ERA-NET PathoGenoMics July 2008

NEWSLETTER - 3

Hungary Finland Spain Germany Israel France Austria Portugal Slovenia Latvia

ERA-NET PathoGenoMics:
PARTNERING WORKSHOP

The Partnering Workshop of the second joint call brought together potential applicants from across Europe

The second Partnering Workshop of the ERA-NET PathoGenoMics took place in Barcelona, in January 2008. The workshop offered a meeting ground for scientists wishing to apply to the second multinational call of the ERA-NET PathoGenoMics – "Applied pathogenomics: Prevention, diagnosis, treatment and monitoring of infectious diseases". Nine countries participated in the event: Austria, Finland, France, Germany, Hungary, Israel, Portugal, Slovenia and Spain.

The Barcelona meeting enabled its participants to exchange research ideas and form multinational partnerships. Altogether, 54 researchers from academia (67%), industry (20%) and the clinic (13%) participated in the meeting. The program included four sessions comprising 19 oral and eight poster presentations.

In addition to emphasizing the transnational, collaborative nature of eligible projects, the call also underscored the need for a partnership between academia, industry and the clinic. Therefore, a special session was dedicated to technology transfer issues, such as IPR and licensing.

The workshop was declared successful by its participants, who found it to be a helpful and fruitful environment for the formation of multinational consortiums.

ERA-NET PathoGenoMics announces 2008 PhD Award winners

The EU-sponsored network ERA-NET PathoGenoMics named three winners of the 2008 PhD Award for outstanding PhD theses in pathogenomics. The young scientists, from France, Portugal and Germany, received the award during the "Genomes 2008" conference held in April at Paris.

The ERA-NET PathoGenoMics, established in 2004, is a network of 15 funding organizations from Austria, Finland, France, Germany, Hungary, Israel, Latvia, Portugal, Slovenia and Spain, which promotes European genomic research on pathogenic microorganisms (pathogenomics). It is one of about 70 ERA-NET networks aimed at encouraging unified, collaborative research across Europe, as well as at the national and regional level in the member states. The ERA-NET was contrived as a funding instrument of the European Commission, first introduced in the Sixth Framework Program.

One of the primary goals of the PathoGenoMics network is to enhance the development of future diagnostic tools and treatments for infectious diseases by supporting European research on pathogenic microorganisms, with emphasis on analysis at the genetic level. In line with this goal, The ERA-NET PathoGenoMics awards prizes for outstanding PhD theses in pathogenomics.

This year, during the "Genomes 2008" conference (Paris, April 8-11), the third PhD Award, at the amount of 2000 Euros, was granted to three young researchers: Marie Bouvier, (France), Cristina Dias Rodrigues (Portugal) and Sascha Thewes (Germany). The winners were chosen by a review board of internationally renowned experts in the field of microbial research.
Marie Bouvier carried out her thesis at the Pierre & Marie Curie University in Paris, France, focusing on the analysis of single strand recombination in integrons. Integrons are small genetic sequences in bacteria that can capture other DNA fragments from chromosomes or plasmids and thus may play a role in horizontal bacterial gene transfer. Marie Bouvier is now working at the Max Planck Institute for Infection Biology in Berlin.

Cristina Dias Rodrigues from the University of Lisbon in Portugal, studied liver infections caused by Plasmodium parasites at the University of Lisbon, Portugal. In the course of her research, she discovered important, host-related factors that influence the infection process. Cristina Dias Rodrigues is now working at the Institute of Molecular Medicine in Lisbon.

PathoGenomics PhD Award 2008 - Award Presentation
From left to right: Mag. Nicole Firnberg (ERA-NET), Dr. Marion Karrasch (ERA-NET), Dr. Marie Bouvier, Dr. Christina Rias Rodrigues, Dr. SachaThewes, Prof. Eliora Ron (Tel Aviv University)

Sascha Thewes, working at the Robert Koch Institute in Germany, analyzed, on a molecular level, the ability of the normally harmless yeast, Candida albicans, to enter the bloodstream, invade and penetrate all organs of the body, resulting in a life-threatening systemic infection. Sascha Thewes is now working at the Free University of Berlin.

More information: www.pathogenomics-era.net
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ERA-NET PathoGenoMics: First joint call

List of project summaries at the end of the first funding year taken from the second status seminar in Paris 7.4.2008 by Sandra Wirsching, biocom

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

Seven research teams from four different countries have come together to shed light on how Helicobacter pylori can cause infections or diseases such as cancer. One of our principle areas of focus was analysis of the genetic variance of H. pylori both on a global scale and within a given patient. To achieve the former, the scientists assembled the first global genetic variance database, using five main strains across the world. On the other end of the genetic spectrum, another group is analysing mutations in sequential isolates from over twenty patients, in order to understand how the genome of H. pylori changes during the infection process. Yet another direction focuses on the role of the cytotoxin associated gene pathogenicity island (cag) in bacteria-host interactions and in cancer.

Contact: Dr. Eduardo Dei-Cas (France)
Consortium: Pneumocystis in the lung

Three groups from three different countries are focusing on the biological changes that enable Pneumocystis fungi to become pathogenic and to spread extensively in the lungs. Using comparative proteomics, the researchers analysed the different host reactions to Pneumocystis species in isolates from patients suffering from various Chronic Pulmonary Diseases (CPDs) and Pneumocystis pneumonia (PcP). These studies revealed an especially high activity of inflammatory substances during Pneumocystis jiroveccii infection. These insights assist in creating in vitro and in vivo models that will allow further analysis of Pneumocystis infections. Another team within the consortium is investigating the proliferation of Pneumocystis in the body via cystic and trophic forms. The first step was to develop basic tools for obtaining pure cystic or trophic forms for further studies.

Contact: Prof. Karl Kuchler (Austria)
Consortium: FunPath

The FunPath Initiative comprises seven researchers from four different countries, aiming to understand 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 yeast genome sequence, the scientists were able to knock-out hundreds of genes, as a first step towards identifying the most interesting disease-relevant genes. Consequently, 10 important sets of genes were already detected, and are now undergoing a more elaborate functional analysis. In the future, the most interesting candidate genes will be analysed in in vivo mouse models. In parallel, another group within the consortium is shedding light on the recognition process and immunological response of the host toward this pathogen.

Contact: Prof. Matthias Maass (Austria)
Consortium: ECIBUG

Nine groups from four different countries are studying Chlamydia, and searching for ways to treat, chronic infections. The scientists identified 60 proteins released into the host cell by the bacterium, some of which may initiate pathological reprogramming of the host cell. Two proteins that may enable the bacteria to enter the host cell were also detected. This novel protein inventory likely contains targets for new therapies. The consortium further studied the intracellular signalling pathways enabling Chlamydia to regulate host cell growth, and that may explain the bacteria's role in atherosclerosis and asthma. On the therapeutic front, the researchers identified eight new compounds with an antibiotic effect on Chlamydia. Finally, the researchers are generating Chlamydia mutants through chemical mutagenesis, a challenging approach, because this bacterium is not amenable to classical molecular genetic analyses.

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 studied using proteomic methods. Currently, the scientists are still establishing efficient techniques for obtaining a sufficient amount of matrix proteins from each model. The next stage will include a thorough functional analysis of selected proteins. Another group is focusing, using in silico methods, 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

Fungal cell-surfaces are the focus of the Glycoshield initiative, comprising seven researchers from three different countries. The first year was dedicated to establishing general strategies and experimental methodologies for achieving the project defined goals. The fungal species Cryptococcis neoformans and Candida albicans, among others, were used to create different surface mutants that enabled the scientists to identify gene products involved in protein secretion and that affect the fungal glycosylation process. Some of these proteins, both known and novel, are now undergoing further analysis with regard to their specific role during the infection process. Another team is analysing a special gene family important in forming 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 mechanisms underlying Enterococcus-related diseases. The biggest challenge during the first year of funding was to establish a suitable mutant library as a starting point for cloning genes that control virulence of Enterococcus faecalis. The initial animal model of choice was Caenorhabditis elegans, but eventually the scientists chose the moth Galleria mellonella, which shows a high degree of structural and functional similarity to the innate human immune system. After screening about 20,000 insect larvae, the researchers established a library of around 140 mutants, comprising more than 200 gene deletions. The next stage, which already begun, involves screening and cloning of candidate genes.

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. The researchers have begun by constructing a genome-wide mutant library, deleting more than 100 kinases and phosphates. The function of each enzyme in this collection will be analysed using different characteristics of the fungi, such as filamentous growth, biofilm formation, cell cycle, change of cell form (white opaque switching), survival in infection-related stress conditions, and drug sensitivity. The aim is to look at around 180 protein kinases and phosphatases in order 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 joined to study the pathogenesis of Streptococcus pyogenes (GBS) and Streptococcus agalactiae (GAS), with emphasis on the role of cell surface molecules. The consortium constructed a GBS/GAS surface protein mutant library, which provided the starting point for high-throughput screening, using sera from a variety of patients. Another group is concentrating on the cell envelope, studying the regulatory network involved in biofilm formation and in adhesion processes through targeted inactivation of important pathways. This has already resulted in the identification of significant differences between GBS and GAS regarding adherence to epithelial cells. The consortium's ultimate goal is to develop an integrative streptococcal database that allows the genome-wide prediction of genetic regulatory networks based on the incorporation of published and experimental results.

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

The reaction of the host to bacterial infections is the focus of 16 research groups from seven different countries. Using RNAi as its core methodology, the consortium is developing high-throughput systems for the targeted silencing of relevant genes in different bacterial species. The scientists have discovered that Helicobacter pylori-derived factors are able to induce host cells to detach from neighbouring cells and migrate into surrounding tissues. Other researchers discovered that the biofilms produced by certain bacteria not only protect the bacteria from phagocytosis, but also target the regulatory signalling pathways of the host cells. Studying intracellular bacteria such as Chlamydia, the researchers detected a variety of factors that influence bacterial proliferation in the body, indicating that the host cells play an active role in the infection process.

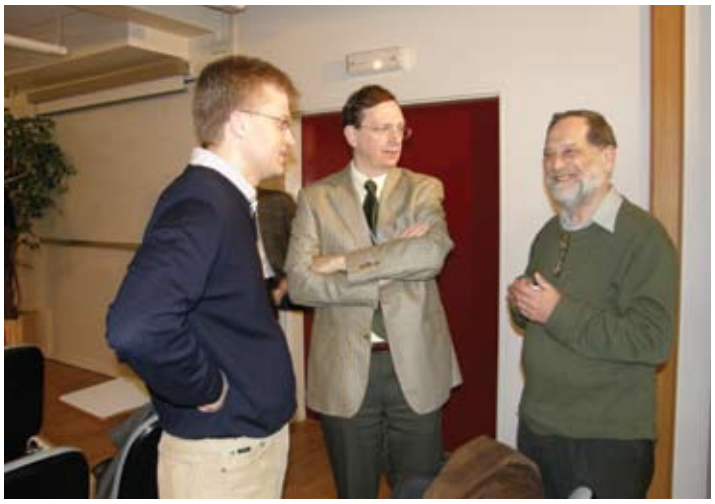
Contact: Prof. Jörg Hacker/ Dr. Ulrich Dobrindt (Germany)
Consortium: Focusing on Escherichia coli

14 groups from seven countries are studying how extraintestinal pathogenic Escherichia coli (ExPEC) strains can become a health risk. The researchers employed genomics tools to compare ExPEC species to commensal or to pathogenic E. coli strains in the gut. They found that ExPEC strains and commensal isolates often behave similarly, which makes it difficult to diagnose ExPEC variants. In contrast to intestinal pathogenic E. coli strains, they do not possess a specific set of virulence factors, yet the expression of virulence-associated genes nevertheless plays a pivotal role in the development of an infection.

The current aim is to develop efficient diagnostic tools. In addition, the researchers identified non-pathogenic strains that might be used to colonise the urinary passage of patients suffering from chronic infections, there by combating bacteria with bacteria.

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

The disease-causing factors of Listeria monocytogenes 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. Genome sequencing of a number of different bacterial strains enabled the scientists to shed some light on specific signalling pathways, surface proteins, and other proteins that play a role in replication, proliferation and adhesion. The first year of funding was dedicated to the creation of a Listeria mutant library in order to test selected virulence gene clusters using a variety of techniques. The first studies produced promising results in terms of the identification and isolation of specific characteristics that help Listeria species to remain shielded from the human immune system.



At the status seminar, some coordinators were interviewed about their experience with their consortium, first results and future goals of their projects:

Consortium: RNAi-Net

Interview with ***Prof. Thomas F. Meyer***

Looking back at the first year of funding, how would you summarize your achievements?

One of our primary achievements was to start shifting the focus of bacteriological research from the bacterial side to the role of the host cells during infection. Our main strategy is performing loss-of-function screens on the host cells using RNAi. This is a challenging approach, but we can already see significant advances. For example, the scientists have discovered that Helicobacter pylori-derived factors are able to induce host cells to detach from neighbouring cells and migrate into surrounding tissues. Another group of researchers has analysed encapsulated bacteria, and found that capsules can negatively influence the regulatory signalling pathways of the host cells, thereby preventing recognition and elimination of the bacteria. These are fascinating initial observations that must undergo further detailed analysis.

What do you perceive as being the biggest challenge within the consortium?

For us, as coordinators, the whole project is a challenge as well as a small revolution in pathogenomics, since it is now evident that the host plays an equally important role during disease development as does the bacterium itself. Much of our data results from applying large scale RNAi screens, an innovative yet challenging approach in the pathogenomics field. Still, some of the participating groups are fully dedicated to the large scale screening approach, whereas other groups have not yet begun with

RNAi screening. The learning curve is therefore immense. In a big consortium like ours, the main challenge is to bring together experienced and less experienced groups in order to spread the existing knowledge and to jointly tackle the most interesting questions in the field.

Are there notable differences between the various bacterial species studied by the consortium?

There are significant differences. One noteworthy example is intracellular versus extracellular bacteria. Studies with intracellular bacteria shed light on host cell mechanisms that play a role during bacterial replication and survival within the host cell. Our initial analysis has revealed numerous host cell factors that support or suppress the proliferation of intracellular bacteria, such as Chlamydia and Salmonella. In contrast, extracellular pathogens can grow in the absence of human cells and the interrelations between host cells and the bacteria are less pronounced, at least in cell cultures. However, studying extracellular bacteria could lead to interesting insights into host cell signalling pathways and innate defence systems activated by the adherent pathogens.

Could you describe the collaboration processes within this ERA-NET consortium?

Collaboration within the consortium takes place at multiple levels. On the most basic level, laboratories studying the same pathogen collaborate intensively within their respective work packages (WPs). On a higher level, the various WPs are laterally connected by four thematic areas (TAs) that stimulate the joint formulation and development of the project design, assay systems, and data analysis. Finally, all partners intensively interact with the central screening facility in Berlin. Several groups already participated in two training courses provided by the centre. Altogether, the laboratories involved are highly engaged, meet regularly, have frequent telephone conferences, and share results and experiences.

Do you think that the RNAi-Net approach has a therapeutic potential?

We are still at the level of basic research within this ERA-NET consortium. Yet, one can think of several cases where conventional antibiotic treatment is insufficient because substances released by the killed bacteria have the potential to exacerbate the disease. One such example is acute meningitis, for which new therapies that could efficiently block intrinsic reactions of the host cell are desperately needed. Another area where the RNAi approach might be beneficial is chronic infections, such as by Chlamydia or Mycobacteria, for which no satisfactory therapies exists to date. A promising approach would be to target human signalling pathways or cellular components that are exploited by the microorganism for their own advantage.

What are your goals for the future?

The RNAi-NET consortium is an important catalyser for studying the host cell in greater depth than previously thought. The RNAi-Net will also provide a platform for comparing host cell influences on different bacteria. Most importantly, however, we wish to extend our analysis to the clinically most relevant pathogens, including those which belong to the BSL3 risk group. This undertaking would require a screening facility at the corresponding safety level. Another long-term goal is to include research on fungi, parasites and viruses in conjunction with other research centres in Europe. To realise this goal, we are currently establishing a joint European research network called FuGID (Functional Genomics in Infectious Disease), which also includes colleagues from non-ERA-NET countries.

Consortium:

Focusing on extraintestinal pathogenic E. coli

Interview with ***Prof. Jörg Hacker and Dr. Ulrich Dobrindt***

Looking back at the first year of funding, how would you summarize your achievements?

One of our most important results is that the so called extraintestinal pathogenic (ExPEC) and non-pathogenic strains are actually part of one continuum. Both strains have the potential to become pathogenic, depending on how they exchange or activate their genes. This is in contrast to pathogenic E. coli strains in the gut, which have a very specific set of virulence factors. Another important result is that virulence-associated genes, such as certain genetic loci encoding colonisation and fitness factors, may play, under certain conditions, a role in the infection process. Examples are adhesin and polyketide associated genes as well as some iron-related genes. However, we are only slowly beginning to understand the detailed mechanism underlying the processes of E. coli infection and pathogenesis.

Do you think that your results have therapeutic potential?

Our results touch upon two clinical applications: diagnostics and bio-therapy of infections. Efficient and reliable diagnostic markers are the basis for successful therapy. However, it is extremely difficult to differentiate between ExPEC and non-pathogenic strains. We hope our results will help to develop better diagnostic tools for identify the dangerous strains. On the therapeutic front, certain non-pathogenic strains could be used to combat more virulent strains. For example, benign strains could be used to displace pathogenic ones in patients suffering from chronic urinary tract infections. The task at hand is to better characterise these strains, in order to tailor them to our needs, and to understand the mechanisms underlying such a potential displacement, in order to prevent the pathogenic bacteria from developing into even more dangerous strains.

Your consortium is a basically a multinational European network. Did this contribute to your work?

This kind of research is an excellent example of the whole being more than the sum of its parts. Our network enables blending of different intellectual approaches, methods and specific competencies. For example, our colleagues in Sweden specialise in the colonisation of urinary tract infection patients, whereas our strength lies in the field of chip technology. Other teams, in France, Israel and Hungary, are involved in analysing the repertoire of strains in animals that do not exist in Germany. The power of the network stems from this unique combination of different teams with different expertise, which come together under one roof and contribute their skills to the group as a whole.

What are your goals for the future?

It is our belief that E. coli bacteria will become increasingly relevant as pathogenic agents, as they are accumulating drug resistances at an alarming rate. We are in genuine need of good diagnostics, and we will try to reach this goal within the ERA-NET funding period. Another goal within reach is the evaluation of the therapeutic potential of colonisation of the urinary tract to treat urinary tract infections. I am confident we will make significant progress in both areas.

Consortium: ECIBUG

Interview with ***Prof. Matthias Maass***

Looking back at the first year of funding, how would you summarize your achievements?

Our foremost achievement is the creation of a consortium of nine academic groups that have coordinated their methodologies and techniques to assemble an integrative international team that communicates on an almost daily basis. This has modified our theoretical framework as well as aided in the establishment of a Chlamydia research network that has never before existed in this form.

Could you elaborate on some of your scientific results?

Firstly, we identified a list of 60 effector proteins released into the infected host cell by the bacterium. Among them are, presumably, proteins that initiate the pathological reprogramming of the host cell. We also detected two proteins that may enable the bacteria to enter the host cell. This novel protein inventory will likely contain targets for new antimicrobial therapies.

We gained a better understanding of the intracellular signalling pathways enabling Chlamydia to regulate host cell growth, and that may also explain the bacteria's role in atherosclerosis and asthma. Another insight bears on autoimmune reactions that may occur after Chlamydia infections. We discovered that Chlamydia infection triggers host cells to express an intracellular human heat shock protein on the surface of the cell, a mis-expression that might trigger autoimmune reactions. Our studies are to be complemented by the development of Chlamydia mutants using chemical mutagenesis, a challenging approach, because this bacterium cannot be manipulated using classical molecular genetic tools.

What is the therapeutic relevance of this consortium's work?

One therapeutic approach is to generate new compounds with antibiotic effects on Chlamydia using chemical databases. We already identified eight such compounds that will be further analysed for their clinical relevance against chronic infections.

Another strategy is to analyse how Chlamydia reprograms the host cell via its secreted effector proteins, and then to tailor new therapies inhibiting this process. This approach would perturb the interaction between bacterial and human gene products in order to prevent a chronic infection. At this point, we are still at a basic research level, and it would be a great success if, by the end of this ERA-NET funding period, we will identify one compound that shows potential for further evaluation in animal models.

What do you think are the advantages of such an ERA-NET consortium?

This sort of cooperation is desperately needed, particularly in our field of research, due to the difficulties in manipulating this bacterium using standard molecular genetic approaches. The consortium enables us to share information and combine expertise in proteomics, genomics, animal models and clinical experiences. We are convinced that only a large integrative network, which we have begun to form under the roof of the ERA-NET framework, can effectively handle Chlamydia research.

Hungary Finland Spain Germany Israel France Austria Portugal Slovenia Latvia

Consortium: Glycoshield initiative

Interview with ***Dr. Jesus Pla***

Looking back at the first year of funding, how would you summarize your achievements?

Most of the first year effort has been devoted to the establishment of general strategies and experimental methodologies to achieve the project defined goals. For example, some groups developed novel methodologies to analyse cellular surfaces, whereas others concentrated on the establishment of in vitro and in vivo model systems to analyse virulence and fungal-host interactions. In a concerted effort, all the groups are trying to identify novel surface components and to understand the mechanisms underlying glycosylation in the fungus.

What do you perceive as the biggest challenge of the consortium?

I think the biggest challenge is trying to identify essential components of the cell wall that have clinical value and that could become potential therapeutic targets. The problem is that pathogenicity is not normally the result of a single trait. Therefore, we need to study the regulatory networks and interactions among the genes and processes involved and to understand their role in constructing the cell wall “glycoshield”.

What do you think could be the therapeutic output of your research when the ERA-NET project is finished?

When dealing with surface molecules of a pathogen, there are, generally speaking, two clinical approaches: using defined structures or molecules to prevent infections, or alternatively, targeting them to cure infections. These two basic strategies are the ultimate goal of our project, but we will of course accumulate a great deal of information before evaluating the results to see if they fit into any one of the slots. It should be emphasized that glycosylation processes have a considerable clinical relevance. Therefore, although we are using yeast and fungi as models, the information we obtain will surely be relevant to human cells.

What do you think is the advantage of a European network under the roof of the ERA-NET PathoGenoMics?

I think this kind of cooperation is very important for maintaining contact with colleagues, sharing ideas and defining common goals. In our case, the groups knew each other beforehand, but under the umbrella of the consortium, our cooperation has been enhanced and strengthened. The biggest advantage is the ability to integrate different approaches and methodologies, such as structural biology and molecular genetics. Everyone stands to profit from this endeavour, and we are able to advance in a greater pace than before. We participated in two training courses provided by the centre. Altogether, the laboratories involved are highly engaged, meet regularly, have frequent telephone conferences, and share results and experiences.