EUROPEAN SCIENCE FOUNDATION

RESEARCH NETWORKING PROGRAMME

Functional genomics in *Aspergillus fumigatus* and new strategies to fight against the first fungal pathogen in Europe: FUMINOMICS

Second Annual Meeting report on Proteomics and Bioinformatics tools, Oct 22-25, 2009, Nouan-le-Fuzellier, France.

Group picture of the participants of the FUMINOMICS workshop at the Ferme de Courcimont, Nouan-le-Fuzellier.

The second annual meeting of FMINOMICS took place in Nouan-le Fuzellier, France. Thirty five members of the consortium, including senior and junior scientists from each of the fifteen partner laboratories, met to discuss their experience and results about proteomics and bioinformatics tools. The program of the meeting is included below.

Second Annual Meeting on Proteomics and Bioinformatic Tools, *Nouan-Le-Fuzelier, France, Oct 22-25, 2009,* Program.

Thursday October 22nd, 2009	
Arrival in the Ferme de Courcimont Nouan-le-Fuzelier	
Day 1 - Friday October 23rd, 2009	
Introduction	*Jean-Paul Latgé*
Transcriptome (chair JP Latgé)	
Comparative transcriptomic analysis on 3 types of microarrays (AF genechip - PFGRC microarrays and amplicon microarrays)	*Jean-Marie François* *Véronique Le Berre*
Experiences with the Toulouse microarrays (1) Exploration of *Aspergillus fumigatus* response to oxidative stress using microarrays and qRT-PCR approaches	*Emilie Frealle*
Experiences with the Toulouse microarrays (2)	*Anne Beauvais*
The future of Transcriptome (Round Table)	*E.Frealle, A.Beauvais,* *JM François,* *V. Le Berre, E. Bignell*
Protein Families in *A. fumigatus* (chair: H. Haas)	
PrtT, a regulator of extracellular proteolytic activity, is dispensable for virulence	*Anna Bergmann*
PrtT and Proteases	*Shelly Hagag*
Investigating the role of glycoside hydrolases in cell wall remodeling	*Lukas Hartl*
The superoxide dismutase family in *Aspergillus fumigatus*	*Karine Lambou*
Application of the Split-Ubiquitin Membrane Yeast Two-Hybrid (MYTH) System to analyse membrane protein complexes involved in *A. fumigatus* sensory perception	*Margherita Bertuzzi*
Histidine kinases in *A. fumigatus*	*Gwenaël Ruprich-Robert*
Open Discussion	*All*
Day 2 - Saturday October 24th, 2009	
Proteome(Chair: Axel Brakhage)	
Proteomics of *Aspergillus fumigatus* physiology and pathogenicity-2D-gel electrophoresis and other techniques	*Olaf Kniemeier*
Proteome studies of the immunosuppressive Gliotoxin family during *A. fumigatus* biofilm formation	*Marc Seidler*
The data warehouse OmniFung and associated tools for the analysis of transcriptomic and proteomic data of *Aspergillus fumigatus*	*Daniela Albrecht*
The Future of Proteome (Round Table)	*A.Brakhage, H. Haas,* *F-M Mueller*
Interactions with the Host and news in the field (Chair: E. Bignell)	
SidL, an acetyltransferase involved in biosynthesis of the intracellular siderophore ferricrocin in *Aspergillus fumigatus*	*Michael Blatzer*
Characterization of the interaction between human immature dendritic cells and recombinant Aspergillus fumigatus antigens	*Michael Ok*
Expression analysis of the interaction of Aspergillus fumigatus with dendritic cells and neutrophils	*Oliver Morton*
Platelets and *A. fumigatus*	*Susanne Perkhofer*
The Scientific paper(s) of the year I liked	*John Morrissey*
News in Anti-fungal Drugs	*Emilia Mellado*
The thoughts of a specialist in immunology	*Dorel Lucian Radu*
Conclusion and Questions	
Is Fuminomics going to start a mutant library of *A. fumigatus?* - Interest of the participants? - If yes, when? Grant Applications?	*All*
Sunday October 25th, 2009 Departure	

Twenty talks were presented mainly by junior scientists (fourteen out of twenty). A lot of time was kept for discussions that are essential for the success of such network and have been indeed very fruitful scientifically.
Large scale biological studies in *A. fumigatus* are based on the classical flowchart presented in Figure 1 that is the main topic of FUMINOMICS. Although the second meeting was based on Proteomics and Bioinformatics tools, other topics (especially the follow up of our Transcriptome discussion during the first meeting) have been discussed. The most significant scientific presentations and discussions have been selected to be included in this report.

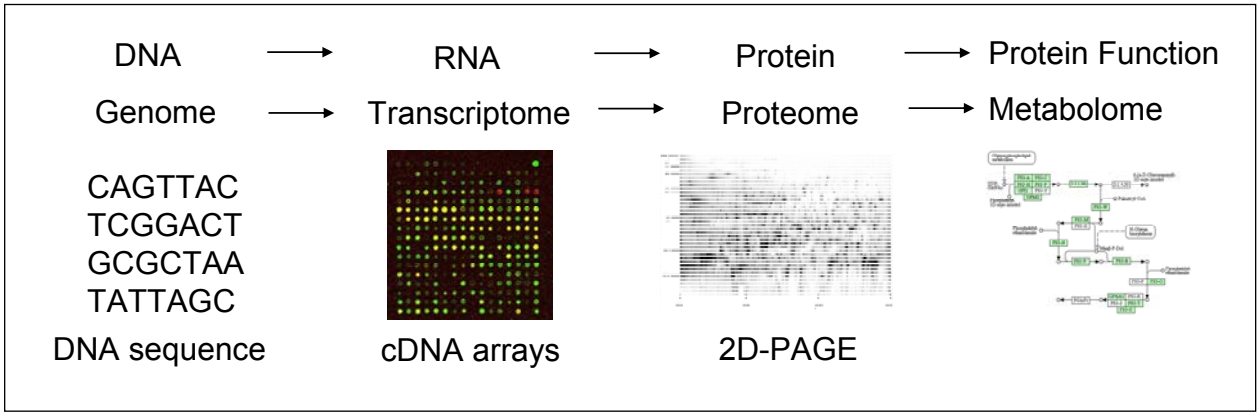


Figure 1: Steps involved in the global analysis of the A. fumigatus physiology.

A. Transcriptome

Major problems in the transcriptome results have been highlighted during the first meeting in Giens (Sept 18-21, 2008): in particular 1) differences in gene expression observed after analysis of the slides from Toulouse were not correlated with QRT-PCR data; 2) important discrepancies were obtained between data obtained with the Toulouse and TIGR chips. A conclusion of the meeting was that RNA extraction methods, labelling and hybridization conditions were different between laboratories making the comparison very difficult. In Giens, two decisions were taken to validate the *A. fumigatus* microarray: 1) a protocol was set up and agreed by all the participants for a final validation of the European slides; 2) a comparison with the American chip has been undertaken. For that purpose, V. Le Berre went to JCVI laboratories in Washington (were TIGR biochips are designed), thanks to an ESF grant covering the trip and stay at the JCVI, from the 26th to the 30th of January 2009. The comparative transcriptomic analysis was started earlier in January by Omar Loss (partner 11), who prepared five RNA biological replicates after calcium treatment of *A. fumigatus* for 15 min. Each RNA batch was separated into three pools (Figure 2). Three types of microarrays were tested:

1- The *Aspergillus fumigatus* oligonucleotides microarrays (70 mers) designed and spotted by the Pathogen Functional Genomics Ressource Center (PFGRC) of the U.S. NIH. (=TIGR slides)

2- The Toulouse Biochip microarrays (50 mers).

3- The amplicon arrays fabricated and spotted in the laboratory of Infectious Diseases (W. C. Nierman) at the JCVI. The amplicons are PCR products around 700 bp. These slides were already used at E. Bignell's laboratory (Partner 11). It was anticipated that 18 Genes (6 genes only expressed on TIGR + 6 genes only expressed on Biochip + 6 genes expressed on both slides) up and down regulated (total number of up- and down- regulated gene equal to 36) would be analyzed by QRT-PCR by using the same RNA batch (pool 3) soon.

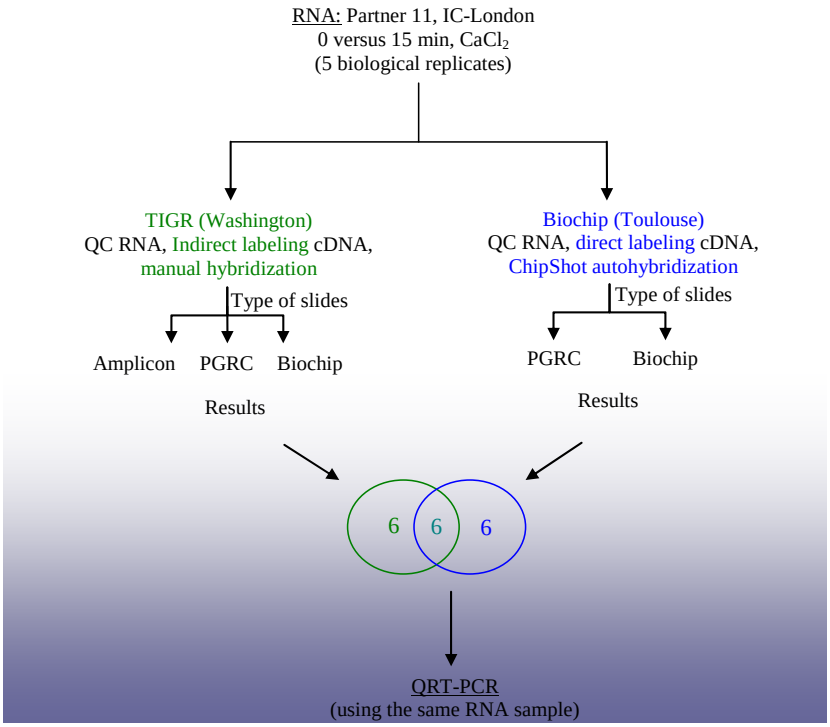


Figure 2: Validation of the transcriptomic analysis of *A. fumigatus* gene expression on Biochip (Toulouse). First part of the validation has been completed (Quality Control of the RNA samples, optimization of the labelling and hybridization method). Final biological validation by RT-PCR will be completed before the end 2009.

V. Le Berre has shown that the slides of Toulouse and JCVI were correctly spotted. However major discrepancies were seen in the transcriptome data (Figure 3). There was almost no correlation between the results obtained with the PGRC and Biochip slides when hybridization and labelling were performed at Genotoul plateform, while correlation was although low and better between the two chips when processed at JCVI (30 – 70 % of modulated genes were common to PGRC and Biochip). These results highlight that the discrepancies were due to the labelling and hybridizing steps, which were different in both platforms. The importance of these two steps seems to be specific of *A. fumigatus* since it was not observed in other spp. such as *Saccharomyces cerevisiae* or bacteria.

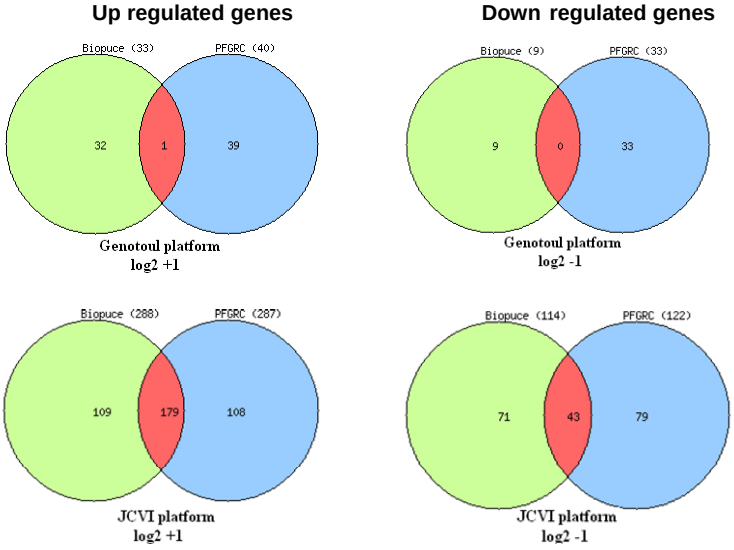


Figure 3: Summary of the transcriptomic results obtained on the two platforms (Genotoul (Toulouse) and JCVI (Washington)).

In parallel to the stay of V. Le Berre at JCVI, another comparative study was undertaken by partners 1, 2 and 4, while analysing gene expression during growth in shaken conditions versus static agar conditions. RNAs samples were extracted by Partner 1, half of them were analysed in Toulouse (Partner 2), and the other part was analysed in Madrid (Partner 4). In Toulouse, direct labeling of RNA was performed during retrotranscription with fluorescent dCTP (Cy3 vert ou Cy5 rouge) followed by automatic hybridization at 42°C with the commercial buffer ChipHybe containing 20% formamide. In Madrid, indirect labeling was obtained by incorporation of amino-allyl dCTP (ou UTP) during retrotranscription, and labeling of the cDNA with Cy3- or Cy5-NHS esters which bind to amino-allyl, followed by manual hybridization at 42°C in the presence of 30% formamide. Low overlapping of the modulated genes was found between the two centres (32 genes were found in common, Figure 4). These data also pointed out a problem in the labelling method, as the main differences between the two methods consisted in the labelling method (direct or indirect).

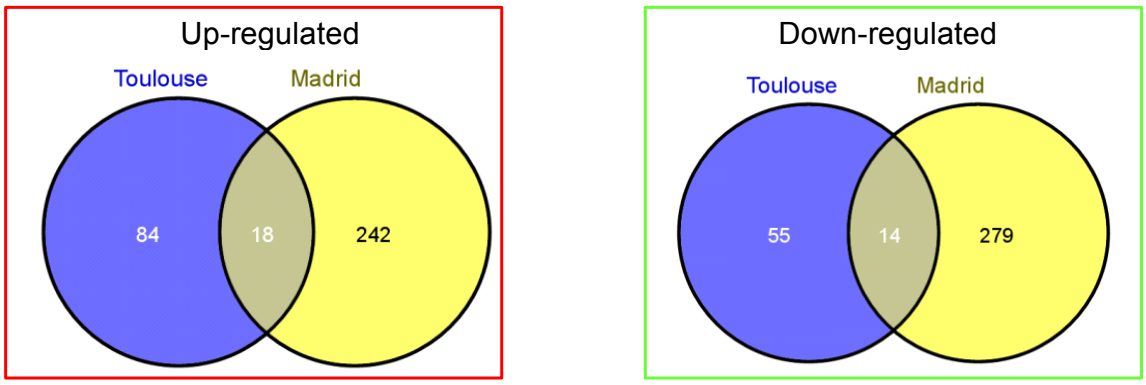


Figure 4: Gene expression of *A fumigatus* during growth in shaken conditions versus static agar conditions and comparison of the results obtained with the Biochip in Toulouse and in Madrid.

Partner 12 explored the role of anti-ROS mechanisms, especially SODs, during *in vitro* oxidative stress by menadione at low (0.08 mM) or high (0.8 mM) concentration at 31°C for 30 min followed by transcriptomic analysis in Toulouse with the Biochip. The comparative data obtained by QRT-PCR and microarrays confirmed the poor correlation between both data sets. The use of the Illumina system was proposed by Partner 12 and discussed by the *A. fumigatus* community. Although it was well received by the community, the price of the sequencing makes this approach impossible to date (more than 20 000 euros per one experimental condition).

Table 1 presents the transcriptome analysis steps which were optimized during the first year of the ESF program. The best protocol will be established before end 2009. The definition of the best labelling protocol (coloured in yellow) will be undertaken by Partner 2 with RNA samples from Partner 11, and subsequently from Partner 1. RNA samples from two conditions will be processed by (1) direct labeling, (2) indirect labeling, with only polyT primers. Two hybridization buffers, available in the commercial kit, will be tested: the first one contain 20% formamide and the second, 40% formamide. It will allow the final definition of the best and unique protocol for transcriptome analysis of *A. fumigatus* at the Genopole in Toulouse to be agreed by all participants.
Regarding the costs, the price for two biological conditions analysed by Biochip in Toulouse reaches 600 to 800 euros.

Improving the annotation of the genome thanks to transcriptomic or pyrosequencing method is possible. All members of the consortium confirmed there are still lots of annotation errors.

On this purpose, a library of cDNA is required. S. Krappman (Partner 15) and A. Bakhage (Partner 3) proposed to lead the curation of the annotation.

Step	Q	A	Comment
RNA samples	Quality	Yes	The best quality of RNAs was obtained by Trizol method.
	Amplification	No/Yes	Amplification of RNAs should not be used except if it is required (samples with very low concentration in Af such as biological tissues). The limit of RNAs detection has to be validated for indirect and direct labelling with either 500 ng or 5-15 µg respectively.
Labeling	Direct		Primers: polyT and Random Method: Chip Shot labelling kit (Promega) at 42°C
	Indirect		Primers: polyT Method (1, partner 2): Manual labelling at 42°C Correlation was good (30 – 70 % of modulated genes in common) between the three chips (PGRC, Amplicon and Biochip). Cost was 50 euros/slide. Method (2, partner 3): Universal Labeling System arrayCGH Labeling Kit (with Cy-dyes, KREATECH). Cost was 70 euros/slide.
Hybridization	Automatic	Yes	Hybridization is performed at 42°C, automatic hybridization was preferred to a manual technique to allow a standardization of the experiment.
	Formamide	20-50 %	% formamide seems not to be important as shown by partner 1 and 2, but this will be validated.
Image analysis	Bioplot software	Yes	Bioplot (Genepix) is the best software to analyse images. Spotfinder or Midas can be also used. One centre has to be defined to store all the images and associated analysis in the same database (see chapter 3.C)

Table 1: Summary of the transcriptome analysis steps tested during the first year of the ESF program.

B. Proteome

1. Proteomics: where are we ?

Partner 3 has pioneered the use of 2D-PAGE and mass spectrometry for *A fumigatus*. O. Kniemeyer (Partner 3) presented a state of the art review of the methods to be used to date for the proteomic analyses of *A. fumigatus*. Currently, the most suitable protein separation techniques are based on 2D-gel electrophoresis, in which proteins are solubilised, separated by charge and size in a gel matrix to obtain a two-dimensional image. Images can be analysed by using image quantification software. To identify proteins, spots of interest are excised, submitted to tryptic digestion and final peptides are analysed by MALDI-TOF. The MASCOTT score gives the probability of matching with a know protein. Several proteomic studies performed in Jena were presented.
The first intracellular soluble protein map (published in 2006 (Kniemeyer O. et al. 2006 Current Genet, 49; 178) shown 381 protein spots. Most of them (22%) were associated to metabolic process. The second protein map described secreted proteins during cultivation on AMM medium. In the third proteome map, Partner 3 analysed the antigenic properties of the proteins through western blot analysis of the 2D gels with serum samples from patients. O. Kniemeyer (Partner 3) presented proteomic analyses of an *A. fumigatus* strain in response to low oxygen partial pressure. To maintain a constant oxygen partial pressure, an oxygen-controlled chemostat for cultivation was used. Proteome analysis by using DIGE method

(DIfferential Gel Electrophoresis), which main advantage is to analyse samples obtained from two different conditions in the same gel by labelling proteins with Cy dyes (Figure 5), revealed an increased expression of proteins involved in glycolysis, amino acid biosynthesis, stress response and respiration under hypoxic growth conditions. In addition, molecular oxygen incorporating monooxygenases of the ubiquinone biosynthesis pathways were up-regulated under hypoxic growth conditions as well. In contrast, proteins involved in sulphate assimilation and acetate activation were down-regulated. These results were discussed in the context of pathogenicity and an outlook will be given for potential future proteome projects of *A. fumigatus*.

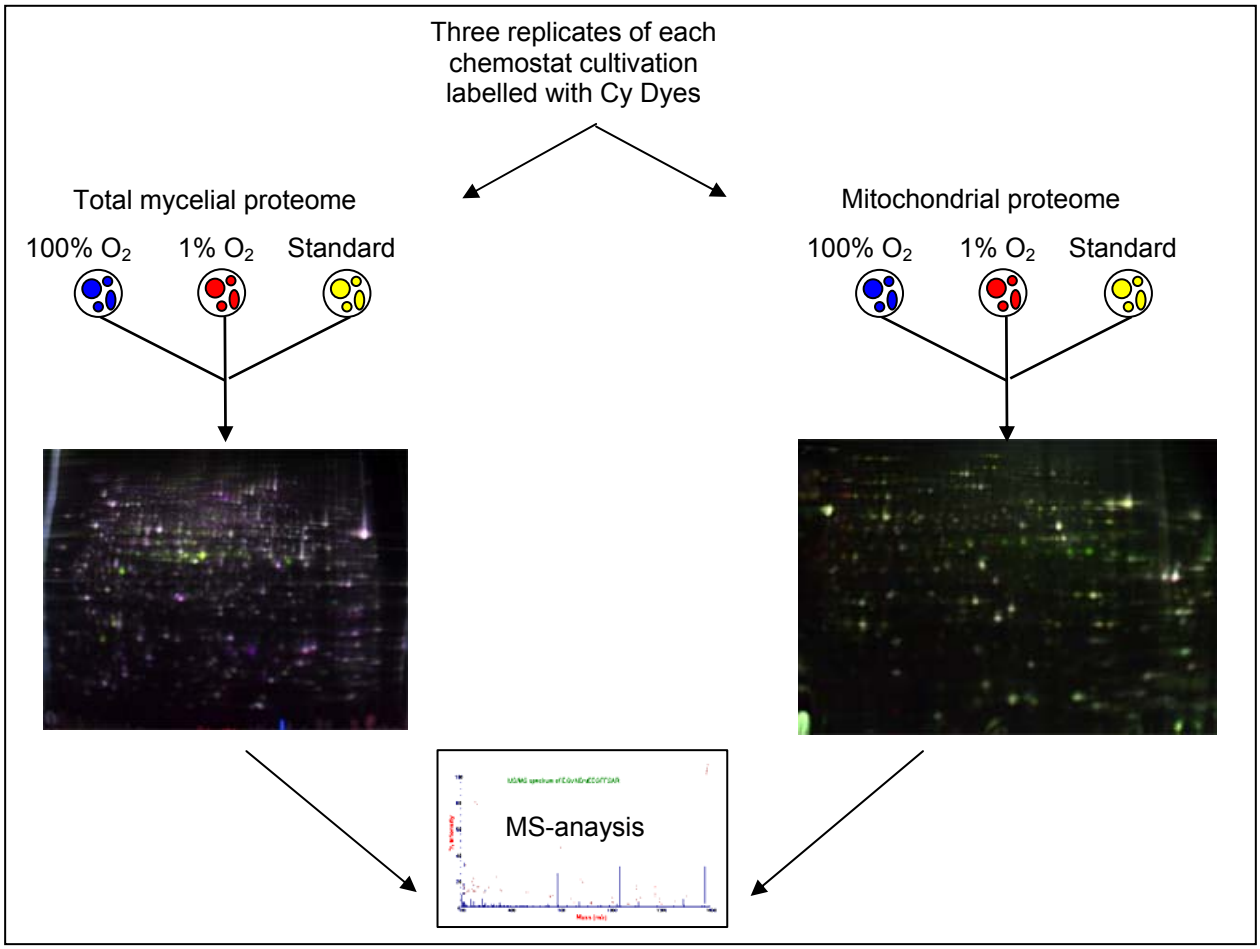


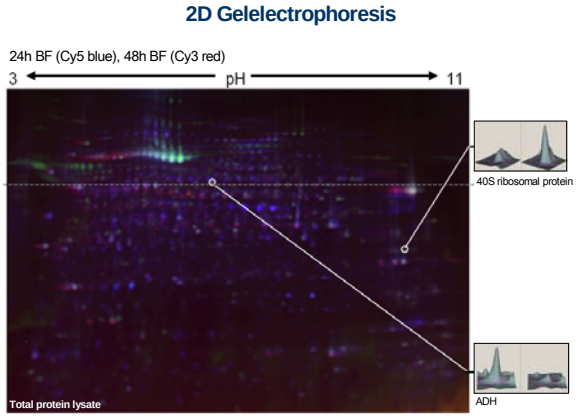
Figure 5: DIGE proteome analysis of the hypoxic response of *A. fumigatus*.

Partner 3 is developing new strategies to investigate proteins present in low amount and membrane proteins by playing with pH range, subcellular fractionation, enrichment strategies or pre-fractionation method based on physiochemical properties.

2. Combination of transcriptome and proteome data: the example of the cluster of the immuno-suppressive Gliotoxin during *A. fumigatus* Biofilm Formation (Partner 17 and 3)

Collaboration between Partner 17 and Partner 3 produced a complete study of *Af* biofilm formation by using a combination of transcriptome and proteome analysis. M. Seidler (Partner 17) described the proteome and transcriptome of planktonic and biofilm grown *A. fumigatus* mycelium after 24h and 48h. Proteins were isolated and a 2D DIGE gel was performed followed by MALDI-TOF analysis. JCVI microarrays were also performed after DNA

isolation of the biofilm. The most striking result was the significant upregulation of proteins and genes of the secondary metabolite cluster (e.g Gliotoxin). Gliotoxin, an epipolythiodioxopiperazine metabolite, is a sulfur-containing antibiotic produced by *A. fumigatus*, which possesses immunosuppressive properties and can cause apoptosis in certain types of cells of the immune system and displays anti-inflammatory activity in vivo. The glutathion S-transferase GliG showed a 1.5 fold increased protein level in biofilm in comparison to planktonic growth after 48h (Figure 6). The thioredoxin reductase GliT, showed a 2.1 fold increased level over time. Among the genes of the gliotoxin cluster in 48h biofilm, GliZ, AFUA_6G09570 and AFUA_6G09580 were slightly upregulated in a time dependant manner. Only GliJ and GliO were upregulated more than 2-fold. The results were confirmed by RT-PCR. These data showed that proteome and transcriptome data can be very complementary.



Putative Function	Ratio	
	24+/24-	48+/48-
AFUA_6G09690 Glutathion S-transferase **GliG**	-2.1	1.5

Putative Function	Ratio	
	48+/24+	48-/24-
AFUA_6G09740 Thioredoxin Reductase **GliT**	2.1	1.2
AFUA_6G09690 Glutathione S-transferase **GliG**	3.5	1.1

Figure 6: Proteome and transcriptome analysis of planktonic and biofilm grown *A. fumigatus* mycelium after 24h and 48h with respect to the regulation of gliotoxins.

3. Future of proteomics

Proteome mapping takes 3-6 month. As a consequence, the number of proteome experiments that can be performed at Jena is limited (four to six experiments per year). Partners from the FUMINOMICS consortium can send their sample directly to O. Kniemeyer (Partner 3) to perform proteomic analysis and inform the community of their investigation. To increase the number of proteome maps to be analysed, E. Bignell (Partner 11) has proposed to start a collaborative discussion with JCVI, where proteomics projects about various pathogens are also initiated. Various proteome maps have already been undertaken by members of the community. The studies are related to three topics critical for Invasive Aspergillosis: 1) growth stages: conidia/hyphae/germlings/biofilm; 2) in vivo samples: rabbit/BAL and 3) temperature, pH or oxidative stress. See below a list of proteome maps achieved or under investigation in Jena (Partner 3) and the corresponding partner:

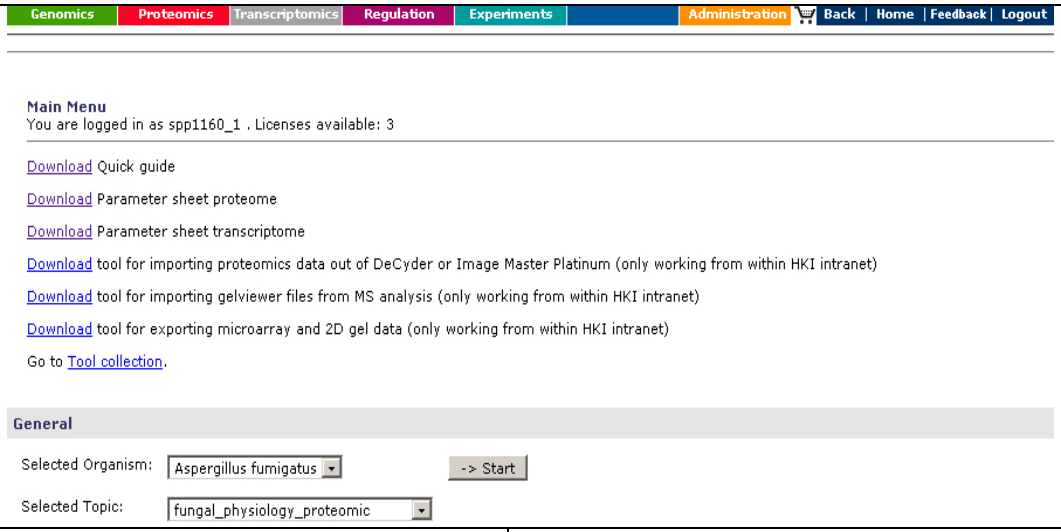
1. Intracellular versus secreted proteome (published – Partner3)
2. ROI inhibitors (published – Partner 3)
3. Iron limitation (Partner – 14)
4. Neutrophils versus Fungus treated with neutrophils (Partner 3)
5. Biofilms (Partner 6, partner1)
6. Mutant deletion: PMT4 (Partner 1), cAMP and MAP kinases (Partner 3)
7. High temperature/ heat shock (partner 3)
8. Hypoxia (Partner 3)
9. Alkali / Acid pH stress (Partner 11)

Proteomic of the plasma membrane proteins is still missing because of their insolubility. LC-MS/MS strategies could be used in this case. Efforts in the construction of subcellular proteomic maps will be undertaken with a special focus on plasma membrane, through collaboration between Partners 3 and 1. Proteomic of the cell wall is very difficult because there will be lots of contaminant protein like transient proteins or membrane proteins. Concerning "hypothetical" proteins, it has been proposed to gather all the results associated to the expression of an unknown gene obtained in various conditions in Omnifung database. Image acquisition and analysis has also to be standardized, if members of the community want to compare their pictures with pictures from the database. Partners interested in developing a proteome map for their own study must contact O. Kniemeyer that will rank the project after discussions with the Proteome team in Jena.

C. Data warehouse

To date, there are only four databases containing *A. fumigatus* data: CADRE, NCBI and e-fungi are genome database; ArrayExpress collect transcriptome data, while there was no data warehouse to collect proteome results. One more difficulty is that there is not a single database containing *A. fumigatus* data from all three cellular levels, while it is already well developed for other fungi such as *Saccharomyces cerevisiae*. To solve this problem A. Brakhage and coworkers have established a data warehouse at HKI Jena, collecting data from microarrays and 2D gels of human-pathogenic fungi (*Aspergillus fumigatus* and the model fungus *Aspergillus nidulans*): www.omnifung.hki-jena.de. Omnifung aimed to be suitable for storing experimental data of microarray or 2D gel experiments, re-analyse experiments with new technique or new point of view, compare your own results to results of other investigations, look for information on special gene / protein such as protein function, gene cluster and category and improve the annotation. All the web links are summarized in the following table (Table 2).

Web link	Comments
http://www.omnifung.hki-jena.de/csp/protecs/login.csp	Main menu - Login: Public User or personal - General: select an organism (Aspergillus fumigatus) and a topic (transcriptome or proteome) and press start - Project: select a project ID and get information about the publication, culture condition and strain, - Proteome map: protein 2D gel can be downloaded onto your own computer as a mastergel.mel or .jpeg file - Protein identification is visible, AFU numbers are provided for each protein which has been indentified - By following the same, transcriptome maps can be obtained



Tool collection - Web links	Comments
www.omnifung.hki-jena.de/Rpad/DIGE_analyzer.Rpad	DIGE analyzer gives you the known available proteins. It does normalization for each gel. (Z scores are fixed at 95%. Confidence level: -1.06; 1.10 means a two fold change)
www.omnifung.hki-jena.de/Rpad/kaplan.Rpad	Survival analysis is a tool to perform Kaplan-Meyer analysis of your virulence screening in an animal model
http://www.omnifung.hki-jena.de/Rpad/Distance_Scan62145298745/index.htm	Distance scan is a tool for prediction of potential functional combinations of transcription factor binding sites
www.omnifung.hki-jena.de/Rpad/server/FungiFun/FungiFun.pl	FungiFun helps you in choosing the most adapted categorization of fungal proteins Categorization method: **FunCat** is the best ranking system for annotated protein at the moment for *A fumigatus* Level/Branch: level 2 provide more pathways. A value below 0.01 tells you that your protein belong to this process / category

Table 2: Web links and tools associated to Omnifung database.

The data warehouse is still under development in Jena but proteome and transcriptome data have started to be stored in. It is planed that all data obtained in Europe on the *Systems biology* of *A. fumigatus* will be stored in Omnifung and available for all the FUMINOMICS Partners. Six partners of the FUMINOMICS consortium (partners: 1, 3, 9, 14, 18 and S. Perkhofer) proposed to participate actively to the Jena database.

D. New advancement in the analysis of protein families and host fungus interaction

Conidial germination and resistance to phagocytes are critical and essential events in initiating disease, which are under the control of multigenic processes and multigenic families. It is essential to be able to analyse individually or together all the genes of *A. fumigatus* to understand the putative compensatory reaction occurring in this fungi. *ΔKu70*/*ΔKu80* strains and the use of PCR fusion have shortened the cloning and transformation duration. Partner 15 developed a recyclable deletion cassette containing a Cre/loxP system, to overcome the limited number of selection markers available for *A. fumigatus.*

1. Analysis of protein families

The following table presents some of the families discussed (Table 3).

Family name	Number of genes	Deletion method	Phenotype / function
PrtT (Zn2Cys6-binuclear cluster protein family)	~50 secreted proteases	Deletion cassette ble/lox (partner 15) or Deletion using a hygromicin selective marker (partner 9)	PrtT is required for transcription of several genes encoding secreted proteases PrtT seems not to be necessary for virulence of this opportunistic pathogen Culture filtrate from the *AfPrtT*-deleted strain exhibits reduced killing of lung epithelial cells and reduced lysis of erythrocytes in vitro.
Hydrolases	18 Chitinases 10 β(1,3)glucanases 9 α(1,3)glucanases	Deletion using different antibiotic selective markers (partner 1) of - 5 ChiA, - 4 β(1,3)glucanases	 No phenotype No phenotype
Superoxide dismutases -SODs	4 genes	Deletion using different antibiotic selective markers (partner 1)	SOD1 and 2: role in intracellular oxidative stress, elevated temperature, germination SOD3 no hypersensitive to intra- and extracellular superoxide anions SOD4 is essential
Histidine kinase	13 genes	Deletion of Chk1, Nik1 and Sln1 using different antibiotic selective markers (partner 10)	Sensitivity to oxidative stresses and some antifungals
pH sensing proteins (Pal)	6 genes	MYTH (Yeast two hybrid system)	The split ubiquitin screen will permit identification of novel interacting partners of PalH and PalI, whose role in virulence could be further tested in *Af*

Table 3: News in protein family studies

2. Understanding of multi-partnership during interaction with the host

Siderophores are molecules that allow iron transport and their biosynthesis is important for virulence. M. Blatzer (partner 14) presented the study of *sidL* gene, one of the six genes involved in siderophore biosynthesis in *A. fumigatus* (*sidA, I, H, D, G, F, C*). *sidL* is required for biosynthesis of hyphal ferricrocin and conidial hydroxyl-ferricrocin under iron repleted conditions. Deletion of *sidL* gene causes smaller conidia size, defects in conidial germination and higher sensitivity to oxidative stress. Cellular localization was nicely assessed by using a *ΔsidL* strain complemented with a functional copy of *sidL* fused to *gfp.*

A new alternative in vivo model was proposed to study virulence of *A fumigatus* mutants: the Zebrafish model (Partner5 - J. Morrissey).

Resistance of *A. fumigatus* to phagocytic attack is also typically a multigenic phenomenon. The ability of *A. fumigatus* to activate, suppress, or subvert host immune response during life cycle in vivo through dynamic changing of cell wall structure and secretion implicates discriminative immune sensing of distinct fungal components. In the study of M. Ok and co-

workers (Partner 7), authors have comparatively investigated secreted protein Aspf1 and membrane-anchored protein Crf1 for the ability to induce maturation of human DC.

Partner 6 applied transcriptomic analysis to study interaction of *A. fumigatus* with DC. Monocyte-derived DC were co-incubated with *A. fumigatus* (Af293) resting conidia over 12 hours in RPMI + 5% FCS. Cells were stained with neutral red to determine their viability, *A. fumigatus* RNA was extracted using RiboPure Yeast Kit (Ambion). DC RNA was extracted using RNeasy Kit (Qiagen). *A. fumigatus* gene expression was analysed with *A. fumigatus* whole genome amplicon arrays (JCVI). DC expression was analysed using immune array (AG Loeffler). Response to host immune cells is a complex process involving changes in metabolism, cell wall synthesis, and transporter activity (Figure 7). No obvious "smoking-gun" could be isolated from the results, but pathogenesis related genes were upregulated.

Af + DC	Af + Neutrophils
•Catabolism ↑	• Fatty Acid Catabolism ↑
•Conidial catalase ↓	•Catalases and MnSOD ↑
•Oxidoreductase Activity	•Reductive Fe Transport/ Response to oxidative stress
•Transporter Activity – Pathogenesis/resistance	
•Asp F1, Asp F2, Asp F4	•Asp F4

Figure 7: Comparison of ***A. fumigatus*** interaction with **DC** and interaction with neutrophils.

S. Perkhofer (Invited partner) showed her recent investigations in the antifungal effects of platelets against *Aspergillus* fungal germination and hyphal elongation (Figure 8), both of which are of major importance in evolving invasive disease. After incubation of *A. fumigatus* hyphae with platelets at 37°C for 30 min, protein changes were analyzed by 2-D Electrophoresis.

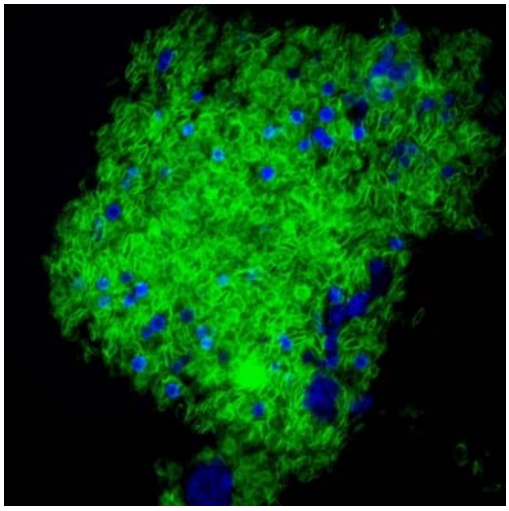


Figure 8: Platelets strongly adhere to A. fumigatus cells and reduce fungal germination and elongation. Conidia (blue, Calcofluor white), Platelets (green, FITC-anti-CD42b), incubation 15min at 37°C (Perkhofer et al., JID 2008)

Expression levels were confirmed by northern blot analysis and quantitative real-time RT-PCR. Incubation of platelets with *A. fumigatus* revealed a change of numerous proteins mainly associated to mitochondria. Their results indicated that fungal mitochondria are targeted by human platelets and that the turnover rates of the entire citric acid cycle are massively diminished. Mitochondria are the energy machines and their impairment is associated with decreased cell function and cell death.

Finally, "Emergence of *Aspergillus fumigatus* azole resistance was presented by E. Mellado" (Partner 16). *Aspergillus fumigatus* azole resistance was first detected in 1997. The underlying molecular mechanisms of resistance have been thoroughly studied and characterized and resistance patterns depend on specific mutations in the azole target: Cyp51A. Although *A. fumigatus* azole resistance detection started as a trivial problem, recent reports are worrisome with increasing percentages of resistant strains going up to 5 to 12 % in different European countries (PLoS Medicine | November 2008 | Volume 5 | Issue 11 | e219) (Figure 9).

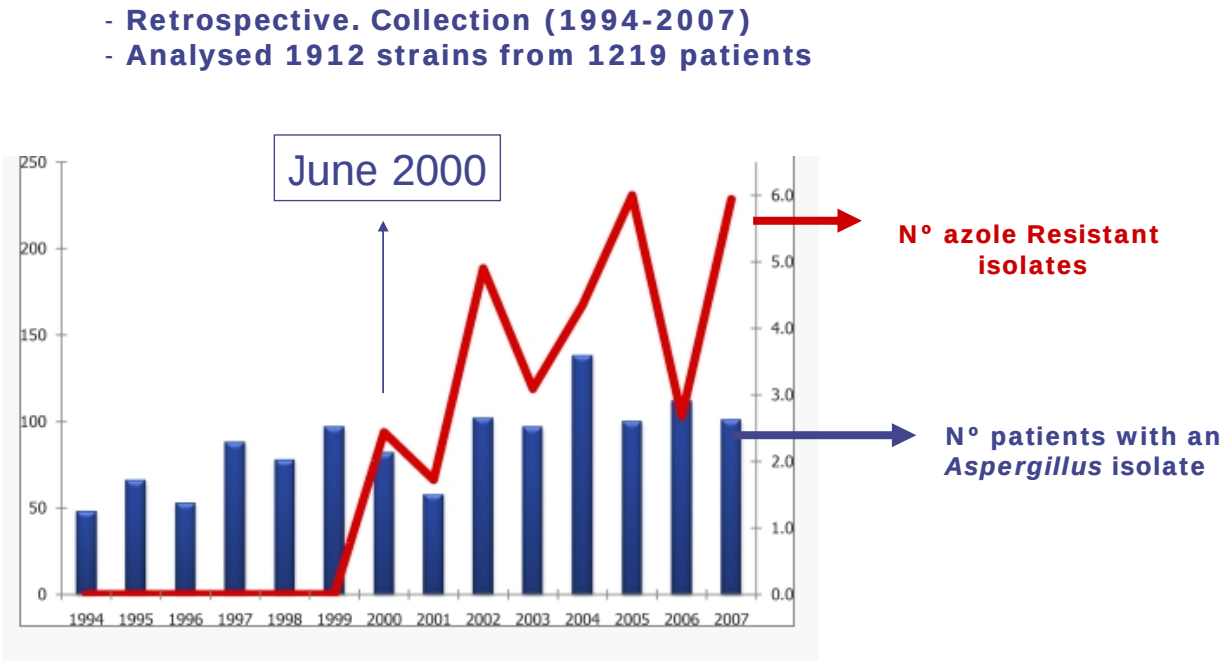


Figure 9: Recent reports show increasing percentages of resistant strains going up to 5 to 12 % in different European countries.

Even in new azoles: Isavuconazole, Ravuconazole and Albaconazole, a cross-resistance is expected. Partner 16 highlighted the fact that we need more epidemiological studies and a System Biology of *A. fumigatus* could be of the best use to analyse the response of wild-type and mutant strains to antifungals.

E. Impact of the event on future research directions in the study of *A. fumigatus*

Thanks to ESF, 15 European research groups have openly shared their experiences in transcriptomic, proteomic and genomic study and join their comments. Like last year, the second annual meeting offered the opportunity to junior scientists to present their research data in transcriptomic, proteomic and genomic analysis. Collaborations at the EU level were strengthened at this meeting in a very friendly and cooperative atmosphere. A major achievement of the meeting was the management of all the data and protocols recently developed in the *A. fumigatus* transcriptomic and proteomic science. Unique protocol will be validated soon and will allow Partner 2 and Partner 3 to receive samples from members of the FUMINOMICS community to perform transcriptome and proteome analysis respectively. Results will be published further on a public website. Major achievements discussed during the meeting are the following:

1- Validation of the *A. fumigatus* Biochip from Toulouse is near to completion. The experiments undertaken by Partner 2 will define in the near future the best labelling and hybridization protocols, which was the major "bottle neck" for the transcriptome project. The optimized protocol will be then used by Toulouse Genopole on all samples received from each FUMINOMICS partners.

2- Proteomic methodologies have been developed and adapted to *A. fumigatus*. However, due to the difficulty in performing many Proteome maps, contacts will be undertaken by Partner 3 and 11 with American consortium, also setting up proteomics at JCVI.

3- Transcriptomic and genomic data on *Af*, obtained by each partner, have started being gathered in a common data warehouse OmniFung. In addition, bioinformatics tools (page 9-10) for the analysis of transcriptomic and proteomic data of *Af* will be published in the website. Since last year, Partner 3 has adapted the user interface for all the partners to allow a quick and easy access to this the database.

4- New in vitro systems were presented to study immune response or virulence of fungal components and facilitate gene disruption testing.

5- Four exchange grants were sponsored this year indicating the willingness of all the FUMINOMICS participants to collaborate at the bench level.

6- The necessity to undertake a mass gene disruption program has been seriously discussed this year. Two major approaches were proposed: either undertake a deletion of all genes to have fully comprehensive library of mutants or start with a subset of genes (e. g. the transcription factors family) to validate first the most appropriate deletion strategies. As many questions were addressed concerning the technical difficulties in terms of molecular biology and the funding of this program, a close workshop will be organized before end 2010, to be sponsored by ESF, to define a strategy. Experts already involved in the establishment of whole mutant libraries in *C. albicans, S. cerevisiae* and *N. crassa* will be invited.

Next annual meeting will be held in summer 2010. It will be organized by Partner 1 in France and focused on Cell biology and Biochemistry. Molecular, biochemical and imaging technologies to be developed for protein functional analysis will be also discussed.

Annexes:

- p15, Participants List
- p17, ESF exchange grants (RNP)

Partner #	Title	Surname	First name	Institution	Dpt	Street Address	Postal code	City	Country
Member of Steering Committee	Prof.	**Radu**	Dorel Lucian	National Instit. of R&D Microbiology and Immunology "Cantacuzino"	Immunology Department	Spl. Independentei, Nr. 103, Sector 5	050096	Bucarest	Romania
P1 coordinator	Prof.	**Latgé**	Jean-Paul	Institut Pasteur	Aspergillus Unit	25 rue du Dr Roux	75015	Paris	France
P1	Admin& Scient. Coord.	Clavaud	Cécile	Institut Pasteur	Aspergillus Unit	25 rue du Dr Roux	75015	Paris	France
Invited	Dr	Beauvais	Anne	Institut Pasteur	Aspergillus Unit	25 rue du Dr Roux	75015	Paris	France
Invited	Dr	Hartl	Lukas	Institut Pasteur	Aspergillus Unit	25 rue du Dr Roux	75015	Paris	France
P1	Dr	Lambou	Karine	Institut Pasteur	Aspergillus Unit	25 rue du Dr Roux	75015	Paris	France
P2	Dr	Le Berre	Véronique	INSA	Biochips Platform	135 avenue de Rangueil	31077	Toulouse Cedex	France
P2 associated coordinator	Prof.	**François**	Jean-Marie	INSA	Biochips Platform	135 avenue de Rangueil	31077	Toulouse	France
P3	Dr	**Brakhage**	Axel	Hans-Knoell-Institute	Depmt Molecular and Applied Microbiology	Beutenbergstrasse 11a	07745	Jena	Germany
P3	PhD student	Kniemeyer	Olaf	Hans-Knoell-Institute	Depmt Molecular and Applied Microbiology	Beutenbergstrasse 11a	07745	Jena	Germany
P3	PhD student	Albrecht	Daniela	Hans-Knoell-Institute	Depmt Molecular and Applied Microbiology	Beutenbergstrasse 11a	07745	Jena	Germany
P5	Dr	**Morrissey**	John	University College of Cork	Department of Microbiology			Cork	Ireland
P6	Prof.	**Rogers**	Tom	Trinity College Dublin	Department of Clinical Microbiology	St James's Hospital	8	Dublin	Ireland
P6	Dr	**Morton**	Oliver	Trinity College Dublin	Department of Clinical Microbiology	St James's Hospital	8	Dublin	Ireland
P6	Dr	Kenny	Claire	Trinity College Dublin	Department of Clinical Microbiology	St James's Hospital	8	Dublin	Ireland
P7	Dr	**Loeffler**	Juergen	University Medical Center Würzburg	Department of Internal Medicine II Gebäude C1	Josef-Schneider-strasse 2	D-97080	Würzburg	Germany
P7	PhD student	OK	Michael	University Medical Center Würzburg	Department of Internal Medicine II Gebäude C1	Josef-Schneider-strasse 2	D-97080	Würzburg	Germany
P9	Dr.	**Osherov**	Nir	Tel Aviv University	Department of Human Microbiology	Sackler School of Medicine	69978	Tel Aviv	Israel
P9	PhD student	Hagag	Shelly	Tel Aviv University , Sackler School of Medicine	Department of Human Microbiology	Sackler School of Medicine	69978	Tel Aviv	Israel
P11	PhD student	Bertuzzi	Margherita	Imperial College London	Department of Molecular Microbiology and Infection	Armstrong Road	SW7 2AZ	London	United-Kingdom
P11 Workshop supervisor	Dr	**Bignell**	Elaine	Imperial College London	Department of Molecular Microbiology and Infection	Armstrong Road	SW7 2AZ	London	United-Kingdom
P12	PhD student	Frealle	Emilie	CHRU Faculté de Médecine de Lille	Laboratoire de Parasitologie-Mycologie		59037	Lille cedex	France
P13	Dr	Ruprich-Robert	Gwenaël	Université Paris Descartes	Faculté des Sciences Pharmaceutiques et Biologiques de Paris	4, Avenue de l'Observatoire	75270	PARIS Cedex 06	France
P13	Dr	Chapeland-Leclerc	Florence	Université Paris Descartes	Faculté des Sciences Pharmaceutiques et Biologiques de Paris	4, Avenue de l'Observatoire	75270	PARIS Cedex 06	France
P14	Prof.	**Haas**	Hubertus	Innsbruck Medical University	Division of Molecular Biology/Biocenter	Fritz-Pregl-strasse 3	A-6020	Innsbruck	Austria
P14	PhD student	Blatzer	Michael	Innsbruck Medical University	Division of Molecular Biology/Biocenter	Fritz-Pregl-strasse 3	A-6020	Innsbruck	Austria
P15	PhD student	Bergmann	Anna	University of Würzburg	Research Center for Infectious Diseases	Röntgenring 11	D-97070	Würzburg	Germany
P15 Workshop supervisor	Dr.	**Krappmann**	Sven	University of Würzburg	Research Center for Infectious Diseases	Röntgenring 11	D-97070	Würzburg	Germany
P16	Dr.	**Mellado**	Emilia	Instituto de Salud Carlos III (ISCIII) - Centro Nacional de Microbiologia	Micology Reference Laboratory	Carretera Majadahonda-Pozuelo, Km2	28003	Madrid	Spain
P16	PhD student	Alcazar Fuoli	Laura	Imperial College London	Department of Molecular Microbiology and Infection	Armstrong Road	SW7 2AZ	London	United-Kingdom
P17	Prof.	**Müller**	Franck-Michael	University of Heidelberg	Dept of Pediatrics III	Im Neuenheimer Feld 430	D-69120	Heidelberg	Germany
P17	PhD student	Seidler	Marc	University of Heidelberg	Dept of Pediatrics III	Im Neuenheimer Feld 430	D-69120	Heidelberg	Germany
Invited Partner	Dr.	**Perkhofer**	Suzanne	Innsbruck Medical University	Department of Hygiene, Micropbiology and Social Medicine	Fritz-Pregl. Str. 3	A-6020	Innsbruck	Austria

ESF grants (RNP)

1) Dr. V Le Berre

Reference Number: 2721
Report submitted: 20/02/2009
Title of the proposed research project: Biological validation of the transcriptomic analysis of *A. fumigatus* gene expression using the Toulouse biochip (A. fumigatus amplicon array) and the A. fumigatus oligo array distributed by the Pathogen Functional Genomics Resource Center of the U.S. NIH.
Date of visit (starting date): 26/01/2009
Duration: 4 day(s)
HOST INSTITUTE(s) : Dr. William C. Nierman, Rockville, MD USA

2) Dr. T Hartmann

Reference Number: 2846
Report submitted: 18/09/2009 09:56:16
Title of the proposed research project: Testing oligopeptide transporter (OPT) mutants of Aspergillus fumigatus
Date of visit (starting date): 10/08/2009
Duration: 15 day(s)
HOST INSTITUTE(s) : Dr. Elaine Bignell, London, United Kingdom

3) Dr. J Wong Sak Hoi

Reference Number: 2661
Application Submitted: 24/09/2009 17:23:03
Title of the proposed research project: Subcellular localization of DPRV proteins in Aspergillus fumigatus
Date of visit (starting date) : 15/10/2009
Duration: 8 week(s)
HOST INSTITUTE(s): Dr. Nick Read, Edinburgh, United Kingdom

4) Dr. M Seidler

Reference Number: 2713
Application Submitted: 06/10/2009 14:15:13
Title of the proposed research project: Aspergillus biofilm deletion mutants
Date of visit (starting date) : 23/11/2009
Duration: 4 week(s)
Applicant's Name: Mr. Marc Jens Seidler, Heidelberg, Germany
HOST INSTITUTE(s) : Prof. Jean-Paul Latgé, Paris, France