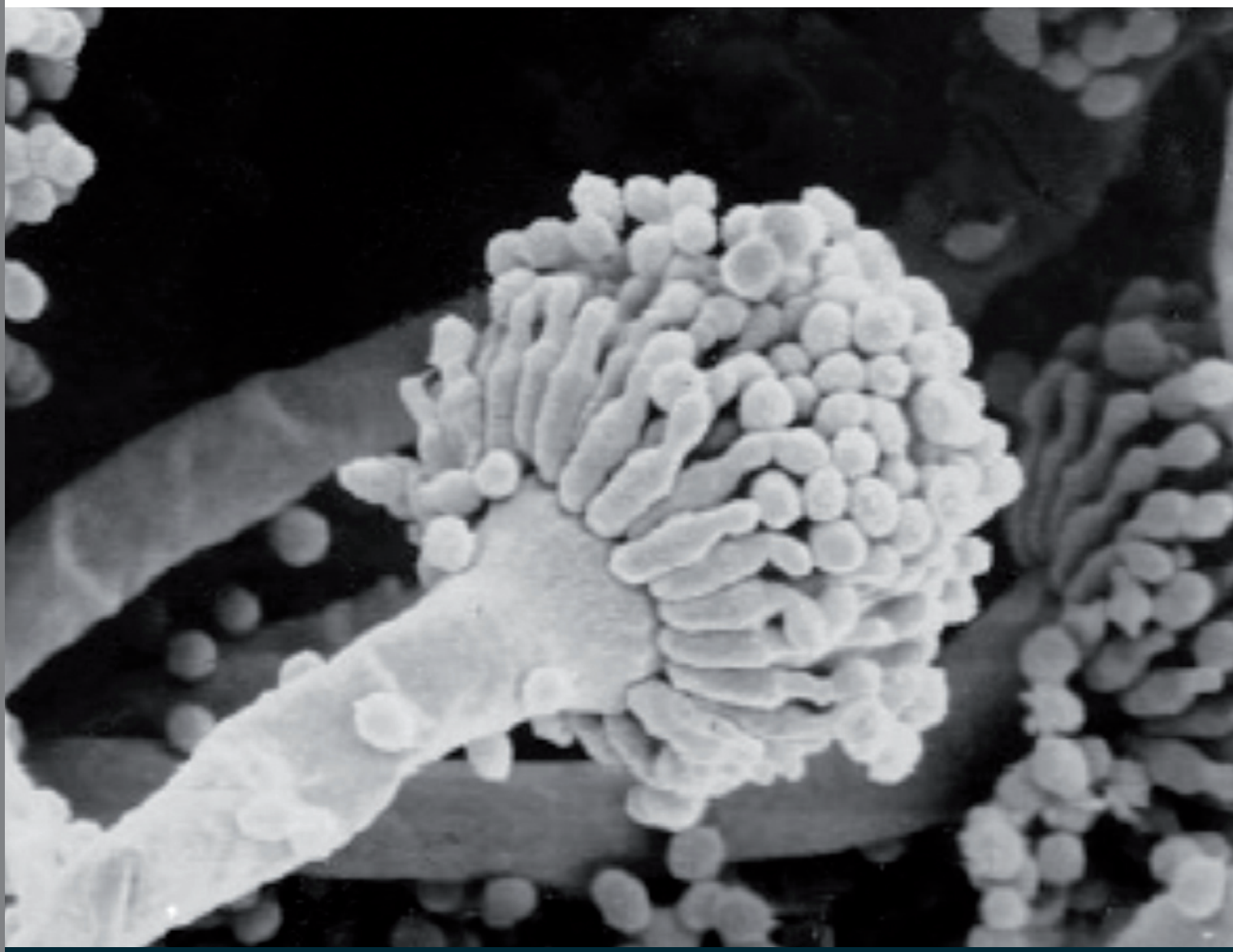
EUROPEAN SCIENCE FOUNDATION

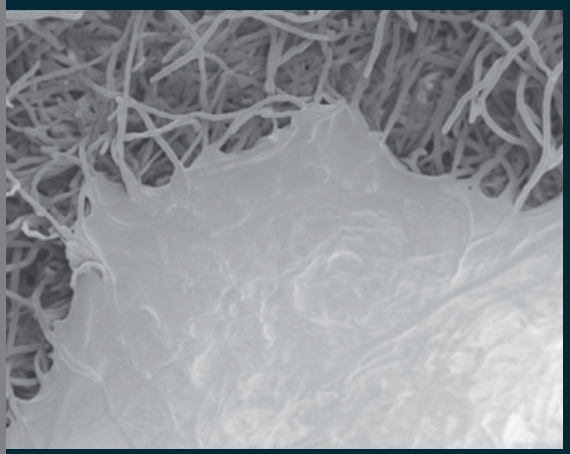
SETTING SCIENCE AGENDAS FOR EUROPE

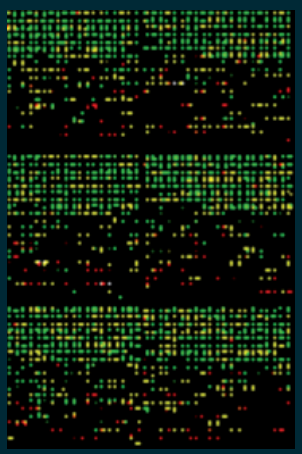
RESEARCH NETWORKING PROGRAMME

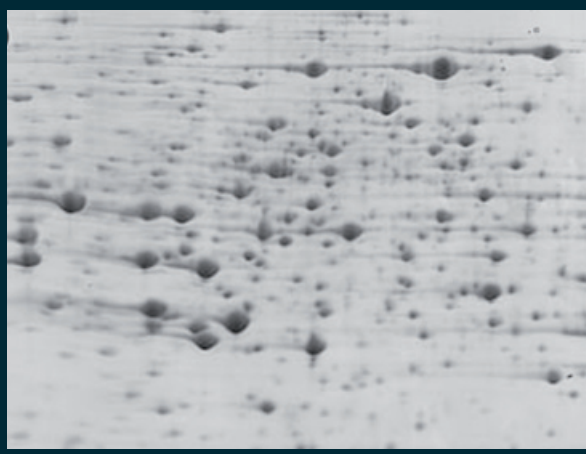
THE FUNCTIONAL GENOMICS IN *ASPERGILLUS FUMIGATUS* AND NEW STRATEGIES TO FIGHT AGAINST THE FIRST FUNGAL PATHOGEN IN EUROPE (FUMINOMICS)

Standing Committee for the Medical Sciences
(European Medical Research Counc ils, EMRC)
Standing Committee for Life, Earth and Environmental Sciences (LESC)









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Introduction

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The strength of ESF lies in the influential membership and in its ability to bring together the different domains of European science in order to meet the challenges of the future.
Since its establishment in 1974, ESF, which has its headquarters in Strasbourg with offices in Brussels and Ostend, has assembled a host of organisations that span all disciplines of science, to create a common platform for cross-border cooperation in Europe.
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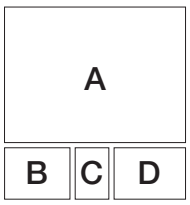

Cover pictures:
A) and B): cryo-scanning electron microscopy images of *A. fumigatus*,
A) conidial head, B) extracellular matrix from *A. fumigatus* covering its own filaments
(from Beauvais A., unpublished data);
C) detail of *A. fumigatus* genechip
(from Mowat E., personal communication);
D) gel electrophoresis for mapping *A. fumigatus* proteins
(from *Genetics*, 49: 178-189, 2005).

In the past ten years, fungal pathogens have emerged as the major eukaryotic pathogens in Europe and other industrialised countries. The opportunistic pathogen *Aspergillus fumigatus* is a saprophytic soil mould that can cause invasive pulmonary diseases in immunocompromised hosts. Aspergillosis now represents the most common invasive mould infection world-wide: one in 25 immunocompromised patients who die in modern European teaching hospitals have been found to suffer from invasive aspergillosis[1]. Invasive aspergillosis affects between 10 and 25% of all leukaemic patients with a high mortality rate of 85%. Invasive aspergillosis, most commonly due to *A. fumigatus,* has risen in incidence through the 1990s to the point that many infectious disease physicians now regard aspergillosis as their major microbial problem. With increases in the number of immunosuppressed patients in modern clinical practices, it is clear that the dramatic increase in the most severe systemic fungal infections, such as disseminated aspergillosis, will continue to represent a major clinical and economic burden.

One of the reasons for the increasing burden due to *A. fumigatus* is the poor understanding of the metabolic pathways controlling the multigenic virulence of the fungus in its host. The ESF Research Networking Programme (RNP) FUMINOMICS is aimed at a multidisciplinary and fully integrated functional genomics analysis of the basic mechanisms of the opportunistic traits developed by *A. fumigatus* to infect host cells. Understanding how the fungal pathogen perceives and adapts to its host environment requires undertaking fundamental research into fungal gene expression and regulation. This objective has now become feasible because (1) the genome of *A. fumigatus* is publicly available; (2) gene expression analysis via microarrays that have been produced in Toulouse can be conducted; (3) proteome analysis that has been established in Jena where the bioinformatics of the project will be coordinated, is available; and (4) recent methodological improvements make it possible to generate knockout mutant strains on a large-scale basis.

1. Denning et al., *Lancet Infect. Dis*, 2: 251, 2002.

Scope and Aims

FUMINOMICS gathers the major laboratories studying *A. fumigatus* in Europe (18 laboratories from eight different countries) and was initiated by the partners of this consortium with their own budgets. This ESF Research Networking Programme has increased the visibility of research on *A. fumigatus* at the European level. The goal of FUMINOMICS is to raise this research through coordination within Europe to the highest competitive level by integrating bioinformatics, transcriptomics, proteomics, physiology, molecular genetics and medicine.

Support provided by the ESF will allow better communication between partners over the four-year period and will speed up progress in *A. fumigatus* research. Activities to be supported include: (1) annual workshops; (2) exchange of PhD students and post-doctoral fellows; (3) the maintenance of a website and databases; and (4) a general final conference that will address the fungal pathogenicity of *A. fumigatus* comparatively with other fungal pathogens.

The running period of the ESF Research Networking Programme FUMINOMICS is for four years from June 2008 to June 2012.

A. fumigatus produces uninucleate haploid conidia, which can be continuously inhaled on a daily basis during routine activities, the lung being the obvious site of infection. In immunocompetent individuals, mucociliary clearance and phagocytic defence normally prevent the disease. Alveolar macrophages (AM), along with neutrophils (which are actively recruited during inflammation), are the major cell types participating in the phagocytosis of *A. fumigatus*. In an immunocompetent host, killing of conidia is extremely slow and can last for several days. The first stage of germination, i.e., the isodiametric swelling of the conidium that occurs intracellularly during the first six hours, is not inhibited (Figure 1). When conidia reach the swollen stage, killing by the AM occurs, and, thereby, polarised growth of conidia (germ tube formation) is not observed. In the immunocompromised host, however, conidia germinate and germ tubes escaping from alveolar macrophages grow out and are attacked by neutrophils, the second line of phagocytic cells. Neutrophils adhere to the surface of the hyphae, as hyphae are too large to be engulfed. In contrast to conidia, killing of the mycelium by neutrophils is fast (two hours are needed to kill 50% of hyphae). Conidial germination and resistance to phagocytes are critical and essential events in initiating the disease.

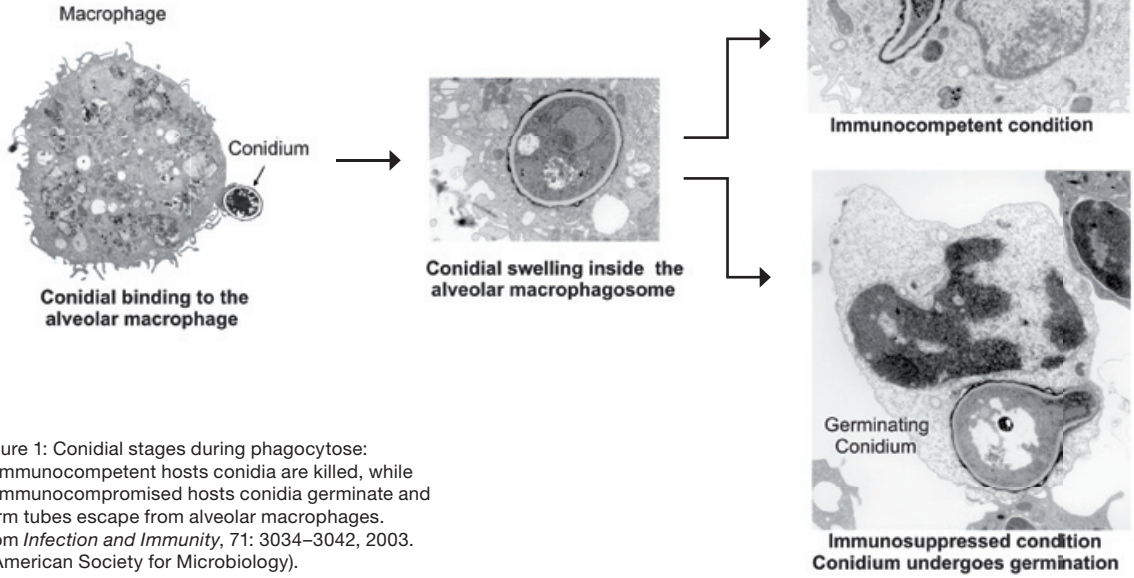

Figure 1: Conidial stages during phagocytose: in immunocompetent hosts conidia are killed, while in immunocompromised hosts conidia germinate and germ tubes escape from alveolar macrophages. (from *Infection and Immunity*, 71: 3034–3042, 2003. ©American Society for Microbiology).

These events are of course under the control of multigenic processes. To globally understand such events and identify novel antifungal targets, post-genomic tools are required, involving interdisciplinary research in the area of *A. fumigatus*, and experts in genomics, transcriptomics, proteomics, as well as cellular biology and biochemistry.

FUMINOMICS is aimed at developing a functional genomics analysis of the basic mechanisms of the opportunistic traits developed by *A. fumigatus* (Af) to infect host cells. The principal areas of scientific interest are how the fungal pathogen perceives and adapts to its host environment. A network giving rise to FUMINOMICS was initiated in 2005 by 11 research groups among the 18 in the consortium. They agreed to combine their financial efforts in raising the design and production of European Af genechips. The analysis of gene and protein expression studies will increase the visibility of FUMINOMICS and will require the integration of technologies including transcriptomics, proteomics, physiology, cell imaging, molecular genetics, mathematics, computing, and medicine. This project will lead to the establishment of a huge genomic database that will become the basis of *A. fumigatus* post-genomic science. Due to the multigenic character of the virulence of *A. fumigatus*, only large-scale genomic analysis will be able to pinpoint the pathways conditioning the early establishment of the disease. Accordingly, the use of post-genomic methods will significantly change our understanding of the interactions between humans and *A. fumigatus*. We also believe that this project will contribute to the promotion of European research integration by networking the most relevant laboratories working in this area, representing eight major European countries.

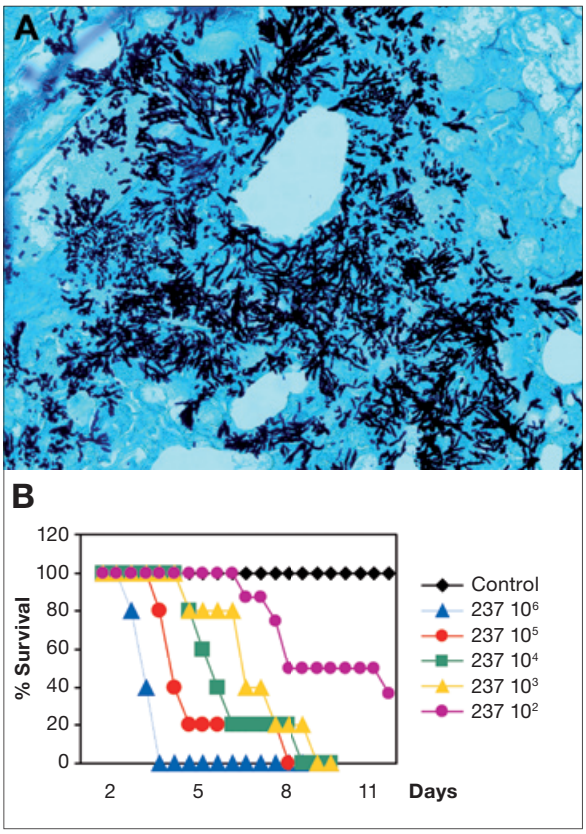

Figure 2: Inocula standardisation for screening virulence in an animal model. Mice CD1 were immunosuppressed by injecting cyclophosphamide and cortisone acetate followed by intranasal inoculation of spores (10^2 to 10^9 suspended in Tween-saline solution).
A) Presence of fungi in the lung (Latgé J.-P., unpublished image).
B) Survival following inoculation of spores (Alcazar L., personal communication).

The FUMINOMICS project will focus on four different aspects:

1) Use of microarray-based data to analyse the modulation of gene expression during early stages of growth *in vitro* and *in vivo*. Common experimental conditions were selected and agreed upon unanimously at a meeting that took place in September 2008. The strategy has been agreed upon by all partners.
2) Establishment of proteome maps, resulting from gel-based methods for the analysis of the proteome of *A. fumigatus* in conditions very similar to the ones used in the transcriptome studies.
3) Storage of data from transcriptome and proteome experiments obtained from members of the consortium in a central "data repository" accessible to every member of the consortium for analysis.
4) Generation of deletion mutants by using new technologies developed in our laboratories, to gain hints on the cellular function and role of genes identified as being differentially expressed under the conditions described in this proposal. All mutants of *A. fumigatus* will be tested in an animal model to characterise their potential role in fungal virulence.

An incentive of this RNP will be to generate a set of common practices and standards in the field of functional genomics of *A. fumigatus* to easily exploit and compare the accumulation of post-genomic data that will be generated by the different teams of this consortium over the next four years. Past experiments with model systems like the yeast *Saccharomyces cerevisiae* have demonstrated how important it is to develop and share common tools, methods and language. In the FUMINOMICS project, several scientific meetings and workshops will be organised and the exchange of scientists between labs will be promoted to disseminate these common methods and to firmly establish standard procedures like those that have been already

implemented in DNA arrays (e.g., MIAME: http://www.mged.org). The major achievement of the study will be the identification of pathways associated with fungal pathogenicity, which ultimately should lead to a better management and treatment of patients with disseminated aspergillosis.

This coordinated project should greatly increase knowledge of the most life-threatening eukaryotic nosocomial infections in Europe, with a potentially major impact on health management processes in fungal infections that are mostly acquired in the hospital setting and are of major socioeconomic concern to all European health systems. The annual cost of treating fungal infections in Europe is currently estimated to amount to hundreds of millions of euros. Systemic mycoses are normally treated by commercially available antifungal drugs. Unfortunately, despite their high price (an average estimated at €50 000 per patient from first treatment to the time of discharge), these are inadequate. This is of specific relevance to the citizens of developed nations where the number of immunocompromised patients is not only increasing due to new clinical treatments opening the door to fungal infections but also to demographic shifts that predict increasing numbers of vulnerable elderly people. Collectively, we expect advances, building on the outcomes from this project, in two areas: (1) identification of therapeutic targets and (2) identification of potential new diagnostic markers.

• Annual meetings

Three annual general meetings will be organised, with each partner's laboratory in the consortium being represented by two participants. Each meeting should take place over at least three full days. The Steering Committee will meet at the same time to discuss new workshops and support for short visits or PhD exchange between laboratories. Minutes of each meeting, after approval by the participants, will be uploaded on the FUMINOMICS website.

Each meeting will be organised in two parts. The first part will be a discussion of scientific achievements in the field by each partner and by invited speakers recognised as experts in the area; such a set-up will lead to the best update on current knowledge as well as getting advice from scientists outside of the consortium. The second part of the annual meeting will be organised as a workshop to define the most common practice, standardised methodologies and experimental designs to be used by the ESF consortium. This part must be attended by all the consortium's PhD students and postdoctoral fellows.

Three main topics for the meetings have been selected:

1. *Transcriptomics and genetic tools.* The first meeting will be chaired by J.-M. François from the Biochips Platform team (Toulouse, France) and E. Bignell from Imperial College (London, United Kingdom). The Af genechips, bearing 9 588 oligos that cover 9 200 coding sequences from the genome of AF293 sequenced by JCVI[2], were developed by the Biochips Platform team. During this meeting, a one-day workshop will be addressed to biologists from the different teams interested in learning about key features in transcriptomics, namely experimental design, the DNA arrays process workflow and the use of the Bioplot-Bioclust software. Another one-day workshop will focus on new tools and strategies for the molecular manipulation of *A. fumigatus*.
2. *Proteomics and bioinformatic tools.* The second meeting will be chaired by V. Schroeckh from the Hans-Knoell Institute (Jena, Germany), who has pioneered the use of 2D-PAGE and mass spectrometry for *A. fumigatus*. In addition, V. Schroeckh already established a database environment "Protecs" for the storage of transcriptome and proteome data. During this second meeting, a two-day workshop will be centred on the standardisation of the proteomic protocols and on the use of the data repository platform for efficient storage of transcriptome and proteome data produced by all partners.

2. AF293: *Aspergillus fumigatus* strain 293.
JCVI: J. Craig Venter Institute

Activities

3. *Cell biology and biochemistry*. The third meeting will be organised by J.-P. Latgé from the Institut Pasteur (Paris, France) around molecular, biochemical and imaging methodologies to be developed for a better functional analysis and localisation of the proteins of interest. Various 2D and 3D light and electron microscopy technologies will be presented in a one-day workshop following the meeting.

• Publications

Public awareness of the project will be addressed through the website. This website has direct links to the web pages of the different laboratories of the partners as well as >50 sites associated with medical resources, culture collections, sequencing centres, organisations and societies, medical mycology centres and glycobiology.

Scientific dissemination will be through national or international scientific and clinical meetings, through oral presentations and posters and by publications in high impact factor peer-reviewed journals.

Publications from the project, including patent applications, the inclusion of data in publicly accessible databases and conference presentations will all be used to disseminate the advances achieved in fundamental knowledge and practical technologies.

• Exchange of PhD and post-doctoral fellows and invitation of external experts

A major issue of our project will be to stimulate exchange of scientists between member laboratories. Scientific exchanges of PhD students and post-doctoral fellows in the consortium will provide a major educational resource, exposing young scientists to new expertise beyond the scope of their individual laboratories. These exchanges should reinforce communication within the project and are an absolute requirement for practical standardisation of the methods to follow in each laboratory. Travel grants will be decided by the Steering Committee after consultation of all members of the consortium.

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- **Ministerio de Educación y Ciencia (MEC)**
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- **Medical Research Council (MRC)**
  United Kingdom

and by:

- **Institut Pasteur***
  France

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