First Status seminar of the ongoing research projects of the 1st PathoGenoMics joint call, which took place on the 7th April 2008 at the Institut Pasteur in Paris.

Microorganisms can be both useful inhabitants of the body and dangerous pathogens. This dual role has long made them a fascinating branch of scientific research. Particularly on a genetic level, disease-causing microorganisms are increasingly being analysed in the hope of identifying critical factors that might be therapeutically applicable. Under the umbrella of the *ERA-NET PathoGenoMics* 12 European research consortia started in 2007 transnational research projects dealing with genome research on pathogenic bacteria or fungi.

On the 8th April 2008 a first status seminar took place at the Institut Pasteur in Paris. All 12 project coordinators were present and gave a presentation about the progress of their project within the first year.

## Summary of the achievements of the first project year:

### Contact: Prof. Sebastian Suerbaum (Germany)
### Consortium: HELDIVNET

Seven researcher teams from four different countries have come together under the umbrella of the HELDIVNET consortium to shed light on how *Helicobacter pylori* can get out of control and cause infections or diseases such as cancer. During the first year, the scientists managed to create the first global map of the bacterium's genetic diversity. They identified the basic structure of five main populations, one of which appears to have its indigenous origins in a Namibian ethnic group known as the Khoisan. Sequencing and deeper analysis is still in progress for the South-East Asian strains. To understand how the genome of *H. pylori* changes during the infection process, another group is analysing and comparing both mutations and recombinations in sequential isolates from a group of more than twenty patients. The ultimate aim is to create a mathematical model to predict the most disease-relevant factors during infection. In another study, a team within the HELDIVNET consortium is focusing on a chromosomal cluster of bacterial genes known as the cytotoxin associated gene pathogenicity island (cag), and its influence on host interactions. Furthermore, the scientists are observing the role of the cag system using a specific animal model, the Mongolian gerbil, to reveal its relevance for cancer.

**Contact: Dr. Eduardo Dei-Cas (France)**
**Consortium: Pneumocystis in the lung**
Three academic groups from three different countries have founded a consortium to focus on the biological changes undergone by *Pneumocystis* organisms that allow them to spread extensively in the lungs, and to develop pathogenic potential. Using comparative methods, the researchers have analysed the different host reactions of *Pneumocystis* species in isolates from patients that suffering various diseases such as Chronic Pulmonary Disease (CPD) and Pneumocystis pneumonia (PcP). With the help of these proteomic level-insights into these fungi, which show an especially high activity regarding inflammatory substances during *Pneumocystis jiroveccii* infection, the scientists are now on the path to creating *in vitro* models, as well as *in vivo* mice models, that will allow the further analysis of *Pneumocystis* infections. Another team within the consortium is aiming at a better understanding of the *Pneumocystis* lifecycle, and its proliferation in the body via cystic and trophic forms. Here, the scientists' first step was to develop basic tools to make available both pure cystic and pure trophic forms for further studies.

**Contact: Prof. Karl Kuchler (Austria)**
**Consortium: FunPath**
Seven researchers from four different countries have come together to start the FunPath Initiative, with a special focus on how the yeast *Candida glabrata* is able to shield itself from the human immune system. Aided by genetic engineering techniques, large scale screening methods and the sequenced genome of the yeast, the scientists were able to delete hundreds of genes in an established knock-out protocol, as a first step towards identifying the most interesting disease-relevant genes. As a result, the researchers have already detected ten important sets of genes, which are now undergoing deeper functional analysis. Furthermore, the scientists are comparing their deletion results with a phenotyping screening to get an overview of all involved genes. In parallel, another group within the consortium is shedding some light on the pathogenic factors on the host side regarding recognition and immunological response. When the scientists have revealed all of the most interesting candidate genes, they will be analysed in a suitable mouse model, which has been created to complete the received data via *in vivo* virulence studies.

**Contact: Prof. Matthias Maass (Austria)**
**Consortium: ECIBUG**
The researcher consortium ECIBUG, which consists of nine academic groups from four different countries, is aiming to bring about a better understanding of the pathogenicity of the intracellular *Chlamydia* species in the body on a molecular level, and to find a way to medicate chronic infections, for which no therapy exists to date. With the help of the different methods employed by these nine research groups, the scientists have already made some significant steps forward. Firstly, they have identified a list of 60 effector proteins that are released into the infected host cell by *Chlamydia*. Among these, the scientists anticipate finding the proteins that contribute most strongly to a pathological reprogramming of the host cell, which will then serve as candidate targets for new antibiotic therapies. Secondly, the consortium has been able to detect two important proteins that potentially mediate the bacteria's access into the host cell. Thirdly, the researchers have identified eight newly defined compounds that show an effect on *Chlamydia* and that will be further analysed for their clinical relevance. Fourthly, the scientists have reached a better understanding of complex intracellular signalling pathways through which *Chlamydia* species regulate host cell growth and proliferation. The scientists assume that these studies will help to explain the bacteria's role in artherosclerosis and asthma. Finally, the researchers are also moving towards the development of random mutants of *Chlamydia* species through chemical

mutagenesis. With this innovative and risky approach, the scientists are optimistic about becoming the first researchers to generate *Chlamydia* mutants.

### Contact: Dr. Jean Marc Ghigo (France)
### Consortium : EPS-Matrix initiative

Under the umbrella of the EPS-Matrix initiative, five researchers from two countries are analysing the role of non-structural proteins in the biofilm matrix of bacterial communities. The consortium is focusing on four model organisms, which are being continuously studied with the help of proteomic methods. At this time, the scientists are still establishing efficient techniques for all the respective models, which will be used to generate a sufficient amount of matrix proteins. In a next step, these proteins will undergo deeper functional analysis. In another group, *in silico* studies are focussing on the so-called type-2-secretion system in the different bacteria species, which is assumed to be responsible for delivering the proteins into the matrix.

### Contact: Dr. Jesus Pla (Spain)
### Consortium : Glycoshield initiative

The surface of fungal organisms is in the focus of the Glycoshield initiative, which was started by seven researchers from three different countries. Most of the first year effort has been devoted to the establishment of general strategies and experimental methodologies to achieve the project defined goals. Based on the fungal species *Cryptococcis neoformans* and *Candida albicans*, which have been used, among others, to create different mutants with defined changes in the surface construction, the scientists were able to identify many different sets of genes that are relevant for protein secretion routes that have an effect on the fungal glycosylation process. Among these, the scientists found both known and unknown proteins that are now undergoing further analysis with regard to their specific role during the infection process in the different stages of fungal pathogenicity. Another team within the consortium has analysed a special gene family present on the GPI anchor. Again, mutants have been created to delete all of the involved genes and to analyse characteristic phenotypes.

### Contact: Prof. Axel Hartke (France)
### Consortium: Enterococcus faecalis infections

Five researcher groups from four different countries have come together to uncover the most important disease-causing mechanisms in the development of *Enterococcus* infections. The strongest challenge during the first year of funding was to establish a suitable mutant library as a starting point for large-scale screenings of the most relevant genes involved in virulence transmission of *Enterococcus faecalis*. After months of research, the scientists decided to stop working with *Caenorhabditis elegans* as the animal model, and eventually chose the moth *Galleria mellonella,* which shows a high degree of structural and functional homology to the innate immune system of humans. Based on about 20,000 insect larvae, the researchers have now established a library of around 140 mutants, comprising more than 200 gene deletions. In a next step, screenings have now begun, which will be the focus of the coming months.

### Contact: Dr. Robert Arkowitz (France)
### Consortium: Pathogenicity of Candida albicans

Three academic groups from three different countries have founded a consortium to reveal the role of *Candida albicans* enzymes (protein kinases and phosphatases) during infection and proliferation. In a first step, the researchers have constructed a genome-wide mutant library, deleting more than 100 kinases and phosphates. Based on this collection, they have

established methods for a phenotypic screening assay, with which the functional activity of each enzyme can be analysed for its different characteristics; among others, filamentous growth, biofilm formation, cell cycle, change of cell form (white opaque switching), survival capabilities in infection-related stress conditions, or drug sensitivity. The aim is to test around 180 protein kinases and phosphatases to get a deeper understanding of the regulatory and adaptive pathways used by the fungal pathogen, and to identify novel drug targets.

**Contact: Dr. Patrick Trieu-Cuot (France)**
**Consortium: Streptococcus pathogenesis**

Six researcher teams from three different countries have initiated a joint consortium to shed some light on the two species *Streptococcus pyogenes* (GBS) and *Streptococccus agalactiae (GAS)*. The key question that they hope to answer is: Why and how are these related bacteria responsible for different pathologies? Especially important in this investigation is the role of cell surface molecules. In a first step, the consortium has established a GBS/GAS mutant library, with different strains expressing different surface proteins. During the first year of funding, these mutants have provided the starting point for high-throughput-screenings, using sera from a variety of patients. Another group is concentrating on the cell envelope, and is studying the regulatory network involved in biofilm formation, as well as adhesion processes through the targeted inactivation of important pathways. As a result, the researchers have already identified significant differences between GBS and GAS regarding adherence to epithelial cells. The consortium's ultimate aim is to develop an integrative streptococcal database that allows the genome-wide prediction of gene regulatory networks based on the incorporation of published and experimental results. The structure and web interface of the database, containing the sequences of 13 GAS genomes and 3 GBS genomes, as well as enzyme data, has now been established.

**Contact: Prof. Thomas Meyer (Germany)**
**Consortium: RNAi-Net**

The host reaction during bacterial infections is at the focus of the RNAi-Net initiative, which is made up of 16 research groups from seven different countries. Using RNA interference as its core methodology, the consortium has begun to develop high-throughput systems for the targeted silencing of relevant genes in different bacterial species. Because most of the involved microbiologists have more experience in studying the bacteria themselves than the host cell, the first months of work were characterised by intensive training to establish suitable and valuable test systems. Initial studies have been conducted in a minority of the groups, for example with *Helicobacter pylori*. Thereby, the scientists have found that bacterial factors are able to influence host cells in such a way that they detach from neighbouring cells and migrate into tissue. Another group of researchers has analysed bacteria with capsules. Here, first analyses showed that capsules can negatively influence the regulatory signalling pathways of the host cells, thereby preventing recognition and elimination of the bacteria. As a result of studies with intracellular bacteria such as *Chlamydia*, the researchers have detected many host cell factors that have an influence on bacterial proliferation in the body.

**Contact: Prof. Jörg Hacker/ Dr. Ulrich Dobrindt (Germany)**
**Consortium: Focusing on Escherichia coli**

14 researcher groups from seven different countries have come together under the roof of the ERA-NET to reveal how extraintestinal *Escherichia coli* species, known as ExPEC strains, can become a health risk. During the first year of funding, the researchers have employed a wide range of genomics tools to shed more light on the molecular activity of ExPEC species in comparison to more useful and harmless *E. coli* species, as well as to pathogenic *E. coli* species in the gut. As a result, they have found that pathogenic ExPEC

strains and useful species share many characteristics, and often behave similarly under certain conditions. In contrast to disease-causing *E. coli* species in the gut, they clearly do not possess a specific set of virulence factors, which makes it more difficult to diagnose dangerous ExPEC variants. Nevertheless, the researchers have revealed that the expression of virulence-associated genes, as well as the presence and activity of regulator elements and fitness factors, play a vital role in the development of an infection. With the help of further experiments, the current aim is to lay the groundwork for the development of efficient diagnostic tools. Furthermore, the researchers have found non-pathogenic strains in the urinary tract that might be useful for therapeutic strategies. One approach could be to use these – or a genetically modified variant – to colonise the urinary passage of patients suffering from chronic urinary infects, thereby combating bacteria with bacteria.

**Contact: Prof. Trinad Chakraborty (Germany)**
**Consortium: SPATELIS initiative**

The disease-causing factors of *Listeria monocygetes* are at the focus of a group of ten researcher teams from five different countries, who have founded the SPATELIS initiative under the umbrella of the ERA-NET. Based on the analysis of genome sequences of a number of different bacterial strains, the scientists have shed some light on specific signalling pathways, surface proteins, and other proteins that play a role in replication, proliferation and adhesion. During the first year of funding, they have optimised their tools for the creation of a *Listeria* mutant library to test selected virulence gene clusters using a variety of techniques. The first studies have produced promising results in terms of the isolation and identification of specific characteristics that help *Listeria* species to remain shielded from the human immune system.