lucas, *Univ California, California, USA*; Keith Roberts, *John Innes Centre, Norwich, UK*; Ben Scheres, *Univ Utrecht, Utrecht, The Netherlands*; Anthony Trewavas FRS, *Univ Edinburgh, Edinburgh, Scotland*;



Students: Riyaz Ahmad Bhat, *Max Planck Institute, Koeln, Germany*; Maria José Carrascosa Gomez, *Univ Freiburg, Freiburg, Germany*; Patricia Carneiro, *Univ Washington, Washington, USA*; Felisberta Cunha, *Univ. Plymouth, Plymouth, UK*; Danilo D. Fernando, *Suny College of Environmental Science and Forestry, NY, USA*; Laura Fraysse, *Univ. Lund, Lund, Sweden*; Rachel Hemsely, *John Innes Centre, Norwich, UK*; Olga Koroleva, *Univ. Wales Bangor, UK*; Birigit Linkohr, *Univ. York, York, UK*; Judith Nardmann, *Univ. Koeln, Koeln, Germany*; Marisa Silvina Ortegui, *Univ. Colorado at Boulder, Colorado, USA*; Alexandra Castilho de Vitória Pereira, *ITQB/UNL, Lisbon, Portugal*; Zhang Pingzu, *Univ. Singapore, Singapore*; Marco Possenti, *Istit. Nazionale Ricerca Alimenti e Nutrizione, Rome, Italy*; Vera Quecini, *Univ. Wageningen, The Netherlands*; Björn Sieberer, *Wageningen Univ., The Netherlands*; Vanda Sunderlikova, *Inst. Plant Genetics and Biotechnology, Slovak*.

## ***March***

### **Conferences of the IGC Scientific Advisory Board Instituto Gulbenkian de Ciência 19 March 2001**

*Reflections on the European dimension in biomedical science policy*  
Kai Simons (Max Planck Institute, Dresden, Germany)

*A clock and trail model for somite development*  
Lewis Wolpert (University College of London, London, UK)

*How many genes do we have?*  
Sydney Brenner (The Molecular Sciences Institute, Berkely, CA, USA)

## ***May***

### **Immunology, Theory and Experiments Convento da Arrábida 17-19 May 2001**

Organisers: Melvin Cohn (Salk Institute, USA) and António Coutinho (IGC, Oeiras, Portugal)

This meeting was organized in an effort to have immunology as a part of medicine confronted by immunology as a part of biology. This meeting was organized in the context of the “Laboratoire Européen Associé au CNRS (France)” that was launched in 1998 at the IGC, and operates in cooperation with several French laboratories. The participants chosen have widely divergent conceptual frameworks and are representatives of a spread of disciplines in medicine and biology. The goal was less to decide whose position is right or wrong but rather to make crystal clear what the formulation is and

what it does and does not explain. A unique characteristic of the meeting was the participation of theoreticians whose interaction with the experimentalists proved salutary for both. We are being inundated by an avalanche of data coming from many sources. The complexity and sheer volume of this information means that most of it would be lost if we do not have a school of computational biologists who spend their time organizing these data into heuristic quantitative models based on transparent sound fundamentals. This meeting was small allowing ample time for discussion and reflection.

*Participants:* Jorge Carneiro, IGC, Oeiras, Portugal; Pierre-Andre Cazenave, Inst. Pasteur, Paris, France; Jocelyne Demengeot, IGC, Oeiras, Portugal, Nicole Le Douarin, College de France /Academ. Sciences, Paris, France; Constantin Fesel, IGC, Oeiras, Portugal; Carlos Penha Gonçalves, IGC, Oeiras, Portugal; Zvi Grossman, IGC/Tel Aviv Univ, Israel; Werner Haas, Linden Technologies, USA, Francois Huetz, Inst. Pasteur, Paris, France; Philippe Druet, Hopital, Purpan, INSERM, Paris, France; Paola Minóprio, Inst. Pasteur, Paris, France; Sylviane Pied, Inst. Pasteur, Paris, France, John Stewart, IGC/COSTECH, Univ. Technol. Compiègne, France, Astrid Vicente, IGC, Oeiras, Portugal, Nelson Vaz, Univ. Federal de Minas Gerais, Minas Gerais, Brazil.

## **July**

### **Programme for the Promotion of Scientific Interaction with Mozambique**

This programme aims to stimulate young researchers, technicians and students to a career in Science by promoting discussion and solving problems on chosen topics of Life Sciences. The programme is organized in annual cycles consisting of two types of activities: training and exchange of students and staff. The first year took place in Mozambique but in the future it may be extended to other African countries. In the medium-term, part of the organizing effort is to be transferred to local institutions.

#### **Annual Training Course:**

**“Fronteiras da Biologia Moderna no contexto de Países em Vias de Desenvolvimento”**

**Maputo, Mozambique**

**23 July- 3 August 2001**

*Organisers:* Mónica Bettencout Dias, (IGC, Oeiras, Portugal/Univ College of London, London, UK); Margarida Trindade, (IGC, Oeiras, Portugal/National Institute for Medical Research, London, UK); José Pereira Leal, (IGC, Oeiras, Portugal/Imperial College School of Medicine, London, UK); Nuno Arantes Oliveira, (IGC, Oeiras, Portugal/Univ California at San Francisco, S. Francisco, USA), José Mário Leite (IGC, Oeiras, Portugal).

Approximately ten researchers at PhD or post-doctoral level went to Maputo to lecture a series of intensive workshops and seminars on chosen topics to approximately 20 pre-selected participants. The course took two weeks and started on 23 July, the date coinciding with the end of the academic year in Mozambique. Portuguese was the main

language used during the course, as suggested by the Mozambican organisers; however support material was also presented in English. The first course focused on Molecular Biology and Biotechnology, and its applications to Medicine, Veterinary, Nutrition, Agriculture and the protection of Biodiversity. Emphasis was given to the role of scientific knowledge and technology to solve particular problems of developing countries. The course also included more general discussions on the career in Science and Science and Technology policies in developing countries. Subjects were approached in an interactive way, including open discussions and problem solving. In the future, and according to the experience acquired in previous years, courses may focus on other areas.

## ***October***

### **IV Gulbenkian Autumn Meeting / I Portuguese Meeting on Theoretical and Computational Biology Instituto Gulbenkian de Ciência 23- 26 October 2001**

Organisers: José Leal (*IGC, Oeiras, Portugal*), Rui Alves (*IGC, Oeiras, Portugal/ICRF, , London, UK*) and Fernando Antunes (*FCUL, Lisbon, Portugal*).

For the past 3 years, the Instituto Gulbenkian de Ciência has organized “Autumn Meetings” that brought together a dozen or so post-doctoral fellows and young group leaders developing state-of-the-art research on “Evolution and Development”, “Adaptation by Horizontal Gene Transfer” and “Cellular Differentiation and Commitment”, respectively.

An area of interest that had not yet been addressed is that of Computational and Theoretical Biology. Furthermore, Bioinformatics has been progressing rapidly and will certainly play an increasingly important role in this age of genomics, proteomics and other large data sets, as well as biomedical automation. The 2001 Gulbenkian Autumn Meeting was thus dedicated to this theme. This meeting was the first Portuguese Meeting on Theoretical and Computational Biology. A considerable number of Portuguese researchers, both inside and outside the country, have been producing world class work in this area, and it was felt that it would be useful to bring them together, in order not only to exchange information about the work being done and foster novel interactions, but also to discuss questions related to the education, academic and industrial applications of work in this area, and to draw common goals for developing and supporting this type of work in Portugal.

This type of research often produces tools that lead to commercial applications and thus attract some of the funds necessary to sustain research programs themselves. In addition, developing competitive research programs in this area is generally not as expensive as an equivalently good wet lab research program. The Gulbenkian Autumn Meeting on Theoretical and Computational Biology aimed, therefore, at bringing together, in addition to the scientists themselves, representatives of the educational, medical, and industrial communities in Portugal

Participants: Jonas Almeida, *Univ. South Carolina, South Carolina, USA*; Filipa Alves, *IST, Lisbon, Portugal*; Juan Camacho, *Univ. Autònoma Barcelona, Barcelona, Spain*; Jorge Carneiro, *IGC, Oeiras, Portugal*; Pedro Coutinho, *IST, Lisbon, Portugal*; Leonor Cruzeiro-Hansson, *Univ. Algarve, Faro, Portugal*; Rui Dilão, *IST, Lisbon, Portugal*; Francisco Dionísio, *IGC, Oeiras, Portugal*; Tiago Domingos, *IST, Lisbon, Portugal*; Patricia Faisca, *Univ Warwick, Coventry, UK*; Pedro Fernandes, *IGC, Oeiras, Portugal*; Antonio Ferreira, *FCUL, Lisbon, Portugal*; João Gomes Ferreira, *FCT/UNL, Lisbon, Portugal*; Claudio Gomes, *ITQB/UNL, Lisbon, Portugal*; André Melo, *FCUP, Porto, Portugal*; Paulo Martel, *ITQB/UNL, Lisbon, Portugal*; Henrique Pereira, *Univ. Stanford, Stanford, USA*; Maria João Romão, *FCT/UNL, Lisbon, Portugal*; Armindo Salvador, *Univ. Michigan, Michigan, USA*; Claudio Soares, *ITQB/UNL, Lisbon, Portugal*; João Sousa, *IGC, Oeiras, Portugal*; Carlos Cunha, *UNL, Lisbon, Portugal*; Rute Fonseca, *FCUP, Porto, Portugal*; Sara Pinto Garcia, *ITQB/UNL, Lisbon, Portugal*; Gabriela Gomes, *Univ. Warwick, Warwick UK*; Ana Margarida Martins, *FCUL, Lisbon, Portugal*; Raquel Tavares, *Inst. Pasteur, Paris, France*; Victor Teixeira, *ITQB/UNL, Lisbon, Portugal*.

#### **Debate: Institutional Policies on Bioinformatics**

**26 October 2001**

#### **Instituto Gulbenkian de Ciência**

Within the context of the IV Gulbenkian Autumn Meeting, a debate was organised by Antonio Coutinho, entitled: Institutional Policies on Bioinformatics.

Participants: Luís Magalhães, *FCT-MCT, Lisbon, Portugal*; Maria do Carmo Fonseca, *IHE-FML, Lisbon, Portugal*; Manuel Nunes de Ponte, *ITQB-UNL, Lisbon, Portugal*; Manuel Santos, *Univ. Aveiro, Aveiro, Portugal*; Mário Seixas, *IPATIMUP, Porto, Portugal*; Alexandre Quintanilha, *IBMC, Porto, Portugal*; Antonio Coutinho, *IGC, Oeiras, Portugal*; Gabriel Pires, *IST, Lisbon, Portugal*; João Branco, *IST, Lisbon, Portugal*; Sousa Ramos, *IST, Lisbon, Portugal*; José Fachada, *IST, Lisbon, Portugal*; Carlos Rocha, *IST, Lisbon, Portugal*; Adelia Sequeira, *IST, Portugal*; Amílcar Sernadas, *IST, Lisbon, Portugal*.

## **TEACHING**

### **POST-GRADUATE EDUCATION**

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

### **GULBENKIAN PhD PROGRAMME IN BIOMEDICINE**

#### **Governing Bodies of the PGDB**

##### **Board of Trustees**

Prof. Luis Magalhães, for the Ministry of Science and Technology

Prof. Carlos Portas, for the Ministry of Education

Dr. Idalina Salgueiro, for the FLAD

Prof. João Caraça, for the FCG

##### **Board of Directors**

Prof. António Coutinho, IGC (Chairman)

Prof. António Xavier, Universidade Nova de Lisboa

Prof. Arsélio Pato de Carvalho, Universidade de Coimbra

Prof. Alexandre Quintanilha, IBMC, Univ. do Porto

Prof. Maria do Carmo Fonseca, Faculdade de Medicina da Univ. Lisboa

##### **Executive Direction**

Prof. Miguel Seabra (Director)

Prof. Sukalyan Chatterjee (Deputy Director)

##### **Staff**

Ms. Dolores Oliveira (Laboratory technician)

Ms. Manuela Cordeiro (Secretary)

Students of the Gulbenkian PHD Programme in Biomedicine for 2000/2001

Joana Martins Vicente Aguiar Câmara  
Célia da Conceição Duarte Cruz  
Fernando António da Costa Ferreira  
Rui Pedro Capelo de Abreu Galvão  
Carlos Miguel da Costa Afonso Lino Gaspar  
Paula Raquel Moreira de Araújo Gomes  
Mónica Rodrigues Fortunato Hilário  
Isabel Cristina Colaço Farias Jaco  
Joana Alexandra Ferraz Teixeira Loureiro  
Venessa Alexandra Zuzarte Luís  
César Miguel Pereira Soares Mendes  
Maya Losa Mendiratta  
Vitor Manuel Bordona de Sousa Paixão  
Artur Filipe Dias de Castro Rodrigues  
Teresa Patrícia Gonçalves dos Santos  
Luis Miguel Mendes Soares  
Cláudia Susana de Lima Vieira

Students of the Gulbenkian PHD Programme in Biomedicine for 2001/2002

Maria João Lopes Gonçalves de Brito Amorim  
Ricardo Filipe Gonçalves de Sousa Carvalho  
Sílvia da Conceição Santos Pereira da Costa  
David Zeferino d'Azevedo Cristina  
Álvaro Gil Araújo Ferreira  
Ana Isabel de Oliveira Franco  
Nuno Duarte Caixinha Geraldo  
Ana Lúcia Gomes Almeida Pereira Mena  
Maria Inês Paula C. Canavarro de Morais  
Alexandre Alves Neves  
Filipa Susana Caldas Pinto  
Eliana Patrícia Coelho Real  
Clara Pinheiro Vieira Correia dos Reis  
Eduardo Alexandre Barros e Silva  
Susana Augusta dos Santos Silva  
André Guilherme V. V. Rodrigues da Silva  
Helena Isabel Martins Soares  
Vivian Leite de Oliveira  
Claudia Istrate

## **Gulbenkian PhD programme in Biomedicine for 2001**

Programme for 2001/2002 conducted at the IGC in 2001:

### **25 September: Introduction day IGC**

*Faculty:*

Sukalyan Chatterjee (IGC, Oeiras, Portugal)

Pedro Fernandes (IGC, Oeiras, Portugal)

### **26-27 September: Introduction to Genetics**

*Faculty:*

John Stewart (COSTECH, Compiègne, France; IGC, Oeiras, Portugal)

### **28 September: Introduction to Biochemistry**

*Faculty:*

Sukalyan Chatterjee (IGC, Oeiras, Portugal)

### **1-12 October: Structure and Function of Proteins**

*Faculty:*

António Xavier (ITQB, Oeiras, Portugal)

Thomas Schneider (Goettingen Univ., Goettingen, Germany)

Maria Arménia Carrondo (ITQB, Oeiras, Portugal)

Miguel Teixeira (ITQB, Oeiras, Portugal)

Lígia Saraiva (ITQB, Oeiras, Portugal)

Carlos Frazão (ITQB, Oeiras, Portugal)

Margarida Archer (ITQB, Oeiras, Portugal)

David Aragão (ITQB, Oeiras, Portugal)

Isabel Bento (ITQB, Oeiras, Portugal)

Francisco Enguita (ITQB, Oeiras, Portugal)

Eurico Melo (ITQB, Oeiras, Portugal)

Cláudio Gomes (ITQB, Oeiras, Portugal)

Inês Cardoso Pereira (ITQB, Oeiras, Portugal)

Rita Lemos (ITQB, Oeiras, Portugal)

Célia Romão (ITQB, Oeiras, Portugal)

António Baptista (ITQB, Oeiras, Portugal)

Francisco Morais (ITQB, Oeiras, Portugal)

Ana Melo (ITQB, Oeiras, Portugal)

Manuela M. Pereira (ITQB, Oeiras, Portugal)

Ricardo Louro (ITQB, Oeiras, Portugal)

Cláudio Soares (ITQB, Oeiras, Portugal)

Pedro Matias (ITQB, Oeiras, Portugal)

### **15-19 October: Transcription**

*Faculty:*

Sukalyan Chatterjee (IGC, Oeiras, Portugal)

Eric Lam (Ludwig Institute for Cancer Research, London, UK)  
Valeria Poli (Torino, Italia)  
Maria Marone (Catholic University, Roma, Italia)  
Leonor Parreira (FMUL, Lisboa; IGC, Oeiras, Portugal)  
João Ferreira (Lisboa, Portugal)

### **22-24 October: Post-Transcriptional Processing**

#### *Faculty:*

Alexandra Moreira do Carmo (IBMC, Porto, Portugal)  
Margarida Gama-Carvalho (IHEFM-UL, Lisboa, Portugal)

### **25-26 Oct: Bioinformatics and Theoretical Biology**

Symposium: José Leal – org. (Imperial College School of Medicine, London, UK)

### **29 October- 2 November: Cytoskeleton**

#### *Faculty:*

Helena Soares (IGC, Oeiras, Portugal)  
J. Victor Small (Institute of Molecular Biology, Salzburg, Austria)  
Peter Jordan (INS Dr. Ricardo Jorge, Lisboa, Portugal)

### **5-9 November: Signal Transduction**

#### *Faculty:*

Marcus Thelen (Institute for Research in Biomedicine, Bellinzona, Switzerland)  
Gioacchino Natoli (Institute for Research in Biomedicine, Bellinzona, Switzerland)  
Oreste Acuto (Institut Pasteur, Paris, France)

### **12-16 November: Apoptosis**

#### *Faculty:*

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

### **19-23 November: Membrane Traffic**

#### *Faculty:*

Miguel Seabra (Imperial College School of Medicine, London, UK; IGC, Oeiras, Portugal)  
Daniel Cutler (UCL, London, UK)  
Margarida Barroso (University of Virginia, Charlottesville, USA)  
Graça Raposo (Université Pierre et Marie Curie, Paris, France)

### **26-30 November: Cell Adhesion and Extracellular Matrix**

#### *Faculty:*

John Couchman (Imperial College, London, UK)  
Hinka Multhaupt (Imperial College, London, UK)  
Vânia Braga (Imperial College, London, UK)



### **3-7 December: Cell Cycle**

#### *Faculty:*

Álvaro Tavares (IGC, Oeiras, Portugal)

Claudio Sunkel (IBMC, Porto, Portugal)

Rui Gomes (FCL, Lisboa, Portugal)

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

Miguel Ferreira (Univ. of Colorado Health Sciences Center, Denver, USA)

Carl Smythe (University of Dundee, Dundee, UK)

Jonathon Pines (Wellcome/CRC Institute, Cambridge, UK)

### **10-14 December: Immunology I**

#### *Faculty:*

Jocelyne Demengeot (IGC, Oeiras, Portugal)

Francesco Colucci (Institut Pasteur, Paris, France)

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

António Coutinho (IGC, Oeiras, Portugal)

Jan Andersson (Dept. Immunology, University of Stockholm, Sweden)

Dan Holmberg (Umea University, Sweden; IGC, Oeiras, Portugal)

Thiago Carvalho (IGC, Oeiras, Portugal)

### **17-21 December: Cancer**

#### *Faculty:*

Carlos Caldas (CRC, Cambridge, UK)

Samuel Aparício (University Department of Oncology, Cambridge, UK)

Paul Pharoah (CRC, Cambridge, UK)

Tony Kouzarides (Univ. of Cambridge, Cambridge, UK)

Ermanno Gherardi (Univ. of Cambridge, Cambridge, UK)

## **SCIENTIFIC OPEN DAY**

The Gulbenkian Ph.D programme (PGDB) and the IGC, in close collaboration with some Portuguese laboratories, organised a scientific open day on 17 February 2001.

This interaction was held with the intention of providing information on the ongoing scientific projects of the laboratories where the PGDB students could opt to rotate and/or carry out their dissertation.

### Selection of Labs for rotations

	<b>1<sup>st</sup> ROTATION</b> <b>17 April-27 June</b>	<b>2<sup>nd</sup> ROTATION</b> <b>4 July-12 September</b>
<b>Cláudia Vieira</b>	<b>Salvador Martinez</b> Univ. Alicante, Spain	<b>Domingos Henrique</b> IHEM, FM-UL
<b>Joana Loureiro</b>	<b>João Gonçalves</b> Dept. Microbiology, FF-UL	<b>Pedro Simas</b> Viral Pathogenegis, IGC
<b>Joana Câmara</b>	<b>Astrid Vicente</b> Human Genetics, IGC	<b>Carmo Fonseca</b> IHEM, FM-UL
<b>Isabel Jaco</b>	<b>Rui Victorino</b> FM-UL	<b>Carmo Fonseca</b> IHEM, FM-UL
<b>Maya Mendiratta</b>	<b>Rui Appelberg</b> IBMC, UP	<b>Patrícia Maciel</b> IBMC, UP
<b>Artur Rodrigues</b>	<b>Maria Arménia Carrondo</b> ITQB, UNL	<b>Deolinda Lima</b> IBMC, UP
<b>Vítor Paixão</b>	<b>Jorge Carneiro</b> EAO, IGC	<b>António Xavier</b> ITQB, UNL
<b>Célia Cruz</b>	<b>Stephen McMahon</b> King's College London, UK	<b>Deolinda Lima</b> IBMC, UP
<b>Paula Raquel Gomes</b>	<b>Jocelyne Demengeot</b> Lymphocyte Physiology, IGC	<b>Rui Appelberg</b> IBMC, UP
<b>Vanessa Luís</b>	<b>Isabel Palmeirim</b> Vertebrate Segmentation, IGC	<b>José Feijó</b> Plant Development, IGC
<b>CarlosMiguel Gaspar</b>	<b>Carmo Fonseca</b> IHEM, FM-UL	<b>Claudio Sunkel</b> IBMC, UP
<b>César Mendes</b>	<b>Miguel Soares</b> Inflammation, IGC	<b>Joaquin León</b> Organogenesis, IGC
<b>Teresa Santos</b>	<b>José Feijó</b> Plant Development, IGC	<b>Leonor Parreira</b> IHEM, FM-UL
<b>Rui Galvão</b>	<b>Carmo Fonseca</b> IHEM, FM-UL	<b>Domingos Henrique</b> IHEM, FM-UL
<b>Mónica Hilário</b>	<b>José Feijó</b> Plant Development, IGC	<b>Miguel Castelo-Branco</b> CNBC, UC

### **Annual Meeting in Curia ( 16-22September 2001)**

As in the past years, the Annual PGDBM/PGDB Meeting of Curia took place from 16 to 22 September, thereby completing the first working year of the newly created Gulbenkian PhD Programme in Biomedicine. The scientific sessions included oral presentations from around 80 students and a poster session. In addition to the students, Portuguese University Professors who were involved in the teaching of the graduate courses or in the supervision of laboratory rotation and theses work, participated in the meeting.

The last day of the Meeting was dedicated to the distribution of diplomas to students who completed their theses in 2001 with merit, and to pay homage to António Coutinho, Patron of the 1<sup>st</sup> Class of the PGDB, whose commendation was made by Dan Holmberg (Umea University Medical School, Umea, Sweden). Also in this session, there was a plenary conference given by Philippe Kourilsky (Collège de France, Directeur-Général de l' Institut Pasteur, Paris, France) entitled "Science policy at the Institut Pasteur in the new European and international context".

## TEACHING PROGRAMME ON BIOINFORMATICS

The IGC operates de EMBnet node in Portugal and develops a specific educational programme in Bioinformatics.

### **Bioinformatics Training Courses**

Local organization: Pedro Fernandes and Isabel Marques

In 2001 another 100 attendees have received formal training within the Gulbenkian Training Program in Bioinformatics. Five training courses, two of which at the introductory level, were held at the Instituto Gulbenkian de Ciência.

#### ***April***

##### *A Practical Introduction to Bioinformatics*

Faculty: David P. Judge, University of Cambridge, UK; Lisa Mullan, HGMP-RC, Hinxton, Cambridge, UK.

#### ***July***

##### *DNA and Protein Sequence Evolution*

Faculty: James McNerny, National University of Ireland, Maynooth, IE Christopher Creevey, Dept. of Biology, National University of Ireland, Maynooth, IE.

#### ***November***

##### *Functional and Comparative Genomics*

Faculty: Martin Bishop, HGMP-RC, Hinxton, Cambridge, UK; Marc Botcherby, HGMP-RC, Hinxton, Cambridge, UK; Sergi Castellano, INIM-UPF, Barcelona, SP; Joaquin Dopazo, CNIO-ISCI, Madrid, SP; Genis Parra, INIM-UPF, Barcelona, SP.

#### ***December***

##### *Microsatellite Markers Bioinformatics*

Faculty: Mark Beaumont, University Reading, UK; Lounes Chikhi, University College, London, UK.

##### *Biological Sequence Analysis*

Faculty: David P. Judge, University of Cambridge, UK; Lisa Mullan, HGMP-RC, Hinxton, Cambridge, UK.

## **SCIENCE AND SOCIETY**

### **CIÊNCIA VIVA**

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

#### **Project “Scientific occupation for teenagers during holidays”**

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

Within the context of this programme the IGC, in 2001, participated with the following training sessions and students:

##### **Estudo do Desenvolvimento em Plantas**

***IGC Member: José Feijó***

30 July- 11 August 2001

*Maria Inês Correia Espinha*

Escola Secundária de Linda-a-Velha

##### **Manutenção dos Animais de Laboratório do Biotério do IGC**

***IGC Member: Bruce Lenhart***

6-17 August 2001

*Joana Oliveira dos Santos*

Escola Secundária de Sebastião da Gama

*Sara Isabel Veiga Martins*

Escola Secundária da Moita

*Ana Cristina Coelho Sousa Parreira*

Colégio S. José do Ramalhão, Sintra

##### **Estudo do desenvolvimento dos membros em galinhas**

***IGC Member: Joaquín Rodríguez León***

6-17 August 2001

*João Filipe Caetano Rosa*

Escola Secundária de Santa Maria

3-14 September

*Magda Inácio Barreto Faria*

Escola Secundária de Stuart Carvalhais

### **Estudo da selecção natural na evolução de bactérias**

**IGC Member: *Francisco Dionísio***

27 August- 14 September 2001

*Ana Sílvia Cunha Coelho*

Escola Secundária de Camilo Castelo Branco

*Ana Catarina Rodrigues Almeida*

Escola Secundária de Camilo Castelo Branco

### **Filosofia e História da Ciência**

**IGC Member: *Francisco Dionísio***

27 August- 14 September 2001

*Ana Cristina Bandarra da Silva*

Escola Secundária Francisco Simões

*Cláudia Lúcia de Jesus Faria da Costa*

Escola Secundária Augusto Cabrita

### **Estudo do Ciclo e da Divisão Celulares**

**IGC Member: *Alvaro Tavares***

3-14 September 2001

*Pedro Ferreira*

Escola Secundária de Cascais

*Mauro Lenadro Conde*

Escola Secundária de Santo António

*Sara Viana Rodrigues da Silva*

Escola Secundária Manuel da Fonseca

### **Project “The Human Genome: Perspectives for Public Health”**

An international project on the perspectives for prevention, diagnosis and treatment of genetic diseases in light of the recent progress of the Human Genome Project, involving secondary school students and teachers, Health Sciences researchers and journalists, was created.

This international project aimed to create opportunities for Portuguese and foreign students (secondary education) to learn more about the recent progress in the elucidation of the Human Genome, and its applications in the prevention, diagnosis and treatment of genetic diseases.

This project was a follow up from the project “Health in the XXI Century: a vision from the European Youth” that was carried out in the preceding year, in close collaboration between the youngsters and the Portuguese scientific community, who provided support throughout the project.

This interaction between students and scientists involved laboratory visits, meetings and interviews with IGC members, the creation of products and presentations of the students’ views on the most recent scientific research and technological developments related to health issues.

**Conferences**

Astrid Vicente

*Genetics of Autism*

Escola Secundária de Cascais

**Visits to the IGC**

Marta Barreto

*Genetics of SLE*

Escola Secundária da Baixa da Banheira

June 2001

**National week for the disclosure of scientific activities:**

**19 –25 November 2001**

**IGC Open Day**

23 November 2001

With reference to the “National week for the disclosure of Scientific Activities” promoted by the Ministry of Science and Technology during the period 19 – 25 November, the IGC had an open day on the 23<sup>rd</sup> November. The IGC was visited by:

St. Julian’s School, Carcavelos – 28 students

Escola Secundária Sebastião e Silva, Oeiras – 30 students

**Workshop on science and technology at the internet**

During the “National week for the disclosure of Scientific Activities” the IGC participated in this initiative by answering to some of the questions related to Health Sciences.

## **SCHOOL VISITS AT THE IGC**

### **Secondary Schools**

Within the IGC's objectives of promoting "scientific culture" and scientific research, regular visits of students from various schools are organised. In 2001, the following visited the IGC:

Colégio Sagrado Coração de Maria – Lisboa (40 students)  
Colégio Valsassina - Lisboa (76 students)  
Escola Secundária de S. João do Estoril – S. João Estoril (20 students)  
Escola Secundária Quinta do Marquês – Oeiras (51 students)  
Escola Secundária Sebastião da Gama - Setúbal (68 students)  
Escola Secundária Santa Maria – Sintra (79 students)  
Colégio Marista - Carcavelos (24 students)  
Escola Secundária Alves Martins – Viseu (60 students)

**(total: 418 students)**

### **Other Schools**

Instituto Superior de Estudos Interculturais e Transdisciplinares, Instituto Piaget, Almada (20 students)  
Life Science Lab of Heidelberg, Germany (20 students)  
Holiday camp of the Câmara Municipal de Almada (26 students)

**(total: 66 students)**



## THESES

The following Theses were prepared in part at the IGC and were presented in 2001:

### *PhD Theses:*

**Fernando Afonso** “Neuropéptidos nas glândulas anexas do aparelho genital masculino dos mamíferos”, Faculty of Veterinarian Medicine, Technical University of Lisbon, Lisbon, Portugal, February 2001.

**Vasco Barreto** “Allelic exclusion of the murine immunoglobulin heavy chain”, University of Paris VI, Paris, France, May 2001.

**Vera Alexandra Lucas Teixeira** “A Importância da dopamine e 5-hidroxitriptamina no transporte de água e electrólitos a nível do epitélio jejunal”, Faculty of Medicine, University of Porto, Porto, Portugal, June 2001.

**Deborah Braun** “Influence de l’Interferon-alpha/beta sur le developpement, la selection des repertoires et les responses lymphocytaires B” University of ParisVI, Paris, France, September 2001.

**Ana Teresa Tavares** “Embryonic Development of the Vertebrate Limb: Analysis of the Role of Rel/NF-Kb Transcription Factors and Co-factors”, Faculty of Medicine, University of Lisbon, Lisbon, Portugal, November 2001.

**Rui Marques da Silva** “Estudo das vias neurobiológicas de lesão cerebral durante a hiperbilirrubinémia neonatal. Comportamento e vulnerabilidade celulares.”, Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal, November 2001.

### *BsC Theses:*

**Inês Ribeiro Martins Alves** ,”Aspectos Celulares e Moleculares da Segmentacao dos Vertebrados”. Faculty of Sciences, University of Lisbon, Lisbon, Portugal, July 2001.

**Susana Rodrigues Pascoal**, “Determinação dos ligandos dos receptores Toll Like receptors KB7 e KB8 do ratinho”. Instituto Superior de Estudos Interculturais e Transdisciplinares de Almada, Lisbon, Portugal, July 2001.

**Sofia Martinho Simões**. Expressão e regulação do VEGF-D no desenvolvimento das extremidades em embriões de galinha. Faculty of Sciences, University of Lisbon, Lisbon, Portugal, July 2001.

**Vania Parelho** “Identificação e caracterização de mutantes mitóticos” Faculty of Sciences, University of Lisbon, Lisbon, Portugal, October 2001.

**Susana Silva**, “Estudo de Factores Genéticos e Autoimunes no Autismo”. Faculty of Sciences, University of Lisbon, Lisbon, Portugal, October 2001.

## **PARTICIPATION IN ACADEMIC COMMITTEES**

### **António Coutinho**

Member of the Jury of the Ph.D Thesis, Deborah Braun, University Paris VI, Paris, France.

### **Jocelyne Demengeot**

Member of the Jury of the Ph.D Thesis, Isabelle Coquillot, University Paris VI, Paris, France.

Member of the Jury of the Ph.D Thesis, Deborah Braun, University Paris VI, Paris, France.

### **José A. Feijó**

Member of the Jury of the Ph.D. Thesis, Ana M. Sánchez, Nijmegen University, Nijmegen, Holland.

### **Sérgio Gulbenkian**

Member of the Jury of the Ph.D Thesis, Fernando Afonso, Faculty of Veterinarian Medicine, Technical University of Lisbon, Lisbon, Portugal.

### **Isabel Palmeirim**

Member of the Jury of the Ph.D Thesis, Ana Teresa Tavares, Faculty of Medicine, University of Lisbon, Lisbon, Portugal.

### **Sylviane Pied**

Member of the Jury of the Ph.D Thesis, Ana Margarida Vigario, University of Paris V, Paris, France.

### **Álvaro Tavares**

Member of the Jury of the PhD. Thesis, Jane Blackburn, University of Dundee, Dundee, Scotland. June 2001.

Member of the Jury of the PhD. Thesis, Renata Basto, Faculty of Sciences, University of Lisbon, Lisbon, Portugal. September 2001.

Member of the Jury of the PhD Thesis, Elsa Logarinho, ICBAS, University of Porto, Porto, Portugal. December 2001.

### **Sólveig Thorsteinsdóttir**

Member of the Jury of the M.Sc. Thesis, Sofia Martinho Simões, Joana Cristóvão Oliveira Martins and Inês Ribeiro, Faculty of Sciences, University of Lisbon, Lisbon, Portugal.

## **PARTICIPATION OF IGC PERSONNEL IN CONFERENCES, SEMINARS AND SCIENTIFIC MEETINGS**

### ***January***

Coutinho A.

*From innate to adaptive immunity.*

Cours d'Immunologie Approfondie de l'Institut Pasteur, Institut Pasteur, Paris, France

Coutinho A.

*Dominant tolerance: implications for cancer therapy.*

Scientific Meeting CIBO/IPATIMUP and Faculdade de Medicina do Porto, IPATIMUP, Porto, Portugal

Coutinho A.

*A Investigação e a Clínica*

Hospital de Santa Maria, Lisbon, Portugal

Feijó J.A.

*Microscopia confocal e multifotónica: o estado da arte da imagiologia de células e moléculas.*

Msc. in Biochemistry, Faculty of Sciences, University of Lisbon, Lisbon, Portugal

### ***February***

Borges A.C., Marques S. and Belo J.A.

*Mechanisms of head induction in vertebrates: the role of BMP inhibition by secreted factors.*

Poster. Conference: Common molecules in development and Carcinogenesis, Fundacion Juan March, Madrid, Spain.

Carneiro, J.

*How regulatory T cells and their targets talk to each other.*

Department of Immunology, Institut Pasteur, Paris, France.

Coutinho A.

*Tolerância Natural*

Conference at the University of Vigo, Vigo, Spain

Feijó J.A.

*Reprodução sexual e crescimento do tubo polínico: paradigmas biológicos do desenvolvimento em plantas.*

Mestrado em Produtividade Vegetal. Instituto Superior de Agronomia, Lisbon, Portugal

Fernandes P.  
*Bioinformatics: converting data to knowledge*  
XXXVII Conferences in Genetics, Porto, Portugal

### **March**

Bagot S., S. Campino, C. Behrschmidt, O. Gorgette, D. Mazier, P.A. Cazenave and S. Pied

*Loci genetiques murins impliquees dans la resistance a la mort par neuropaludisme ou par anemie consecutive a l'infection par Plasmodium Berghei ANKA.*

3<sup>ème</sup> Reunion Biennale de Parasitologie. Lille, France

Collette A., S. Bagot, D. Mazier, P.-A. Cazenave, A. Six and S. Pied.

*Etude du repertoire TCR? lors du neuropaludisme murin.*

3<sup>ème</sup> Réunion Biennale de Parasitologie. Lille, France.

Coutinho A.

*All about Instituto Gulbenkian de Ciência.*

IBMC, Porto, Portugal

Coutinho A.

*Genoma, clonagem e outras questões da vida.*

Lesson to the 4th year of the “Comunicação Social” Course

Universidade Católica de Lisboa, Lisbon, Portugal

Fernandes P.

*Bioinformática, o que é e como se usa?*

Faculty of Sciences, University of Lisbon, Lisbon, Portugal

### **April**

Coutinho A.

*Dominant versus recessive tolerance in autoimmunity.*

Symposium “Autoimmunity and autoimmune pathological manifestations”, Journées de l'Ecole Doctorale, Faculté de Médecine de Rangueil, Toulouse, France

Coutinho A.

*Identidade Molecular e auto-imunidade.*

Conferences “O Estranho Terrível Outro”, Biblioteca Almeida Garrett, Porto 2001, Porto, Portugal

Demengeot J.

*Early death and severe lymphopenia caused by the ubiquitous expression of Rag1 and 2 genes in mice.*

Centre d'Immunology de Marseille Luminy, France

Demengeot J.

*Control of acute inflammatory responses by Regulatory CD4+CD25+ T cells.*

IBMC, Porto, Portugal.

Feijó J.A.

*Pólen e reprodução sexual: modelos de comunicação celular e de desenvolvimento em plantas.*

Mestrado em Biotecnologia, Instituto Superior Técnico, Lisbon, Portugal

Fernandes P.

*Bioinformática*

IV Jornadas de Engenharia Biológica/II Encontro Nacional de Jovens Biotecnólogos –

University of Minho, Braga, Portugal

Filipe M.R., Steinbeisser H. and Belo J.A.

*Study of the evolutionary conservation of specific regulatory genomic regions involved in the mechanisms of vertebrate head induction.*

Poster. Conference: Comparative Developmental Biology, Sant'Angelo di Schia, Italy.

Rodriguez-Leon, J., Diaz-Trelles, R., Carvalho, C.R., Francisco-Morcillo, J., Simões, S. And Izpisua Belmonte, J.C.

*Study of limb identity using microarray technology*

Poster. Workshop: Embryonic Organizer Signaling: The next frontiers. EMBL, Heidelberg, Germany

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

*Comparative study of the transcriptional regulation of several cerberus-like family genes.*

Poster. Conference: Comparative Developmental Biology, Sant'Angelo di Schia, Italy.

## **May**

Coutinho A.

*Infection and immunity at the ICBAS: 1978-2000.*

Chairman of the last session and closing of the Tribute to Mário Arala-Chaves, ICBAS, Porto, Portugal

Borges A.C., Marques S. and Belo J.A.

*Study of the genetic interactions with mouse cerberus-like.*

Poster. EMBO Workshop on Embryonic Organizer Signaling: The Next Frontiers. Heidelberg, Germany.

Freitas C., Rodrigues S., Charrier J-B., Teillet M-A. and Palmeirim I.

*Lateral PSM cells, isolated from medial cells, do not form somites.*

Poster. EMBO Workshop: Embryonic Organizer Signaling: The Next Frontiers, Heidelberg, Germany.

Marques I.

*Genetic characterisation and phylogenetic analysis of five portuguese strains of FIV*

Poster. First International Symposium on Research in Veterinary Science, Lisbon, Portugal

Tavares A.

*Cytokinesis in Drosophila.*

Cytokinesis Workshop, Institute of Molecular Pathology, Vienna, Austria.

Tavares A.

*A Drosophila no estudo do cancro.*

I Encontro de Biotecnologia, Univ. Lusofona, Lisbon, Portugal

## **June**

Becker J.D., Feijó J.A.

*Study of gene expression patterns in developing pollen by Arabidopsis genechips.*

Poster, FEBS 2001, Lisbon, Portugal

Fernandes P.

*Multimedia Teaching in Bioinformatics*

II Jornadas de Bioinformática, Malaga Spain

Haury M.

*4 Color Highspeed Cell Sorting*

Iberian Flowcytometry Society (SIC), Coimbra, Portugal

Parkhouse R.M.E.

*Evasion of host cell responses by African Swine Fever Virus.*

VII Venezuelan Congress of Genetics, Maracay, Venezuela

## **July**

Bajanca F. and Thorsteinsdóttir S.

*Integrin expression patterns during the development of mouse limb skeletal muscle.*

27<sup>th</sup> Meeting of the Federation of European Biochemical Societies (FEBS), Lisbon, Portugal

Borges A.C., Marques S. and Belo J. A.

*Study of genetic interactions with mouse cerberus-like.*

Poster. 27<sup>th</sup> Meeting of the Federation of European Biochemical Societies (FEBS), Lisbon, Portugal

Carneiro J.

*Similar T cell turnover in HIV2 and HIV1 infections in spite of distinct viral load and progression rate.*

International Congress of Immunology, Stockholm, Sweden.

Carneiro J.

*Self-tolerance: Lessons from Mathematical Modelling.*

International Conference on Mathematical and Theoretical Biology. Annual Meeting of the Society for Mathematical Biology Joint with Japanese Association for Mathematical Biology, Hilo, Big Island, Hawaii.

Coutinho A.

*Autoimmunity is not a normal consequence of adequate immune reactivity.*

Current Controversies. 11<sup>th</sup> International Congress of Immunology, Stockholm International Fairs Convention Centre, Stockholm, Sweden

Domingues C., Florindo C., Lima B., Wainman A., Glover D and Tavares A.

*Cloning and characterization of new centrosomal proteins in higher eukaryotes.*

27<sup>th</sup> Meeting of the Federation of European Biochemical Societies (FEBS), Lisbon, Portugal

Dionisio F., Matic I., Radman M., Taddei F.

*How plasmids take advantage from bacterial diversity: a case of phage-driven polymorphism? A case of parasites taking advantage from host diversity.*

Gordon Research Conference “Microbial Population Biology”, Williamstown, USA

Faria M., Parelho V., Gomes R., Deak P., Glover D. And Tavares A.

*Characterization of a mitotic mutant affecting cytokinesis.*

27<sup>th</sup> Meeting of the Federation of European Biochemical Societies (FEBS), Lisbon, Portugal

Fernandes P., Judge D. and Cunningham P.

*Analysis of biological sequences: introduction to bionformatics*

Life and Health Sciences International Postgraduate Courses, University of Minho, Braga, Portugal

Filipe M.R., Tavares A.T., Steinbeisser H. and Belo J.A.

*Study of the evolutionary conservation of the regulatory genomic regions in the Cerberus-like family.*

Poster. 14<sup>th</sup> International Congress of Developmental Biology, Kyoto, Japan.



Filipe M.R., Marques S., Belo J.A.

*Isolation of novel head-inducing genes expressed in the Anterior Visceral Endoderm, by selective EGFP-labeled cell sorting.*

Poster. 14<sup>th</sup> International Congress of Developmental Biology, Kyoto, Japan.

Freitas C., Rodrigues S., Charrier J-B., Teillet M-A. and Palmeirim I.

*Cycling genes at somitic prospective territory define two regions differently committed to somite segmentation.*

Poster. Segmentation Meeting in Nara, Nara, Japan.

Freitas C., Rodrigues S., Charrier J-B., Teillet M-A. and Palmeirim I.

*Cycling genes at somitic prospective territory define two regions differently committed to somite segmentation.*

Poster. 14<sup>th</sup> International Congress of Developmental Biology, Kyoto, Japan.

Parkhouse R.M.E.

*Biology of the WC1 receptor and its possible role in Bluetongue virus persistence.*

VI International Veterinary Symposium, Uppsala, Sweden

Rodrigues S., Freitas C., Charrier J-B., Teillet M-A. and Palmeirim I.

*Cycling genes define two regions differently committed to form somites*

Poster and oral communication. 27<sup>th</sup> Meeting of the Federation of European Biochemical Societies (FEBS), Lisbon, Portugal.

Tavares A.

*Mobs: a growing family.*

Chromosome Segregation and Aneuploidy, Chartres, France.

Zuzarte V., Liaubet L., Pituello F. and Palmeirim I.

*Colloid-like1 gene function during somite differentiation*

Poster and oral communication. 27<sup>th</sup> Meeting of the Federation of European Biochemical Societies (FEBS), Lisbon, Portugal.

## **August**

Seixas C., Nolasco S., Casalou C. and Soares H.

*Isolation and characterization of a Tetrahymena tubulin complex: functional relationships with microtubule assembly and dynamics under hyperthermic stress.*

16<sup>th</sup> Annual Meeting of the European Cytoskeleton Forum, The Netherlands.

Casalou C., Nolasco S., Melo L.V., Brogueira P. and Soares H.

*Chaperonin CCT subunits are present in cilia basal bodies and oral apparatus of the ciliate Tetrahymena: implications in cilia biogenesis.*

16<sup>th</sup> Annual Meeting of the European Cytoskeleton Forum, The Netherlands.

## ***September***

Bajanca F. and Thorsteinsdóttir S.

*Integrin expression patterns during early skeletal muscle development in the mouse.*

European Research Conferences: Fifth Research Conference on Molecular Biology of Cellular Interactions: Cell Adhesion Molecules and Receptor Cross Talk, Giens, France.

Bandarrinha, R., Feijó J.A.

*Proton flux patterns correlate with callose plug formation in Nicotiana tabacum pollen.*

Poster, Microscopy, Barcelona , Spain

Belo J.A.

*Mechanisms of head induction in vertebrates: biochemical and genomic approaches.*

III Congreso de la Sociedad Española de Biología del Desarrollo (SEBD), Málaga, Spain.

Borges A.C., Marques S. and Belo J.A.

*Study of the genetic interactions with mouse cerebrus-like.*

Poster. III Congreso de la Sociedad Española de Biología del Desarrollo (SEBD), Málaga, Spain.

Borges A.C., Marques S. and Belo J.A.

*Subtractive screen for the isolation of novel AVE genes.*

Poster. III Congreso de la Sociedad Española de Biología del Desarrollo (SEBD), Málaga, Spain.

Cachaço A.S., Chuva de Sousa Lopes S.M., Kreft M., Bajanca F., Mummery C.L., Sonnenberg A. and Thorsteinsdóttir S.

*Integrin  $\beta 1D$  supports embryonic cell migration in vivo and some  $\beta 1D$  knock-in embryos reach birth on a predominantly FVB background.*

European Research Conferences: Fifth Research Conference on Molecular Biology of Cellular Interactions: Cell Adhesion Molecules and Receptor Cross Talk, Giens, France.

Carneiro J.

*Nature and mechanism of action of CD4+CD25+ regulatory T cells. Lessons from mathematical modeling.*

Hands on meeting in Modeling Immune systems, Amsterdam, The Netherlands.

Cordeiro M.S. and Feijó J.A.

*Ion fluxes and their relative contribution to the total current generated during early germination of pollen tubes of Lilium longiflorum.*

Microscopy, Barcelona, Spain

Costa S, Chen C., Cheung A. Y., Feijó J.A.

*Actin cytoskeleton plays a crucial role in the regulation of extracellular proton fluxes in pollen tubes.*

Microscopy, Barcelona, Spain

Díaz-Trelles, R., Rodríguez-León, J., Rocha-Carvalho, C., Simões, S., and Izpisua-Belmonte, J.C.

*Chicken vascular endothelial growth factor D revealed in limb development using microarray technology.*

III Congress of the Spanish Society for Developmental Biology. Málaga. Spain.

Feijó J.A.

*Ion dynamics influences growth and morphogenesis of plant cells.*

International Conference on ion channels “Canaux ioniques”, Lalond sur maure, France

Fernandes P.

*EMBER, a Multimedia Bioinformatics Education Resource*

Concertation Meeting, EC IST Program, - Luxembourg, Luxembourg

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

*Genomic approaches to the study of the mechanisms of vertebrate head induction.*

Poster. III Congreso de la Sociedad Española de Biología del Desarrollo (SEBD), Málaga, Spain.

Freitas C., Rodrigues S. And Palmeirim I.

*Medial and Lateral PSM cells are differently committed to somite segmentation.*

Poster. III Congress of the Spanish Society of Developmental Biology, Málaga. Spain.

Prado, A. M. M; Porterfield D. M. and Feijó J.A.

*When pollen tubes say no to NO.*

Microscopy, Barcelona, Spain

Rodrigues S., Freitas C., Charrier J-B., Teillet M-A. and Palmeirim I.

*The molecular clock at the chicken organizer.*

Poster. III Congress of the Spanish Society of Developmental Biology, Málaga., Spain.

Rodríguez-León, J., Díaz-Trelles, R., Rocha-Carvalho, C., Simões, S., and Izpisua-Belmonte, J.C.

*Expression and regulation of the chick tolloid metalloprotease, colloid, during limb bud development.*

III Congress of the Spanish Society for Developmental Biology, Málaga. Spain.

Vieira A. M. and Feijó J. A.

*Water entrance into the pollen grain during early hydration in Eucalyptus globulus: do hydrogels play a role?*

Microscopy, Barcelona, Spain

## **October**

Barreto M., Vicente A., Vassilevskaia T., Duarte N., Gardete-Correia L., Boavida J., Duarte R., Raposo F., Mendes J., Penha-Gonçalves C. and Holmberg D.

*Análise genética da região dos genes CTLA-4/CD28 na Diabetes de tipo 1.*

V Annual Meeting of the Portuguese Society for Human Genetics, Aveiro, Portugal.

Barreto M., Vicente A., Vassilevskaia T., Duarte N., Gardete-Correia L., Boavida J., Duarte R., Raposo F., Mendes J., Penha-Gonçalves C. and Holmberg D.

*Analysis of the CTLA-4/CD28 Gene Region in Type I Diabetes.*

Annual Meeting of the American Society of Human Genetics, San Diego, USA.

Belo J.A.

*Mechanisms of Vertebrate Head Induction.*

IBMC, University of Porto, Porto, Portugal.

Belo J.A.

*Culturas celulares e manipulação genética dirigida.*

IST, University of Lisbon, Lisbon, Portugal.

Coutinho A.

*Conceitos e abordagens actuais na compreensão e tratamento das doenças autoimunes.*

Scientific Conference of the Portuguese Society for Rheumatology Meeting, Caramulo Hotel, Caramulo, Portugal

Coutinho A.

*A imunologia do “self”: da patologia à fisiologia da autoimunidade.*

Autumn days. Opening of the academic year. Scientific Conference of the Portuguese Society for Rheumatology Meeting, Caramulo Hotel, Caramulo, Portugal

Coutinho A.

*Les chercheurs scientifiques: professionnels du doute.*

Colloquium: Itinerários da Dúvida, 18e rencontres image et science, CNRS, Museu de Física, Universidade de Coimbra, Coimbra

Coutinho A.M., Morgadinho T., Marques C., Ataíde A., São Miguel T., Bento C., Mota-Vieira L., Macedo T., Oliveira G., and Vicente A.

*O sistema serotoninérgico e o autismo: variação genética de receptores e do transportador da serotonina e correlação com níveis de serotonina plaquetária.*

V Annual Meeting of the Portuguese Society for Human Genetics, Aveiro, Portugal.

Silva S., Fesel C., Marques C., Ataíde A., São Miguel T., Bento C., Oliveira G. and Vicente A.

*Autismo e reacções autoimunes: screening dos perfis de autoanticorpos contra proteínas de cérebro humano por “Panama Immunoblot”.*

V Annual Meeting of the Portuguese Society for Human Genetics, Aveiro, Portugal.

Silva S., Marques C., Ataíde A., São Miguel T., Bento C., Oliveira G. and Vicente A.

*Autismo : estudos de associação com o gene Brain Derived Neurotrophic Factor.* V V V

V Annual Meeting of the Portuguese Society for Human Genetics, Aveiro, Portugal.

## **November**

Barreto M., Fesel C., Fontes F., Tam A., Reis I., Andreia R., Faro Viana J., Crespo F., Vasconcelos C., Mota-Vieira L., Ferreira C., Coutinho A. and Vicente A.

*The Genetics of Autoantibody Repertoires in Systemic Lupus Erythematosus (SLE).*

EURESCO Conferences – Inherited Disorders and Their Genes in Different European Populations. Barcelona, Spain.

Caramalho I.

*Growth of regulatory CD25<sup>+</sup> T cells depends on naïve CD25<sup>-</sup> T cells and is enhanced by inflammatory signals.*

27<sup>th</sup> Annual Meeting of the Portuguese Society of Immunology, FMV/UTL, Lisbon, Portugal

Caramalho I.

*Towards the therapeutic use of regulatory T cells: in vitro production of suppressor CD4 cells.*

27<sup>th</sup> Annual Meeting of the Portuguese Society of Immunology, FMV/UTL, Lisbon, Portugal

Caramalho I., Castro A.G., Carneiro J., and Demengeot J.

*Towards the therapeutic use of regulatory T cells: in vitro production of suppressor CD4 cells.*

27<sup>th</sup> Annual Meeting of the Portuguese Society of Immunology, FMV/UTL, Lisbon, Portugal

Caramalho I., Carvalho T., Oliveira V., Carneiro J., and Demengeot J.

*A quantitative analysis of T lymphocyte proliferation.*

27<sup>th</sup> Annual Meeting of the Portuguese Society of Immunology, FMV/UTL, Lisbon, Portugal

Carvalho T.

*Arrested B Lymphopoiesis and Persistence of Activated B Cells in Adult IL 7 <sup>-/-</sup> Mice*

The 27<sup>th</sup> Annual New England Immunology Conference, Woods Hole, Mass., USA.

Carvalho T.

*CD4+CD25+ regulatory T-cells and the control of inflammatory responses.*

27<sup>th</sup> Annual Meeting of the Portuguese Society of Immunology, FMV/UTL, Lisbon, Portugal

Coutinho A.

*The contribution of Immunology to vaccine development.*

Chairman of the Round Table. XXVII Annual Meeting Sociedade Portuguesa de Imunologia. "Infection and Immunity: Perspectives for Vaccination", Faculdade de Medicina Veterinária, UTL, Lisbon, Portugal

Coutinho A.

*Ciências e Tecnologias da Vida.*

Round Table at the International Congress "Ciência e Tecnologia de Portugal e Espanha na Viragem do Milénio", CSIC, Room: Roca solano, Madrid, Spain

Coutinho A.

*Olhares Cruzados: perspectivas para o século XXI: Física/Biologia.*

Debate with António Coutinho and Jorge Dias de Deus.

Fundação Calouste Gulbenkian, Lisbon, Portugal

Coutinho A.

*Estratégias vacinais anti-parasitárias: dificuldades, fracassos e perspectivas.*

Round Table at the Hôpital Pitié-Salpêtrière, Paris, France

Coutinho A.

*O papel do estado: a cooperação intersectorial e internacional*

Seminar: VIH/SIDA e os Direitos Humanos, promoted by the President of Portuguese Republic, Parque das Nações, Pavilhão do Futuro, Lisbon, Portugal

Coutinho A.

*From the Lab bench to the bedside; progress in immunology and the management of allergy and autoimmune disease.*

Conference within the context of the Cerimónia Solene da Assembleia Geral da Federação das Academias de Medicina da União Europeia, Aula Magna da Faculdade de Medicina de Lisboa, Lisbon, Portugal

Demengeot J.

*Regulatory T cells control immune responses targeted at both self and non-self antigen.*

27<sup>th</sup> Annual Meeting of the Portuguese Society of Immunology, FMV/UTL, Lisbon, Portugal

Feijó JA

*Advanced imaging methods: applications to plant cell development.*

Leica Course on Confocal and Digital Microscopy, Condeixa, Portugal

Feijó JA

*Ion dynamics influences growth and morphogenesis of plant cells.*

International Congress on Systems Biology, Caltech, Los Angeles, USA

Leon K. and Carneiro J.

*A quantitative analysis of T lymphocyte proliferation.*

27<sup>th</sup> Annual Meeting of the Portuguese Society of Immunology, FMV/UTL, Lisbon, Portugal

Pied S., Roland J., Louise A., Idrissa Boubou M., Voegtle D., Soulard V., Mazier D. and P.-A. Cazenave.

*Liver CD4-CD8-NK1.1+ TCRab cells increase during experimental malaria and are able to exhibit inhibitory activity against the pre-erythrocytic stages.*

49<sup>th</sup> Annual Meeting of the American Society of Tropical Medicine and Hygiene, Houston, Texas, USA.

Rebelo M.

*Role of development genes in the regulation of lymphocyte homeostasis.*

27<sup>th</sup> Annual Meeting of the Portuguese Society of Immunology, FMV/UTL, Lisbon, Portugal

Sousa J. and Carneiro J.

*Tuneable activation thresholds and homeostasis of lymphocytes*

27<sup>th</sup> Annual Meeting of the Portuguese Society of Immunology, FMV/UTL, Lisbon, Portugal

## ***December***

Dionisio F., Matic I., Radman M., Rodrigues O.R., Taddei F.

*High speed of plasmid spread among heterogeneous bacterial populations.*

Microbiology Congress (Micro2001), Póvoa de Varzim, Portugal

Duarte A., Marques M. I., Taveira L. and Fevereiro M.

*Phylogenetic analysis of five Portuguese isolates of Feline Immunodeficiency Virus (FIV)*

Poster. Microbiology Congress (Micro2001), Póvoa de Varzim, Portugal

## OTHER ACTIVITIES OF THE IGC PERSONNEL

### *February*

Parkhouse M.

Collaborative visit (Cysticercosis project) to Universidad San Marcos and Universidad Peruana Cayetano Heredia, Peru.

### *March*

Fernandes P., Marques I., Brandão A.

Startup Meeting of the EMBER (EC funded project), Oeiras, Portugal

Fernandes P.

Coordination meeting on Teaching Bioinformatics. Madrid, Spain

### *April*

Coutinho A.

Visit with Prof. Eduardo Marçal Grilo, Trustee of the FCG, and Prof. João Caração, Director of the Science Service of the FCG, to the Wellcome Trust, London, UK.

Coutinho A.

Phase Display Technologies

Cambridge Healthtech Institute Conference

University Park Hotel at MIT, USA

### *May*

Marques I.

SRS Workshop – Sequence Retrieval System Server Administration , Cambridge, UK

### *June*

Coutinho A.

Mateus Meeting on the Human Genome, Casa de Mateus, Vila Real, Portugal



## ***July***

Coutinho A.

Editorial Board meeting “Current Opinion Immunology”, Stockholm, Sweden

## ***August***

Assessor to the Ramon y Cajal Programme of the Spanish Ministry of Science and Technology, Madrid, Spain.

## ***September***

Coutinho A.

Estudos Gerais da Arrabida, 10º Encontro Nacional de Prospectiva. O desenvolvimento da investigação biomédica em Portugal e a criação do Instituto Nacional de Investigação Biomédica, Convento da Arrabida, Arrabida, Portugal

Coutinho A.

EMBO Council Meeting , Heidelberg, Germany

Fernandes P. and Marques I.

Annual General Meeting of the EMBnet , EMBER, meeting Public Relations and Publications of the EMBnet, Vienna Biozentrum, Vienna, Austria

Haury M.

Cytomation User Meeting, Freiburg, Germany

Parkhouse M.

Collaborative visit (Cysticercosis project) to Universidad Autonoma Gabriel Rene Moreno, Bolívia.

## ***November***

Fernandes P.

Meeting: Public Relations and Publications of the EMBnet, Uppsala, Sweden

Haury M.

Member of the EU evaluation committee, EU 5<sup>th</sup> Framework Program Key action “Control of Infectious Diseases”, Brussels, Belgium.

Parkhouse M.

Member of the Organization Committee 27<sup>th</sup> Annual Meeting of the Portuguese Society of Immunology, FMV/UTL, Lisbon, Portugal.

***December***

Coutinho A.

Workshop on “Molecular Basis of Human Congenital Lymphocyte disorders”, Instituto Juan March de Estudios e Investigaciones, Madrid, Spain

Coutinho A.

EMBO World Programme Meeting, Heidelberg, Germany

Coutinho A.

Scientific Advisory Board Meeting, Igeneon, Vienna, Austria

Parkhouse M.

Collaborative visit (Cysticercosis project) to Universidad Autonoma Mexico, in December.