

# **INSTITUTO GULBENKIAN DE CIÊNCIA ANNUAL REPORT**

## **2000**

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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 2000 were:

Doutor Victor de Sá Machado (Chairman)

Dr. José Blanco

Dr. Pedro Tamen (retired September 2000)

Dr. Mikhael Essayan

Dr. Emílio Rui Vilar

Prof. Doutor Diogo de Lucena

Dra. Isabel Mota

Prof. Doutor Eduardo Marçal Grilo (from October 2000)

## **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 2000 were:

Dr. Emílio Rui Vilar (Chairman till September 2000)

Prof. Doutor Eduardo Marçal Grilo (Chairman from October 2000)

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 21 February 2000.

## **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 2000 were:

Prof. Sydney Brenner (Chairman)  
Prof. Nicole Le Douarin  
Prof. Martin Raff  
Prof. Susumu Tonegawa  
Prof. Hans Wigzell  
Prof. Lewis Wolpert  
Prof. Kai Simons  
Prof. Philippe Kourilisky (from 2001)

The Scientific Advisory Board met at the IGC on 28-29 February 2000.

## **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.

Jord 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 (FCT)

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

Sukalyan Chatterjee (FCG/FCT)

Mário Arala Chaves (ICBAS)

Melvin Cohn (Salk Inst./FCT)

Suzanne Bourgeois Cohn (Salk Inst.)

António Coutinho (CNRS/FCG)

Nandita Das (UCSC)

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/FCG)

Carlos Penha Gonçalves (Minist.Defesa/FCG)

Sérgio Gulbenkian (FCG)

Matthias Haury (FCG)

Dan Holmberg (Umea Univ./FCT)

Shohei Hori (Tokyo Univ./FCG)

Rodney Langman (Salk Inst./FCT)

Javier Morcillo (FCG)

Guilherme Horácio Neves (FCMUNL/FCG)

Isabel Palmeirim (FCG)  
Michael Parkhouse (FCT)  
Sylviane Pied (INSERM/FCT)  
Joaquin Rodriguez (FCG/FCT)  
Helena Soares (Esc. Sup. Tec. Saúde Lisboa/FCT)  
João Pedro Simas (ICBAS/FCT)  
John Stewart (Univ. Tech. Compiègne/CNRS/FCT)  
Ana Teresa Tavares (FCG)  
Alvaro Augusto Tavares (IST)  
Alexandra Teixeira (FCT)  
Vera Lucas Teixeira (FCG)  
Solveig Thorsteinsdottir (FCUL)  
Ramon Trelles (FCG/FCT)  
Tatiana Vassilevskaia (Associação de Protecção do Diabético de Portugal)  
Nelson Vaz (Univ. Fed. Minas Gerais)  
Astrid Moura Vicente (Instituto Piaget/FCG)  
Luisa Mota Vieira (Hospital do Dívino Espírito Santo, Ponta Delgada)  
Paulo Vieira (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 (FCG/FCT)  
Fernanda Maria Bajanca (FCUL/FCT)  
Vasco Barreto (Univ. Paris VI/PGDBM)  
Catja Behrschmidt (Univ. Cologne)  
Marie-Louise Bergman (Univ. Umea)  
Leonor Boavida (FCUL/FCT)  
Ana Cristina Borges (FCUL/IEFP)  
Déborah Braun (Univ. Paris VI/École Normale Supérieure)  
Alexandre Michelato Bubel (Univ. São Paulo)  
Ana Sofia Cachão (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/IEFP)  
Célia Domingues (FCUL/FCT)  
Mário Rui Filipe (FCUL/FCT)  
Cláudia Florindo (UNL/FCT)  
Catarina Freitas (FCUL/FCT)

Sandra Penélope Freitas (FERNUA/FCT)  
Ana Cristina Gaspar (FCUL/FCT)  
Susana Godinho (FCUL/FCT)  
Patrícia Madureira (ICBAS/FCT)  
Sofia Pinto Guia Marques (ICBAS/FCT)  
Joana Monteiro (FCUL/FCT)  
Kalet León Monzón (Univ. Havana/FCG)  
Sofia Nolasco (FCUL/FCT)  
Leonor Orge (LNIV)  
João Pedro Pereira (ICBAS/FCT)  
M<sup>a</sup> Gabriela Rodrigues (FCUL)  
Mark Jan Seldom (IGC)  
Valerie Soulard (Inst. Pasteur Paris, Hosp. Pitié-Salpêtrière)  
João Sousa (FCUL/Inst. Rocha Cabral/FCT)

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

Nuno Duarte Afonso (FCUL)  
Ana Paula Alexandre (FCUL/IEFP)  
Inês Martins Alves (FCUL)  
Ricardo Bandarrinha (FCUL/IEFP)  
Lara Costa (FCUL)  
Sílvia Pereira Costa (FCUL)  
Pedro Geraldès (FCUL)  
Nuno Geraldo (Univ. Évora)  
Vanessa Zuzarte Luís (FCUL)  
Anders Morberg (Umea Univ)  
Vânia Parelho (FCUL)  
Susana Pascoal (Instituto Piaget)  
Ana Margarida Prado (FCUL/IEFP)  
João Pedro Preto (FCUL)  
Ana Catarina Silva (FCUL)  
Susana Silva (FCUL)  
Sofia Simões (FCUL)  
Rui Soares (FCUL)  
Ana Maria Vieira (FCUL)  
Cláudia Vieira (FCTUNL)  
Joana Vital (FCUL)

### **Technical Support Students**

Nádia Silva Duarte (BTI/FCT)  
Mariana Faria (FCUL/IEFP)  
Lídia Fonseca (BIC/FCT)  
Brigitte de Lima (FCUL/IEFP)  
Sara Lopes Marques (IEFP)  
Vanessa Oliveira (BIC/FCT)  
Sofia de Albuquerque Rodrigues (IEFP)  
Ana Cecília Seixas (BIC/FCT)



## **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 2000.

Anne-Frederique Antoine (Education Nationale, ENS Lyon, Lyon, France)  
Jean-Pierre Cabaniols (Institut Pasteur, Paris, France)  
Sussana Cardell (University of Lund, Lund, Sweden)  
Alexis Collette (Institut Pasteur, Paris, France)  
Ana Mafalda Cumano (Institut Pasteur, Paris, France)  
Nandita Das (Institute di Ginecologia, Universita Cattolica Del Sacro Cuore, Rome, Italy)  
Alf Grandien (Stockholm Univ., Sweden)  
Zvi Grossman (Tel Aviv Univ., Israel)  
Werner Haas (Linden Techonologies, Weburn, USA)  
Veronique Havelange (Univ. Tech. Compiègne, France)  
Gerard Hoyne (Rayne Lab., University of Edinburgh Medical School, Edinburgh, UK)  
François Huetz (Institut Pasteur, Paris, France)  
Moises Mallo (Max-Planck Institute of Immunobiology, Freiburg, Germany)  
Maria Maroné (Tecnofarmaci S.C.p.A., Pomezia, Rome and Institute di Ginecologia, Universita Cattolica Del Sacro Cuore, Rome, Italy)  
Hércules Menezes (Instituto de Biociências, UNESP, São Paulo, Brazil)  
Petros Mirilas (Lab. Anatomy & Embryology, Fac. Medicine, Univ. Crete, Crete, Greece)  
Josselyne Salaun (Inst. d'Embryologie Cellul. et Moleculaire, Nogent-sur-Marne, France)  
Herbert Steinbeisser (Max-Planck Inst. fuer Entwicklungsbiologie, Tuebingen, Germany)  
Anne Sundblad (Karolinska Institutet, Sweden)  
Ari Waisman (Institute for Genetics, University of Cologne, Cologne, Germany)

## 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 Católica 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 2000.

### **Administrative and Secretarial Staff**

Ana Paiva Brandão  
Manuel Carvalho  
Manuela Cordeiro  
Jorge Costa  
Alcino Gonçalves (left April 2000)  
Fátima Mateus  
Maria Matoso  
Greta Martins  
João Nunes  
Ana Lícia Pires  
Manuela Ramalho (left July 2000)  
Ana Maria Santos  
M<sup>a</sup> Eduarda Santos (left December 2000)  
Abílio Simões  
Teresa M<sup>a</sup> Sousa  
Lurdes Torres  
Maria Vasconcelos

### **Laboratory Technical Staff**

M<sup>a</sup> Ressurreição Alpiarça  
Magda Carlos  
Dolores Constantino  
Ana C. Homem  
Bruce Lenhart  
Júlia Lobato  
Isabel Marques  
Rute Marques  
Nuno Moreno  
Rosa M<sup>a</sup> Santos  
Sónia Torres (left December 2000)

### **Technical Support Staff**

José A. Correia (left September 2000)  
António Gomes  
António C. Ligeiro  
João Carlos Lopes  
Paulo J. L. Martinho  
Carlos Nunes  
João António B. Pires  
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 (by interim)

### **Animal Facility**

Bruce Lenhart

### **DNA Sequencing and Genotyping**

Júlia Lobato

### **Bioinformatics**

Pedro Fernandes

### **Library and Scientific Information**

Pedro Fernandes

### **Antibody**

Jocelyne Demengeot

## CELL IMAGING UNIT

The Cell Imaging Unit provides cell and tissue imaging facilities, as well as flowcytometry and histology services. The following services were available: Flowcytometry (4 color analysis and highspeed cell sorting); Conventional transmitted and fluorescence light microscopy (epifluorescence, Normarsky, phase-contrast and dark field microscopy); Confocal 4 color multi-photon microscopy; Transmission electron microscopy; Image analysis and acquisition; Phospho-imager and densitometry; Cryostat, parafin, resin and vibratome sectioning for histology and immunohistochemistry; Ultrathin resin (epoxy and acrylic) and cryo-sectioning for electron microscopy

In the year 2000 the high-speed cell-sorter as well as the multiphoton confocal and the transmission electron microscope were fully functional and two engineers were trained to ensure the day to day operation.

The cell sorter is now being upgraded and new fluorochromes (5-7 colors) will soon be introduced. During the year 2000 the histology service processed more than 2400 microscope slides.

The antibody production service was launched together with the Antibody Unit and several monoclonal antibodies have already been produced in high quantities using a new in vitro system. Custom labeling of monoclonal antibodies has been started (FITC, Biotin, PE, APC), and additional color combinations tested for future production.

## **GENETIC MANIPULATION OF MICE AND RATS / ANIMAL FACILITY**

Due to the major infrastructure reform of the Animal House during the year 2000, only a limited amount of work could be performed. Nevertheless, a total of 25 microinjections and 31 rederivations were carried out.

Paulo Vieira resigned from his duties as head of the Genetic Manipulation Unit and Bruce Lenhart accumulated this responsibility, by interim, to that of the Animal Facility.

## **GENOTYPING AND SEQUENCING UNIT**

The Genotyping and Sequencing Unit worked at full capacity of its 3 sequencing machines and has sequenced, in the year 2000, a total of 4222 samples for in-home 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 the sequencing machines was also used for genotyping in a “self-service” mode by IGC users.

## **BIOINFORMATICS UNIT**

The improvements in connectivity of the IGC and the expansion of online storage space have allowed to reestablish the regular update of the major Molecular Biology databases, daily when applicable. More programmes were installed, especially in the Human Genetics area. The Bioinformatics service is delivered to a total of 450 users.

During the year 2000 the Gulbenkian Training Programme in Bioinformatics provided six courses, two of which were co-financed by the EMBnet, at which 120 attendees have received more than 170 hours of hands-on tuition on Bioinformatics.

## **CIDC – IGC Library**

The number of registered readers increased to 1120 and there were 7600 visits throughout the year. Of the current 139 titles in the Library, 67 are complemented with online access within the IGC premises. Reorganization of the storage space was started, aiming at a sensible use of old titles and optimization of reading areas.

## **ANTIBODY UNIT**

This Unit started operating in the year 2000. Large scale production of monoclonal antibodies for in vivo and in vitro experiments has been achieved successfully together with the Cell Imaging Unit.

The “immunoscope” technique, a tool to evaluate the genetic repertoires of B and T cell antigen receptors, has been introduced successfully in the IGC. Collections of primers are now available. The “panama blot” technique used for the global evaluation of reactive immunoglobulin repertoires, has also been introduced and is currently being adapted to the analyses of reactivities directed towards nuclear proteins.

The first fusion was conducted to produce specific monoclonal antibodies directed towards microbial antigens.

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

### **European Mouse Mutant Archive (EMMA)**

The Unit for Genetic Manipulation of Mice and Rats joined the European Mouse Mutant Archive (EMMA) in 1997, as one of its 5 “nodes”: CNR, Montorotondo, Italy; CNRS, Orleans, France; MRC, Harwell, UK; IGC, Oeiras, Portugal; Karolinska Institute, Huddinge, Sweden. The specific mission of the IGC Unit within EMMA is the transfer to germ-free conditions of targeted mouse lines with immunological defects. These lines are then made available, upon request from interested scientists in Europe. In order to facilitate the analysis of such animals, notably avoiding costly special transportation at the risk of losing the “germ-free” condition, the IGC decided to open its laboratories and facilities to external scientists engaged in these studies.

Intense discussions were conducted in 2000 with all EMMA members and representatives of the EU Commission XII, in order to define the future financial basis of EMMA and to appoint its Technical Director, Dr. Martin Hrabé de Angelis.

### **Laboratoire Européen Associé CNRS “Génétique et développement de la tolérance naturelle “**

The LEA CNRS (Centre National de Recherche Scientifique) was created at the IGC on September 21, 1999, by an agreement between several French (Institut Pasteur and Université Pierre et Marie Curie) and Portuguese (Fundação para a Ciência e Tecnologia, Instituto para a Cooperação Científica e Tecnológica Internacional, and Fundação Calouste Gulbenkian) organisations, aiming at synergising human competences and resources in research on the genetic and developmental aspects of immunological tolerance. The LEA was created for a 4-year period and António Coutinho was appointed Director.

The activities of the LEA entered a normal rhythm in 2000, notably through the exchange of a dozen scientists and students in both directions, for periods of one to several weeks.

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

### **Unidade de Investigação da Fundação para a Ciência e a Tecnologia “Tolerância natural”**

This Unit created by the end of 1999 began operation in 2000. The research programme of the Unit, which involves some 15 scientists at the IGC and several collaborations in other institutions, concerns immunology, virology, and genetics of autoimmune diseases in mouse and man. The Unit launched collaboration programmes with several Portuguese institutions (Associação de Protecção do Diabético de Portugal, Associação dos Doentes de Lupus, Hospital Pediátrico de Coimbra and Núcleo de Investigação at the Hospital do Divino Espírito Santo, Ponta Delgada, Azores).

### **Laboratório Associado do Ministério da Ciência e da Tecnologia entre o ITQB, o IGC/FCG e o IBET.**

One of the first four “Laboratórios Associados” created by the Ministry of Science and Technology was launched at the Oeiras campus, through a cooperative agreement between the IGC and two other institutions: the ITQB (Instituto de Tecnologia Química e Biológica) of the Universidade Nova de Lisboa and the IBET (Instituto de Biologia Experimental e Tecnológica).

The founding contract, celebrated with the Fundação para a Ciência e a Tecnologia for a renewable 5 years period, defines the missions and areas of competence of the laboratory (biologically active molecules; molecular medicine and veterinary medicine; developmental biology in animals and plants; biological risk; plant and forest improvement), and provides for a total of 34 research positions along the next 5 years.

The various agreements involved were signed in Oeiras, on November 24, 2000, in the presence of the Minister of Science and Technology, the Rector of the Universidade Nova de Lisboa, the President of the Fundação para a Ciência e a Tecnologia, the member of the board of Fundação Calouste Gulbenkian in charge of Science, and the Directors of the three participating institutions.



## **INSTITUTIONAL AGREEMENTS**

### **Faculdade de Medicina da Universidade de Lisboa and FCG/IGC**

Since 1999, a cooperation agreement exists between the Faculdade de Medicina da Universidade de Lisboa and the IGC. This protocol, aiming at improving the utilisation of resources at each of the institutions in various scientific and pedagogic projects was enriched in 2000 by the appointment of the group directed by Prof. Leonor Parreira, at the CEBIP, Instituto de Histologia e Embriologia, Faculdade de Medicina de Lisboa, as an “external group at the IGC.

### **Protocol between the IGC and the Centro Regional de Sangue de Lisboa (CRSL)**

A protocol between IGC and the Centro Regional de Sangue de Lisboa (CRSL) was established with the aim of obtaining blood samples from healthy individuals to be used as controls in genetics research projects being developed at the IGC.

Blood samples are collected at the CRSL and all participants sign an informed consent, which is kept at the CRSL. The identity of participants is unknown to the research team. The studies performed are not used for diagnosis and therefore there are no results given back to the participants.

## INTRODUCTION

The Instituto Gulbenkian de Ciência is fully dedicated to research and education in biomedical science; it is all the more natural, therefore, that we aim at following biological principles in our development and growth as a living organism.

First of all, the principles of diversity and cooperativity. As coined by Dobzhansky, “nothing makes sense in biology except in the frame of evolution”. The basic mechanisms of evolution, everyone knows, are variation & selection that allow for adaptation, together with the emergence of novel cooperative processes that generate higher levels of organization, which some designate as “order” or “complexity”. Organisms are the result of cooperativity amongst many genes, however “selfish” these might be; the biosphere emerges through cooperation amongst many different organisms and species. All gains in complexity, therefore, require increased levels of cooperativity, balancing the preferential survival/reproduction of variants that reads out as competitive advantage. If only competition counted, life would probably be nothing else than replicating ribozymes. Cooperativity requires diversity amongst cooperating components. Variation is, therefore, the very essence of evolution, as it provides for the only source of novelty. In the biological world, variation concerns the structure of genes and of the sequences that regulate their expression. Genes are the stuff that specifies the structures and the mechanisms making up for evolving organisms. Obviously, the physical world also varies, providing “the other side” of adaptation, but genes “take for granted” a lot of things that do not vary (e.g., that oxygen with hydrogen make water), such that all living organisms carry genes which can only function within the boundaries of a set of physical conditions that allowed life to appear and those genes to evolve.

In biological evolution, the processes that generate diversity are entirely purposeless, actually the result of mistakes in the enzymes that ensure faithful reproduction of the genetic information. What an extraordinary “destiny” for living organisms: we are all the result of consecutive “mistakes” accumulating along a few billion years, ever since the first ribozyme duplicated in the original soup. Mistakes that turned out to improve survival and duplicative (reproductive) efficiency, sometimes in a changing environment, sometimes by allowing for “tinkering”, together with previously existing genes, a novel function that, in turn, improves performance, survival and reproduction. And how extraordinary are some of the attributes of living organisms that were derived by “mistakes”: the precision of the eagles’ eye and the sensitivity of the mouse smell, the navigation of migrating birds, the human language, the flowering plants, the myriad shapes of living beings. Above all, this fantastic diversity that is the major characteristic of the living world, accumulated by successive errors in the duplication of genetic information ! Because there is no general plan, blueprint or “architect” of biological evolution (designated by Dawkins as “the blind watch-maker”), the diversity that allows it can only be purposeless. If we think about it, however, this seems to be the best possible strategy for life to cope with environments that potentially change in unpredictable manners. In other words, the best strategy for unpredictability is to cover as many possibilities as possible: hence, purposeless diversity is the solution to the unknown. Particularly, if the novelty represents variations of what existed before and had thus been tested for convenient operation. This is why life is “an arrow in time” with an irreversible history, and, in a nutshell, the core of the Darwinian legacy.

A characteristic of vertebrate evolution is the development of centralized systems for the control of body functions and behavior (a large brain, a proper circulation with a central pump, immune and endocrine systems, etc.). The extraordinary development of the brain is so striking that evolutionarily and developmentally “new” bones, blood vessels and other structures had to be “invented” to protect and feed this sort of “evolutionary tumor”. Ever since the brain of some mammals grew out of proportion, it reached the threshold of functional capabilities that allowed language and a universal grammar to emerge. Language, in turn, improved communication to such an extent that it became possible for men to transfer detailed information of previous experiences and descriptions of brain activities like “inner states” and novel thoughts. It thus became possible to learn and educate, to invent and create. The invention of agriculture (through the “genetic manipulation” of plants and animals, by the way), allowed for the sedentary concentration of large numbers of individuals, and for the historical accumulation of past experiences that could be transmitted to new generations. Humans, therefore, fell into the realm of another sort of evolution, conventionally designated by “cultural” evolution. Since cultural evolution is based on somatically acquired information that is transmitted to new generations, it is essentially Lamarckian in its nature. Accordingly, cultural evolution, in contrast with Darwinian evolution, is not purposeless, but definitely embodied by goals and plans projected into the future, by beliefs and codes, by many expectations on individual and collective behaviors. Cultural, human evolution is thus produced by “architects” who think they know what is best, by “watch-makers” looking at the future, proceeding, nevertheless, through variation & selection that generate adaptation and the emergence of higher levels of cooperativity. Being Lamarckian and purposeful, cultural evolution is very much faster than Darwinian evolution. In contrast, except for very rare “genetic catastrophes”, biological evolution is based on the novelty generated by mutation rates (errors in duplicating DNA) that only occur once in a thousand million “letters” copied. Since many such mistakes do not alter the “meaning” of the word or the sentence, and we are specified by some 30,000 genes for a total of 3,000,000,000 letters in our genome, it is easy to understand the long time it took to accumulate significant mistakes to turn a mouse into a man, or a fly into a mouse, or a bacteria into a fly or a lily. Some three and a half billion years. In contrast, cultural evolution has been extremely rapid along the 300,000 years or so since Homo sapiens exists. Moreover, being Lamarckian, cultural evolution is prone to positive feedback in its own rates. It is well known that along the last 100 years or so, we have accumulated more “knowledge” than in the whole rest of our 299,900 years of life as a species. Some evolutionists do think that the recent technologies for storing and transmitting information in very large scales may be as relevant for human evolution as the invention of written language, or even the emergence of language all together. The only serious concern with the recent progress in biological science is centered in this very problem: are we prepared to deal with our novel powers of interfering with the purposeless Darwinian evolution, following objectives that are derived from our purposeful cultural evolution?

Humans as we are, we can not, and do not want to, abdicate either side of our nature. We are both “nature and nurture”, we represent the products of a long biological evolution, transformed by the short cultural history of mankind. We are of the same nature as eucalyptus and elephants, eagles and crows, cockroaches and lady bugs, algae and bacteria, but in addition, we have a grammar to think and talk, we have had Picasso, Mozart, Newton, and Leonardo, we build cities and reach to the moon, we cure disease

and prolong life, above all, we make sense of the universe, of the world and of ourselves. The drive to understand these essential problems is probably one of the first things “secreted” by the evolutionary tumor of neurons that our brain represents. This is the business of science and scientific research; or, as Sydney Brenner puts it, “the business of scientists is solving problems”.

We live days of extraordinary excitement in biology and biomedical sciences that will, sooner or later, make a strong impact in day-to-day medical care. Many of us in the trade had been born, and some were already quite active in research, when Watson & Crick discovered the structure of DNA; few, I dare to say, could imagine that less than 50 years later, in our life time, we would have at hand the complete genome sequences of humans, of several other animals and plants, and of a sizeable number of microorganisms. What a feast for the youngsters who start now a life-long dedication to science. The hard work is done, it is now time to make the “interesting” discoveries. Knowledge in biology is said to duplicate every 2 years, and many thousands of scientific articles, describing new advances, facts or ideas, appear every year. Of the 100 most influential scientific journals in the world, over 80 are devoted to biology and medicine. More than the massive quantity of data, however, the excitement comes from the increasing understanding of highly complex biological processes from organism physiology to cellular organization, to the level of molecular interactions and gene functions. Genetics and physiology are finally coming together. Diseases can finally be understood at this molecular level, in what they have of genetic pre-disposition and of response to environmental interactions, such that we can start dreaming of a “predictive” (and a fortiori, preventive) medicine, and of correcting disease processes by specific biological interventions, rather than by using pharmacological “drugs” as in conventional therapeutics. Having now gained a scientific basis, medicine will undergo the greatest revolution ever since its founding fathers, only comparable to the revolution in life style that the progresses in physics and chemistry promoted along the last two centuries.

Vitalism is stone dead, and the essential laws of life have been principally established. This has been a major step in the coming of age of our culture in the second half of the 20<sup>th</sup> Century (that, surprisingly, is not widely celebrated), and the road is now open to understanding fundamental biological processes and their evolution. For example, there is no doubt that the genome contains instructions for shape & size of the bodies: a frog’s egg develops into a frog, but an apple seed develops into an apple tree. This is true even to the smallest details: our noses are short or long, flat or thin, according to the shape & size of the noses of our parents and other ancestors. Moreover, the genome contains instructions to “produce” a nose all together, and to put in the right place, in the middle of the ventral face of the anterior extremity of the body. And this is not for esthetical reasons only, of course: noses are used to smell, this requires olfactory neurons in the nasal mucosae which must transmit “information” (electrical potentials) to the first neuronal relay centers, and these are located in the anterior extremity of the body, making it most convenient that noses are around there as well. The interesting question here is to find out the precise nature of the determination of shapes & sizes, and how the linear sequence information in the genome is “converted” into three-dimensional volumes, in such a precise manner that the process seems capable of dealing with cartesian coordinates. Thus, from very early in their development, eggs – that are single round cells, must “be clear” about what and where their head and tail are going to be, what is dorsal and ventral, where is right and left. If it is hard to imagine how the “axis” of the

body are defined, the “construction” of forms is also extraordinarily complex, as it involves multiple “controls” of the differentiation of various types of cells, as well as of the respective number of divisions and of their differential migration ... to produce, precisely, a nose just like that of our mother or father. But also, to produce hearts and kidneys and brains of the “right” size for the species, with all the “right” types and numbers of cells to function properly, to resist environmental aggressions and “usage”, and to last for the “right” length of time. We have little or no understanding why a mouse can only live for 2-3 years, while a canary of roughly the same size can live for 10 or 12. These have been most relevant questions in embryology and continue to be focus of interest in modern developmental biology. Proper understanding of these questions, on the other hand, requires at least three complementary aspects: genetics (the transmission of information in the genomes from generation to generation), development (the “reading” of the genomic information along the development of each individual), and evolution (the accumulation of that information in the genomes of the species).

The beauty of this extraordinarily complex process of the emergence of size & shapes, certainly the most complex in the realm of higher organisms, animals and plants alike (enough to think of the extreme variety of flower forms, shapes and colors), it is also that it goes wrong so rarely. In the overwhelming majority of cases, new individuals have the right size & shape for their species. This indicates that the process is robust and strongly selected throughout evolution to be so. For example, it is amazing that, if an embryo is longitudinally sectioned early enough, two individuals will be produced, with the right shapes and roughly the right size, overcoming all complications that the “left-right” problem, at least, would seem to pose. The evolution of development is interesting, also because it can only proceed from something (organisms and their genomes) “that is already there”. Not any body shape & size is possible; organisms and genes are never “invented anew” but evolve from previous ones. It is a frequent assertion that “nature is perfect” in finding extraordinary solutions for difficult questions. This is not true. First of all, because having no “blue print”, evolution can only proceed by adaptation of pre-existing structures to survival and reproductive success. Second, if the architecture of an eye in a fly or in a human being is truly astonishing in precision and functional adaptation, it is also true that nature is always “on the way” to achieve an ever increasing adaptation. As Susumu Ohno used to point out, anyone who has had children knows that nature has yet to resolve the problem of fitting all the teeth we have in the space we have for them; many a dentist actually make a living on this problem.

Even bacteria that only dream of dividing – as François Jacob once put it – must deal with the same, though much simpler, type of problem: bacteria must “know” when they are big enough (and have twice the amount of each of their thousands of components) to divide, and must “know” where about is their “middle”, in order to produce two “new” bacteria of about the same size that keep the shape of the species (round like a coccus, or long like a bacillum, featuring a long flagellum or many pilli). No doubt that all of this is “in the genes”, but the recent advances in the composition of the genomes of men, mice, fruit flies, and worms, even of several plants, came to tell us that those genes are apparently much less different, after all, then the shapes and sizes of the respective species. We are not humans because we are all made of special “human” genes and proteins, and the flies are not what they are just because of “special” fly genes. We know now that we are all “cousins”, and share with worms and flies, mice and frogs, many of the same genes that are active in embryonic development, making it even more

interesting to find out how it all works. We have now the proof, if necessary it was, that we reached to “the top” of the living world by power and not by right.

Underlying all this, there is the fantastic problem of “counting time”: development, acquisition of shape & size, follows a strict time schedule for the overall length of the process, but also for each specific step, all ordered in a precise succession. As all development is based on differential regulation of gene expression, and this is all done by proteins, counting time for an embryo must have to do with the time of “survival” of proteins (rates of production, persistence, accumulation or degradation) inside or outside cells, and this is likely to provide the “clock” that evolution “tinkered” to tick with the highest precision. The current excitement with “pulsating” genes in the embryo – discovered by one of the group leaders at the IGC, is only at the start, I believe, for it adds the “fourth dimension” to the cartesian coordinates that the embryo must read in the process of constructing its own size & shape.

How are then the forms & sizes of the bodies determined and established? And the form & size of each of their organs? How do kidneys and hearts, for example, come to be bean-shaped or pear-shaped, respectively? And how do real beans and pears get the form they have? A good fraction of our work at the IGC deals with these questions: from very early molecular interactions in the mammalian embryo which distinguish “heads and tails”, to the genetic mechanisms that allow the embryo to form its segments and “count” them, to the genes that participate in the emergence of identity in a limb-bud such that it will become a leg or an arm, to the mechanisms that regulate cell division and growth. One of the simplest systems to study the emergence of form is the growth of pollen tubes. As the name indicates, these are long structures that result from the germination of pollen grains in plants. Obviously, the system is very simple: all there is to it is the growth (extension) of one cell on one direction. It is precisely this simplicity, however, that enables us to analyze in great detail the molecular and biophysical basis of the process, and may allow for deriving general rules that are applicable to the “generation of form” and “guidance” in 3-dimensional space. After all, we think because neuronal axons do similar things, in order to find connections with other neurons.

Cell communication is the essence of multicellular organisms, as indeed we are, embodying a wealth of complementarities that make up for the body and its physiology. Obviously, there is no molecule in our body that does not have at least one complementary correspondent inside ourselves. Evolutionarily, this reflects previous gains in complexity and functional possibilities: specialization in cells functions and division of labor, for example, but also the emergence of properties that ensembles of cells can do but single cells do not (such as thinking, for instance). Most interestingly, this also corresponds to the loss of the individual “projects” of single cells. Thus, unicellular organisms that only dream of dividing must, once they engage in cooperativity and evolve to form an organism with differentiated tissues, “give up that dream” and also “decline” to use many of the genes they carry in their nucleus. As Maynard-Smith has often stressed, cooperativity is the essential feature in all major transitions in evolution: evolution of complex multicellular organisms could only happen because of the appearance of a bacterial variant that lost its wall. Fragile and vulnerable as it had become, such an apparently non-competitive mutant “invented” – by cooperativity – a strategy that gained tremendous competitive advantages... and produced the extraordinary diversity of forms & sizes of living organisms. This is another

biological principle that we intend to follow closely in the development of the size & shape of the IGC.

Every year, a good fraction of the research topics pursued at the IGC have to be left out of this introduction, because of my limited abilities to describe the interest of them all in a simple manner. Thus, the IGC already counts on 14 independent research groups, and we expect to launch another 5 in the current year, as soon as new laboratory spaces become available. Most of these are small groups, lead by very young scientists who have here their first opportunity to show capabilities for scientific leadership. Another 3 “external groups” at the Science and the Medical Faculties of Lisbon University are also an integral part of our projects, as they share everything with the others, except laboratory spaces within our walls. The research activities of our groups, naturally slow at the outset of their “incubation”, are now beginning to bring results, excitement and publications, and we witness increasing levels of “cooperativity” amongst the different groups. In January 2000, the recently approved FCT Research Unit on “Natural Tolerance” was launched, and later in the year the IGC became, together with ITQB and IBET in the Oeiras campus, one of the first four “Laboratorios Associados” to the Ministry of Science and Technology in Portugal. We plan to submit to the FCT in the current year the project of another Research Unit, this time on Developmental Biology.

A major event in the frame of our educational activities in 2000 was the final evaluation of the Gulbenkian PhD Programme in Biology and Medicine by an international expert committee, appointed by the authorities that supported the Programme. Prepared and launched as an experimental program, the PGDBM was then completing its 7<sup>th</sup> year of activity, and its future evolution depended upon that evaluation. The Committee produced a report that led the authorities to the decision of “institutionalizing” a similar program, adapted to the rapid evolution of the scientific community in Portugal, that should now operate with an unlimited time frame and be open to foreign students. The protocol creating the Gulbenkian PhD Programme in Biomedicine (PGDB) was signed in September at the PGDBM’s Curia retreat, in the presence of the Minister for Science and Technology, the Secretary of State for High Education, the President of the National Research Council (FCT), the Director-General of High Education at the Ministry of Education, and the member of the Board of Administration of the Gulbenkian Foundation for Science. The PGDB has meanwhile entered its first year of activity and has just organized the first exchange meeting of the Portuguese laboratories that were credited to receive the program’s students in “rotations”. In 2000, the IGC organized a number of modular courses open to external and international attendance, giving a particular attention to bioinformatics, where 6 courses at distinct levels of progression were organized. The 3<sup>rd</sup> Gulbenkian Autumn Meeting was organised at the IGC, this time on “Cell Commitment & Fate”, with the attendance of numerous “post-doctoral” young scientists who constitute the primary target of such meetings. Together with the Science Section of the Gulbenkian Foundation, we have also organised, in collaboration with the Orient Foundation, three “closed” Symposia at the Arrabida Monastery, where world experts come to reflect on difficult topics: Consciousness, Immune Diversity, and Dominant Tolerance. The IGC has also mediated the organisation and activities of the Gulbenkian Chairs at the Federal Universities of Rio de Janeiro and Minas Gerais in Brazil that have now completed their 3<sup>rd</sup> year of existence.

All the IGC activities are supported by the institutional services, notably administrative, and by the technological and scientific support units. Eight such units are already in operation, most of them having been strengthened in the past year, in terms of personnel, heavy equipments or infrastructures. The renewal of the institute's buildings and the reconstruction of the various spaces have been continued, with the main areas of the animal house together with the lecture and seminar rooms receiving most of our attention.

The Instituto Gulbenkian de Ciência represents the direct intervention of the Fundação Calouste Gulbenkian in science – one of its four statutory areas. In last years' report, I have considered the arguments justifying the Foundation's decision of selectively investing in basic biomedical sciences at the IGC, and discussed some of the principles that informed our mode of organization and operation. Thus, given the very large and ever increasing public and private investments in biomedical sciences all over the world, the contribution of the IGC must be thoroughly considered such that it may be significant. Furthermore, as the Foundation's investment in the Institute represents a very small fraction of the public spending in this area in Portugal, the strategic missions and the organisational model of the IGC, rather than duplicate other public institutions, must attempt to provide what those do not. The flexibility and the risks that can be assumed by a private institution may be used for experimenting in the search for novelty and excellence, characteristics of the Foundation's profile. The IGC has assumed the strategic mission of attracting to Portugal promising young scientists, including those educated in our own graduate programs, and provide conditions that allow them to prove their competence in a fully autonomous manner. This may foster the emergence of new leaderships – a fundamental objective in a country with a very poor scientific tradition, but it can only be done within the frame of governmental (or European) programs, and in close cooperation with the public science structures, notably the Universities that should be the first to profit of new scientific leaders.

It is not the intention of IGC, therefore, to grow through the stability of “departments” and the permanency of all its members: this would simply reproduce University departments or institutes and their difficulties. The IGC must grow and live with a dynamic human structure, as a platform of encounters, where scientists and students come and stay for a few weeks or a few years, where they will always come back to, because they can find here intellectual excitement and unique peer figures, diversity of approaches and questions, but also rigorous quantitative thinking and, to the limit of our possibilities, excellence in facilities, equipments and services; above all, where all can find a pleasant and warm environment of openness in human interactions. What must become stable is this spirit and not the persons; what must define all IGC members is not their rank or status, not even their location in the world, but the uniqueness of each one and of her/his interests, ideas and expertises, wherever they are; what must define the IGC is not the wall around the garden, but the conviction of belonging to a common project, regardless of working in Oeiras or elsewhere, sharing the same spirit and relevant bits of history; what characterizes the IGC is not a pyramidal hierarchy or an organization in divisions and departments, but the profound sense of sharing excitement, ideas and resources in a common endeavor.

Niels Jerne used to say that, with money available, it is easy to start a very good institute: all that is necessary is to go around in the world and recruit some of the best scientists. Without much money, however, it becomes quite difficult, he also added, before



concluding, that this is not what we want to do, after all: first, because anybody can do it (with a lot of money, that is); second, because, paying no attention to complementarities, we would not have generated anything new, but only move people around. To create conditions where a unique “spirit” can develop and be nurtured, to ensure “stability” of an intrinsically unstable group of people, these are the challenges for all of us, objectives that can be possibly reached only if each and everyone fully participates in the enterprise that is his or her own. It would certainly be easier to fill up the laboratories with “permanent” scientists and let them do their thing; the job would be finished in a few years, but the “new IGC” would also be over, regardless of how good those would be. Most scientists at the IGC must, therefore, be here for some years only, such that the project can profit to, and be shared by, a larger number, and its “youth” be maintained by turnover of components, just like in any living system. Those who will have to stay longer and ensure stable competence in scientific profile and technological structures, must also dedicate their efforts to that very objective, often forgetting about their personal careers and their own scientific contributions. All the others will acknowledge their generous contribution, I am sure. We need, therefore, that all in the project exercise exemplary generosity, for they are so poorly rewarded. Thus, there is a tremendous social injustice in the scientist’s salaries. We all seem to agree that science is fundamental for modern societies, in terms of the robustness of culture, as well as the basis of technological innovation and motor of socio-economic development. Yet, scientists are not paid accordingly. Take the best youngsters from the intellectual “elite” of the country, let them study, say, Medicine, for 6 years and work for 2 more as a hospital intern; then, attract and stringently select the very best into a PhD program where they will spend another 4 or 5 years working hard; then, encourage them to go for a “post-doc” for 2 or 4 years; and, finally, after some 17 years of the highest worldwide education, try to bring them back to set up their own group in Portugal. At present, all we can offer them is a few years fellowship just above 1,000 Dols/month that any young bank clerk, informatics “hacker” or physiotherapeutics technician with 3 years university education would refuse outrightly, even as a permanent position with social security, health insurances and dental care. Let me add that, along those 15-17 years, the science student or young scientist lived with even less money, mostly away from the confort of home, family and friends, often sacrificing her/his own family plans for the sake of science. Moreover, when he/she finally becomes responsible for a research group, trouble is just starting: a group is some sort of small enterprise with 4-5 “employees” for whom he/she has to assume full responsibility in terms of education, of salaries or fellowships, and of their future career; furthermore, the underpaid group leader will also have to find the money to buy reagents and test tubes, pencils and software, to travel to meetings and conferences, let alone his/her own miserable salary. It is clear that nobody comes to science for the money, but if the present situation is maintained we risk losing all the good ones (the brain drain of biomedical scientists to countries that have understood their value is just starting). And, with time, science and the motor of progress would be left in the hands of those who could not find a better job.

Having adopted the strategy of establishing small research groups with full autonomy to let them work in peace for a handful of years, we must know that they will face the tremendous competition by much larger and “well-established” groups all over the world, particularly within institutions with resources and means that are incomparably above ours. So competitively limited as we are, shall we then give up ideas of contributing in our own right to the progress of science and to the future of the world? Shall we retreat to

“small” scientific problems, or to second-rate research to be published in obscure journals? Well, although serious thinking is becoming rare, it is still not expensive, and perhaps we can invest instead on projects that are more based on thinking than on accelerated performance, that are a little less obvious than others, that require a little longer than what is allowed by competitive granting, that are a little more interdisciplinary than the extreme specialization growing in most laboratories. Above all, we can certainly invest in individual creativity and in the differences between individuals: many heads will always think better than a single one, such that full scientific autonomy will always pay off in the long run. Hence, the advantages of an “horizontal” organization with many fully autonomous scientists, as compared to “vertical” structures in which the top of the hierarchy thinks and the youngsters execute. These are the risks that a Foundation can take, for it is clear at the outset that only a fraction of all of us will ever “make it” to a strong impact and a world-wide leadership. In attempting to increase the competitiveness of small groups, the institution must develop a few instruments: first, a good level of intellectual life and scientific exchange, based on full openness and sharing of ideas and projects, fostered by investing on short- and medium-term visits, seminars and conferences, workshops and symposia, graduate and post-graduate courses, and an active theoretical biology sector; second, we must support the operation of the groups by installing centralised infrastructures, equipments, services and human competencies. These two aspects should represent most of the institutional investments, all the more so as scientists and groups outside the walls of the IGC can also use them. The costs of the scientific projects themselves, if these are good enough, are likely to be often found elsewhere, by competitive granting at national and international agencies, whenever the ideas are not too innovative to fall in the frame of current evaluation methods. The institutional finances, however, must also cover the salaries a few stable scientists, as well as the “starting-up” of new groups, and the most truly innovative and medium-term projects of “high” risk. Obviously, this is nearly as difficult as to make fluffy omelets with no, or very few, eggs. Clearly, however, if we would think this is impossible, nobody would be here. First, we are already producing some omelets, demonstrating the viability of this model and strategy. Second, we believe that the difficult times of today will get better, as we trust the vision of the Board of Administration of the Gulbenkian Foundation and the new policy that it has repeatedly announced over the last 2 years: investments in science will grow to levels that will be comparable to current spending in arts at the Foundation. Moreover, we witness the increasing role of science in the activities of various sectors of the Foundation (Health, Education, Cooperation), and we applaud the novel policy of specifically fostering cooperative projects across them. We also trust the support of the public authorities and of many colleagues in Portugal and abroad, who see the interest of having a “different” institution complementing their own plans and evolution.

In the name of all of us at the IGC, I want to thank very warmly the Board of Administration of the Calouste Gulbenkian Foundation for understanding, support and encouragement, and all others at the Headquarters that help us in making this project work. The project has been imagined by Dr. V. de Sá Machado, and then led by two other members of the Board who were successively in charge of science: Dr. E. R. Vilar and Prof. E. Marçal Grilo. We also want to express our sincere gratitude for the constant attention, advice and support that we are privileged to get from our Scientific Advisory Board. I should thank very specially all the colleagues at the IGC, scientists and students,

engineers and technicians, administration and service personnel, for their constant and extraordinary efforts to make this a place where we feel proud and happy to work.

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In the past year, we lost a very good friend and a first-rate scientist. Mario Arala Chaves was an immunologist who contributed fundamental insights to the immunobiology of infections, through the structural and functional characterization of microbial VIPs (Virulence-associated Immuno-suppressive Proteins). This work has opened entirely new perspectives in the field of vaccination against chronic infections that are now beginning to bear fruits. Mario Arala Chaves was the Professor of Immunology at the Instituto de Ciências Biomédicas Abel Salazar where he established his research group and educated a number of students. Since 1999, he was a visiting scientist at the IGC, where he entertained active collaborations and many a discussion with most of us; he published several papers with the IGC's affiliation. Above all, Mario was a constant example of a truly dedicated scientist, creative and ingenious, generous and enthusiastic, critical and rigorous, crystal clear in his integrity, fully uncompromised with social influences, powers and conveniences. Only science and friendship counted for his independent style of life. We will certainly miss him very much, but his unique example will guide us for long.

## 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

### **Rag1 and Rag 2 overexpression leads to a severe defect in early lymphopoiesis**

Member: Jocelyne Demengeot

Student: Vasco Barreto

Recombination activating genes (Rag) proteins involvement in late events of lymphocyte development has been reported (from VH region reshuffling to light chain editing). To investigate further the function of the Rag genes we generated double transgenic mice for Rag1 and Rag2 such that expression of the genes will be ubiquitous and sustained at all stages of lymphocytes development. Analyses of those transgenic mice revealed that ectopic expression of Rag1 and/or Rag2 during development or at early stage of life was lethal. In addition, overexpression of those genes in the lymphoid lineage is associated with severe lymphopenia due to a developmental block at the stage where the first event of VDJ recombination normally occurred. These results lead us to investigate the function of the Rag in mediating unconventional DNA breaks as well as controlling cell cycle.

### **Arrested B lymphopoiesis and persistence of activated B cells in adult IL 7 <sup>-/-</sup> mice**

Members: Tomaz Mota-Santos, Jocelyne Demengeot and Paulo Vieira

Student: Thiago Carvalho

We examined the development, persistence and activation of B lymphocytes in IL7<sup>-/-</sup> mice. We showed that the bulk of B lymphocytes in IL7<sup>-/-</sup> mice had a fetal and perinatal origin, because B cell development in adult bone marrow was arrested at a very early stage. In agreement with this finding, the majority of B lymphocytes found in the spleen of adult IL7<sup>-/-</sup> mice carried little or no N sequence additions at their VDJ borders. The pool of peripheral mature B lymphocytes in these mice was stable in numbers, at some 10% of control levels, and consisted predominantly of large activated cells with a phenotype that closely resembled that of MZ cells. Interestingly, the lymphopenia caused by the lack of IL 7 did not affect the B1 population nor the levels of serum immunoglobulin, as IL7<sup>-/-</sup> mice had actually more circulating antibodies than wild type controls. These results provided a clear evidence of a distinct pathway of B cell development during embryonic and adult life. Further evaluation of the repertoire requirement for survival of these differential waves of lymphopoiesis is under investigation.

## **IFN1 and threshold of antigen receptor triggering**

Member: Jocelyne Demengeot

Students: Déborah Braun, Pedro Geraldès and Iris Caramalho

We demonstrated that IFN 1 is a regulator of B cell responses to BCR ligation and act as an amplifier of BCR signal. Consequences of this effect on the fate of a B cell during development and activation are under evaluation. In that frame, we reassessed the principle of regulation of terminal differentiation in natural immunity as well as upon infection. In addition, an increasing body of evidence indicated that IFN1 promoted the survival of activated T cells and we are currently reevaluating this finding in light of our experience with B cell physiology. Finally, novel analyses of the *lpr* mutant mice revealed that IFN1 plays a major role in controlling the onset of a SLE like autoimmune disease.

## **Specificities of regulatory T cells**

Members: Jocelyne Demengeot and Shohei Hori

Students : Thiago Caravvalho and Iris Caramalho

Elucidation of the mechanisms of tolerance will require identification of the specificities (reactivities) of regulatory T cells. As demonstrated by Lafaille et al. (Cell 78:399; 1994) mice carrying a transgene encoding an MBP-specific TCR develop spontaneous EAE if monoclonal (Rag-1-deficient), while the transfer of a small number of peripheral T cells from normal animal prevents emergence of the disease. In addition, in normal TCR anti-MBP transgenic mice, few T cells bearing endogenous encoded TCR can be produced, preventing emergence of EAE. In such system, manipulation of subsets of CD25+ T cells purified at different stages of development, according to the nature of their TCR, will provide understanding of the specificity requirements for regulatory cell function. We already evidenced that transgenic T cells bearing the CD25 marker are potent regulatory cells when assessed in vitro. Extension of these results to in vivo situation is under investigation. In addition, using this knowledge we are currently developing in vitro assay to generate antigen specific regulatory T cells.

## **Control of immune pathology by regulatory T cells**

Members: Jocelyne Demengeot and Shohei Hori

Student: Thiago Carvalho.

A subpopulation of T cells (CD4+ CD25+) has been reported to suppress CD4+ T cell proliferation induced by commensal bacteria in the intestine. The same population seems to orchestrate dominant tolerance to self-components. In order to elucidate the paradox of effective immune responses versus active tolerance, the boundaries of this suppressive effect are followed in mouse models presenting local inflammation. The nature of the cellular targets (CD4, CD8 and B cells) submitted to suppression are being investigated. We have shown that lung inflammation by *Pneumocystis Carinii* targets a massive and

fatal local T cell expansion that can be controlled by CD4+CD25+. Moreover, degrees of local inflammation were directly correlated with a hierarchy in T cell expansion. Finally, sterile inflammation was enough to induce lymphoproliferation, which was also controlled by CD4+CD25+ T cells. Extension of this work will assess whether sterile inflammation is an targeting event in organ specific autoimmune disorder, and whether immune response versus tolerance results from the regulation of acute versus chronic infection.

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

Members: Jorge Carneiro, Zvi Grossman and José Faro

Student: João Sousa

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 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 main results of the model were that the kinetics of TCR down-regulation required the existence of two pools of TCR with distinct down-regulation kinetics and the requirement for a high co-operativity in TCR triggering. The later result indicated that TCR triggering may be a complex mechanism. Further study of the model indicated that the most probable explanation for the high cooperativity in TCR triggering was the concerted activity of a kinase and of phosphatase acting directly on the TCR or on Zap-70 — the TCR-triggering cycle model. This model can reproduce the cooperativity required to fit the experimental data on TCR down-regulation, contrary to the simple models of TCR cross-linking tested [Sousa J. & Carneiro J., *in preparation*]. Thus our study of the kinetics of TCR triggering and down-regulation favoured a monovalent mechanism of triggering of TCR. Additionally, the model of TCR down-regulation displayed properties compatible with the concept of tuning of activation thresholds as formulated by Zvi Grossman and colleagues [Grossman Z. (1993) *Immun. Rev.*, 133, 45; Grosman & Paul (2000) *Semin Immunol* 12, 197].

In collaboration with Zvi Grossman and Jose Faro we are developing further the concept of tuning aiming to understand what is the contribution of tuning in the regulation of homeostasis of lymphocytes and in self-tolerance. To address this question, first we must design a plausible model of tuning at the cellular level and secondly, population dynamics of lymphocytes displaying and not displaying tuning must be compared.

Two models of tuning at the single cell level were developed 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-Golbetter hypersensitive mechanism. The simple model is able to produce adaptation and tuning of a lymphocyte to a constant stimulus and displays both anergy and a threshold of activation. The threshold of activation is set by the gradient of stimulation in time. A slow increase in stimulation will lead to adaptation but a fast increase will lead to activation. A decrease in the stimulus will induce a transient state of anergy in the system

for a period of time that depends on the magnitude of the change in the stimulus. The implications of this single cell based model on the dynamics of lymphocyte populations are being explored using both cellular automata and ODE formalisms.

Cytokine receptor signalling pathways share a considerable number of components, from the receptors down to genome 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  $\gamma_c$  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 demonstrated that under physiological conditions IL-2 may act as an inhibitor of IL-4 signalling. The model also predicted that IL-4 did not significantly inhibit IL-2 signalling. These results on cytokine responsiveness were used in a population dynamics model of Th cell proliferation and differentiation. Phase plane analysis of this model showed that inhibition of IL-4-dependent differentiation by IL-2 ensured the coexistence of both pools of precursors Th cells and committed Th2 cells. Without inhibition by IL-2, Th2 cells will outgrow and exhaust the precursor pool. These results suggest a simple mechanism for preventing commitment of an entire lymphocyte population to a particular differentiation pathway, and therefore to ensure functional pluripotentiality.

### **Quantitative analysis of paracrine and juxtacrine modes of action of cytokines**

Member: Jorge Carneiro

Student: Kalet Leon

Collaborator: Rob de Boer, University of Utrecht, Netherlands

The immune system is regulated by T-lymphocytes that interact with other cells through cytokines. Cytokines are intercellular messengers that control mitosis, survival and differentiation of cells. Their actual modes of action are still unclear. Cytokines may act in paracrine mode, diffusing from the source cell to the target cell through the medium, or in juxtacrine mode, being exchanged during direct cell-cell contact. With the aim of further the understanding of this field, we investigated the quantitative implications of these two modes of action.

Three models were studied that describe the population dynamics of two interacting T-cell types stimulated by a population of antigen presenting cells (APCs). In the first model there is a paracrine interaction between the two Th cells types, whereas in the second and third models, the interaction is juxtacrine. In the second model the juxtacrine signal is delivered from one T-cell to the APC and, subsequently, from the APC to the second T-cell. In the third model, a juxtacrine signal is directly delivered from one T-cell to the other, while both are "linked" by an APC. The dependency of the cytokine effect on several control parameters was studied. We demonstrated that paracrine and juxtacrine modes imply qualitatively different response curves as a function of the number of APCs.

The results have direct bearing observations on Th1 and Th2 differentiation and on Th cell mediated immunosuppression. The dependence on the APC number offers a simple way of determining on which mode a cytokine is operating. Also, the predominance of either paracrine or juxtacrine modes may explain the discrepancy between reports on cytokine functions in vitro and in vivo.

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

Members: Jorge Carneiro and Jocelyne Demengeot

Student: Kalet Leon

Evidence for T cell-mediated suppression has been derived from adoptive transfer experiments where the tolerant state of an animal is transferred to a naïve recipient by a particular subset of regulatory CD4 T cells. These regulatory cells also suppress the proliferative response of naïve T cells *in vitro*. Recent evidence suggested that the suppression required linked recognition of antigen and simultaneous conjugation of both regulatory and naïve T cells with a single antigen-presenting cell (APC). Despite this strong requirement, it is not yet clear what is the nature of the signals exchanged between regulatory and naïve cells in multi-cellular conjugates, and what are the consequences of these interactions for dynamics of the two cell populations. In this work we investigate this issue by a mixed modelling and experimental approach.

We proposed a general mathematical formalism describing the formation of multi-cellular conjugates, from where we derived particular models representing alternative mechanisms of T cell-mediated suppression. We significantly reduced the number of plausible mechanisms by: 1) Performing phase plane and bifurcation analysis of the generic properties of these models and relating these properties to the results of adoptive transfers [Leon et al. 2000. *J.Theor. Biol.* 207, 231]; 2) Fitting the models to the results of in vitro immunosuppression assays, based on inhibition of thymidine-incorporation [Leon et al., submitted]. However, because several parameters are unconstrained, the candidate solutions are still very degenerate.

In order to further narrow down the hypotheses, we optimised a proliferation assay in which the number of cycling cells as well as the number of rounds of division were quantified, by the decrease in fluorescent intensity of CFSE-stained cells measured by flow cytometry. We developed software for the analysis of populations of CFSE-labelled cells — available at <http://www.igc.gulbenkian.pt/eao/ti> link “Only4U”—, and also algorithms that allow to estimate the number of naïve and regulatory cells conjugated with APCs, as well as the maximal and effective proliferation rates of the populations of these cells. Using this new approach we were able to further discriminate between competing models for linked suppression. As a whole our results indicated that linked suppression was based on a mechanism in which regulatory cells prevented the proliferation of naïve cells and the proliferation of regulatory cells was dependent on naïve cells.

## **Modeling the process of somatic hypermutation in Ig genes during the germinal center reaction (GCR)**

Members: José Faro and Jorge Carneiro

Collaborator: Africa González-Fernández, Univ. of Vigo, Spain

According to experimental data on hypermutation of antibodies (Ab) anti-2-phenyloxazolone (phOx), during the humoral immunoresponse to phOx there are two main point mutations that increase, each of them, the affinity of anti-phOx Abs expressing the VkOx1-Jk5 light chain. Those two particular mutations, when



simultaneously present rise even more the affinity of the mutated Abs. Since the distribution of B cells with different mutations and its change with time is more or less known in that particular instance, we set to model the dynamics of hypermutations during a GCR. One of the goals is to test different hypothesis in respect to the probability distribution of the mutational process along a variable region-encoding nucleotide sequence. To our initial model of the dynamics of hypermutations we have added explicitly competition for Ag as the selective force. So far we have tested the hypermutation model basically for calibration purposes. Calculations were carried out for parameter values within ranges estimated experimentally. The model predicts a considerable lag in the onset of the double specific mutants. Also, it predicts a counterintuitive net flux of cells in the direction of accumulating slightly impairing mutations in spite of their lower survival and reproductive rates. Preliminary results suggested that the model can indeed be used to analyze the experimental data of González-Fernández et al. (*Immunology*, 93:149, 1998) on the accumulation of hypermutations during the humoral immunoresponse to phOx.

### **Modeling T cell dynamics under a sub-model of tunable T-cell activation**

Members: José Faro, Zvi Grossman and Jorge Carneiro

Student: João Sousa

T cells very likely conjugate to and de-conjugate from APCs several times before getting activated and induced to proliferate. The binding/unbinding time-history of a T cell is a crucial element of the tunable activation threshold (TAT) hypothesis because it will determine whether that cell will be activated or will become refractory to activation. In order to incorporate this hypothesis into a kinetic population model of T cells we propose a tuning mechanism (based on the activation of a kinase and a phosphatase which operate on a Koshland-Goldbeter cycle of activation) that allows the quantification of the activation state of T cells as a function of their binding/unbinding time-history. This permits to determine the distribution of activation states among T cells after a given period of time. The obtained distribution of activation states of T cells converge to an equilibrium distribution when the time allowed for the system to evolve increases. Assuming that the processes of binding and unbinding are much faster than the death and division of T cells, then the distribution of activated states is at quasi-equilibrium (and hence is a fixed one) in the dynamical population system of T cells. The fraction of T cells in that distribution with activation states above the dynamic threshold provides the fraction of cells that will divide at any time in the T-cell dynamical population model.

### **HIV2 as a attenuated model of AIDS virus infection**

Member: Jorge Carneiro

Collaborators: A. Sousa, A. Loureiro and Rui Victorino, CEBIP-Faculdade de Medicina de Lisboa, Portugal

The rate of CD4 decline during the course of HIV2 infection is known to be slower than in HIV1 infection and this is associated to a lower mortality and morbidity. The aim of this study was to compare two cohorts of HIV2 (n=25) and HIV1 (n=24) infected patients

with similar degrees of CD4 depletion in what concerns “lymphocyte turnover” and state of T cell activation. Lymphocyte turnover was measured by the expression of the proliferation marker Ki67 antigen within the memory and naïve CD4 and CD8 T cell subsets. A panel of markers of cell activation and differentiation (CD69, Fas, HLA DR, CD38, CD40L, OX40, CD28, CD27) was measured in parallel by flow cytometry. As previously described in HIV1 infection, HIV2 patients exhibited an increase in the proportion of Ki67+ cells within the CD4 subset, which is inversely correlated with the CD4 counts. This increase is mainly due to proliferation of CD4 cells with a memory phenotype. Moreover, the proportion of Ki67+ cells in the CD4 subset was clearly correlated with level of CD4 T cell activation. Intriguingly, the quantitative relationship between the turnover rate and the numbers of CD4 cells is similar in HIV1 and HIV2 infected individuals, despite the fact that the rate of progression of the diseases is very different. A mathematical model is being developed to understand the mechanism underlying these observations.

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

Member: Robert Michael Evans Parkhouse  
Student: Sílvia Almeida

Projects have been written and now assure funding for 3-5 scientists and 1-2 students. One student has started and the screening of viral cDNA libraries for viral “evasion” genes has commenced. The remaining staff necessary to put the project into active investigation will be recruited shortly, when the grant money becomes available.

### **Transforming growth factors beta (TGF?) and integrins in mouse cardiac and skeletal muscle development**

Member: Sólveig Thorsteinsdóttir  
Students: Ana Gaspar, Fernanda Bajanca and Lara Costa  
Collaborators: Christine Mummery, Hubrecht Laboratory, Univ. Utrecht and Arnoud Sonnenberg, Netherlands Cancer Institute, Amsterdam, The Netherlands

The differentiation of skeletal muscle is governed by myogenic determination factors and equivalent cardiac regulatory molecules are likely to exist. The differentiation of both types of muscle can, however, be regulated or modulated by growth factors, among them TGFbeta. Cell adhesion molecules (e.g. integrins) probably also play an important role in muscle differentiation and morphogenesis, and their expression can be regulated by growth factors.

In this project, we have mapped the expression pattern of several integrins during the development of cardiac and skeletal muscle, which can be related to the different stages of development in both of these two muscle types.

We will now address the question of the role of TGF? in the differentiation of skeletal muscle by producing and analysing embryos that express a truncated (dominant negative) TGF? type II receptor construct in skeletal muscle cells only. These embryos will be analysed in terms of morphology as well as the expression of differentiation markers and

integrins. Changes in the expression of differentiation markers can tell us if the lack of TGF $\beta$  signalling affects the differentiation of skeletal muscle, while the analysis of integrin expression patterns might give us insight into the mechanisms by which growth factors affect the development of muscle. This approach, i.e. blocking TGF $\beta$  signalling specifically in the tissue under study, will hopefully advance our understanding of TGF $\beta$ 's in skeletal muscle development.

### **Analysis of epithelium to mesenchyme transitions in integrin beta1D knock-in embryos**

Member: Sólveig Thorsteinsdóttir

Students: Ana Sofia Cachão and Fernanda Bajanca

Collaborators: Christine Mummery, Hubrecht Laboratory, Univ. Utrecht and Arnoud Sonnenberg, Netherlands Cancer Institute, Amsterdam, The Netherlands

In this project, we are determining the expression pattern of integrins and their ligands during mesenchyme to epithelium and epithelium to mesenchyme, concentrating on somite formation and differentiation in the mouse embryo. Furthermore, we are performing a detailed analysis of somite formation and differentiation in embryos obtained from the beta1D integrin knock-in strain (Baudoin et al., 1998). Embryos homozygous for the beta1D knock-in allele express exclusively the muscle-specific beta1D integrin splice variant instead of the ubiquitous beta1A variant, thus all their beta1 integrins are of the beta1D type. We have found that somite formation is often abnormal in these embryos, and we are characterising those defects in detail. Furthermore, embryos homozygous for the knock-in allele all die in mid-late gestation and we are attempting to determine the cause of death in these animals. Our data support the notion that the beta1D variant is able to support some, but not all, developmental processes where beta1 integrins are involved.

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

Member: Álvaro Tavares

Students: Suzana Godinho Paulo Alves, Célia Domingues, Claudia Florindo and Vânia Parelho.

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

extracts or from *Xenopus* egg extracts, bind to several proteins forming different stable complexes. We are now on the process of identifying the complex's components in total embryo extracts and in centrosome preparations. We want to characterize these proteins, sorting which are polo substrates and which are activators. *Drosophila* and *Xenopus* are used as working models due to their convenience for genetic and biochemical studies. Taking advantage of *Drosophila* genetics we have also searched and isolated new genes required for spindle assembly and centrosome function, some of which code for proteins with high degree of homology with the *Saccharomyces cerevisiae* proteins. We are now on the process of characterising these genes.

We are also starting a new line of research, using the chicken cell line DT40 to identify the function of the vertebrate homologues of the centrosomal kinases polo and aurora, and of other centrosomal components. We hope to create null lines and specific mutation lines to analyze the role of the conserved aminoacids in the non-catalytic conserved regions of the kinases. We will take advantage of the cell line ability to be synchronized to look for substrates at specific phases of the cell cycle.

## **Genetics of autism**

Members: Astrid Moura Vicente and Luísa Mota Vieira

Student: Suzana Silva

The objective of this research project is the identification and characterization of genes predisposing to autism spectrum disorders (ASD). In one approach we are gathering genetic epidemiological data for autism spectrum disorders in Portugal. With the support of the Serviço de Saúde e Protecção Social (FCG), we conducted an epidemiological survey in public, private and special program schools, to determine the prevalence of ASD in school aged children in mainland Portugal and the Azores. The response to our survey reached nearly 90%, with close to 200 children identified. The identified children will be evaluated and diagnosed by Dr. Guiomar Oliveira at the Hospital Pediátrico de Coimbra (HPC), following a predefined comprehensive protocol. This protocol includes a structured interview for diagnosis (Autism Diagnostic Interview-Revised) that was translated into Portuguese by our team, and for which clinicians and other health technicians involved in this work received training at the IGC. Family history will be collected and the families will be invited to participate in the genetic study. In parallel, and again in collaboration with the HPC, we have collected clinical data, biological samples and family history of 85 autistic children and their close relatives. The family history is being further analyzed to identify the pedigrees that may be most interesting for a familial genetic study of ASD and mental illness. For this a protocol for the evaluation of adult relatives is being defined. In this sample we are searching for endophenotypes, physiological and behavioral, that may be transmitted in a less complex way and therefore easier to map. We are also testing multiple candidate genes and gene regions. A positive association has been found in this sample with the serotonin transporter gene, a strong candidate gene for ASD.

## **Genetics of SLE**

Members: Carlos Ferreira, Astrid Moura Vicente, Luísa Mota Vieira, Constantin Fesel, Dan Holmberg and Carlos Penha Gonçalves

Student: Marta Barreto

Collaborator: Associação de Doentes com Lupus

With the goal of identifying susceptibility genes for SLE, we have initiated the collection of multiplex pedigrees from mainland Portugal and the Azorian islands. In mainland Portugal, this work proceeds in collaboration with the Associação de Doentes com Lupus, which has nearly 1800 associates and through which 82 multiple affected families have been identified. The first twelve families, including 22 patients and 67 healthy relatives, have already been evaluated following a predefined protocol. This protocol includes a structured interview, a clinical examination and a classical battery of serological tests for SLE, providing an accurate overall clinical assessment. Biological samples have been collected for the establishment of a cell/serum/DNA bank. Patients and their families are being screened for endophenotypes associated with the disease, with particular focus on clinical data, prevalence of different types of autoantibodies, lymphocyte physiology and apoptosis. A genome wide screening will be conducted for SLE and for identified endophenotypes.

## **Genetics and biology**

Members: John Stewart, Jorge Carneiro, Astrid Vicente and Tatiana Vassilevskaia

Work has continued on the genetic analysis of multifactorial genetic diseases, with especial application to Type-1 Diabetes, SLE and autism. Two complementary lines have been explored and developed: i) The calculation of expected frequencies of disease and subphenotypes in sibs, parents and offspring, as a function of: the heritability, the number of loci and the threshold for disease; ii) Computer simulation of population dynamics, based on a simplified but physiologically plausible mechanism of auto-immune disease.

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

Member: Lisete Fernandes

Student: Joana Monteiro

Eukaryotic cells respond to both intrinsic (originating within the cytoplasm) and extrinsic (extracellular) stimuli like oxidative stress, metal ion concentration, and temperature fluctuations, by activating cascades, which are diverse but with discrete specificity. 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 *S. cerevisiae* contains eight members and it has been described as central player in cellular responses to stress

challenges. Our major aim is to understand how yeast cells cope with distinct stimuli in a Yap1-dependent manner.

A biochemical approach will be undertaken to understand how specific extracellular signals propagate to impinge on Yap proteins. For that purpose polyclonal antibodies against some Yap proteins were raised and tested.

In order to elucidate the mechanisms of signal propagation downstream to Yap1, non-overlapping targets genes, which are required for Yap1-dependent cellular response to distinct stress agents are being selected. The role of other relevant members of Yap family in this control was also addressed and studies were also extended to conditions of differential status of mitochondria utilization.

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

Member: Isabel Palmeirim

Student: Catarina Freitas

Like insects and worms, vertebrates are segmented organisms and the somites constitute the framework on which the segmental pattern of the vertebrae, body muscles and peripheral nervous system is established. 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 mesenchymal cells enter the posterior end of this tissue as a consequence of gastrulation (1). Previous work showed that the avian homologues of the *Drosophila hairy* gene (*c-hairy1* and *chairy2*) are expressed in a wavy fashion in PSM cells providing the first evidence for the existence of a molecular clock underlying somite formation (2, 3). It has been also shown that the cycling expression of these genes are an autonomous property of PSM independent of any signal coming from the most posterior part of this tissue (2, 3).

In this work, we used a tridimensional *in vitro* culture system to evaluate the *c-hairy* genes expression autonomy of each portion of the PSM in order to detect any controlling signal coming either from the anterior, lateral or medial part of PSM.

The analysis of the results showed that each isolated piece of the PSM expressed the *c-hairy1* and *c-hairy2* genes in a pattern identical to the corresponding region in the control half. This showed that the expression pattern of this gene was completely independent of any signal coming either from the posterior or anterior part of the presomitic mesoderm.

Strikingly, a different set of explants demonstrated that lateral PSM cells were not able to form somites by themselves losing PSM markers expression when cultured isolated from more medial PSM cells. These results strongly indicate that cells from the medial part of PSM are the only ones that have the information of segmentation likely if medial ones must recruit most lateral cells in order to form somites.

### **The cycling genes at the chicken organizer**

Member: Isabel Palmeirim

Student: Catarina Freitas

Somitogenesis is a very well time and space controlled process in which metameric structures are formed, every 90 minutes, in the Chick embryo. Concomitantly with

epithelial somite formation at the anterior end of presomitic mesoderm (PSM), gastrulation is taking place in the most posterior part of the embryo, at the level of Hensen's node and primitive streak, causing progressive embryo elongation. Subsequently, development allows somites to give rise to all the muscles of the body, except those of the head, as well as the dermis of the back and the axial skeleton (vertebrae, intervertebrae discs and ribs) (reviewed by Gossler and Hrabe de Angelis, 1998). In a previous work (Palmeirim *et al.* 1997 and Jouve *et al.*, 2000) evidences for the existence of a molecular clock underlying this process have been provided showing that presomitic cells undergo several cycles of expression of the *c-hairy1* and *c-hairy2* genes until they incorporate a somite. Catala (1996) showed that somites originate from the anterior third of the primitive streak in 6-somite stage embryos (HH). We now carefully analysed the *c-hairy1*, *c-hairy2* and *lunatic fringe* expression patterns at the Hensen's node/primitive streak region in 6-somite stage embryos. Correlation between this whole-mount and cross-sections detailed analysis and the results of a more precise quail/chick fate map of this region, performed by the authors, allow us to precisely determine where such oscillatory behaviour occurs.

### **What is colloid function 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 of the astacin family was identified in different organisms and named *tolloid* in fly, fish and mammals and *xolloid* in frog. This metalloprotease acts in several developmental processes by a double inhibition mechanism to establish the graded activity of BMPs, genes involved in the establishment of the vertebrate embryonic dorsoventral axis. Tolloid-like genes specifically cleave Chordin allowing the activity of BMP proteins (Piccolo *et al.*, 1996). The chick-*tolloid*-like gene (*colloid*) has been identified and its role in the patterning of neural tube described (Liaubet *et al.*, 1998).

In the vertebrate embryo, somites constitute the basis for the segmental pattern of the body and they give rise to the axial skeleton, the dermis of the back and all striated muscles of the body, except those of the head (Gossler and Hrabe de Angelis, 1998). During differentiation of the somites they became polarised according to a mediolateral (dorsoventral) axis. BMP4 is produced by the tissue that flanks the somites laterally and has been proposed to play an important role in lateral somite specification (Pourquié *et al.*, 1996). Therefore determination of the dorsomedial mesodermal lineages require the inhibition of BMP4 activity by proteins such as Noggin, Chordin or Follistatin.

We carefully analysed *colloid*'s pattern of expression during the process of 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* chick grafts we determined the role of surface ectoderm and neural tube in the induction and maintenance of *colloid* expression.

## Mechanisms of head induction in vertebrates

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

Students: Mário Rui Filipe, Ana Cristina Borges and Sandra Freitas

The *Xenopus cerberus* gene encodes a secreted factor expressed in the Spemann organizer that induces ectopic head formation when its mRNA is injected into *Xenopus* embryos. We reported the isolation of the related mouse *cerberus-like* (*cer-l*), a gene encoding a novel secreted protein that is specifically expressed in the Anterior Visceral Endoderm (AVE) and mesendoderm that underlies the presumptive anterior neural plate. Both factors function in the extracellular space as multivalent growth-factor antagonists inhibiting BMP, Nodal and Wnt proteins. The specific aim of our research is to further contribute to the understanding of the role of the anterior visceral endoderm and the *Cerberus/Dan* family of secreted factors in the specification of vertebrate embryonic structures. We have genetically inactivated by homologous recombination in ES Cells the *cer-l* gene. Currently we are studying possible genetic interactions of *cer-l* with these 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*. They are expressed in the node and its derivatives and interact for correct forebrain development. A case of genetic interaction was not verified in when we generated *cer-l;noggin* double mutants, leading to a view of a complex genetic network at the level of the several BMP antagonists in the patterning of the early mouse embryo. By this, a model of how head and trunk development might be regulated can be discussed.

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. For this end we have generated a transgenic mouse line expressing EGFP under the control of the mCer-l promoter. By sorting out these EGFP positive cells we can generate a differential cDNA library leading to the isolation of novel genes expressed in the AVE. Interesting genes will be studied at the biochemical, expression and genetic levels. An already identified novel gene related to *cer-l* is being inactivated by homologous recombination in ES cells, in order to access its role during embryonic development.

## Tradeoffs in the evolution of virulence

Member: Francisco Dionisio

Virulence, defined as the amount of damage 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.

Another important process in the evolution of virulence concerns the "social" aspect of parasite interactions. Suppose that prudent exploitation of a host maximizes a parasite's fitness. Selection then favors each parasite phenotype, when alone in a host, to follow the



prudent strategy. However, what happens when two or more genotypes occupy the same host? If one genotype extracts host resources rapidly and reproduces quickly, then the host may die in a short time. A prudent genotype would have relatively low fitness when paired in a host with a "greedy" genotype because, for both genotypes, the host is short lived, and the greedy genotype reproduces more rapidly than the prudent one.

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.

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

Member: Matthias Haury

Student: Dinis Calado

We are currently in the process of creating a new mouse 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 these IL-10 expressing T regulatory cells.

We are also in the process of characterising in more detail the immuno-subphenotype of regulatory T-cells and their localisation using multicolor (5-6 colors) flowcytometry and multiphoton confocal microscopy.

These studies will be carried out in collaboration with the laboratory of Dr. Antonio Bandeira, Pasteur Institute Paris.

### **Cytoskeleton mediated signaling (taxol and menedione) in the induction of apoptosis under oxidative stress**

Member: Sukalyan Chatterjee and Nandita Das

Student: Mario Grãos

Collaborators: Maria Maroné and G. Scambia, Univ. Cattolica del Sacro Cuore, Rome, Italy and Mark Grimes, Massey University, NZ

Cells respond to both intrinsic (originating within the cytosol) and extrinsic (extracellular) stimuli by activating signaling cascades with discrete specificity. What cross-talk exists between such cascades and how are they regulated? Our aim is to conduct a molecular study of how alterations in microtubules under oxidative stress challenge affect associated proteins and signaling events in the control of apoptosis. We hypothesize that microtubules are a dynamic scaffold for regulating and directing signal transduction. Apoptosis is a mechanism of programmed cell death is the outcome of a signaling cascade whose initiating stimuli originate within the cell or from the external environment, and is a highly conserved mechanism present in organisms ranging from *C. elegans* to humans. The discrete specificity of the signaling cascades is determined in the early steps prior to converging to the later death-blowing event. What these signaling cascades are and how they achieve specificity are still open questions. There is mounting evidence that anchoring and scaffolding proteins play a decisive role in signal

transduction, serving to bring kinases or phosphatases proximal to substrates and assemble sequential components through the formation of multiprotein complexes. The goal of this project is to understand the mechanisms of microtubule-mediated signaling in mammalian cells addressing issues like how the cytoskeleton exerts their effects with respect to structural and signal transduction-mediated events. Using genetic, biochemical and cell biology approaches we aim to elucidate the mechanism of these signaling events. Our initial study with microtubule perturbation showed that the microtubule-perturbing agent Taxol had multiple effects on this signaling pathway and was a potent inducer of apoptosis. The specific pathways activated by low, clinically relevant concentrations of the drug are still largely unknown and are dependent on cell type and drug concentration. In this work, we have investigated why HeLa respond to Taxol by undergoing complete apoptosis whereas MCF-7 remained in an intermediate phase with reduced death. Three phases were distinguished in these apoptotic pathways. The initial phase, characterized by cellular detachment, was followed by a second phase, which included the onset of apoptotic morphology, and p38 and Bcl-2 phosphorylation. These two phases were common to both cell lines. HeLa then proceeded to the third and final execution phase, which culminated in death, whereas MCF-7 did not progress. Interestingly, the isoflavonoid Quercetin, a known general kinase inhibitor and an antioxidant, was able to prevent the onset of Taxol-induced cellular detachment and to protect from cell death. Moreover, it blocked Taxol-induced phosphorylation of p38 and Bcl-2, and prevented a Taxol-induced change in relative mobility of the apoptosis signal-regulating kinase 1 (ASK1). Our data elucidated the specific signaling pathways activated by Taxol at low clinically relevant concentrations.

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

Members: Sukalyan Chatterjee, Matthias Haury and Paula Parra

The major goal of this study will be 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 are differentiated 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. The major goal of this study will be 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 are differentiated 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 a pathway of differentiation or apoptosis. 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. This project is focusing on this issue and more specifically is addressing questions like: 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.

### **Function of the components of the tubulin folding pathway in microtubule dynamics and signal transduction in *Tetrahymena* and mammalian cells**

Member: Helena Soares

Students: Cristina Casalou and Sofia Nolasco

The main goal of this study is to understand the mechanism/s underlying microtubules (Mts) polymerizing/depolymerizing dynamics and its relationship with signal transduction pathways triggered by oxidative stress in *Tetrahymena* and mammalian cells. Our immediate aim is to investigate: (i) How 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 tubulin folding machinery to control Mt dynamics. Cellular responses to stress events require cytoskeleton re-arrangements 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 this context we report:

- a) Tubulin exists in *Tetrahymena* exponentially growing cells in several different complexes with molecular masses ~90 to ~500 kDa as revealed by native gel analysis and confirmed by gel filtration chromatography. We decided to characterize these protein complexes containing tubulin in *Tetrahymena* exponentially growing cells and cells submitted to a hyperthermic stress (28°C to 34°C). Using sucrose density gradients combined with gel filtration chromatography we have partially purified a molecular mass of about 500 kDa. This complex contains at least 12 distinct proteins with molecular masses ranging from 30 kDa to 150 kDa. Remarkably, besides tubulin we were able to identify three of these proteins as CCTa, CCTe, CCT? and CCTd. This complex seems to be more abundant in cells submitted to a heat-shock as compared with control cells.
- b) During *Tetrahymena* cilia recovery we observed a clear decrease in the amounts of 900 kDa chaperonin complex whereas CCT micro-complexes are increasing. These results suggest the existence of a correlation between the abundance of the intact chaperonin and the CCT micro-complexes. This correlation is affected by the physiological state of the cell. Unexpectedly, after cell fractionation we found that the insoluble fraction, that in *Tetrahymena* also contain cilia, basal bodies and remaining parts of the cort x structure, CCTe seems to be part of a complex of about 300 kDa

that contains other unidentified proteins. We are trying to establish a functional relationship of this complex with the referred *Tetrahymena* structures.

- c) We started to characterize several mouse positive clones encoding the p14 cofactor A (a molecular chaperone that accepts  $\gamma$ -tubulin after the folding assistance by CCT) in order to understand how many functional genes are present in mammalian cells.

### **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 continuously pumped out biological specificities that we aim to explore on a fundamental perspective.

### **Specification of vertebrate limb identity**

Members: Juan Carlos Izpisua-Belmonte, Cláudia Rocha Carvalho, Ramón Díaz Trelles, Javier de Francisco Morcillo and Joaquín M<sup>a</sup> Rodríguez León

Student: Sofia Martinho Simões

In the last years several groups have been 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 discriminate the genes expressed either in the hindlimb or in the forelimb. Then we will process these data and we will study the differential expression patterns of these genes during limb development. Next step will be the functional analysis of these genes and their interactions with other genes involved in growth, determination and specification of limbs.

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

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

Students: Sébastien Bagot and Valérie Soulard

The objective of the project is to analyse the T-cell response involved in pathogenesis of Cerebral Malaria (CM). Using a murine experimental model of CM, B10.D2 mice infected with erythrocytic stages of *Plasmodium berghei* ANKA (PbA), we have previously shown that occurrence of the neuropathology is linked to  $\alpha\beta$  T cells. In this mouse strain highly susceptible to CM, an increase of TCRV $\beta$ 8<sup>+</sup> cells was observed in the blood of mice developing the disease. Most of these TCRV $\beta$ 8<sup>+</sup> cells belong to the CD8 subset and express the phenotypic markers CD69<sup>+</sup>, CD44<sup>high</sup> and L-Selectin<sup>low</sup>. The link between expansion of T cells bearing V $\beta$ 8 and the pathological consequences of PbA infection was further confirmed by the fact that CM was significantly reduced both in mice rendered deficient in V $\beta$ 8.1<sup>+</sup> and V $\beta$ 8.2<sup>+</sup> T cells either by treatment with antibodies or by infection with a mouse mammary tumor virus (MMTV) which by itself presents no interference with the parasite. During our investigation at the IGC, in collaboration with the group headed by Pr. Virgilio Do Rosario, CMDT, Lisboa, we developed a new model of CM by initiating the infection with sporozoites. The Sporozoite is the parasite developmental stage inoculated during the bite of an infected mosquito. They invade hepatocytes where they differentiate and multiply to initiate the intrahepatic stage which release merozoites able to initiate the erythrocytic stage by infecting red blood cells. The latter is responsible for the pathology during malaria. This model is more similar to the human situation in which CM develops generally during primary infection and permits us to analyse the control of the hepatic phase of the parasite life cycle in the development of pathogenesis. In these models of CM, our research is centered on 1) characterisation by cytofluorometry and microscopy of T-cell populations present in the Central Nervous System (CNS) in normal and pathological situations; 2) reconstitution experiments using mice lacking T cell population in order to study the key mechanisms of selection and recruitment of T- cells involved in pathogenesis of CM and 3) *in vitro* co-culture model to analyse effector functions leading to CM that take place between the infiltrated T cells and the other cell components of the CNS such as endothelial cells, astroglia, microglia and neuronal cells. Results obtained show an important infiltration of CD8 T cells in the

brain of susceptible mice developing CM and a role for NK T cells in the control of the pathogenesis. We are currently analysing these T-cell responses.

### **Molecular pathogenesis of murine gammaherpesvirus-68 in mice and latent infection of B-lymphocytes**

Members: Pedro Simas and Alexandra Teixeira

Students: Patricia Madureira and Sofia Marques

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. MHV-68 has 14 unique such genes, designated M1 to M14, and several cellular homologues, including a complement control protein, a D-type cyclin and an IL8 receptor. In addition to these cellular homologues two of the  $\text{CM}^1$  genes, M1 and M11, show low level similarity to serpins and bcl2 cellular protein, respectively.

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

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

### **Study of immunogenic proteins of mycoplasma mycoides subsp. mycoides small colony type**

Members: Pedro Simas

Collaborators: Jose Regalla and Rosario Goncalves, Laboratório Nacional de Investigação Veterinária de Lisboa, Portugal

Mycoplasma mycoides subsp. mycoides (Mmm SC) is the causative agent of contagious bovine pleuropneumonia (CBPP) in domestic cattle. Due 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 Laboratório Nacional de Investigação Veterinária (LNIV) and the Instituto Gulbenkian de Ciência (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), iv) to develop improved serological reagents to serological tests for CBPP (LNIV) and to develop improved serological tests for CBPP.

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

Member: Pedro Simas

Student: Leonor Orge

Collaborator: Alexandre Galo, Laboratório Nacional de Investigação Veterinária de Lisboa, Portugal

This project is part of a formal collaboration between the Laboratório Nacional de Investigação Veterinária (LNIV) and the Instituto Gulbenkian de Ciência (IGC). The objectives of this project are: i) to strain type the current agent of bovine spongiform encephalopathy in Portugal and ii) to determine Prnp gene polymorphism in selected Portuguese sheep populations.

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

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

Student: Susana Campino

The identification of genetic factors controlling resistance to clinical forms of malaria will provide an important contribution to the understanding of malaria pathogenesis and will suggest therapeutic and vaccine strategies to improve resistance to disease. This project aims to identify genetic factors that control resistance to clinical forms of malaria in mouse models. To identify malaria resistance loci, we have exploited the appearance of novel phenotypes and the higher frequency of polymorphisms in wild derived mouse strains compared to laboratory strains. We have studied the WLA strain, which is highly resistant to cerebral malaria. Genetic analysis crossing WLA and a reference strain for susceptibility (C57BL/6) has revealed a novel phenotype of resistance to both cerebral malaria and hyperparasitemia (HP) in 17% of F2 animals. Further, genome wide screening of F2 cohorts has identified two loci linked to cerebral malaria resistance and two loci linked to resistance to hyperparasitemia. Using a congenic mouse approach we are currently aiming to identify and characterize candidate genes in these chromosomal regions.

## **Genetics of murine IDDM**

Members: Dan Holmberg and Carlos Penha Gonçalves

Students: Marie Louise Bergman and Suzana 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. Previous quantitative trait locus analysis has identified the chromosomal location of a locus contributing to the apoptosis resistance of immature CD4+8+ thymocytes. More recently, we found that immature thymocytes of female NOD mice proliferate with a relatively low rate compared to non-autoimmune C57Bl/6 mice and mapped this trait to the same region on chromosome 6. We will now continue our search of genes that mediate these effects by fine mapping using congenic mouse strains established over the distal region of mouse chromosome 6.

## **Genetics of human autoimmune diabetes**

Members: Dan Holmberg, Luisa Mota Vieira, Carlos Penha Gonçalves, Tatiana

Vassilevskaia and Astrid Vicente

Student: Marta Barreto

The overall goal of this project is to localise, molecularly clone, and investigate the functions of genes predisposing to human insulin dependent diabetes mellitus type 1. To overcome the genetic heterogeneity problem of these complex traits, the approach used is the genetic mapping of disease-associated phenotypes that are likely to contribute to the pathogenesis of the disease. These are under a more simple genetic control and, thus, more easily subjected to positional cloning strategies for identifying contributing genes. Immunologically related traits associated with the disease are identified, and chromosomal regions with candidate genes for these "sub-phenotypes" are sought for, aiming at the genetic mapping and positional cloning of individual risk genes.

## **Genetics of lymphoid homeostasis**

Member: Carlos Penha Gonçalves

Student: Nadia Duarte

The goal of this project is to characterize the genetic control of the number of lymphocytes along the development of the lymphoid organs in mouse. The work plan includes (1) to study the establishment of lymphocyte homeostasis during the ontogenesis of the lymphoid organs in different laboratory mouse strains and (2) to genetically map and to identify the genetic factors involved in the homeostatic mechanisms that control the number of lymphocytes in the lymphoid organs of the mouse.

The total number of cells in the tissues and organs of multicellular organisms is under strict control but little is known on the homeostatic mechanisms that control the number of



lymphocytes. On the other hand, it is known that a number of immune dysfunctions, such as immunodeficiencies and certain autoimmune diseases, occur with lymphopenia. The notion that certain lymphocyte populations can regulate the size of other lymphocyte populations raises the interesting possibility that maintenance of lymphocyte cellularity is a function of the immune system that is important for the responsiveness towards foreign antigens and to the maintenance of self tolerance.

During year 2000 we investigated the variation of the number of cells within several lymphocyte populations both in the thymus and in the spleen of two mouse inbred strains BALB/c and C57Bl/6. We observed a higher proportion of CD4 single positive thymocytes in Balb/c mice when compared to the C57BL/6 strain and we found that this cellularity difference is genetically controlled by the MHC locus. As the C57BL/6 strain is known to lack the expression of one MHC class II molecule we are now exploring the hypothesis that the polygenism of the MHC class II locus is implicated in the efficiency of selection from DP to CD4 mature T cells.

Another avenue of this project is the genetic mapping of cellularity differences we found in splenic lymphocyte populations.

### **Molecular characterization and preliminary biological activities of two novel toll-like receptors**

Member: Paulo Vieira

Students: João Pedro Pereira and Susana Pascoal

Collaborators: Rob Kastelein and Fernando Bazan, DNAX Research Institute, Palo Alto California

Recently, we have accomplished the molecular characterization and preliminary biological activities of two new TLR molecules, provisionally designated TLRKB6 and TLRKB7. Their amino acid (a.a.) sequence identity is 47% and the overall a.a. sequence identity to *Drosophila* Toll is 38%. The human homologues of mouse TLRKB6 and TLRKB7 are located in chromosome Xp22, in a region where several human genetic diseases associated with developmental problems (e.g. ocular albinism, mental retardation) have been mapped. Synthenic studies map both mouse genes also to chromosome X, in a region near Oa1. BLAST search in mouse Expressed-Sequence Tags (EST) database using the intracellular domain and 3' untranslated sequence revealed two ESTs from the mouse two cell-stage of embryo development. This result suggests a possible role for these genes during embryonic development. Expression analysis by quantitative PCR showed similar patterns of expression, for TLRKB6 and TLRKB7, almost exclusively in splenic macrophages and B lymphocytes. We are currently investigating potential ligands for these receptors, and also generating mouse strains with inactivated copies of these genes.

## **CD4+ T Cells producing IFNg are essential for the rejection of thyroid allotransplants**

Members: Paulo Vieira and António Gil Castro

To understand the molecular mechanisms involved in allograft rejection, we assessed the production of IFNg and IL4 in lymphocytes infiltrating allogeneic thyroid lobes. Interestingly we observe that early after transplantation (day 5) a small percentage of cells infiltrating the graft produce IL4 but not IFNg. By day 10 there is an increase in the percentage of cells expressing IFNg and no IL4. Neutralization of IFNg production leads to an increase in the acceptance of the transplanted thyroid. The data show that CD4+ T cells, in particular those of the Th1 phenotype, play a major role in allotransplant rejection of thyroid lobes.

## **Immunologic tolerance to allotransplantation by induction of IL-4 and IL-10 expression in transgenic mice**

Members: Paulo Vieira and António Gil Castro

Student: Rui Soares

To study the implications of a Th1 to Th2 shift in the rejection of thyroid allografts we generated lines of transgenic mice with inducible expression of IL-4 and IL-10. Preliminary characterisation of these mice shows that the levels of mRNA coding for IL-4 and IL-10 in the thyroid and the brain can be upregulated and also that serum IL-4 and IL-10 are detected after the induction of the transgenes. Interestingly, induction of transgenic IL-10 also induces expression of IL-4. Conversely we also noted an increase in the expression of IL-10 after induction of the IL-4 transgene. We are now using these transgenic mice as recipients and as donors of allogeneic thyroids. In this system, we are now attempting to induce local expression of IL-4 and IL-10 and evaluate its effects in the acceptance or rejection of the transplanted organ.

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## SEMINARS AT THE IGC

### **January**

João Pedro Pereira (IGC, Portugal)  
*Two Toll Pieces for the Innate Immunity Puzzle*

Pablo Pereira (Institut Pasteur, Paris, France)  
*Development and possible functions of IL-4-producing gamma delta T cells*

### **February**

Elliot Meyerowitz (California Institute of Technology, EUA)  
*Cell-cell communication and the control of plant growth*

Roberto Perris (The National Cancer Institute, University of Parma, Italy)  
*Role of the extracellular matrix during neural crest cell migration*

Alexandra do Carmo (IBMC, Porto)  
*Polyadenylation signals: ending a message is not so simple*

Martin Catala (URA, CNRS, France)  
*FGF8 and its regulation in the tail bud*

### **March**

Stefano Piccolo (Univ. Padova, Italy)  
*Biochemical properties of xenopus cerberus as a model for head induction in vertebrates*  
*Establishing asymmetries in early Vertebrate development*  
*Regulation of Dorso-Ventral patterning during embryogenesis*

Michael Kessel (Max-Planck Institute, Goettingen, Germany)  
*Aspects of ectodermal patterning, epidermis-neural plate-and prospective neural crest in between*  
*The Nieuwkoop-organizer problem in amniota*

José António Belo (IGC, Portugal)  
*Targeted inactivation of the mouse chordin gene*

Luis Puelles (Univ. Murcia, Spain)  
*Telencephalic molecular subdivisions and morphogenesis*

Ivan Lefkovits (Basel Institute for Immunology, Basel, Switzerland)  
*From Proteins to genes, from genomics to proteomics*

James McInerney (Dept. Biology, Nat. University of Ireland, Maynooth, Ireland)  
*Mutational bias and codon usage: lessons from completed microbial genomes*

Isabel Palmeirim (IGC, Portugal)  
*c-hairy 2, a second hairy like gene regulated by the clock linked to somito genesis - study of c-hairy expression in the Hansen's node/primitive streak region*

Lionel Jaffe (Marine Biological Laboratory, Woods Hole)  
*Calcium waves*

## ***April***

Jorge Pedrosa (IBMC, Porto)  
*Neutrophils: neglected partners in the defense mechanisms against tuberculosis and other mycobacterial infections*

Laura Zonia (Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Prague)  
*Signals and mechanisms regulating pollen development*

Hércules Menezes (Instituto de Biociências, UNESP, Brazil)  
*Defense mechanisms and evolution of the immune system*

## ***May***

Susanna Cardell (Immunology Section CMB, Lund University, Sweden)  
*CD1-autoreactive natural killer (NK) T lymphocytes*

Ana Mafalda Cumano (Institut Pasteur, Paris, France)  
*Identification and characterization of committed T cell precursors in hematopoietic organs*

Sergiy Bobrovnyk (IGC, Portugal)  
*Transformation of specific antibodies into polyreactive immunoglobulins*

Herbert Steinbeisser (Max-Planck-Institut fuer Entwicklungsbiologie, Germany)  
*Getting in shape; axis formation in xenopus embryos*

Fernando Catarino (FCUL, Lisboa)  
*Flower power*

Sukalyan Chatterjee (IGC, Portugal)  
*Receptor tyrosine kinase mediated apoptosis*

Gerard Hoyne (University of Edinburgh Medical School, UK)  
*A role for Notch signalling in peripheral tolerance*

Frank Gannon (EMBO, Heidelberg, Germany)

*Functional genomics: will all genes be as complex as the estrogen receptor?*

Francisco Dionísio (IGC, Portugal)

*When parasites profit from host's diversity*

Enrique Montero (Weizmann Institute of Science, Rehovot, Israel)

*Plasticity of the immunoresponse against self-antigens after transient immunosuppression*

Alexis Collette (INSERM U 511, Institut Pasteur, Paris)

*Analysis of the TCR $\beta$  repertoire during malaria using the immunoscope method*

Peter Pfeffer (Institute of Molecular Pathology, Vienna, Austria)

*Regulation of the Pax2 and Pax5 genes at the midbrain-hindbrain boundary*

Derek Wakelin, Murray Selkirk and Michael Parkhouse (Univ. of Nottingham, UK;  
Imperial College, London; IGC, Portugal)

*Three parasites for the price of one*

## **June**

Jacques Louis (WHO/University of Lausanne, Switzerland)

*Early events shaping TH2 cell development in BALB/C infected with leishmania major*

Ramón Díaz Trelles (IGC, Portugal)

*Effects of the antihistaminic Terfenadine (Seldane) in rat cerebellar neurons in culture*

Jocelyne Demengeot (IGC, Portugal)

*Numbers, diversity and specificity: an attempt to find the coherence in repertoire selection*

Melvin Cohn (Salk Institute, USA/IGC, Portugal)

*Alice in the wonderland of the T-cell receptor*

## **July**

Álvaro Tavares (IGC, Portugal)

*Mitosis - new genes for old ideas*

Alexandra Teixeira (IGC, Portugal)

*Characterisation of the molecular mechanisms controlling astrocyte proliferation in response to endothelin-1*

Shohei Hori (IGC, Portugal)

*Towards a consistent model for self/non-self discrimination made by CD4<sup>+</sup> regulatory T cells*

John Stewart (Université de Technologie de Compiègne, France/IGC, Portugal)

*Elementary genetics from an advanced point of view*

*I Mendel: genetics and heredity*

*II The Elston-Stewart algorithm*

*III Genetics, disease and biology*

Eric Lam (Ludwig Institute for Cancer Research, Imperial College School of Medicine, UK)

*The role and regulation of the pRB/E2F Pathway in B-cells*

Maria Maroné (Università Cattolica del Sacro Cuore, Istituto di Clinica Ostetrica e Ginecologia, Rome, Italy)

*Role of TGF $\beta$  in the differentiation of hematopoietic*

Marat Alimzhanov (Institute for Genetics, University of Cologne, Germany)

*A transgenic mouse strain for Cre/loxP-mediated gene modifications in mature B cells*

Tatiana Novobrantseva (Institute for Genetics, University of Cologne, Germany)

*Characterisation of B cells expressing natural poly-autoreactive immunoglobulin receptor in vivo*

## **September**

John Parrington (Department of Anatomy and Developmental Biology, UCL, London, UK)

*New insights into the molecular mechanisms of egg activation and fertilization*

Jorge Carneiro (IGC, Portugal)

*Towards a taxonomy of the mechanism of T cell mediated tolerance*

Ludo Pagie (Santa Fe Institute, New Mexico, USA)

*Diversity in bacterium populations; a population-based mode and an individual-based mode*

Can Kesmir (Utrecht University, The Netherlands)

*How do cells slaughter, transport and sell microbes? The sequence analysis story*

## **October**

Ari Waisman (Institute for Genetics, University of Cologne, Germany)

*The B cell receptor is necessary to regulate responses from innate immunity receptors*

Deborah Braun (IGC, Portugal)

*Type I interferon, B cell activation and autoimmunity*

Michael Rose (University of California, Irvine, USA)

*Evolution of immortality*

Paul Rainey (University of Oxford, UK)

*Ecological and genetic causes of diversification in experimental bacterial populations*

## **November**

Oliver Annacker (Institut Pasteur, Paris, France)

*Regulatory T cells in lymphocytes homeostasis*

Joana Desterro (Univ St. Andrews, Scotland)

*Role of SUMO-1 modification in transcriptional activation*

Geraldo Fernandes (UFMG, Brazil)

*Plant elicitation of hypersensitive reaction against tumor-inducing insects: from cells to biodiversity*

Giovanna Chimini (CIML, Marseille, France)

*ATP binding cassette transporter 1 and membrane lipid architecture*

Elsa Seixas (National Institute for Medical Research, London, UK)

*Role of pro-inflammatory responses to infection with plasmodium chabaudi chabaudi (AS).*

Thiago Carvalho (IGC, Portugal)

*Ontogeny, lymphopoiesis and differentiation: lessons from the IL7 <sup>-/-</sup> mouse*

Miguel Soares (Immunobiology Research Center, Boston, USA)

*Expression of heme oxygenase-1 in endothelial cells: a protective response to injury in organ transplantation*

Pedro Fernandes (IGC, Portugal)

*Bioinformatics: converting data to knowledge*

Ethan M. Shevach (National Institute of Allergy and Infectious Diseases Bethesda, USA)

*Control of autoimmunity by regulatory T cells*

Pierre-Olivier Couraud (Directeur CNRS UPR 415, Institut Cochin de Génétique

Moléculaire, Paris, France)

*Blood-brain barrier and signal transduction*

Carl Smythe (Univ. Dundee, UK)

*Analysis of checkpoint kinases in mammalian cells*

Moisés Mallo (Max-Planck Institute for Immunobiology, Freiburg, Germany)

*Signalling and patterning processes controlling skeletal development in the face*

Jonathan Pines (Univ. Cambridge, UK)  
*Regulating mitosis by proteolysis*

### ***December***

Magdalena Zernicka-Goetz (Univ. Cambridge, UK)  
*Establishing polarity in the mouse embryo*

David Glover (Univ. Cambridge, UK)  
*Centrosomes and mitotic kinases*

Jean-Baptiste Charrier (Institut d'Embryologie CNRS et College de France, Nogent sur Marne , France)  
*Experimental approach of gastrulation in avian embryo*

Leonor Parreira (Inst. de Histologia e Embriologia, Faculdade de Medicina de Lisboa)  
*I. The third dimension of the genome, leukemogenesis and cellular differentiation.*  
*II. Cell fate decision genes in hematopoiesis.*

Ramon Trelles (IGC, Portugal)  
*Microarray from chicken: an overview.*

### **Gulbenkian Lectures : Perspectives in 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 new “Gulbenkian Lecture” series was launched in 2000. Selected invited speakers give conferences 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.

### ***October***

Avrion Mitchison (University College London Medical School, UK)  
*cis-regulatory polymorphisms provide flexibility and identify checkpoints open to intervention*

Jan Andersson (Basel Institute for Immunology, Switzerland)  
*B lymphocyte differentiation and regulation*

### ***November***

Mike Owen (Imperial Cancer Research Foundation, London, UK)  
*Molecular mechanisms of the regulation of T cell*

Fabienne Pituello (Centre de Biologie du Développement, Université P. Sabatier,  
Toulouse, France)

*Early events in spinal cord development*

## **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.

#### **GULBENKIAN PHD PROGRAMME IN BIOLOGY AND MEDICINE**

The Gulbenkian PhD Programme in Biology and Medicine (PGDBM) was launched and is conducted by the Fundação Calouste Gulbenkian together with the Secretaria de Estado do Ensino Superior, the Fundação para a Ciência e Tecnologia e a Fundação Luso-Americana para o Desenvolvimento. The Programme is developed in collaboration with a large number of Universities and Institutes in Portugal and abroad, under the responsibility of the IGC.

The PGDBM recruits students from very diverse university curricula (from Medicine to Physics, Agronomy and Economy) on a fully egalitarian basis. The Programme provides one full year of graduate courses, followed by three years of supervised research work, leading to a doctoral thesis. The graduate courses aim at providing the students with the “common language” of modern biology, and at exposing them to some of the most active research areas and respective leaders. They are organised in 4 “blocks” of 6 weeks each (structural biology, cell biology, genes & development, evolution), followed by a number of one- or two-weeks thematic “modules”, the subject of which may vary (e.g., hematopoiesis, visual system, apoptosis, signalling, genetics of complex diseases). Students are required to take all 4 blocks and 12 thematic modules that they may choose amongst the 20 or so proposed. Teaching takes place at the IGC, at the Instituto de Biologia Molecular e Celular (Universidade do Porto) and at several other University Institutes in Lisbon, Coimbra and Porto, according to the respective course-leaders. A Faculty of over 120 includes a majority of foreign scientists and is also considerably renewed every year (see listings). A limited number of places are available for students outside the Programme for every thematic module. Students who successfully complete the first year of graduate courses proceed to prepare and submit a 3-year research project in a topic of their preference, to be conducted at an institution of their choice, under the supervision of one or two scientists credited by the Programme. The resulting Thesis can be presented at a Portuguese or a foreign University.

The graduate courses in the year 2000 were the last of PGDM's. Already from the summer, the new Gulbenkian PhD Programme in Biomedicine (PGDB, see below), selected its own students who initiated activities in September. The PGDBM, however, will continue to operate until the last of its 103 students completes her or his Thesis.



## **Governing Bodies of the PGDBM**

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### Students of the Gulbenkian PhD Programme in Biology and Medicine for 1999/2000

Filipa Carreira de Avelar Barbosa  
Ana Margarida Gonçalves Campilho  
Tiago Daniel Basto Linhares Carneiro  
Nuno Miguel Maçarico Amorim da Costa  
Ricardo Manuel Benites Costa  
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Andrea Lages Lino Vala

## **Gulbenkian PhD programme in Biology and Medicine for 2000**

Programme for 1999/2000 conducted at the IGC in 2000

### **BLOCK 3**

#### **6-7 January: Introduction to Embryology and Developmental Biology (Oeiras)**

Heads: José António Belo and Isabel Palmeirim (IGC, Portugal)

Faculty: Solveig Thorsteindottir (FCUL, Portugal), Carlos Plancha (IHE-FML, Portugal)

#### **10-21 January: Immunology (Oeiras)**

Head: António Coutinho (IGC, Portugal)

Faculty: Paulo Vieira (IGC, Portugal), Jocelyne Demengeot (IGC, Portugal)

#### **24-28 January: Hematopoiesis (Oeiras)**

Head: Françoise Dieterlen (CNRS, France)

Faculty: Ana Mafalda Cumano (CNRS-Institut Pasteur, France), Thierry Jaffredo (CNRS, France), Paul-Henri Romeo (INSER-CHU, France)

#### **14 February-3 March : Developmental Biology Block (Oeiras)**

Heads: José António Belo and Isabel Palmeirim (IGC, Portugal)

Faculty: Kenneth Kemphues (Cornell Univ., USA), Rolando Rivera Pomar (Max-Planck-Institute, Goettingen, Germany), Herbert Steinbeisser (Max-Planck-Institute, Tuebingen, Germany), Eric Agius (Centre de Biologie du Développement, France), Derek Stemple (NIMR, UK), Martin Catala (URA/CNRS, France), Paulo Vieira (IGC, Portugal), Luis Puelles (Universidad de Murcia, Spain), Michael Kessel (Max-Planck-Institute, Goettingen, Germany), Stefano Piccolo (Universita di Padova, Italy), Bruce Lenhart (IGC, Portugal)

#### **6-10 March : Evo-Devo (Oeiras)**

Heads: Élio Sucena (Univ. Museum of Zoology, UK), Patrícia Beldade (Univ. of Leiden, The Netherlands) and Henrique Teotónio (Univ. California at Irvine, USA)

Faculty: Paul Brakefield (Univ. of Leiden, The Netherlands), David Stern (Univ. Museum of Zoology, UK), Miodrag Grbic (Univ. Western Ontario, Canada), Anthony Long (Univ. of California at Irvine, USA) and Margarida Matos (FCUL, Portugal)

### **BLOCK 4**

#### **13-24 March : Evolution (Oeiras)**

Head: Mário Ramirez (ITQB-UNL, Portugal)

Faculty: Günther Theissen (Max-Planck-Institute, Koeln, Germany), James McInerney (Natural History Museum, UK), Claudio Gomes (ITQB-UNL, Portugal), Francisco Dionísio (Univ. René Descartes-Paris V, France) and Isabel Gordo (Univ. of Edinburgh, UK)

## ***OPTIONAL MODULES***

### **3-7 April : Introduction to the use of computers in molecular biological research - An introduction to bioinformatics (Oeiras)**

Head: Pedro Fernandes (IGC, Portugal)

Faculty: David P. Judge (Univ. of Cambridge, UK) and Jack Leunissen (Univ. of Nijmegen, The Netherlands)

### **17-21 April : Apoptosis - causes and mechanisms of cell death (Oeiras)**

Head: Luis Miguel Martins (ICRF, UK)

Faculty: William C. Earnshaw (Univ. Edinburgh, UK), Michael Hengartner (Cold Spring Harbour Lab., USA), Scott H. Kaufmann (Mayo Medical School, USA) and Stuart Milstein (Cold Spring Harbor Lab, USA)

### **24 April-5 May: From cells to perception – an overview of nervous system structure, function and development at multiple levels (Oeiras)**

Heads: Miguel Castelo-Branco (Max-Planck-Institute, Germany), Guilherme Neves (MRC-Univ. Cambridge, UK) and Miguel Vaz Afonso (Max-Planck-Institute of Neurobiology, Munich, Germany).

Faculty: Tobias Bonhoeffer (Max-Planck-Institute of Neurobiology, Munich, Germany), Yves-Alain Barde (Max-Planck-Institute of Neurobiology, Munich, Germany), Jeff W. Lichman (Washington University School of Medicine, St. Louis, USA), Silvia Arber (Columbia Univ., Columbia, USA), Rainer Goebel (Univ. of Maastricht, The Netherlands), David Fitzpatrick (Duke Univ. Medical Center, North Carolina, USA), Marty Usrey (Harvard Medical School, USA), Clay Reid (Harvard Univ., USA), Simon Laughlin (Univ. of Cambridge, UK) and Mandeep Sagoo (MRC-LMB de Cambridge, UK)

### **8-19 May: Neurobiology of learning and memory (Oeiras)**

Head: Marta Moita (Center for Neural Science, New York Univ., N.Y., USA)

Faculty: Yadin Dudai (Weizman Institute, Israel ), Timothy Bliss (NIMR, Mill Hill, UK), Erich Jarvis (Duke Univ., USA), Thomas J. Carew (Univ. of California, Irvine, USA), Michael S. Fanselow (Univ. of California, Los Angeles, USA) and Alcino Silva (Univ. of California, Los Angeles, USA)

### **22-26 May: Mathematical modeling in biology (Oeiras)**

Head: Rui Alves (Medical School Michigan University, USA)

Faculty: Michael Savageau (Univ. of Michigan, USA), Eberhard Voit (Medical Univ. of South Carolina, USA), Albert Sorribas (Univ. de Lleyda, Spain), António Ferreira (Univ. de Lisboa, Portugal), Armindo Salvador (Univ. Of Michigan, USA)

### **29 May-2 June : Immunity and parasitism (Oeiras)**

Head: Sylviane Pied (INSERM Paris, France; IGC, Portugal)

Faculty: Geneviève Milon (Institut Pasteur Paris, France), Pierre-André Cazenave (Institut Pasteur Paris, France), Serge Morand (CNRS Perpignan, France), Virgilio do Rosário (Inst. Higiene Medicina Tropical, Portugal), Paola Minoprio (Institut Pasteur Paris, France), Laurent Rénia (INSERM Paris, France), Jacques Louis (WHO, Univ. of Lausanne, Switzerland), Derek Wakelin (Univ. of Nottingham, UK), Leslie Harrison (Univ. of Edinburgh, UK) and Murray Selkirk (Imperial College of Science, Technology and Medicine, London, UK)

### **12-16 June: Viral pathogenesis (Oeiras)**

Head: Pedro Simas (IGC, Portugal)

Faculty: Antonio Alcamí (Univ. Cambridge, UK), Covadonga Alonso (INIA, Spain), Helena Browne (Univ. Cambridge, UK), Nick Davis-Poynter (Animal Health Trust, UK), Paul Digard (Univ. Cambridge, UK), Linda Dixon (Institute for Animal Health, UK), Stacey Efstathiou (Univ. Cambridge, UK), Martha Holley (Univ. Cambridge, UK), John McCauley (Institute for Animal Health), Mike Parkhouse (IGC, Portugal), Margarida Saraiva (Univ. Cambridge, UK), Kenneth Smith (Univ. Cambridge, UK)

### **26-30 June: Mutation (Oeiras)**

Head: Matthias Walb (Univ. of California, San Francisco, USA)

Faculty: Graham Walker (MIT, USA), Bruce Ames (Univ. of California, Berkeley, USA), Hein te Riele (Univ. of Amsterdam, The Netherlands).

### **Annual Meeting in Curia (17-23 September 2000)**

As in the past years, the Annual PGDBM Meeting of Curia took place from 17 to 23 September, thereby completing the seventh working year of the Gulbenkian PhD Programme in Biology and Medicine. The scientific sessions counted on the participation of around 100 students, as well as Portuguese University Professors who are involved in the teaching of the graduate courses or in the supervision of theses work.

The last day of the Meeting was dedicated to the distribution of diplomas to students who completed their “graduate courses year” in 2000 with merit, and to pay homage to Prof. Charles Steinberg, Patron of the 7<sup>th</sup> Class of the PGDBM, whose commendation was made by Prof. Matthias Walb (University of California, S. Francisco, USA). In this session, the traditional plenary conference was given by Prof. Gunther Stent (University of California, Berkeley, USA) entitled “Paradoxes of Free Will”.

## **GULBENKIAN PHD PROGRAMME IN BIOMEDICINE**

The PGDBM was launched in 1993 as an experimental programme limited in time, designed to educate 100 PhD's. It had been positively evaluated at mid-term and was again submitted to continuity by an international evaluation committee appointed by its Board of Trustees. The evaluation committee was composed by Drs. Michael Ashburner (University of Cambridge, UK), Matthias Hentze (EMBL, Heidelberg, Germany) and Luis Salicrup (National Institute of Health; Bethesda, MA, USA).

After a 4-day long site-visit, the committee produced a report (see Annex) that provided the basis for the decision of the founding entities to launch another similar programme, now on a regular operating basis without time limits, adapted to the rapidly changing reality of the portuguese scientific committee. The new programme is designated as Programa Gulbenkian de Doutoramento em Biomedicina (PGDB) and the founding protocol was signed at the closing session of the 2000 Curia Retreat of the PGDBM which thus provided the natural link between the two.

The Protocol of the new PGDB was signed by the President of the Fundação para a Ciência e a Tecnologia, Prof. Luís Magalhães, the Director-Geral do Ensino Superior, Prof. Manuel Brandão Alves, the Trustee of the Fundação Luso-Americana para o Desenvolvimento, Dr. Rui Chancerelle de Manchete and the Trustee of the Fundação Calouste Gulbenkian, Dr. Emílio Rui Vilar.

The formal agreement also counted on the presence of the Minister of Science and Technology, Prof. Mariano Gago, of the Secretary of State for High Education, Dr. José Dinis Reis, the Board of trustees of the Programmmes, members of the Scientific and Pedagogic Council of the PGDBM and other University personalities.

### **Introduction and Description**

The Programme provides one full year of graduate courses and laboratory rotations, followed by three years of supervised research work, leading to a doctoral thesis to be submitted at a Portuguese or foreign University.

The graduate courses aim at providing the students with the “common language” of modern biology, and at exposing them to some of the most active research areas and respective leaders. The themes covered include protein chemistry and structural biology, cell and molecular biology, developmental biology, genetics and evolution, and more specialised subjects such as neuroscience, immunology and human disease. Current listings are available here. The courses are based at the IGC and last for the first six months (October - March) of the first year. Then, students engage in 2 laboratory rotations of approximately two and a half months each. Each year, laboratories involved are selected by the Scientific Board and include laboratories in the centres associated with PGDB (Instituto Gulbenkian de Ciência, Instituto de Tecnologia Química e Biológica - Universidade Nova de Lisboa, Instituto de Biologia Molecular e Celular - Universidade de Porto, Instituto de Histologia e Embriologia - Faculdade de Medicina de Lisboa, Centro de Neurociências e Biologia Celular - Universidade de Coimbra). The first year begins and ends at the Annual meeting of the Programme in late September.

Students who successfully complete the first year of graduate courses proceed to prepare and submit a 3-year research project in a topic of their preference, to be conducted at an institution of their choice, under the supervision of scientists credited by the Programme. The resulting thesis can be presented at a Portuguese or a foreign University. In the case of foreign nationals, the research project must be conducted in Portugal.

The Programme provides students with a 4-year fellowship, travel allowances, as well as with university and bench fees. All students report their year's work at the Annual meeting of the Programme.

## **History and Background**

The PGDB is a natural progression from an experimental programme launched in 1993 called the Gulbenkian PhD Programme in Biology and Medicine (PGDBM). This was an initiative of the Instituto Gulbenkian de Ciência aimed at training a limited number of young scientists in particularly relevant areas of modern biomedical sciences. As PGDB, this Programme was funded by the Calouste Gulbenkian Foundation (FCG), by the Ministries of Education and of Science and Technology, and by the Luso-American Foundation for Development (FLAD). This was the first PhD Programme in Portugal, but others have now appeared with similar formats. The Programme lasted for 7 years and enrolled over 100 students and alumni.

The Instituto Gulbenkian de Ciência has a solid tradition of graduate and post-graduate education in biomedical sciences, initiated in the 1960's under the leadership of Prof. N. Van Uden who pioneered these activities in Portugal at the Estudos Avançados de Oeiras. In addition to the PhD Programme, the Institute organises regular scientific meetings of various types on research subjects within its own area of interest (genetic bases of development and evolution of complex systems), namely, genetics of complex traits, neurosciences, immunology, developmental biology; symposia with leaders in the respective fields, "post-doc" meetings and courses, workshops and seminar series.

## **Conditions of eligibility and selection process**

- ?? a 4-5 years undergraduate university education in any specific area, completed within 3 years prior application, at a foreign or Portuguese University; all areas of higher education are accepted;
- ?? exclusive dedication to the Programme for the next 4 years;
- ?? availability for mobility and permanency up to three years at national and foreign centres; foreign citizens, non-resident in Portugal at the date of entry into the Programme, must join laboratories at portuguese institutions;
- ?? candidates are selected on the basis of information submitted in the application and of a personal interview that is strictly necessary for admission.

Sixteen students are selected every year. The strategy of the Programme is to leave a very large degree of personal freedom and span of choices to the individual students, while providing them with a maximum of opportunities, with advice in their decisions, and with support to follow-up their choices.

## **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  
Vítor 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

## **Gulbenkian PhD programme in Biomedicine for 2000**

Programme for 2000/2001 conducted at the IGC in 2000

### **2 October: Introduction to course and IGC - I**

Sukalyan Chatterjee, *IGC, Portugal*

### **3 October: Introduction to Computers**

Pedro Fernandes, *IGC, Portugal*

### **4 October: Introduction to course and IGC - II**

Sukalyan Chatterjee, *IGC, Portugal* / Jorge Carneiro, *IGC, Portugal*

António Coutinho, *IGC, Portugal*

### **5 – 7 October: Elementary Formal Genetics**

Faculty: John Stewart, *IGC, Portugal*

### **9-14 October: Theoretical Biology**

Head: Jorge Carneiro, *IGC, Portugal*

Faculty: S. Bobrovnik, *IGC, Portugal*, José Faro, *IGC, Portugal*, Gabriela Gomes, *University of Warwick, UK*, Rui Dilão, *IST, Portugal*, Jonas Almeida, *FCT-UNL, Portugal*, Denis Thieffry, *Univ. de la Méditerranée, France*

### **16-20 October: Introduction to Cell Biology**

Faculty: Maria do Carmo Fonseca, *IHEFM-UNL, Portugal*

### **23-28 October: Transcription and control of Gene Expression**

Faculty: Sukalyan Chatterjee, *IGC, Portugal*, Eric Lam, *Ludwig Inst. for Cancer Research, UK*

### **30 October – 3 November: Post –transcriptional events**

Faculty: Maria do Carmo Fonseca *IHEFM-UNL, Portugal*

### **6-10 November: Protein Traffic**

Head: José Leal, *Imperial College, UK*

### **13-17 November: Signal Transduction**

Head: Marcus Thelen, *Inst. for Research in Biomedicine, Switzerland*

Faculty: Gioacchino Natoli, *Inst. for Research in Biomedicine, Switzerland*, Matthias Peter, *Inst. Suisse Recherche Experimentale sur le Cancer, Switzerland*, Edgar da Cruz e Silva, *Aveiro, Portugal*

### **20-24 November: Cytoskeleton and Molecular Motors**

Head; Helena Soares, *IGC, Portugal*

Faculty: Vic Small, *Institute Molec. Biol., Austria*, Peter Jordan, *Portugal*, Jesús Avila, *CBM Spain*, Lisete Fernandes, *IGC, Portugal*



**27 November–1 December: Cell Cycle**

Heads: Alvaro Tavares, *IGC, Portugal* / Claudio Sunkel *IBMC, Portugal*

Faculty: David Glover, *Univ. Cambridge, UK*, Magdalena Zerricka-Goetz *Univ. Cambridge, UK*, Jon Pines, *Wellcome/CRC Institute, UK*, Rui Gomes, *FCL, Portugal*

**4-8 December: Cell Death**

Faculty: Yuri Lazebnik, *CSHL, USA*

**11-22 December: Structure and Function of Proteins I & II**

Head: António Xavier, *ITQB-UNL, Portugal*

Faculty: M<sup>a</sup> Arménia Carrondo, *ITQB-UNL, Portugal*, Inês Cardoso Pereira, Miguel Teixeira, *ITQB-UNL, Portugal*, Lúcia Saraiva, Claudio Gomes, *ITQB-UNL, Portugal*, Rita Lemos, Carlos Frazão, Margarida Archer, Francisco Enguita, Francisco Morais, Bauke Dijkstra, *Univ. Groningen, The Netherlands*, Jeffrey Stock, *Princeton University, USA*, Ana Margarida Damas, Claudio Soares, António Baptista, *ITQB-UNL, Portugal*

## TEACHING PROGRAMME ON BIOINFORMATICS

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

### **Bioinformatics Training Courses**

Local organisation: Pedro Fernandes and Isabel Marques

Place: IGC

#### ***June***

##### *Introduction to Sequence Analysis*

Faculty: David Judge, University of Cambridge, UK and Phil Cunningham, Kings College, London, UK

#### ***October***

##### *Gene Identification and Protein Functional Analysis*

Faculty: Genís Parra Farré, IMIM-UPF, Barcelona, Spain; Enrique Blanco García, IMIM-UPF, Barcelona, Spain and Alfonso Valencia, CNB-UAM, Madrid, Spain

#### ***November***

##### *Molecular Evolution*

Faculty: James McInerney, Nat. Univ. of Ireland, Maynooth, Ireland and T. Martin Embley, Natural History Museum, London, UK

##### *Gene Identification and Linkage Analysis*

Faculty: Bernard North, Royal London Hospital, London UK and Gilareh Koochaki, Human Genome Mapping Project Resource Centre, Cambridge, UK

##### *Human Genome Bioinformatics*

Faculty: Martin Bishop, HGMP-RC, Cambridge, UK; Phil Cunningham, Kings College, London, UK; Roderic Guigo, IMIM-UPF, Barcelona, Spain; Luciano Milanesi, CNR, Italy and Clare Sansom, University of Cambridge, UK

#### ***December***

##### *Sequence Analysis Techniques*

Faculty: David Judge, University of Cambridge, UK and Phil Cunningham, Kings College, London, 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 to be 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 participated with the following training sessions and students:

##### **Genética das Doenças Humanas**

**IGC Member: Astrid Moura Vicente**

3-14 July

*Maria João Soares*

Escola Secundária da Cidade Universitária - Lisboa

*Joana Martins*

Escola Secundária Luís de Freitas Branco - Paço d'Arcos

17 a 28 de Julho

*Nuno Jacinto*

*Lara Lourenço*

Escola Secundária Camilo Castelo Branco - Carnaxide

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

**IGC Member: Bruce Lenhart**

7-18 August

*Sónia de Castro Girante*

Escola Secundária de Santo António - Barreiro

*Susana Monteiro*

Escola Secundária de Cascais

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

**IGC Member: Alvaro Tavares**

28 August – 15 September

*Sara Isabel Morais Silva*

Escola Secundária de S. João do Estoril

*Rute Vanessa Alves Martins*

Escola Secundária da Cidade Universitária - Lisboa

## **Estudo do Desenvolvimento em Plantas**

**IGC Member: José Feijó**

28 August – 15 September

*Catarina Torre do Vale*

Escola Secundária de Cascais

*Sofia Margarida Baptista Leite*

Escola Secundária Carolina Michaelis, Porto

## **Project “Health in the XXI Century: a vision from the European Youth”**

**January – May 2000**

The project “Health in the XXI Century: a vision from the European Youth” aimed to create opportunities for German and Portuguese students (secondary education) to learn about recent developments of Science and Technology in what concerns their capacity to give answers to problems in different Health areas.

This project was carried out 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 and resulted in the creation of products with the students' views on the most recent scientific research and technological developments related to health issues.

### **IGC Member:**

João Pedro Simas

### **Schools in the project:**

Cascais Secondary School

Christian Gymnasium Hermannsburg (Germany)

Gymnasium Bad Essen (Germany)

Virtually all-living organisms, when studied carefully, have viral parasites, and so these smallest of living entities exert significant forces upon all living forms, including themselves. The medical, veterinary and plant consequences of viral infections have altered our history and have resulted in extraordinary efforts on the part of virologists to study, understand, and eradicate these agents. In order to survive in nature a virus must be able to spread quickly from host to host or survive outside the host or survive inside the host.

Three general conditions must be fulfilled for a virus to persist long-term in a host. The virus must be able to infect host cells without being overtly cytolytic, there must be mechanisms for maintaining the viral genome in the host cells and the virus has to avoid detection and elimination by the host immune system.

Our research efforts concentrate in a group of viruses, herpesviruses, that once infecting a host can evade the host defence response and persist for the lifetime of the host.

The virus we specifically study, designated murine gammaherpesvirus 68, has targeted a cell of the immune system, that produces antibodies to combat microbial infections, to live in.

**IGC Member:**

Astrid Moura Vicente

**School in the project:**

Baixa da Banheira Secondary School

Certain chronic diseases, such as cystic fibrosis, Duchenne's muscular dystrophy, diabetes or schizophrenia, are inherited within families. This means that there are one or more modified – mutated - genes, which are transmitted and are responsible for the disease. The gene mutations lead to structural and/or functional changes in the encoded proteins, which in turn are responsible for the physiological disturbances expressed as disease symptoms.

Genes and mutations leading to some diseases have been found. As an example, cystic fibrosis is caused by mutations in the CFTR gene, which encodes a cell membrane protein involved in ion transport. The defective protein produced by the gene mutations leads to deficient ion transport across epithelial cells in airways, pancreas and gut, and consequently to chronic pulmonary, pancreatic and gastrointestinal disease. The identification of the CFTR gene and mutations nowadays allows the genetic testing of individuals at risks, and will eventually lead to gene therapy effective for the disease.

The identification of genes for multifactorial disorders such as diabetes and schizophrenia will require more sophisticated genetic strategies. There are multiple genes involved in these complex disorders, which individually may increase susceptibility, but are likely not necessary or sufficient to cause the disease. As an example, some genes involved in diabetes have been identified and mapped to specific genome regions (the MHC complex genes and the insulin gene). However, these genes are responsible for only a fraction of the heritability of diabetes, and their biological mechanisms for influencing susceptibility have not been properly understood. Other susceptibility genes with minor impact for the disease must be found to allow for a precise understanding of its pathophysiology. Similar pictures are drawn for other complex disorders like Systemic Lupus Erythematosus, or schizophrenia or autism. The more complex the phenotype, the harder it will be to disentangle the individual contribution of genes for the disease. This type of genetic research will certainly benefit greatly from the human genome sequencing programme, likely to be completed within the next two years.

**IGC Member:**

Jocelyne Demengeot

**Schools in the project:**

Amadora Secondary School

Gymnasium Bad Essen

From neurons in the brain to muscle fibers, all the body's cells are genetically identical, with two exceptions: spermatozooids and ovules that together form a new embryo, and the lymphocytes, the cellular protagonists of the immune system.

Poisoning, stabbing, confinement, or simply swallowing and digesting- the body has many different ways of ridding itself of undesirable guests or unruly residents. The problem consists in knowing who is who, and leaving the law-abiding inhabitants in peace. The bacteria, viruses, and tumors (among many others) that can cause serious, even lethal, damage reproduce at a much higher speed than us, a bacterial cell can duplicate in 20 minutes, a human being (or two) can reproduce only after almost 20

years, and even then only once a year. The immune system tracks and reacts to this in a fascinating and dangerous way: it creates its own diversity, without waiting for the rest of the body to do so, by shuffling the genes that it uses to recognize pathogens. To understand how these randomly generated genes acquire meaning, how the system decides if each one of them will recognize a part of a pathogen, or a part of the body, or neither, is one of the main challenges of modern immunology. Immunologists look for specific genes and cells that influence this decision-making process, and the process of making new lymphocytes (including how genes are actually shuffled). Powerful tools in this search are our ability to generate mice with or without certain genes, and to identify and separate different cell types. In these animal systems we can induce lymphocytes to make the wrong decisions, leading to self-destructive processes, mimicking human conditions which we try to comprehend, like multiple sclerosis or diabetes. In this way we hope to be able to manipulate the immune system to either boost it when it is inefficient (as in Malaria or AIDS) or to tune it down when it is harmful (as in auto-immunity or immunity to organ transplants).

**IGC Member:**

Isabel Palmeirim

**School in the project:**

Pinhal Novo secondary school

In Human beings, Mice, Chickens, Flies or any other organism, the time for the formation of the respective embryos is constant and carefully regulated. Each step of the embryonic development only produces the wanted effect if it takes place in the adequate place and the adequate moment. Somites are embryonic structures in pairs, repetitive structures which originate the segmented structures of the vertebrae (vertebrae, intervertebral disks and ribs) and all the skeletal muscles of the body. With a well controlled pace, every hour and a half, a pair of somites appears in the chicken embryo (every two hours and a half in the human embryo). The time control mechanism of this periodic and symmetric phenomenon which leads to the formation of the somites was completely unknown until the discovery of a gene that through its cyclic expression, with hour and a half cycles, reveals the existence of an embryonic clock which commands the pace of some events during the formation of the chicken's embryo.

Within the context of this project the following debates and conferences took place:

**Debates:**

30 May

João Pedro Simas

*Health Sciences - from research to its application: difficulties and obstacles*

Pavillion of Knowledge Ciência Viva – Parque da Nações, Lisbon, Portugal

5 October

João Pedro Simas

*Future Challenges for the Health Sciences: Sorting Fact from Fiction*

Pavillion of Portugal in Hannover, Germany

## **Conferences:**

28 October

João Pedro Simas

*Herpes*

Pavillion of Knowledge Ciência Viva – Parque da Nações, Lisbon, Portugal

11 November

Astrid Moura Vicente

*Diseases of Behaviour*

Pavillion of Knowledge Ciência Viva – Parque da Nações, Lisbon, Portugal

## **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 types of schools are organised. In 2000, the following have been at the IGC:

Escola Secundária da Bela Vista – Setúbal (25 students)

Escola Secundária Fernando Lopes Graça – Parede (18 students)

Escola Secundária Fernando Lopes Graça – Parede (24 students)

Escola Secundária Fernando Lopes Graça – Parede (24 students)

Escola Secundária de Carcavelos - Carcavelos (20 students)

Escola Secundária Miguel Torga – Queluz (54 students)

Escola Secundária Sebastião da Gama – Setúbal (55 students)

Escola Secundária N<sup>a</sup> S<sup>a</sup> da Encarnação – Benedita (52 students)

Escola Secundária Alves Martins – Viseu (50 students)

Escola Secundária de Santa Maria – Sintra (20 students)

Escola Secundária de Santa Maria – Sintra (21 students)

Colégio Marista de Carcavelos – Carcavelos (21 students)

Escola Secundária de Carcavelos – Carcavelos (20 students)

Escola Secundária de Miraflares – Miraflares (45 students)

(total: 450 students)

### **Other Schools**

ISCSP - Instituto Superior de Ciências Sociais e Políticas, Lisbon - (20 students of Antropobiology)

## THESES

The following Theses were prepared in part at the IGC and were presented in 2000:

### *PhD Theses:*

**Francisco Campos Pereira Dionísio** "Horizontal gene transfer in enterobacteria: homologous recombination and dynamics of conjugative plasmids", University of Paris VII-Denis Diderot, France, December 2000.

**Brigitte de Lima** "Contribuição para a caracterização molecular de proteínas tipo Mob1 em *Drosophila melanogaster*", Universidade de Lisboa, Lisbon, Portugal, July 2000.

**Mariana Faria** "Novos mutantes mitóticos em *Drosophila melanogaster*", Universidade de Lisboa, Lisbon, Portugal, July 2000.

### *BsC Theses:*

**Ana Cristina Borges** "Estudo de genes da família *cerberus-like* na padronização do cérebro de ratinho *Mus musculus*", Faculdade de Ciências da Universidade de Lisboa, Lisbon, Portugal, July 2000.

**Rui Soares** "Tolerância à alotransplantação pela indução da expressão de IL-4 e IL-10 em ratinhos transgénicos", Faculdade de Ciências da Universidade de Lisboa, Lisbon, Portugal, December 2000.



## PARTICIPATION IN ACADEMIC COMMITTEES

### **José A. Belo**

Member of the Jury of the M.Sc. Thesis, Ana Rolo, Universidade Lusófona de Humanidades e Tecnologias, Lisbon, Portugal

### **Sukalyan Chatterjee**

Member of the Jury of the Ph.D. Thesis, Nandita Das, Jadavpur University, Calcutta, India

### **António Coutinho**

Member of the Jury of the Ph.D. Thesis, Jorge Pedrosa, Universidade do Porto, Porto, Portugal

Member of the Jury for the Concurso para Prof. Associado Área Biologia Celular e Biotecnologia Vegetais, FCUL, Lisbon, Portugal

Member of the Jury of the Ph.D. Thesis, Rui Alves, FCUL, Lisbon, Portugal

Member of the Jury of the Ph.D. Thesis, Ana Sousa, Faculdade de Medicina de Lisboa, Lisbon, Portugal

### **Jocelyne Demengeot**

Member of the Jury of the Ph.D. Thesis, Bernardo Rodrigues Peixoto, Universidade do Porto, Porto, Portugal

### **José A. Feijó**

Member of the Jury of the Ph.D. Thesis, Teresa Valdiviesso, Estação Florestal Nacional, Instituto Nacional de Investigação Agrária, Lisbon, Portugal

Member of the Jury of the Ph.D. Thesis, Clélia Neves, Instituto Superior de Agronomia, Universidade Técnica de Lisboa, Lisbon, Portugal

### **João Pedro Simas**

Member of the Jury of the Ph.D. Thesis, Manuela Florido, Universidade do Porto, Porto, Portugal

Member of the Jury of the Ph.D. Thesis, Ana Figueiredo, Universidade do Porto, Porto, Portugal

Member of the Jury of the Ph.D. Thesis, Alexandra Teixeira, Universidade do Porto, Porto, Portugal

### **Álvaro Tavares**

Member of the Jury of the Ph.D. Thesis, Isabel Salazar, FCUL, Lisbon, Portugal

Member of the Jury of the M.Sc Thesis, Sandra Martins. Universidade Lusófona de Humanidades e Tecnologias, Lisbon, Portugal

## SYMPOSIA, CONFERENCES AND MEETING ORGANISED BY THE IGC

### *February*

#### **Conferences of the IGC Scientific Advisory Board 27-29 February 2000**

*Lipid rafts in membrane traffic and signalling.*

Kai Simons (EMBL/Max-Planck Dresden, Germany)  
Instituto Gulbenkian de Ciência, Oeiras

*Chlamidia pneumoniae immune protection with paradoxical components*

Hans Wigzell (Karolinska Institute, Sweden)  
Instituto Gulbenkian de Ciência, Oeiras

*Perspectives for young researchers*

Sydney Brenner (Molecular Sciences Institute, Berkeley, USA)  
Fundação Calouste Gulbenkian, Lisbon

### *March*

#### **Workshop “Autism Diagnostic Interview – Revised” Instituto Gulbenkian de Ciência, 27-30 March 2000**

Organisers: Astrid Moura Vicente (*IGC, Portugal*) and Guiomar Oliveira (*Hospital Pediátrico de Coimbra, Portugal*)

A workshop entitled “Autism Diagnostic Interview - Revised” (ADI-R) took place at the Instituto Gulbenkian de Ciência (IGC). This workshop was the first to be carried out within the research project Genetic Epidemiology of Autism, established at the IGC in collaboration with the Hospital Pediátrico de Coimbra and the Hospital do Divino Espírito Santo, the main hospital in the Azores. The participants were 12 health professionals involved in the diagnosis, evaluation and education of autistic children, including medical doctors, psychologists, special education teachers and speech therapists from various hospitals in Portugal.

The goal of this workshop was to prepare these health professionals in the use of a structured diagnostic interview specific for autism spectrum disorders (ASD). This instrument allows a uniform diagnosis of ASD, which is fundamental for research purposes. The ADI-R will be used in the assessment and diagnosis of ASD children in the above referred epidemiological and genetic study, and will allow the direct comparison of results with international research groups on the genetics of autism. The ADI-R workshop was taught by Professor C. Lord from the University of Chicago, a leading researcher in autism and a co-author of this interview.

The initiative was completed with a one-day seminar entitled “Outro dia com o autismo infantil”, where communications were presented by Professor Lord and by several members of the clinical and research team from the IGC and Hospital Pediátrico de

Coimbra. This seminar took place on March 30<sup>th</sup>, 2000, at the Grande Hotel das Termas do Luso, and was open to the general public. The high rate of attendance was an indication of the interest of clinicians, psychologists, educators and parents on new research developments for autism.

## *April*

### **The First Gulbenkian Antibody Workshop Symposium Convento da Arrabida, 8-12 April, 2000.**

The creation of modernism in Immunology owes a lot to the series of "Antibody Workshops", which were implanted in the discipline of the principles and approaches of the birth of molecular biology at the time. With a very limited participation, the "workshops" marked an age not only by their impact but also by the style of open discussion, the importance of the topics approached, the quality of the participants, the intellectual rigour.

Professor Melvin Cohn, of the Salk Institute for Biological Studies, USA, responsible for the initiative and organisation of these historic conferences proposed to initiate a new Antibody Workshop series in Portugal.

The first of the series took place from 8 to 12 April at the Convento de Arrabida, organised by the IGC and the Science Department of the FCG, with the support of the Foundation for Science and Technology, the Associação dos Doentes com Lupus e da Fundação Oriente, on the generation of diversity in the immune system, which involved the participation of 16 specialists of great international repute.

Organiser: Melvin Cohn (*Salk Institute, USA/IGC, Portugal*)

Participants: Melvin Cohn, *The Salk Institute, San Diego, USA*; Antonio Coutinho, *Instituto Gulbenkian de Ciência, Oeiras, Portugal*, Martin Flajnik, *Dept Microbiol & Immunol, Baltimore, USA*, Stephen M. Hedrick, *U Cal-San Diego, La Jolla, USA*, H. Hengartner, *UniversitätsSpital Zürich, Zürich, Switzerland*, Katherine L. Knight, *Loyola University, Maywood, USA*, Phillippe Kourilsky, *Institut Pasteur/INSERM U 277, Paris, France*, Don Mason, *Medical Research Council, University of Oxford, Oxford, UK*, Ed Palmer, *Basel Institute For Immunology, Basel, Switzerland*, Peter Parham, *Stanford University, Stanford, USA*, Martin Weigert, *Princeton University, Princeton, USA*, J.C. Weill, *INSERM U373, Institut Necker-Université Paris 5, Paris, France*, Ian A. Wilson, *Scripps Res Inst, La Jolla, USA*, Hans G. Zachau, *Univ Munich, Munich, Germany*.

*July*

### **III Gulbenkian Seminar on Science and Consciousness**

**Fundação Calouste Gulbenkian, Lisbon, 5 July**

Series of public Conferences organized jointly with the Serviço de Ciência of the Fundação Calouste Gulbenkian:

**António Coutinho**

Instituto Gulbenkian de Ciência, Portugal

*Opening remarks*

**Jean-Pierre Changeux**

Collège de France / Institut Pasteur, Paris, France

*Development of the Brain, Selection vs Instruction*

**Miguel Castelo-Branco**

F. Psychologie, Neurocognitie, University of Maastricht, Maastricht, The Netherlands

*From Neural Representations to Conscious Perception*

**Francisco J. Varela**

LENA ( Neurosciences Cognitives et Imagerie Cérébrale, Paris, France)

*Can Mind be a Causal Agent*

**Jaak Panksepp**

Dept. of Philosophy and Cognitive Science, Bowling Green State University, Ohio, USA

*How the Brain creates Emotional Feelings*

**Michel Le Moal**

Psychologie des Comportements Adaptatifs, University of Bordeaux, Bordeaux, France

*Drug Dependence: the Quintessential Biobehavioural Disorder*

### **III Gulbenkian Symposium on Cognitive Neuroscience: Consciousness**

**Convento da Arrábida, 6-8 July**

Every year since 1998 the IGC, together with the Serviço de Ciência of the Fundação Calouste Gulbenkian and the Fundação Oriente, organizes a regular Symposium series aimed at convening some of the world' specialists in various aspects of Cognitive Neurosciences/Consciousness. Retreated for a few days into a pleasant and quiet setting, the group informally discusses current problems, approaches and progresses, recent developments and difficulties, eventually drawing a chart of essential steps to accomplish in the field.

Organisers: Jean-Pierre Changeux (*Collège de France/ Institut Pasteur, Paris, France*) and Francisco Varela (*LENA-Neurosciences Cognitives et Imagerie Cérébrale, Paris, France*).

Participants: João Caraça, *Serviço de Ciência, FCG, Lisbon, Portugal*, Miguel Castelo-Branco, *University of Maastricht, The Netherlands*; Jean-Pierre Changeux, *Collège de France/ Institut Pasteur, Paris, France*; Gaetano di Chiara, *Dept. Tossicologia, U. Cagliari, Italy*; António Coutinho, *Instituto Gulbenkian de Ciência, Oeiras, Portugal*, Shaun Gallagher, *Canisius College, New York, USA*; Thomas Gisiger, *Institut Pasteur,*

Paris, France; Marc Jeannerod, *INSERM U.94, Vision et Motricité, Bron, France*; Michel Le Moal, *University of Bordeaux, Bordeaux, France*; Jaak Panksepp, *Bowling Green State University, Ohio, USA*; Alvaro Pacual-Leone, *Beth Israel Hospital, Boston, USA*; Edmund Rolls, *University of Oxford, UK*; Yves Rossetti, *INSERM U.94, Espace et Action, Bron, France*; Jacques Vaclair, *University of Provence, Provence, France*; Francisco Varela, *LENA-Neurosciences Cognitives et Imagerie Cérébrale, Paris, France*.

## ***September***

### **Annual Retreat of the Immunology Groups at the IGC**

The first annual retreat of the Immunology groups at the IGC was held from the 15<sup>th</sup> to the 17<sup>th</sup> of September in Obidos. This retreat was organized by IGC immunologists, as a weekend-long brain storming on the state of Immunology and the position of the institute in the field. A month in advance, questions of interest were collected from all students and group leaders, from which the following sessions were organised.

Lymphocytes commitment and fate determination. Homeostasis of the immune system;

Establishment and selection of antigen receptor repertoires;

(Autoimmune) disease: approaches for diagnostic and therapy;

Cytokines and immune responses;

Autoimmunity and Tolerance to self components.

For each session, a senior scientist presented a summary of the state of the art, giving emphasis on open questions. Discussions followed, illustrated by students' short presentations of ongoing studies at the IGC. The overall format was informal and friendly. Twenty seven people attended the event, 21 IGC members and 5 visiting scientists who provided numerous and pertinent external comments. This first IGC retreat was a success both on the strengthening of interactions and for the establishment of a common language. More importantly, it reinforced the intellectual and experimental links between the different groups.

Organisers: IGC Immunologists

Participants *from the IGC*: Thiago Carvalho; John Stewart; Antonio Coutinho; J Pedro Pereira; Jose Faro; Sergey Bobrovnyk; Deborah Braun; Astrid Vincente; Kalet Leon; Dinis Calado; Matthias Haury; Joao Sousa; Jorge Carneiro; Iris Caramalho; Shohei Hori; Jocelyne Demengeot; Magda Carlos; Nadia Duarte; Manuel Rebelo; Tatiana Vasssilevskaia; Gil Castro; Claudia Vaz.

Visitor Scientists at the IGC: Nelson Vaz, *Universidade federal de Minas Gerais, Brasil*; Constantin Fesel, *Weisman Institute, Israel*; Werner Haas, *Linden Technologies, USA*; Ludo Pagie, *Santa Fe Institute, New Mexico, USA*; Can Kesmir *Utrecht University, The Netherlands*.

## *October*

### **III Gulbenkian Autumn Meeting: Cellular Differentiation IGC, Oeiras, 15-17 October 2000**

In a determined strategy for modernity and internationalisation, attention is being given to emerging fields in biology and to identifying young and creative scientists. To make this possible, and in order to promote interactions with the Portuguese scientific community, the Institute organises scientific workshops open to all. This year 16 young scientists from Europe and the USA interacted and gave lectures on Cellular Differentiation to an audience composed of students and scientists from the IGC and other institutes and universities in Lisbon (Instituto Ricardo Jorge, Instituto de Citologia e Embriologia da Universidade de Medicina, Faculdade de Agronomia), Oporto and Evora. The meeting was sponsored by the Gulbenkian Foundation and the FCT.

Organiser: Vasco Barreto (*IGC, Portugal/Institut Pasteur, Paris, France*)

Participants: Philip Benfey, *NY University, NY, USA*; Andrea Brand, *Wellcome/CRC Institute, Cambridge, UK*; Francesco Colucci, *Institut Pasteur, Paris, France*; Marie Dellatre, *Institut Jacques Monod, Paris, France*; Paul Fairchild, *University of Oxford, Sir William Dunn School of Pathology, UK*; Bob Goldstein, *University of North Carolina, Chapel Hill, USA*; Adriano Henrique, *ITQB, Oeiras, Portugal*; Stephan Jungbluth, *King's College, London, UK*; Barbara Kee, *University of California at San Diego, La Jolla, CA, USA*; Rueyling Lin, *Southwestern Medical Center, Dallas, USA*; Perpetua do O, *University of Umea, Umea, Sweden*; Isabel Palmeirim, *IGC, Oeiras, Portugal*; Fernando Roch, *University Museum of Zoology, Cambridge, UK*; Shahragim Tajbakahsh, *Institut Pasteur, Paris, France*; Elly Tanaka, *Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany*; Aaron Zorn, *Wellcome/CRC Institute of Cancer and Developmental Biology, Cambridge, UK*

## *November*

### **II Gulbenkian Symposium on Dominant Tolerance Convento da Arrábida, 1-5 November**

For several years, a small number of research groups have been calling the attention to immunological phenomena in which tolerance can be transferred by T cells from tolerant donors. This form of tolerance is thus designated by “dominant”, and it obviously pertains to the whole animal, rather than individual lymphocytes. The experimental systems showing this behaviour are quite heterogeneous, ranging from protection of autoimmune diseases that spontaneously develop in monoclonal TCR transgenic mice, to induction of tolerance to allogenic skin grafts by thymic epithelium in embryos or by anti-CD4 antibodies in the adult, to prevention of inflammatory disease caused by lymphopenic states. The biomedical importance of this topic derives from its potential value in strategies for intervention in a number of increasingly frequent autoimmune diseases and in cancer immunotherapy. The novelty of this area called for a second meeting gathering essentially all specialists, aiming at drawing the limits of current theory, approaches and results. The IGC organised the second Symposium in this series

which was supported by the Associação Portuguesa de Doentes com Lupus and the Fundação Oriente.

Organiser: António Coutinho (*IGC, Portugal*)

Participants: Rita Andreia, *Associação de Lupus, Portugal*; Bernd Arnold, *Institut für Immunologie und Genetik, Germany*; Jorge Carneiro, *IGC, Portugal*; Irun Cohen, *Weizmann Institute of Science, Israel*; Jocelyne Demengeot, *IGC, Portugal*; Jean Charles Guéry, *INSERM U28, Hôpital Purpan, France*; Werner Haas, *Linden Technologies, USA*; Matthias Haury, *IGC, Portugal*; Gerard Hoyne, *University of Edinburgh Medical School, UK*; Shohei Hori, *IGC, Portugal*; Frederik Ivars, *Lund University, Sweden*; Juan Lafaille, *NYU Medical Center, Skirball Institute, USA*; Don Mason, *University of Oxford, UK*; Shimon Sakaguchi, *Kyoto University, Japan*; Eli Sercarz, *La Jolla Institute for Allergy and Immunology, USA*; Herman Waldmann, *Sir William Dunn School of Pathology, UK*.

Students: Oliver Annaker, *Institut Pasteur, France*; Ricardo Araujo, *Institut Pasteur, France*; Iris Caramalho, *IGC, Portugal*; Thiago Carvalho, *IGC, Portugal*; Luis Graça, *Sir William Dunn School of Pathology, UK*; Francisco Quintana, *Weizmann Institute of Science, Israel*; Manuel Rebelo, *IGC, Portugal*; João Sousa, *IGC, Portugal*; Leigh Stephens, *University of Oxford, UK*.

## ***December***

### **III UNESCO/Gulbenkian/UFRJ Research Course**

#### **The biology of embryonic and adult stem cells**

#### **Rio de Janeiro, 11-15 December**

The Gulbenkian Professorship at the Department of Anatomy of the Federal University of Rio de Janeiro (UFRJ), continued to operate in close collaboration with the UNESCO chair and with various Brazilian Universities and research laboratories in Latin America. In its 3<sup>rd</sup> year of activity, a lecture course was organised on “Stem cells” with the attendance of over 120 students.

Organisers: Vivaldo Moura-Neto (*UFRJ, Brazil*) and Nicole le Douarin (*Academie des Sciences de l’Institut de France, France*)

Participants: Nicole le Douarin, *Academie des Sciences de l’Institut de France, France*; Charles Babinet, *Institut Pasteur, Paris, France*; Jean David, *CNRS, France*; Elisabeth Dupin, *Institut d’Embryologie Cellulaire et Moléculaire du CNRS, France*; Fernando G. de Mello, *UFRJ, Brazil*; Clive Svenden, *Univ. Cambridge, UK*; Arturo Alvarez Buylla, *The Rockefeller University, New York, USA*; Jonas Frisen, *Karolinska Institute, Sweden*; Fiona Watt, *Imperial Cancer Research Fund, London, UK*; Roberto Lent, *UFRJ, Brazil*; Françoise Dieterlen, *Institut d’Embryologie Cellulaire et Moléculaire du CNRS, France*; Irving Weissman, *Stanford University School of Medicine, USA*; Laurie Milner, *The Fred Hutchinson Cancer Research Center, Seattle, USA*; Radovan Borojevic, *UFRJ, Brazil*; Paolo Bianco, *La Sapienza University, Rome, Italy*; Jose Minguell, *University of Chile, Santiago, Chile*; Antonio Coutinho, *IGC, Portugal* and Vivaldo Moura-Neto, *UFRJ, Brazil*.

**III UNESCO/Gulbenkian/UFGM Symposium**  
**Conservation Biology**  
**Belo Horizonte, 13-14 Dezembro**

The Gulbenkian Professorship at the Instituto de Ciências Biológicas of the Federal University of Minas Gerais (UFGM) in Belo Horizonte, Brazil, co-organized this last year a Symposium of Conservation Biology, attended by some 60 students from various Brazilian Universities.

Organisers: Geraldo W. Fernandes (*UFGM, Brazil*) and Thiago Carvalho (*IGC, Portugal*)

Participants: Otto Gottlieb, *Fiocruz, Rio de Janeiro, Brazil*; James Sanderson, *Conservation International, Washington DC, USA*; Gustavo Fonseca, *Conservation International, Washington DC, USA*; Geraldo W. Fernandes, *UFGM, Belo Horizonte, Brazil* and Bráulio Sousa Dias, *Ministério do Meio Ambiente, Brasília, Brazil*.



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

### ***January***

Coutinho A.

*Conclusion.*

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

Palmeirim I.

*Mechanisms of embryonic development.*

Escola Secundária D. João de Castro, Lisbon, Portugal

### ***February***

Coutinho A.

*Natural immunological tolerance.*

Course on Selected Problems in Immunology 2000, Stockholm University, Sweden

Coutinho A.

*Vacinação: do empirismo ao cientifismo.*

I Congress "A Vacinação e a Saúde em Portugal: Novas Vacinas – Novo Plano Nacional de Vacinação" Fundação Calouste Gulbenkian, Lisbon, Portugal

Coutinho A.

*O que sabemos e o que falta saber sobre a génese da diabetes auto-imune.*

IV Congresso Português de Diabetes, Funchal, Portugal

Cox G., Dibbayawan T., Harper J., Marc J. and Feijó J.A.

*Multiphoton Microscopy Of Cell Division In Plant Cells.*

Australian Conference on Electron Microscopy, Canberra, Australia

Parkhouse R.M.E.

*Protection and diagnosis of cysticercosis.*

Faculdade de Medicina Veterinária, UTL, Lisbon, Portugal

### ***March***

Coutinho A.

*A Life in Science.*

2nd Workshop "Educação pela Ciência", Faculdade de Medicina Universidade de Lisboa, Lisbon, Portugal

Coutinho A.

*Tolerance and autoimmunity.*

Excellence in Immunology Lecture Series, The University of Texas Southwestern Medical Center at Dallas, Dallas, USA

Fernandes P. and Marques I.

*Bioinformática e biotecnologia.*

Encontro Nacional de Biotecnologia, Núcleo de Engenharia Biotecnológica, Algarve, Portugal

Fernandes P.

*Sistemas de informação em biodiversidade.*

Encontro Nacional de Biotecnologia, Núcleo de Engenharia Biotecnológica, Algarve, Portugal

## ***April***

Boavida L.C. and Feijó J.A.

*Quercus suber L.: Pre and post-fertilization mechanisms following intraspecific and interspecific pollinations.*

XVIth International Congress on Sexual Plant Reproduction, Banf, Canada

Coutinho A.

Chairman of the Session “Engenharia de Tecidos e Transplante de Órgãos” at the conference “Manipulação da Vida Humana”, FLAD - Fundação Luso-Americana para o Desenvolvimento, Lisbon, Portugal

Coutinho A.

*A unidade da vida e medicina preventiva.*

Auditorium of the Santa Casa da Misericórdia de Vila Flor, supported by the Town Hall of Vila Flor, Portugal

Cordeiro M. S. And Feijó J.A..

*Chloride and potassium fluxes and their role in pollen tube growth.*

XVIth International Congress on Sexual Plant Reproduction, Banf, Canada

(Awarded The Hf Linskens Prize For The Best Talk.)

Feijó J.A.

*A biophysical approach to cell growth: the pollen tube paradigm.*

Instituto Superior Técnico, Lisbon, Portugal

Simas J.P.

*Latency related gene expression and a novel strategy of chemokine blockade by a lymphotropic herpesvirus.*

Nephrology, Immunology and Transplantation Seminars, Cambridge Institute for Medical Research, Cambridge, UK

Tavares A., Avides M.C. and Glover D.  
*Centrosome microtubule nucleation by polo and asp proteins.*  
The Cell Division Cycle, CNRS Conference Jacques Monod, Roscoff, France

## **May**

Belo J.A.  
*Mechanisms of head induction in vertebrates.*  
Departamento de Anatomia do Instituto de Ciências Biomédicas, Universidade Federal Rio de Janeiro, Brazil.

Coutinho A.  
Comentator of Prof. Jay Moskowitz's Conference (Senior Associated Dean; Wake Forest University School of Medicine, USA) *O passado e o futuro da investigação clínica nos Hospitais Universitários*, Fórum de Lisboa de Administração de Saúde, Fundação Calouste Gulbenkian, Lisbon, Portugal

Coutinho A.  
*Autoimunidade.*  
6º Congresso Nacional Medicina Interna, Porto, Portugal

Feijó J.A.  
*Ion dynamics and its possible role during in vitro pollen germination and tube growth.*  
University of Salzburg, Austria

Fernandes P.  
*Acesso aos recursos Bioinformáticos do IGC.*  
Instituto Nacional Saúde Dr. Ricardo Jorge, Lisbon, Portugal

Parkhouse R.M.E.  
*Immune evasion by African Swine Fever Virus.*  
Kennedy Institute, London, UK

Tavares A., Avides M.C. and Glover D.  
*Polo kinase and asp are required for centrosome microtubule nucleation.*  
The Cell Cycle meeting, Cold Spring Harbor, USA

## **June**

Bajanca F. and Thorsteinsdóttir S.  
*Expression of integrins during the development of limb skeletal muscle in the mouse.*  
V Jornadas de Biologia do Desenvolvimento, Lisbon, Portugal

Belo J.A.  
*Mechanisms of head induction in vertebrates.*  
Department of Morphological Sciences, Faculty Medicine, University Murcia, Spain

Cachaço A.S., Goumans M.-J., Bajanca F., Mummery C.L., Sonnenberg A. and Thorsteinsdóttir S.

*Integrins in the formation and differentiation of the somites in the mouse: An analysis of the beta1D integrin knock-in embryos.*

V Jornadas de Biologia do Desenvolvimento, Lisbon, Portugal

Certal A.C., Rongcai M., Boskovic R., Sonneveld T., Tobutt K.R., Feijó J.A. and Oliveira M.M.

*A study on the diversity and expression of almond S-Alleles.*

Plant Molecular Biology Congress, Quebec, Canada

Feijó J.A.

*Ion dynamics and the control of tip growth in pollen tubes.*

"NATO workshop on tip development", Siena, Italy

Gulbenkian S.

*O Instituto Gulbenkian de Ciência.*

Conferências Nicolau Van Uden 2000 "Biologia experimental e biomedicina; novos institutos autónomos, novas oportunidades", Instituto de Biologia Experimental e Biomedicina, Universidade de Coimbra, Coimbra, Portugal

Tavares A.

*Regulation of cell division in Drosophila.*

EMBO Workshop, Cortona, Italy

Zonia L, Cordeiro M.S. and Feijó J.A.

*Chloride efflux oscillations and osmoregulation during pollen tube apical tip growth.*

NATO workshop on tip development, Siena, Italy

## **July**

Belo J.A.

*Mechanisms of head induction in vertebrates.*

V Meetings on Developmental Biology, Universidade Lusófona de Humanidades e Tecnologias, Lisbon, Portugal

Carneiro J.

*Mathematical models of tolerance: Beyond "Immunocartoonology".*

IBMC, Porto, Portugal

Carneiro J.

*Mathematical modelling and in vitro proliferation assays provide insights on the mechanism of linked suppression.*

Cell Cycle in Immunology and Pathology. University of Warwick, Warwick, UK

Carneiro J.

*Mathematical modelling and in vitro proliferation assays provide insights on the mechanism of linked suppression.*

Immunology, Hammersmith Hospital, London, UK

Coutinho A.

Chairman of the session on “Autoimmunity” of the XIXth Congress of the European Academy of Allergology and Clinical Immunology, Lisbon, Portugal

Fernandes P.

*Bioinformática.*

Universidade do Minho, Departamento de Biologia, Braga, Portugal

Freitas C.

*Forming vertebrae in a embryo.*

Science Workshops at the Portugal Pavillion, Hannover Mondial Exposition 2000, Hannover, Germany

Palmeirim I.

*A molecular clock linked to vertebrate embryonic development.*

Science Workshops at the Portugal Pavillion, Hannover Mondial Exposition 2000, Hannover, Germany

## ***August***

Carneiro J.

*Assessing the paracrine and juxtacrine effects of cytokines involved in lymphocyte regulation.*

SMB Annual Meeting, Utah, USA

Carneiro, J.

*Towards a taxonomy of the mechanisms of dominant tolerance.*

Santa Fe Institute, Santa Fe, New Mexico, USA

Leon K, Perez R., Lage A. and Carneiro J.

*Modelling T cell mediated suppression involving multi-cellular conjugates with APC.*

Poster, SMB Annual Meeting, Utah, USA

Sousa J. and Carneiro J.

*Interference between IL-2 and IL-4 receptors signalling and its functional consequences.*

Poster, SMB Annual Meeting, Utah, USA

## ***September***

Braun D., Geraldes P. and Demengeot J.

*Type I interferon enhances B cell activation.*

EFIS 2000 (European Federation of Immunological Societies) Poznan, Poland

Coutinho A.

*A shift in paradigm may be necessary for solving autoimmune diseases and immunity to infection*

Symposium New Challenges for Research in Biology: Pasteurian Missions for the 21<sup>st</sup> Century, Salines d'Arc et Senans, France

Faria M., Gomes R. and Tavares A.

*New mitotic mutants in Drosophila melanogaster.*

Poster, XII Congresso Nacional de Bioquímica, Póvoa do Varzim, Portugal

Fernandes P. and Marques I.

Workshop: "Mining the Human Genome" within the "EMBnet Annual General Assembly Meeting", Lausanne, Switzerland

Freitas C.

*Somite information is segregated within presomitic mesoderm.*

Poster, XII Congresso Nacional de Bioquímica, Póvoa do Varzim, Portugal

Lima B., Godinho S. and Tavares A.

*Cloning and characterisation of Mob1-like proteins in Drosophila melanogaster.*

XII Congresso Nacional de Bioquímica, Póvoa do Varzim, Portugal

Rodrigues S.

*The node clock is ticking.*

Poster, XII Congresso Nacional de Bioquímica, Póvoa do Varzim, Portugal

Sousa A.E., Carneiro J., Loureiro A. and Victorino R.

*T Cell turnover and activation during HIV2 and HIV1 infections.*

Poster, International Meeting of the Institute of Human Virology, Baltimore, Maryland, USA

Vieira P.

*Arrested B lymphopoiesis and persistence of activated B cells in adult IL 7<sup>-/-</sup> mice.*

Institut für Genetik, University of Cologne, Cologne, Germany

Zuzarte V.

*New insights into colloid's role during somitogenesis.*

Poster, XII Congresso Nacional de Bioquímica, Póvoa do Varzim, Portugal

## **October**

Borges A.C., Marques S., De Robertis E.M. and Belo J.A.

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

Poster, Conference Jacques Monod "Cellular and Molecular Basis of Morphogenesis", Aussois, France

Cachaço A.S., Bajanca F., Mummery C.L., Thorsteinsdóttir S. and Sonnenberg A.  
*Integrins in the formation and differentiation of the somites in the mouse: an analysis of the beta1D integrin knock-in embryos.*  
Cellular and molecular basis of morphogenesis, Aussois, France

Coutinho A.  
*Os Investigadores “Torna-Viagens”.*  
“Os Torna-Viagens: Ontem e Hoje” - IV Conferência do Equinócio, IPATIMUP, Porto, Portugal

Dumas C., Antoine A-F., Aldon D., Digonnet, C., Faure, J.-E., Feijó, J.A.  
*Fertilization in flowering plants.*  
Frontiers in Sexual Plant Reproduction, International Symposium, Albany, NY, USA

Feijó J.A.  
*Ion dynamics and the control of tip growth in pollen tubes.*  
Frontiers in Sexual Plant Reproduction, International Symposium, Albany, NY, USA

Freitas C.  
*Lateral PSM cells count on medial ones to form somites.*  
Poster, Jacques Monod Conference “Cellular and Molecular Basis of Morphogenesis”, Aussois, France

Nolasco S., Casalou C. and Soares H.  
*Isolation and characterization of the Tetrahymena tubulin complexes functional relationships with microtubule assembly and dynamics.*  
European Research Conference on Frontiers of Cellular Microbiology and Cell Biology- Euroconference on signaling and Cytoskeleton Plasticity”, France

Palmeirim I.  
*Lateral PSM cells count on medial ones to form somites.*  
Conference Jacques Monod "Cellular and Molecular Basis of Morphogenesis", Aussois, France

Rodrigues S.  
*c-hairy waves of expression - a closer view!*  
Poster, Jacques Monod Conference “Cellular and Molecular Basis of Morphogenesis”, Aussois, France

Rougier M., Antoine A.-F., Faure J.-E., Cordeiro S., Dumas C. and Feijó J.A.  
*Calcium and fertilization in flowering plants.*  
Conference Hong-Kong-France, Ca<sup>2+</sup> in development: from ion transients to gene expression, Hong-Kong, China

Vicente AM., Coutinho A., Mota-Vieira L., Marques C. and Oliveira G.  
*Genetic variation of serotonin system genes in a sample of autism families from Portugal.*  
50th Annual Meeting of the American Society of Human Genetics, Philadelphia, USA

Zuzarte V.

*Understanding Colloid during somitogenesis.*

Poster, Jacques Monod Conference “Cellular and Molecular Basis of Morphogenesis”, Aussois, France

## **November**

Belo J.A.

*Mechanisms of head induction in vertebrates.*

III Reunion Cientifica “Mecanismos Comunes em el desarrollo Embrionario”, Universidade de Extremadura, Badajoz, Spain

Braun D., Geraldles P. and Demengeot J.

*Type 1 Interferon enhances B cell activation and autoimmunity development.*

XXVI Reunião Anual da Sociedade Portuguesa de Imunologia, Porto, Portugal

Carneiro J.

*Mathematical modelling and in vitro proliferation assays provide insights on the mechanism of linked suppression.*

Immunotherapy for the New Century, Havana, Cuba

Carvalho T.

*The evolution of the immune system.*

Immunology course for Biology students, University of Braga, Braga, Portugal

Carvalho T., Mota-Santos T., Cumano A., Demengeot J. and Vieira P.

*Arrested B lymphopoiesis and persistence of activated B cells in adult IL 7 <sup>-/-</sup> mice.*

XXVI Reunião Anual da Sociedade Portuguesa de Imunologia, Porto, Portugal

Carvalho T.

*Neoteny in the immune system: the origin and fate of B cells in IL7<sup>-/-</sup> mice.*

IBMC, Porto, Portugal

Casalou C., Cyrne L., Nolasco S. and Soares H.

*Dynamic relationship between CCT and CCT micro-complexes in Tetrahymena reciliating cells.*

Workshop “Chaperonins: structure and function”, Instituto Juan March de Estudios e Investigaciones, Madrid, Spain

Coutinho A.

*Synthesis Session*

Co-chairman with François Kourilsky (Previous General Director of CNRS), “Rencontres Science et Société”, Universidade de Coimbra, Coimbra, Portugal

Coutinho A.

*O que ganhamos com os progressos da descoberta do genoma humano?*

III Encontro Nacional das Tecnologias da Saúde, Grande Auditório de Congressos de Lisboa, Lisbon., Portugal



Demengeot J.

*Control of T cell expansion by regulatory CD4+CD25+ cells.*

International workshop, "Immunotherapy for the new century", CIM, Havana, Cuba

Feijó J.A.

*Ion dynamics in pollen tube growth and morphogenesis.*

Plant Biology Seminar Series, Univ. Massachussets, Amherst, USA

Hori S., Carvalho T., and Demengeot J.

*Phenotypically distinct subsets of CD4+ T cells induce or protect from acute pneumonia in Pneumocystis carinii infected immunodeficient mice.*

XXVI Reunião Anual da Sociedade Portuguesa de Imunologia, Porto, Portugal

Oliveira V., Hori S. Leon K., Carneiro J. and Demengeot J.

*T cell mediated tolerance: Growth of regulatory CD25+ T cells depends on naïve CD25-T cells.*

XXVI Reunião Anual da Sociedade Portuguesa de Imunologia, Porto, Portugal

Palmeirim I.

*Ethical impact in the study of embryonic development.*

Escola Secundária da Cidade Universitária, Lisbon, Portugal

Soares R., Castro A.G. and Vieira P.

*Immunologic tolerance to allotransplantation by induction of IL-4 and IL-10 expression in transgenic mice.*

XXVI Reunião Anual da Sociedade Portuguesa de Imunologia, Porto, Portugal

Sousa J. and Carneiro J.

*Interference between IL-2 and IL-4 receptors signalling and its functional consequences.*

XXVI Reunião Anual da Sociedade Portuguesa de Imunologia, Porto, Portugal

## ***December***

Braun D.

*Interferon de type I, activation B et autoimmunité.*

Unité INSERM 131, Clamart, France

Carvalho T.

*The limits of diversity: lymphocyte repertoires and development.*

Immunology graduate program, Biology Department, State University of Sao Paulo, Campinas (UNICAMP), São Paulo, Brazil

Coutinho A.

*Stem cells and the immune function.*

Stem Cells, Third Unesco Course, Department of Anatomy, UFRJ, Rio Janeiro, Brazil

Parkhouse R.M.E.

*Strategies for immune evasion by African Swine Fever Virus.*

British Society for Immunology Annual Meeting, Harrogate, UK

## OTHER ACTIVITIES OF THE IGC PERSONNEL

### *January*

Coutinho A.

Special Committee of the Research Council of Norway for Evaluation of the Country's Science and Technology System on Life Sciences, Oslo, Norway.

### *February*

Coutinho A.

Review Committee for Research Grants on “Molecular mechanisms”, HFSP - Human Frontier Science Program, Strasbourg, France

### *April*

Haury M.

Organization of the 2nd USP Flowcytometry Course, University of São Paulo, São Paulo, Brazil

### *June*

Coutinho A.

EMBO Fellows Meeting, Heidelberg, Germany

### *July*

Haury, M.

Member of the EU evaluation committee, EU 5<sup>th</sup> Framework Program Key action, Brussels, Belgium

### *September*

Coutinho A.

EMBO Council Meeting, Heidelberg, Germany

## ***November***

Soares H and Fernandes L.

Organization of the "*III Encontro Nacional das Tecnologias da Saúde*" held in Lisbon, Portugal

Haury M.

Organization of 1st Caribbean Flowcytometry Course, Institute of Molecular Immunology, Havana, Cuba

Haury, M.

Member of the EU evaluation committee, EU 5<sup>th</sup> Framework Program Key action, Brussels, Belgium

Parkhouse R.M.E.

Collaborative Project Evaluation, Univ. Carabobo, Venezuela

## ***December***

Parkhouse R.M.E.

Collaborative Project Evaluation, Instituto Biomédicas, Universidad Autónoma, México, México

## ATTENDANCE OF COURSES BY IGC PERSONNEL

### ***March***

*Names:* Ana Paiva Brandão and Manuel Carvalho

*Course:* “Apple Mac OS9”

*Place:* FLAG, Lisbon

### ***May***

*Names:* Ana Homem and Dolores Oliveira

*Course:* Curso de gestão da Segurança em Laboratórios

*Place:* SOQUIMICA, Lisbon

### ***October***

*Name:* Ana Paiva Brandão

*Course:* “Linguagem SQL em Oracle”

*Place:* ORACLE, Lisbon

### ***December***

*Name:* Ana Paiva Brandão

*Course:* “Administração Oracle8i: Arquitectura”

*Place:* ORACLE, Lisbon

## **Annex**

# **REPORT OF THE FINAL EVALUATION OF THE GULBENKIAN PROGRAMME FOR THE PREPARATION OF PH.D. DEGREES IN BIOLOGY AND MEDICINE.**

## **Introduction**

Concern about opportunities for training in Portugal in the general fields of biology and medicine lead to the establishment, in 1993, of a novel four year Ph.D. Programme that involved a year of course work followed by three years of research typically, but not always, in an overseas laboratory. This Programme was jointly developed and financed by the Calouste Gulbenkian Foundation, the Luso-American Foundation and the Portuguese Ministries of Science and Education. The Programme accepted students in seven successive years (1993-1999) and will close when the last year's intake graduates in three to four years time. During this period 103 students have been members of the Programme.

Drs Ashburner, Hentze and Salicrup were asked to prepare an evaluation of the Programme for its funders. They visited Portugal during April 17 - 19, 2000 to meet with the funders, teachers and students of the Programme, as well as members of its Board of Trustees and Scientific Board, in both Lisbon and Porto. They received a report on the Programme written by Professor Antonio Coutinho, documentation concerning the courses given in successive years of the Programme and information about all of the students.

## **Executive Summary**

We provide a brief summary of our conclusions. Justification for these will be found in the Full Report which follows.

## **Retrospective**

The major objective of the Programme was to prepare about 100 Ph.Ds in modern biomedical sciences, with a strong emphasis on cellular and molecular biology. This was to be done by selecting students after a national competition, subjecting them to an intense first year of teaching and course work, much of which was given by international scholars, and then placing the students in the very best laboratories, usually overseas, for their research.

The Programme was designed to be highly selective and to train a new generation of leaders in Portuguese science. It was also designed to promote the internationalisation of Portuguese biomedicine. Finally, the Programme was designed to be a model for the close co-operation between private foundations and government in promoting science in Portugal.

Recognising that a final judgement will not be possible for many years – that is when all of the students on the Programme have completed their Ph.Ds and entered the professions - we judge the Programme to have been very successful at meeting all of its objectives and we would here like to congratulate all of those who have worked so hard to achieve this success.

## **The Future**

(a). There is an urgent need to establish a programme in Portugal to provide attractive career opportunities for young scientists. This has been recognised in a number of other countries and the general solution has been to establish competitive grants for non-tenure track but independent research positions. We recommend that such a scheme be established in Portugal as soon as possible and that this be very broadly advertised.

(b). Although the situation of training in the biomedical sciences in Portugal now is very different from that in 1992, when the Programme was conceived, there remains the need for high quality graduate education. We recommend that one or more new Ph.D. training programmes be initiated and that they should meet the following criteria:

- selection of programmes by open call and competition,
- open access,
- a culture of excellence,
- interdisciplinarity & breadth in the life sciences,
- flexibility & freedom of choice for students,
- collegiality,
- international perspective for both teaching and training,
- a strong institutional context,
- funding by private/public partnerships.

## **Full Report**

### **Retrospective**

#### **The Programme and its students**

The major objective of this Programme was to increase the opportunities for, and the general standard of, Ph.D. training in biology and medicine in Portugal. The major features of the Programme which would allow this objective to be achieved were: rigorous selection of students; high quality courses, from both domestic and international teachers, in the first year; placement of students in first class groups, predominantly overseas, for their experimental studies and, close mentoring of students throughout their studies.

We have little doubt that the great success of the Programme has been the result of the first of these features - the rigorous selection of first class students. The Programme was widely advertised and, in recent years at least, has attracted well over 100 applicants. All of these have been interviewed, by either (or both) Professors Coutinho and Quintanilha. Unusually, no personal references were required. Professors Coutinho and Quintanilha

deliberately aimed to select "interesting" students, irrespective of their background. The primary criteria used for selection were: motivation, curiosity and seriousness of purpose. Excellent academic promise was a major criterion, whereas past performance, in the narrow sense, was not.

This selection has been a labour of love by both Professors Coutinho and Quintanilha, and one that is unlikely to be repeated in any other programme. It has, nevertheless, been remarkably successful. We make this statement on the basis of both objective and subjective criteria. Objectively, the drop out rate has been very low; objectively, all of the 1993 class have successfully defended their Ph.Ds; subjectively, we were all enormously impressed by all of the students that we met during our visit. We talked at some length with 25 students from the 1993 to 1999 classes. Without exception they were articulate and intelligent. Without exception they all had high praise for the Programme.

One objective of the Programme has been to be interdisciplinary, that is to attract students whose background has not been in the life sciences. Although the majority of students have come from the traditional background of biochemistry, a sufficient number of others, physicists, chemists, economists, etc, have been recruited to make a very interesting balance of students in each year's class. It is interesting that many of the students have already spent study time abroad, through the EU's ERASMUS Programme.

There has been criticism that the Programme has not been successful in attracting students from medicine. The Programme has only attracted, on average, just over one medical student per year. Despite some suggestions to the contrary we see no evidence for any bias against medical students at the time of selection. The problem is that these students do not apply, at least not in sufficient numbers. The rather rigid structure of clinical education, and the lack of any strong tradition of research within clinical medicine, in Portugal, are two of the reasons for their failure to apply to the Programme. There is clearly some confusion as to whether medical students should be encouraged to apply to programmes such as this before or after their speciality. There is concern too that a Ph.D. degree is simply not valued within the medical community in Portugal, so that the four years spent in research will simply not contribute to career advancement, should the student wish to return to medicine.

Another criticism has been that the selection has been very elitist. This is probably true, but we point out that only 10% of the Ph.Ds in biology in Portugal have been selected by this means. There have been plenty of opportunities for other students to do a Ph.D. in this area.

The first year of the Programme has been dedicated to course work, a mixture of obligatory and elective courses. The majority of teachers have been from overseas. This has had several positive consequences. The first is that it has ensured a very high quality of teaching; second is that it has exposed the students to international science at its best; third it has exposed the international teachers to the best of Portuguese students and to Portuguese science; fourth it has created, or strengthened, contacts between these international scholars and Portuguese scientists. Many of the students subsequently chose to do their experimental studies with a mentor that they had met during one of these first year courses.



We had some discussion as to whether or not (a) one year of course work had been too long and, (b) some practical studies should have been included in this first year, e.g. as laboratory rotations. We raise these questions because both should be considered carefully should new Ph.D. programmes be designed. It has been suggested to us that a one-year course is too long for those who have a background in biochemistry and that good students have been put off applying for this reason. We accept that there are two valid models: (i) a full year of course work or (ii) six months (or so) course work plus six months (or so) of laboratory rotations.

At the end of their first year the students present their proposals for their experimental work. This is done in the context of the Programme's annual retreat, an occasion at which all students and teachers get together for an intensive week of discussions and reports. This retreat has clearly been a most valuable aspect of the Programme. It has brought to the Programme a great sense of collegiality, as well as providing a great platform for constructive criticism and the exchange of information.

[We note some criticism that the proposals presented by the students at the end of their first year suffer from too much input from the intended supervisor. This is probably inevitable, and can be countered by a vigorous questioning of the student.]

The choice of host laboratory for the student's experimental work is clearly very critical. While the majority of students choose to go overseas, a significant number have remained in Portugal. The Directors of the Programme, course teachers and other students all influence the choice of host laboratory, and we were impressed with the advice available to students. This is not to say that mistakes have not been made, but they have been rare.

Of the 16 students who entered the Programme in 1993 all now have been awarded their Ph.D. degree. All remain in scientific research and six have returned to Portugal. For the 1994 class nine students have obtained their Ph.D.s and two have already returned to Portugal. This is a magnificent achievement.

### **Administrative aspects**

This Programme has been very novel, not only in Portugal but also in the broader European context. Its novelty comes from the partnership between two private foundations and government ministries in both promoting and funding it. In the Portuguese context it was novel in being the first taught Ph.D. course in that country and in the fact that the Ministry of Science agreed to the course organisers selecting the students for fellowships. The fact that most of the students did their research abroad is not unusual in Portugal; about half of the Ministry of Science's Ph.D. fellowships (which are awarded *ad hominem*) go to students who study abroad.

The two major aspects of the funding of this Programme - the partnership between the private and public sectors and the selection for Ministry of Science fellowships by the course organisers - contributed to its success.

The role of the Gulbenkian and Luso-American Foundations in this partnership goes far beyond providing money. These private foundations are in a position to catalyse change

and to respond very flexibly to changes in circumstance and needs. They can be more elitist and more experimental than even the most enlightened Government Ministry.

The Programme has quite a complex governance. Its day to day activities have been under the supervision of Dr. Paulo Vieira at the Gulbenkian Institute of Science. It has three Directors of whom one has been inactive (we are not criticising this person). It has a Scientific and Pedagogic Board (Conselho Cientifico e Pedagogico) of five members, representing the major universities and institutes involved. We understand that this Board has only met four or five times, although as individuals its members have been available and generous with their time and advice. Finally, a Board of Trustees (Comissão de Acompanhamento), to whom we report, is the ultimate body and consists of representatives of the four funding bodies, the Ministries of Education and Science, and the Gulbenkian and Luso-American Foundations.

### **Prospective**

We have, we hope, made it clear that we are enthusiastic in our praise for the present Programme and we congratulate all that have made it possible. This Programme will, however, come to its end when the current students have all graduated; no further students will be accepted. It is, therefore, time to look to the future. We wish to consider two very different future activities - the first is support for further Ph.D. programs, the second is support for young scientists within Portuguese Universities and other academic institutes.

### **Ph.D. programmes**

We are strongly of the opinion that the experience of the present Programme must not be lost, nor must the lessons be ignored. The Programme has demonstrated beyond any doubt that Portuguese universities are producing graduates who are as capable of competing in the international arena of scientific research as those from countries with longer and stronger academic traditions. The Programme has demonstrated that a very hierarchical graduate training, that had been characteristic in Portugal, can be replaced by a vibrant and exciting alternative.

The demand for well-trained Ph.Ds in the biomedical sciences sees little sign of declining. Indeed, the revolution now underway in the life sciences, a consequence, largely, of advances in genomic research, is only now beginning to have its impact on fundamental science, on medicine and on agriculture. The synergistic interactions between the biological sciences and computing, on the one hand, and biological sciences and engineering, on the other will have an enormous impact on society in the next decades. Although Portugal may lag behind other European states in the commercial exploitation of genomics, and other aspects of biotechnology, and although Portugal may lag in the exploitation of advances in molecular medicine, this is not a situation that can be allowed to continue. It is for these reasons that we see the education, to the highest international level, of scientists in the life sciences and medicine to be a very high national priority. The present Programme has clearly demonstrated proof of principle, there are young Portuguese students who can take maximum advantage of a high quality programme.

For these reasons we strongly recommend that new Ph.D. training programmes, close in spirit, if not in detail, to the Gulbenkian Programme, be started. We have attempted to analyse the factors that have made the present Programme such a success. It is on the basis of this analysis that we offer several criteria for any new programme.

**Selection of programmes by open call and competition.** By this we mean that there should be an open call to interested parties to submit proposals for new Ph.D. programmes. These could be broad in scope (as the present Programme) or more narrow. They could be offered by a single university or institute or by a consortium. They should, in our view, be open to a minimum of ten and a maximum of twenty students a year and should be funded for only five years (of intake) in the first instance. Proposals should be subject to peer review, preferably by an international committee. The following should be taken into consideration when reviewing proposals.

**Open access.** Access to the new programmes should be open to all, and should be based on national (if not international) competition.

**A culture of excellence.** Selection of students should be rigorous and independent of patronage. A new proposal should be very explicit about the mechanisms, and criteria, that it will use to select students.

**Interdisciplinarity & breadth in the life sciences.** The selection of students should not be constrained by a narrow view of their prior education. A determined effort should be made to select, and then to nurture, students trained in disciplines other than biochemistry. On the other hand the training offered should be equally broad and not narrowly constrained to sub-disciplines of the life sciences.

**Flexibility & freedom of choice for students.** The course should offer as much flexibility to students as possible. They should not be constrained by red tape. The students should have complete freedom of choice of where to do the Ph.D., this may be in Portugal or abroad, this may be in the same institution as offers the Ph.D. programme or elsewhere.

**Collegiality.** The course should encourage collegial relations between students, not only within but also between years. An annual retreat is one obvious way of promoting this.

**International perspective for both teaching and training.** It is very important that the students be exposed to teachers and researchers from both a broad intellectual spectrum but also from teachers and researchers who have high international reputations. This not only exposes Portuguese biology to these visitors but, as importantly, exposes the students to the broad international context of modern science.

[We are aware of some new Ph.D. programmes that have recently been started in Portugal which lack this strong internationalist spirit; we do not consider these to be a substitute for the very high quality programmes we recommend now be established.]

**Strong institutional context.** The present programme has benefited enormously from its strong institutional contexts, particularly that of the Gulbenkian Institute of Science. Such a context, and commitment, is essential for the success of a programme such as this.

**Funding by private/public partnerships.** The model of a mix of private and public funding is a very good one. It offers flexibility and encourages innovation and experiment. We would expect those who propose new programmes to look beyond the traditional private foundations as partners.

It must be quite explicit in any new proposals just who will be responsible for the formal award of the Ph.D. degree. We are well aware of some tensions about this in respect of the current Programme.

We have one final recommendation. The Gulbenkian Programme is allotted a given number of Fellowships from the Ministry of Science. Each student is assured a fellowship from the time he/she enters the Programme. This should be the model for any new Programmes. It puts the onus of selection on the organisers and avoids any competition for fellowships between students.

### **New research positions**

We were somewhat surprised to discover that (until very recently) no effort had been made to capitalise on the Gulbenkian Programme by offering attractive opportunities for research in Portugal to its graduates. We repeatedly heard fears that no suitable positions would be open, and that this would force the most ambitious to remain overseas. Although it is true that there are posts available, particularly in some of the newer private universities, it is clear from what we learned that these are not environments for first class research at an international level, largely because of the very heavy teaching loads of their staff.

Other countries have faced very similar problems and have solved them in similar ways: by having "junior research fellowships" or "career development awards" open to young scientists. These typically have the following features:

They are open to all by open competition, normally once or twice a year. Selection is very stringent and is by a mixture of peer review and interview. The application includes a detailed research proposal. Applicants have typically had two or three years of post-doctoral research.

Appointments are made typically for five years and are non-tenure track; in some countries (e.g. those of the Wellcome Trust in the UK) there are schemes for more senior researchers that can be used after five years.

The fellowships include not only the fellow's own salary but also those of, for example, a postdoctoral student, graduate students as well as funds for set up, consumables and other expenses.

The fellowships can be held at any approved institute or university. The applicants normally have obtained a letter of support from a suitable host. The host institution cannot exploit the fellow, for example by demanding teaching or administration.

Such schemes have had a major impact on research capability in many countries. As an example the small Department in which one of us (Ashburner) works in Cambridge normally hosts six or seven small independent research groups funded by schemes similar to this. These not only offer great opportunities for young researchers, but they add critical mass of researchers.

In this context we wish to draw attention to the Young Investigator Scheme that is about to be launched by EMBO. This scheme may prove to be particularly helpful in recruiting the most outstanding young Portuguese researchers back to Portugal.

[At our last meeting with the Comissao de Acompanhamento we learned from Professor Luis Magalhaes that the Ministry is just about to announce a new scheme, funded by Cohesion funds from the EU. We very much welcome this.]

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