The Fourth *Aspergillus* Meeting

March 18-20, 2007
Asilomar Conference Center
Pacific Grove, California

Organized by the
***Aspergillus* Genomes Research Policy Committee**

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 this widest sense, 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 future 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).

2006 AGRPC:

Scott Baker (*A. niger)*, 2006-08
Pacific Northwest National Laboratory, USA; scott.baker@pnl.gov
Gerhard Braus (*A. nidulans*), 2005-07
Georg-August-University Goettingen, Germany; gbraus@gwdg.de
Paul Dyer (*A. fumigatus*), 2004-06
University of Nottingham, UK; Paul.Dyer@Nottingham.ac.uk
Gustavo Goldman (Special Appointment), 2006
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Michael Hynes (*A. nidulans*), 2004-06
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Michelle Momany, Chair (*A. nidulans* and *A. fumigatus*), 2006-08
University of Georgia, USA; momany@plantbio.uga.edu
Gary Payne (*A. flavus*), 2005-2007
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Arthur Ram (*A. niger* and *A. nidulans*), 2006-08
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THANKS TO OUR MEETING SPONSORS!!!!!

The Fourth *Aspergillus* Meeting

March 18-20, 2007
Asilomar Conference Center

All sessions in Merrill Hall

March 18, Sunday

3:00- 6:00 Registration

6:00 ***Dinner***

7:00-9:00 ***Welcome Reception—Sponsored by Novozymes***

March 19, Monday

7:30-9:00 ***Breakfast***
9:00-9:15 **Welcome, introductions and announcements**

Session I:
9:15-10:15 **Genetics and Cell Biology** **Chair: Gerhard Braus**

Dr. Norio Takeshita, Univ. of Karlsruhe. Cell end factors in *A. nidulans*

Dr. Jae Hyuk Yu, Univ. of Wisconsin. GPCRs, RGSs and velvet-like proteins

Dr. Kerstin Helmstaedt, Univ. of Göttingen. The nuclear migration protein NUDF associates with BNFA and NUDC at spindle pole bodies in *Aspergillus nidulans*.

10:15-10:45 ***Coffee Break –Sponsored by Monsanto***

Session II:
10:45-11:45 **Phylogenetics/Evolutionary Biology** **Chair: Paul Dyer**

Dr. Arun Balajee, Centers for Disease Control and Prevention, Atlanta. MLST and the molecular phylogeny of the *Aspergillus* section *Fumigati*.

Dr. Peter J. Cotty, United States Department of Agriculture, University of Arizona. Vegetative incompatibility in *Aspergillus flavus*.

Dr. Rolf F. Hoekstra, Wageningen University.
Experimental means to study evolutionary processes within *Aspergillus*

12-1:00 ***Lunch***

Session III:

1:00-2:00 **Annotation, Comparative Genomics, Databases**
Chair: Masayuki Machida

Dr. Jennifer Wortman, The Institute for Genomic Research. Comparative genomics as a tool for annotation improvement and analysis of related *Aspergillus* genomes.

Dr. Gavin Sherlock, Candida Database, CGD. Using GBrowse to visualize multiple genome assemblies.

Drs. Jennifer Wortman, Steve Oliver, Dave Ussery, Jaap Visser and Cees van den Hondel. The *A. nidulans* annotation project: a global community effort.

2:00-2:30 **Community Directions Discussion**
Moderator: Michelle Momany

2:30-2:45 ***Coffee Break –Sponsored by Monsanto***

Session IV:

2:45-5:00 **Talks selected from Abstracts Chair: Gary Payne**

Dr. Özgür Bayram, Georg August University, Institut of Microbiology & Genetics Goettingen. The *Aspergillus nidulans* F-box Protein Project.

Dr. Jakob B. Nielsen, Technical University of Denmark, CMB, BioCentrum-DTU Kgs. Lyngby. A transiently disrupted non-homologous end joining pathway in *Aspergillus nidulans* allows simple and efficient gene targeting.

Dr. Peter J. Punt, TNO Quality of Life, Microbiology Zeist. New screening approaches for fungal strain development.

Dr. Natalie D. Fedorova, The Institute for Genomic Research, Microbial Genetics, Rockville, MD. Comparative analysis of secondary metabolism gene clusters from two strains of *Aspergillus fumigatus* and closely related species *Neosartorya fischeri* and *Aspergillus clavatus*.

Dr. Ronald de Vries, Utrecht University Microbiology, Utrecht. Colonies from the filamentous fungus *Aspergillus niger* are highly differentiated in spite of cytoplasmic continuity.

Dr. William Steinbach, Pediatrics, Molecular Genetics, and Microbiology, Duke University Medical Center. The benefits and real future of calcineurin inhibition against invasive aspergillosis.

Dr. William C. Nierman, The Institute for Genomic Research. A pilot project for a high throughput *Aspergillus fumigatus* mutant strain Resource

Dr. Steven Harris, The University of Nebraska. Proposed tiling project

5:15-6:00 **Pontecorvo Lecture: Joan Bennett, Rutgers University**
"On being Aspergillus-o-centric: From girl geek to fungus freak"

6:00-7:00 ***Dinner***

7:00-10:00 **Posters and drinks**
Poster session and outstanding student poster sponsored by DSM

March 20, Tuesday

7:30-9:00 ***Breakfast***

9:00-9:15 Elections

Session V:

9:15-10:15 **Genomics/Proteomics/transcriptomics/metabolomics updates**
Chair: Gustavo H. Goldman

Dr. Manda Gent, Faculty of Life Sciences, Manchester University A comparison of the expression of secreted hydrolases in *Aspergillus fumigatus* and *Aspergillus nidulans* under phospholipid-rich conditions

Dr. Olaf Kniemeyer, Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie Hans-Knöll-Institut. Comparative proteomics of the human-pathogenic fungus *Aspergillus fumigatus*

Dr. Jens Frisvad, Center for Microbial Biotechnology, BioCentrum-DTU, Technical University of Denmark. The exometabolomics of *Aspergilli*

Dr. Noel van Peij, DSM. *A. niger* genome update

10:15-10:30 ***Coffee Break–Sponsored by Monsanto***

Session VI:

10:30-11:30 **Industrial Applications** **Chair: Scott Baker**

Dr. Ken Bruno, Chemical & Biological Processes Development Group, Pacific Northwest National Laboratory. Cell Biology and Citric Acid Production in *Aspergillus niger*.

Dr. Mikael Rørdam Andersen, Technical University of Denmark. Metabolic network driven interpretation of transcription data from *Aspergillus* cell factories.

Dr. Adrian Tsang, Concordia University. *Aspergillus niger* as a host for global expression of secreted proteins.

11:30 **Announce election results and take any further discussion**
Announce winner of DSM student poster prize

12:00-1:00 ***Lunch***

Poster Abstracts

CELL BIOLOGY

1. The *Aspergillus nidulans cetA* and *calA* genes are involved in conidial biogenesis and germination. Ravit Balaish, Chaim Sharon, Emma Levdansky, Shulamit Greenberg, Yana Shadkchan and Nir Osherov[1] Department of Human Microbiology, Sackler School of Medicine, [1]Tel-Aviv University Tel-Aviv, Israel.E-mail: nosherov@post.tau.ac.il

Recently, we characterized the *cetA* (AN3076) gene, whose transcript is highly expressed in dormant conidia of *A.nidulans*1,2. CetA, is similar to plant thaumatin-like (TL) genes encoding proteins which have an antifungal activity. We found that the cetA transcript is rapidly translated during early germination and secreted into the culture medium. It was also found that cetA transcription is subject to glucose-mediated carbon catabolite repression (CCR) and is activated by the PKA pathway. However, *cetA* gene-disruption showed no obvious phenotypic growth defects, as compared to the wild-type strain. *A. nidulans* contains an additional cetA-like gene, *calA* (AN7619), whose transcript is not expressed in dormant conidia but only during germination. We hypothesized that *cetA* and *calA* are redundant and that deletion of both genes may reveal their function. We therefore prepared knockout *cetA, calA* and *calA/cetA A. nidulans* strains and analyzed their phenotype. The mutants in which *calA* and *cetA* were deleted were phenotypically identical to the wild-type strain. In contrast, the *cetA/calA*-double mutant showed a lethal phenotype. Most of the conidia in the double mutant were completely inhibited in germination. Many collapsed and underwent autolysis. A few showed abnormal germination characterized by short swollen hyphae, and abnormal hyphal branching. Furthermore, the ungerminated conidia contained a single condensed nucleus suggestive of a defect in initiating the cell life-cycle (mitosis did not occur). This is the first study to analyze the function of the novel *cetA/calA* family of thaumatin-like genes and their role in the germination of *A. nidulans* conidia. We show that *cetAp* and *calAp* are redundant proteins that together play an essential role in the development of the conidial cell wall.
1)Osherov et al. FGB 37:197-204, 2002. 2)Greenstein et al. FGB 43:42-53, 2006.

2. Characterization of the *Aspergillus nidulans* septin *AspA/Cdc11*. Rebecca Lindsey, Susan Cowden and Michelle Momany* Department of Plant Biology, University of Georgia, Athens, Georgia 30602 USA lindsey@plantbio.uga.edu

The septin proteins form filamentous rings at the mother-bud neck in yeast. Septins are found in microsporidia, fungi, and animals and are absent from plants. In addition to their original role in cell division, septins have been shown to have roles in cytoskeletal organization, coordination of nuclear division and trafficking across membranes. In *Aspergillus nidulans* there are five septins *aspA/cdc11, aspB/cdc3, aspC/cdc12, aspD/cdc10*, and *aspE*. We have conducted studies on the *A. nidulans* septin *aspA/cdc11*. The null mutant of *aspA/cdc11* is viable and shows uncoordinated germ tubes, hooked hyphae, split tips, hyperbranching, disorganized conidiophores and hypersensitivity to the actin depolymerizer Cytocholasin A. Localization of AspA/Cdc11-GFP is dynamic. AspA/Cdc11-GFP is visible as a spot on conidia, throughout the cortex in swelling conidia, at the polarizing surface when germ tubes emerge, at septa in germ tubes and branches, and as filaments at tips and in early branches. In *delta aspA/cdc11* SepA-GFP and BimG-GFP localization are lost, but TubA- GFP localization is normal. Our results suggest that AspA/Cdc11 interacts with actin as well as the formin, SepA and the protein phosphatase BimG, possibly in the growing hyphal tip.

3. Characterization of Protein *O*-mannosyltransferases in *Aspergillus nidulans.* Thanyanuch Kriangkripipat and Michelle Momany* Department of Plant Biology, University of Georgia, Athens, Georgia 30602 USA tkriang@plantbio.uga.edu

Protein *O*-mannosyltransferases (PMTs) are found in bacteria, fungi, and animals but are not present in plants. In fungi, PMTs are divided into three subfamilies, PMT1, PMT2 and PMT4 and each species has 3-7 PMTs. *Aspergillus nidulans* possesses three PMTs, Pmt1, Pmt2 and Pmt4. Single *pmt* deletion mutants are viable. Each Δ*pmt* mutant exhibits different phenotypes when characterized by growth at different temperatures, morphology and sensitivity to chemicals disturbing cell wall synthesis. Double mutants show additive phenotypes. The Δ*pmt1* mutant has hyphal tip lysis and produces aberrant conidiophores at 42^{O}C. The Δ*pmt2* mutant cannot send out germ tubes at 42^{O}C. The Δ*pmt4* mutant has swollen hyphae and produces aberrant conidiophores at 42^{O}C. The Δ*pmt1*Δ*pmt2* double mutant is viable and has additive phenotypes of Δ*pmt1* mutant and Δ*pmt2* mutant. The Δ*pmt2*Δ*pmt4* double mutant is viable but very sick and forms a microcolony only when an osmotic stabilizer is added to the medium. Lower temperatures and osmoticum can partially restore wildtype hyphal growth and conidiation of these Δ*pmt* mutants except for the Δ*pmt2*Δ*pmt4* double mutant. Our results suggest that protein *O*-mannosylation is important for cell wall integrity of *A. nidulans*.

4. Characterization of the *Aspergillus nidulans* septin mutant *ΔaspB/cdc3.* Yainitza Rodriguez and Michelle Momany Department of Plant Biology, University of Georgia, Athens, Georgia 30602 USA *yrodriguez@plantbio.uga.edu*

Septins are filament forming P-loop GTPases found in microsporidia, fungi and animals. Septins play important roles in a variety of processes such as cellular and nuclear division, membrane trafficking and organization of the cytoskeleton. This family of proteins was first discovered in a screen for temperature sensitive cell cycle mutants in *Saccharomyces cerevisiae*. In yeast, septins form filamentous rings at the mother-bud neck which are necessary for completion of cytokinesis and normal morphogenesis. In *Aspergillus nidulans* there are five septins *aspA/cdc11*, *aspB/cdc3*, *aspC/cdc12*, *aspD/cdc10*, and *aspE*. The *A. nidulans* septin *aspB/cdc3* was previously shown to localize at septation and branching sites and at interface layers of the conidiophore. This gene was reported to be essential. We have now found that deletion of *aspB/cdc3* is not lethal, but causes severe defects in asexual reproduction and aberrant morphology in several developmental stages. The mutant shows emergence of multiple germ tubes, hyperbranching and hooked branches and disorganized conidiophores. *ΔaspB/cdc3* also shows sensitivity to calcoflour, but not benomyl or cytochalasin A, which suggests that the mutant phenotype is not directly associated with the actin or microtubule cytoskeleton, but perhaps with cell wall stability.

5. The Nuclear Migration Protein NUDF Associates with BNFA and NUDC at Spindle Pole Bodies in *Aspergillus nidulans.* Kerstin Helmstaedt, Karen Meng, Silke Busch, Özgür Bayram, Oliver Valerius and Gerhard H. Braus. Institut für Mikrobiologie und Genetik, Georg-August-Universität Göttingen, Grisebachstr. 8, D-37077 Göttingen, Germany. Tel. +49-551-3919693, Fax +49-551-393820, khelmst@gwdg.de

In *A. nidulans*, nuclear migration depends on microtubuli, the motor dynein and nuclear distribution proteins like NUDF. Applying tandem affinity purification, we isolated a unique NUDF-associated protein, which we named BNFA (Binding of NUDF). An *A. nidulans bnfA* deletion strain did not show a *nud* phenotype indicating that a protein with redundant function might exist. A GFP-BNFA fusion localized to spindle pole bodies (SPBs) throughout the cell cycle. This position depended on NUDF, since in a *nudF6* strain BNFA localized mainly to dots in the cytoplasm. In a yeast two-hybrid screen using BNFA as bait, we found that BNFA is a dimer and that a link might exist to the septation signalling pathway. In a candidate approach, we analysed the putative NUDC-NUDF interaction in *A. nidulans*. Although NUDC-GFP alone was localized to immobile dots at the cortex, we found a direct interaction between NUDF and NUDC in yeast two-hybrid experiments, which depended on NUDF's WD40 domain. Applying bimolecular fluorescence complementation microscopy, we showed that *in vivo* NUDF and NUDC interact also at spindle pole bodies throughout the cell cycle and at immobile dots at the cortex.

6. Analysis of two cell end marker proteins, TeaA and TeaR, in *Aspergillus nidulans*. Norio Takeshita, Sven Konzack, Yuhei Higashitsuji and Reinhard Fischer. Applied Microbiology, University of Karlsruhe, Germany.

The interplay of the actin and the microtubule (MT) cytoskeleton in polarized growth of fungi has recently been revealed. In *Schizosaccharomyces pombe*, Tea1 is a key protein in this process. Tea1 is transported to the plus ends of MTs by the kinesin Tea2, and is delivered to cell ends by hitchhiking with the growing MTs. Mod5, which is posttranslationally modified by prenylation, anchors Tea1 at the cell ends. These three proteins were indentified by screening for polarity mutants. At the cell ends, Tea1 recruits formin which initiates actin assembly and the establishment of cell polarity. Tea1 and Tea2 homologues were identified in the *Aspergillus nidulans* genome (named TeaA and KipA), whereas Mod5 could not be identified due to sequence similarity. *kipA* mutants showed mislocalization of the Spitzenkorper and hence curved hyphae. GFP-KipA accumulated at the MT plus ends. *teaA* mutants showed a similar but not identical phenotype to *kipA* mutants. GFP-TeaA localized to one point in the apex of hyphal tips. To test whether the function of *S. pombe* Mod5 was conserved, we searched the *A. nidulans* genome for proteins with a C-terminal prenylation motif (CAAX). From 22 identified proteins one (536 amino acids) was likely to serve a TeaA-anchorage function. We named it TeaR. *teaR* mutants indeed displayed a phenotype similar to the *kipA* mutant. GFP-tagged TeaR localized to the membrane at hyphal tips. Their putative interactions will be analyzed.

7. Secondary metabolism and sporulation signalling in *Aspergillus nidulans*. Olivia Márquez[1], Angel Trigos[1], J. Luis Ramos[2], Gustavo Viniegra[3], Holger Deising[4], Nallely Cano[2] and Jesús Aguirre[2]*. [1]Universidad Veracruzana, México. [2]Universidad Nacional Autónoma de México, México. [3]Universidad Autónoma Metropolitana, México. [4]Martin-Luther University Halle-Wittenberg, Germany. *E-mail: jaguirre@ifc.unam.mx

We characterized *Aspergillus nidulans* strains carrying conditional (*cfwA2*) and null (*DcfwA*) mutant alleles of the *cfwA/npgA* gene, encoding an essential phosphopantetheinyl transferase (PPTase). We identified the polyketides shamixanthone, emericellin and dehydroaustinol, as well as the sterols ergosterol, peroxiergosterol and cerevisterol in extracts from developmental cultures. The PPTase CfwA/NpgA was required for production of polyketide compounds, but dispensable for fatty acid biosynthesis. The asexual sporulation defects of *cfwA, DfluG* and *DtmpA* mutants were not rescued by the *cfwA*-dependent compounds identified here. However, *cfwA2* mutation drastically enhanced the sporulation defects of both *DtmpA* and *DfluG* single mutants, suggesting that unidentified CfwA-dependent PKS and/or NRPS are involved in production of unknown compounds, required for sporulation. In addition, we show that *tmpA* and *tmpB* genes define two new *fluG*-independent sporulation pathways.
Supported by CONACYT-SAGARPA 2002-C01-1713, México.

8. Functional analysis of histidine-containing phosphotransmitter gene *ypdA* in *Aspergillus nidulans*. Natsuko Sato, Kentaro Furukawa, Tomonori Fujioka, Osamu Mizutani, and Keietsu Abe. Molecular and Cell Biology, Tohoku University, Sendai, Japan.

The high-osmolarity glycerol (HOG) response pathway responding to osmotic stimuli has been well studied in *Saccharomyces cerevisiae*. Sln1p-Ypd1p-Ssk1p proteins organize a two-component signalling (TCS) unit in the upstream of the HOG pathway, and negatively regulate the downstream Hog1p mitogen-activated protein kinase (MAPK) cascade. We previously revealed that a filamentous fungus *Aspergillus nidulans* possesses all counterparts of the components of *S. cerevisiae* HOG pathway. Deletion of Ypd1p, the TCS histidine-containing phosphotransmitter of *S. cerevisiae*, is known to cause lethality because of constitutive activation of Hog1 MAPK. While, *S. cerevisiae* possesses only one set of TCS unit consisted of Sln1p and Ypd1p, *A. nidulans* has been predicted to have 15 histidine kinases and some of them are thought to interact with the unique YpdA. Thus, the TCS pathway of *A. nidulans* might be more complex and robust than that of yeast . In addition, *YPD1* is essential in *S. cervisiae* but not in *Shizosaccharomyces pombe*. In the present study, in order to examine in vivo functionality of *A. nidulans ypdA* gene, we constructed an *ypdA* delta strain conditionally expressing the *ypdA* gene under the control of *A. nidulans alcA* promoter and investigated its phenotypes under the *ypdA*-repressed condition. Downregulation of *ypdA* transcription caused sever growth inhibition. We observed a constitutive phosphorylation of HogA MAPK in *A. nidulans ypdA* delta. These results suggest that YpdA is a essential component of the upstream of *A. nidulans* HOG (AnHOG) pathway, and the growth inhibition caused by *ypdA* delta would be attributed to disorder of signalling through the AnHOG pathway.

9. Colonies from the filamentous fungus *Aspergillus niger* are highly differentiated in spite of cytoplasmic continuity. Ronald P de Vries[1], Ana M Levin[1], Ana Conesa[2], Hildegard H. Menke[3], Manuel Talon[2], Noel NME van Peij[3], Han AB Wösten[1]
[1]Microbiology, Institute of Biomembranes, Utrecht University, Padulaan 8, 3584 CH Utrecht, The Netherlands; [2]Centro de Genomica, Instituto Valenciano de Investigaciones Agrarias, Moncada, Valencia, Spain; [3]DSM Food Specialties, PO Box 1, 2600 MA Delft, The Netherlands.

Filamentous fungi play an important role in element cycling by degrading organic material. The unexplored substrate at the periphery of the fungal mycelium and the partially degraded substrate in the mycelial centre are expected to have a major impact on local gene expression. However, the continuity of the cytoplasm in a fungal mycelium and the phenomenon of cytoplasmic streaming raise the question whether there are indeed spatial differences in the mRNA composition and physiology. This was studied in 7-day-old maltose and xylose grown colonies of *Aspergillus niger*. Differential changes were detected with respect to growth, protein secretion and gene expression. Differential gene expression was not limited to specific functional categories, but occurred genome-wide. However, some gene groups related to nutrient utilization displayed significantly increased levels of differential gene expression. These data will be presented as well as evidence for the involvement of transcriptional regulators that effect the expression of specific gene groups.

10. The gene for the ubiquitin ligase regulator Cand1 is putatively split into two genes in the filamentous fungus *Aspergillus nidulans*. Elke U. Schwier, Martin Christmann, Krystyna Nahlik, Silke Busch and Gerhard H. Braus. Institut für Mikrobiologie und Genetik, Georg-August-Universität Göttingen, Grisebachstrasse 8, 37077 Göttingen, Germany; e-mail: eschwie@gwdg.de

Ubiquitin dependent proteolysis plays an important role in many cellular processes in eukaryotes. Cullin containing ubiquitin ligases like the SCF (Skp1-Cullin-F-box protein) complex mark proteins for degradation by ubiquitinylation. It has been shown that the protein Cand1 binds to cullins. The Cand1 C-terminus blocks the SKP1 binding site of the SCF complex component Cul1 and affects thereby the assembly/disassembly of the ubiquitin ligase. The N-terminus of Cand1 buries the neddylation site on Cul1, which prevents its modification by Nedd8, an ubiquitin-like protein and alters the activity of the complex. In *A. nidulans* the gene encoding the putative homolog of human Cand1 seems to be split in two, both independent genes having about 20% identity to the human protein. Deletion of the *A. nidulans* gene coding for the protein Cand1_C similar to the c-terminal part of human Cand1 leads to a red hyphae phenotype. Cand1_C is expressed during vegetative growth but not during sexual development. It localizes to the nucleus and interacts with CulA, CulD and Cand1_N in the yeast two hybrid system. Currently we are investigating the function of the smaller protein Cand_N. The split *cand1* gene makes the fungus *A. nidulans* an attractive model organism for studying the putative different functions of the two parts of the Cand1 protein.

11. The *Aspergillus nidulans* nuclear pore complex protein An-Nup-2 plays a novel role in mitosis but is not essential for nuclear transport. Sarine Markossian and Stephen A. Osmani. The Ohio State University, Columbus, Ohio, USA. markossian.1@osu.edu

The nuclear pore complex (NPC) regulates nuclear trafficking and is composed of ~30 subunits called nucleoporins (Nups). In yeast, Nup2p has been shown to facilitate nuclear transport. Unlike yeast Nup2p, *Aspergillus nidulans* Nup2 (An-Nup2) localizes to chromatin during mitosis but to the NPC during interphase. This indicates An-Nup2 may play a role during mitosis. An-*nup2* is essential and its deletion causes mitotic defects. We therefore speculate that the localization of An-Nup2 to mitotic chromatin is important for mitosis. To test this hypothesis, a domain study was performed to define the An-Nup2 domain responsible for its mitotic translocation to chromatin and an antibody was generated against An-Nup2. A domain spanning from aa 400 to aa 1200, which encompasses a basic stretch of amino acids, a coiled coil region, and two potential nuclear localization sequences (NLS), is sufficient to locate An-Nup2-GFP to the NPC during interphase and to DNA during mitosis. The An-Nup2 antibody was used for immunofluorescence and successfully stained the nuclear periphery during interphase and chromatin during mitosis confirming the An-Nup2-GFP localization. Most importantly, the heterokaryon rescue technique was used to define if nuclear transport and/or mitosis is defective without An-Nup2. An-Nup2 deleted cells were not deficient in nuclear transport of NLS-dsRed suggesting that the lethality caused by An-Nup2 deletion is due to mitotic defects and not nuclear transport defects. In conclusion, there is emerging evidence that the localization of An-Nup2 to mitotic chromatin is essential for mitosis although An-Nup2 may not be essential for nuclear transport.

12. A Cytoplasmic Remnant is involved during the Segregation of Nucleolar proteins in *Aspergillus nidulans* . Leena Ukil, Colin De-Souza, Hui-lin Liu, Stephen A Osmani, Department of Molecular Genetics, The Ohio State University, 484 W 12th Ave, Columbus, OH 43210, Email: leenaukil@yahoo.com

The nucleolus is a prominent nuclear structure whose mitotic segregation is poorly understood. During yeast mitosis the nucleolus segregates intact with rDNA. In contrast, during open mitosis the nucleolus is disassembled then reassembled. *Aspergillus nidulans* nuclei undergoes partially open mitosis, which is an evolutionary intermediate between open and closed mitosis. We therefore determined how *A. nidulans* nucleoli are segregated during mitosis. Unlike *Saccharomyces cerevisiae* , few *A. nidulans* nucleolar proteins segregate with DNA. Instead we have defined two patterns by which different nucleolar proteins segregate during mitosis: (1) Dispersal into the cytoplasm at the onset of mitosis but with some protein remaining bound to DNA, (2) A novel pattern in which nucleolar proteins remain in a nuclear remnant, distinct from daughter nuclei, before re-accumulating into daughter nucleoli during G1. Dual labeling of nucleolar proteins and nuclear envelope markers reveal that the nucleolar remnant is generated as a result of a double nuclear envelope fission event. This double fission occurs around a nucleolar protein mass during telophase. This mechanism generates two transport competent daughter nuclei and a very transient nucleolar remnant containing class 2 nucleolar proteins. This study indicates *A. nidulans* undergoes mitotic disassembly then reassembly of its nucleolus, as do higher eukaryotes, and that generation of daughter nuclei occurs via a double fission mechanism, not a single fission as occurs in yeasts. We suggest the novel mitotic nuclear remnant we have defined serves as a storage pool from which equal distribution of nucleolar proteins occur. It may also serve as a sink for unwanted cytoplasmic proteins or RNAs that gain access to nuclei during mitosis and may be a positional cue for the double fission.

13. The NADH oxidase *nadA* and its involvement in oxidative stress in *Aspergillus flavus*. Carrie A. Smith[1], Massimo Reverberi[2], Niki Robertson[1], Gary A. Payne[1] . [1]North Carolina State University, Raleigh, NC, USA, 27606. [2]Università "La Sapienza," Rome, Italy 00165.

nadA, which encodes a predicted NADH oxidase, was identified as part of a sugar utilization cluster that lies adjacent to the aflatoxin biosynthetic cluster in several species of *Aspergillus*. NADH oxidases convert NADH to NAD+, which is a possible coenzyme needed for reactions in the aflatoxin biochemical pathway. In a microarray experiment comparing gene expression between a wild type strain of *A. parasiticus* and a deletion mutant for the pathway regulatory gene *aflR*, *nadA* expression was significantly decreased in the mutant background. Although *nadA* is transcriptionally controlled by AflR, aflatoxin levels were unaffected in *A. flavus nadA* deletion strains under several conditions. NADH oxidases can also be a source of reactive oxygen species formation. Previous reports have shown a relationship between oxidative stress and aflatoxin production in *Aspergillus sp*. The activity of several antioxidant enzymes were examined in *nadA* deletion strains and peak activity was delayed when compared to wild type. Lower levels of lipoperoxide accumulation were also seen in *nadA* deletion strains. These data suggest that *nadA* plays a role in the oxidative stress response in *A. flavus*. Investigations are underway to characterize additional phenotypes of *nadA* deletion mutants.

14. Short term growth rate of *Aspergillus nidulans* hyphae is independent of near-apical cytoplasmic microtubule abundance. Michelle Hubbard, Susan Kaminskyj [1]. [1] Univ Saskatchewan, Saskatoon, SK S7N 5E2, Canada.

Apart from mitosis and nuclear migration, role(s) for the microtubule (MT) cytoskeleton in fungal growth rate vary between reports, likely due to strain and/or experimental differences. Using confocal microscopy, we quantified near- apical cytoplasmic MTs and hyphal growth rates in *Aspergillus nidulans* hyphae with constitutively expressed GFP-alpha-tubulin (a gift of the Oakley lab). For 219 untreated hyphae, all of which were morphologically similar but only about half of which were later found to be growing, we found no correlation between MT abundance and growth rate in the previous 2-5 min. Hyphae were treated for 30-120 min with taxol, benomyl, or latrunculin B, or with solvent controls. Considering only growing hyphae, and compared to their respective controls, MT numbers were significantly increased by DMSO, significantly reduced by benomyl, and moderately increased by latrunculin, but were unaffected by ethanol or taxol. In the same cells, growth rates were significantly increased by ethanol and taxol, unaffected by benomyl and DMSO, and reduced by latrunculin. Average hyphal growth rate in the first 30-120 min following benomyl treatment was similar to control cells, despite the absence of visible MTs after 2 min. Thus, unlike actin, we have not found a role for MTs in determining tip growth rate between 30-120 min after anti-MT treatments. Unexpectedly, growth rates of taxol-treated hyphae decreased significantly following observation by fluorescence microscopy.

15. The *Aspergillus nidulans* F-box Protein Project. Özgür Bayram[1], Heike S. Rupprecht[1], Marc Dumkow[1], Marcia R. v. Z. Kress[1], Thomas Linger[2], Özlem Sarikaya Bayram[1], Gustavo H. Goldman[3], Gerhard H. Braus[1]. [1]Institute of Microbiology & Genetics, Georg-August-University Goettingen, Grisebachstr. 8, 37077 Goettingen, Germany; e-mail: obayram@gwdg.de; hruppre@gwdg.de. [2]Institute of Microbiology & Genetics, Dept. of Bioinformatics, Georg-August-University Goettingen, Goldschmidtstr. 1, 37077 Goettingen, Germany. [3]Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, CEP 14040-903, Ribeirão Preto, São Paulo, Brazil

F-box proteins are part of E3 SCF (Skp1,Cullin,F-box) ubiquitin ligases which are the specificity factors of the ubiquitin dependent protein degradation machinery. They contain the F-box as conserved N-terminal domain which acts as interface to the Skp1 protein. In addition they have a substrate binding domain for the protein to be ubiquitinated. There are at least 70 F-box-like proteins encoded by the *Aspergillus nidulans* genome. To address the cellular functions of F-box proteins in *A. nidulans*, we have started to systematically disrupt F-box encoding genes via homologous gene replacement method. The characterization of the F-box mutants, which has been carried out so far, will be presented.

16. Expression and transposition of DNA transposon *Crawler* in *Aspergillus oryzae*. Hironobu Ogasawara[1], Hiroshi Obata[2], Yoji Hata[2], Saori Takahashi[1], and Katsuya Gomi[3]. [1]Akita Research Institute for Food and Brewing, Akita, Japan. [2]Research Institute, Gekkeikan Sake Co. Ltd., Kyoto, Japan. [3]Graduate School of Agricultural Science, Tohoku University, Sendai, Japan.

An active DNA transposon *Crawler* has been isolated and characterized from the industrially important fungus *Aspergillus oryzae*. The transposition events of *Crawler* were induced by various stress treatments such as $CuSO_4$ or heat shock. The existence of two or more transcripts in different size of *Crawler* was shown under standard culture conditions. In the present study, we analyzed the transcripts of different size by 3'- RACE analysis. Moreover, relationship between the transposition activity and the proportions of *Crawler* mRNA molecules was also studied to clarify the control mechanism for transcription of exogenous gene *Crawler*. The smaller transcribed fragments were resulted from premature polyadenylation and in some cases erroneous intron splicing within the transposase. The erroneous splicing tends to be inhibited by stress treatment of $CuSO_4$, which stimulated the transposition events in conidia allowing the full-length and active transposase to be produced. These results indicate that *A. oryzae* has a defense system against the exogenous active genes like transposons by mRNA quality control system such as undesirable splicing or polyadenylation resulted in nonstop mRNA decay.

17. The *Aspergillus nidulans snxA1* and *nimA5* mutations interact to affect mitotic spindle structure. Yulon Stewart, Ryan Day, Kirk Jackson, Michael Jackson, and Sarah Lea McGuire, Millsaps College, 1701 N. State St., Jackson, MS, 39210.

Both the *nimA* and *snxA* genes interact with $nimX^{cdc2}$ to affect mitosis in *Aspergillus nidulans*. *nimA* affects the nuclear import of $nimX^{cdc2}$, while the *snxA1* mutation is a cold-sensitive suppressor of the $nimX2^{cdc2}$ mutation. *snxA1* leads to abnormal nuclear morphology at 17°C. To better understand effects of the *snxA1* mutation on cells and the relationship between *snxA* and *nimA*, we generated strains expressing GFP- *tubA* (alpha-tubulin) and various combinations of *snxA* and *nimA* mutations. At 17°C *snxA1*/GFP-*tubA* cells had severe nuclear defects, thickened hyphae, abnormal spindle structures, and abnormal interphase microtubule arrays. Mitotic spindles were highly variable in length. Some spindles had no nuclei attached to them, while others were bifurcated or trifurcated and had fragmented, variably condensed nuclei along their lengths. Similar abnormal nuclei and spindle structures were observed when *snxA1/nimA1/*GFP-*tubA* cells and *snxA1/nimA5/*GFP-*tubA* cells were germinated at 32°C and upshifted to 44°C for 3 hours, suggesting that the effects of *snxA1* on *nimA* are not allele-specific. After 3 hours at 44°C, 69% of *snxA1/nimA1/*GFP-*tubA* cells had abnormal nuclei, and 56% had abnormal spindles; similar results were obtained with *snxA1/nimA5/*GFP-*tubA* cells. Confocal microscopy of the abnormal spindles shows highly unusual spindle structures, which are more severe in cells carrying the *snxA1/nimA5* double mutant; *snxA1/nimA1* double mutant cells often have significantly shortened spindles. Efforts to clone the *snxA* gene are ongoing and should aid in the understanding of the interactions of the *snxA* and *nimA* genes in mitotic control. Supported by NIH R15GM55885 and NIH RR016476 from the MFGN INBRE program of the NCRR.

18. Role of vesicular trafficking in nutrient sensing. Margaret E. Katz[1], Cara J. Evans[1], Joan M. Kelly[2] and Brian F. Cheetham[1], [1]University of New England, Armidale, NSW Australia and [2]University of Adelaide, SA, Australia. mkatz@une.edu.au

The *xprG* gene encodes a putative transcriptional activator involved in the response to nutrient limitation. Strains carrying an *xprG*$^-$ null mutation do not produce extracellular proteases in response to carbon limitation. We have isolated revertants of an *xprG*$^-$ mutant. The revertants carry mutations in genes which we have named suppressors of *xprG* (*sogA*, *sogB* and *sogC*). Two of the revertants carry chromosome rearrangements. A combination of genetic mapping and Southern blot analysis was used to show that the translocation breakpoint in the *sogA1* mutant was located in a gene encoding a sorting nexin (Vps5). Transformation with a wild type copy of the gene confirmed that the *sogA* gene encodes Vps5. Mapping data indicated that *sogB* was tightly linked to a gene on chromosome VII encoding another component of the multivesicular body pathway, Vps17 and complementation of *sogB1* with the gene encoding the *A. nidulans* Vps17 homolog confirmed the identification. Vps17 and Vps 5 are part of a complex that has been shown to be involved in sorting of vacuolar proteins (*e.g.* intracellular proteases) in yeast and regulation of cell-surface receptors in mammals. Two hypotheses could account for the ability of mutations in *sogA* and *sogB* to suppress loss-of-function mutations in *xprG*. Mutations in *sogA* and *sogB* may permit secretion of intracellular proteases (as in *S. cerevisiae*) or an alteration in cell-surface receptors may trigger an XprG-independent mechanism for activation of extracellular protease gene expression.

19. A RasGAP protein involved in polarity establishment and maintenance in *Aspergillus nidulans.* Laura Harispe[1,2,3], Lisette Gorfinkiel[2], Cecilia Portela[2], Miguel A. Peñalva[3], and Claudio Scazzocchio[1]. [1]IGM, Univ. Paris-Sud XI. Bat 409 Centre d´Orsay. 91405 Orsay(France). [2]F. Ciencias, Univ. de la República. Iguá 4525. 11400 Montevideo(Uruguay) [3]CIB, CSIC, Ramiro de Maeztu 9. 28040 Madrid(Spain)

Filamentous fungi represent an extreme example of polarised growth[(a)]. We report on the identification and characterisation of GapA, an *A. nidulans* RasGAP involved in polarity establishment and maintenance. GapA was identified after serendipitously isolating a partial loss-of-function mutation, designated *gapA1*, in a genetic screen. Phenotypic characteristics resulting from *gapA* deletion include compact colony morphology, a marked delay in polarity establishment during conidial germination, impairment of polarised hyphal extension, a conspicuous developmental defect typically manifested by the absence of one layer of sterigmata in the conidiophore and a defect in the otherwise polarised distribution of the actin cytoskeleton. GapA-GFP protein fusion expressed from a gene replacement allel appears localises to hyphal tips and septa. This localisation suggests that a Ras protein(s), whose activity is antagonised by GapA plays a role in the regulation of the actin cytoskeleton at the hyphal tip and that this abnormal regulation underlies the polarity phenotypes associated with *gapA* loss-of-function.

[(a)] Momany M. (2002),Curr. Opinion in Microbiology, 5:580–585.

20. A link between N-myristoylation and proteasome activity. Soo Chan Lee and Brian D. Shaw Program for the Biology of Filamentous Fungi/Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX, 77840

Ubiquitin dependent proteolysis is a fundamental biological process regulating the half-life of proteins. The interaction between the 19S and 20S proteasome particles is essential for this activity. A subunit of the 19S particle, RptA in *A. nidulans*, has a conserved N-myristoylation motif. Orthologs of RptA are known to control substrate entry and gate the channel of 20S particle, but less is known about the role of N-myristoylation of the protein on its function. In our analyses of genetic suppressors of an A. nidulans N-myristoyl transferase mutant (*swoF1*), we found a mutation in a 20S proteasome alpha subunit that partially bypasses phenotypic defects of the *swoF1* mutant. To investigate the mode of suppression, we used monoclonal anti-ubiquitin antibody to measure the amount of the ubiquitinated proteins. The *swoF1* mutant accumulates fewer ubiquitinated proteins than does wild-type. The suppressor mutant, however, accumulated more. In addition, the abnormal hyphal growth phenotypes of the *swoF1* mutant were partially bypassed in the presence of MG132, a proteasome inhibitor. These data suggest that N-myristoylation negatively regulates the proteasome activity. We will discuss our ongoing investigation of the role of myristoylation of RptA protein on i) function of the proteasome, ii) the interaction between 19S and 20S proteasome and iii) the localization of 19S proteasome.

21. The role of ADP-ribosylation factors in cell morphogenesis of *Aspergillus nidulans.* Soo Chan Lee and Brian D. Shaw Program for the Biology of Filamentous Fungi/Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX, 77840

ADP-ribosylation factors (ARFs) are small GTPase proteins with several biological activities including vesicle formation and trafficking and, in yeast, bud site selection. In filamentous fungi, numerous vesicles are found at the growing tips and in the Spitzenkorper where they are thought to be active in secretion of cellular components, endocytosis, and maintenance of tip growth. The exact roles of the ARFs in filamentous fungi have not been established. ArfA::GFP localizes to cellular compartment which may be Golgi. ArfA::GFP was not co-localized with endocytosis machineries indicating ArfA is involved in exocytosis. ArfB::GFP localized to septa, a new cell wall synthesis site. Disruption of ArfB by transposon insertion resulted in loss of polarity during germ tube emergence and hyphal growth. A compromised Spitzenkorper was observed in the mutant. In addition, the arfB::Tn strain displayed delayed endocytosis. The Arf proteins have a conserved N-myristoylation motif. In *swoF1* (N-myristoyl transferase) mutant cells, ArfA::GFP and ArfB::GFP showed non-specific localization. In wild type cells, $ArfA^{G2A}$::GFP and $ArfB^{G2A}$::GFP, each with a G2A amino acid substitution that disrupts myristoylation, mislocalized. Interestingly overexpression of ArfA protein partially rescues the polarity defect of the *swoF1* mutant. These observations suggest that both endocytosis and exocytosis by the Arf proteins play a critical role in hyphal polarized growth in filamentous fungi and N-myristoylation determines subcellular localizations for ArfA and ArfB.

22. A putative MAP-kinase, MpkB, regulates natural product biosynthesis in *Aspergillus nidulans.* Dapeng Bao and Ana M. Calvo Department of Biological Sciences, Northern Illinois University, 1425 W Lincoln Hwy., Dekalb, Illinois. 60115, USA

Mitogen-activated protein kinase pathways transduce a large variety of external signals. This essential funtion is conserved in many eukaryotes, including fungi. In this work we studied the role of *mpkB*, encoding a putative homolog of the *Saccharomyces cerevisiae* FUS3 MAP-kinase, on the biosynthesis of natural products in the model filamentous fungus *Aspergillus nidulans*. We found that the *mpkB* loss-of-function mutant not only presented reduced production of the mycotoxin sterigmatocystin but also showed alterations in the biosynthesis of other natural products. The *mpkB* mutant only produced trace amounts of penicillin under conditions that promoted the production of this antibiotic in the wild type (approximately 27-fold greater). Furthermore, we found that expression of *ipnA*, encoding an isopenicillin synthase, is substantially reduced in the *mpkB* mutant. The complemented strain restored sterigmatocystin and penicillin wild-type levels. These results suggest that the MAPK signaling cascade regulates secondary metabolism in an adaptive response to environmental stimuli.

23. Effect of osmotic stress on sclerotial and aflatoxin production in *Aspergillus flavus*. Rocio M Duran and Ana M Calvo Department of Biological Sciences, Northern Illinois University, 1425 W Lincoln Hwy, DeKalb, Illinois.

Fungal cells respond to osmotic stress with molecular adaptations that result in chemical and morphological changes. We examined the effect of environmental osmotic stress on the fungus *Aspergillus flavus*. We found that hypertonic media differentially affected the formation of resistant structures, sclerotia, and the biosynthesis of the carcinogenic mycotoxin aflatoxin. While aflatoxin biosynthesis slightly increased in media with high concentration of sodium chloride or sorbitol, production of sclerotia was greatly reduced. The strong reduction of sclerotia was accompanied by an increase in colony growth and conidiophore formation. Search of the *A. flavus* genomic database allowed us to identify *AFhogA*, a gene encoding a putative homolog of the mitogen-activated protein kinase HOG1 that mediates the osmotic stress response in *Saccharomyces cerevisiae*. Additional components of this signaling pathway were also found in the *A. flavus* genomic sequence. We are further investigating the implications of this pathway in the regulation of resistant structure formation in this important plant pathogen.

24. A CLASP-related protein acts at the spindle pole body and spindle midzone during mitosis in *Aspergillus nidulans*. C. Tracy Zeng, and Bo Liu. Section of Plant Biology, University of California, Davis, CA 95616, USA.

In *A. nidulans*, microtubules undergo rapid reorganization during mitosis inside growing hyphae. Proteins like CLASP (CLIP170-associated protein) are known to play critical roles in regulating microtubule dynamics in mitotic spindles in animal cells. CLASP, also known as Orbit/Mast, is among a class of microtubule plus end-tracking proteins or +TIPs, and plays a critical role in attaching the kinetochore to microtubules during mitosis. In the *A. nidulans* genome, the AN0995 locus codes a polypeptide with limited sequence homology with the mammalian CLASP. In order to learn the function of this CLAA, for CLASP-like protein A, we attempted to knock out the *claA* gene by homologous recombination. Because none of over 100 transformants was viable, the result indicated that *claA* could be an essential gene. To further elucidate the function of CLAA, a strain was created in which a CLAA-GFP fusion protein was expressed under the control of the native promoter. CLAA-GFP only exhibited distinct localization pattern during mitosis. It first appeared at the spindle pole body. In addition to decorating the spindle pole body, at anaphase CLAA-GFP was associated with spindle midzone. In contrast to CLASP localization in animal cells, CLAA did not act as a typical +TIP in *A. nidulans*. Results of testing interactions between CLAA and other microtubule-associated proteins will be presented.

25. The roles of fimbrin, *fimA*, and alpha-actinin, *acnA*, in hyphal growth. Srijana Upadhyay[1], Aleksandra Virag[2], Soo Chan Lee[1], Steven D. Harris[2], and Brian D. Shaw[1]. [1]Dept of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas, 77803, USA. [2]Plant Science Initiative, University of Nebraska-Lincoln, N234 Beadle Center, 1901 Vine Street, Lincoln, NE 68588, USA.

We investigated the roles of actin (ActA) binding proteins fimbrin (FimA) and alpha-actinin (AcnA) in hyphal growth in *A. nidulans*. We have used live cell imaging to examine the distribution of ActA::GFP, FimA::GFP and AcnA::GFP. In actively growing hyphae cortical ActA::GFP and FimA::GFP patches are highly mobile and are concentrated near the hyphal apex, but a patch depeleted zone occupies the hyphal apex. FimA::GFP localizes transiently to septa. Localization of both ActA::GFP and FimA::GFP was disrupted after cytochalasin treatment. AcnA::GFP localizes to septal rings and has not been visualized at hyphal apices. A transposon insertional strategy was used to disrupt *fimA* resulting in germinating conidia with an extended isotropic growth phase followed by simultaneous emergence of multiple germ tubes. Colonies of the *fimA* disruptants are compact and conidiate poorly. Deletion of *acnA*, results in a severe hyphal growth defect leading to compact colonies that not sporulate. Endocytosis was severly impaired in the *fimA* disruption strain but was unaffected in the *acnA* deletion strain. ActA::GFP distribution in the *fimA* disruption strain, results in abnormal ActA::GFP distribution. A model for the roles of these proteins in hyphal growth is proposed.

26. A mutation in a GDP mannose pyrophosphorylase encoding gene leads to aberrant hyphal growth. *Brian D. Shaw, Gustavo Rebello, Soo Chan Lee, Srijana Upadhyay and Melissa Long Program for the Biology of Filamentous Fungi, Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas, 77803, USA.

The temperature sensitive *swoN1* mutant results in an aberrant hyphal growth pattern that differs significantly from wild type. Growth from conidia results in germlings with pronounced swollen sub-apical cell compartments as much as 20 micron in diameter. Growth and development at hyphal apices appears to proceed similarly to wild type. The *swoN1* mutant was complemented using a genomic library, in which two genes were identified with potential to restore growth of the mutant to wild type levels. These genes are AN1911.3 and AN5586.3 as designated by the Broad Institute *A. nidulans* genomic database; each encode proteins with predicted similarity to GDP mannose pyrophosphorylase. We designate AN1911.3 *swoN* since it contains a point mutation at position 1240 bp after start of genomic sequence and 1093 bp after the start codon of coding sequence. This point mutation results in a predicted residue change of serine to a phenylalanine at amino acid 365 of the protein. The *swoN1* mutant also exhibits an altered staining pattern of the mannoprotein stain Alcian Blue, relative to wild type. This gene along with *swoM*, *manA* and *swoA* represents the fourth identified in *A. nidulans* that is likely to participate in protein mannosylation. A model for the role of protein mannosylation in hyphal growth is discussed.

27. The *Aspergillus nidulans snoA* inhibitor of cell division associates with the BRDF checkpoint domain of *nimO*^{Dbf4}. Steve James, James Barra, Megan Campbell, and Matthew Denholtz. Biology Department, Gettysburg College, Gettysburg, PA. sjames@gettysburg.edu

In *Aspergillus nidulans*, *nimO*^{Dbf4} and *cdc7* encode regulatory and catalytic subunits of the conserved DBF4-dependent kinase (DDK). DDK initiates DNA synthesis by phosphorylating the replicative DNA helicase to trigger DNA unwinding at origins of replication. In addition, DBF4 plays an important role in the DNA damage response. This role is mediated by an N-terminal BRDF motif (*BR*CT and *D*B*F*4 similarity domain), as revealed by mutations in yeast homologs that confer enhanced sensitivity to DNA damage agents and failure to restrain DNA synthesis during genotoxic stress. In *Saccharomyces cerevisiae*, *RAD53/CHK2* kinase is the only checkpoint mediator known to associate directly with the *DBF4* BRDF motif. We identified a novel inhibitor of *nimO*^{Dbf4} called *snoA* (*s*uppressor-of-*n*im*O*). Loss of *snoA* rescues *nimO*18 ts-lethality and hypomorphic *nimO*+ expression. Conversely, *snoA* overexpression confers a dose-dependent, lethal interphase cell cycle arrest in *nimO*18 cells. Here we report a novel interaction between the *nimO* BRDF motif and *snoA*. Using yeast two-hybrid analysis, we demonstrate that a short (~100 amino acid) serine- and proline-rich region in the *snoA* C-terminus can associate with the *nimO* BRDF. This novel discovery suggests that *snoA* may act to regulate normal DNA synthesis or to exert S phase checkpoint control by direct association with the *A. nidulans* DBF4-dependent kinase. (Supported by NSF-RUI #01-14446 to SJ)

28. *Aspergillus oryzae atfA* encodes a transcription factor, which is required for vigorous growth in the solid-state fermentation. K. Sakamoto[1], O. Yamada[1], Y. Okita[1], K. Iwashita[1], O. Akita[2], K. Gomi[3], S. Mikami[1] 1 National Research Institute of Brewing, 3-7-1 Kagamiyama Higashi-Hiroshima, Hiroshima, Japan. 2 Jissen women's University, 4-1-1 Osakaue, Hino, Tokyo, Japan. 3 Tohoku University, 1-1 Tsutsumidori-Amamiyatyou Aoba Sendai, Miyagi, Japan

In the solid-state culture, *Aspergillus oryzae* exhibits phenotypes such as the high production of enzymes and conidiophore development. Though these characteristics should involve various gene expressions, the only a few regulatory systems have been understood. From the EST database of *A.oryzae*, we found a gene encoding transcription factors that show high homology to *atf1* of *Shizosaccharomyces pombe* and named it *atfA*. We constructed *atfA*-deletion strain (DelA51) to analyze the function. The germination ratio of DelA51 conidia was reduced to 13.7%, while that of wild type (wt) conidia was over 90 %. Furthermore the DelA51 conidia were more sensitive to stress than wt. Although DelA51 grew as fast as wt in the submerged liquid culture, the growth of DelA51 was attenuated in solid-state fermentation. High humidity of atmosphere restored the growth of DelA51. Thus we concluded that *atfA* is necessary for the vigorous growth on the low water activity substrate. To determine *atfA*-regulated genes, we identified 34 cDNAs down regulated in DelA51 under osmotic stress condition using mycroarray.

29. Putative mannose transporters complement a branching/septation defect in *Aspergillus nidulans*. Loretta Jackson-Hayes, Lauren Fay, Terry W. Hill and Darlene M. Loprete Departments of Biology and Chemistry, Rhodes College, Memphis, TN 38112. jacksonhayesl@rhodes.edu

In order to identify novel genes affecting cell wall integrity, we have generated mutant strains of the filamentous fungus *Aspergillus nidulans*, which show hypersensitivity to the chitin synthase inhibitor Calcofluor White (CFW). The phenotype of one of these strains (R205) also shows morphological abnormalities related to branching and septation. We have cloned two DNA fragments from an *A. nidulans* genomic DNA library which improve resistance to CFW and restore a more normal phenotype. One fragment is gene AN8848.3, “MT1”, which shows homology to GDP- mannose transporters. The second fragment is gene AN9298.3, “MT2”, which is a similar but distinct gene also homologous to GDP-mannose transporters. When separately cloned, the putative GDP-mannose transporters restore normal phenotype including full restoration of subapical hyphal compartment length and branch density in the mutant. Sequencing reveals a genetic lesion in Exon 5 of MT1 which causes an alanine to proline substitution and no mutation in MT2 in mutant strain R205. Cloned R205 MT1 containing the Exon 5 mutation does not complement the R205 phenotype. Attempts to produce null mutants of MT1 did not produce viable transformants, suggesting that AN8848.3 is an essential gene. MT2 null mutants grow normally under normal growth conditions and show wild type CFW resistance.

30. Characterization of *radC*, the *Aspergillus nidulans* homolog of *RAD52*, a key gene for DNA repair by homologous recombination. Michael Lynge Nielsen, Gaëlle Lettier, Jakob Blæsbjerg Nielsen and Uffe Hasbro Mortensen

Repair of DNA double-strand breaks (DSBs) is crucial for maintaining genome integrity and failure to repair even a single DNA DSB is lethal as it causes loss of part of a chromosome during cell division. In the yeast, *Saccharomyces cerevisiae*, Rad52 plays a fundamental role in the repair of DSBs by homologous recombination (HR) and among genes involved in DNA DSB repair and HR deletion of *RAD52* produces the most dramatic phenotype. Hence, *S. cerevisiae* cells lacking Rad52 are impaired in DSB repair and all types of HR including targeted integration of transforming DNA. Orthologs of *RAD52* have been identified in other organisms, including *Schizosaccharomyces pombe*, *Aspergillus nidulans*, chicken, mouse, and human. However, in contrast to *S. cerevisiae*, the absence of *RAD52* in higher eukaryotes does not result in a severe phenotype, due to the evolution of additional repair pathways and the role of Rad52 in DNA DSB repair and HR remains unclear. To address the role of Rad52 in higher eukaryotes, we have recently constructed a *radC* (homolog of *RAD52*) deletion mutant in *A. nidulans* and performed an initial characterization of the mutant strain. Unlike in *S. cerevisiae*, where Rad52 is essential for the repair of all types of DNA lesions that require HR, we show that in *A. nidulans* it is only required for the repair of a subset of these lesions. Hence, in an evolutionary perspective, HR repair in *A. nidulans* may represent an intermediate state between *S. cerevisiae* and human and *A. nidulans* may therefore be a useful model to further the understanding of HR in higher eukaryotes.

COMPARATIVE AND FUNCTIONAL GENOMICS

31. Chemogenomics of antioxidant inhibition of aflatoxin biosynthesis. Jong Kim[1], Jiujiang Yu[2,3], Noreen Mahoney[1], Kathleen Chan[1], Deepak Bhatnagar[2], Thomas Cleveland[2], Russell Molyneux[1], William Nierman[3,4] and Bruce Campbell[1*]. [1]Plant Mycotoxin Research, USDA, Albany, CA 94710 USA. bcc@pw.usda.gov. [2]Food and Feed Safety Research, USDA, New Orleans, LA 70124 USA. [3]The Institute of Genomic Research, Rockville, MD 20850 USA. [4]The George Washington University, Washington, DC 20006 USA.

Natural compounds were used to probe the functional genomics (chemogenomics) of aflatoxin biosynthesis. Caffeic acid (12mM) treatment of *Aspergillus flavus* inhibited aflatoxin biosynthesis without affecting growth. Microarray analysis showed genes in the aflatoxin biosynthetic cluster were completely down-regulated (log2 ratio –0.04 to –3.13). However, aflatoxin pathway regulator genes, *aflJ* or *laeA*, and sugar utilization cluster genes showed only minor changes in expression (log2 ratio 0.08 to –0.58). Genes in amino acid biosynthetic and aromatic compound metabolism were up-regulated (log2 ratio > 1.5). Most notable was up-regulation (log2 ratio 1.08 to 2.65, qRT-PCR) of four peroxiredoxin genes orthologous to the *AHP1* gene (alkyl hydroperoxide reductase) of *Saccharomyces cerevisiae*. Antioxidants trigger induction of *ahp* genes in *A. flavus* to protect the fungus from oxidizing agents, e.g., lipoperoxides, reactive oxygen species, etc. This detoxification attenuates upstream oxidative stress-response pathway signals, thus suppressing aflatoxigenesis.

32. Transcriptional activators of *Aspergillus nidulans* sulfur metabolism. Sebastian Pilsyk, Jerzy Brzywczy and Andrzej Paszewski. Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawiñskiego 5A, 02-106 Warsaw, Poland e-mail: Seba@ibb.waw.pl

Aspergillus nidulans metR gene encodes a bZIP transcription factor specific for activation of several sulfur metabolism genes including these encoding the sulfate assimilation pathway enzymes. METR protein level and/or activity is negatively regulated by SCF ubiquitin ligase complex which consists of the proteins encoded by the *scon* (sulfur controller) genes. Both *metR* and *scon* genes constitute a regulatory system known as sulfur metabolite repression (SMR). Loss of function mutations in the *metR* gene cause methionine auxotrophy while mutations in the *scon* genes lead to a derepression of the sulfate assimilation pathway enzymes. All *metR* and *scon* mutants isolated so far were recessive. Recently, we have isolated three dominant suppressor mutants in a diploid strain homozygotic for *metB3* allele. Genetic and molecular analysis showed that Phe48 codon of the *metR* gene is affected in every of the dominant mutants obtained (*MetR20*, *21* and *22*). The orthologs of the *metR* gene were also found in other fungal species and Phe48 appears to be one of the most conserved amino acids of the N-terminal domain which suggests that this residue is important for METR protein function and/or stability. *MetR20* mutant has elevated acivity of the sulfur metabolism enzymes in comparison to the wild type. Suppressor *metR* mutants have also an increased level of sulfur compounds in mycelia which suggests that they are resistant to SMR. By searching of the *A. nidulans* genome sequence, we have found a paralog of the METR protein that encoded protein has similar basic region and leucine zipper sequences. The gene we named *metZ* is well conserved among other *Aspergilli* and it has apparently unusual large conserved intron as long as intron in the *metR* gene. Northern blot analysis indicates that the *metZ* gene is regulated by SMR.

33. Relative Protein Quantification through Stable Isotope Labeling by Amino Acids in *Aspergillus flavus*: Temperature Regulation of Aflatoxin Biosynthesis. D. Ryan Georgianna[1,3], David C. Muddiman[2], and Gary A. Payne[1]. [1]Department of Plant Pathology and Center for Integrated Fungal Research. [2]Department of Chemistry. [3]Functional Genomics Graduate Program. North Carolina State University,Raleigh, NC 27606 USA.

Aflatoxin biosynthesis is inhibited at 37C, the optimum temperature for growth of *Aspergillus flavus*. Transcriptional analysis has shown that all aflatoxin biosynthetic genes except the pathway regulatory genes *aflR* and *aflS* are down regulated at 37C relative to 28C, suggesting that AFLR may be modified at 37C. To quantify the response of AFLR and other proteins to high temperature we adapted a stable isotope labeling by amino acids (SILAC) strategy for relative protein quantification in *A. flavus*. SILAC relies on the quantitative incorporation of labeled amino acids into proteins to provide a powerful mass spectrometry based proteomics tool useful for both the rapid quantification of proteins and identification of interactions. This technique has been used in several systems; including yeast, mammalian cells, and *Arabidopsis* cell culture. Samples were prepared using in-gel trypsin digestion of selected 1D-PAGE gel slices and analyzed on an ESI LTQ linear ion trap mass- spectrometer directly coupled to RP-HPLC. The *A. flavus* labeling strategy was optimized to provide a homogenously labeled sample with ~90% incorporation of [13C6] arginine. Furthermore, we found that the relative abundance of aflatoxin pathway enzymes compared between 28C and 37C is consistent with the relative abundance of their encoding transcripts at the respective temperatures. This is the first report of SILAC being used to quantify proteins in a filamentous fungus as well as a multi-cellular free-living prototrophic organism.

34. Comparative and functional genomics in identifying aflatoxin biosynthetic genes. Jiujiang Yu[1,7], Jeffery Wilkinson[2], Gary Payne[3], Masayuki Machida[4], Bruce Campbell[5], Joan Bennett[6], Deepak Bhatnagar[1], Thomas Cleveland[1], and William Nierman[7,8]. [1]USDA/ARS, Southern Regional Research Center, New Orleans, LA 70124, USA; [2]Mississippi State University, Mississippi State, MS 39762, USA; [3]North Carolina State University, Raleigh, NC 27695, USA; [4]National Institute of Advanced Industrial Science and Technologies (AIST), Tsukuba, Ibaraki, Japan; [5]USDA/ARS, Western Regional Research Center, Albany, CA 94710, USA; [6]Rutgers University, New Brunswick, NJ 08901, USA; [7]The Institute for Genomic Research, Rockville, MD 20850, USA; [8]The George Washington University School of Medicine, Washington, DC 20037, USA

Identification of genes involved in aflatoxin biosynthesis through *Aspergillus flavus* genomics has been actively pursued. *A. flavus* Expressed Sequence Tags (EST) and whole genome sequencing have been completed. Groups of genes that are potentially involved in aflatoxin production have been profiled using microarrays under different culture conditions and during fungal infection of corn. Preliminary annotation of the sequence revealed that there are about 12,000 genes in the *A. flavus* genome. Many genes in the genome, which potentially encode for enzymes involved in secondary metabolite production, such as polyketide synthases, non-ribosomal peptide synthases, cytochrome P450 monooxigenases, have been identified. Comparative analysis of *A. flavus* genome with food grade industrial fermentation organism *A. oryzae* can help understanding the mechanism of aflatoxin biosynthesis and solving the problem of aflatoxin contamination.

35. Growth and developmental control in *Aspergillus nidulans* and *A. fumigatus*. Jae-Hyung Mah and Jae-Hyuk Yu Department of Bacteriology, University of Wisconsin, Madison, WI 53706

The opportunistic human pathogen *Aspergillus fumigatus* reproduces by forming a large number of asexual spores. We studied the mechanisms regulating asexual development in *A. fumigatus* via examining functions of four key controllers, GpaA (G alpha), AfFlbA (RGS), AfFluG and AfBrlA. Expression analyses of gpaA, AfflbA, AffluG, AfbrlA and AfwetA revealed that, whereas transcripts of AfflbA and AffluG accumulate constantly, AfbrlA and AfwetA are specifically expressed during conidiation. Both loss of function AfflbA and dominant activating GpaAQ204L mutations resulted in reduced conidiation coupled with increased hyphal mass, indicating that GpaA mediates signaling that activates vegetative growth while inhibiting conidiation. As GpaA is the principal target for AfFlbA, the dominant interfering GpaAG203R mutation suppressed the phenotype resulting from loss of AfflbA function. These results corroborate the idea that parimary roles of G proteins and RGSs are conserved in aspergilli. Functions of the two major developmental activators AfFluG and AfBrlA are then examined. While deletion of AfbrlA eliminated conidiation completely, deletion of AffluG did not cause severe defects in *A. fumigatus* sporulation in air-exposed culture, implying that, whereas the two Aspergillus species may have a common key downstream developmental activator, upstream mechanisms activating brlA may be distinct. Finally, both AffluG and AfflbA mutants showed reduced conidiation and delayed accumulation of AfbrlA mRNA in developmental induction, indicating that these upstream regulators are associated with the proper progression of conidiation.

36. Integrated database for functional analysis in *Aspergillus flavus*.C. P. Smith[1], C. P. Woloshuk[2], N. P. Keller[3], J. Yu[4], and G. A. Payne[5] [1]North Carolina State University, Raleigh, USA. chris@statgen.ncsu.edu. [2] Purdue University, West Lafayette, IN, USA. woloshuk@purdue.edu. [3]University of Wisconsin, Madison, WI, USA. npk@plantpath.wisc.edu. [4]USDA/ARS/SRRC, New Orleans, LA, USA. jiuyu@srrc.ars.usda.gov. [5]North Carolina State University, Raleigh, USA. gary_payne@ncsu.edu.

Aspergillus flavus is a plant and animal pathogen that also produces the carcinogen, aflatoxin. Because of its economic importance and well characterized pathway of aflatoxin biosynthesis, several labs are studying the development, metabolism, ecology, and pathogenicity of this fungus. To facilitate the research efforts in these areas and to identify potential genes and pathways for functional analysis, we are developing a database to integrate multiple categories of data. This database resource will serve two important functions: 1) it will provide a platform for the deposition of data from individual experiments; and 2) it will permit the ready analysis of composite data from all experiments enabling researchers to mine a larger data set. It will include phenotypic measurements, gene expression data from microarrays, and metabolic profile information and will be flexible enough to allow the addition of new types of measurement in the future. Users will interact with the database through a web based interface and will be able to: describe experiments; upload data gathered during those experiments; run analyses on the data; select and download raw data; select and download the results of analyses. Recently acquired lab and field data will be used to highlight the structure and utility of this database. These measurements will include fungal growth, aflatoxin concentrations, and gene expression data acquired from lab studies and infected developing maize seeds. This research was funded by USDA/NRI/CGP 2006-35604-16666.

37. Aspergillus Comparative Database: a web-based tool for comparative analysis. Vinita Joardar, Jonathan Crabtree, Rama Maiti, Natalie Fedorova, Paolo Amedeo, Samuel Angiuoli, William Nierman, Owen R. White, and Jennifer R. Wortman. The Institute for Genomic Research, Rockville, MD, USA. vinita@tigr.org

Comparative genome analysis in the genus Aspergillus has been facilitated by the availability of genome sequences for multiple species. Ortholog clusters were computed based on the mutual best blastP hits between the Aspergillus proteomes. Syntenic blocks were identified by searching for collinear orthologs, with allowances for gaps and rearrangements, along the full chromosomes and/or supercontigs. The results of the genome and proteome level computes for the Aspergillus genomes were stored in the Aspergillus Comparative Database (asp), a chado relational database. Sybil, a web-based software package developed at TIGR, was used for visualization and analysis of comparative genomics data. Sybil uses a graphical user interface to present and navigate the information stored in asp. The interactive graphical displays allow the user to navigate from global genome views down to specific protein reports. Protein cluster reports, lists of singletons, comparative sequence displays and publication-quality figures can be customized based on user specifications. The Sybil package also leverages the comparative data for annotation improvement. We present an overview of the Sybil package applied to the comparative analysis of aspergilli (http://www.tigr.org/sybil/asp/index.html). The Sybil visualization software is freely available for download from http://sybil.sf.net/

38. A comparison of the expression of secreted hydrolases in *Aspergillus fumigatus* and *Aspergillus nidulans* under phospholipid rich conditions. Manda Gent, Karin Lanthaler, David Denning, Michael Wilson, Stephen Oliver and Geoffrey Robson. Faculty of Life Sciences, University of Manchester, Oxford Road, Manchester M13 9PT, UK.

Aspergillus fumigatus is an opportunistic pathogen of man and is a particular risk for immunocompromised patients. Considerable research is being directed to the identification of pathogenicity determinants which may explain the virulence of *A. fumigatus* over other common environmental fungal species. Extracellular phospholipases have been shown to be important virulence determinants in the pathogenesis of several bacterial infections where they cause tissue damage and necrosis. It was recently demonstrated that clinical isolates of *A. fumigatus* produce significantly higher levels of extracellular phospholipase C activity compared with environmental isolates [Birch *et al*., 2004]. This may be important in disease development, as infection usually occurs through the inhalation of airborne conidia and the surface of the alveoli is coated in surfactant which is composed of >80% phospholipid. We have examined gene regulation in the presence and absence of lecithin, a phosphatidylcholine phospholipid, similar to that found in the lung, using the TIGR microarrays of the *A. fumigatus* and *A. nidulans* genomes. Analysis showed significant differential regulation of a number of secreted lipases as well as known allergens and virulence-related genes in *A. fumigatus* and the far less pathogenic *A. nidulans* under the same conditions.

39. Revealing components of the glucose sensing and repression pathways in *Aspergillus niger.* Margarita Salazar*, Michael L. Nielsen, Jens Nielsen Center for Microbial Biotechnology, BioCentrum, Technical University of Denmark, DK 2800 Kgs. Lyngby, Denmark. *masa@biocentrum.dtu.dk

Carbon repression is a global regulatory mechanism in which the presence of glucose or other readily metabolized carbohydrates represses expression of genes involved in the utilization of less-favored carbon sources. Mig1 is the major transcription factor responsible for carbon catabolite repression in *Saccharomyces cerevisiae* and its homologue, CreA is present in several aspergilli. Nevertheless, no mechanisms have been identified by which CreA is regulated, as well as no components for glucose sensing have been recognized. Comparative analysis of proteins involved in glucose sensing and repression pathways in *S. cerevisiae*, *A. niger*, *A. nidulans* and *A. oryzae* was performed. The analysis showed that some proteins involved in carbon catabolite repression in yeast may also play a role in aspergilli. The sensors Snf3 and Rgt2 from *S. cerevisiae* seem to be present in *A. niger*, as well as homologues to the regulatory protein Grr1 (e-value = $3E^{-92}$, score = 335) and the protein phosphatase Glc7 (e-value = $1E^{-149}$, score = 523) involved in regulating the activity of the Snf1-complex. A disrupted *grr1* mutant was constructed through the bipartite gene targeting method. The deduced amino acid sequence from the *grr1* locus showed 59 % identity to the *Aspergillus nidulans* FGSC A4 gene product (1576 aa). According to the Joint Genome Institute, the coding sequence is 4.8 kb long with the capacity to express a 1615 aa protein interrupted by five introns. Characterization of the *grr1* mutant is being performed and the morphology compared to the wild type was different during growth on several media plates. This study will be complemented by transcriptome analysis.
The authors acknowledge Conacyt and SEP Mexico for providing research fellowship to Margarita Salazar.

40. Dual genome microarray: *Fusarium verticillioides* and *Aspergillus flavus* gene expression in co-culture. Daren W. Brown Mycotoxin Research Unit, U.S. Department of Agriculture-ARS, Peoria, IL 61604

Aflatoxins produced by *Aspergillus flavus* and fumonisins produced by *Fusarium verticillioides* are prominent among the mycotoxins associated with economic losses to the maize grain industry worldwide. *F. verticillioides* is also recognized as a systemic endophyte of maize that prevents opportunistic saprotrophs such as *A. flavus* from spreading within the ear and rotting the seeds. Protective endophtyes are potential sources of gene products that can suppress fungal growth or silence genes critical to mycotoxin synthesis that are *a priori* adapted to function *in planta*. *F. verticillioides* genes that serve to augment maize host defenses are likely to exhibit patterns of expression that are correlated with exposure to fungal competitors. We have designed a NimbleGen Systems microarray based on over 25,000 *A. flavus* and *F. verticillioides* genes derived from genomic and EST data. Each probe set consists of up to twelve 60-mer oligonucleotides specific to each gene sequence. This report describes gene expression changes in both *F. verticillioides* and *A. flavus* due to co-culture on a maize based medium.

41. Comparative analysis of secondary metabolism gene clusters from two strains of *Aspergillus fumigatus* and closely related species *Neosartorya fischeri* and *Aspergillus clavatus*. Natalie Fedorova[1], Vinita Joardar[1], Jonathan Crabtree[1], Rama Maiti[1], Paolo Amedeo[1], David Denning[2], Jennifer Wortman[1], Geoffrey Turner[3], and William Nierman[1]. [1]The Institute for Genomic Research, Rockville, MD, USA; [2]School of Medicine and Faculty of Life Sciences, The University of Manchester, Manchester, UK; [3]Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield, UK. Email: natalief@tigr.org

Genes responsible for biosynthesis of secondary metabolites are often referred to as the most variable segment of fungal genomes. Comparative analysis of secondary metabolism gene clusters from *A. fumigatus* and the two closely related species confirmed that these organisms are indeed relentlessly updating their repertoire of mycotoxins. Out of 23 clusters identified in *A. fumigatus*, only seven have putative orthologs in the other two aspergilli. Most of these 'core' clusters are highly conserved and appear to be under strong negative selection. The rest of the clusters, however, are species- or even strain-specific, implying that the aspergilli must utilize specific mechanisms to ensure a quick divergence and turnover of clusters in the course of evolution. These mechanisms may involve de-novo assembly, segmental duplication, translocation, accelerated differentiation, and differential loss of clusters in various lineages.

42. Improvements in *Aspergillus fumigatus* annotation. Paolo Amedeo, Natalie Fedorova, Rama Maiti, Vinita Joardar, Crabtree Jonathan, Samuel Angiuoli, William Nierman, Owen White, Jennifer Russo Wortman The Institute for Genomic Research, 9712 Medical Center Dr., Rockville, MD 20850

Aspergillus fumigatus has been one among the first fungal genomes to be fully sequenced and annotated. The almost complete absence of genes belonging to closely-related organisms, in public databases, had influenced negatively the quality of annotation. Recently, several other *Aspergillus* species have been fully sequenced and annotated. Moreover, during these past years, annotation tools have been considerably improved. Leveraging on comparative genomic analysis we have revised the annotation of this genome. Here we describe the process taken and demonstrate the improvements.

43. Metabolic network-driven analysis of genome-wide transcription data from *Aspergillus nidulans*. Helga Moreira David[1], Gerald Hofmann[2], Ana Paula Oliveira[2], Hanne Jarmer[3], and Jens Nielsen[2]. [1]Fluxome Sciences A/S, Diplomvej 378, 2800 Lyngby, Denmark [2] Center for Microbial Biotechnology, Technical University of Denmark, 2800 Lyngby, Denmark [3] Center for Biological Sequence Analysis, Technical University of Denmark, 2800 Lyngby, Denmark

Aspergillus nidulans is a model organism for aspergilli, an important group of filamentous fungi that encompasses human and plant pathogens, as well as industrial cell factories. Aspergilli have a highly diversified metabolism and, because of their medical, agricultural and biotechnological importance, it is valuable to understand how their metabolism is regulated. We therefore performed genome-wide transcription analysis of *A. nidulans* grown on glucose, glycerol, and ethanol with the objective of identifying global regulatory structures. We furthermore reconstructed the complete metabolic network of this organism, which resulted in linking 666 genes to metabolic functions, as well as assigning metabolic roles to 472 genes that were previously uncharacterized. Through combination of the reconstructed metabolic network and the transcription data, we identified subnetwork structures that pointed to coordinated regulation of genes involved in many different parts of the metabolism. Thus, for a shift from glucose to ethanol, we identified a coordinated regulation of the complete pathway for oxidation of ethanol, as well as up-regulation of gluconeogenesis and down-regulation of glycolysis and the Pentose Phosphate (PP) pathway. Furthermore, upon a change in the carbon source from glucose to ethanol, the cells shift from using the PP pathway as the major source of NADPH for biosynthesis to use of the malic enzyme. Our analysis indicated that some of the genes are regulated by common transcription factors, making it possible to establish new putative links between known transcription factors and genes, through clustering.

FUNGAL-HOST INTERACTIONS

44. Translating and Coping with Stress: eIF2? kinases and the Cross-Pathway Control System of *Aspergillus fumigatus*. Christoph Sasse[1], Elaine M. Bignell[2], Stanley Kim[3], Gerhard H. Braus[1], Sven Krappmann[1]. [1]Georg-August University Goettingen, Grisebachstr. 8, D-37077 Goettingen, Germany; [2]Imperial College London, UK; [3]The Institute for Genomic Research, USA

Aspergilli represent unique pathogens with *Aspergillus fumigatus* being the predominant perpetrator. We are interested in nutritional requirements sustaining propagation and supporting virulence. Fungal amino acid (aa) biosynthesis is regulated on various levels; besides pathway-specific systems, one global regulatory network has evolved that acts on aa metabolism as a whole. In its very components, this *C*ross-*P*athway *C*ontrol is made up by an eIF2 kinase sensing aa deprivation and translating it into increased levels of the transcriptional activator protein CpcA, which in turn elevates transcription for the majority of amino acid biosynthetic genes. As deduced from profiling studies, the scope of the CpcA-directed transcriptome exceeds amino acid biosynthesis. To elucidate the role of the CPC signal transduction cascade, the gene of the sensor kinase CpcC was cloned. In contrast to *cpcAdelta* mutants, strains deleted for *cpcC* are not impaired in virulence, indicating that the basal expression level of CpcA is necessary and sufficient to support pathogenesis. Western blot analyses indicate that the *cpcC*-encoded kinase is not required exclusively to phosphorylate the eIF2 subunit. Upon inspection of the *A. fumigatus* genome, the presence of a related gene (*ifkB*, for initiation factor kinase) could be revealed. The *ifkB* gene was deleted in and corresponding mutant strains are currently evaluated.

45. Defense of *Aspergillus fumigatus* against reactive oxigen species mediated by Afyap1. Franziska Lessing, Olaf Kniemeyer and Axel A. Brakhage. Leibniz Institute for Natural Products and Infection Biology – Hans-Knoell-Institute Friedrich Schiller University, Jena, Germany Contact: franziska.lessing@hki-jena.de

With the increasing number of immunocompromised individuals *Aspergillus fumigatus* has become one of the most important opportunistic fungal pathogens. During infection *A. fumigatus* is confronted with a number of defence mechanisms in the host, particulary neutrophiles and macrophages, which kill conidia by producing reactive oxygen species. We identified a homologe of the AP1 like transcription factor *Yap1* from yeast in *A. fumigatus* which we designated *Afyap1*. In yeast, Yap1p was found to be a global regulator for oxidative stress response and required for the protection of the cell against H_2O_2 and other reactive oxygen species. Yap1 is transported in and out the nucleus under nonstressed conditions. The nuclear export is inhibited by oxidative stress and Yap1 induces the transcription of target genes. Nuclear loaclisation of an Afyap1-eGFP fusion in *A. fumigatus* was dependent on the presence of H_2O_2 and diamide. To identify new targets of *Afyap1*, we compared the proteome pattern from H_2O_2 induced and uninduced wild-type mycelia and a Afyap1 deletion strain by 2D-gel analysis.

46. Proteome analysis of the response of *Aspergillus fumigatus* to iron limitation. André D. Schmidt[1], Olaf Kniemeyer[1], Hubertus Haas[2] and Axel A. Brakhage[1]. [1]Leibniz Institute for Natural Product Research and Infection Biology (HKI) / Friedrich-Schiller-University Jena, Germany [2]Division of Molecular Biology/Biocenter, Innsbruck Medical University, Austria

The acquisition of iron is known to be an essential step in any microbial infection process due to iron-limiting conditions in the human host. This iron limitation is caused by high-affinity iron-binding proteins like transferrin or lactoferrin in the host. Since iron plays an essential role in key metabolic processes like DNA synthesis, oxidative phosphorylation or electron transport *A. fumigatus* has to overcome the iron deficiency by the synthesis of siderophores, which chelate iron. It was shown that an *A. fumigatus* strain unable to synthesize siderophores was attenuated in virulence in a murine infection model. To understand the cellular processes, induced by iron starvation, we analysed the proteome of *A. fumigatus* strain ATCC 46645 grown under iron-deficiency conditions. Under iron depletion, proteins involved in siderophore biosynthesis are upregulated, e.g. L-ornithine N^5-oxygenase (SidA), and iron cluster-containing proteins as aconitase or 3-isopropylmalate dehydratase are down-regulated. In addition, proteins involved in the heme biosynthesis are less abundant under iron-deficiency. Further proteins analysed under different non-linear pH-scales will be presented and their putative role will be discussed.

47. Putative G protein-coupled receptors GPRC and GPRD are involved in development and morphogenesis in *Aspergillus fumigatus.* Alexander Gehrke[1], Thorsten Heinekamp[1] and Axel A. Brakhage[1,2]. [1]Leibniz Institute for Natural Product Research and Infection Biology - Department of Molecular and Applied Microbiology - Hans-Knoell-Institute, [2]Friedrich-Schiller-University, Jena; eMail:alexander.gehrke@hki-jena.de

The opportunistic human-pathogen *Aspergillus fumigatus* was subject to recent studies on cAMP signal transduction with regard to morphogenesis and virulence. To date, one of the most important questions is still unanswered: what are the external signals and the corresponding proteins sensing those ligands or stimuli which enable the fungus to grow in a wide variety of different ecological niches? In a first approach, two genes encoding putative G protein-coupled receptors *gprC* and *gprD*, designated as carbon source-sensing receptors, were deleted in *A. fumigatus*. The physiological characterisation of the mutants revealed altered growth on solid media. However, various growth conditions, which included the use of different carbon- and nitrogen-sources, did not restore the defect of the mutants. Virulence of the mutant strains, as tested in a low-dose murine infection model, was attenuated. The function of the putative GPCRs was further investigated by analysing fluorescent protein-fusions *in vivo* by confocal microscopy. Recent data on the function of the receptors will be presented.

48. Transcriptional profile of *Aspergillus fumigatus* conidia in response to human neutrophils. Sugui, J.[1], Zarember, K.[1], Kim, S.[2], Chang, Y.[1], Nierman, W.[2], Gallin, J.[1] and Kwon-Chung, K.[1]. [1]NIH/NIAID, Bethesda, MD 20892 and [2]The Institute for Genomic Research, Rockville, MD 20850.

Neutrophils are cells of the innate immune system recruited to the sites of infection to phagocytose and kill microbial pathogens. Phagocytosis triggers the production of reactive oxygen species (ROS) as well as the fusion of cytoplasmic granules with the phagolysosome. We have previously shown that neutrophils from CGD patients, which generate little to no ROS, are capable of inhibiting *Aspergillus fumigatus* (AF) conidial growth as efficiently as neutrophils from healthy individuals. This suggests that the inhibition depends on non-oxidative mechanisms. We challenged conidia with normal and CGD neutrophils and analyzed the transcriptional profile. Analysis of the microarray data identified a group of 245 genes that were up-regulated in response to neutrophils. The majority of these genes were up-regulated in conidia but not in hyphae. A general classification of these genes showed that 26% are involved in transport, 17% in transcriptional regulation, 13% are related to peroxisomes, 12% in C1-C3 metabolism and 9% in tricarboxylic/glyoxylate cycles. The increase in sugar transport and C1-C3 carbon metabolism suggests an increased requirement of carbon sources when conidia are challenged with neutrophils and fatty acids might be used as carbon source via the tricarboxylic and glyoxylate cycles. Real time PCR data confirmed the up-regulation of 3 genes involved in C1-C3 metabolism; isocitrate lyase (a key enzyme of the glyoxylate cycle), a peroxisomal biogenesis factor and a NAD-dependent formate dehydrogenase. Studies are underway to investigate the biology of AF conidia inside human neutrophils.

GENOME STRUCTURE AND MAINTENANCE

49. Transcriptome analysis of *Aspergillus nidulans* exposed to camptothecin-induced DNA damage. Iran Malavazi[1], Marcela Savoldi[1], Sônia Marli Zingaretti Di Mauro[2], Carlos Frederico Martins Menck[3], Steven D. Harris[4], Maria Helena de Souza Goldman[5], and Gustavo Henrique Goldman[1]. [1]Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Brazil [2] Universidade de Ribeirão Preto, São Paulo, Brazil, [3] Instituto de Ciências Biomédicas, Universidade de São Paulo, Brazil, [4] Plant Science Initiative, University of Nebraska, Lincoln, Nebraska, [5]Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto Universidade de São Paulo, Brazil.

We have used an *Aspergillus nidulans* macroarray carrying sequences of 2,787 genes from this fungus to monitor gene expression of both wild-type and $uvsB^{ATR}$ deletion strains in a time course exposure to camptothecin (CPT). The results revealed a total of 1,512 and 1,700 genes in the wild-type and $uvsB^{ATR}$ deletion strain respectively that displayed a statistically significant difference in at least one experimental time point. We characterized six genes that have increased mRNA expression in the presence of CPT in the wild-type strain relative to the $uvsB^{ATR}$ mutant strain: *fhdA* (forkhead associated domain protein), *tprA* (hypothetical protein that contains a tetratrico peptide repeat), *mshA* (MutS homologue involved in mismatch repair), *phbA* (prohibitin homologue), $uvsC^{RAD51}$ (RAD51 homologue), and *cshA* (homologue of the excision repair protein ERCC-6 [Cockayne's syndrome protein]). The induced transcript levels of these genes in the presence of CPT require $uvsB^{ATR}$. These genes were deleted, and surprisingly, only the delta *uvsC* mutant strain was sensitive to CPT; however, the others displayed sensitivity to a range of DNA-damaging and oxidative stress agents. These results indicate that the selected genes when inactivated display very complex and heterogeneous sensitivity behavior during growth in the presence of agents that directly or indirectly cause DNA damage. Moreover, with the exception of UvsC, deletion of each of these genes partially suppressed the sensitivity of the delta *uvsB* strain to menadione and paraquat. Our results provide the first insight into the overall complexity of the response to DNA damage in filamentous fungi and suggest that multiple pathways may act in parallel to mediate DNA repair.
Financial Support: FAPESP and CNPq Brazil

INDUSTRIAL BIOLOGY

50. New screening approaches for fungal strain development. Peter J. Punt[1], Xavier O. Weenink[2], Marc van der Maarel[1], Jan Jore[1], Arthur Ram[2], Cees van den Hondel[2]. [1]TNO Quality of Life, Zeist, the Netherlands,[2]Leiden University, Leiden, the Netherlands

Since the development of recombinant DNA technologies for yeast and filamentous fungi, a considerable part of the strain development programs was diverted to the use of molecular genetic tools. Whereas these approaches have exerted considerable success, recent developments in our laboratory have shown that new developments in classical biological screening approaches, or a combination of both, can still be very useful. A first purely classical approach is based on the discovery of a so-called suicide (SUI) substrate, which we have successfully used for the selection of protease deficient fungal host strains. These protease deficient strains show an increased resistance to the SUI substrate allowing their selection. The advantage of this non-GMO approach is that it can be applied to new and already established production strains. A combination of a molecular and classical approach is based on the use of the so-called glucoamylase carrier approach. Combining this approach with fungal strains unable to use starch as a carbon source allowed us to select for hyper secretive fungal strains generated by classical mutagenesis (Weenink et al., 2006). Moreover, the same approach also allows for selection of the highest producers in a collection of primary transformant strains expressing a glucoamylase-fusion gene.

51. Engineering of a novel protein secretion pathway in *Aspergillus niger*. Robbert Damveld[1], Miranda Hartog[1], Peter ten Haaft[1], Inge Minneboo[1], Cees Sagt[1], Han de Winde[1], Thibaut Wenzel[1]. [1]DSM Food Specialties, P.O. Box 1, 2600 MA Delft, The Netherlands.

Production of enzymes on an industrial scale is often limited to extracellularly enzymes. When enzymes are produced intracellular, the downstream processing is costly and process robustness is not guaranteed. We have developed a technology which enables the secretion of enzymes which are normally localized in the cell. The folding conditions of intracellular proteins differ from those of secreted proteins. In the cytosol proteins fold under relative reducing conditions compared to the oxidizing environment in the ER. Moreover, many secreted proteins undergo extensive N- glycosylation and disulphide bridge formation. The set of folding enzymes and chaperones in the secretory pathway and in the cytosol are different as well. Therefore the simple solution of forcing intracellular proteins through the secretory pathway does in many cases fail to result in biologically active secreted enzymes. We have developed a method to secrete intracellular enzymes in an active form. This technology allows the intracellular enzymes to be folded in their native environment in the cytosol using cytosolic folding enzymes and chaperones. After folding the intracellular proteins are translocated to a modified cell compartment. After translocation in this modified cell compartment the content is released into the medium by a specific process. Using controlled fermentations we have established that the physiology of cells containing this novel secretory system is not dramatically different form non-modified cells. We will show the recently obtained proof of principle of this concept with the Green Fluorescent Protein (GFP). In addition we have demonstrated the concept for intracellular enzymes, which are secreted in an active form using this novel approach. Moreover we also have preliminary evidence that this technology can be used to secrete metabolites, which are normally localized intracellular. This is the first report that describes the introduction of a completely novel secretory pathway in eukaryotic cells, which allows the production of intracellular enzymes in a secreted active form enabling industrial application on an economically feasible scale.

52. Applied genome-scale modelling of *Aspergillus niger*. Mikael Rørdam Andersen, Michael Lynge Nielsen, Jens Nielsen. Center for Microbial Biotechnology, Build. 223, Technical University of Denmark, DK-2800 Lyngby, Denmark, jn@biocentrum.dtu.dk

The filamentous fungus *Aspergillus niger* has through the years fascinated academic and industrial researchers alike due to its innate ability to produce a large number of enzymes and high concentrations of acids. However, the regulatory mechanisms governing these processes are not thoroughly understood. We are combining genome-scale stoichiometric modeling, transcriptomics and novel tools for graphical analysis to elucidate these mechanisms.
A genome-scale model was constructed based on literature of *A. niger* and related aspergilli. Completion of pathways was performed using pathway databases. Genomic information was added based on the sequencing and annotation provided by DSM Food Specialities (Pel *et al*, manuscript accepted). The model comprises 1230 biochemically unique reactions (isoenzymes not included). 850 are supported by literature and 873 are backed by the genomic evidence of 1024 open reading frames. The remaining reactions are transport reactions and other reactions added for connectivity. Using a digital pathway map of the model, the computed metabolic fluxes were analysed for a large number of substrates. The results are in accordance with the available literature.
Using a fast PCR-based cloning-free approach (Nielsen *et al,* 2006) adapted for *A. niger*, deletion mutants of carbon metabolism regulators of *A. niger* have been constructed. These are to be analysed using steady-state cultures and DNA arrays for *A. niger*.

53. A transiently disrupted non-homologous end joining pathway in *Aspergillus nidulans* allows simple and efficient gene targeting. Jakob Blæsbjerg Nielsen, Michael Lynge Nielsen, and Uffe Hasbro Mortensen. Center for Microbial Biotechnology, BioCentrum-DTU, Technical University of Denmark, Denmark jbn@biocentrum.dtu.dk

Gene targeting was developed more than a decade ago for high-throughput gene deletions in the unicellular *Saccharomyces cerevisiae* owing to the fact that DNA double strand breaks are effectively repaired by the homologous recombination (HR) pathway. In multicellular eukaryotes, from filamentous fungi to man, DNA double strand breaks are preferentially repaired by the non-homologous end joining (NHEJ) repair pathway. When performing gene targeting in such multicellular organisms, the influence of NHEJ compromises the efficiency by causing random integration of the gene targeting DNA. Elimination of NHEJ by deleting one of the main genes in the pathway, e.g. *ku70*, *ku80* or *lig4* has been done in several fungal species including *Aspergillus fumigatus*, *A. nidulans*, *Sordaria macrospora* and *Neurospora crassa*. In such strains, the gene targeting efficiency is often as high as 100%. The phenotype of NHEJ strains includes altered colony morphology and an increase in sensitivity to various genotoxins. This raises the concern that phenotype of NHEJ deficient strains may produce synthetic effects with other mutations introduced in this background. This may hamper direct interpretation of the phenotype of this new mutation. In line with this, authors often recommend as a safety measure to reconstitute the NHEJ pathway, e.g. by retransforming with the functional gene or sexual crossing with wild-type strains, before analyzing the effects of novel mutations. In both cases, strain reconstruction constitutes a bottle neck in large scale gene-targeting experiments. To bypass these problems, we have developed an effective alternative in *A. nidulans*. The system employs a strain with a transiently disrupted *ku70* homolog as the starting point for efficient gene targeting. The *ku70* mutation in this strain can via a simple selection scheme be reverted to wild type after the desired genetic manipulation(s) have been carried out. The system can easily be adapted to other filamentous fungi.

54. Isolation of isoosmotic up-regulated genes in *Aspergillus oryzae* and use of its promoters for protein expression system. Ken Oda[1], Kazutoshi Sakamoto[2], Toshihide Arima[3], Yuka Okita[2], Dararat Kakizono[2], Osamu Yamada[2], Shinichi Ohashi[1], Osamu Akita[5], Kazuhiro Iwashita[2,4]. Kanazawa Institute of Technology[1],3-1 Yatsukaho, Hakusan, Ishikawa, Japan tel D+81(76)274-7500, fax+81(76)274-7511, e-mail Fodaken@neptune.kanazawa-it.ac.jp National Research Institute of Brewing[2], Prefecture Univ. of Hiroshima[3], Hiroshima Univ.[4], Jissen Women's Univ.[5], Japan

The mechanism of response to osmotic pressure exists in eukaryotes. In *A. orzyae*, response to osmotic pressure is significant factor for environment recognition in koji-making (solid-state culture), and in fact it was suggested that osmotic pressure regulation component, such as *AtfA*, or *HogA*, is important. We analyzed the genes which respond to isoosmotic pressure (0.8M NaCl and 1.2M sorbitol) by microarray analysis. In isoosmotic condition 96 genes in 3000 spots array were 2-fold up- regulated from 30 to 90 min after induction. Some genes that are needed to respond to osmotic stress, such as *atfA*, , *gpd*, , and *srk1*, were isolated. 19 genes were over 5-fold up-regulated, and named as Isoosmotic Up-regulated Genes (IUG). 5Õ- upstream region of 10 genes in IUG were cloned and applied to GUS reporter assay to evaluate induction ability in isoosmotic condition. The promoters of two genes (IUG2 and IUG9) were strictly regulated. To construct protein expression system the promoter region of high expression vector pNGA142 was substituted with IUG2 and IUG9 promoters. EGFP as a model of heterologous protein was expressed after 2hr only in induction conditions, suggesting that response speed of this system is fast and control of expression is strict. We constructed new protein expression system which protein expression was induced by osmotic pressure and their level of expression are controlled by NaCl concentration.

55. Highly efficient gene targeting in *Aspergillus oryzae* deficient in DNA ligase IV ortholog (LigD). Youhei Kudo[1], Osamu Mizutani[1], Akemi Saito[1], Tomomi Matsuura[1], Hirokazu Inoue[2], Keietsu Abe[1], Katsuya Gomi[1]. [1]Graduate School of Agricultural Science, Tohoku University, Sendai, [2]Faculty of Science, Saitama University, Saitama, Japan. gomi@biochem.tohoku.ac.jp

Disruption of the gene that encodes Ku70 or Ku80 involved in the non-homologous end joining (NHEJ) has significantly increased the frequency of gene targeting in filamentous fungi, and thus the mutants deficient in Ku70/Ku80 are suitable hosts for comprehensive gene knockout. However, even though using *ku70/ku80* mutant, we sometimes failed to obtain an expected gene disruptant in *Aspergillus oryzae*, probably dependent on the gene of interest. Thus, we constructed the disruptant deficient in DNA ligase IV ortholog (LigD) involved in the final step of NHEJ in *A. oryzae* and examined the effect of *ligD* disruption on gene targeting frequency. The *ligD* disruptant showed no apparent growth defect and a similar sensitivity to DNA-damaging agents. Gene replacement of the *prtR* gene encoding a transcription factor for extracellular proteolytic genes using *A. nidulans sC* gene as a selectable marker resulted in 100% of gene targeting frequency in the *ligD* disruptant. In addition, gene replacement of five MAP kinase genes found in *A. oryzae* genome database also showed the targeting rates as high as 100%. Consequently, the *ligD* deletion mutants are quite excellent tools for gene targeting in *A. oryzae*.

OTHER

56. Analysis of four putative beta–oxidation genes in *Aspergillus nidulans*. Kathrin Reiser, Meryl A. Davis and Michael J. Hynes. Department of Genetics, University of Melbourne, Parkville 3010, Australia

Filamentous fungi are able to use fatty acids as sole carbon sources via beta-oxidation. The enzymes required are present in both peroxisomes and mitochondria (Maggio-Hall and Keller 2004 Mol. Microbiol. 54:1173–1185).
Two putative fatty acyl-CoA dehydrogenases, AcdA and AcdB, and two putative fatty acyl-CoA oxidases, AoxA and AoxB, were identified in the genome of *A. nidulans*. Because of their homology to Fox1p of *S. cerevisiae* and a peroxisomal dehydrogenase of *N. crassa*, they are thought to be involved in the first step of beta-oxidation. Homologues for each were found in other fungal species. Three of the proteins have a clear PTS1 (peroxisomal targeting sequence), while AcdA has a PTS1-like sequence. Hence, all four proteins are predicted to be peroxisomal. GFP fusion proteins have been constructed to investigate this hypothesis.
In the 5' promoter region (1kb) of each gene, a 6bp sequence (CCGAGG/ CCTCGG) was found at least once. This sequence is predicted to be the core of a fatty acid depending regulation site (Hynes et al. 2006 Eukaryot. Cell. 5:794- 805) suggesting positive regulation by fatty acids. Promoter-lacZ fusions have shown that at least two genes are induced by short- and long-chain fatty acids and induction is altered in the absence of the predicted regulatory proteins.
Deletions of all four genes do not give a severe fatty acid growth phenotype. Only the *aox*A-deletion phenotype is clearly visible. This implies a great redundancy amongst peroxisomal proteins involved in the first step of beta- oxidation.

57. Mitotic recombination accelerates adaptation in *Aspergillus nidulans*. Sijmen Schoustra, Fons Debets, Marijke Slakhorst & Rolf Hoekstra, Laboratory of Genetics, Wageningen University, e- mail: rolf.hoekstra@wur.nl

We have performed an experimental study to explore the specific advantages of haploidy or diploidy in the fungus *Aspergillus nidulans*. Comparing the rate of adaptation to a novel environment between haploid and isogenic diploid strains over 3000 mitotic generations, we demonstrate that diploid strains which during the experiment have reverted to haploidy following parasexual recombination reach the highest fitness. This is due to the accumulation of recessive deleterious mutations in diploid nuclei, some of which show their combined beneficial effect in haploid recombinants. Our findings show the adaptive significance of mitotic recombination combined with a flexibility in the timing of ploidy level transition if sign epistasis is an important determinant of fitness. We believe that our results justify a rehabilitation of Pontecorvo's view that the parasexual cycle has an important evolutionary role in fungi, because we show that in initially homozygous diploids sufficient genetic variation is generated by mutation to make parasexual recombination effective.

58. Identification of AatB, a new component of the penicillin biosynthesis pathway of *Aspergillus nidulans*. Petra Sproete [1], Michael J. Hynes [2], and Axel A. Brakhage [1]. [1] Leibniz Institute for Natural Product Research and Infection Biology (HKI) / Friedrich-Schiller-University Jena, Germany [2] Department of Genetics, University of Melbourne, Australia

The acyl coenzyme A:isopenicillin N acyltransferase (IAT) of *A. nidulans*, which is encoded by the *aatA* gene, catalyzes the final step of the penicillin biosynthesis, i.e., the exchange of the hydrophilic L-alpha-aminoadipic acid side chain of isopenicillin N for a hydrophobic acyl group. By analyzing a GFP-IAT protein fusion it could be shown that in *A. nidulans* – as in *Penicillium chrysogenum* – the IAT and therefore the enzymatic reaction is located in the peroxisomes. Further studies indicated a PTS1 dependent transport of the enzyme since the deletion of the rather untypical putative peroxisomal targeting sequence 1 (PTS1) Ala-Asn-Ile at the C terminus of the protein led to cytoplasmic localization of the IAT. Nevertheless, unlike the IAT of *P. chrysogenum*, such a mislocated enzyme seems to be functional because both, an *A. nidulans* strain lacking the PTS1 transporter and a strain possessing a mislocated IAT still produced about 50% and 80% of penicillin, respectively, compared to wild-type levels. Because an *aatA* disruption strain still was able to produce small amounts of an inhibitory substance, the *A. nidulans* database was searched for a putative redundant protein. A gene displaying a very similar exon distribution and a 58% similarity with the *aatA* gene but lacking the PTS1 encoding sequence was named *aatB*. First analyses of an *aatB* disruption strain indicated a participitation of AatB in penicillin biosynthesis of *A. nidulans*.

59. CoA-Transferase from *Aspergillus nidulans* is required to forward the CoA-moiety from propionyl-CoA to acetate. Christian Fleck, Matthias Brock. Leibniz Institute for Natural Product Research and Infection Biology e.V. – Hans-Knöll-Institute (HKI) Contact: christian.fleck@hki-jena.de

Metabolism of amino acids and propionate leads to the toxic intermediate propionyl-CoA. Efficient removal via the methylcitrate cycle is guaranteed by a functional methylcitrate synthase. Deletion of the coding region of this enzyme leads to the inability to grow on propionate or ethanol/propionate but not acetate/propionate. This led to the assumption that the level of propionyl-CoA may become reduced by direct transfer of the CoA-moiety to acetate. Therefore, we purified a CoA-transferase from *Aspergillus nidulans* and characterised the enzyme biochemically. The CoA-transferase was specific for the CoA-esters succinyl-CoA, propionyl-CoA, and acetyl-CoA as CoA-donors and the corresponding acids as acceptors. No other donors or acceptors suited as substrates. To elucidate the role of the enzyme under *in vivo* conditions the corresponding gene was deleted. The deletion mutant only showed mild phenotypes when tested for growth and development on propionate containing media. Therefore, a double mutant with a methylcitrate synthase deletion strain was created by sexual crossing. In contrast to the methylcitrate synthase deletion strain, the double mutant was neither able to grow on ethanol/propionate nor on acetate/propionate media. Thus, CoA-transferase is essential for removal of toxic propionyl-CoA in the presence of acetate as a CoA-acceptor.

60. Gene Silencing by RNA Interference in the Koji Mold *Aspergillus oryzae*. Osamu Yamada, Ryoko Ikeda, Yuka Ohkita, Risa Hayashi, Kazutoshi Sakamoto, and Osamu Akita. National Research Institute of Brewing, 3-7-1 Kagamiyama, Higashi-Hiroshima 739-0046, Japan

Aspergillus oryzae is an important filamentous fungus in the Japanese fermentation industry, used in the manufacture of such products as sake, soy sauce, and miso, as well as in commercial enzyme production. We found the orthologous genes required for RNA interference (RNAi) in the *A. oryzae* genome database, and constructed a set of tools for gene silencing using RNAi. This system utilizes compatible restriction enzyme sites so that only a single target gene fragment is required to create the hairpin RNA cassette. For ease of handling, we also separated the construction of the hairpin RNA cassette for the target gene from its subsequent introduction into the expression vector. Using the *brlA* gene as a target for RNAi, we detected decreased mRNA levels and a delayed conidiation phenotype in the transformants. Furthermore, even though *A. oryzae* possesses three copies of the Taka- amylase gene, a single copy of a Taka-amylase RNAi construct was sufficient to downregulate the mRNA levels and decrease the enzymatic activity to 10% of control levels. Gene silencing by RNAi should provide a powerful genetic tool for post-genomic studies of the industrially important fungus *A. oryzae*.

61. Easy ways to identify *veA1* mutation in *Aspergillus nidulans*. Kap-Hoon Han[1], Hyoun-Young Kim[1], Jong Hwa Kim[1], Hee-Seo Kim[2], Keon-Sang Chae[2] and Dong-Min Han[3]. [1]Dept. of Pharmaceutical Engineering, Woosuk Univ, Wanju, 565-701, [2]Div. of Biological Science, Chonbuk Nat'l Univ, Jeonju, 561-756, [3]Dept. of Life Science, Wonkwang Univ, Iksan, 570-749, Korea.

The *veA* gene in *Aspergillus nidulans* plays an important role in light response and the balance of sexual and asexual sporulation. So, the presence of the *veA1* mutation gave us difficulty to study sexual development as well as photobiological research. Because of the *veA1* mutation was caused by a single nucleotide substitution, nucleotide sequencing of the region was the only way for the verification. Here, we present a few simple ways to identify the *veA* allele easier than the genome sequencing. First, incubation of the *A. nidulans* strains on the medium containing oxalic acid provides clear colony distinguishment between the *veA*+ and *veA1* strain. Secondly, PCR mediated verification allowed us to distinguish more clearly. The PCR products could only be obtained in the wild type *veA* allele using double mismatched primers. Finally, restriction enzyme digestion followed by the PCR amplification of the *veA* allele resulted in the clearest result. Since the *veA1* mutation eliminated a restriction enzyme site, only the PCR product containing *veA*+ allele is subjected to be digested by the enzyme. These methods provided fast and cost-saving ways for identifying the *veA* allele.

62. Analysis of *pceA*, a regulatory gene for early stage of conidiation in *Aspergillus oryzae*. Kenichiro Matsushima[1], Osamu Hatamoto[2], Genryou Umitsuki[1] and Yasuji Koyama[1]. [1]Noda Institute for Scientific Research, [2]Product Development Division, Kikkoman Corporation. Japan.

The *pceA* gene of *A. oryzae*, encoding a putative transcription factor with a GAL4-like Zn(II)2Cys6 motif, is an ortholog of the *A. nidulans nosA* gene, which is involved in regulation of sexual development. KN16-10, a *pceA* overexpressing strain of *A. oryzae*, exhibited an earlier and increased conidiation compared to the wild-type strain. In addition, KN16-10 produced conidiospores in liquid medium, whereas the wild-type strain did not produce them under the same conditions. The expression of conidiation-related gene homologs, such as *brlA* and *flbA*, and that of the *pceB*, a paralog of *pceA*, were investigated in KN16-10 and the wild-type strain. The Real-Time PCR analysis showed that expression of *brlA*, a regulatory gene for the early stage of conidiation in *A. nidulans*, increased in KN16-10 both in liquid and solid medium. At the same time, the expression of *pceB* increased in parallel with the expression of *pceA*. These findings suggest that *pceA* plays an important regulatory role in the early stage of conidiation in *A. oryzae* by upregulating the expression of *brlA*. Finally, considering that the expression of *pceB* seems to be also upregulated by *pceA*, *pceB* might be involved in conidiation as well, although its exact function remains unclear.

63. Functional analysis of the putative transcription factor CrzA in *Aspergillus nidulans*. A. Spielvogel[1,2], U. Stahl[1], E.A. Espeso[2],V. Meyer[1]. [1]Technische Universität Berlin, Institut für Biotechnologie, Fachgebiet Mikrobiologie und Genetik, 13355 Berlin, Germany [2]Departamento de Microbiología Molecular, Centro de Investigaciones Biológicas CSIC, Madrid 28040, Spain A.Spielvogel@lb.tu-berlin.de

Calcium signaling mechanisms are employed by eukaryotic cells to regulate gene expression in response to various environmental stimuli. In *Saccharomyces cerevisiae*, the Ca2+/calmodulin regulated protein phosphatase calcineurin promotes yeast survival during stress conditions such as alkaline pH, salinity, ER stress and high temperature. The transcription factor Crz1p is a major mediator of calcineurin-dependent genes and regulates different proteins that participate in ion homeostasis, cell wall integrity, membrane trafficking and signal transduction. Crz1p is a C2H2 zinc-finger protein and binds to specific elements (CDRE) within the promoter regions of target genes. Using a reverse genetics approach, we searched the *A. nidulans* genome for the presence of a Crz1p homologue and identified an ORF that displays 66% identity over the zinc-finger region of Crz1p. Based on this sequence similarity, we designated the corresponding gene *crzA*. The zinc-finger domain of CrzA contains three zinc-finger motifs, whereby the first two meet the C2H2 consensus. The entire zinc-finger domain is able to form specific DNA-protein complexes with promoter fragments harbouring CDRE's, indicating that CDRE's represent binding sites that are conserved among yeast and filamentous fungi. Using site-directed mutagenesis, we could show that the non-consensus zinc-finger three is essential for efficient DNA binding. Deletion of *crzA* had no influence on the viability of *A. nidulans*, but rendered the strain sensitive towards calcium, salt stress (Na+, K+) and alkaline pH, indicating that CrzA plays a crucial role in stress adaptation. Northern analyses are currently underway to identify genes regulated by CrzA in response to salt and alkaline stress.

64. Deleting *Aspergillus nidulans* checkpoint regulators in an undergraduate molecular genetics course. Steve James. Biology Department, Gettysburg College, Gettysburg PA sjames@gettysburg.edu

Recent advances in gene targeting and fusion PCR technology make it feasible for undergraduates to design, perform, and characterize gene deletions in filamentous fungi as part of a semester-long laboratory course. For the past two years, undergraduates in the Molecular Genetics course at Gettysburg College have deleted *Aspergillus nidulans* genes involved in cell cycle checkpoint control. Working in pairs, students applied bioinformatic tools to design a set of six PCR primers for creating a gene replacement construct. They used these primers in three-way fusion PCR to join the *A. fumigatus pyrG* selectable marker with sequences flanking the target gene. In subsequent steps, students (1) generated *pyr+* transformants in strains bearing a deletion of the KU70 homolog, (2) isolated genomic DNA and performed Southern blots using *pyrG A.f.* as a probe, (3) tested their targeted deletions for increased sensitivity to genotoxic agents, and (4) amplified and cloned the coding region into an *alcA*-based overexpression vector. Students completed this capstone experience by writing an as-for- publication paper in which they used bioinformatic tools and current literature to integrate their findings with the broader fields of cell cycle regulation and DNA damage checkpoint control. As well as providing rich opportunities for original, investigative research, this course serves as a wellspring of new projects for students wishing to continue into independent research. (Supported by Gettysburg College)

65. Aspergillus at the FGSC: Expanding with the community. Kevin McCluskey, Sheera Walker and Aric Wiest. Fungal Genetics Stock Center, University of Missouri- Kansas City, School of Biological Sciences

The Fungal Genetics Stock Center has endeavored to support research efforts with a number of fungi for almost fifty years. The first *Aspergillus nidulans* strain was deposited in October of 1962. In 2007 the FGSC holdings totaled include nearly 900 *A. nidulans* strains and 129 are *A. niger*. Recent deposits have included many *A. fumigatus* strains as well as several strains from genome sequencing programs. 37 of the genetically marked Aspergillus strains in the FGSC collection have only one marker while 178 strains have two markers (including veA+). 260 strains have three markers while 168 strains have 4 markers. Fifty eight strains have ten or more markers. Two strains share the record of the greatest number of markers with 15 each. (A591 and 593). The average Aspergillus strain has 8 markers and the most common markers are biA1 and yA2. There are 869 different loci represented among strains in the FGSC collection

The experience that the FGSC gains from being part of functional genomics programs makes us uniquely qualified to offer the same services to the Aspergillus community. As new effort is dedicated to unraveling the genomes of Aspergillus species, the FGSC can assure that resources are put to their greatest benefit, drawing new scientists to the field of Aspergillus research. Key technologies include arraying mutants, international distribution and support of information dissemination.

Supported by National Science Foundation Grant DBI 0235887

66. Development and evaluation of an Affymetrix array for *Aspergillus flavus*. Andrea Dolezal[1], David Ryan Georgianna[1], Greg OBrian[1], Charles Woloshuk[2], Nancy Keller[3], Jiujiang Yu[4], Dahlia Nielsen[1], Gary Payne[1]. [1] North Carolina State University, Raleigh, NC. [2] Purdue University, West Lafayette, IN. [3] University of Wisconsin, Madison, WI. [4]USDA/ARS/SRRC, New Orleans, LA.

A multi-species Affymetrix GeneChip array was developed to study development, metabolism, and pathogenicity of *A. flavus*. This chip, based on the whole genome sequence of *A. flavus*, contains 13,000 *A. flavus* genes, 8,000 maize genes, and 25 human and mouse innate immune response genes as well as the fumonisin and trichothecene clusters from *Fusarium*. These arrays were used to monitor gene expression of *A. flavus* during aflatoxin biosynthesis in defined media and during infection of developing maize seeds. A parallel study comparing this array with a 5002 element cDNA array showed the same expression profile for the aflatoxin biosynthetic genes when *A. flavus* was grown in culture on defined media at conducive and non conducive temperatures for aflatoxin production. Gene expression was also monitored in *A. flavus* during the infection of field grown maize seeds. The profile of aflatoxin gene expression by *A. flavus* in infected maize kernels was similar to that observed for *A. flavus* grown in the lab under conductive temperatures for aflatoxin production. In addition, several genes encoding enzymes for the metabolism of complex carbohydrates and for transporters were also elevated during infection of maize seeds. Nonspecific hybridization across species has not been observed in any of our experiments using the Affymetrix GeneChip. These initial observations show that these multi-species arrays will be a powerful tool for studying the complex ecology and metabolism of *A. flavus*. This research was funded by USDA/NRI/CGP 2006-35604-16666.

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