EUROPEAN SCIENCE FOUNDATION

RESEARCH NETWORKING PROGRAMME

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

First Annual Meeting report on Transcriptomics and Molecular tools, Sept 18-21, 2008, Giens, France

Thirty eight members of the consortium gathered at the Giens VVF (France).

The first annual meeting took place in Giens, south of France. Thirty eight members of the consortium, including senior and junior scientists from each of the eighteen partner laboratories, met to discuss their experience and results about transcriptomic analysis and genomic tools recently developed.

European Science Foundation (ESF)
Research Networking Programme on
The Functional genomics in *Aspergillus fumigatus* and new strategies to fight against the first fungal pathogen in Europe (Fuminomics)
First Annual Meeting

Transcriptomics and Molecular tools

Giens, France
Sept 18-21, 2008

Thursday September 18th, 2008		
Arrival in Giens		
Day 1- Friday September 19th, 2008		
	Introduction	*Jean-Paul Latgé*
	ESF Presentation	*Thomas Brühn*
A. Microarrays		
	The Toulouse experience	*Véronique Le Berre*
	Transcriptional consequences of cytoplasmatic calcium spiking and basis of alkaline adaptation: the JCVI (TIGR) and TOULOUSE experiences	*Omar Loss*
	The identification of the *A.fumigatus* SREA regulon by microarray analysis	*Hubertus Haas*
	The Spanish experience	*Sara Alvarez*
	The German experience	*Volker Schroeckh* *Marc Seidler*
	The Scottish experience 'Development of an in vitro model of Aspergillosis'	*Eilidh Mowat*
	The Irish experiences	*Jim Morrissey* *Oliver Morton*
	The French experience	*Anne Beauvais* *Emilie Frealle*
The List of "To Do" to validate/use Toulouse microarrays		
	Oligo Design	*All*
	Spotting	*All*
	RNA extraction protocols	*All*
	Hybridization protocols	*All*
	Experiment design	*All*
	Reading slides	*All*
	Data storage	*All*
	Conclusion	*All*

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Day 2 – Saturday September 20th, 2008		
B. Molecular Tools		
	'No gene left behind – Targeting the *opt* gene family using the Cre/loxP system'	*Thomas Hartmann*
	Resistance/Auxotrophic markers	*Shelly Hagag*
	xylP promoter-driven gene expression in Aspergillus	*Michael Blatzer*
	Large scale gene disruption in *A. fumigatus?*	*Joanne Wong Sak Hoi*
	How can we use *A. nidulans* techniques to analyse *A. fumigatus?*	*Nir Osherov*
	Recombinant Proteins	*Isabelle Mouyna*
	The most adapted animal model for screening virulence	*Laura Alcazar* *Emilia Mellado*
	Discussion	
	Which stress to study ?	*To be discussed at the next meeting*
	XFP-labeling	*To be discussed at the next meeting*
Sunday September 21st, 2008 Departure		

After a brief introduction by the coordinator of the consortium, Pr. Latgé, Science Officer Dr. Brühn introduced European Science Foundation and the pillars of ESF activity (Figure1). He presented also a portfolio of activities of the European Medical Research Councils.

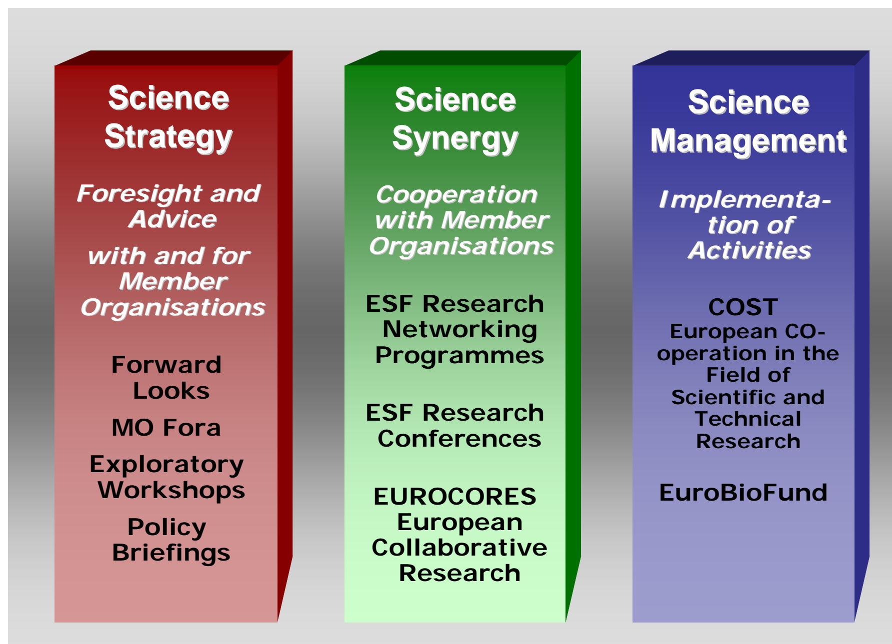
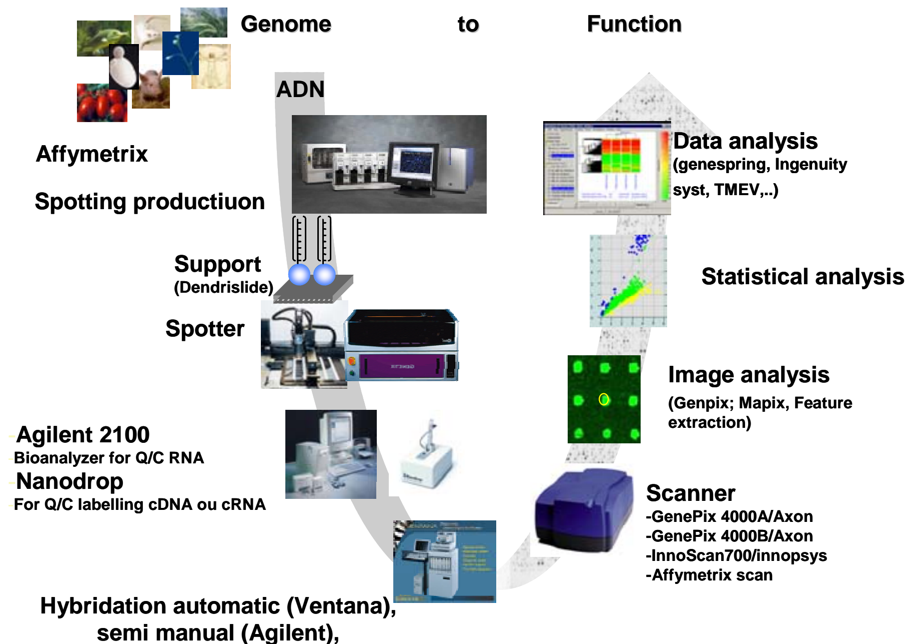


Figure 1 : Pillars of ESF Activity.

A. Microarrays – transcriptomic analysis

1. Biochip (Toulouse experience)

The Toulouse Genopole has built up the AF genechip “Biochip” that covers about 9600 Open Reading Frames from genome of strain AF293, sequenced by JCVI (TIGR). Evaluation of the performances of *A. fumigatus* Biochip microarray compared to JCVI microarray was initiated in response to difficulties encountered by some of the partners while performing transcriptomic analysis. The Toulouse Genopole platform is equipped with spotter machine for production of DNA chips, with Affimetrix machinery, Agilent 2100 system to check RNA quality, a hybridizing automate, a high resolution scanner and the Genepix software to analyse images (Figure 2). DNA Biochips from Toulouse bear 9.588 oligos of 50 bp length designed by the ROSO software, whereas the JCVI chips are made with 70 bp oligos for 9.654 genes. Toulouse biochip sequences match well with cDNA (93%) and genomic DNA (87%). For the technical validation three steps were followed: 1) total RNA of several samples (different origins) were pooled, 2) labelled with Cy3 and Cy5 dyes and 3) co-hybridized on the same slide for each type of microarray. On both chips, more than 92% of the spots were visible with respect of gene intensity, experiments were reproducible with slight gene-dye effect and targeted genes intersection included 8.967 genes for both chips. Biochip and JCVI chip at least gave similar responses, suggesting they were equally appropriate for biological validation.



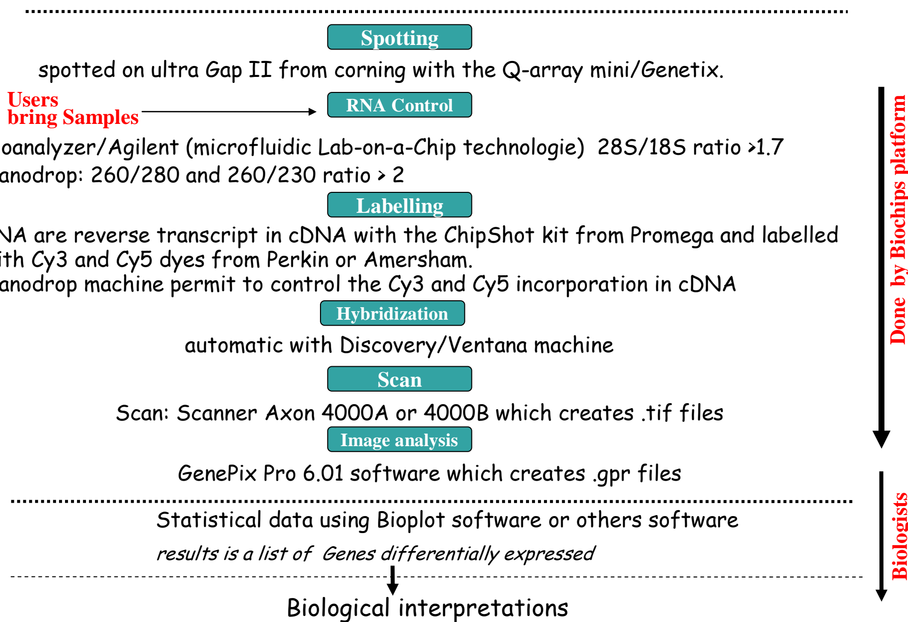


Figure 2 : Equipment and flowchart of the Biochips processing in Toulouse

2. From RNA extraction to slide preparation

A high variation between experimental conditions was seen for RNA extraction, labelling and hybridization among the different laboratories. RNA extraction was done either by using phenol procedure (partner 11), Trizol (partner 1 and 16), which seemed to produce less DNA contaminations, RiboPure Yeast kit (Applied Bioscience; partner 3 and 6) or MasterPure

(Epicentre; partner 3). Protocols which include a column purification step, such as the Quiagen column, allow minimal DNA contamination. Quality was controlled by Nanodrop measurement followed by Bioanalyser (Agilent). Labelling was performed by using a kit such as Amersham CyScribe or Chipshot kit Promega. Cy3 and Cy5 are mainly used through a "dye swap design". Finally labelled DNAs are hybridized on the slides at either 42 or 45 °C.

3. Transcriptomic analysis on microarrays from Toulouse Genopole and JCVI

Omar Loss from Imperial College London (partner 11) has given the first example of biological comparison between the JCVI and the Toulouse experience, by analysing transcriptomal consequences of cytoplasmic calcium spiking and alkaline pH. Calcium signalling is one of the key pathways to the versatility of *A. fumigatus* to adapt to different environmental conditions. *A. fumigatus* was grown in liquid minimal medium (MM) pH 5.0 for 14h and mycelia was then shifted to MM containing calcium chloride and/or pH 8.0. RNAs were extracted by using the phenol procedure. Transcriptomic analysis of the regulation process was performed on the Biochip in parallel with the JCVI chips at various times after calcium addition, until 60 min. Calcium effect happens shortly after addition (10 min), in contrast pH effect is seen after 1h. Different results could be observed; more genes were found to be down or up regulated with JCVI chip compared to Biochips (Figure 3).

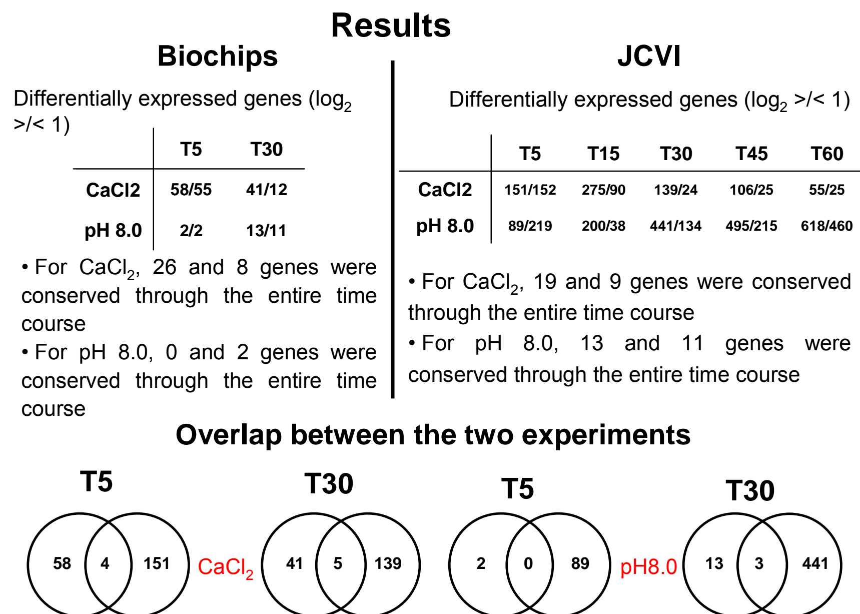


Figure 3: Genomic response of *A fumigatus* following cytoplasmic calcium spiking at pH 8.0 and comparison of the results obtained with the Biochip (Toulouse) and the JCVI (TIGR) method. More genes were found to be differentially expressed with JCVI.

Very few common genes were observed from one chip to the other and some genes presented opposite regulations. JCVI results were validated by QRT-PCR and correlated well (65%) with a previous study published by Goldman and colleagues (Molecular Microbiology, 2008, **67**, 1274–1291). On the contrary Biochip results, obtained with a different experimental design, could not be validated by QRT-PCR.
Collaboration between partner 1 (Institut Pasteur - Paris) and partner 4 (Complutense University –Madrid) was undertaken to analyse gene expression during growth in shaken conditions versus static agar conditions. RNAs were extracted by using Trizol (Invitrogen manufacturer protocol slightly modified), and controlled with Agilent bioanalyser, labelled

and hybridized, either in Toulouse or in Madrid. Acquisition of the image was performed with GenePix microarray scanner. 497 and 550 genes were induced on Biochip in Toulouse and in Madrid respectively, this may be due to a low labelling efficiency with Cy3 and Cy5 (Table 1). In addition, overlapping of the genes was too limited (80 genes were found in common). Despite a purification step of the RNAs, labelling efficiency was low (less with *A. fumigatus* than with *S. cerevisiae*). To go further on gene expression analysis a better way of labelling has to be used.

	Toulouse	Madrid
Gene number	497	550
Genes in common	80	
Upregulated	314 (ratio : ≤ 9)	292 (ratio : ≤ 146)
Upregulated in common	47	
Repressed	182 (ratio : ≥ 0.0244)	257 (ratio : ≥ 0.0164)
Repressed in common	29	
opposite	4	
Genes in commun in all analysis (Toulouse + Madrid)	**49 (27 upregulated + 22 repressed)**	

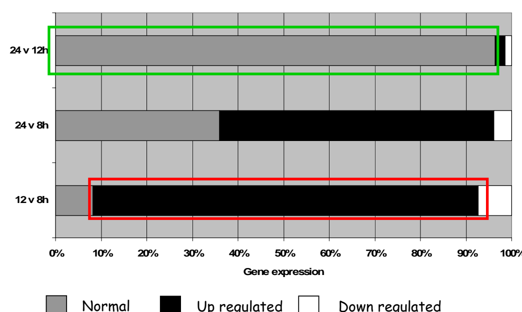
Table 1 : Gene expression of *A fumigatus* during growth in shaken conditions versus static agar conditions and comparison of the results obtained with the Biochip in Toulouse and in Madrid.

Gene expression during biofilm formation was investigated after 12h versus 8h and 24h versus 12h of culture by using the Biochip (partner 16). RNAs were extracted with the Trizol method and checked by Agilent analyser. During the first step, from 8h-12h, mainly metabolism related genes were up regulated, while biogenesis genes were most differentially regulated during 12h-24h (Figure 5). Thirty genes only were specific for the 12h-24h period. 17 differentially expressed genes on microarrays could be validated by QRT-PCR.

Data analysis

Gene Expression	8h			12h			24h		
	Normal	Up regulated	Down regulated	Normal	Up regulated	Down regulated	Normal	Up regulated	Down regulated
8h				8.2 (599)	7.3 (140)	84.5 (1611)	35.9 (599)	1.6 (65)	25.1 (1003)
12h	8.2 (156*)	84.5 (1611)	7.3 (140)				96.4 (1317)	1.4 (19)	2.2 (30)
24h	35.9 (599)	60.2 (1003)	3.9 (65)	96.4 (1317)	2.2 (30)	1.4 (19)			

* Total number of genes



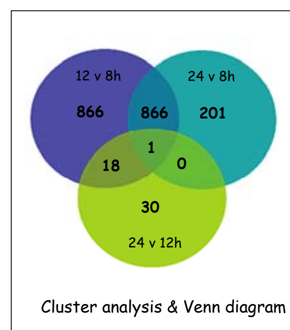


Figure 5: Gene expression analysis by using the Biochip during biofilm formation after 12h versus 8h and 24h versus 12h of culture.

Partner 18 has investigated the development of biofilm on bronchial epithelial cells. Transcriptomic analysis of the genes, as well as 2D gel electrophoresis followed by MALDI-MS analysis were performed to elucidate proteins involved in biofilm formation after 48h culture versus 24h culture. Following static culture (biofilm formed) 614 and 259 genes were found to be up-regulated by using Biochip and JCVI and 95 genes only were common to both experiments. Although most of the genes have not been identified and matched with proteomic analysis, JCVI results of induced genes fitted well with the 2D gel results (7 genes out of 9 were found to be up-regulated with JCVI and 2D gel electrophoresis method). On the contrary, there was no correlation with Biochip transcriptomic results.

By using Toulouse Biochip, secondary metabolite production or low oxygen condition effects on gene expression (for example co-culture with bacteria) were investigated by partner 3 (Hans-Knoell Institute – Jena), who has good expertise in *Aspergillus nidulans* arrays. Extraction of the RNAs was done by using RiboPure Yeast kit (Applied Bioscience) or MasterPure Yeast kit (Epicentre). After quality control by the Nanodrop and Bioanalyser (Agilent), cDNA were amplified, labelled with Cy dyes and further hybridized at 45°C. GenePix microarray scanner was also used for image acquisition. Proteomic analysis of the same samples showed a difference in expression of a higher number of proteins (101 proteins were differentially expressed), suggesting a lower sensitivity for the transcriptomic method.

Transcriptomic analysis was used to look for genes differentially expressed at different time periods after exposure to granulocytes (partner 6). RNA were extracted by RiboPure kit in Trinity College - Dublin and sent to Toulouse, where they were further processed. Results are still in processing phase.

Biochip (Toulouse) was used to analyse gene expression during nitrogen starvation and inhibition of TORC1 (Target of Rapamycin Complex 1). TORC1 plays a central role in mediating cellular response to nutrient availability and has a prominent role in cell growth and proliferation. RNA were extracted at the University College of Cork (partner 5) and sent to Toulouse for further processing (conversion to cDNA and hybridization). They obtained a good quality signal. 111 genes were up regulated and 104 genes were down regulated during nitrogen starvation (Ammonium versus Urea). 57 genes were up regulated and 32 genes were down regulated when TORC1 pathway was inhibited by Rapamycin. Results were validated on ten genes by semi quantitative RT-PCR. Some expected genes, such as nitrogen transport associated genes, were not seen in this analysis, while MEPA and GAP1 genes were expressed differently like in *S. cerevisiae*. In this study, hybridization signals observed were less intense in *A. fumigatus* compared to *S. cerevisiae* and changes in levels of gene expression are lower in *A. fumigatus* than in *S. cerevisiae*.

Oxidative stress is important during early phases of infection in the macrophage and the neutrophil (partner 12). An oxidative stress was simulated by addition of menadione (0.08mM) at 37°C for 30 min and transcriptomic analysis was performed in Toulouse with the Biochip. Results showed induction of 69 genes, 33 genes were down regulated, 36 genes were up regulated (genes involved in transport, gene expression regulation, oxidative stress, cell wall biogenesis, cellular lipid metabolic process, acetyl-CoA metabolic process). Surprisingly, SOD genes, which are known to be associated to oxidative stress regulation, were not modulated. However, results were not checked by QRT-PCR.

Hubertus Haas and colleagues (partner 14) made use of JCVI microarray analysis to study genes involved in iron regulation in a *A. fumigatus* mutant that exhibits altered siderophore

biosynthesis (*ΔsreA*). Siderophores are molecules that allow iron transport and their biosynthesis is important for virulence. Transcriptomic experiments showed 8 clusters, among which 7 were belonging to iron regulatory system and siderophore biosynthesis. In addition, gene expression was validated on six genes by northern blot analysis.

4. Achievements and perspectives

Table 2 summarise the transcriptome analysis data obtained to date.

Partner #	Biochip Toulouse	JCVI	Transcriptome results
P11	✓	✓	Experimental design were different Correlation between QRT-PCR and transcriptome was good with JCVI, but not with Toulouse, (2x6 genes checked) Number of differentially expressed genes common to both experiments was very low
P1+4	✓		Hybridisation and analysis of the microarray, performed in two different places gave results with very low overlapping No validation with QRT-PCR
P16	✓		Very good results, more than 500 genes were showing variation of expression Validation with QRT-PCR on 7 genes
P18	✓	✓	More than 500 genes showed varying expression Very low overlapping between two arrays Validation by 2D-MS was obtained on 7 proteins for JCVI chips only No QRT-PCR verification
P3	✓		Lack of correlation with proteome data Complaints on the spotting and hybridization data
P6	✓		Correlation with QRT-PCR has not been analysed yet
P12	✓		Low number of genes with variation of expression. No validation with QRT-PCR
P14		✓	Results in agreement with iron metabolism Good correlation with northern (6 genes checked)
P5	✓		Nitrogen starvation gave good results Validation with QRT-PCR on 10 genes

Table2: First transcriptomic analysis data.

Major problems have been highlighted during the presentations: 1) differences in gene expression observed by analysing slides from Toulouse were not correlating with QRT-PCR (partner 11, 12 and 18); 2) gene expression changes observed by using Biochips were less intense in *A. fumigatus* compared to *S. cerevisiae* and changes in gene expression were low (partner 1,4, 5 and 15). Results obtained by using JCVI chips seemed better validated by QRT-PCR or 2D-gel electrophoresis than Toulouse array data. It has to be pointed out that validation has been performed on a very limited number of genes (always less than 10 genes). The low level of gene expression suggested a low level of cDNA labelling and/or a possible contamination of the RNA with DNA. Experiments that have been performed using Biochip and JCVI chip in parallel (partner 1, 3, 4, 11), were achieved with different experimental

design, various RNA extraction methods, labelling kits and hybridization conditions, making efficient comparison very difficult. As a result, all the participants agreed for the need of setting up a very stringent protocol to validate Toulouse biochip. After lengthly and animated discussion, a protocol was set up and agreed by all the participants for a final validation of the European slides. This validation will include a comparison with the American chip (figure 6).

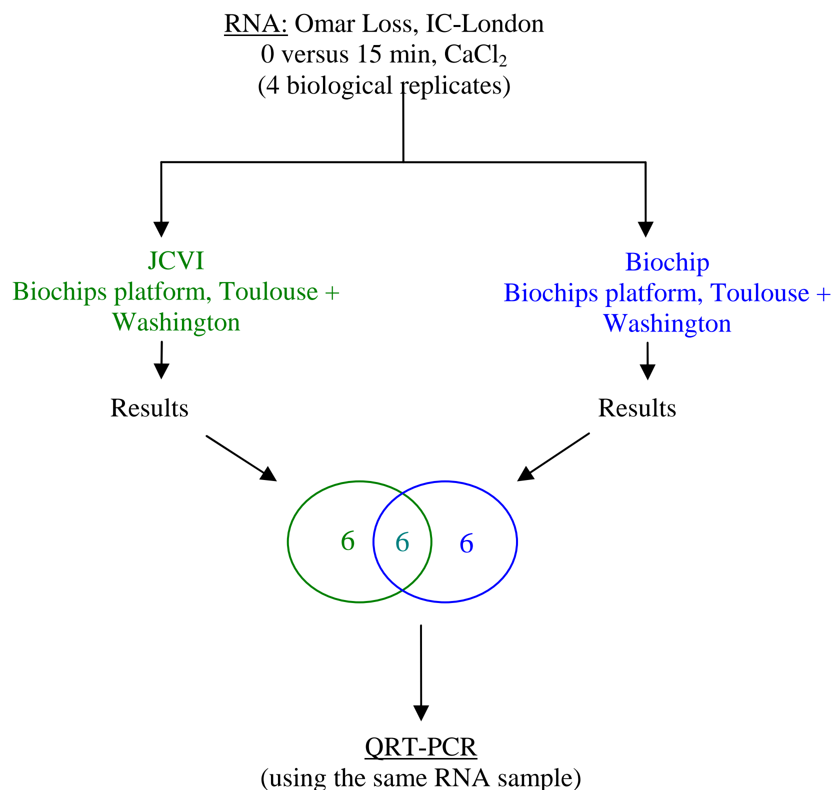


Figure 6: Biological validation of the transcriptomic analysis of *A. fumigatus* gene expression on Biochip (Toulouse).

Validation will be achieved following two steps. First step will be a comparative analysis of the quality of the microarray produced in Europe and USA using a single set of RNA, prepared by partner 11, and further analysed by JCVI in USA and at the Biochips platform of Toulouse (France). After discussion between W. Nierman's group and E. Bignell, JM François and JP Latgé, it was decided that to fulfil the first step, V. Le Berre (from The Biochips platform – partner 2) will go to JCVI at the end of January to perform the first assays in collaboration with the Nierman's team. Based on the data obtained, a second step will involve the analysis of different biological replicates. For the second step it is anticipated that four RNA biological replicates will be analysed. All RNA samples are prepared by Omar Loss (partner 11) and each RNA batch will be separated into three pools. First pool will be analysed by JCVI and second pool by Toulouse platform. For each step, 18 genes (6 genes only expressed at on JCVI + 6 genes only expressed on Biochip + 6 genes expressed on both slides) up and down regulated (total number of gene is will be 36) will be validated by QRT-PCR by using the same RNA batch (pool 3). Experimental designs, labeling and hybridisation protocols will be the ones currently used by the Toulouse and JCVI QRT-PCR method will be standardized. Laboratories in Europe (partner 1, 3, 5, 6, 7, 9, 11, 12, 15, 16 and 17) agreed to participate and share the work for QRT-PCR showing the willingness of this community to work together. Next meeting to validate the array will be held in Paris in late spring in Paris.

B. Molecular tools – overexpression or silencing of the genes

Recent methodological improvements from partners of our consortium have been made to generate knockout mutant strains on a large scale and study essential genes.

1. Large scale gene disruption

Deletion with a resistance cassette is the current way to produce deletion mutants. *ΔKu70*/*ΔKu80* strains and the use of PCR fusion have shortened the cloning and transformation duration. Hygromycin and phleomycin are dominant antibiotics for transformant selection, while glufosinate, nourseothricin, pyrithiamin are less efficient for *A. fumigatus* and auxotrophic markers can affect virulence. Deletion with resistance cassette is limited by the number of selection markers, especially when the gene of interest belongs to a family, which members display functional redundancy (Figure 7).

Selection Markers in
Aspergillus fumigatus

Dominant
hph - Hygromycin
ble - Phleomycin
bar - Glufosinate
nat1 - Nourseothricin
ptrA – Pyrithiamine

Counter selection
pyrG Blaster
sC
niaD (possible)
Cre/lox⁻

Auxotrophy
pyrG
argB
lysB
lysF

Figure 7 : Resistance and auxotrophic markers in *Aspergillus fumigatus*.

Partner 15 has developed a recyclable deletion cassette containing a Cre/loxP system that allows successive multiple disruptions. To accomplish excision by site-specific recombination at *loxP* acceptor sites, Cre recombinase is transiently expressed, from a module integrated into the autonomously replicating vector, which carries *AMA1* and the *ptrA* marker allele for plasmid maintenance in the presence of pyrithiamine. After removal of the deletion cassette, the same resistance marker can be reused for another gene deletion (Figure 8).

Making a knockout in a nutshell

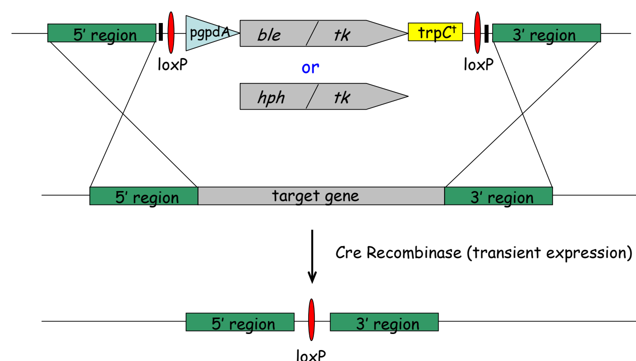


Figure 8: Deletion cassette containing a Cre/loxP system.

2. Study of essential genes

Modulation of gene expression through an inducible promoter can be used to study essential genes. The endogenous promoter can be replaced with an inducible promoter, such as pNiiA (*NiiA*), pAlc (*alcA*), pGla (*glaA*), pAcu (*acuD*) or pCbh (*cbhB*) (Figure 9). Thus, the expression of the gene is switched off by the addition of a specific nutrient to the growth medium. More recently, partner 14 has successfully applied the pXyl (*xylP*) promoter to *A. fumigatus* to study *AfZrfB* gene overexpression. They observed a tight repression in glucose containing culture medium and induction of the gene expression in the presence of xylose. However these promoters are all leaky and no promoter is tight enough to study essential genes.

Inducible promoters used in *A. fumigatus*

promoter	organism	inducer
alcA	*A. nidulans*	ethanol / threonine
acuD	*A. fumigatus*	acetate / C_2 sources
cbhB	*A. fumigatus*	carboxymethylcellulose
glaA	*A. niger*	maltose
xylP	*P. chrysogenum*	xylose / xylan

Figure 9 : Inducible promoters used in *A. fumigatus*.

Techniques, initially developed to study essential genes in *Aspergillus nidulans*, were presented and the possibility and the feasibility to transpose them to *A. fumigatus* were investigated: 1) AMA1 self replicating vector or gene overexpression, AMA1 library is available at The Fungal Genetics Stock Center (FGSC); 2) heterokaryon rescue for essential genes, which is facilitated by working in a NHEJ-deficient genetic background, and 3) parasexual rescue is performed to prove that a gene is essential (the last one is not useful for *A. fumigatus* because of important deletion during transformation). (Figure 10)

What's out there in the A. nidulans world?

1. Inducible promoters (i.e alcA, niiA)
(Waring et al. Gene 79:119 1989; Punt et al. Gene 104:119 1991)
2. Selectable markers (auxotrophic, dominant)
(Nayak et al. Genetics 172:1557 2006; Cullen et a. Biotechnology 10:419 1988; Johnstone Microbiol Sc. 2:307 1985)
3. Rapid vector construction (GATEWAY, overlap PCR)
(Toews et al. Curr. Genet. 45:383 2004; Szewczyk et al. Nat Protoc. 1:3111 2006)
4. AMA1 self replicating vectors: overexpression library,complementation, gene overexpression
5. Heterokaryon rescue
6. Parasexual genetics?

Figure 10: Transposable genomic tools from *A. nidulans* to *A. fumigatus*.

The conclusion of these presentations was that overexpression of genes in *A. fumigatus* has been under looked and may be very useful functional analysis of genes.

3. In vivo analysis and medicinal applications

All mutants of *A. fumigatus* have to be tested in an animal model. The most adapted animal model for screening virulence was developed at partner 17 and agreed by all the partners. Mice CD1 were immuno-suppressed by injecting cyclophosphamide and cortisone acetate followed by intra-nasal inoculation of spores (10^4 suspended in 30 µl Tween 0.01% saline solution). Cell count was checked by plating onto solid medium. Presence of the fungi was checked in lung. Then survival was measured (Figure 11).

INOCULATION BY SPORES SUSPENSION

Anesthetics

❖ KETAMINE

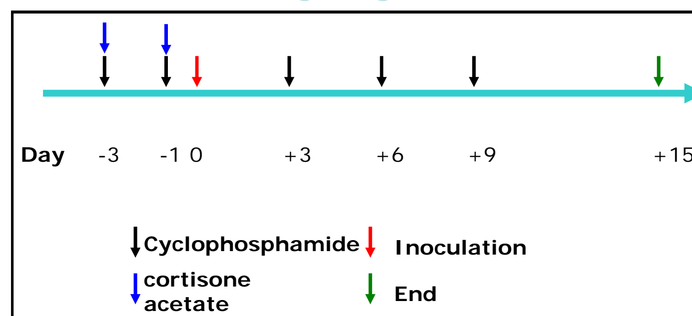
❖ XYLAZINE

Mixture of 9:1 (v/v), 12.5 mg/ml ketamine + 2% xylazine

100µl/mouse

Intramuscular injection

Flowchart of dosage regimen



Inocula standardization

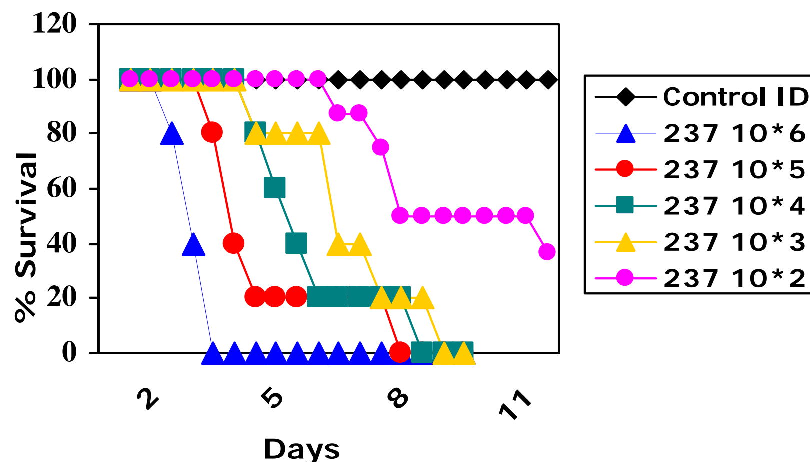


Figure 11: The most adapted animal model for screening virulence

Production of recombinant proteins is useful for further characterization of the protein structure, analysis of enzymatic activity, antifungal drug/inhibitors testing or antibody preparation. Partner 1, who has a good expertise in recombinant protein preparation, has presented the *Pichia pastoris* system. This system allows high quantity and purity of the protein produced and was successfully applied to several *A. fumigatus* antigen and enzymes production (Gelp family, Catalase, Dipeptidypeptidase…).

C. Impact of the event on the future direction of the field

Thanks to ESF, 18 European research groups have joined their effort to develop new transcriptomic and genomic tools to better understand the establishment of aspergillosis and improve the management of Invasive Aspergillosis in patients. The first annual meeting offered the opportunity to all participants, especially young scientists, to expose their transcriptomic and genomic studies. A major achievement of the meeting was the sharing of all protocols, problems and advances in the *A. fumigatus* science. Collaborations at the EU level were either started or fastened at this meeting in a very friendly and cooperative atmosphere.

Main effort was made to improve the *A. fumigatus* Biochip from Toulouse. Transcriptomic and genomic data on *Af*, obtained by each partner, will be gathered in a common database. Partner 3 has already established a catalogue covering 80% of the proteins and providing functional information on *Af* proteome. A script will be further adapted for all the partners to have a quick and easy access to this the database. New genomic solutions were proposed to analyse essential genes or multigenic families (recyclable deletion cassette loxP, interest of the Gateway system). Proteomic and bioinformatics advancements will be the topic of the next scientific meeting.

The question has been asked (but not solved yet) of the necessity to undertake a mass disruption program. A preliminary project would be centred on the disruption of specific *A. fumigatus* transcription factors (378 putative transcription factors were found in the genome of *Af*, 47 were specific to *Af*), in which four partners were interested.

Next meeting will be held in 2009 and focused on Proteomic and Bioinformatics' tools.

Annexe : Participants List

ESF Fuminomics Meeting in Giens – September 17-21, 2008
"Transcriptomics and Molecular Tools"
Participants List

Partner #	Title	Surname	First name	Institution	Dpt	Street Address	Postal code	City	Country
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
ESF Science Officer	Mr	Bruhn	Thomas	ESF		1 quai Lezay-Marnésia - BP 90015	67080	Strasbourg cedex	France
P1 coordinator	Prof.	Latgé	Jean-Paul	Institut Pasteur	Aspergillus Unit	25 rue du Docteur Roux	75015	Paris	France
P1	Admin & Scient. Coord.	Clavaud	Cécile	Institut Pasteur	Aspergillus Unit	25 rue du Docteur Roux	75015	Paris	France
P1	Dr.	Lambou	Karine	Institut Pasteur	Aspergillus Unit	25 rue du Docteur Roux	75015	Paris	France
P2 associated coordinator	Prof.	François	Jean-Marie	INSA	Biochips Platform	135 avenue de Rangueil	31077	Toulouse	France
P2	Dr	Le Berre	Véronique	INSA	Biochips Platform	135 avenue de Rangueil	31077	Toulouse Cedex	France
P2	Dr	Trouilh	Lidwine	INSA	Biochips Platform	135 avenue de Rangueil	31077	Toulouse Cedex	France
P2	PhD student	NGuyen	Antoine	INSA	Biochips Platform	135 avenue de Rangueil	31077	Toulouse Cedex	France
P12	PhD student	Frealle	Emilie	CHRU Faculté de Médecine de Lille	Laboratoire de Parasitologie-Mycologie		59037	Lille cedex	France
P12	Dr	Delhaes	Laurence	CHRU Faculté de Médecine de Lille	Laboratoire de Parasitologie-Mycologie		59037	Lille cedex	France
P13	Dr	Berkova	Nadejda	Ecole Nationale Vétérinaire - Unité de Parasitologie-Mycologie	UMR 956 BIPAR	22 rue Curie	94700	Maisons-Alfort	France
Invité	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
Invité	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
P3	Dr	Schroeckh	Volker	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
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
P15 Workshop supervisor	Dr.	Krappmann	Sven	University of Würzburg	Research Center for Infectious Diseases	Röntgenring 11	D-97070	Würzburg	Germany
P15	PhD student	Hartmann	Thomas	University of Würzburg	Research Center for Infectious Diseases	Röntgenring 11	D-97070	Würzburg	Germany
P18	Prof.	Müller	Franck-Michael	University of Heidelberg	Dept of Pediatrics III	Im Neuenheimer Feld 430	D-69120	Heidelberg	Germany
P18	PhD student	Seidler	Marc	University of Heidelberg	Dept of Pediatrics III	Im Neuenheimer Feld 430	D-69120	Heidelberg	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
P9	Dr.	Osherov	Nir	Tel Aviv University	Department of Human Microbiology	Sackler School of Medicine	69978	Tel Aviv	Israel

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Participants List

P9	PhD student	Hagag	Shelly	Tel Aviv University , Sackler School of Medicine	Department of Human Microbiology	Sackler School of Medicine	69978	Tel Aviv	Israel
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
	Dr	Malic	Luminita-Iuliana	National Instit. of R&D Microbiology and Immunology "Cantacuzino"	Immunology Department	Spl. Independentei, Nr. 103, Sector 5	050096	Bucarest	Romania
P4	Prof.	Arroyo	Javier	Complutense University	Department of Microbiology II, Faculty of Pharmacy	Plaza Ramón y Cajal s/n	28040	Madrid	Spain
P4	PhD student	Alvarez	Sara	Complutense University	Department of Microbiology II, Faculty of Pharmacy	Parque Científico de Madrid-UCM	28040	Madrid	Spain
P17	Dr.	Mellado	Emilia	Instituto de Salud Carlos III (ISCIII) - Centro Nacional de Microbiologia	Micology Reference Laboratory	Carretera Majadahonda-Pozuelo, Km2	28003	Madrid	Spain
P17	PhD student	Alcazar	Laura	Instituto de Salud Carlos III (ISCIII) - Centro Nacional de Microbiologia	Micology Reference Laboratory	Carretera Majadahonda-Pozuelo, Km2	28003	Madrid	Spain
P11 Workshop supervisor	Dr	Bignell	Elaine	Imperial College London	Department of Molecular Microbiology and Infection	Armstrong Road	SW7 2AZ	London	United-Kingdom
P11	PhD student	Loss	Omar	Imperial College London	Department of Molecular Microbiology and Infection	Armstrong Road	SW7 2AZ	London	United-Kingdom
P16	Dr	Ramage	Gordon	Glasgow Dental School and Hospital - The University of Glasgow	Section of Infection and Immunity	378 Sauchiehall Street	G2 3JZ.	Glasgow	United-Kingdom
P16	PhD student	Mowat	Eilidh	Glasgow Dental School and Hospital - The University of Glasgow	Section of Infection and Immunity	378 Sauchiehall Street	G2 3JZ.	Glasgow	United-Kingdom