The Eleventh International *Aspergillus* Meeting

Asperfest 11

March 22-23, 2014

Hotel Silken-Al Andalus, Seville, Spain

Aspergillus Genomes Research Policy Group (AGRPG)

An *Aspergillus* Genomics workshop was held at the March 2003 Asilomar Fungal Genetics meeting. From discussions in that workshop it was obvious that our community needed to organize to fully exploit genomics resources. A provisional *Aspergillus* Genomes Research Policy Committee (AGRPC) was conscripted and charged with creating a structure for community-wide coordination and organizing an annual meeting. The First *Aspergillus* Meeting was held in Copenhagen, April 21, 2004, as a satellite meeting of the European Congress on Fungal Genetics-7. In addition to scientific presentations, bylaws were approved, community research directions were discussed and the 2004 AGPRC was elected. The name *Aspergillus* Genomes Research Policy Group was adopted for the community. The objectives of the AGRPG are: (1) Provision of an educational and discussion forum for issues pertaining to *Aspergillus* genomics, in its widest sense, and for the various *Aspergillus* research communities; (2) Influencing grant making bodies and other institutions on behalf of the various *Aspergillus* research communities; (3) Coordinating research activities internationally, as and when required, to further the science base of the *Aspergillus* genus. For more information on the activities of the AGRPG and other *Aspergillus* news see our homepage at FGSC (http://www.fgsc.net/Aspergillus/asperghome.html).

2013 AGRPC

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The Eleventh International *Aspergillus* Meeting
Asperfest 11
March 22-23, 2014
Hotel Silken-Al Andalus, Seville, Spain

March 22 (Saturday)

17:00 - Registration
19:00-22:00 Posters and Welcome Reception (sponsored by Novozymes)
19:00-20:30 Students with even number posters
20:30-22:00 Students with odd number posters
All posters must be removed by lunch on March 23 (Sunday)

March 23 (Sunday)

9:00 Welcome, introductions and announcements **Michelle Momany**

9:15 Session I **Gerhard Braus and Richard Todd**

Miguel Peñalva Centro de Investigaciones Biológicas, Spain
Intimacies between autophagy and exocytosis in *Aspergillus nidulans*

Vera Meyer Berlin University of Technology, Germany
Aspergillus niger as expression platform for secondary metabolite production

Philipp Wiemann University of Wisconsin-Madison, USA
Regulation of the fumagillin/pseurotin secondary metabolite supercluster in *Aspergillus fumigatus*

10:15 -10:45 Coffee Break

10:45 Session II: Genome Projects **Mikael Andersen and Ronald deVries**

Mikael Andersen Technical University of Denmark
Aspergillus whole genus genome project

Jane Gilsenan University of Manchester, UK
An update of on-going work with CADRE and AsperCyc

Axel Brakhage Leibniz-Institute for Natural Product Research and Infection Biology, Germany
Michael Bromley University of Manchester, UK
Aspergillus fumigatus knockouts

Ronald de Vries CBS-KNAW Fungal Biodiversity Centre, The Netherlands
Update on comparative *Aspergillus* project

Michelle Momany University of Georgia, USA
AspGD update

12:00-13:30PM Lunch

13:30 Community directions discussion; Elections **Michelle Momany**

13:45 Session III: Talks from Abstracts **Hubertus Haas and Clay Wang**

Jun-ichi Maruyama University of Tokyo, Japan
Hyphal fusion in *Aspergillus oryzae* as evidenced by a detection system for heterokaryon formation

Isabelle Benoit CBS-KNAW Fungal Biodiversity Centre, The Netherlands
Bacillus subtilis attachment to *Aspergillus niger* hyphae results in mutually altered metabolism and defense mechanisms

Falk Hillmann Leibniz-Institute for Natural Product Research and Infection Biology, Germany
A complex interplay of *Aspergillus fumigatus* with the social amoeba *Dictyostelium discoideum supports the virulence school concept*

Georgios Tzelepis Swedish University of Agricultural Sciences, Sweden
Studying the role of the C-II subgroup killer toxin-like chitinases in the filamentous ascomycete *Aspergillus nidulans*

Saturnino Herrero de Vega Karlsruhe Institute of Technologies, Germany
KipA of no return: Regulation of kinesin-7 turnover by the F-Box protein RcyA in *Aspergillus nidulans*

Miguel Hernandez-Gonzalez Centro de Investigaciones Biológicas, Spain
Rapid disorganization of the Golgi apparatus by blocking the exit of COPII traffic from the endoplasmic reticulum

15:15 – 15:45 Coffee Break

15:45 Pontecorvo Lecture (sponsored by the British Mycological Society)

Jean-Paul Latge Institut Pasteur, France
Aspergillus fumigatus, a bad guy or a new model?

16:15 Election results; poster prize; other discussion items

17:00 Dismiss

Abstracts

All posters must be removed by lunch on March 23 (Sunday).
*Denotes student presenting poster.
Students must be at their posters for Novozymes Student Poster Prize judging as follows:
19:00-20:30 Students with even number posters; *20:30-22:00 Students with odd number posters*

1. Interaction of I Aspergillus oryzae[i] hydrophobin RolA with solid surfaces
Takumi Tanaka, Hiroki Tanabe[1] Toru Takahashi[2] Toshihiko Arita[3], Fumihiko Hasegawa[2] and **Keietsu Abe U[1], [2]**
[1]Laboratory of Applied Microbiology, Department of Microbial Biotechnology, Graduate School of Agricultural Science, Tohoku University, [2]Microbial Genomics Laboratory, New Industry Creation Hatchery Center, Tohoku University, [3]Institute of Multidisciplinary Research for Advanced Materials, Tohoku University

Hydrophobins are amphipathic proteins secreted by filamentous fungi, and play several important roles in fungal physiology, for example, in fungal adhesion to hydrophobic surfaces, in the formation of a protective surface coating, and in the reduction of water surface tension; these roles support the growth of fungal aerial structures such as hyphae and conidiospores. Furthermore, when the industrial fungus Aspergillus oryzae[i] is grown in a liquid medium containing the polyester polybutylene succinate co-adipate (PBSA), the fungus produces RolA, a hydrophobin, and CutL1, a PBSA-degrading cutinase. Secreted RolA attaches to the surface of the PBSA-particles and recruits CutL1, which then condenses on the particles and stimulates the hydrolysis of PBSA. Because the enzyme recruitment requires RolA adsorption onto the solid surfaces precedent to the recruitment, kinetic properties of RolA-adsorption to solid surfaces are important. In order to examine kinetic properties of RolA-adosorption onto solid surfaces, we constructed self-assembly monolayers (SAM) of 1-undcanethiol (UD), 11-amino-1-undecanethiol (AUD, and 10-carboxy-1-undecanethiol (CUD) on the electrodes of Quartz Crystal Microbalance (QCM) and analyzed kinetics of RolA-adsorption to the SAM-electrodes. The Kd values of RolA to AUD were larger than Kd values to UD at pH 4 and 7, and was decreased at pH 10. The amounts of adsorbed RolA to CUD were significantly smaller than those to UD or AUD at the three pH points. RolA indicates pI=4.8 and large negative zeta-potentials at pH 7 and 10. These results suggest that RolA-adsorption to SAMs depends on pH and electrical properties of SAMs. Overall, hydrophobicity and positive charges of solid surfaces contribute to adsorption of RolA to the surface and negative charges of solid surfaces leads to repulsion between the surface and RolA.

***2. MUTATIONAL ANALYSIS OF THE PH-SENSING RECEPTOR PalH**
Daniel Lucena Agell[1*], América Hervás-Aguilar[2], Herbert N. Jr Arst[1,3], Miguel Ángel Peñalva Soto[1]
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There is a well-conserved signalling pathway among ascomycete fungi to mediate environmental pH sensing. This pathway, involving six proteins (PalA, PalB, PalC, PalF, PalH and PalI) in *Aspergillus nidulans*, mediates the activation of the transcription factor PacC under alkaline conditions. PalH is a seven-transmembrane domain protein reminiscent of G Protein Coupled Receptors (GPCR) but, instead of signalling through a heterotrimeric G protein, it is coupled to the positive-acting, arrestin-like protein PalF through the two PalF Binding Domains located in its cytosolic tail. PalH is phosphorylated exclusively under alkaline pH. However, this phosphorylation is not essential for pH signalling, and hence its physiological role remains unclear.

In addition, site-directed mutagenesis analyses of PalH interhelical loops and transmembrane helices have been carried out. This screening has led to the identification of several loss-of-function mutations and, importantly, of weak gain-of-function mutations, a class of mutations in the pH signalling receptor that had not been previously described. In this mutant background, a certain degree of PacC proteolytic processing activation is detectable under acidic conditions. With previously reported data indicating that PalH acts upstream of all other Pal proteins with the exception of PalI (a traffic 'policeman'), these results strongly indicate that PalH is the pH sensing receptor in the Pal/pH signalling pathway.

3. Development of a high throughput gene expression platform for expression of transcription factors in *Aspergillus nidulans

Diana C. Anyaogu[1*], Dorte K. Holm[2], Ali Altintas[1], Chris Workman[1], Uffe Hasbro Mortensen[1]
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Aspergillus nidulans is a well characterized model organism for which many genetic engineering tools have been developed. This makes it an ideal system to study and uncover the mechanisms that control fundamental physiological aspects of fungal life forms i.e. cell differentiation, signaling and metabolism. In order to address fungal physiology in a systems perspective we are in the process of making an overexpression library of all 490 putative and annotated transcription factors (TF) contained in the *Aspergillus* genome.
To facilitate the construction of this overexpression library we have developed a high throughput (HTP) gene expression platform with background free cloning vectors and background free integration systems. Furthermore, to limit the manual work most of the construction of gene targeting substrates has been automated, including the validation of PCR fragments.

Here we present the results of the first generation of the library, which is composed by all 52 TF on chromosome I expressed under the control of the inducible Tet-on promoter. The initial characterization identified several strains with an altered metabolite profile. Hence, some showed up-regulation, or down-regulation, of a few secondary metabolites indicating that the library contains both activators and repressors. Among the up-regulated compounds some are potentially novel. Interestingly, overexpression of some of the TFs had significant impact on the morphology, conidiation and growth rate.

Together our results show that the high throughput gene expression platform is an efficient and suitable way to construct a TF library, which can be used to study the regulation of the secondary metabolism as well as various other aspects of fungal physiology like cell cycle regulation, cell differentiation and signaling.

4. Efficient Generation of *Aspergillus niger* Knock Out Strains by Combining NHEJ Mutants and a Split Marker Approach

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To generate gene deletion mutants in *Aspergillus niger*, we combined the use of Non-Homologous End Joining (NHEJ) mutants (*ku70* mutant) and the split marker approach. The combination of both tools resulted in efficient PCR amplification because of the reduced length of the PCR fragments and efficient homologous recombination frequencies. A set of five selection markers, two dominant selection markers (*hph*; hygromycin B resistance and BLE; phleomycin resistance) and three auxotrofic markers (*pyrG*, *argB* and *nicB*) were successfully used in a split marker approach to obtain *amyR* knock outs with high efficiency. *AmyR* encodes a transcription factor that is required for the expression of starch degrading enzymes and disruption of *amyR* results in the inability to grow on starch. The strategy to generate the gene deletion constructs is such that with one set of four gene specific primers, a gene deletion mutant can be generated with either one of the five selection markers. The strategy is based on fusion PCR and omits the necessity for cloning the disruption cassettes. This accelerates the process of generating gene deletion cassettes which can now be accomplished within eight hours. The split marker approach can also be used to make gene deletions in a wild-type background in stead of a *Δku70* background. In this chapter, we present protocols and considerations that we used to generate gene knock out constructs by fusion PCR and to obtain and verify gene knock outs with any of the five marker genes using the split marker approach. The method is easily transferable to other filamentous fungi.

5. The transcriptional activators AraR and XlnR from Aspergillus niger regulate expression of pentose catabolic and pentose phosphate pathway genes

Evy Battaglia, Miaomiao Zhou, Ronald P. de Vries

The pentose catabolic pathway (PCP) and the pentose phosphate pathway (PPP) are required for the conversion of pentose sugars in fungi and are linked via xylulose-5-phosphate. Previously, it was shown that the D-xylose and L-arabinose release, as well as the PCP is regulated by the transcriptional activators XlnR and AraR in *Aspergillus niger*. In this study we assessed whether XlnR and AraR also regulate the PPP to evaluate to how deep into central carbion metabolism the influence of these regulator extents. Expression of two PPP genes, *rpiA* and *talB*, was reduced in the Δ*araR*/Δ*xlnR* strain and increased in the *xkiA*1 mutant on D-xylose and/or L-arabinose, similar to what was found for PCP genes. This expression profile indicates that at least these two genes are under direct regulation of AraR and/or XlnR. It also demonstrates the strong regulatory connection between sugar release and sugar catabolism, that continues into the central part of carbon catabolism in *Aspergillus*.

***6. Use of ChIP-seq technology for the functional characterization of the mating type protein MAT1-1-1 from the industrial penicillin producer *Penicillium chrysogenum*.**

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Penicillium chrysogenum is the major industrial producer of the ß-lactam antibiotic penicillin which is widely used in the treatment of infections caused by gram positive bacteria. We have studied the regulation of secondary metabolism and morphology of this filamentous fungus to broaden our current understanding of these fundamental processes. Recently we were able to show that the MAT1-1-1 mating-type protein, playing a crucial role in governing sexual identity, also is involved in the regulation of a wide range of genes with biotechnological relevance ranging from regulators of penicillin production, hyphal morphology and conidial formation [1].

In order to understand the regulatory functions of MAT1-1-1, chromatin immunoprecipitation combined with next generation sequencing (ChIP-seq) was performed. Bioinformatic analysis was used for the identification of putative DNA-binding regions of MAT1-1-1 as well as for *de novo* motif analysis. qPCR confirmed the enrichment of MAT1-1-1 specific DNA-regions in ChIP-DNA compared to input-DNA and was used for the verification of peak quality scores generated during bioinformatic analysis. Furthermore, we performed qRT-PCR analyses to distinguish genes that are specifically regulated by MAT1-1-1 from those that are unaffected by overexpression and deletion of the MAT1-1 gene. In order to conduct electrophoretic mobility shift assays (EMSA), we fused the MAT1-1 gene to the glutathione S-transferase (GST) gene for overexpression in *E. coli*. The purified fusion protein was used to verify the binding of MAT1-1-1 to putative DNA binding sites.

Taken together, our data from ChIP-seq analysis extends the current knowledge of the regulatory network controlling both penicillin biosynthesis and morphogenesis in *P. chrysogenum*. Hence, our experiments will identify new starting points for targeted genetic engineering of *P. chrysogenum,* which is crucial for further optimization of industrial production strains.

[1] Böhm et al. (2013) Sexual reproduction and mating-type-mediated strain development in the penicillin-producing fungus *Penicillium chrysogenum*. PNAS 22;110:1476-81

***7. The influence of regulatory and metabolic mutations on growth of *Trichoderma reesei* on plant biomass related carbon sources**

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Many regulators and pathways are involved in fungal growth on plant biomass. In this study we have analysed regulatory and metabolic mutants of *Trichoderma reesei* (*Hypocrea jecorina*) with respect to growth on pure and complex carbon sources.

Growth and enzyme production of wild-type and knock-out strains were compared on 33 different plant biomass related carbon sources, ranging from monosaccharides to crude plant biomass. This data helps to understand the biotope specificity of this fungus. Differences in growth on carbon sources suggest which metabolic pathways and regulators are important for the utilization of the different substrates. Highlights from this study will be presented.

8. ***Bacillus subtilis* attachment to *Aspergillus niger* hyphae results in mutually altered metabolism and defense mechanisms.**

Isabelle Benoit[1,2,4]; Marielle H. van den Esker[3]; Aleksandrina Patyshakulieva[2]; Miaomiao Zhou[2]; Jan Dijksterhuis[2]; Oscar P. Kuipers[3,4]; Ronald P. de Vries[1,2,4], Ákos T. Kovács[3,5]
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Interaction between microbes affects the growth, metabolism and differentiation of members of the microbial community. While direct and indirect competitions, like spite and nutrient consumption have a negative effect on the members of the population, microbes also evolved in nature not only to fight, but in some cases to adapt or support each other, while increasing the fitness of the community. The presence of bacteria and fungi in soil results in various interactions, such as mutualism. *Bacilli* attach to the plant root and form complex communities in the rhizosphere. *Bacillus subtilis*, when grown in the presence of *Aspergillus niger* interacts similarly with the fungal partner, by attaching and growing on the hyphae. Using a dual transcriptome experiment, we suggest that both fungi and bacteria alter their metabolism during the interaction. Interestingly, the transcription of genes related to the antifungal and antibacterial defense mechanism of *B. subtilis* and *A. niger*, respectively, are decreased upon attachment of bacteria to the mycelia. When in *Aspergillus nidulans* for instance, it has been shown that some intimate bacterial–fungal interaction triggers biosynthesis of archetypal polyketides [1].

Previous studies on dual transcriptome of *A. niger* and the genus *Collimonas* were restricted to indirect interaction of bacteria and fungi via chemical communication [2]. In our study, we present for the first time the transcriptional changes that occur in the fungal and bacterial partners during their direct interaction.

[1] Schroeckh V, Scherlach K, Nützmann HW, Shelest E, Schmidt-Heck W, Schuemann J, *et al.* (2009). Intimate bacterial–fungal interaction triggers biosynthesis of archetypal polyketides in *Aspergillus nidulans*. Proc Natl Acad Sci USA 106:14558-14563.

[2] Mela F, Fritsche K, de Boer W, an Veen JA, de Graaff LH, van den Berg M, Leveau JHJ (2011). Dual transcriptional profiling of a bacterial/fungal confrontation: *Collimonas fungivorans* versus *Aspergillus niger*. ISME J 5:1494-1504.

9. The role of ERAD and autophagy in the degradation of misfolded proteins in *Aspergillus niger

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Folding of secretory and transmembrane proteins is tightly controlled by a stringent ER quality control system which only allows correctly folded proteins to leave the ER for subsequent delivery to their site of action. Misfolded proteins are sent back into the folding cycle or targeted for destruction via the ER-associated degradation (ERAD) pathway. It has been demonstrated that deletion of the ERAD gene *derA* in *Aspergillus niger* only slightly reduces growth and conidiation, even when the mutant strain contains multiple copies of a

Glucoamylase-Glucuronidase (mcGlaGus) gene fusion (Carvalho *et al*., 2011). This present study investigates whether autophagy might serve as an alternative process to remove misfolded proteins from the ER. Autophagy is an intracellular degradation process functioning in the targeting of cytoplasmic content and organelles to vacuoles. We generated *A. niger* double knock-out mutants in which genes were deleted that are essential for ERAD (*derA*) or autophagy (*atg1* and *atg8*) and assessed their growth both under normal and under ER stress conditions. It was observed that deletion of genes required for autophagy in combination with deleting the *derA* gene had no effect on growth, not even in the mcGlaGus background. Furthermore, sensitivity to the ER stressor dithiothreitol was not increased in the double knock-outs in comparison to the single knock-out mutants. Functional redundancy between ERAD and autophagy could thus not be observed in *A. niger*; deleting both processes did not affect the growth phenotype or conidiation in this species.

10. The velvet complex protein, VeA, regulates production of novel *Aspergillus flavus* secondary metabolites involved in morphogenesis and survival.

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Analysis of the *Aspergillus flavus* genome has identified 55 putative secondary metabolic gene clusters predicted to encode metabolites derived from polyketide synthases (PKSs), non-ribosomal peptide synthetases (NRPSs), hybrid PKS-NRPS and prenyltransferases (PTRs). Very few of the metabolites capable of being produced by these gene clusters have been characterized. By whole genome microarray transcript profiling we confirmed that expression of a number of genes associated with these putative secondary metabolic gene clusters in *A. flavus* are regulated by *veA*, a global regulator of fungal development and secondary metabolism. Focusing on two of these *veA*-dependent gene clusters, 23 and 27, we created gene deletion and overexpression strains and used comparative metabolomics to identify these gene cluster-associated metabolites. Cluster 27 harbors a PKS involved in the production of the anthraquinone, asparasone A (358 Da), as well as three related anthraquinones of molecular weight 374, 340, and 316 Da. The 316 Da anthraquinone is particularly interesting because it is most likely formed by incorporation of 7 malonyl-CoA units rather than the 8 units required for biosynthesis of asparasone A. Inactivation of the cluster 27 *pks* (*pks27*) affected sclerotial pigmentation but not conidial pigmentation. Sclerotia produced by *pks27* mutants were significantly more susceptible to insect predation and damage by ultraviolet light and heat than were the sclerotia produced by the isogenic control. Cluster 23 is one of only two gene clusters in *A. flavus* that harbor a hybrid PKS-NRPS, the other being the cyclopiazonic acid (CPA) cluster. While no significant differences in phenotype or metabolic profiles were observed in a *pks-nrps* deletion mutant compared to the isogenic control strain, overexpression of one of three cluster 23 Zn(2)-Cys(6) transcription factors resulted in transformants producing a novel 335 Da pyridone, subsequently identified as demethoxyleporin A by mass spectral and nmr analysis and by comparison to leporin A from *Aspergillus leporis*. Like leporin A, demethoxyleporin inhibits insect feeding. Our results show that biosynthesis of metabolites required for protection of *A. flavus* sclerotia, like the production of the sclerotia themselves, is inhibited in *veA* mutants, suggesting that a major role of VeA is its affect on sclerotial development and survival.

11. Oxygen and an Extracellular Phase Transition Independently Control Central Regulatory Genes and Conidiogenesis in *Aspergillus fumigatus*.

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Conidiogenesis is the primary process for asexual reproduction in filamentous fungi. As the conidia resulting from the conidiogenesis process are primarily disseminated via air currents and/or water, an outstanding question has been how fungi recognize aerial environments suitable for conidial development. In this study, we

documented the somewhat complex development of the conidia-bearing structures, termed conidiophores, from several *Aspergillus* species in a subsurface (gel-phase) layer of solid media. A subset of the isolates studied was able to develop conidiophores in a gel-phase environment, but exposure to the aeriform environment was required for the terminal developmental transition from phialide cells to conidia. The remaining Aspergilli could not initiate the conidiogenesis process until they were exposed to the aeriform environment. Our observations of conidiophore development in high or low oxygen conditions in both aeriform and gel-phase environments revealed that oxygen and the aeriform state are positive environmental factors for inducing conidiogenesis in most of the aspergilli tested in this study. Transcriptional analysis using *A. fumigatus* strain AF293 confined to either the aeriform or gel-phase environments revealed that expression of a key regulatory gene for conidiophore development (*AfubrlA*) is facilitated by oxygen while expression of another regulatory gene controlling conidia formation from phialides (*AfuabaA*) was repressed regardless of oxygen levels in the gel-embedded environment. Furthermore, by comparing the conidiation-defective mutants lacking genes controlling various regulatory checkpoints throughout the conidiogenesis pathway, we propose that this aerial response by the fungus requires both oxygen and the phase transition (solid to aeriform), with these environmental signals integrating into the upstream regulatory pathway and central regulatory pathway of conidiogenesis, respectively. Our findings provide not only novel insight into how fungi respond to an aerial environment to trigger development for airborne conidia production but also the relationship between environmental factors and conidiogenesis regulation in aspergilli.

Keywords: *Aspergillus fumigatus*, asexual development, conidiogenesis, oxygen, environmental recognition

12. A possible explanation of the variegated phenotype of the *Aspergillus nidulans brlA12* mutant

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Colonies of the variegated *brlA12* mutant of *Aspergillus nidulans* bear bristle-type barren conidiophores intermingled with a few near normal conidial heads. The mutation is associated with a translocation fusing the *brlA* gene (chromosome VIII) downstream of a putative cytochrome P450 gene on chromosome III. It is suggested that rare transcription of the p450 gene, coupled to positive feedback engendered by brlA alpha and beta transcripts could explain variegation.

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13. The responses of A*spergillus niger* to different lignocellulosic substrates highlight both similarities and substrate-specific differences

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Fungi are major degraders of lignocellulose in nature and are the main sources of enzymes used to saccharify lignocellulose in the production of second generation biofuels. The cost of producing these enzyme cocktails is a major barrier to cheaper biofuels. One path to reducing costs is through a better understanding of the response of fungi to lignocellulose. *Aspergillus niger* has an extensive repertoire of genes encoding enzymes that hydrolyse the components of lignocellulose (cellulose, hemicelluloses and pectin) as well as various accessory proteins. This repertoire of genes makes *A. niger* an excellent model to investigate fungal response to lignocellulosic substrates over time.

Previously we investigated the global transcriptional response of *A. niger* to wheat straw (Delmas et al., 2012) and more recently have compared this response with the response to stems from a willow tree at a single time point. At the transcriptional level, there are many similarities in the responses to the two substrates such as the large increase in carbohydrate active enzyme (CAZy) transcripts from glycosyl hydrolase (GH) families. Some of the differences in the responses can in part be explained by the differences in composition of the substrates. At the biochemical level, enzymes prepared from *A. niger* exposed to wheat straw were used in a saccharification assay and were more effective at saccharification of wheat straw than willow.

In further work at the transcriptional level, we are investigating with the Joint Bioenergy Institute (JBEI) and the Joint Genome Institute (JGI) the responses of *A. niger* to untreated and pre-treated (ionic liquid or hydrothermal) *Miscanthus* stems and wheat straw at nine time points from three hours until five days after transfer from a simple carbon source. Here we aim to elucidate the transcriptional response of *A. niger* as a function of time and lignocellulosic substrate and we will present some preliminary results from our study.

In terms of fungal biology, our work shows that *A. niger* has some ability to distinguish between different lignocellulosic substrates and that is likely to be related to the differences in inducing molecules derived from these different substrates and the evolutionary selective advantage to the fungus of preferentially synthesising appropriate enzymes for available carbon sources. In terms of applications, our work could improve functionality of enzyme cocktails and reduce the costs of their production.

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14. How transporter specificity is determined: an emerging role of channel-like gating domains (lessons from *Aspergillus nidulans*)

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Transporters are proteins mediating the translocation of solutes and drugs across the cell membrane of all cells, a biological process implicated in nutrition, signaling and cell communication with its environment in general. The biological importance of transporters is reflected in several genetic diseases and phenomena of drug resistance or sensitivity, neurotransmission or metabolite redistribution in heterotrophic tissues. Similarly to enzymes, transporters have a major substrate binding site interacting specifically with a single molecule of the substrate in each transport cycle. Consequently they are characterized, in most cases, with Michaelis-Menten kinetics. Substrate binding elicits consequential conformational changes which eventually produce a transporter conformer open to the opposite side from that from where the substrate originally bound the transporter. This rocking-switch mechanism, implicating alternate outward- and inward-facing transporter conformers, has gained significant support not only from structural studies but also from a plethora of genetic, biochemical or biophysical approaches[1,2]. Most transporters are rather specific for a given substrate or a group of substrates with similar chemical structure (e.g. amino acids, purines), but their degree of substrate specificity and affinity can vary dramatically, even among phylogenetically related members of a transport family showing high overall similarity. How then substrate affinities and specificities are determined or evolve? The current dogma is that transporter specificity is determined by the interactions a given solute can make within a specific binding site. However, genetic, biochemical and *in silico* modeling approaches from our lab, using the *A. nidulans* purine transporter UapA, have challenged this dogma[3-5]. We are going to highlight results leading to a novel concept stating that substrate specificity and transport kinetics are determined by subtle intramolecular interactions between a major substrate binding site and independent outward- and cytoplasmically-facing, gating domains, analogous to those present in channels[6]. The significance of this finding will be discussed in relationship to transporter turnover regulation and the development of novel transporter-specific antifungals.

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Key words*:* structure-function relationships/mutational analysis/uptakes/substrate docking

15. Exploring the Enzymatic Mechanism and Biological Function of Gliotoxin S-methylation in *Aspergillus fumigatus

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Gliotoxin is a redox active molecule secreted by *Aspergillus fumigatus*. It is an epipolythiodioxopiperazine class fungal toxin containing a disulphide bridge which is essential to its biological activity. GliT, an oxidoreductase, developmental behavior of
has been shown to be essential for self-protection against gliotoxin. *A. fumigatus* also produces an inactive bis-*S*-methylated form of gliotoxin. This modification of the active dithiol form has been hypothesised by others as a backup plan to protect the producer from dithiol end-products/toxic biosynthetic intermediates. Despite the identification of *S*-methylated forms for the majority of ETPs, to date, no enzyme has been demonstrated to catalyse this modification. Bioinformatic analysis of the *A. fumigatus* genome identified three methyltransferase encoding genes which may be responsible for this enzymatic conversion: the *gli* cluster genes *gliN*, *gliM* and a previously unclassified non-*gli* cluster encoded methyltransferase termed gliotoxin thiomethyltransferase A (*gtmA)*. Disruption of *gtmA* completely abrogated organismal ability to biosynthesize and secrete bis-methyl gliotoxin (BmGT), while gliotoxin production and secretion were unaffected. This result also excludes a role for either GliM or GliN in gliotoxin thiomethylation. Surprisingly, exposure of *A. fumigatus* Δ*gtmA* to exogenous gliotoxin or H_2O_2 did not reveal the acquisition of a sensitive phenotype compared to wild-type. Thus, GtmA-mediated gliotoxin bismethylation is not essential for self-protection. Subsequent recombinant expression of GtmA as a GST fusion protein in *Escherichia coli*, led to the assessment of activity of the purified enzyme. Recombinant GtmA was shown to bismethylate dithiol gliotoxin using *S*-adenosyl methionine as methyl donor, *via* a novel LC-MS enabled activity assay. GtmA activity was detectable in *A. fumigatus* protein lysates obtained only under conditions permissive for BmGT formation. The *S*-methylation of endogenous and exogenous bioactive natural products was originally proposed as a host strategy for detoxification, shared amongst multiple species of bacteria and fungi. Our data unambiguously identify the first enzyme involved in ETP *S*-methylation and demonstrate that this mechanism is not essential for self-protection in *A. fumigatus*. Additionally, the location of this enzyme outside of the gliotoxin biosynthetic cluster is an exception to the rule that genes involved in the production of a particular metabolite are contiguously aligned.

Keywords: Gliotoxin, Aspergillus fumigatus, Epipolythiodioxopiperazine, S-Methyltransferase, Detoxification

16. Quantitative Proteomic and Targeted Metabolomic Analysis Reveal Unexpected Effects of Interference with Gliotoxin Biosynthesis in *Aspergillus fumigatus*.

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Previous work has shown that deletion of the *gliK* gene from the *gli* cluster in *A. fumigatus* resulted in the abrogation of gliotoxin biosynthesis and led to a significant increase in sensitivity to exogenous gliotoxin, relative to the parent strain (≥ 1 µg/ml gliotoxin; $p < 0.001$). Comparative proteomic profiling of the gliotoxin-sensitive mutant, Δ*gliK*, revealed perturbation of translation in response to gliotoxin and also indicated dysregulation of the unfolded protein response (UPR) to endoplasmic reticulum (ER)-associated stress. This informs on the mechanisms involved in gliotoxin-mediated toxicity and may extend to other gliotoxin-sensitive species. Additionally, enzymes involved in the methyl/methionine cycle, including methylenetetrahydrofolate reductase (MTHFR) and methionine synthase, were significantly up-regulated in Δ*gliK* upon exposure to gliotoxin ($p < 0.05$). Up-regulation of the methionine cycle in response to gliotoxin, as revealed by comparative proteomics, may therefore indicate an attempt to compensate for disruption in mechanisms required for gliotoxin methylation in Δ*gliK*. Metabolomic investigation of Δ*gliK* revealed a reduced capacity to produce bis-

methyl gliotoxin (BmGT), or *S*-methylate exogenous gliotoxin, relative to the parent strain. However, this inefficient conversion of gliotoxin to BmGT does not appear to specifically contribute to the increased sensitivity of Δ*gliK* to gliotoxin. LC-MS analysis, using ^{13}C-labelled-methionine (via *S*-adenosylmethionine; SAM) revealed Met as the source of the methyl groups on BmGT. Additionally, deletion of *gliK*, but not *gliT*, from *A. fumigatus* also resulted in over-production of two diketopiperazines (6-methoxyspirotryprostatin B (*m/z* 394) and 18-oxotryprostatin A (*m/z* 396) which are unrelated to gliotoxin and previously only detected in a marine species, *Aspergillus sydowi*. This suggests that disruption of gliotoxin biosynthesis, and unexpected *trans* effects, specifically alters the biosynthesis of additional secondary metabolites and may represent a novel strategy for activating silent gene clusters.

17. Identification of the inducer of the *bgaD* (beta-galactosidase-encoding) gene in *Aspergillus nidulans* upon growth on D-galactose

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Fungal beta-D-galactosidase is an elementary enzyme in biotech industry on account of its ability to hydrolyse lactose to produce galactose and glucose. Fungal beta-galactosidases can be distinguished into extracellular enzymes characterised by an acidic pH optimum, and intracellular ones, that function optimally at neutral pH. In *Aspergillus nidulans*, only a lactose- and D-galactose inducible intracellular activity with a neutral pH optimum has been described. Functionally characterised intracellular (pH-neutral) □-galactosidases belong to the Glycosyl Hydrolase family 2 (GH2).

Genome annotation predicts nine genes for GH2 enzymes, out of which four were mapped to chromosome VI, but only one (AN3201) appeared to specify a gene big enough to produce a peptide of 120 kDal, the experimentally estimated molecular weight of the □-galactosidase subunit. This gene was assigned the abbreviation *bgaD*.

In this study we report on the expression characteristics of the *A. nidulans bgaD* gene upon growth on D-galactose. The potential inductors can be identified by testing loss-of-function mutants defective in a single defined step of D-galactose catabolism. In case of a galactokinase (*galE*) mutant, *bgaD* is strongly induced by D-galactose, indicating that the intermediates of the Leloir pathway are dispensible for the induction. The expression profile of *bgaD* was similar in L-arabitol dehydrogenase (*araA1*) and hexose kinase (*frA1*) negative backgrounds, indicating that intermediates of the oxido-reductive pathway downstream of galactitol are not necessary for *bgaD* induction either. An *frA1/galeE* double mutant (which cannot grow on D-galactose) still produced *bgaD* transcript upon transfer onto D-galactose. Together, these results suggested that the true inducer of *bgaD* upon D-galactose induction is either D-galactose itself or its poliol form (galactitol).

To test this hypothesis, we grew the wild-type as well as the *galE* mutants on galactitol as a sole carbon source. No *bgaD* induction was observed at any time-points tested, e.g. galactitol is not an inducer of the major intracellular beta-galactosidase in *A. nidulans*. We therefore concluded that the true inducer of *bgaD* in *A. nidulans* upon growth on D-galactose is the sugar itself, and its catabolism is not needed to achieve sufficient level of transcript formation.

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***18. The cell wall stress response of *Aspergillus niger* involves the activity of at least two transcription factors: RlmA and MsnA**

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Comprehensive understanding on how fungi adapt and survive cell wall stress conditions is still missing. Recently, we have shown the importance of the cell wall integrity pathway and its downstream targets RlmA (transcription factor) and AgsA (α-1,3 glucan synthase) for *Aspergillus niger* to survive sublethal concentrations of caspofungin, fenpropimorph and the antifungal protein AFP (Meyer et al 2007, Hagen et al. 2007) . In this study, we extended these transcriptomic and physiologic analyses to study the response of *A. niger* towards aureobasidin A (AbaA), an inhibitor of the sphingolipid biosynthesis and FK506, an inhibitor of the calcium-calcineurin signaling pathway. Upon AbaA treatment, 237 responsive genes were found which were mainly assigned to function in (i) lipid metabolism, (ii) cell wall remodelling, (iii) vesicle transport, (iv) nutrient transport and (vi) proteasomal degradation. Expression of 96 genes changed upon FK506 treatment, which are predicted to function in (i) ion homeostasis, (ii) calcium signaling, (iii) protein folding and maturation and (iv) vesicle trafficking. In silico analysis of all responsive genes and their promoter regions predicted that beside RlmA, another transcription factor, MsnA, might guard *A. niger* against these cell wall stressors. Analysis of the phenotype of *A. niger* when depleted for MsnA indeed confirmed that MsnA is important for *A. niger* to withstand cell wall stress.

Key words: *Aspergillus niger*, RlmA, MsnA, cell wall integrity, antifungals

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19. Impact of G protein-coupled receptors on sensing and regulation of growth in the human-pathogenic fungus *Aspergillus fumigatus

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The asexual conidia of the ubiquitous fungus *Aspergillus fumigatus* are a major thread to immunocompromised patients[1]. Until now, little is known about the pathophysiology of *A. fumigatus*, especially with regard to the function of the 15 G protein-coupled receptors (GPCR's) that are encoded in the genome. Therefore, we investigate the signalling function of GPCR's and their contribution to physiology and virulence of *A. fumigatus*. To characterise the function, we generated single knock-out mutant strains that were phenotypically characterised. Furthermore, we applied the BIOLOG-System to investigate putative ligands as well as the Split-Ubiquitin-Yeast-Two-Hybrid-System to verify protein-protein interaction with cytoplasmic signal transduction proteins. With the methods mentioned, we could show that the deletion of some GPCR-encoding genes leads to a decrease in spore formation, whereas the germination rate and the response towards reactive oxygen-inducing agents remained unaffected. The deletion of *gprG* additionally led to a significant delay in radial growth. Using the BIOLOG-System, we showed that the lack of certain GPCR's alters the growth of the respective mutant strains. Interestingly, addition of the identified metabolites in excess complemented the growth defect of the respective mutants, indicating the link between the ability to sense certain molecules *via* GPCR's and the effect of these molecules on the physiology of *A. fumigatus*. In summary, GPCR's of *A. fumigatus* are involved in sensing of nutrients, as it has been shown recently for *A. nidulans*[2]. Furthermore, they contribute to the regulation of growth and spore formation as indicated by the changed phenotypes of single-ko strains. Current investigations focus on the link of GPCRs to signal transduction proteins and on their contribution to virulence of *A. fumigatus.*

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[2] de Souza *et al.*; PLOS One, 2013

***20. KdmB, a histone H3 lysine K9/K36 demethylase modulates gene expression of secondary metabolite gene clusters**

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Chromatin remodelling at secondary metabolite (SM) gene clusters is an important mechanism regulating the biosynthesis of natural products. Mechanisms such as histone acetylation/deacetylation, methylation/demethylation and the associated binding of non-histone proteins, such as heterochromatin protein-1 (HepA) profoundly alter the chromatin landscape and associated gene expression profiles in toxigenic *Aspergilli* or pathogenic *Fusarium* species. These processes proofed to be important for controlling the ON/ OFF state of SM biosynthetic genes as well as the fine tuning of their expression.

Here, we present the dynamic process of histone H3 demethylation by a jumonji-type demethylase termed KdmB. The purified enzyme removes the methyl mark from tri-methylated H3K9me3 and H3K36me3, but not from H3K4me2 *in vitro*. Chromatin immunoprecipitation (ChIP) revealed that KdmB is involved in counteracting the heterochromatic state mediated by H3K9 methylation at several SM gene clusters, e.g. penicillin (PEN) or sterigmatocystin (ST) during transition from silent to actively transcribed loci. Transcriptome analysis of a *kdmB* null mutant exhibits repression of several SM gene clusters with concomitant loss of corresponding SM production. Our data revealed surprising differences in the chromatin-mediated mechanisms regulating ST and PEN cluster gene expression in respect to the function of HepA and the global regulator of SM gene expression, LaeA. Moreover, *kdmB* deletion is associated with the loss of H3 serine 10 phosphorylation, a histone mark usually associated with actively transcribed genes. Strikingly, *ΔlaeA* strains showed the same effect at ST gene cluster indicating a genetic interaction between KdmB and the complex networks regulating SM cluster gene expression.

Key words: demethylation, chromatin, secondary metabolism, gene clusters, *Aspergillus*

21. An update of on-going work with CADRE and AsperCyc

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The Central *Aspergillus* Data Resource (C*A*DRE; www.cadre-genomes.org.uk) gathers automated and manual annotation efforts for this genus, providing enriched data for each genome. This information flows into AsperCyc (www.aspercyc.org.uk), an online resource of predicted metabolic pathways for the *Aspergillus* genus. Recently, much work has been going on that will filter into these online resources. We have recently been involved in an EC-FP7 funded systems biology study of fungal pathogens (Sybaris) during which we sequenced and annotated nine *Aspergillus fumigatus* strains, including CEA10 and AF300, and one *A. nidulans* strain (F8226) using data from C*A*DRE. Currently, we are involved in another EC-FP7 funded project (NOFUN; www.nofunproject.org) that builds on our earlier work and aims to identify novel drug targets and to develop novel classes of antifungal drugs. Both projects have and will involve RNAseq analyses, data that we can make available, along with the strains, within C*A*DRE.

22. Self-excising Cre/mutant *lox* marker recycling system for multiple gene integrations and consecutive gene deletions in *Aspergillus oryzae*

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We have previously developed the Cre-mediated marker recycling with mutant *lox* sequences to introduce a number of biosynthetic genes into *A. oryzae*, and achieved a high yield production of kojic acid by sequential introduction of genes for oxidoreductase and its transporter [1]. In this study, we further attempted to improve the marker recycling system in more convenient manner by applying a self-excising marker cassette that allows the removal of selectable marker together with Cre expression construct.

We used the *adeA* gene as a selectable marker, because the *adeA* deficient mutant shows an orange colony, which allows us to easily discriminate the marker-rescued strains. The *Aspergillus nidulans adeA* gene was used to replace the *Neurospora crassa pyr4* flanked by mutant *lox* sequences in the expression plasmid

previously constructed [1]. Cre expression construct was designed to place the *cre* gene downstream of *A. oryzae* xylanase G2-encoding *xynG2* promoter or *Penicillium chrysogenum* xylanase-encoding *xylP* promoter. These gene promoters are repressed in the presence of glucose resulting in the silence of Cre expression, and induced in the presence of xylose to drive Cre expression. The Cre expression construct was inserted between the *adeA* gene and mutant *lox* sites in the promoter expression plasmid, but construction of the plasmid was failed probably due to a slight expression of *cre* gene under fungal gene promoter in *Escherichia coli*. Hence, to avoid such an undesirable excision of the cassette in *E. coli* cells, an intron of 72 bp in the *xynG2* gene was inserted into the *cre* gene and the resulting plasmid was successfully obtained. *A. oryzae adeA* deletion mutant was transformed by using the resulting plasmid in the presence of glucose, and then the transformants were transferred to the medium containing xylose as a sole carbon source. PCR analysis of genomic DNA from colonies that grew on the xylose medium revealed the excision of both the marker and Cre expression construct, indicating that a self-excising marker cassette was efficiently function to remove the selectable marker. By using the plasmid constructed here, both multiple-gene integrations and consecutive gene deletions can be readily carried out in *Aspergillus* fungi.

[1] Ebara et al., 9th *Aspergillus* meeting, Marburg, Germany (2012).

23. The metal transporter FetD is involved in low-affinity iron uptake in *Aspergillus fumigatus*

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Iron is an essential nutrient for all eukaryotes. Iron uptake has been investigated extensively in various fungi including the opportunistic human pathogen *Aspergillus fumigatus*. To satisfy its cellular iron demand, *A. fumigatus* employs three main iron uptake mechanisms. When iron is scarce iron import is mediated mainly by two high-affinity systems, reductive iron assimilation and siderophore-assisted transport. At high iron concentrations (a) so far uncharacterized low-affinity transport system contributes to supply the need for iron. In this study we identified a protein, termed FetD, which is part of the low-affinity system. *fetD* transcription is activated at harsh iron starvation and iron excess while lack of *fetD* in wt (Δ*fetD*) results in decreased cellular iron content. Nevertheless, *fetD* deletion had no effect on *A. fumigatus* growth rate, which demonstrates that the two high-affinity systems can compensate *fetD* deficiency. However, deletion of *fetD* in a strain lacking both high-affinity systems (Δ*sidA*Δ*ftrA*Δ*fetD*) results in severe growth reduction, especially under hypoxic conditions. Taken together, our data show that FetD is involved in low-affinity uptake. Moreover, FetD activity is of major importance during hypoxic conditions.

24. The CCAAT-Binding-Complex mediates Iron Regulation in *Aspergillus fumigatus*

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Iron is essential for a wide range of cellular processes but its excess is toxic. Therefore, microorganisms evolved fine-tuned mechanisms for uptake and storage of iron, to sustain iron homeostasis. In the opportunistic fungal pathogen *Aspergillus fumigatus,* the bZIP-type transcription factor HapX mediates adaption to iron starvation by activating siderophore biosynthesis and repressing iron-dependent pathways. HapX-deficiency attenuates the virulence of *A. fumigatus* underlining the importance of adaptation to iron starvation in pathogenicity. The HapX N-terminal amino acid sequence predicts interaction with the DNA-binding, heterotrimeric CCAAT-binding complex (CBC), which is conserved in all eukaryotes and believed to co-regulate up to 30% of all genes. Here, we characterized the role of the CBC in iron regulation of *A. fumigatus* by analysis of the phenotypic consequences of genetic inactivation of the CBC subunit HapC.

HapC-deficiency was deleterious during both iron starvation as well as iron sufficiency, demonstrating iron-independent regulatory functions of the CBC. In contrast, HapX is important during iron starvation only. Whole transcriptome sequencing during iron starvation conditions revealed that HapC-deficiency upregulates 562 genes, upregulated by HapX-deficiency, and down-regulates 635 genes, of which 397 (60 %) are also

downregulated by HapX-deficiency. Deficiency in both HapX and CBC derepressed genes involved in iron-consuming pathways, but decreased production of siderophores as well as certain secondary metabolites. Inhibition of reductive iron assimilation by ferrous iron chelation blocked colony formation of both HapC-deficient and HapX-deficient conidia. Moreover, inactivation of HapC was epistatic to HapX-deficiency. Taken together, these data indicate that the CBC mediates both the activating and repressing functions of the iron-regulatory transcription factor HapX. The central role of the CBC in environmental adaptation is underlined by HapC-deficiency rendering *A. fumigatus* avirulent in a murine model of aspergillosis.

25. The effects of cultivation temperature on characteristics of conidia in pathogenic fungus *Aspergillus fumigatus*

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Filamentous fungi vigorously produce asexual spores (conidia) under appropriate conditions. Conidia are reproductive structures that are important for both distribution and survival for fungi. From a view of food preservation, the conidia are the main factor of food contamination and deterioration. From the viewpoint of infection, in some pathogenic fungi including *Aspergillus fumigatus*, inhalation of the conidia is the main route of infection. Not only understanding of conidial long-term survival and germination, but also characterization of conidia produced under divergent environmental conditions is undoubtedly useful for us to develop new systems for protecting foods, crops, and susceptible patients from the problematic fungi.

To understand the effect of temperature during conidiation on characteristics of the conidia, we took *A. fumigatus* as an example to show the differences in conidia color, the stress tolerance, content of compatible solutes in the conidia, and germination rates. We harvested conidia produced after 7days-cultivation on PDA agar medium at 25, 37, and 45°C. The color of conidia from 25°C-culture was darker than those from 37 and 45°C-culture. The conidia from 25°C-culture showed a lower tolerance to heat stress (60°C, 15 min) compared to the others. Trehalose is a component that plays an important role in heat tolerance as a compatible solute. In consistent with the result of heat stress test, accumulation of trehalose was lowest in the conidia from 25°C-culture. Furthermore, when conidia were harvested from cultivation on glucose minimal medium (GMM) at 25°C, the sensitivity to heat stress and reduction of trehalose accumulation were increased compared with those cultured on PDA. To gain more insight into characteristics of conidia harvested from different temperature cultivation, we are going to reveal the transcriptome in the conidia by RNA-sequencing technology, and to compare them. Based on these results, we will discuss the stress tolerance mechanisms of conidia and effects of the surrounding environment during conidiation.
of which 214 (38 %) are also

26. The conserved MAP kinase MpkB affects developmental processes but not secondary metabolism in *Aspergillus flavus*

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In eukaryotes, MAP kinase pathways play important roles in regulation of growth, development, and various stress responses. To characterize the function of MAP kinase in an important pathogenic and toxigenic fungus *Aspergillus flavus*, the *AflmpkB* gene (AFL2G_02589), an orthologue of the yeast *fus3* gene, was deleted. In *Aspergillus nidulans*, previous studies revealed that MpkB positively regulates the sexual and asexual differentiation as well as secondary metabolite production. In this study, deletion of *AflmpkB* resulted in no mycelial growth change, while the conidial production was reduced about 60% comparing to the wild-type. Moreover, the mutant produced immature and abnormal conidiophores such as vesicular dome-immaturity in the conidiophore head, decreased number of the phialides and very short stalks, although expression of the *brlA* gene, a key regulator of conidiation, was up-regulated in the mutant. Also, Δ*AflmpkB* couldn't produce any sclerotia, suggesting that the *AflmpkB* gene is important to conidiophore and sclerotia development. However, *AflmpkB* mutants produced normal level of aflatoxin B_1. Taking together, *A. flavus* MpkB plays a positive regulatory role in the production of the conidiation and the sclerotia but not in the production of the secondary

metabolite such as aflatoxin B_1. [This work was supported by the National Research Foundation of Korea (NRF) grants NRF-2012R1A1A4A01012864 and NRF-2011-0014718.]

27. NGS analysis revealed new mutations in NSDA sterile mutant of *Aspergillus nidulans*

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Sexual development and fruiting body production of fungi play pivotal roles in production of ascospores by meiosis as well as adaptation of various environmental changes. In a homothallic fungus *Aspergillus nidulans*, many environmental factors and genes affecting sexual development have been elucidated. One of the first and important attempts for understanding the sexual development of *A. nidulans* was isolation of NSD mutants, which are defective in the process. NSD mutants are divided into four different complementation groups, NSDA-D, and the two genes responsible for the *nsdC* and *nsdD* mutation have already been isolated and characterized. However, *nsdA4* and *nsdB5* mutations from NSDA and NSDB mutants, respectively, are remained to be unveiled. Since classical complementation experiments by transforming genomic DNA library to the mutants were not successful, we analyzed the whole genome sequence of NSDA mutant obtained from Next Generation Sequencing (NGS) to identify the *nsdA4* mutation. As a result of analysis of mutation sites, we previously found several NSDA mutant-specific mutations and confirmed the mutations by PCR followed by sequencing analysis. Three mutations including AN3939 locus, which encodes SCF ubiquitin ligase subunit CulC, were investigated but none of the knock-out of the candidate genes showed *nsd*$^-$ phenotype. However, recent intensive mutation analysis revealed that the NSDA mutant strain carries missense mutations both in the *nsdD* and *nsdC* ORF region, suggesting that phenotype of NSDA mutant might be derived from the two simultaneous mutations of the previously identified important genes for sexual development. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (NRF-2012R1A1A4A01012864).

28.The forkhead gene *fkhB* is required for proper conidiophore morphogenesis in *Aspergillus nidulans*

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Developmental process in a model filamentous fungus *Aspergillus nidulans* is controlled by multiple regulatory systems including conserved signal transduction pathways and transcription factors. Previously, we identified forkhead type transcription factors, which are conserved in fungi, by using genome sequence screening. Here, we isolated and identified the *fkhB* gene encoding a putative forkhead transcription factor containing both of conserved forkhead domain (FH) and forkhead associated domain (FHA). The *fkhB* gene expression was up-regulated after 24 h induction of asexual development and GFP tagged FkhB protein was localized in nucleus, suggesting that the *fkhB* gene is a transcription factor related in asexual developmental process. To know the function of the *fkhB* gene, we generated *fkhB* deletion strain in *A. nidulans*, which resulted in abnormal conidiophore formation under standard conditions and delayed sexual development process, suggesting that the *fkhB* gene plays an important role in conidiophore morphogenesis as well as in coordinating sexual development. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (313-2008-2-C00804).

29. Production of a novel immunomodulatory compound by a protein kinase A regulated gene cluster of *Aspergillus fumigatus*

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Aspergillus fumigatus is a common soil inhabiting mould but also an opportunistic human pathogen that can cause life threatening infections in immunocompromised individuals. Its survival in these entirely different habitats depends on effective mechanisms for signal perception and transduction. One of these signal transduction cascades is the cAMP/protein kinase A pathway that represents also an important virulence determinant of *A. fumigatus*.

In a transcriptomics approach putative targets of this signaling cascade were identified. Among these were several transcriptional regulators and a transcription factor, which showed highest upregulation by PKA. This transcription factor is part of a secondary metabolite gene cluster. Deletion of the corresponding gene as well as a deletion of the non-ribosomal peptide synthetase (NRPS) encoding gene in the cluster led to reduced fungal growth and sporulation. Overproduction of the transcription factor resulted in formation of a brown substance, whose structure was elucidated. The novel identified compound was named fumipyrrole. Remarkably, fumipyrrole inhibits interleukin-1β secretion by human peripheral blood mononuclear cells, suggesting a role in pathogenicity of *A. fumigatus*.

***30. Rapid disorganization of the Golgi apparatus by blocking the exit of COPII traffic from the endoplasmic reticulum**

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The mechanistic bases of the biogenesis and maintenance of the cisternae of the Golgi apparatus are still subject to debate. Proteins and lipids synthesized at the endoplasmic reticulum (ER) are sent to the Golgi apparatus into vesicles. The formation of these vesicles is a crucial step of the secretory pathway involved in Golgi function and maintenance. Coat protein complex II (COPII) is a set of highly conserved proteins that mediates the biogenesis of those membrane vesicles. Sar1 is a p21 GTPase that triggers and regulates the assembly of COPII. *sarA,* the *Aspergillus nidulans sar1* orthologue, is essential. We generated a saturated library of mutant alleles by random PCR combined with gene replacement and selected a collection of temperature-sensitive alleles that are useful to specifically block traffic from the ER to the Golgi apparatus. Following a temperature shift-up, we found that Sec23, a subunit of COPII, shifts its localization from the ER to the cytosol, suggesting that COPII vesicles do not form. In addition, an acute and rapid disorganization of the Golgi apparatus occurs: $RerA^{Rer1}$, a Golgi resident protein that cycles between Golgi and ER to retrieve proteins to the ER, abnormally labels ER membranes, indicating that its steady-state equilibrium has changed, and the late Golgi marker PH^{OSBP} becomes cytosolic. Our data seem incompatible with the stable cisternae model, supporting instead cisternal maturation.

31. KipA of no return: Regulation of kinesin-7 turnover by the F-Box protein RcyA in *Aspergillus nidulans*

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Filamentous growth needs to the continuous delivery of proteins, mRNAs and secretory vesicles to the growing tip. The growing tip is determined by specific proteins, the cell-end markers, and differentiated plasma membrane domains. To keep intact the specific PM-domain is necessary that the secretion is coupled with endocytosis. We present the characterization of RcyA, the *A. nidulans* homolog of Rcy1 in yeast. RcyA plays a role in coupling endo- and exocytosis. The cell-end markers and the endocytic ring are spatially disturbed in the absence of *rcyA*. In addition, RcyA is necessary for the degradation of the kinesin motor protein KipA to keep it under physiological levels suggesting that RcyA is the substrate adaptor for KipA in a SCF-ubiquitin ligase complex.

32. Diversity of feruloyl esterase activity in 14 *Aspergilli* species

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Feruloyl esterases (ferulic acid esterases, EC 3.1.1.73) are a subclass of carboxylic acid esterases, which catalyze the hydrolysis of ester linkage of ferulic acid and other cinnamic acids from plant cell wall polymers. Feruloyl esterases cleave phenolic acids (ferulic acid and p-coumaric acid) and their dimers from naturally occurring hemicelluloses and pectins. Hemicelluloses represent about 20–35% of the lignocellulosic biomass, and therefore there is a large potential for industrial applications of feruloyl esterases.

The presence of feruloyl esterase encoding genes varies significantly in fungi, even between species of the same genus. For instance, orthologs for *Aspergillus niger faeA* were only found in some *Aspergilli*, while the number of candidate feruloyl esterases of carbohydrate esterase family 1 varies between one and four in these species.

Plant cell wall derived substrates have been reported to induce feruloyl esterase activity in fungi. We studied the ability of 16 *Aspergilli* to release ferulic acid from sugar beet pulp and wheat bran. Total feruloyl esterase activity was detected by using different methyl esters as substrates. Feruloyl esterase activity profile of *Aspergilli* strains on different growth media and substrate specificity will be discussed.

33. A complex interplay of *Aspergillus fumigatus* with the social amoeba *Dictyostelium discoideum* supports the virulence school concept

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Aspergillus fumigatus can be regarded as a classical example of an environmentally acquired pathogen with a broad host range covering humans and various animals. The disease pattern may vary greatly depending on the immune status or other predispositions of the patient, ranging from the sole mucus colonization in the lungs of cystic fibrosis patients to a deleterious, tissue invasive growth in immunocompromised individuals. Originally focused on the identification of single virulence factors, research in the last years has established that virulence is rather driven by multifactorial processes on the host and pathogen side. However, the evolutionary basis between these complex interplays are yet to be identified. It has long been proposed that the general mechanisms of fungal virulence must have emerged long before the appearance of innate immune systems and fostered the hypothesis that it could at least partially result from the selection pressure imposed by amoeba predation. A central question is therefore how the interaction with predatory amoeba could also have stimulated the development of virulence mechanisms. We have exploited the social amoeba *Dictyostelium discoideum* as a model organism to study its interaction with conidia of *A. fumigatus.* Although not a regular food source, fungal conidia were readily taken up by the amoeba within the first hours of exposure, as seen by transmission electron microscopy and discriminant fluorescence of intracellular conidia. Interestingly, non-melanized conidia of a *pksP*-deletion mutant of *A. fumigatus* were taken up at much higher rates than the wild type, leading to a massive intracellular accumulation. These results are also in agreement with the well documented role of melanin in the protection against macrophage killing. Despite the observation of phagolysosomal fusions, the viability of the fungal conidia was not impaired after phagocytosis and intracellular germination initiated after 24 hours. Up to this time point amoeba also remained fully viable and only fungal germination coincided with an increased number of dead amoeba. We hypothesize that both, fungus and amoeba also interact chemically and present first results on the cross inhibition of the two species.

34. The novel sensor-globin fungoglobin is involved in low oxygen adaptation of *Aspergillus fumigatus*

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Infection with conidia of the pathogenic fungus *Aspergillus fumigatus* is a frequent health threat for immunocompromised human individuals. While classic virulence factors have not been identified, it has become increasingly clear that its physiological versatility as a saprophyte may largely contribute to the establishment of invasive growth. Low oxygen partial pressures is a property which transiently occurs in most natural growth environments, but also defines deep layers of infected human tissue. *A. fumigatus* survives and prospers in such hypoxic areas and, as other fungi, exploits ergosterol biosynthesis as an essential measure for oxygen. However, the direct metabolic and energetic consequences of low O_2 availability are less understood, and we hypothesized that the fungus could also sense and react to O_2 directly. In a first approach, we used Next Generation Sequencing to study the dynamic and short term response to a transient exposure to low O_2. Deprivation of O_2 triggered a more than threefold induction of 680 genes after only 15 min while 420 genes were down regulated at the same time point. Among the highest upregulated genes we identified a gene encoding a hypothetical protein which appears to be conserved in filamentous fungi. In sharp contrast, reoxygenation of the growth medium resulted in the complete repression of its mRNA. This transcriptional dynamics was verified by Northern Hybridization, and interestingly, hypoxic induction was also observed in an *srbA* deletion mutant. Low iron also induced its expression, but in a HapX-independent mode, indicating that this gene is not under direct control of either of these two regulators. The encoded protein comprised a globin-like N-terminal domain and was identified as a member of the large protein family of sensor globins. The function of these proteins is largely unknown but heterologous expression and purification gave evidence for a functional heme binding site. Furthermore, the deletion of the gene led to an impaired growth of *A. fumigatus* in low oxygen atmospheres and hence, the putative role of this “fungoglobin” during such conditions will be discussed.

35. Elucidation of the biosynthetic pathway of the antibiotic yanuthone D in *Aspergillus niger

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In this study we elucidated the genetic and biosynthetic pathway of meroterpenoid yanuthone D originating from *Aspergillus niger*. Feeding *A. niger* labeled 6-methylsalicylic acid (6-MSA), we showed that this building block is the precursor of yanuthone D. Moreover, we identified 10 genes, involved in the production of yanuthone D, including *yanA*, the gene encoding 6-MSA synthase. The deletion of all genes within this cluster and analyses of the constructed strains allowed for the identification restored by co-injecting larvae with iron.

We postulate that, at least in the larval model, it is PPTAs role in siderophore biosynthesis and not the activation of other secondary metabolism pathways that is critical for the virulence of *A. fumigatus*. The loss of pptA appears to affect the immune recognition of spores by human and murine cells which may be due to the role it plays in melanin biosynthesis. *In vivo* studies challenging human dendritic cells against fixed ΔpptA conidia lead to an increase in the release of proinflammatory cytokines Il-1B and Il-6. Furthermore, intranasal challenge of the ΔpptA mutant in an immunocompetent murine model leads to a reduction in neutrophil recruitment in the lung which suggests a more rapid clearance of the fungus by macrophages.

36. The sfp-type phosphopantetheinyl transferase, PPTA, is critical for the virulence and important in host detection of *Aspergillus fumigatus*

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Aspergillus fumigatus is the leading cause of invasive aspergillosis (IA), a fungal disease which is increasing annually on a global scale. IA poses as a common threat to patients with a weakened immune response due to disorders such as leukaemia, HIV, AIDS and also persons undergoing chemotherapy treatments. The ability of *A. fumigatus* to produce a wide array of secondary metabolites is thought to contribute to the pathogenicity of this organism. We have identified an enzyme, PPTA that plays a key role in secondary metabolism in *A. fumigatus*. PPTA is a sfp-type phosphopantetheinyl transferase and is required to activate non-ribosomal peptide synthases, polyketide synthases and a protein required for lysine biosynthesis aminoadipate reductase (AARA). Disruption of pptA renders the fungus avirulent in both insect and murine infection models. To investigate which aspects of pptA activity are essential to virulence a series of knock out mutant strains were generated; ΔaarA, ΔpksP and ΔsidA. These genes play a vital part in lysine, melanin and siderophore biosynthesis pathways respectively. The sidA gene proved vital to virulence in the insect model whereas the ΔaarA and ΔpksP mutants were unaffected. The pathogenicity of both the pptA and sidA knock out strains was restored by co-injecting larvae with iron. We postulate that, at least in the larval model, it is PPTAs role in siderophore biosynthesis and not the activation of other secondary metabolism pathways that is critical for the virulence of *A. fumigatus*. The loss of pptA appears to affect the immune recognition of spores by human and murine cells which may be due to the role it plays in melanin biosynthesis. *In vivo* studies challenging human dendritic cells against fixed ΔpptA conidia lead to an increase in the release of proinflammatory cytokines Il-1B and Il-6. Furthermore, intranasal challenge of the ΔpptA mutant in an immunocompetent murine model leads to a reduction in neutrophil recruitment in the lung which suggests a more rapid clearance of the fungus by macrophages.

37. Lactose induction of sterigmatocystin formation in *Aspergillus nidulans*

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Seed contamination with polyketide mycotoxins such as aflatoxin (AF) and sterigmatocystin (ST) produced by *Aspergillus* spp., is an agricultural, economic, and medical issue worldwide. ST is the penultimate intermediate in the biosynthesis of AF; moreover, in several fungi including the model fungus *A. nidulans*, it is the end product of the AF pathway. The AF/ST biosynthetic pathway is well-characterized in *A. nidulans*, but many of the regulatory aspects including those related to the carbon source available for the fungus are still enigmatic. For example, *A. nidulans* mutants unable to produce ST on glucose medium have been observed to produce ST on lactose as a carbon source.

One of the genes important for ST production is the so-called “velvet” gene (*veA*). The wild type allele is *veA* while *veA1* is the mutation. In *A. nidulans*, VeA has been shown to control the AF/ST regulatory gene *afl*R and, subsequently, ST production. To address the relative importance of VeA and its interaction with carbon source, we initiated a project of lactose metabolism vs. ST production in *A. nidulans* in both a *veA* and *veA1* background.

Since environmental factors influence ST formation, we employed well-controlled submerged cultivation methods where temperature, pH and DO levels could be kept at preset values, and light intrusion could be

prevented. To isolate and detect ST from fungal cultures, we developed an extraction protocol suitable to extract ST with an efficiency of 98.5%, and a rapid, reliable HPLC–UV method.

Under these conditions, independently of the carbon source available, *A. nidulans* was not able to produce any ST in a *veA1* background. In contrast, *veA* strain was capable of producing ST on both glucose and lactose in a concentration of up to 0.5 mg per liter. Time-profiles of ST formation were markedly different, however: on D-glucose, ST could be detected only after glucose was depleted from the medium, while on lactose, ST appeared in the early stages of the rapid growth phase. We concluded that ST-formation in *A. nidulans* may either be mediated by a carbon catabolite regulatory mechanism prominent on D-glucose, or induced by the low specific growth rate attainable on lactose. We are currently testing this hypothesis employing chemostat-type continuous cultures.

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*38. Carbon metabolism of *Aspergillus nidulans* during growth on plant biomass

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Plant biomass is the most abundant and renewable carbon source for many fungal species and it consist of polysaccharides, lignin and proteins. Plant polysaccharides come from a variety of plants, and the composition depends not only on the plant, but also on the growth conditions, on the part of the plant, and on the season. The average composition is 40-45% cellulose, 20-30% hemicellulose, and 15-25% lignin. Monosaccharides are the main components of the biomass and an important carbon source for many fungi. These monosaccharides are converted through a variety of carbon catabolic pathways.

Several carbon metabolism mutants of *A. nidulans* were generated that were (partially) impaired in glycolysis and therefore are reduced in their ability to use hexoses as carbon source. In addition, strains were generated in which these mutations were combined with a mutation in CreA, the major regulatory system involved in carbon catabolism. Growth of these mutants on plant biomass was performed and compared to the wild type, while enzyme assays were performed to study the production of extracellular enzymes involved in degradation of polysaccharides. The results highlight the interplay between hexose release and catabolism and the central role CreA plays.

39. Flow cytometric analysis of *Aspergillus niger* conidia

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Aspergillus niger is an important host organism for the production of organic acids and proteins. The germination of conidia is the first step in the fermentation process and a highly relevant process, because the molecular mechanisms during germination directly influence the morphology of the culture. The germination process can be followed by flow cytometry until the germ tube is formed. Flow cytometry is a powerful single cell analysis tool, which amongst others allows to simultaneously monitor cell growth and fluorescent biomarkers. We set out to establish flow cytometry as a tool for the analysis of fungal conidia and the germination process. With this technique it is possible to analyze on a single cell level the underlying principles of germination and to investigate the dynamic behavior of conidia populations. In this work, we analyzed the impact of age and media composition on the size and swelling of *Aspergillus niger* conidia. Potato dextrose agar and malt extract agar - two commonly used sporulation media - were compared. Thereby, the media used for conidiation has a direct influence on the further development in the swelling/growth medium. The size distribution of the conidia populations between the two conidiation media differed significantly. Furthermore, the conidia showed different kinetics during swelling on the same media.

***40. Interaction of *Aspergillus nidulans* GalR, XlnR and AraR in D-galactose and L-arabinose catabolism**.

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The xylanolytic regulator XlnR and the arabinanolytic regulator AraR control pentose catabolism in *A. niger* and *A. nidulans*. The role of AraR differs between these species as *A. niger ΔxlnRΔaraR* is not able to grow on L-arabinose, whereas *A. nidulans ΔxlnRΔaraR* still grows on this substrate. The D-galactose oxido-reductive pathway in *A. nidulans* make use of pentose catabolic pathway enzymes and one gene encoding such an enzyme has been shown to be under control of the galactose-responsive regulator GalR. GalR is unique to *A. nidulans*, and a role for GalR in L-arabinose catabolism could explain the observed difference between *A. niger* and *A. nidulans*. In this study the interactions of XlnR, AraR and GalR were investigated in more detail by studying the phenotype of double and triple disruptant strains of these regulators in *A. nidulans*. Interactions between all three regulators with respect to pentose catabolic and D-galactose catabolic gene expression were observed.

41. Superfamily of actinoporin-like proteins in fungi

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Superfamily of actinoporin-like proteins (ALPs) comprises diverse protein families that show structural similarity to actinoporins. Actinoporins (20 kDa) are potent cytolytic toxins isolated from sea anemones, structurally defined by a rigid beta sandwich flanked by two alpha helices (PF06369). We determined the distribution of ALP sequences in fungi for some ALPs families like fungal fruit body lectins, necrosis inducing proteins and aegerolysins.
Fungal fruit body lectins are mostly fungal proteins. Their domain fold is named FB lectin (PF07367) and it is not related to any other of several lectin folds, however, it shows significant structural similarity to actinoporins. The lectin XCL from *Boletus chrysenteron* induces drastic changes in the actin cytoskeleton after sugar binding at the cell surface and internalization and has potent insecticidal activity (Birck *et al*., 2004).

Necrosis inducing proteins belonging to NPP1 domain (PF05630) are present in oomycetes, fungi and bacteria. Infiltration of NPP1 into leaves of *Arabidopsis thaliana* plants result in transcript accumulation of pathogenesis-related genes, production of ROS and ethylene, callose apposition and cell death (Fellbrich *et al*., 2002).

Aegerolysins were discovered in fungi, oomycetes and bacteria. They share common aegerolysin fold (PF06355). The bacterial members of this family are expressed during sporulation. Aegerolysins have been described to exhibit pleiotropic functions; some of them are haemolytic in the presence of another MACPF-domain containing protein (Tomita *et al.*, 2004; Ota *et al.*, Shibata *et al.*, 2010). Aegerolysin and ostreolysin are expressed during formation of fungal primordia and fruiting bodies, and may play an important role in the initial phase of fungal fruiting (Berne *et al.*, 2002).

The occurrence of ALPs in fungi was highly heterogeneous, with aegerolysins and NPP1 overrepresented, while the other ALPs were more rarely identified and we observed no obvious correlation to taxonomy or pathogenous lifestyle. At least part of ALPs may be considered as small secreted proteins, even though sometimes no recognizable signal peptide was observed. ALPs from *Aspergillus niger* were chosen for cloning and further characterisation studies, NPP1 protein represented here and aegerolysins elsewhere (poster M. Novak).

Key words: actinoporin, aegerolysin, fungal fruit body lectin, necrosis inducing protein, *Aspergillus niger*

42. Regulation of cellulase genes through pH signaling pathway in *Aspergillus nidulans*

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In *Aspergillus nidulans*, expression of cellulase genes is cooperatively regulated by the pathway specific transcription factor ManR/ClrB and the wide-domain transcription factor McmA. The expression appeared to be also under control of the *pal-pacC* pH signaling, as the *palC* mutant displayed decreased cellulase productivity. Higher productivity of endoglucanases A and B (EglA and EglB) at neutral pH compared to acidic pH and impaired production of the enzymes in the *pacC* deletion strain confirmed the involvement of the *pal-pacC* signaling. qRT-PCR analysis indicated that expression of not only *eglA* and *eglB* but also the other cellulase genes such as *cbhA* and *cbhD*, which are under control of ManR/ClrB and McmA were rapidly induced by cellobiose at alkaline pH in the wild type strain, while their expression was delayed and decreased by the *pacC* deletion. PacC appeared to regulate indirectly transcription of *eglA* based on mutational analysis of its promoter, but expression of the transcription factors known to date to be involved in cellulase regulation, including *manR*/*clrB* and *mcmA*, was not regulated by PacC. Nevertheless, genome-wide identification of PacC-dependent genes under cellobiose-induced conditions at alkaline pH demonstrated that the genes highly-expressed in a PacC dependent manner were significantly overlapped with those regulated by ManR/ClrB. PacC might control uptake of cellobiose, the most possible inducer of ManR/ClrB-mediated regulation, because cellobiose consumption was delayed in the *pacC* deletion strain.

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keyword: cellulase, *pacC*, pH signaling, *Aspergillus nidulans*

43. Cell wall stress induced genes use as new targets for antifungals in *Aspergillus fumigatus*

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A. fumigatus infections are a demanding problem for immunocompromised patients. Invasive aspergillosis has a poor prospect with mortality up to 90%. Upcoming problem is resistance to antibiotics of pathogenic fungi. It is important to identify new targets for antifungals. The cell wall is an essential organelle for fungi and not present in mammalian cells. Similar to S. cereviseae, cell wall stress induces cell wall related genes via the cell wall integrity (CWI) pathway in A. fumigatus.

The role of PkcA and RlmA in the cell wall integrity pathway was studied in a loss-of-function pkcA mutant (Campos Rocha et. al. 2012) and a rlmA deletion mutant (Malavazi, unpublished data). The mutants are used to study expression of cell wall stress related genes upon calcofluor white stress by Northern blot analysis, RNAseq and introduction of cell wall stress reporter constructs.

This study presents the involvement of PkcA and transcription factor RlmA in the CWI pathway in *A. fumigatus*. The results show also new RlmA induced gene(s), thereby presenting the first indication of RlmA regulated gene(s) in *A. fumigatus*. The RlmA induced genes are useful tools for development of an *A. fumigatus* cell wall stress reporter strains.

***44. RNA-Seq analysis of the interaction of *Aspergillus fumigatus* with human neutrophils revealed nitric oxide as negative feedback regulator of neutrophil extracellular traps**

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Aspergillus fumigatus is a saprophytic mold that can cause life-threatening infections in immunocompromised patients. In the lung, conidia are challenged by immune effector cells. Among them, neutrophils attack fungal hyphae using various mechanisms including production of antimicrobial proteins, degranulation or neutrophil extracellular trap (NET) formation. Moreover neutrophils form nitric oxide (NO) and reactive nitrogen intermediates (RNI) whose function in defence against *A. fumigatus* is still a matter of debate. By applying a dual transcriptomics approach, we were able to identify genes expressed during the interaction of *A. fumigatus* with human neutrophils. Here, we show that *A. fumigatus* produces several enzymes potentially involved in RNI detoxification, namely two flavohemoglobins, FhpA and FhpB, and the S-nitrosoglutathione reductase GnoA. To elucidate the role of these enzymes, single and double deletion mutants of FhpA, FhpB and GnoA encoding genes were generated.
Our data indicate that FhpA and GnoA are the primarily responsible enzymes in *A. fumigatus* to counteract RNI. Interestingly, based on the finding that human neutrophil granulocytes showed a significant reduction in NETosis when either confronted with hyphae of *A. fumigatus* mutants deficient for RNI-detoxification or by addition of exogenous NO donors, we strongly suggest that NO derivates play a role as potential regulator of NET formation.

45. Ergothioneine is involved in antioxidative defense in *Aspergillus fumigatus

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Ergothioneine (EGT) is a naturally occurring thiourea derivative of histidine. While Saccharomycotina species lack the EGT biosynthetic pathway, it is produced by filamentous fungi, *Actinomycetales*, cyanobacteria and it is present in higher eukaryotes due to nutritional uptake *via* a specific EGT-transporter. Although various human diseases, such as inflammatory processes, rheumatoid arthritis and Morbus Crohn are linked to raised EGT-levels, the function of EGT is still elusive. Similar to thiols such as glutathione, neuroprotective effects in mouse models and oxidative stress resistance in fungi have been found to be associated with EGT.
The airborne human-pathogenic saprophytic mould *Aspergillus fumigatus* causes life-threatening diseases in immunocompromized patients and the protection against oxidative stress is crucial for virulence of *A. fumigatus*. To examine the function of EGT in *A. fumigatus,* we generated a mutant strain (Δ*egtA)* lacking the putative EGT biosynthetic enzyme AFUA_2G15650, termed EgtA. Consistent with the proposed function, inactivation of EgtA eliminated EGT production. EGT-deficiency was phenotypically inconspicuous and did not affect resistance against oxidative or metal stress. To circumvent the redundancy of oxidative stress defense, we next generated a mutant strain (ΔΔ*egtA*/*yap1*) lacking EgtA and Yap1, a transcription factor that orchestrates oxidative stress defense. In this background, EGT-deficiency decreased superoxide resistance (e.g. hydrogen peroxide, menadione) demonstrating that EGT is involved in protection against oxidative stress in its producer. EGT-deficiency decreased the accumulation of the intracellular siderophore ferricrocin in Δ*egtA*. Interestingly, in the Yap1-lacking background, EGT-depletion affected production of extracellular siderophores: it increased fusarinine C but decreased triactylfusarinine C biosynthesis. The effects of EGT-deficiency on siderophore production indicate a link between the cellular redox system and iron homeostasis.

46. Regulation of cellulolytic and hemicellulolytic genes by McmA and ManR/ClrB in *Aspergillus nidulans

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Fungal cellulolytic and hemicellulolytic enzymes are promising tools for industrial hydrolysis of cellulosic biomass. The regulatory system involved in their production had not been well understood, but recent discovery of new transcriptional activators are now providing the keys to solve the complexity of the regulatory system. In *Aspergillus*, XlnR regulates xylanase and cellulase genes, and ManR/ClrB does mannanase and

cellulase genes. McmA also regulates cellulase genes in *A. nidulans*. We report here that cellulase and mannanase genes are regulated by ManR/ClrB, McmA, and the ManR paralog ManS and that the factor(s) responsible for the regulation differs genes to genes.

Transcription of highly expressed cellulolytic genes including endoglucanse genes *eglA* and *eglB*, cellobiohydrolase genes *cbhA* and *cbhD*, were regulated by both McmA and ManR/ClrB in response to cellobiose. Cellobiose also induced some mannanase genes (*manB*, *manC*, *mndB*) in a ManR/ClrB dependent manner, while galactomannan induced a different set of mannanase genes (*manC*, *manE*, *manF*), which were under control of ManS. McmA was not required at least for expression of the β-mannosidase gene *mndB*.

CeRE (Cellulose Responsive Element, $CCGN_2CCN_6GG$) is the sole *cis*-element responsible for the induction of *eglA*. We have shown that McmA binds to CeRE on the *eglA* promoter by EMSA (electrophoretic mobility shift assay). In the assays to detect DNA binding of ManR/ClrB, DNA binding domain of ManR fused to Flag-tag (ManR-DBD) was used. ManR-DBD displayed very weak binding to the *eglA* CeRE by itself, but significantly increased binding was detected in the presence of McmA. Examination of binding to the *eglB* promoter exhibited the similar results, that is, ManR could not bind to the *eglB* CeRE without assistance of McmA. These observations indicate that cellulase genes are regulated by cooperative binding of ManR/ClrB and McmA to CeRE.

The expression of *mndB* was not McmA dependent and its promoter lacked CeRE. This suggested that ManR/ClrB can bind to the promoter without assistance of McmA. EMSA using various probes with intact and mutated sequences revealed that ManR/ClrB should bind to $CGGN_8CCG$. This discovery provided a reference to locate the binding sites on other ManR/ClrB-dependent genes. The arising question is why cellulase genes require McmA for its expression.

***47. Coordination between BrlA regulation and secretion of the oxidoreductase, FmqD, to the cell wall directs selective accumulation of fumiquinazoline C to the spores of *Aspergillus fumigatus*.**

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Aerial spores, crucial for propagation and dispersal of the Kingdom Fungi, are commonly the initial inoculum of pathogenic fungi. Natural products (secondary metabolites) have been correlated with fungal spore development and enhanced virulence in the human pathogen *Aspergillus fumigatus* but mechanism(s) for metabolite deposition in the spores is unknown. The fumiquinazolines (Fqs) comprise a related, sequentially generated family of cytotoxic peptidyl alkaloids that are signature metabolites from *A. fumigatus*. Metabolite profiling of clinical *A. fumigatus* isolates reveal that the first two products of the Fq cluster, FqF and FqA, are produced to comparable levels in all fungal tissues but the final enzymatically-derived product, FqC, selectively accumulates in the fungal spore. Loss of the sporulation-specific transcription factor, BrlA, yields a strain incapable of FqA and FqC production. However, loss of two sequentially downstream transcription factors involved in conidiophore development and maturation namely AbaA and WetA did not affect selective accumulation of FqC in the spores. *In silico* analysis of FmqD, the oxidoreductase required to generate FqC, predicted an N-terminal secretion signal on this enzyme. Fluorescence microscopy showed that FmqD is secreted via the Golgi apparatus to the cell wall in an actin-dependent manner. Removal of the signal peptide abolished FmqD cell wall localization and significantly reduced FqC production. In contrast, all other members of the Fq pathway including the putative transporter, FmqE – which had no effect on Fq biosynthesis – were internal to the fungal hyphae with varying subcellular localization. The coordination between BrlA-mediated tissue specificity with FmqD secretion to the cell wall presents a previously undescribed mechanism to direct localization of specific secondary metabolites to spores of the differentiating fungus.

48. Citric acid production in 24-well plates is an efficient screening platform for *Aspergillus carbonarius*

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Conventional citric acid batch-fermentation by filamentous fungi is often linked with large variations in production levels. The most used citric acid batch-fermentation method described in literature is based on Erlenmeyer flasks with cotton-stoppers in a heated shaker. Our preliminary studies using Erlenmeyer flasks in sextuplicates showed a relative standard sample deviation of 54 % citric acid produced with the filamentous fungi *Aspergillus carbonarius*, thereby confirming these large variations. The objective of this study was to propose a different and more reliable screening method for citric acid production from filamentous fungi, using *A. carbonarius* as test organism in a 24 well plate system. The two systems where run simultaneously with only the mechanical parameters as difference between them. All experiments were performed in sextuplicates to be able to give a higher statistical impact than the conventional triplicates. After 5 days fermentation, the citric acid produced in the Erlenmeyer system had a relative standard deviation of 48% compared to the 24-well system that gave a relative standard deviation of 6%. The statistical analysis showed that the difference between the replicates in the 24 well plate system was significantly smaller than in the conventional Erlenmeyer flask setup. This allows for a more precise determination of effects caused by for example changing fermentation parameters or making genetic alterations. We therefore conclude that fermentation in 24-well plates is a more reliable screening method for citric acid production by filamentous fungi, and likely for screening in general. Furthermore by screening in a 24 well plate system, it is possible to screen the same amount of samples as in a conventional flask system, using less space and less materials.

Keywords: Optimisation. Fermentation. Aspergillus carbonarius

***49. Unlocking the lignin degrading potential of ascomycete fungi**

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Lignin degradation involves several classes of oxidative enzymes, such as peroxidases and laccases. Traditionally, lignin degradation is considered to be an ability of mainly basidiomycete fungi, in particular white rot fungi. Many oxidative enzymes from these fungi have been characterised and genome analysis of white rot fungi demonstrated the presence of gene families encoding these enzymes. In contrast, ascomycete fungi are generally considered to be incapable of degrading lignin and their genomes lack (most of) the traditional lignin-related oxidases. However, many ascomycete fungi live in biotopes that contain high amounts of lignin (e.g. forest soils, leaf litter), suggesting at least a tolerance to the presence of aromatic compounds.

We have analysed several ascomycete fungi for their ability to grown on medium with lignin as the sole carbon source. Some species displayed good growth even on lignins that were unable to support growth of most other species, suggesting a lignin degrading ability for these fungi. In contrast, other species, such as the industrially relevant fungus *Aspergillus niger*, were unable to grow on these media. Using a novel approach we have isolated mutants from *A. niger* that were able to grow on lignin as the sole carbon source. This growth was similar or possibly even better than growth of the basidiomycete *Phanerochaete chrysosporium*. The genome of *A. niger* contains several laccases as well as genes encoding other oxidative enzymes, which may explain this phenotype. Highlights of these studies will be presented.

50. Regulation of SNAREs in the endovacuolar system of *Aspergillus nidulans*

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Aspergillus nidulans presents many advantages for the study of the traffic within the endocytic pathway. Probably, the main one is the ease to differentiate by fluorescent microscopy highly motile early endosomes (EEs) from late endosomes (LEs), larger and relatively static, and from vacuoles, spherical, static and with an optically visible lumenal space. Previous work in our lab defined Rab5 and Rab7 domains in EEs and

vacuoles, respectively, and also provided evidence of maturation of the former into the latter [1,2,3]. Maturation of EEs into LEs is essential but, once EEs has matured, homotypic fusion of LEs/vacuoles is not vital. In the present work we address the study of endosomal maturation focusing in the regulation of syntaxins, one kind of t-SNAREs, in the endovacuolar system. Pep12 is the sole syntaxin of *A. nidulans* in this context, which lacks a Vam3 homolog. Vps45 and Vps33 are SM proteins that positively regulate syntaxins. Vps45 binds tightly to the late-Golgi/endosomal syntaxin Tlg2 but surprisingly, inactivation of Tlg2 gave rise to no growth alterations while a *vps45* null mutant is markedly affected. These data suggest that Vps45 must be regulating an additional syntaxin and we hypothesize that Pep12 is this second target. In fact, there is evidence of functional [4] and, debatably, physical [5,6] interaction between both proteins in yeast and deletion of *A. nidulans pep12* recapitulates the *vps45*Δ phenotype. We have proved experimentally that rabbit polyclonal antiserum against Pep12 is able to specifically immunoprecipitate Vps33 and Vps45 but not Sly1, the SM of the early-Golgi syntaxin Sed5, while Tlg2 antiserum exclusively and strongly immunoprecipitate Vps45. We believe that Pep12 must be regulated at three different levels by two SM proteins: Vps33, in the EEs CORVET and LEs/vacuole HOPS contexts, and Vps45, in the Golgi-to-endosomes traffic. Additionally, we are identifying all the SNARE complexes formed in the endovacuolar system to establish a physical map of interactions. Even though much more effort is required to understand the source of all interactions our results indicate that Pep12 is regulated by two different SM proteins along the endovacuolar system.

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***51. How does gliotoxin inhibit the growth of *Aspergillus niger*?**

Lara Manzanares*, Rebecca Owens, Grainne O'Keeffe, Gary Jones, Sean Doyle

Aspergillus fumigatus makes an epipolythiodioxopiperazine-type toxin, called gliotoxin (GT), which has deleterious effects in immunocompromised humans. GT contains a characteristic disulphide bridge, which it employs in two damaging activities; (i) cross-linking with proteins via thiol residues and (ii) generation of reactive oxygen species (ROS) through redox cycling. The mechanism of GT cytotoxicity in fungi has not been fully characterised. Previously, identification of genes associated with increased resistance and sensitivity to gliotoxin have been performed in *Saccharomyces cerevisiae* in order to elucidate the molecular mechanisms of GT action in humans, however little has been done to understand GT affects in fungi. Our objectives were (i) to investigate *A. niger* as a model to explore GT sensitivity, as it does not produce gliotoxin and (ii) to improve our understanding of GT cytotoxicity and reveal new metabolic systems interactions in filamentous fungi. Inhibition assays in *A. niger* showed significantly ($p < 0.001$) impaired growth in a dose-dependent manner when exposed to gliotoxin. Exposure of *A. niger* to exogenous gliotoxin (2.5 µg/ml), for 3 h followed by LC-MS enabled comparative proteomic analysis, resulted in the identification of 30 proteins that were differentially regulated ($p < 0.05$) in response to exogenous gliotoxin. Specifically, proteins involved in the methyl/methionine cycle were differentially expressed in *A. niger* in response to gliotoxin. Expression of S-adenosylmethionine (SAM) synthetase was down-regulated (3.1 fold) and homoserine dehydrogenase was up-regulated (1.5 fold). Furthermore, SAM levels were decreased when *A. niger* was exposed to gliotoxin in accordance with the aforementioned proteomic results. Interestingly, we have also found that exogenous gliotoxin (2.5 µg/ml) is taken up by *A. niger* and is converted to bis-methyl gliotoxin (BmGT) over a 3 h period. Our current hypothesis is that *S*-methylation of gliotoxin may result in the production of *S*-adenosyl homocysteine (SAH), and homocysteine, both are toxic towards *A. niger*, which could explain why the organism is sensitive to gliotoxin. Further investigation will be focused on elucidating the mechanism of gliotoxin methylation and determination of cellular homocysteine levels in *A. niger*.

Keywords: SAM, SAH, methylation, proteomics, gliotoxin

***52. Immunoassay for the detection of Fusarinine C produced by Aspergillus spp.**

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Aspergillus fumigatus is an opportunistic pathogen responsible for a number of clinically relevant diseases in immunocompromised individuals, including invasive aspergillosis (IA). IA is the most lethal type of infection with mortality rates ranging from 30 – 90 % depending on the patient primary condition causing the immunosuppression. Conventional diagnosis of IA can be laborious, subject to poor sensitivity or specificity of detection, or unable to differentiate between live versus dead organisms. Detection of siderophores (triacetylfusarinine C or fusarinine C) which may be produced by infectious microorganisms represents an alternative method to diagnose fungal infections. We have developed a fungal-siderophore ELISA to detect fusarinine C (FusC) as a biomarker of IA in animals. FusC was purified from culture supernatants of *A. fumigatus* ATCC46645 by passage through Sep-Pak C_{18} cartridges. Quantification of FusC was measured photometrically using a molar extinction co-efficient of 2996 M^{-1} cm^{-1} at 435nm. FusC-KLH immunogen (6 mg) was generated using a UV crosslinking method. As this cross-linker had not previously been used for immunogen synthesis several molar ratios of hapten to carrier protein and UV exposure times were evaluated in order to maximise conjugation. Western blotting, spectrophotometry and RP-HPLC were utilised to confirm the formation of these conjugates. These techniques were also employed to investigate the hapten densities attached to the carrier protein. Rabbit immunisations using the FusC-KLH immunogen were carried out by commercial arrangement. The resulting polyclonal antisera contained highly specific antibodies against FusC. Purified FusC was then used to develop a fusarinine C-specific competitive ELISA, based on the competition between immobilized and free FusC for IgG [anti-FusC]. Availability of the FusC ELISA facilitated evaluation of analyte presence in normal-state and disease-state specimens of animal origin. FusC concentration in specimens were calculated by comparison to a FusC calibration curve (0 – 50 µg/ml). FusC was detectable in the urine and sera of immunocompromised guinea pigs while no detectable FusC was present in uninfected guinea pigs. In summary we have developed the first fungal-siderophore specific ELISA to detect siderophores as biomarkers of IA in animals.

Keywords: Fusarinine C, ELISA, siderophore, conjugation, diagnostic

53. Hyphal fusion in *Aspergillus oryzae* as evidenced by a detection system for heterokaryon formation

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Hyphal fusion is involved in the formation of an interconnected colony in filamentous fungi. It is the first process in sexual/parasexual reproduction, which is biotechnologically important for crossbreeding. In the industrial filamentous fungus *Aspergillus oryzae*, a parasexual cycle has been reported, and its potential sexuality was suggested. However, as *A. oryzae* possibly enters into hyphal fusion with a much lower frequency than *Neurospora crassa*, it was difficult to detect the hyphal fusion in *A. oryzae*.

In order to evaluate the hyphal fusion ability in *A. oryzae*, we developed a detection system for heterokaryon formation by differentially labeling strains with auxotrophies and fluorescent proteins. With mixed culture, it was demonstrated that AoSO and AoFus3 are required for heterokaryon formation, and the efficiency of heterokaryon appearance was varied by media composition. In paired culture, we detected formation of heterokaryotic sclerotia in the hyphal contact region between two auxotrophic strains. Sclerotia were reported to be capable of acting as repositories for a sexual reproductive structure ascocarp in other Aspergilli, but *A. oryzae* strains have no or much lower ability to form sclerotia. Overexpression of the *sclR* gene, which encodes a transcription factor promoting sclerotial formation, enhanced the formation of heterokaryotic sclerotia. This enhancing effect for heterokaryotic sclerotia was observed independently of the mating-type pairing combinations.

Collectively, these findings would help for understanding of the colonial physiology in industrial processes and for an efficient crossbreeding with sexual/parasexual reproduction in *A. oryzae*.

Keywords *Aspergillus oryzae* · hyphal fusion · heterokaryon· sclerotium · mating type

***54. Signalling process and activation of SltA, a transcription factor involved in cation/alkalinity stress response**

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Many microorganisms, including fungi, have developed genetic strategies to survive to environment stresses, such as variations in pH, temperature, nutrient availability, reactive oxygen or diverse saline concentrations. In the filamentous fungus and model organism *Aspergillus nidulans*, tolerance to an alkaline ambient pH requires the activities of three high hierarchy transcription factors: PacC, CrzA and SltA. We have described the role of SltA, a C_2H_2 zinc-finger transcription factor, in tolerance to alkalinity and to high concentrations of certain mono and divalent cations. Although PacC and CrzA homologues are widely distributed among fungal kingdom, SltA homologues are found only in filamentous fungi.

Here we present our latest results in the signalling process and the activation of SltA, in addition to its transcriptional regulatory activity. Signalling of SltA requires its proteolytic processing, an extreme post-translational modification mechanism that shares with PacC. To understand how SltA is signalised and mediates its regulatory action we have isolated mutations affecting this cation/pH response pathway. A source of new *slt*$^-$ mutations was the isolation of extragenic suppressor mutations of the lethal phenotype caused by certain null *vps* alleles. Several of these mutations mapped in *sltA* and others allowed the identification of a novel member of this pathway. The new locus has been denoted as *sltB*.

sltB gene encodes for a protein of 1272 amino acids, also specific to filamentous fungi, with two putative functional domains. The N-terminal pseudokinase domain is involved in the proteolysis of native SltA 78 kDa to a 32 kDa form. A second domain is similar to a trypsin-like protease, and our data suggest that SltB is auto-proteolysed through this protease activity. Finally, we have determined that SltB is expressed in a SltA dependent manner. A model of regulation of SltA through SltB activity is presented for this novel cation/alkaline pH regulatory pathway in filamentous fungi.

55. *Aspergillus niger* as expression platform for secondary metabolite production

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Filamentous fungi can each produce dozens of different secondary metabolites which are attractive to become exploited as therapeuticals, drugs, antimicrobials, flavour compounds, and other high-value chemicals. Although the genomes of filamentous fungi contain numerous gene clusters that encode for enzymes involved in the biosynthesis of so far uncharacterized secondary metabolites (SM), many of these gene clusters are silent under laboratory growth conditions. Where known, SM production is regulated by complex networks and involves intricate multi-step biosynthetic machineries, as well as major reorganization of primary metabolic fluxes to redirect cellular metabolic resources towards their biosynthesis.
As SM expression varies considerably with the host and enzymes catalyzing SM are often difficult to express, we aim to establish the filamentous fungus *Aspergillus niger* as heterologous host for SM production. We used a multifunctional non-ribosomal peptide synthetase (NRPS), catalyzing the formation of a cyclic peptide and expressed its encoding gene under control of a bacterial-fungal hybrid promoter in *A. niger*. The peptide was isolated and purified from *A. niger* shake flask cultures using EtOAc extraction and liquid chromatography. The purity of the peptide was proven by MS/MS and NMR analyses. The initial yields of 1 mg / litre were increased at least 100 fold using a DOE approach, which addressed feeding conditions and the morphology of *A. niger*. This peptide titre was found to be significantly higher than that reached in bacterial expression hosts, demonstrating that *A. niger* is a promising host for NRPS expression.

***56. The transcriptomic and physiologic consequences of RacA activation and inactivation for polar growth of Aspergillus niger**

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RacA is the main Rho GTPase in *Aspergillus niger* regulating polarity maintenance via controlling actin dynamics. Both deletion and dominant activation of RacA (Rac^{G18V}) provoke an actin localization defect and thereby loss of polarized tip extension, resulting in frequent dichotomous branching in the Δ*racA* strain and an apolar growing phenotype for Rac^{G18V}. In the current study the transcriptomics and physiological consequences of these morphological changes were investigated and compared with the data the morphogenetic network model for the dichotomous branching mutant *ramosa-1*. This integrated approach revealed that polar tip growth is most likely orchestrated by the concerted activities of phospholipid signaling, sphingolipid signaling, TORC2 signaling, calcium signaling and CWI signaling pathways. The transcriptomic signatures and the reconstructed network model for all three morphology mutants (Δ*racA*, Rac^{G18V}, *ramosa-1*) imply that these pathways become integrated to bring about different physiological adaptations including changes in sterol, zinc and amino acid metabolism and changes in ion transport and protein trafficking. Finally, the fate of exocytotic (SncA) and endocytotic (AbpA, SlaB) markers in the dichotomous branching mutant Δ*racA* was followed, demonstrating that hyperbranching does not *per se* result in increased protein secretion.

Key words: *Aspergillus niger*, morphogenesis, polar growth, Rho, Rac, TORC2

***57. Detection of Aspergillosis By GliT-specific IgG ELISA.**

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Aspergillus fumigatus is the predominant causative agent of Aspergillosis and Invasive Aspergillosis (IA), indeed the latter exhibits a notoriously high mortality rate, largely due to poor diagnosis. The critical condition of many IA patients necessitates non-invasive serological diagnostic methods, which require standardisation. To this end, antibody prevalence against GliT (AFUA_6G09740), an oxidoreductase, normally required for gliotoxin production and resistance in *A. fumigatus*, was investigated. Recombinant GliT was expressed at high level in *Escherichia coli* and a robust indirect enzyme-linked immunosorbent assay (ELISA), for the detection of GliT-specific antibodies was developed. Efficacy of this ELISA has been demonstrated for diagnosing Aspergillosis in a canine model system whereby Receiver Operator Characteristic (ROC) analysis confirmed that the assay is highly sensitive (90%) and specific (86%) using 59 sera (n = 10 positive; n = 49 negative) from canines with Sinonasal Aspergillosis. Moreover, analysis of immune-competent human sera has revealed a range of GliT-specific antibody titres, in all samples tested (n = 42). We have also identified GliT-specific IgE in selected human sera and purified the GliT-specific IgG which is immunoreactive with both native and recombinant GliT. Others have suggested GliT-specific IgG detection as an immunodiagnostic for Aspergillosis, but clearly the occurrence of anti-GliT IgG in immune-competent individuals necessitates further investigation of this proposal. To summarise, the high GliT-specific IgG prevalence observed herein indicates that exposure to *A. fumigatus* spores is common and that GliT is highly antigenic, as widespread antibody responses are not evident against other *A. fumigatus* proteins. We suggest that this response is initiated in the upper respiratory tract or alveoli, whereby inhalation and germination of spores may expose individuals to the gliotoxin oxidoreductase, GliT. Ultimately, this research supports GliT-specific IgG as a promising diagnostic marker for *A. fumigatus* infection in humans, however, further assay validation is necessary.

Keywords: *Aspergillus fumigatus,* Aspergillosis, GliT, diagnosis, gliotoxin

58. Functional complementation and ethylene response of plant-fungal fusion histidine kinase in yeast and filamentous fungi.

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The two-component signal transduction system (TCS) has been conserved widely in bacteria and eukaryotes, including plants and fungi. TCS typically consists of two types of common signal transducers: histidine kinase (HK), a response regulator (RR). In plant *Arabidopsis thaliana*, the ethylene receptor AtETR1 acts as HK and their HK activities are regulated by ethylene. AtETR1 contains (i) an ethylene-binding domain (EBD) consisting of three transmembrane helices (TM) in the N-terminal half, (ii) an HK domain (HKD) containing HK, and (iii) the receiver domains of RR in the C-terminal half. As well as AtETR1, fungal HKs also consist of an N-terminal sensor detecting environmental stimuli, an HKD, and a RR in the same order of functional domains in AtETR1. Signal transduction pathways caused by these fungal sensors control cellular responses to extrinsic and intrinsic signals. If these fungal sensor domains are replaced by EBD of AtETR1 and fungal pathways can be controlled by ethylene, the hybrid HKs would be useful as a new gene regulation system in fungal industry. To create a novel system of gene regulation by ethylene, we constructed and examined expression systems of plant-fungal fusion HKs in yeast and filamentous fungi. Here, we report functional complementation and ethylene response of plant-fungal fusion HKs in a temperature-sensitive *sln1* yeast mutant and *Aspergillus nidulans*.

***59. Heterologous Expression and modification of a 30-kb gene cluster in *Aspergillus nidulans* for the production of novel secondary metabolites**

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Fungal secondary metabolism is the source of a large number of structurally diverse natural products that possess a wide variety of biological activities. With the continuous increase in the number of filamentous fungi that have their genome sequenced, it has been become clear that the full potential of fungi as a producer of natural products has yet to be explored. The genes responsible for the synthesis of a given metabolite are most commonly collected in clusters that, in many cases, also encode transcription factors that will specifically induce expression of the genes in the cluster. However, clusters are not always expressed under standard laboratory conditions, thereby preventing metabolite production and pathway characterization.

Characterization of unexplored biosynthetic pathways is also hampered by the lack of genetic tools for many fungi. These problems can be addressed by the heterologous expression of single genes or entire gene clusters in your favorite fungal hosts. Recently, we developed a novel approach for heterologous production of secondary metabolites, involving the two-step transfer of a 25-kb gene cluster from *Aspergillus terreus* to *A. nidulans* enabling synthesis of the polyketide geodin (Nielsen *et al.*, 2013). Inspired by this success, we apply this strategy for heterologous expression of the 30-kb *ccs* gene cluster from *A. clavatus* for characterization of the pathway. Subsequently, we will use this cluster as a platform for engineering in *A. nidulans* with the aim of producing an array of related products with differentiated bioactivities.

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Nielsen, M. T., Nielsen, J. B., Anyaogu, D. C., Holm, D. K., Nielsen, K. F., Larsen, T. O., & Mortensen, U. H. (2013). Heterologous reconstitution of the intact geodin gene cluster in Aspergillus nidulans through a simple and versatile PCR based approach. *PloS one*, *8*(8), e72871. doi:10.1371/journal.pone.0072871

60. Aegerolysins and proteins with MACPF domain in Filamentous Fungus *Aspergillus niger

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Aegerolysins and MACPF domain containing proteins (Pfam06355 and 01823 protein families, respectively) comprise of more than 350 and 500 proteins found in various kingdoms of life. While biological roles of MACPF domain containing proteins are well-known (plant and animal defence mechanism, virulent factors, development...), biological roles of aegerolysins remain to be elucidated. So far it has been shown that they play an important role during sporulation of bacteria and development of primordia and fruiting bodies of fungi from Basidiomycota phylum. They were also suggested to act as virulence factors in filamentous fungi. Various members of both protein families display hemolytic activity and have been shown to form pores in biological and artificial lipid membranes, either sole or in combination with one another (aegerolysin - MACPF domain containing protein). While organisms containing only aegerolysins or only proteins with MACPF domain are abundant, those containing members of both protein families are scarce.

Aspergillus niger is a saprophytic, filamentous fungus found throughout the world. When screening various strains of genus *Aspergillus* for hemolytic activity, we found that two *Aspergillus niger* strains displayed hemolytic activity on sheep blood agar plates and their mycelial ethanolic extracts lysed bovine erythrocytes in suspension. This fungus is also one of a few organisms whose genome contains both aegerolysin and MACPF domain containing homologues. In order to determine the biological role of these proteins in filamentous fungi and to find the possible link between hemolytical activity and the presence of these proteins we are now using various approaches, such as QPCR for monitoring the expression of the target proteins in different time points and different conditions of growth, deletion mutants for target proteins and their combinations for morphological and transcriptome studies. We are also trying to obtain recombinant proteins for studies of hemolytical activity, membrane interactions and production of antibodies that will be used further for immunolocalization studies.

Key words: aegerolysins, MACPF domain containing proteins, biological role, QPCR, recombinant proteins.

61. RNA-Seq Analysis of the Effects of Gliotoxin on *A. fumigatus* wild-type and *A. fumigatus* Δ*gliT* Reveal a Dysregulation of Cysteine and Methionine Metabolism.

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Aspergillus fumigatus produces a number of secondary metabolites, one of which, gliotoxin, has been shown to exhibit anti-fungal activity. Thus, *A. fumigatus* must be able to protect itself against gliotoxin. Indeed one of the genes in the gliotoxin biosynthetic gene cluster in *A. fumigatus*, *gliT*, is required for self-protection against the toxin- however the global self-protection mechanism deployed is unclear. RNA-Seq was employed to identify genes differentially regulated upon exposure to gliotoxin in *A. fumigatus* wild-type and *A. fumigatus* Δ*gliT* (hypersensitive to gliotoxin). A total of 164 genes were differentially regulated ($\log_2$ fold change of 1.5) in *A. fumigatus* wild-type when exposed to gliotoxin, consisting of 101 genes with up-regulated expression and 63 genes with down-regulated expression. Interestingly, a much larger number of genes, 1700, were found to be differentially regulated ($\log_2$ fold change of 1.5) in *A. fumigatus* Δ*gliT* when challenged with gliotoxin. These consisted of 508 genes with up-regulated expression, and 1700 genes with down-regulated expression. In both strains primary metabolic functions, including amino acid metabolism and carbohydrate metabolism were affected. Both strains exhibited differential regulation of genes involved in secondary metabolism, in particular gliotoxin biosynthesis and helvolic acid biosynthesis, protein binding functions, cellular transport, and expectedly, cell rescue and defence. Interestingly, genes involved in cysteine and methionine metabolism along with sulphur metabolism were differentially expressed in *A. fumigatus* Δ*gliT* upon addition of exogenous gliotoxin. Quantitative determination of S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) levels revealed a significant depletion of SAM ($p < 0.05$) in *A. fumigatus* Δ*gliT* upon exposure to gliotoxin and a significant increase ($p < 0.05$) in SAH levels, not observed in *A. fumigatus* wild-type. Comparative proteomics

identified the presence of two isoforms of S-adenosylhomocysteine hydrolase differentially expressed in *A. fumigatus* Δ*gliT* following exogenous gliotoxin addition. One isoform is increased in intensity while the other decreases compared to the control, indicating a possible switch in activity due to post-translational modification. In the absence of GliT, addition of exogenous gliotoxin results in a dysregulation of cysteine and methionine metabolism, as evidenced particularly by the increased SAH levels in *A. fumigatus* Δ*gliT*, consequently resulting in a hypersensitive phenotype to gliotoxin.

62. **IDENTIFICATION AND CHARACTERIZATION OF THE *Aspergillus nidulans* $Zn(II)_2Cys_6$ TRANSCRIPTION FACTOR *rhaR* THAT MEDIATES L-RHAMNOSE UTILIZATION AND THE PRODUCTION OF □-L-RHAMNOSIDASES**

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L-Rhamnose is a naturally occurring deoxyhexose sugar that can be assimilated by numerous yeasts and filamentous fungi when preferred carbon sources such as glucose are limited or absent. L-Rhamnose is widely distributed in plants where it is commonly found glycosydically bound to other sugars and organic moieties including the primary cell wall pectic polysaccharides rhamnogalacturonan I and II, hemicellulose, glycoproteins and diverse secondary metabolites, some of the latter being important bioactive compounds. L-rhamnose released from the degradation of these plant materials induces the production of diverse enzymes appropriate for the continued depolymerisation/modification and utilization of these substrates.

L-rhamnose and rhamnosides are promising candidates for use in the fields of food, cosmetics, agriculture and health. Thus, □-L-rhamnosidases - catalyse the hydrolysis of terminal non-reducing L-rhamnose residues in oligosaccharides and □-L-rhamnosides - find a variety of uses in industry that include the reduction of citrus juice bitterness, improvement of the release of aromas in musts and wines, increase the bioavailability of food ingredients, drug development, etc. In addition, these enzymes also are involved in the detoxification of plant secondary metabolites and hence they could play a role in evading plant defences against fungal attacks.

In this study we have indentified in *Aspergillus nidulans* and *Neurospora crassa* the *rhaR* gene that encodes a putative $Zn(II)_2Cys_6$ DNA-binding protein. Genetic evidences indicate that the product of *rhaR* acts in a positive manner to induce transcription of the *A. nidulans* L-rhamnose regulon. *rhaR*-deleted mutants showed a reduced ability to induce the expression of the □-L-rhamnosidase genes *rhaA* and *rhaE* and concomitant reduction in □-L-rhamnosidase production. The *rhaR* deletion phenotype also results in a significant reduction in growth on L-rhamnose that correlates with reduced expression of the L-rhamnonate dehydratase gene *lraC*, evidencing that RhaR also controls the expression of the L-rhamnose catabolic pathway. Expression of *rhaR* alone is not sufficient for induction since its mRNA accumulates even in the absence of L-rhamnose, therefore the presence of both functional RhaR and L-rhamnose are absolutely required. In *N. crassa*, deletion of *rhaR* also impairs growth on L-rhamnose.

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63. Cisternal maturation within the *Aspergillus nidulans* Golgi visualized *in vivo*

Areti Pantazopoulou, Mario Pinar, Miguel Hernández-González, Herb N Arst and Miguel A. Peñalva

115 years after Camillo Golgi´s description of the homonymous organelle, the mechanism by which proteins and lipids traffic in the secretory pathway, undergoing ordered modifications before being distributed to their target organelles, remains elusive and debated.

Over the last few years, we have established that Golgi cisternae of the filamentous fungus *Aspergillus nidulans* are not stacked and are thus optically resolvable, as opposed to the mammalian Golgi, which is organized in stacks of sub-resolution cisternal distance. In hyphal cells, Golgi cisternae display polarized distribution towards the growing apex; however, the late Golgi is absent from a ≈3μm region immediately below the apex, where secretion predominates. This region is populated by both microtubules and the actin mesh emerging from the Spitzenkörper, where secretory membranes accumulate, awaiting fusion with the plasma membrane.

According to the cisternal maturation model for cargo transport, acute impairment of traffic in the ER-Golgi interface would lead to rapid disorganization of both the early and the late Golgi cisternae, while the vesicular transport model anticipates that stable Golgi cisternae would not be affected under these conditions, at least not promptly. We have constructed appropriate conditional mutants and, using *in vivo* fluorescence microscopy, we observed that a reversible block in the ER-Golgi traffic results in the reversible disorganization of both the early and late Golgi cisternae within minutes, as predicted by the cisternal maturation model. Indeed, we have found that Golgi cisternae in growing hyphae are transient entities. By employing multidimensional microscopy, we are able to directly observe cisternal maturation; that is the *de novo* formation of an early Golgi compartment and its subsequent enrichment in a late Golgi marker with concomitant loss of the early Golgi marker, until the formation of a late Golgi compartment. In turn, the late Golgi eventually diminishes.

***64. Strategic screening of filamentous fungi for their production of lignocellulose degrading enzymes**

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Filamentous fungi possess a high unexplored potential for production of lignocellulose degrading enzymes. There are a few fungi, such as *Trichoderma reesei* and *Aspergillus niger*, which are widely used in industry. But there is a huge interest in discovery of novel and more efficient enzymes, and therefore other fungi should be evaluated. The goal of this study was to strategically screen selected filamentous fungi, which are potentially good producers of lignocellulose degrading enzymes, and to link their genomic information to the experimental data.

We picked 9 fungi for which genomic data is available and screened for their growth abilities on cellulose and lignocellulose (steam exploded spruce). The five best performing fungi were chosen for further cultivations for evaluation of their enzyme profiles and enzymatic hydrolysis efficiency. SDS-PAGE showed large differences between the secretomes of the different fungi. For enzyme screening different enzyme assays were designed based on the CAZymes in the genomes of these fungi and the characteristics of the growth substrates. Enzymes produced by the fungi were evaluated for their hydrolytic efficiency on the different substrates. All in all, our experimental approach provided insight into the mechanisms of enzymatic hydrolysis by different filamentous fungi.

65. Autophagy in *Aspergillus nidulans*. The ER as a possible source of membranes for autophagy.

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The genetic model *Aspergillus nidulans*, whose multinucleated hyphal cells are notably larger than those of *Saccharomyces cerevisiae*, is ideally suited for in vivo microscopy and intracellular trafficking studies. Therefore, we have exploited these advantages to investigate autophagy.
A protein playing a central role in autophagy in yeast is the ubiquitin-like Atg8, which localizes to the phagophore assembly site (PAS). This location depends on the conjugation of phosphatidylethanolamine (PE) to this molecule, mediated by a set of proteins, including Atg4 cysteine protease and E1-like Atg7. This protein modification is necessary to anchor Atg8 to membranes and plays a key role in autophagosome biogenesis. In *A. nidulans* the localization of Atg8 to the PAS is independent of PE conjugation to the protein, since it is located in this structure in the absence of Atg4 and Atg7.

Under nitrogen starvation conditions, GFP-Atg8 containing pre-autophagosomal puncta give rise to cup-shaped phagophores and circular (0.9-μm diameter) autophagosomes that disappear in the proximity of the vacuoles after their shape becomes irregular and their GFP-Atg8 fluorescence decays.

Autophagy does not require endosomal maturation or ESCRTs, as autophagosomes fuse with the vacuole in a $RabS^{RAB7}$ / HOPS dependent manner. Also, does not require Golgi or post-Golgi traffic since mutations affecting known Golgi resident proteins, or mutations in proteins involved in the post-Golgi trafficking to the

plasma membrane or endosomes do not affect the formation of autophagosomes and their fusion with the vacuole.
By using a *ts* mutation in *rabO*, we have seen that autophagy it is dependent on this Rab protein. $RabO^{RAB1}$ localizes to phagophores and autophagosomes. Additionally TRAPPIII-specific factor Trs85 localizes to the PAS.

The critical role of $RabO^{Rab1}$ in autophagy, combined with the fact that the traffic through the Golgi is not required for this process, suggest that the ER could be a potential source of autophagic membranes. In fact we have detected the presence of omegasome-like structures, similar to those described in mammalian cells, associated with fungal autophagosomes.
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66. Increasing the stability of mitochondria in *Aspergillus nidulans*
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Programmed cell death appears to be a unique feature of all living organisms, including fungi. Physiological changes of mitochondria, *e.g.* elevation of Reactive Oxygen Species (ROS) concentrations plays a pivotal role in the initiation of apoptosis (Osiewacz and Scheckhuber, 2006). This work aims at the stabilization of the integrity and function of the mitochondria of *Aspergillus nidulans* by overexpressing the mitochondrial proteins manganese superoxide dismutase (MnSOD, AN5577), alternative oxidase (AoxA, AN2099), LON-like protease (Pim1, AN6193); and by deleting the gene encoding the dynamin-related protein DnmA (AN8874). Both the deletion and overexpression mutants of the selected genes have been constructed employing DJ-PCR (Yu *et al*; 2004). The deletion of *mnSOD* resulted in a remarkably high sensitivity to menadione in the agar plate assay. The Δ*dnmA* mutant showed reduced growth even without stress treatment in comparison to the control strain. The deletion of *pim1* caused decreased tolerance, whereas overexpression of it elevated the tolerance to $CdCl_2$. Morphology of the mutant mitochondria were studied by Mitotracker Green staining using a laser scanning microscope, but no clear morphological differences were observable. In terms of respiration, manipulation of the alternative oxidase production resulted in changes in the cyanide-resistant phenotype. The cyanide resistant respiration was decreased by the deletion of *aoxA*, but increased by the overexpression of *aoxA*. Ongoing research in our laboratories aims at the comparison of the apoptosis rates of the gene deletion and overexpression mutants. It is our anticipation that data coming from these experiments will shed light on the importance of mitochondrial functions in the protection of *A. nidulans* hyphae against ROS and, consequently, cell death.

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***67. Conservation and diversity of sugar-related catabolic pathways in fungi**

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Plant polysaccharides are among the major substrates for many fungi. After extracellular degradation, the monomeric components (mainly monosaccharides) are taken up by the cell and used as carbon sources to enable the fungus to grow. This would also imply that the range of catabolic pathways of a fungus may be correlated to the composition of the polysaccharides it can degrade.

In this study we have tested that hypothesis by analyzing the presence, absence and redundancy of genes of a number of catabolic pathways in selected fungi from the Ascomycota, the Basidiomycota and the Zygomycota. This involved first the identification of the catabolic pathway genes which was performed by automated ortholog and paralog searches. The expression of the genes was evaluated for those species for which transcriptome data was available.

The results were then compared to growth profiling data of the species on a set of plant-related poly- and monosaccharides to determine to which extent the genome content fits the physiological ability of the species.

*68. **Genetic modification of carbon catabolite repression levels in *Aspergillus niger* for improved hemicellulase production**

Diogo Robl*, Robson Tramontina, Isabelle Benoit, José Geraldo da Cruz Pradella, Gabriel Padilla, Ronald P. de Vries

Aspergillus niger is known worldwide for its ability to produce an extensive range of extracellular glycohydrolases, including xylanases, pectinases, and β-glucosidase. Cultivation and media optimization is one way to improve enzyme production in microorganisms. However, bioprocess approaches have limitations since fungal metabolism involves a complex network control. In *A. niger* the synthesis of hemicellulolytic enzymes is controlled at the transcriptional level mainly by a carbon catabolite repressor protein (CreA) and the activator XlnR, which directs the expression of the hemicellulolytic genes. In this way, this study aimed to improve hemicellulases production by genetic modifications of CreA an XlnR genes in the carbon catabolic repression (CCR) of *A. niger*. Submerged fermentations (SmF) were performed in agro waste residues and pure substrates and several cellulolytic and hemicellulolytic enzymes were measured. The kinetics studies revealed that the genetic tools associated with biochemical engineering increased enzyme production. As so far, these modifications could be the key to develop of an economic process in hemicellulase production applied to biomass saccharification.

***69. The *Aspergillus niger* ChrA protein confers chromate sensitivity participating as a chromate transporter**

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The widespread use of chromium in industrial processes has become a serious pollution problem, which has caused contamination of air, soil and water. Hexavalent Cr, in the form of chromate (CrO_4^{2-}) or dichromate ($Cr_2O_7^{2-}$) oxyanions is considered the most toxic form of chromium, which actively enters biological membranes by means of sulfate uptake pathways in a variety of cells, reflecting the chemical analogy between these two oxyanions. The ChrA membrane protein belongs to the CHR superfamily of chromate ion transporters, which includes homologues from bacteria, archaea and eukaryotes. Bacterial ChrA homologues confer chromate resistance by exporting chromate ions from the cell's cytoplasm. It has been shown that in the genome of filamentous fungi, but not in yeast genomes, there are homologous genes that encode ChrA proteins.

In this work we demonstrated the absence of the *chrA* gene in the chromate-tolerant environmental strain Ed8 of *Aspergillus tubingensis*, isolated from a Cr(VI)-contaminated site; reference strains FGSC A732 of *A. niger*

and NRRL593 of *A. tubingensis* showed the presence of the *chrA* gene and it was observed they show increased expression of the gene in the presence of Cr(VI). With the purpose to determine the function of the ChrA protein in *A. niger*, the *chrA* gene from the reference strain FGSC A732 of *A. niger* was cloned in an expression vector and the construct was introduced into the genome of strain Ed8 of *A. tubingensis*. Transformant LIVG6, expressing the *chrA* gene, was further characterized; LIVG6 showed decreased chromate tolerance, exhibiting an oxyanion sensitivity similar to that observed in the reference strains of *A. niger* and *A. tubingensis*. In addition, Cr uptake assays in cultures incubated with Cr(VI) revealed that chromium incorporation in the biomass was higher in transformant strain LIVG6 and in the *A. niger* and *A. tubingensis* reference strains, as that observed in strain Ed8 of *A. tubingensis*. These results indicate that the ChrA protein from *Aspergillus* strains functions as a cromate transporter, incorporating chromium into the cells.

Key words: *Aspergillus niger*; chromate transport; ChrA protein.

***70. Siderophore utilization determines fungal competitiveness**

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Nature abounds with a rich variety of altruistic and competitive strategies. The most common form of microbial cooperation is the secretion of enzymes and metabolites to the environment, e.g. saprobic decomposition. This benefits not just to the secretor, but anyone in close proximity. Another well-studied altruistic trait is iron mobilization through secreted low molecular-mass iron chelators, termed siderophores. On the other hand however, here we demonstrate that siderophores and their utilization, respectively, determine fungal competiveness. We have previously shown that the mold *Aspergillus fumigatus* secretes the siderophores fusarinine C (FSC) and triacetylfusarinine C (TAFC).

In this study, we found that the growth of *Aspergillus terreus,* secreting the siderophores coprogen and ferrichrysin, is inhibited by desferri-TAFC induced iron deprivation, indicating an inability to utilize TAFC-chelated iron. Subsequent phylogenetic analysis indicated the lack of two putative siderophore transporters in *A. terreus* compared to *A. fumigatus,* termed *Afu*-MirB and *Afu*-MirD. Heterologous expression of the gene encoding *Afu*-MirB, but not *Afu*-MirD, rendered *A. terreus* resistant to desferri-TAFC, revealing its substrate specificity. In cocultivation with *A. fumigatus, Afu*-MirB-mediated TAFC uptake increased the fitness of *A. terreus* as indicated by significantly increased production of conidial offspring.

These data underline that, with respect to competiveness, the optimal strategy is to produce siderophores unusable by competitors, while at the same time accepting xenosiderophores. In line, most fungal species encode multiple siderophore transporters (including *A. fumigatus* and *A. terreus*), even siderophore non-producers such as *Saccharomyces cerevisiae* and *Cryptococcus neoformans*.

71. Secretion pathway engineering of *Aspergillus nidulans

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Recombinant protein production is a multi-billion dollar market and most biopharmaceuticals today are produced from a recombinant host. Therefore, there is a huge demand for optimizing productivity and yields in producing bioactive compounds such as enzymes, antibodies and other biopharmaceuticals from recombinant hosts. To facilitate downstream processing, products are preferably secreted out of the cell. The *Aspergillus* spp. are well known for their ability to secrete proteins at high levels and are therefore commonly applied as cell factories for heterologous protein production.

Research in filamentous fungi is moving in to a systems biology based mode, where more genome sequences are becoming available and transcriptome analyses are more frequently pursued in these hosts. Together with better tools for genetic engineering, this enables exciting new studies that previously have been predestined to

be performed in “easier” model organisms such as *S. cerevisiae.* For example, enzyme secretion is now being extensively studied in *Aspergillus* spp.*,* with detailed characterization of how protein secretion is affecting the host cell. Studies have mainly focussed on assigning function to genes involved in the secretory pathway by investigating deletion mutants. Overexpressing genes involved in the secretion pathway of filamentous fungi has not yet been extensively tested. Thus, metabolic engineering of the secretory pathway in filamentous fungi is a new and interesting way of engineering protein secretion in these organisms.

The aim of this project is to investigate the protein secretion machinery in *Aspergillus spp.,* with *Aspergillus nidulans* as a model organism, through manipulation of key genes in order to increase the capacity of the secretory pathway. Overexpression of secretory transport genes has been performed in order to increase the transport of three recombinant model proteins: Beta-glucosidase and cellobiohydrolase from *Trichoderma reesei*, and red fluorescent protein mRFP1. Targets were based on a number of transcriptomic studies performed in fungal hosts under secretion stress. The mutants were analysed with respect to recombinant protein secretion, total amount of protein secreted, biomass generation and phenotype on plates. This will give new insights into how *Aspergillus* can be further improved for production of recombinant proteins.

Keywords: Secretion, metabolic engineering, recombinant proteins, Aspergillus

72. On the Biosynthesis and Function of the Antioxidant Ergothioneine in *Aspergillus fumigatus.
Authors: Kevin Sheridan*, Grainne O’Keeffe, Aoife McHugh, Rebecca Owens, Beatrix Lechner, Hubertus Haas, David Fitzpatrick, Gary Jones, Sean Doyle

Ergothionine (EGT; 2-mercaptohistidine trimethylbetaine) is a trimethylated and sulphurised histidine derivative which exhibits antioxidant properties due to its ability to exist in both thiol and thione forms. EGT production has been recently demonstrated in *Aspergillus fumigatus* and its biosynthesis was also found to be significantly elevated in a mutant (Δ*gliK*), deficient in gliotoxin biosynthesis. It was therefore hypothesised that the production of these two molecules may be related. Indeed, our analysis has revealed that EGT production in *A. fumigatus* is inversely proportional to that of gliotoxin in *A. fumigatus* strains ATCC26933 and ATCC46645, where high gliotoxin production coincides with low ergothionine levels, and vice versa. Label-free quantitative proteomic analysis has further revealed a $\log_2$ 2.25-fold increase in abundance of a putative EGT biosynthetic enzyme (11 % sequence coverage) in *A. fumigatus* Δ*gliK*, compared to ATCC46645. To further investigate EGT biosynthesis, the predicted ergothionine biosynthesis gene, *egt1*, (2.9 kb; containing 6 introns) was identified by bioinformatic analysis, and deleted from *A. fumigatus* ATCC26933 via a split marker strategy. Deletion was confirmed by Southern analysis and absence of expression demonstrated via RT-PCR. Pre-column derivatisation of EGT using 5’iodoacetamidofluorescein (5-IAF) and subsequent RP-HPLC, plus high resolution LC-MS analysis confirmed that *A. fumigatus* Δ*egt1*, was unable to produce ergothionine, confirming its role in ergothionine biosynthesis. *A. fumigatus* Δ*egt1* could still synthesise gliotoxin, however the level of gliotoxin production was found to be significantly reduced ($p > 0.001$), suggesting that high level gliotoxin production is dependent on EGT presence. *A. fumigatus* Δ*egt1* was also found to be sensitive to 3 mM H_2O_2 compared to wild type ($p > 0.001$), however no sensitivity was observed at lower H_2O_2 concentrations. This suggests that EGT may act as an auxiliary antioxidant, the presence of which is necessary to adapt to growth at higher levels of oxidative stress. As EGT appears to be involved in protecting against oxidative damage, we speculate if gliotoxin production is dependent on cellular redox homeostasis, whereby absence of EGT could result in sub-optimal redox conditions for gliotoxin production and secretion.

***73. New Insights into Gliotoxin Secretion from *Aspergillus fumigatus*.**
Elizabeth Smith*, Stephen Hammel, Sean Doyle, Grainne O’Keeffe, Gary Jones.

Aspergillus fumigatus is an opportunistic pathogen which produces gliotoxin, an epipolythiodioxopiperazine (ETP) which is characterised by an intramolecular disulphide bridge. The *gli* gene cluster is comprised of thirteen genes which are involved in aspects of gliotoxin biosynthesis and self-protection. The cluster includes *gliA* (CADRE locus identifier: AFUA_ 6G09710), a gene which is predicted to encode a transmembrane gliotoxin efflux pump, which is a member of the Major Facilitator Superfamily (MFS). The role of *gliA* in the biosynthesis and secretion of gliotoxin by *A. fumigatus* is unknown, however a previous study in which the *sirA* gene, an ortholog of *gliA,* from *Leptosphaeria maculans* was deleted, resulted in increased sensitivity to both gliotoxin and sirodesmin, and also an increase the secretion of sirodesmin from *L. maculans*. Deletion of *gliA*

was undertaken in *A. fumigatus* ATCC26933, previously shown to produce gliotoxin at high levels, using a split marker strategy and acquisition of pyrithiamine resistance. It was also observed that *A. fumigatus* ATCC26933 secretes bis-methyl gliotoxin (BmGT), a derivative of gliotoxin where both thiols are methylated. Both Southern and qRT-PCR analysis confirmed deletion of *gliA* and absence of *gliA* expression in *A. fumigatus* $\Delta gliA^{26933}$, respectively. Deletion of *gliA* completely abolished gliotoxin secretion, as determined by both RP-HPLC and LC-MS analysis, compared to that from *A. fumigatus* ATCC26933. Interestingly, secretion of the gliotoxin derivative, BmGT was not inhibited, indeed, there was a significant increase in the levels of BmGT secreted by *A. fumigatus* $\Delta gliA^{26933}$ compared to wild type between 48-96 h growth ($p < 0.001$). *A. fumigatus* $\Delta gliA^{26933}$ also exhibited significant sensitivity to 5 µg/ml exogenous gliotoxin at both 24 ($p < 0.001$) and 48 h ($p < 0.0001$) growth. However, determination of the gliotoxin uptake rate revealed no significant difference between that of ATCC26933 and Δ*gliA* ($p = 0.3$). These results strongly suggest a role for *gliA* in the secretion of endogenously produced gliotoxin, but not bis-methyl gliotoxin, and that *gliA* functionality is necessary to protect against exogenous gliotoxin. Finally, we speculate that gliotoxin may undergo bis-methylation, if the secretion pathway is inhibited, but that this process does not facilitate self-protection.

74. Regulation of carbon catabolite derepression through dephosphorylation of arrestin-like protein CreD in *Aspergillus oryzae*

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Aspergillus oryzae has an ability to produce copious amounts of amylolytic enzymes, although transcriptional induction of these enzymes is repressed in the presence of glucose. The carbon catabolite repression (CCR) in filamentous fungi is regulated by the transcription factor CreA. In *Aspergillus nidulans*, CreB, CreC and CreD were also identified as the CCR regulating factors. CreD belongs to a family of arrestin-like proteins and is presumably involved in recruiting a ubiquitin ligase to a target protein for ubiquitination. Although deletion of ubiquitin processing protease CreB resulted in relief of CCR, double deletion of *creB* and *creD* suppresses the *creB* mutant phenotype, suggesting that CreD is required for carbon catabolite derepresson. In this study, we generated the 3FLAG-fused CreD expression strain of *A. oryzae,* and post-translational modification of CreD protein and its involvement in CCR relief were examined.

The CreD-3FLAG was detected as several bands by Western blot analysis, and higher molecular weight bands disappeared by alkaline phosphatase treatment. It has been reported that Rod1, the yeast homolog of CreD, was phosphorylated by Snf1 kinase. Since the two putative target sequences for Snf1 existed within CreD amino acid sequences, these two serine residues were substituted by alanine (CreD-AA). The CreD-AA mutant protein was detected as a sharp band with lower molecular weight, suggesting that CreD is phosphorylated at these two serine residues. To investigate the effect of phosphorylation/dephosphorylation of CreD on carbon catabolite derepression, in addition to CreD-AA mutant, CreD-EE mutant with substitution of these two serine residues for glutamic acids was expressed in the *creB* deletion mutant. On the starch medium supplemented with glucose, formation of clear zones in the *creB* deletion mutant was strongly inhibited by CreD-EE mutation but not by CreD-AA mutation. This result suggested that dephosphorylation of CreD is required for relief of CCR. To examine the interaction of CreD with HECT ubiquitin ligase HulA, co-immunoprecipitation analysis was performed using 3HA-HulA and CreD-3FLAG co-expression strains. Interestingly, both of phosphorylated and dephosphorylated forms of CreD were interacted with HulA, and CreD-EE mutant protein was also interacted with HulA. These results suggested that dephosphorylation of CreD has no effect on interaction with HulA.

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75. Functional analysis of ClbR and ClbR2 controlling the cellulosic biomass degrading enzyme gene expression in *Aspergillus aculeatus*

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Aspergillus aculeatus possesses at least two signaling pathways controlling gene expression in response to cellulosic biomass. The cellobiose- and cellulose-responsive induction of the FIII-avicelase (*cbhI*), FII-

carboxymethyl cellulase (*cmc2*), hydrocellulase (*cel7b*), and FIa-xylanase (*xynIa*) genes are under control of XlnR-independent signaling pathway, while the cellulose-, D-xylose-, and arabinose-responsive induction of the FI-carboxymethyl cellulase (*cmc1*) and FIb-xylanase (*xynIb*) genes are under control of XlnR-dependent-signaling pathway. By screening the T-DNA insertion mutant library in *A. aculeatus*, we have identified a cellobiose response regulator (ClbR), a DNA-binding protein possessing the $Zn(II)_2Cys_6$ binuclear cluster domain. Although ClbR participates in the cellulose-inductive expression regardless of XlnR dependency, the effect of the *clbR* overexpression was limited. It increased the amount of the *xynIa* transcripts, while it decreased the *cel7b* transcripts and had no effect on the others. On the presumption that the multiple effects of the *clbR* overexpression were due to transcription factor titration, we screened ClbR-interactor(s) by Yeast-two hybrid system. ClbR paralog, namely ClbR2 showing 42% identity with ClbR, was isolated. Genetic analysis of *clbR* and *clbR2* revealed that both factors control the expression of *cbhI*, *cmc2*, and *manR*, a transcription factor controlling the expression of not only mannanolytic enzyme genes but also cellulolytic enzyme genes in *A. oryzae*. Although we confirmed that ManR also controlled the cellulose-induced expression of *cbhI* and *cmc2* in *A. aculeatus*, neither ManR nor ClbR2 regulated the *xynIa* expression. We next investigated the binding properties of recombinant MalE-$ClbR_{1-250}$ and ManE-$ClbR2_{1-295}$ proteins to the *xynIa* promoter by electrophoresis mobility shift assay (EMSA). EMSA demonstrated that MalE-$ClbR_{1-250}$ and MalE-$ClbR2_{1-295}$ independently bound to the *xynIa* promoter, while mixture of both proteins did not yield an additional shift band, suggesting that MalE-$ClbR_{1-250}$ and MalE-$ClbR2_{1-295}$ competitively bind to the same region. Now, we are analyzing the binding properties of both recombinant proteins to the *manR* promoter.

76. Studying the role of the C-II subgroup killer toxin-like chitinases in the filamentous ascomycete *Aspergillus nidulans

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Chitinases are hydrolytic enzymes responsible for chitin polymer degradation. Fungal chitinases are involved in different aspects of fungal biology for instance, in hyphal branching and sporulation, in autolysis, in exogenous chitin degradation for nutrient acquisition, and during fungal-fungal interactions. Genome analyses reveal that many soil-borne, highly competitive ascomycetes, such as *Trichoderma* spp., *Fusarium* spp. and *Aspergillus* spp., contain large arrays of chitinase genes. Fungal chitinases belong to glycoside hydrolases family 18 and they are categorized into three phylogenetic groups (A, B and C), which are further divided into subgroups (A-II to A-V, B-I to B-V and C-I to C-II). Subgroup C chitinases display similarity with the α/β-subunit of the zymocin yeast killer toxin produced by Kluyveromyces lactis. The role of these subunits is to facilitate the permeability of the γ-subunit, which is the active toxin, into antagonist cells. Induction of these C-II chitinase genes during interspecific interactions has been reported in mycoparasites such as *T. atroviride* and *T. virens*, and in saprophytes such as *Neurospora crassa*, suggesting a role of these enzymes in interspecific interactions. In this study, we investigated the regulation and function of 4 *Aspergillus nidulans* subgroup C-II killer toxin-like chitinases (chiC2-1 to chiC2-4), by quantitative PCR and by constructing gene deletion strains. Our results showed that all 4 genes were highly induced during interactions with *Botrytis cinerea* and *Rhizoctonia solani*, compared to self-interactions. In addition, *chiC2-2* and *chiC2-3* were also induced during contact with *Fusarium sporotrichoides*, while none of these genes were induced during interactions with the oomycete *Phytophthora niederhauserii*. Phenotypic analysis of chitinase gene deletion strains revealed that *B. cinerea* biomass was significant higher in culture filtrate derived from the ΔchiC2-2 strain compared to biomasses grown in media derived from A. nidulans wild type or the other chitinase gene deletion strains. The analysis also showed that all chitinase gene deletion strains displayed increased biomass production in liquid cultures. In addition, deletion of C-II chitinases altered the abiotic stress tolerance of *A. nidulans* mycelia. In summary, our gene expression data suggest the involvement of *A. nidulans* subgroup C-II chitinases in fungal-fungal interactions, which is further proven for ChiC2-2. Moreover, these chitinases have showed a possible involvement in fungal growth, suggesting a potential multiple and variable role of killer toxin-like chitinases in *A. nidulans* life cycle.

***77. The filamentous fungi *Aspergillus niger* has high industrial value and underexploited, metabolic potential**

Daniel Upton*, Simon McQueen-Mason, Jamie Wood

It has been used in industry since the 1930s, and finds importance in both the bulk production of citric acid and specialist production of enzymes. Citric acid is a valuable chemical, with multiple uses. Its current global production of 7 million tonnes per year, and rapidly rising global demand, adds necessity to developing *A. niger* strains capable of increased citric acid output when grown on cheap feedstocks from biorefinery waste-streams, such as C5 sugars and glycerol, rather than the expensive sugar feedstocks currently used. This project aims to achieve this using a Systems biology approach. A genome-scale metabolic model, iMA871, has been recently developed. The model uses the mathematical method of Flux balance analysis to predict growth rate or output of different metabolites under steady-state and given constraints. The initial aim of the project is to use experimental data from fermentation with different substrates to verify, refine and optimise the iMA871 metabolic model, so it accurately represents the citric acid producing ATCC 1015 strain. The project intends to use the optimised metabolic models to predict mutations *in silico* that give rise to increased production of value-added chemicals, such as citric acid, when *A. niger* is grown on waste-streams. TILLING will then be used to select for these mutations, and the effectiveness of resulting strains tested in bioreactors.

78. The role of carbon starvation in induction of hydrolytic enzymes during exposure of *Aspergillus niger* to wheat straw

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The saccharification of lignocellulosic biomass for the production of second generation biofuels requires cheaper and more effective enzyme mixtures. Understanding the mechanism behind the efficient degradative response of saprophytic fungi generates clues for the improvement of fungal enzyme mixtures. We recently identified the hydrolytic enzymes and accessory proteins produced by the industrially important fungus *Aspergillus niger* in response to wheat straw. Importantly, induction of transcripts encoding hydrolytic enzymes was sequential (Delmas et al., 2012). Carbon catabolite derepression was responsible for the initial induction of three genes that were induced early on straw. We presented a model of how fungi respond to lignocellulose, whereby starvation induced enzymes may produce a molecule that induces expression of the full suite of hydrolytic enzymes required for deconstruction of a particular substrate.
To further elucidate this mechanism, we now investigated the early events in the response of *A. niger* to straw and carbon starvation using genome-wide RNA sequencing. The influence of carbon starvation during lignocellulose degradation was demonstrated by a substantial overlap in transcripts encoding carbohydrate active enzymes induced during early carbon starvation and during early exposure to straw. The majority of these enzymes are predicted to be active on plant-derived polymers. Filtrate from carbon starved cultures indeed contained enzymes active on plant-derived polymers. Our current investigation of the activities of these enzymes towards simple and complex plant polymers, as well as identification of the products formed by these activities, could yield information on transcriptional inducers of genes that encode these enzymes.

Delmas et al (2012) PLoS Genet. 8 (8)e1002875

Keywords: Aspergillus niger, Starvation, Biofuels, Glycoside hydrolase, lignocellulose degradation

***79. The transcriptional activators XlnR and AraR affect physiology of *Aspergillus niger* on solid cultures with wheat bran as carbon source**

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In nature *Aspergillus niger* degrades plant material using extracellular enzymes. One of the main regulatory factors controlling the production of these enzymes is the (hemi-)cellulolytic regulator XlnR. In the presence of D-xylose XlnR activates genes involved in cellulose and hemicellulose degradation, as well as some genes of the pentose catabolic pathway. We have also identified de L-arabinose responsive transcriptional activator AraR in *A. niger*, which controls activation of genes involved in release and conversion of L-arabinose. These

two regulators interact with each other and together are essential for an efficient utilization of plant biomass by *A. niger*.

In this study we analyze the influence of XlnR and AraR in *A. niger* during growth on solid media with wheat bran as carbon source. *A. niger* wild type and disruptants for both transcriptional activators (AraR, XlnR) were analyzed for enzyme activity, gene expression and protein production in 5 concentric zones of the colony from the centre (oldest part of the colony) to the periphery (youngest part of the colony).

Clear differences were visible between the mutants and the wild type, in particular with respect to genes involved in plant biomass degradation.

80. Gene silencing on demand: Establishment of the Tet-off system for *Aspergillus niger

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The tetracycline-expression system is a versatile tool to control and fine-tune gene expression in eukaryotic cells in a metabolism-independent manner. By the addition of doxycycline, genes can either be switched on (Tet-on system) or switched off (Tet-off system). Recently, the Tet-on system has successfully been established for *Aspergillus niger* (Meyer et al., 2011).

In the current study, we tested and evaluated different variations of the Tet-off system for use in *A. niger* by using luciferase as a reporter gene. Transformants with single or multiple copies of the Tet-Off system were generated and their expression levels determined and compared with a respective control strain. By adding various concentrations of doxycycline to the cultivation medium, it could be shown that the expression of the reporter gene could indeed be down-regulated in a concentration-dependent manner. Most importantly, it was possible to completely shut down luciferase expression. Furthermore, the system turned out to be not only tuneable dependent on the concentrations of doxycycline. Also multiple copies of the Tet-off cassette changed the intensity of expression and its respective down-regulation kinetics.

Key words: *Aspergillus niger*, Tet-On, Tet-Off, expression system, gene regulation

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81. Regulation of the fumagillin/pseurotin secondary metabolite supercluster in *Aspergillus fumigatus*

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The hallmark trait of fungal secondary metabolite gene clusters is well established, consisting of contiguous enzymatic and often regulatory gene(s) devoted to the production of a metabolite of a specific chemical class. Unexpectedly, we have found a deviation from this motif in a sub-telomeric region of *Aspergillus fumigatus*. This region, under the control of the master regulator of secondary metabolism LaeA in its entirety, contains the genetic machinery for three natural products (fumitremorgin, fumagillin and pseurotin) where genes for fumagillin and pseurotin are physically intertwined in a single supercluster. Deletions of 29 adjoining genes revealed that fumagillin and pseurotin are co-regulated by the supercluster-embedded transcription factor FapR with biosynthetic genes belonging to one of the two metabolic pathways in a non-contiguous manner. Comparative genomics indicates the fumagillin/pseurotin supercluster is maintained in a rapidly evolving region of diverse fungal genomes. This blended design confounds predictions from established secondary metabolite cluster search algorithms and provides a novel view of natural product evolution.

***82. Enhanced organic acid production of Aspergillus carbonarius by genetic engineering**

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The black fungus Aspergillus carbonarius has potential as a good cell factory for production of organic acids. The attempts have been made to improve the conversion of the renewable carbon sources and the flux into selected organic acids by engineering of the central carbon metabolism. The present work is to evaluate the effect of deleting the gluconic acid producing pathway and inserting an alternative cytosolic pathway on organic acid production. The gox mutant was constructed by deleting the gox gene, which is assumed to encode the glucose oxidase in A. carbonarius, as there is a very high accumulation of gluconic acid as the main byproduct during organic acid fermentation from glucose. It was shown that the glucose consumption and efficiency of citric acid production were improved compared with the wildtype strain, and no gluconic acid could be detected from gox mutants. The pepck and ppc mutant was constructed separately by inserting the gene pepck and ppc, which encode the phosphoenolpyruvate carboxykinase in Actinobacillus succinogenes and phosphoenolpyruvate carboxylase in Escherichia coli, into A. carbonarius, in order to create a new bypass for shunting the carbon flux into the reductive pathway for stimulating the organic acid production. An improvement on both malic acid and citric acid production was observed which may result from the competition on the phosphoenolpyruvate with the original pathway. This may further lead to the enhanced carbon flux towards the cytosolic reductive pathway. Combining the two mutations in one strain shall demonstrate whether the production of organic acids will further increase.

83. Functional Analysis of the α-1,3-Glucan Synthase Genes *agsA* and *agsB* in *Aspergillus nidulans*

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Although α-1,3-glucan is one of the major cell wall polysaccharides in filamentous fungi, the physiological roles of α-1,3-glucan remain unclear. The model fungus *Aspergillus nidulans* possesses two α-1,3-glucan synthase (AGS) genes, *agsA* and *agsB*. For functional analysis of these genes, we constructed several mutant strains in *A. nidulans*: *agsA* disruption, *agsB* disruption, and double-disruption strains. The *agsA* disruption strains did not show markedly different phenotypes from those of the wild-type strain. The *agsB* disruption strains and the double-disruption strains showed increased sensitivity to congo red and lysing enzymes, which are a cell wall stress–inducing compound and a cell wall–degrading enzyme, respectively. In addition, the *agsB* disruption strains formed dispersed hyphal cells under liquid culture conditions, regardless of the *agsA* genetic background. The mycelial dry weight of the *agsB* disruption strains cultured in liquid medium was increased compared with that of the wild-type strain, suggesting that the dispersed hyphal cells observed in the *agsB* disruption strains was suitable to high-density cultivation for high-production of biomaterials. Fractionation of the cell wall based on the alkali solubility of its components, quantification of sugars, and ^{13}C-NMR spectroscopic analysis revealed that α-1,3-glucan was the main component of the alkali-soluble fraction in the wild-type and *agsA* disruption strains, but almost no α-1,3-glucan was found in the alkali-soluble fraction derived from either the *agsB* disruption strain, regardless of the *agsA* genetic background. Taken together, our

data demonstrate that the two AGS genes are dispensable in *A. nidulans*, but that AgsB is required for normal growth characteristics under liquid culture conditions and is the major AGS in this species

84. A new method for fungal genetics: flow cytometry of microencapsulated filamentous microcolonies.

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Genetic analysis of organisms can be achieved by the isolation of consistent, well-defined colonies on solid media. In the case of non-filamentous microorganisms genetic analysis is also facilitated by the handling of individual cells by flow cytometry. In contrast, some filamentous fungi are hard to be analyzed by flow cytometry. In this work we have employed the combination of single spore microencapsulation and large particle flow cytometry as an alternative for the analysis of filamentous fungi. Mycelium proliferation inside the microcapsules can be detected using either microscopy or COPAS™ large particle flow cytometry.
Here we show the successful application of the Flow Focusing® technology to the microencapsulation of filamentous fungi in monodisperse alginate microspheres, using *Aspergillus* and *Trychoderma* as model systems. Using a Cellena® Flow Focusing microencapsulator, we managed to produce monodisperse microparticles containing individual spores and to develop microcolonies of these fungi upon germination in the appropriate conditions. Proliferation inside the particles was monitored by microscopy and large particle flow cytometry without requiring fluorescent labeling. Auxotrophic mutants were utilized to demonstrate the feasibility of the method. This procedure allows for the handling, screening and analysis of clonal colonies in liquid culture. Examples of genetic analysis with conditional mutants are provided.

***85. Role of oligomerization in the trafficking and vacuolar turnover of a purine transporter in a model fungal system**

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The uric acid/xanthine transporter UapA of the model fungus *Aspergillus nidulans* has been used as a prototype cargo for studying membrane trafficking and endocytosis. In the presence of ammonium ions or substrates UapA is ubiquitinated, internalized from the PM and sorted to the MVB/vacuolar pathway. Interestingly, substrate-elicited endocytosis operates only for functional UapA molecules. However, inactive UapA versions are endocytosed if co-expressed with active UapA molecules. The latter phenomenon, designated *in trans* endocytosis, prompted us to investigate whether UapA homo-oligomerizes. Here, we confirm the oligomerization of UapA using two different approaches; *in vivo* bimolecular fluorescence complementation (BiFC) and direct pull-down assays of differentially tagged UapA molecules. We show that functional UapA oligomers are initially formed in the ER membrane and remain stable in PM. Using UapA mutants showing ER-retention, we subsequently identify an N-terminal motif and other elements affecting oligomerization. Finally, we show that substrate-elicited endocytosis, unlike ammonium-induced, coincides with the dissociation of transporter oligomers, prior to their internalization. Our findings suggest that UapA oligomerization, analogously to some plant and mammalian transporters, is critical for ER-exit, sorting to the PM and endocytosis. At present, we examine the possible role of the Sec23/24 ER-exit molecular machinery on UapA oligomerization and *vice versa*.

*86. DUAL DNA BINDING AND COACTIVATOR FUNCTIONS OF ASPERGILLUS NIDULANS TAMA, A ZN(II)2CYS6 TRANSCRIPTION FACTOR

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Transcription factors that contain DNA binding domains generally regulate transcription by direct interaction with DNA. Zn(II)2Cys6 zinc binuclear cluster proteins – the largest family of transcription factors in fungi – regulate genes of diverse pathways, including primary and secondary metabolism and development. For most transcription factors, including Zn(II)2Cys6 proteins, the DNA binding motif is essential for function. However, Aspergillus nidulans TamA and the related Saccharomyces cerevisiae Dal81p protein contain Zn(II)2Cys6 motifs that previous studies have shown are dispensable for function. TamA acts at several promoters as a coactivator of the global nitrogen GATA transcription factor AreA. We now show that the TamA DNA binding motif mediates regulation of the key nitrogen metabolism gene gdhA, which encodes NADP-glutamate dehydrogenase. Therefore TamA has dual DNA binding and non-DNA binding coactivator functions. Using electrophoretic mobility shift assay (EMSA) and chromatin-immunoprecipitation (ChIP) we detect binding of FLAG-epitope-tagged TamA to the promoter region of gdhA and show that the TamA DNA binding motif is required for DNA binding. Additionally, we show that TamA and AreA are reciprocally required for full binding at the gdhA promoter under conditions where AreA activates gdhA but is inactive at most promoters. Finally, using ChIP-seq we map binding of TamA throughout the genome and identify novel TamA targets. The dual functions of TamA provide an additional level of combinatorial control for transcription factors to mediate gene-specific expression.

*87. The Minos transposon as a novel efficient tool for functional genomic analysis in *Aspergillus nidulans*

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Transposons constitute powerful genetic tools for gene inactivation, exon or promoter trapping and genome analyses in general. The *Minos* element from *Drosophila hydei,* related to the Tc1/mariner-like transposons, has proved as a very efficient tool for heterologous transposition in several insects and metazoa. It is characterized by high transposition rates, it does not show insertion site biases and most of its insertions disrupt gene function. Several *Minos*-based vectors which contain exogenous DNA between the inverted terminal repeats of the element have proved suitable for genome-wide screens for unraveling gene function and studying gene expression. In filamentous fungi, only a handful of fungal-specific transposable elements have been exploited as genetic tools, with the *Impala* element from *Fusarium oxysporum* being the most successful. Here, we have developed a system to manipulate *Minos* transposition through a two-component transposition system in *Aspergillus nidulans*. A *Minos* derivative, carrying the *yA*$^+$ and *inoB*$^+$ genes, not carrying the Minos transposase, was constructed and integrated, through homologous integration, in the promoter of the *niaD* gene (nitrate reductase), resulting in *niaD*$^-$ *yA*$^+$ *inoB*$^+$ transformants. A selected transformant was crossed with a strain expressing the *Minos* transposase from the *gpdA*^{m} promoter and selected progeny was analysed for *niaD*$^+$ *yA*$^+$ *inoB*$^+$ revertants. On average, among 10^7 conidiospores plated, we have obtained up to ~10^3 transposition events leading to the expected revertant phenotype (transposition frequency: ~10^{-4}). We are at present investigating the randomness of *Minos* transposition by sequencing integration sites, and how physiological conditions or different genetic backgrounds affect transposition frequencies. *Minos* will also be used in selected specific screens and for obtaining morphological mutants or recessive lethal in diploids. Our work will allow the evaluation of *Minos* as a tool for mutational genomic analysis in *A. nidulans* and fungi.

***88. Whole-genus sequencing of the *Aspergillus* genus**

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The genus Aspergillus is a highly important genus of organic matter-degrading filamentous fungi. This genus is species-rich and is currently composed of more than 300 species. There are few groups of organisms with a higher number of potential products per species than the fungi; for Aspergilli, above 250 carbohydrate-active enzymes and 30-100 secondary metabolites have been identified in each species examined so far. The group is ubiquitous and diverse, and includes key cell factories for enzyme production, model organisms, fermenters of foodstuffs, plant pathogens, producers of animal and human mycotoxins, and degraders of organic matter ranging from dung over plant matter to dried and semidried fruits.

In collaboration with a consortium including the US Joint Genome Institute and the Joint Bioenergy Institute, we are at DTU Systems Biology currently advancing research in the *Aspergillus* species and fungi in general by *de novo* genome sequencing of all unsequenced species (>280) in the genus. This is estimated to identify >75,000 carbohydrate-active enzymes and > 10,000 new biosynthetic gene clusters for secondary metabolism and aims to answer key questions about the linkage between physiology and phylogeny of this important genus.

Here we present the preliminary results for the first app. 50 species of the genus focusing on biological insights and genome dynamics. We will also present the current status of this ongoing work, and the planned order of the next genomes to be sequenced.

89. Elucidating the role of the hyphal tip of in the triggering mechanism of conidiation

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Contact of growing hyphae with the air is a prerequisite for the induction of the asexual development in *Aspergillus nidulans*. The induction process involves a group of proteins called Upstream Developmental Activators (UDAs). One of these UDAs is the bZIP-type transcription factor (TF) FlbB, which directly binds the promoter of the coniciation-specific transcription factor *brlA*. FlbB is located at the hyphal tip and the most apical nucleus. Constitutive expression of *flbB* under the *gpd*A^{mp} promoter altered the nuclear accumulation of FlbB in vegetative hyphae, leading to an even distribution among nuclei. Addition of latrunculin B excluded constitutively expressed GFP::FlbB from the tip and provoked its accumulation at subapical regions.

flbE is a second UDA factor that interacts with FlbB at the tip of hyphae. Through the analysis of a FlbE::GFP chimera constitutively expressed under gpdA^{mp}, we observed that its localization and dynamics also depended on the actin cytoskeleton. Latrunculin B has the same effect on FlbE as it has on FlbB, leading to accumulation of both proteins at the subapical region. Stream acquisitions also showed that both proteins move together to the tip. The FlbB/E interaction is mediated by the bZIP transcriptional regulatory domain of the former, which is sufficient and necessary for its binding to FlbE. FlbE has a signal sequence at its N-terminus, which purportedly targets the protein to the secretory pathway. Deletion of this sequence or the N-terminal GFP tagging of a wild-type FlbE generate a *fluffy* phenotype in which FlbE movement is highly restricted. This suggests that the signal sequence of FlbE is necessary for the transport of both FlbE and FlbB. A new feature

of FlbE is its ability to be imported to nuclei when constitutively expressed under gpd*A*^{mp}. The nuclear accumulation of FlbE is increased in *flbB* mutants that inhibit the tip localization of the signalling complex: C382A and L104A;E105A (being the latter mutation located within the dimerization domain of the bZIP).

Overall, these results suggest that the transport of the FlbB/E complex can be divided into two stages: initial delivery to the subapical region and a final transport to the apex. The second step depends on actin filaments. Furthermore, these findings emphasize the need of FlbE for the apical localization of FlbB, which is a pre-requisite for the correct induction of asexual development at nuclei.

90. A NOVEL SIALIDASE IN THE OPPORTUNISTIC FUNGAL PATHOGEN, *ASPERGILLUS FUMIGATUS*

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We have studied the role of sialic acids in the opportunistic fungal pathogen, *Aspergillus fumigatus*, the most common cause of airborne mould infections worldwide. The pathogenesis of many bacteria and viruses is known to involve sialic acids, negatively charged monosaccharides present on cell surface glycans. We have shown that unsubstituted N-acetylneuraminic acid (Neu5Ac) is present on *A. fumigatus* spores and that the removal of spore Neu5Ac decreases their uptake by murine macrophages and human lung epithelial cells. How *A. fumigatus* presents sialic acids on its cell surface is unknown; to date, no sialic acid biosynthetic genes have been found in the genome. However, a sialidase gene was identified and the protein was expressed in bacteria and a crystal structure obtained. Further analysis of the *A. fumigatus* sialidase revealed an unexpected finding: rather than Neu5Ac, the enzyme prefers KDN as a substrate, an uncommon member of the sialic acid family. KDN is found in almost all types of glycoconjugates in place of Neu5Ac. The crystal structure of the recombinant *A. fumigatus* KDNase enzyme showed that the arginine pocket can accommodate the smaller substitution at the fifth carbon on KDN. To investigate the mechanism of the *A. fumigatus* sialidase (KDNase), we used site-directed mutagenesis to create mutant recombinant enzymes modified at the active site including the catalytic nucleophile (Y358H), the general acid/ base catalyst (D84A), and an enlargement of the binding pocket to attempt to accommodate the N-acetyl group of Neu5Ac (R171L). Crystal structures for all three mutant enzymes were determined. The D84A mutant had a greater effect on decreasing the activity of AfKDNase compared to the same mutation in the structurally similar sialidase from the bacterium, *Micromonospora viridifaciens*. Removal of the catalytic nucleophile (Y358H) significantly lowered the activity of the enzyme but this mutant remained a retaining glycosidase as demonstrated by NMR spectroscopic analysis. This is a novel finding that has not been shown with other sialidases. Kinetic analysis revealed that R171L had higher activity on a Neu5Ac-based substrate compared to wild type KDNase; hence, leucine in place of arginine in the binding pocket improved catalysis towards Neu5Ac substrates. Hence, whether a sialidase is primarily a KDNase or a neuraminidase is due in part to the presence of an amino acid that creates a steric clash with the N-acetyl group.

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