The Eighth International *Aspergillus* Meeting

ASPERFEST 8

March 14-15, 2011

Asilomar Conference Center
Pacific Grove, California

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March 14-15, 2011
Merrill Hall, Asilomar Conference Center, Pacific Grove, California

March 14 (Monday)
7:00 -10:00pm Posters and Welcome Reception *Sponsored by Novozymes*

March 15 (Tuesday)

9:00 Welcome, introductions and announcements Michelle Momany/Gary Payne

9:15-10:15 Session I Michelle Momany

9:15 Signaling the end: mRNA 3' tagging, degradation and translation
Mark Caddick, Institute of Integrative Biology, University of Liverpool, UK

9:35 Impact of glucose metabolism during pathogenesis of *Aspergillus fumigatus*
Matthias Brock, Microbial Biochemistry and Physiology, Leibniz Institute for Natural Product Research and Infection Biology, Germany

9:55 Genomic diversification and evolution of *Aspergillus oryzae* industrial strains
Kazuhiro Iwashita, National Research Institute of Brewing, Hiroshima, Japan

10:15 -10:35 Coffee Break

10:35-11:55 Session II: Genomics Projects Jennifer Wortman/Scott Baker

10:35 Aspergillus Knock Out Project Update
Stephen Osmani, Ohio State University, USA

10:50 Comparative Analysis of Aspergilli
Ronald de Vries, CBS-KNAW Fungal Biodiversity Centre, The Netherlands

11:05 AspGD update
Gavin Sherlock, Stanford University and Jennifer Wortman, Broad Institute, USA

11:20 New Resources for Functional Analysis of Omics Data for the Genus *Aspergillus*
Benjamin Nitsche, Leiden University, The Netherlands

11:35 Pan Fungal Informatics Resources
Jason Stajich, University of California, Riverside, USA

11:50 2012 Community Sequencing Project at JGI
Kevin McCluskey, Fungal Genetics Stock Center

12:00-1:00 Lunch

1:00-1:15 Community directions discussion; Elections Michelle Momany

1:15 - 2:45 Session III: Talks from abstracts Gerhard Braus/Masa Machida

1:30 Asymmetric RNA localization in *Aspergillus fumigatus*
Mara Couto-Rodríguez, University of Georgia, USA

1:45 Genome mining of secondary metabolite genes in *Aspergillus* species
Clay Wang, University of Southern California, Los Angeles, USA

2:00 Why are Aspergilli so different in their expression of secondary metabolites from section to section?
Jens Christian Frisvad, Technical University of Denmark, Denmark

2:15 Nitric oxide (NO) is a morphogenetic signal in fungi
David Cánovas Universidad de Sevilla, Spain

2:30 Gold nanoparticles in *Aspergillus nidulans* hyphae: can we study real-time physiology?
Susan Kaminskyj , University of Saskatchewan, Canada

2:45 Secrelection: A Novel Fungal Expression System For Selection Of Secreted Enzymes
Robbert Damveld DSM Biotechnology Center, The Netherlands

2:45 Coffee Break

3:00-3:45 Pontecorvo Lecture: *Sponsored by AlerGenetica*

Contribution of *Aspergillus* research to green, white and red biotechnology: past, present and future?
Cees van den Hondel, University of Leiden, The Netherlands

3:45 - 4:00 Announcements: election results; poster prizes; any other discussion items

Abstracts for Posters

COMPARATIVE AND FUNCTIONAL GENOMICS

1) Gene expression analysis of *Aspergillus awamori* for metabolism regulation in long term cultivation by SOLiD. Masayuki Machida[1*], Morimi Teruya[2*], Noriko Yamane[1*], Hideaki Koike[1*], Masatoshi Tsukahara[3*], Yuki Satou[4*], Kuniko Teruya[4*], Kazuhito Satou[4*], Hiroko Miyahara[4*], Akino Shiroma[4*], Makiko Shimoji[3*], Ikuya Kikuzato[4*], Kazuhiro E. Fujimori[1*], Yutaka Kawarabayasi[1*], Osamu Yamada[5], Koji Jinno[6], Hiroshi Horikawa[6], Akira Hosoyama[6], Takasumi Hattori[7], Motoaki Sano[8], Koichi Tamano[1], Kazurou Fukuda[9], Takaomi Yasuhara[9], Kenichi Higa[7], Shinichi Ohashi[5], Kotaro Kirimura[4], Masanori Arita[10], Kiyoshi Asai[10], Keietsu Abe[11], Katsuya Gomi[11], Shigeaki Mikami[5], Takeaki Ishikawa[12], Kaoru Nakasone[13], Nobuyuki Fujita[6], Takashi Hirano[1*] ([1]Natl. Inst. Advanced Inst. Sci. Technol., [2]Okinawa Iind. Technol. Center, [3]Tropical Technol. Center, [4]Okinawa Sci. Technol. Promotion Center, [5]Natl. Res. Inst. Brewing, [6]Natl. Inst. Technol. Eval., [7]Waseda U., [8]Kanazawa Inst. Technol., [9]Asahi Breweries, [10]U. Tokyo, [11]Tohoku U., [12]Brewing Soc. Japan, [13]Kinki U., *Okinawa Cutting-edge Genome Project)

Aspergillus awamori is widely used for brewing Japanese traditional spirits, Awamori.. High potential of enzyme production makes this microorganism important for modern biotechnology as well. We have sequenced 34.7 Mb of the *A. awamori* NBRC 4314 (RIB 2604) genome. In this work, we analyzed transcriptome of *A. awamori* by SOLiD to elucidate metabolic regulation. Comparison of closely related species, *Aspergillus niger* and *Aspergillus oryzae*, which have different citric acid productivity, will give us useful information in terms of metabolic regulation. Metabolic regulation in long term cultivation will be also discussed.

2) Genome sequence and comparative analysis of *Aspergillus oryzae* sake strains Takanori Nomura[12], Tomoaki Fujimura[12], Kenta Oda[13], Kazuhiro Iwashita[12], Osamu Yamada[2]. [1]Hiroshima university, Hiroshima, Japan. [2] National Research Institute of Brewing, Hiroshima, Japan. [3] SYSMEX CORPORATION, Japan.

Aspergillus oryzae has been used for Japanese traditional fermentation industry and some different strains were selected and used for each product according to their character. In our previous work, we reported the phylogenic analysis of industrial *A. oryzae* strains using DNA microarray and the correlation between the clade and their use. In this work, we performed genome sequence analysis of two *A. oryzae* sake strains belong to two different clade using high-throughput DNA sequencer and compared with the RIB40 genome. We isolated genome DNA from RIB128 and RIBOIS01 strains and applied for 454 genome sequencer. The genes of assembled contigs were further annotated using Spaln and BLAST. As the result, the genome size of RIB128 and RIBOIS01 strains were 36.9 and 37.8 Mbps, and the homology of conserved genes were 99.42 and 99.65 % in average comparing RIB40. Large deletion and insertion, more than 500 bps, was identified in both genomes and 577 (RIB128) and 337 (RIBOIS01) new genes were founded in the inserted regions. Furthermore, several inter chromosomal recombination was identified in both genome. Now we are examining for the second metabolite gene clusters in these genomes.

3) Sequencing the Black Aspergilli species complex. Kuo, Alan[1*], Asaf Salamov[1], Scott Baker[2], and Igor Grigoriev[1]. [1]DOE Joint Genome Institute, CA, USA. [2]Pacific Northwest National Lab, WA, USA. [*]akuo@lbl.gov.

The ~15 members of the *Aspergillus* section *Nigri* species complex (the "Black Aspergilli") are significant as platforms for bioenergy and bioindustrial technology, as members of soil microbial communities and players in the global carbon cycle, and as food processing and spoilage agents and agricultural toxigens. Despite their utility and ubiquity, the morphological and metabolic distinctiveness of the complex's members, and thus their taxonomy, is poorly defined. We are using short read pyrosequencing technology (Roche/454 and Illumina/Solexa) to rapidly scale up genomic and transcriptomic analysis of this species complex. To date we predict 11197 genes in *Aspergillus niger*, 11624 genes in *A. carbonarius*, and 10845 genes in *A. aculeatus*. *A. aculeatus* is our most recent genome, and was assembled primarily from 454-sequenced reads and annotated with the aid of > 2 million 454 ESTs and > 300 million Solexa ESTs. To most effectively deploy these very large numbers of ESTs we developed 2 novel methods for clustering the ESTs into assemblies. We have also developed a pipeline to propose orthologies and paralogies among genes in the species complex. In the near future we will apply these methods to additional species of Black Aspergilli that are currently in our sequencing pipeline.

4) Aspergillus Stress Database . István Pócsi[1], Márton Miskei[1] and Zsolt Karányi[2] [1] - Department of Microbial Biotechnology and Cell Biology, University of Debrecen, Debrecen, Hungary, e- mail: ipocsi@gmail.com [2] – Department of Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary, e-mail: karanyi@internal.med.unideb.hu

The Aspergillus Stress Database (http://193.6.155.82/AspergillusStress/) was released in May 2008 and contains the description of 3908 stress-response proteins (and translated genes) that may play any role in the stress response of eight *Aspergillus* species with fully sequenced genomes including *Aspergillus clavatus*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus oryzae*, *Aspergillus terreus* and *Neosartorya fischeri*. Each annotated protein was characterized with *Aspergillus locus ID*, its closest homologues in *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Candida albicans* and *Neurospora crassa* (gene locus IDs, protein names), a brief functional description (stress type, gene ontology classes) and links to relevant publications (PMID IDs). The annotation of *Aspergillus* stress response proteins revealed the limits of yeast-based models when genome annotation work is performed in filamentous fungi. Currently, we develop the Aspergillus Stress Database further to a more general Fungal Stress Database.

5) A versatile gene expression and characterization system for filamentous fungi. Bjarne Gram Hansen, Morten Thrane Nielsen, Bo Salomonsen, Jakob Blæsbjerg Nielsen, Niels Bjørn Hansen, Kristian Fog Nielsen, Kiran Patil and Uffe Hasbro Mortensen. Technical University of Denmark, Department of Systems Biology, Center for Microbial Biotechnology, Denmark. E-mail: bgha@bio.dtu.dk

Assigning functions to newly discovered genes constitutes one of the major challenges en route to fully exploit the data becoming available from the genome sequencing initiatives. To facilitate the use of filamentous fungi in functional genomics, we present a versatile cloning system that allows gene of interest (GOI) to be expressed from a defined genomic location of *A. nidulans*. By a single USER cloning step, genes are easily inserted into a combined targeting-expression cassette ready for rapid integration and analysis. The system comprises a vector set that allows genes to be expressed from a range of constitutive promoters or from the inducible PalcA promoter. Moreover, by using the vector set, protein variants can easily be made and expressed from the same locus, which is mandatory for proper comparative analyses. Lastly, all individual elements of the vectors can easily be substituted for other similar elements ensuring the flexibility of the system. We have demonstrated the high-throughput potential of the system by transferring more than 100 genes from filamentous fungi into *A. nidulans*. In addition, we produce defined mutant derivatives of selected GOI which allows an in-depth analysis of the GOI. Importantly, since the vector set is constructed in a flexible manner, it can without problems be modified to allow specific integration of GOI into other fungi. The strategy for gene characterization presented here is therefore widely applicable and should greatly facilitate assignment of gene functions in fungi where the genetic tool-box is poorly developed.

6) Comparative analysis of mitochondrial genomes from *Aspergillus* and *Penicillium* spp. Vinita Joardar, Suman Pakala, Jessica Hostetler, Suchitra Pakala, Natalie Fedorova and William Nierman J. Craig Venter Institute, Rockville MD, USA.

Fungal mitochondrial genes are widely used in population and phylogenetic studies and have been linked to virulence and senescence in some fungi. While multiple nuclear genomes are available for *Aspergillus* spp., few annotated mitochondrial genomes have been published for these organisms. We report here the complete sequence of 6 mitochondrial genomes of *Aspergillus* spp. as well as 3 *Penicillium* spp. obtained by Sanger sequencing. While core gene content and synteny are well conserved within each genus, the genomes sizes range considerably from 24,658 to 35,056 bp in *Aspergillus* spp. and from 27,017 to 36,351 bp in *Penicillium* spp. The core mitochondrial genome includes the 14 genes involved in oxidative phosphorylation, a complete set of tRNAs, the small and large subunits of ribosomal RNA and the ribosomal protein S5, all encoded on the same strand. The differences in size correlate with the number of introns and the number of accessory genes present in the genome. The smallest genomes do not contain introns in the protein-coding genes whereas the larger genomes contain as many as 9 introns. Accessory genes include intron-encoded endonucleases, DNA and RNA polymerases and hypothetical proteins. The comparative and phylogenetic analysis of these and related publicly available mitochondrial genomes are presented. Funding: NIAID

7) Identification and characterization of genes responsible for biosynthesis of kojic acid from *Aspergillus oryzae*. Hideaki Koike[1], Junichiro Marui[1], Noriko Yamane[1],Sumiko Ohashi[1], Yasunobu Terabayashi[1],Motoaki Sano[2], Shinichi Ohashi[2], Eiji Ohshima[3], Kuniharu Tachibana [3], Yoshitaka Higa[3], Marie Nishimura[4], and Masayuki Machida[1] [1] Natl. Inst. Adv. Indus. Sci. Tech. (AIST), [2] Kanazawa Inst. Tech., [3]Sansho Seiyaku Co., Ltd., [4] Natl. Inst. Agrobiol. Sci.

Aspergillus oryzae is one of the most important organisms in Japanese fermented food industry. Kojic acid is produced in large amounts by *Aspergillus oryzae* as a secondary metabolite and is used in the cosmetic industry. Glucose can be converted to kojic acid, perhaps by only a few steps, but no genes for the conversion have thus far been revealed. Using a DNA microarray, gene expression profiles under three pairs of conditions, significantly affecting kojic acid production, were compared. All genes were ranked using an index parameter reflecting both high amounts of transcription and a high induction ratio under producing conditions. After disruption of nine candidate genes selected from the top of the list, two genes of unknown function were found to be responsible to kojic acid biosynthesis, one having an oxidoreductase motif and the other a transporter motif. These two genes are closely associated in the genome, showing typical characteristics of genes involved in secondary metabolism. Regulatory mechanism of the gene cluster will be discussed.

8) A method for accurate prediction of the size of secondary metabolite clusters in *Aspergillus nidulans*. Mikael R. Andersen[1], Jakob B. Nielsen[1], Mia Zachariassen[1], Tilde J. Hansen[1], Kristian F. Nielsen[1], and Uffe H. Mortensen[1]. [1]Center for Microbial Biotechnology, Technical University of Denmark, Denmark.

Fungal secondary metabolites (SMs) are receiving increasing interest due to their role as bioactives, ranging from antibiotics over cholesterol-lowering drugs to food toxins. The identification of SMs and their biosynthetic gene clusters are thus a major topic of interest. Identifying these genes is a tedious and time-consuming affair, with the standard method requiring the knockout of genes on both sides of putative SM synthases. Furthermore, one does not know the number of genes in the cluster and thereby extent of this work before starting the experiment. In this work, we present an algorithm for prediction of the size of SM clusters in *Aspergillus nidulans*. The method is based on an gene expression catalog of >60 transcriptome experiments, using a diverse set of strains, media, carbon sources, and solid/liquid cultivations. Furthermore, the method is independent of the quality of annotation. Application of the algorithm has allowed the accurate prediction of the number of included genes in well-characterized gene clusters. including the 25 genes of the sterigmatocystin cluster and the emericellamide cluster (4 genes). The method has provided strong predictions of unknown clusters, some of which we have verified experimentally and identified the corresponding metabolites.

9) Systematic Deletion Analysis of Aspergillus nidulans Kinase Genes. [1]Colin P. De Souza, [1]Shahr B. Hashmi, [1]Aysha H. Osmani, [2]Carol S. Ringelberg, [2]Jay C. Dunlap and [1]Stephen A. Osmani. [1]The Ohio State University, Columbus, OH, USA. [2]Dartmouth Medical School, Hanover, NH, USA. (osmani.2@osu.edu)

Phosphorylation mediated through kinase enzymes is important to the regulation of virtually all eukaryotic processes. In the filamentous fungi analysis of these important regulatory enzymes has been limited to specific kinases identified through biochemical or genetic approaches or by sequence similarities to kinases in other organisms. However, recent advances in gene targeting and the availability of pre-made deletion constructs makes possible the global analysis of all kinases in Aspergillus nidulans. We report here the deletion and primary characterization of all protein kinase, histidine kinase and PI3/PI4 kinase encoding genes, totaling 130 deletions, in this model fungus. Each gene has been deleted and defined as either essential or not essential. Non-essential haploid deleted strains have been tested for conditional phenotypes in response to numerous cellular stress conditions. In addition, for all 24 of the essential kinase genes, the terminal growth and cell cycle phenotype has been defined using heterokaryon rescue and microscopic analysis of DAPI stained cells. This global analysis has confirmed the phenotypes of previously studied kinase genes and has expanded the number of kinase genes that have now been characterized in A. nidulans by 82. The deleted strains have been deposited at the FGSC and provide a powerful resource for analysis of processes regulated by phosphorylation in fungi. (Funded by NIH Program Project grant GM068087)

10) Alternative annotation of *Aspergillus* genomes with multi-genome Gnomon method. Alexandre Souvorov, Boris Kiryutin, Vyacheslav Chetvernin, Barbara Robbertse, Leonid Zaslavsky, Tatiana Tatusova., The National Center for Biotechnology Information, National Institutes of Health Bethesda Maryland, USA., robberts@ncbi.nlm.nih.gov.

A novel Gnomon annotation method uses comparative multi-genome approach that utilize the fact that functional regions of a genomic sequence, and protein-coding regions in particular, are more conserved than non-functional. This method starts from a single genome Gnomon gene prediction and then uses a comparative analysis among multiple genomes to gradually improve the annotation through the iterative process. The approach has been tested on genomes from eight *Aspergillus* species. Original annotations submitted to GenBank for eight *Aspergillus* species were compared to those predicted by multi-Gnomon using a protein cluster procedure. By design, the multi-genome Gnomon propagates proteins found on one genome into other genomes. The result is an increased number of clusters which included proteins from all eight *Aspergillus* genomes. Overall the clustering results showed that more Gnomon models shared clusters with other fungal proteins than the predicted proteins of these genomes in GenBank. There were 1054 clusters which included a *S. pombe* protein and proteins from all eight *Aspergillus* genomes (Gnomon or existing Genbank annotations). Gnomon predicted proteins were present in almost all of the clusters. In these clusters 545 Gnomon models did not have a GenBank counterpart yet 87% of these Gnomon models had direct (same species) or indirect EST support (from other Aspergilli).

11) New resources for functional analysis of omics data for the genus *Aspergillus* B.M. Nitsche [1], A.F.J. Ram [1,2], V. Meyer [1,2], J.R. Wortman [3] [1] Institute of Biology, Leiden University Sylviusweg 72, 2333 BE Leiden, the Netherlands, [2] Kluyver Centre for Genomics of Industrial Fermentation, Sylviusweg 72, 2333 BE Leiden, the Netherlands and [3] Department of Medicine Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD, USA

Detailed and comprehensive genome annotation can be considered a prerequisite for effective analysis and dissection of omics data. As such, Gene Ontology (GO) annotation has become a well-accepted framework for functional annotation. The genus *Aspergillus* comprises fungal species that are important model organisms, plant and human pathogens as well as industrial workhorses. GO annotation based on computational prediction and manual curation has so far only been available for one of its species, namely *A. nidulans*. Based on protein homology and synteny, we have mapped 97% of the 3,498 *A. nidulans* GO annotated genes to at least one of seven other *Aspergillus* species: *A. niger, A. fumigatus, A. flavus, A. clavatus, A. terreus, A. oryzae* and *Neosartorya fischeri*. Furthermore, we developed the web application FetGOat, which can be used to perform GO enrichment analysis for eight *Aspergillus* species. We have benchmarked the newly mapped GO annotation and the web application FetGOat by analyzing two recently published microarray datasets and comparing the results to those obtained with two freely available analysis tools, Blast2GO and GSEA.

12) Validation of a novel self-excising marker by employing the beta-rec/*six* site-specific recombination system in the human pathogen *Aspergillus fumigatus*. Edyta Szewczyk,[1] Michaela Dümig,[1] Thomas Hartmann,[1] Basem M. Jaber,[3] Patrick Olbermann,[1] Joachim Morschhäuser,[2] and Sven Krappmann[1] Research Center for Infectious Diseases[1] and Institute for Molecular Infection Biology,[2] Julius-Maximilians-University Würzburg, Würzburg, Germany, and Department of Biological Sciences, University of Jordan, Amman, Jordan[3]

Functional studies of genes often rely on methods of their manipulation, most often by means of gene targeting, such as deletion. This is achieved by replacing the sequence of interest with a marker gene, the presence of which can be selected under specific conditions. Many cellular activities in higher eukaryotes are often encoded by multiple and sometimes redundant genes, making the generation of definite null mutants a difficult task. One of the crucial problems is the limited number of selectable markers and mutations, be it nutritional or drug resistance genes. Recyclable marker modules allow repetitive rounds of gene deletion, followed by marker rescue, making the recurring use of resistance cassettes in gene targeting tasks possible. Excision of the selective marker also allows to avoid the potential risk of phenotypic effects caused by expression of additional heterologous genes. In aspergilli, the Cre/*lox* system has recently been established, employing transient expression of the recombinase-encoding gene from an autonomously replicating plasmid, which is later lost under non-selective culture conditions. One drawback of this method is the requirement of two rounds of transformation per gene deletion/marker rescue event. Here, we describe functionality of a bacterial recombination system employing the small beta serine recombinase (beta-rec) acting on *six* recognition sequences in a fungal host, the human pathogen *Aspergillus fumigatus*. By combining a selectable marker and a tightly controllable beta-rec expression module in the same cassette, a novel self- excising resistance marker for serial gene replacement purposes could be validated. As further benefit, uncontrolled chromosomal rearrangements during marker excision are precluded due to the strict *cis* (intramolecular) action of the beta-recombinase. This innovative cassette allows marker rescue in a more convenient manner, requiring only one transformation per deletion/marker excision event.

13) A new mutant phenotype system and the curation of pathogenesis-related phenotypes for *Aspergillus nidulans* and *Aspergillus fumigatus* at the *Aspergillus* Genome Database Diane O. Inglis [1], Martha B. Arnaud [1], Jon Binkley [1], Gustavo Cerqueira [2], Maria C. Costanzo [1], Marcus C. Chibucos [2], Jonathan Crabtree [2], Joshua Orvis [2], Prachi Shah [1], Marek S. Skrzypek [1], Gail Binkley [1], Stuart R. Miyasato [1], Jennifer R. Wortman [2] and Gavin Sherlock [1] [1] Department of Genetics, Stanford University School of Medicine, Stanford, CA [2]Institute for Genomic Sciences, University of Maryland School of Medicine, Baltimore MD

The *Aspergillus Genome Database* (AspGD; www.aspgd.org) collects and displays gene, protein, and genomic information gathered from published literature about the model fungus, *Aspergillus nidulans* and other aspergilli including the pathogen *A. fumigatus*. Much of the curated information in AspGD is described using controlled vocabularies, such as the Gene Ontology, which greatly facilitates searching for specific data and comparison across genomes. We use a new phenotype curation system that conforms to a rigorously controlled-vocabulary system that was developed at the *Saccharomyces* Genome Database (SGD). Each phenotype annotation is broken down into an chr(34)observablechr(34), representing the entity or process that is observed (e.g., conidiation) and a qualifier that describes the effect on that entity or process in the mutant (e.g., decreased, increased, abnormal, normal). Additional fields may contain information about the mutant such as strain background, allele name, conditions under which the phenotype is observed or the animal model used in a virulence assay. To more comprehensively capture host-pathogen interaction phenotypes, we have expanded our phenotype vocabulary to include the additional pathogenesis-related terms, “resistance to killing by host cells” and “resistance to phagocytosis” in addition to terms, such as virulence, that were already in use. A summary of the mutant phenotype information is displayed on the Locus Summary page for each gene, and the complete information is displayed in tabular format on the Phenotype details page for each gene. All of the information is searchable, and may also be downloaded in bulk using AspGD's Batch Download tool or from the Download Data page. AspGD is supported by grant RO1 AI077599 from the NIAID at the NIH.

GENE REGULATION

14) Nitric oxide (NO) is a morphogenetic signal in fungi Ana T. Marcos*, Thorsten Schinko#, Joseph Strauss#, David Cánovas* *Departamento de Genética, Facultad de Biología, Universidad de Sevilla, Spain, davidc@us.es; #Fungal Genetics and Genomics Unit, AIT-Austrian Institute of Technology and BOKU University, Vienna, Austria, joseph.strauss@boku.ac.at

Nitric oxide (NO●) is an important signalling and defence molecule in higher eukaryotes, including plants and mammals. We recently showed that in A. nidulans this short-lived nitrogen oxide radical is generated during the nitrate assimilation process, and that detoxification by flavohemoglobin proteins FhbA and FhbB, which are co-regulated with the nitrate pathway, is required to protect nitrate- and nitrite reductase from nitrosative inactivation under elevated NO● conditions (1). We here report the effect of nitric oxide donors and of mutations in fhbA and fhbB on developmental processes in two fungal genera. In response to external and internal signals, all fungi undergo developmental programs to form specialized structures and in A. nidulans, there is a fine balance between asexual (conidiation) and sexual development. We have found that addition of the NO●-releasing compound DetaNONOate reduced asexual development in A. nidulans. On the other hand, the formation of sexual structures is increased after DetaNONOate supplementation in several fungal species, including species from Aspergillus and Neurospora. (1) Schinko et al. (2010). Transcriptome analysis of nitrate assimilation in Aspergillus nidulans reveals connections to nitric oxide metabolism. Mol. Microbiol. 78: 720-738.

15) Involvement of FarA transcription factor in fatty acid utilization in *Aspergillus oryzae* Sharon Marie Garrido[1], Noriyuki Kitamoto[2], Akira Watanabe[1], Takahiro Shintani[1], and Katsuya Gomi[1]1Graduate School of Agricultural Science, Tohoku University, Japan; [2]Food Research Center, Aichi Industrial Technology Institute, Japan.

This zinc finger transcriptional factor FarA in *Aspergillus nidulans* up-regulates genes required for growth on fatty acids. FarA is widely conserved in fungi and binds to 5'-CCTCGG-3' core sequences in the promoters of their target genes (Hynes et al, 2006). Ctf1 and Por1, orthologs of FarA of *A. nidulans* are required for growth on fatty acids in *Candida albicans* and also for the essential transcriptional activation of genes involved in beta-oxidation and peroxisomal biogenesis in *Yarrowia lipolytica*, respectively (Ramirez and Lorenz, 2009; Poopanitpan et al, 2010). FarA transcriptional factor is also found in the *Aspergillus oryzae* which has 83% homology of all the amino acid sequences and 97.5% homology of Zn2Cys6 motifs with the *A. nidulans*. In this study, *farA* disruptant in *A. oryzae* was characterized and expression levels of genes for fatty acid metabolism and peroxisomal biogenesis and functions were examined. Interestingly, *A. oryzae farA* disruptants showed indistinguishable growth in fatty acid sources compared to the wild-type, inconsistent with the growth phenotype of the *A. nidulans* counterpart. In contrast, expressions of some genes for fatty acid metabolism, such as acyl-CoA oxidase and acyl-CoA dehydrogenase genes, were significantly reduced in the *farA* disruptants. These contradicting results suggested that FarA acts not only the primary transcriptional activator for fatty acid utilization in *A. oryzae* and another transcriptional factor(s) may regulate other fatty acid metabolic genes which can be accounted to the differences on the number of genes between these two *Aspergilli*.

16) Functional characterization of nuclear localization signals in the *Aspergillus nidulans* transcription activator of nitrogen metabolic genes AreA. Richard B. Todd[1], Cameron C. Hunter[1], Kendra S. Siebert[1], Koon Ho Wong[2], Sara Lewis[2], Damien J. Downes[1], James A. Fraser[2], Michael J. Hynes[2] and Meryl A. Davis[2]. [1] Department of Plant Pathology, Kansas State University, Manhattan, KS 66506, USA. [2] Department of Genetics, The University of Melbourne, VIC 3010, AUSTRALIA. Email: rbtodd@k-state.edu

The *Aspergillus nidulans* GATA DNA-binding transcription factor AreA activates transcription of genes for uptake and metabolism of nitrogen nutrients. AreA accumulates in the nucleus during nitrogen starvation but not in the presence of nitrogen sources. The AreA protein contains five putative classical SV40 Large T Antigen-type nuclear localization sequences (NLSs) and one putative non-canonical bipartite NLS conserved with mammalian GATA4. We are using two approaches to determine which of the putative NLSs are functional. First, we constructed epitope-tagged gene replacement *areA* mutants affected in the NLSs to identify sequences required for nuclear localization. Immunofluorescence microscopy experiments show that at least one of the classical NLSs contributes to nuclear import. However, deletion of all five classical NLSs does not affect utilization of nitrogen sources and does not prevent AreA nuclear localization. Mutation of the bipartite NLS confers inability to utilize alternative nitrogen sources. We are determining the effect of this bipartite NLS mutation individually and in combination with deletion of the five classical NLSs on nuclear localization. Second, we fused DNA sequences encoding the putative AreA NLSs to the Green Fluorescent Protein (GFP) gene and introduced these constructs into *A. nidulans* to determine which of the predicted NLSs are sufficient to direct GFP to the nucleus. We will use UV-fluorescence microscopy to determine the subcellular location of the GFP-NLS fusion protein in the transformants.

17) The $Zn(II)_2Cys_2$ binuclear cluster of TamA is required for activation of *gdhA* expression in *Aspergillus nidulans*. Downes, Damien J.[1,2], Brendan L. Taig[1], Sara Lewis[1], Richard B. Todd[2], Meryl A. Davis[1] [1]Department of Genetics, University of Melbourne, Vic, Australia. [2]Department of Plant Pathology, Kansas State University, KS, USA

NADP-dependent glutamate dehydrogenase (NADP-GDH) encoded by *gdhA* is required for the assimilation of alternate nitrogen sources through ammonium in *Aspergillus nidulans*. Previous studies have shown that *gdhA* expression is regulated by three transcription factors: the major transcription activator of nitrogen metabolic genes, AreA, the regulator of leucine biosynthesis, LeuB, and TamA a co-activator of AreA that also interacts with LeuB. At the *gdhA* promoter TamA is the major contributor to gene expression, unlike at most nitrogen assimilation promoters, where TamA plays a minor role in gene activation. We show that mutation of the DNA binding domain in any of AreA, LeuB or TamA reduces activity at the *gdhA* promoter. Significantly this suggests a role for the $Zn(II)_2Cys_2$ binuclear cluster of TamA, which is dispensable for function at other promoters. Using fragments of the *gdhA* promoter fused to the *lacZ* reporter gene we identified two core regulatory regions in the promoter. One regulatory region contains a potential site of action for TamA close to putative binding sites for both AreA and LeuB. At the second regulatory site LeuB acts independently as a modulated activator/repressor to mediate glutamate feedback regulation.

18) Secrelection: A Novel Fungal Expression System For Selection Of Secreted Enzymes. Robbert Damveld [1], Brenda Vonk [1], Cees Sagt [1], Hildegard

Menke [1], Thibaut Wenzel [1]. [1]DSM Biotechnology Center

Modern biotechnology has generated an impressive set of molecular tools: for instance the ability to generate large sets of error prone mutant libraries or cDNA libraries. When these libraries are expressed in a host (e.g. *Aspergillus niger*) not all strains produce a secreted protein. This is mainly dependent on the quality of the library. Here we describe a novel expression system that was developed by using genome expression profiling under different conditions. We were able to identify promoters that fit the required expression profile. These promoters were both up regulated during protein secretion and were not expressed during overexpression of intracellular proteins. By making use of transcriptomics for useful promoter identification, we were able to generate reporter construct(s) that allow us to easily select clones that secrete proteins. This technology can speed up novel protein discovery significantly. Additionally we have shown this approach is not limited to fungi but can also be applied to other production organisms.

19) Effect of primary metabolism on secondary metabolite production in *Aspergillus terreus*. Markus Gressler[1], Christoph Zaehle[2], Kirstin Scherlach[2], Christian Hertweck[2], and Matthias Brock[1] [1]Junior Research Group Microbial Biochemistry and Physiology; [2]Department Biomolecular Chemistry Leibniz Institute for Natural Product Research and Infection Biology (Hans Knoell Institute); D-07745 Jena; Germany; markus.gressler@hki-jena.de

Genome sequencing has shown that *Aspergillus terreus* has the potential to produce a great variety of different natural products. Although several metabolites have been identified, it can be assumed that the potential to produce secondary metabolites is much larger than currently known. Several strategies have been developed to discover new metabolites produced by filamentous fungi. Besides the use of epigenetic modifiers or co-cultivation experiments, targeted overexpression of putative transcription factors provides a promising tool to activate silent gene clusters. Here, we investigated the expression of the only complete PKS-NRPS hybrid gene present in the genome of *A. terreus*. Since overexpression of a putative transcriptional activator adjacent to the PKS-NRPS gene did not activate gene transcription, we constructed a *lacZ* reporter to screen for naturally inducing conditions. Results revealed that expression was activated in the presence of several amino acids at alkaline pH. However, glucose mediated carbon catabolite repression remained as the dominating inhibiting factor. When the adjacent transcription factor, which failed to induce PKS-NRPS expression in initial experiments, was expressed under naturally non-inducing, but also non-repressing conditions, activation of the PKS-NRPS gene was observed. Thus, factors involved in regulation of primary metabolism can override activating effects from cluster specific transcription factors. Finally, product identification revealed that the gene cluster is responsible for producing metabolites of the fruit rot toxin family.

20) Azole drug species-dependent responses of the transcription factor AtrR in *Aspergilli*. Ayumi Ohba[1], Kiminori Shimizu[2], Takahiro Shintani[1], Susumu Kawamoto[2], Katsuya Gomi[1]. [1]Graduate School of Agricultural Science, Tohoku university, Japan [2]Medical Mycology Research Center, Chiba University, Japan

It has hitherto been shown that overexpression of a transcription factor gene (*atrR*) resulted in increased azole drug resistance and also in upregulation of gene expression of PDR-type ABC transporters in *Aspergillus oryzae*. In contrast, deletion of *atrR* led to downregulation of ABC transporter gene expression and consequently resulted in significant increase in azole drug susceptibility. Previously, we revealed that expression of *atrR* and three PDR-type ABC transporter genes (*atrA*, *atrF*, and *atrG*) are upregulated by miconazole in *A. oryzae* wild-type strain. In this study, we investigated the expression profiles of *atrR* and the PDR-type ABC transporter genes in the presence of other azole drugs. The *atrR* and PDR-type ABC transporter genes (at least *atrA*, *atrF*, and *atrG*) were upregulated by clotrimazole, but not by itraconazole, ketoconazole, or fluconazole, although deletion of *atrR* resulted in significant increase in itraconazole and fluconazole susceptibility. This suggested that the transcription factor AtrR shows different responses dependent on azole drug species. To identify the target genes regulated by AtrR in the presence of different azole drug, we performed DNA microarray analyses of wild type and *atrR* disruptant, which will be presented. In addition, expression profiles of the *atrR* ortholog and ABC transporter genes in several azole drugs were also examined in human pathogenic fungus, *A. fumigatus*.

21) Asexual development is regulated by histone H3 acetylation in *Aspergillus nidulans*. David Cánovas*, Yazmid Reyes-Domínguez#, Ana T. Marcos*, Ulrich Güldener‡, and Joseph Strauss# *Departamento de Genética, Facultad de Biología, Universidad de Sevilla, Spain, davidc@us.es; #Fungal Genetics and Genomics Unit, AIT-Austrian Institute of Technology and BOKU University, Vienna, Austria. ‡Institute of Bioinformatics and Systems Biology, Helmholtz Center Munich, Germany

The ascomycete Aspergillus nidulans is a model organism to study fungal development. The expression of the brlA gene triggers the formation of the developmental structures, the conidiophores. The expression of brlA is regulated by a number of upstream regulators, including FluG, FlbA-E. In addition to these regulators, we have found that the product of gcnE is necessary for the expression of the brlA gene. GcnE is a homolog of yeast GCN5p, the catalytic subunit of the conserved SAGA/ADA complexes responsible for the majority of lysine acetylation in histone H3 (H3ac) and subsequent transcription-related chromatin remodelling. In A. nidulans, deletion of gcnE results in a severe defect of asexual development. In this study, we compared wild type and the gcnEΔ mutant by transcriptome analysis and chromatin modification assays. Microarray analysis revealed major effects on the expression of genes involved in primary and secondary metabolism as well as in development. Consistently, the expression of brlA is dramatically reduced and some of the upstream regulators are deregulated. Chromatin immunoprecipitation assays revealed an altered pattern of H3ac in promoters of the conidiation regulators, suggesting that H3 acetylation carried out by GcnE is required for the accurate regulation of conidiation.

22) Evidence that HxkC, an *Aspergillus nidulans* mitochondrial hexokinase-like protein, is anti-apoptotic Margaret E. Katz[1], Rebecca Buckland[1], and Matthias Brock[2], [1]Molecular and Cellular Biology, University of New England, Armidale, NSW 2351 Australia, mkatz@une.edu.au [2]Microbiell Biochemistry, Hans-Knoell-Institut, Beutenbergstr. 11a, Jena 07745, Germany, Matthias.Brock@hki-jena.de

Binding of hexokinase II to mitochondria inhibits Bax-induced cytochrome *c* release from mitochondria and apoptosis in mammalian cells (Pastorino et al, 2002). HxkC, which plays a role in the response to nutrient stress, is the first fungal hexokinase shown to be associated with mitochondria (Bernardo et al. 2007). In a strain lacking functional HxkC, cleavage of DNA into oligonucleosomal fragments, a hallmark of mammalian apoptosis, occurs even in the absence of nutrient stress. This suggests that, as in plants, a fungal mitochondrial hexokinase inhibits programmed cell death even though Bax, a member of the Bcl-2 family, is not present. The *hxkC delta* null mutant shows increased susceptibility to oxidative stress but increased resistance to rapamycin-induced-inhibition of conidiation. Higher levels of intracellular protease activity, which could be the result of autophagy, are detected in the *hxkC delta* mutant. To determine whether HxkC plays a role in autophagy, we have generated mutants that lack both HxkC and AtgA, Although no loss of hexokinase activity was detected in the *hxkCdelta* mutant, purification of HxkC has revealed that the protein possesses low levels of ATPase and glucose-phosphorylating activity. Pastorino J.G., Shulga N., Hoek J.B. (2002) Mitochondrial binding of hexokinase II inhibits Bax-induced cytochrome *c* release and apoptosis. *J. Biol. Chem.* **277:** 7610-7618. Bernardo S.M.H., Gray K.-A., Todd R.B., Cheetham B.F., Katz M.E. (2007) Characterization of regulatory non-catalytic hexokinases in *Aspergillus nidulans. Mol. Genet. Genomics* **277:** 519-532.

23) Activity of AREA and AREB under different carbon and nitrogen regimes. Maria Macios[1], Piotr Wêgleñski[1,2], Agnieszka Dzikowska[1,2] [1]Institute of Genetics and Biotechnology, University of Warsaw, Poland [2]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Poland adzik@ibb.waw.pl; marym@op.pl

Nitrogen metabolite repression modulates the expression of target genes participating in utilization of alternative nitrogen sources, resulting in transcription only when glutamine or ammonium levels are limiting. In *Aspergillus nidulans* this regulatory mechanism depends on a positively acting regulator AREA. At variance with other pathways regulated by AREA, this factor functions as a repressor of arginine catabolism genes, acting concertedly with the other GATA factor: AREB. The activities of AREA and AREB are differentially regulated by the carbon regime: AREA being necessary for the ammonium repression of *agaA* and *otaA* under carbon repressing conditions, while AREB is primarily involved under carbon-limiting conditions. The ability of both AREA and AREB to sense the status of carbon metabolism is most probably dependent on NMRA, and not on the transcription factor CREA, which mediates general carbon catabolite repression is *A. nidulans*. Our current research is focused on investigation of the hypothesis of AREA/AREB cooperation under different carbon and nitrogen regime.

24) A NsdD-dependent novel helix-loop-helix transcription factor is necessary for the conidiation of *Aspergillus nidulans* and *Aspergillus fumigatus.* Kap-Hoon Han1, Seul-A Gu, Jae Sin Park, Lee Han Kim, Dong-Soon Oh1, and Dong Min Han Division of Life Science, Wonkwang University, Iksan, 570-749, Korea 1Department of Pharmaceutical Engineering, Woosuk University, Samrye, 565-701, Korea

The *nsdD* gene encodes a GATA type transcription factor, which controls sexual development positively in *A. nidulans*. According to the transcription profile analysis using WT vs. *nsdD* deletion mutant, more than hundred genes were expressed by NsdD-dependent manner during various stages of life cycle. One of them, named *ndeA* (*nsdD*-dependent expression), which encodes a conserved protein carrying helix loop helix (HLH) domain, showed the preferential development of cleistothecia even under the various stress conditions, when deleted. Overexpression of the gene under *niiA* promoter resulted in the failure of cleistothecia formation, suggesting that the gene controls sexual development negatively or positively controls conidiation. Northern analysis revealed that the gene was transcribed at early stages of both sexual and asexual sporultion. The mRNA levels were increased in *nsdD* or *veA* deletion mutant at same stages, indicating that the gene was repressed by those regulators during early development stages. In *Aspergillus fumigatus*, knock-out of the homologous *AfndeA* gene resulted in aconidiation phenotype, indicating that this gene plays an important role in conidiation process as well as sexual development.

25) LaeA control of velvet family regulatory proteins for light-dependent development and fungal cell-type specificity. Özlem Sarikaya Bayram[1], Özgür Bayram[1], Oliver Valerius[1], Hee Soo Park[2], Stefan Irniger[1], Jennifer Gerke[1], Min Ni[2], Kap-Hoon Han[3], Jae-Hyuk Yu[2] and Gerhard H. Braus[1] [1]Dept of Molecular Microbiology & Genetics, Georg August University, Göttingen, Germany [2]University of Wisconsin, Madison, USA [3] Woosuk University, Wanju, Korea

VeA is the founding member of the velvet superfamily of fungal regulatory proteins involved in light response and coordinates sexual reproduction and secondary metabolism in *Aspergillus nidulans*. In the dark, VeA bridges VelB and LaeA to form the VelB-VeA-LaeA (velvet) complex. Here we show that VelB forms a second light-regulated developmental complex with VosA. LaeA plays a key role not only in secondary metabolism but also in directing formation of the VelB-VosA and VelB-VeA-LaeA complexes. LaeA controls VeA modification and protein levels and possesses additional developmental functions. The laeA null mutant results in constitutive sexual differentiation, indicating that LaeA plays a pivotal role in inhibiting sexual development in response to light. Moreover, the absence of LaeA results in the formation of significantly smaller fruiting bodies. This is due to the lack of a specific globose cell type (Hülle cells), which nurse the young fruiting body during development. This suggests that LaeA controls Hülle cells. In summary, LaeA plays a dynamic role in fungal morphological and chemical development, controls expression, interactions and modification of the velvet regulators.

26) Exploratory survey for potential transposable elements in *Aspergillus oryzae* by a stress-fluctuation cDNA browser. Hironobu Ogasawara[1], Saori Takahashi[1], and Katsuya Gomi[2 1] Akita Res. Inst. Food and Brewing, Akita, Japan. [2] Graduate School of Agricultural Science, Tohoku University, Sendai, Japan. E-mail:hironobu@arif.pref.akita.jp

An active DNA transposon *Crawler* isolated from the genome of industrially important fungus *Aspergillus oryzae* transposes under extreme stress conditions [1]. The mRNA analysis of *Crawler* in the conidiospores revealed that cryptic splicing and premature polyadenylation of the mRNA occurred under the normal culture condition. The increasing in mature mRNA molecules was caused by stress treatment of $CuSO_4$ or heat shock, which could stimulate the transposition events allowing the full-length and active transposase to be produced. @In this study, we carried out direct high-throughput paired-end RNA @sequencing to construct a stress-fluctuation cDNA browser with DOGAN-DB to survey exogenous or transposon-like genes such as *Crawler* in *A. oryzae*. With comparison of expression pattern under extreme stress condition ($CuSO_4$) to the normal condition, several novel transcripts with open reading frames were identified in intergenic regions, where none of genes have been annotated in DOGAN-DB. Full length of DNA sequences encoding transposable elements were frequently identified. Among them, a novel transposable element homologous to *Tan1* from *A. niger* was identified and tentatively designated *AoTan1* that shows multiple characteristics of class II transposon. The elements are present as multiple copies in the genome of the RIB40 strain, suggesting that *AoTan1* is also expected to show a transposition activity. 1)H. Ogasawara *et al*. *Fungal Genet. Biol.* , **46**, 441-449 (2009)

27) SrbA and its Role in Mediating Azole Drug Resistance in *Aspergillus fumigatus* Sara J. Blosser, Sven D. Willger, Robert A. Cramer Jr Department of Immunology & Infectious Diseases, Montana State University, Bozeman, Montana, USA sara.wezensky@msu.montana.edu

Deletion of the transcription factor SrbA results in complete growth inhibition under hypoxic conditions, avirulence in a murine model of Invasive Aspergillosis (IA), and increased sensitivity of *A. fumigatus* to triazoles. The purpose of this study is to investigate the mechanism and role of SrbA in mediating azole resistance. Azole drugs target ergosterol biosynthesis as their mechanism of action, and are the drug class of choice for treatment of IA. Erg11 (*cyp51*), the target of triazoles, is a 14-demethylase, and two functional copies (A/B) are encoded in the A. fumigatus genome. Transcript analysis shows down-regulation of Erg11A in the SrbA mutant, delta-srbA, suggesting regulation by this transcription factor. Induction of Erg11A in delta-srbA by regulatable promoter replacement restores wild-type levels of Erg11A, and ameliorates the azole sensitivity phenotype observed in delta-srbA. Repression of this construct restores azole sensitivity, demonstrating that Erg11A repression is partially or wholly responsible for the delta-srbA-azole phenotype. As SrbA appears to regulate Erg11A and several other key enzymes in ergosterol biosynthesis, understanding the regulon of SrbA could be vital in the development of higher-octane antifungals. Sterol intermediates in delta-srbA indicate a blockage in the Erg25 (C4-desaturase) enzymatic step, which is compounded in the Erg11A-induced strain. Studies investigating the induction of Erg25A with Erg11A in the delta-srbA background are underway.

CELL BIOLOGY

28) Maltose permease-encoding mRNA is cleaved in *Aspergillus oryzae*. Mizuki Tanaka, Takahiro Shintani, and Katsuya Gomi Graduate School of Agricultural Science, Tohoku University, Japan

Eukaryotic mRNA is degraded by two degradation pathways: the 5 L to 3 L degaradation pathway by Xrn1 and the 3 L to 5 L degradation pathway by exosome-Ski complex. To investigate the mRNA degradation mechanism in filamentous fungi, we generated the disruptions of orthologous genes encoding mRNA degradation machinery in *A. oryzae*. Interestingly, the disruptants of *ski2* and *ski3*, components of Ski complex, showed the remarkable growth defect on minimal medium containing maltose or starch as a sole carbon source, whereas they normally grew on the medium with glucose or fructose as a sole carbon source. Northern blot analysis showed that the 3 L-truncated fragment of mRNA encoding maltose permease (*malP*) was accumulated in Ski complex deficient mutants. Circularized RT- PCR analysis revealed that the *malP* mRNA was cleaved at a large stem-loop structure situated within the coding region. Since the 3 L-truncated *malP* mRNA has no translational termination codon, it would be recognized by a certain ribosome releasing factor(s). We thus generated the gene disruptant of HbsA, ortholog of yeast Hbs1 identified as a recognition factor of aberrant mRNA in which ribosome was stalled in translation elongation. In a *hbsA* disruptant, the 3 L-truncated *malP* mRNA was accumulated, and its degradation was suppressed. These results indicate that the *malP* mRNA is cleaved by endonuclease and the 3 L-truncated *malP* mRNA is degraded rapidly by HbsA-dependent 3 L to 5 L degradation pathway.

29) Asymmetric RNA localization in *Aspergillus fumigatus*. Mara Couto-Rodríguez, Susan Cowden, Ken Oda and Michelle Momany. Plant Biology Department, University of Georgia, Athens, Georgia 30602.

Filamentous fungi, such as *Aspergillus fumigatus*, are characterized by a highly polarized growth that occurs mainly by hyphal tip extension. Fungal tip growth involves many processes such as cell wall synthesis, vesicle transport, exocytosis and endocytosis. Even though there has been a reasonable amount of progress understanding these processes in the past few years, the exact mechanisms that regulate establishment and maintenance of polarity are not completely understood. Recent studies of highly polar cells from *Drosophila melanoganster, Candida albicans, Ustilago maydis* and others have demonstrated that RNA localization is used to restrict translation spatially and temporally. Consequently, we investigated asymmetric RNA localization in *A. fumigatus* . Laser microcapture combined with 454 sequencing done in our lab identified many of transcripts that appeared to be asymmetrically localized in polar A. fumigatus cells. In order to validate the level of asymmetry detected by 454 sequencing we performed Fluorescent in situ hybridization (FISH) . Transcripts from tip, base and conidia that showed the greatest asymmetry and the highest expression levels were chosen to synthesize digoxigenin labeled dsDNA probes for in vivo detection in *A. fumigatus* germlings. FISH experiments confirmed that many individual mRNA's are differentially localized to tip, base and conidium.

30) Mutational Analysis of *Aspergillus fumigatus* Calcineurin A reveals critical domains required for its function *in vivo* and targeting to the hyphal septum. Praveen R Juvvadi, Jarrod R Fortwendel, Luise E Rogg, and William J Steinbach. Department of Pediatrics, Division of Pediatric Infectious Diseases Duke University Medical Center, Durham NC, USA.

Calcineurin, a conserved calmodulin-dependent protein phosphatase, is a heterodimer consisting of the catalytic (CnaA) and the regulatory (CnaB) subunits. It is known to play key roles in virulence, growth and stress responses of pathogenic fungi. Critically understanding the calcineurin pathway and identifying the residues indispensible for calcineurin activity *in vivo* will pave way for devising new drug targets for combating Aspergillosis. We previously reported that CnaA localizes at the hyphal septum implicating its importance for septum formation and conidiophore development. By constructing the delta-cnaA delta-cnaB double mutant strain of *A. fumigatus* and utilizing the dual fluorescent labeling technique we provide evidence on colocalization of CnaA-GFP and mcherry-CnaB fusion proteins at the hyphal septum. Surprisingly, while the CnaB-GFP fusion protein mislocalized to the cytosol in the absence of of cnaA, cnaA still localized to the hyphal septum in the absence of cnaB. By site-directed point mutagenesis of several residues in the catalytic domain, CnaB binding helix, and the calmodulin binding domain of CnaA, we identify critical domains essential for its function *in vivo* apart from the absolute requirement of complexing with CnaB for its function at the hyphal septum.

31) Many mRNAs of *Aspergillus fumigatus* are asymmetrically localized in germlings. Ken Oda, Mara Couto-Rodriguez, Susan Cowden, John Kerry, Michelle Momany, Dept. of Plant Biology, Univ. of Georgia, Athens, GA 30602, USA (koda@plantbio.uga.edu)

A.fumigatus is the most common airborne pathogen causing fatal mycoses in immunocompromised patients. Polarized growth is one of the critical factors for establishing fungal pathogenesis, but little is known about the genes involved in early polar growth and their regulation. To understand the spatial distribution of polarity related mRNA, we performed spatial gene expression analysis of germlings. *A. fumigatus* Af293 was cultured in complete medium for 8hr which is the time just before septation. Tip, base, and conidium regions were captured by Laser Microdissection Pressure Catapulting (LMPC) and whole germlings were collected as a reference. Total RNA was extracted and a cDNA library was constructed for each region and for whole germlings. The quality of each cDNA library was confirmed by performing qRT-PCR for highly expressed genes. mRNA sequencing of each library was performed using a Next- Generation Sequencer (454 GS FLX). By comparing each region, we found that more than 1000 mRNAs are asymmetrically localized. To confirm mRNA localization, we performed Fluorescence in situ Hybridization (FISH) with some of the highly expressed genes in each region and found that their distribution was consistent with sequence results. These data suggest that many mRNAs are asymmetrically localized in tip, base, and conidium region of germlings.

32) The Trehalose Pathway contributes to key virulence attribute production in *Aspergillus fumigatus*. Srisombat Puttikamonkul[1], Sven D. Willger[1], Nora Grahl[1], John R. Perfect[2], Navid Movahed[3], Brian Bothner[3], and Robert A. Cramer Jr.[1]. [1]Department of Immunology and infectious diseases, Montana State University, Bozeman, MT 59718. [2] Department of Medicine, Duke University Medical Center, Durham, NC 27713. [3] Department of Chemistry and Biochemistry, Montana State University, Bozeman, MT 59718.

Recently, our studies on OrlA (Trehalose 6 Phosphate (T6P) Phosphatase) in *A. fumigatus* suggest that increases in T6P levels lead to decreased activity of hexokinase, abolished asexual reproduction, cell wall defects, and avirulence in murine models of IPA. However, these phenotypes are not due to loss of trehalose itself since production persists in the absence of OrlA through an unknown mechanism. Moreover, complete loss of T6P and trehalose production only occurs when 2 genes, TpsA and TpsB, encoding trehalose 6 phosphate synthases, are both deleted. Intriguingly, loss of TpsA but not TpsB in strain CEA10 results in cell wall defects that affect fungal pathogenesis further supporting a link between trehalose metabolism and cell wall dynamics in *A. fumigatus*. Consequently, we hypothesize that the trehalose biosynthesis pathway and its intermediates play key roles in regulating fungal cell wall biosynthesis by affecting central carbon metabolism of fungi. These affects consequently have significant ramifications for the ability of *A. fumigatus* to cause disease. Further genome sequence analysis of the *A. fumigatus* genome reveals additional uncharacterized genes predicted to encode proteins likely involved in trehalose biosynthesis and metabolism. Amino acid alignments of these genes suggests that they may play important undefined roles in regulating trehalose production underscoring the complexity and unknown mechanisms of action of this pathway in *A. fumigatus*. Therefore, our results strongly suggest that trehalose biosynthesis and catabolism are important components of Aspergillus biology that directly affect fungal pathogenesis by as yet undefined mechanisms.

33) Quantifying the importance of galactofuranose in Aspergillus nidulans hyphal wall surface organization using atomic force microscopy. Biplab Paul (a), Amira El-Ganiny (b,c), Tanya Dahms (a), Susan Kaminskyj (b) a) Dept Chem&Biochem, Univ Regina, Canada; b) Dept Biology, Univ Saskatchewan, Canada; c) Dept Microbiol, Fac Pharmacy, Univ Zagazig, Egypt. Susan.Kaminskyj@usask.ca

Galactofuranose (Galf), the five-member ring form of galactose, is a minor component of Aspergillus walls. Strains deleted for Galf biosynthesis enzymes UgeA (UDP-glucose-4-epimerase) and UgmA (UDP-galactopyranose mutase) lacked immunolocalizable Galf, and had growth defects and abnormal wall structure. We used atomic force microscopy and force spectroscopy to image and quantify surface elasticity and adhesion of ugeAΔ and ugmAΔ strains and to compare them with two near-isogenic wild type strains, AAE1 and the ugeBΔ. Our results suggest that UgeA and UgmA are important for cell wall surface subunit organization and wall elasticity. The ugeAΔ and ugmAΔ strains had larger surface subunits and lower cell wall viscoelasticity than those of AAE1 or ugeBΔ hyphae. Double deletion strains [ugeAΔ, ugeBΔ] and [ugeAΔ, ugmAΔ] had more disorganized surfaces than single deletion strains. Wall surface structure correlated with wall viscoelasticity for both fixed and living hyphae, with wild type walls being the most viscoelastic and the double deletion strains being the least. The ugmAΔ and particularly the [ugeAΔ, ugmAΔ] strain were more adhesive to hydrophilic surfaces than wild type. We propose that Galf is necessary for proper packing of cell wall components, so its loss gives rise to surface disorder, greater hydrophilic character and reduced viscoelasticity.

34) Aspergillus nidulans GputA (galactose-1-phosphate uridylyltransferase) is not essential for galactose metabolism, but is required for wild type conidiation. Kausar Alam and Susan Kaminskyj, Dept Biology, Univ Saskatchewan, Canada. Susan.Kaminskyj@usask.ca

Saccharomyces galactose-1-phosphate uridylyltransferase (GAL7) mediates transfer of UDP between galactose and glucose and their respective sugar-1-phosphate conjugates, leading to glycolysis or to wall glycan synthesis. Aspergillus nidulans ANID_06182 has 50% amino acid sequence identity with GAL7 and was annotated as a galactose-1-phosphate uridylyltransferase. We named it GputA. Although GAL7 is essential, a confirmed gputA deletion strain, AKA1 grew and conidiated on minimal medium containing glucose or galactose as sole carbon sources, and on peptide-only Difco Nutrient Broth. AKA1 conidiation was reduced compared to a near-isogenic gputA+ strain, AAE1 on complete medium (CM) containing glucose or galactose (CM-Glu and CM-Gal) as sole carbohydrate sources. Scanning electron microscopy showed this was due to failure at the vesicle-metula transition on CM-Glu vs reduced spore production from morphologically normal phialides on CM-Gal. AKA1 spore germination was 78% on CM-Glu but 1% on CM Gal. GputA-GFP fluorescence was cytoplasmic, was more intense in spores than hyphae, and was significantly brighter for cells grown on CM-Gal. Using qRT-PCR, we found that gputA expression was enhanced ten-fold by growth on CM-Gal compared to CM-Glu. These findings suggest that GputA function is distinct from Saccharomyces GAL7, and is consistent with A. nidulans hexose metabolism complexity. AKA1 defects appear to relate to cell polarity establishment but not polarity maintenance.

35) The Aspergillus nidulans UDP-galactofuranose transporter, UgtA roles in wall structure, hyphal morphology, and conidiation. Sharmin Afroz (a), Amira El-Ganiny (a, b), Susan G.W. Kaminskyj (a) a) Dept Biology, Univ Saskatchewan, Canada; b) Microbiology Dept, Faculty of Pharmacy, Zagazig Univ, Egypt. Susan.Kaminskyj@usask.ca

Galactofuranose (Galf) is the 5-member-ring form of galactose found in the walls of fungi including Aspergillus. UDP- galactofuranose mutase (ANID_3112.1) generates UDP-Galf from UDP-galactopyranose (6-member ring form). UgmA is cytoplasmic. UDP-Galf must be transported into an endomembrane compartment before incorporation into wall components. ANID_3113.1 (which we call UgtA) was identified based on its high amino acid sequence identity with GlfB, the UDP galactofuranose transporter in A. fumigatus. UgtA is not essential. The ugtAΔ strain, ASA1 has a phenotype similar to that of ugmAΔ: compact colonies with wide, highly branched hyphae. ASA1 spore production and germination were reduced compared to wild type. Using SEM, some ASA1 metulae produced phialide triplets, rather than pairs. These phialides produced nucleated spores. Using TEM, ASA1 hyphal walls were more than three-fold thicker than wild type strains. Aspergillus nidulans ugtA is predicted to have five exons, which we confirmed by isolating and sequencing its cDNA. The UgtA predicted product is a 400 amino acid integral membrane protein likely to have 11 transmembrane helices. An A. nidulans strain with UgtA GFP under the control of its constitutive promoter had a punctate GFP fluorescence pattern consistent with localization to the fungal Golgi equivalent. We are exploring possible interactions between UgmA and cytoplasmic loops of UgtA.

36) Plasma membrane-compartmentalized activity of *Aspergillus fumigatus* RasA is required for polarized growth and virulence. Jarrod R. Fortwendel, Praveen R. Juvvadi, Luise E. Rogg, and William J. Steinbach Duke University Medical Center, Durham, NC USA

Ras homologs are multifunctional proteins that are localized to specific sub-cellular membranes via post-translational addition of farnesyl and palmitoyl lipid moieties. Farnesylation of Ras directs the nascent Ras protein to the endomembrane system, whereas plamitoylation drives localization to the plasma membrane. This "compartmentalization" of activity allows for specificity in signal transduction. We have previously shown that deletion of *A. fumigatus rasA* causes slowed growth, malformed hyphae, and reduced cell wall integrity. However, the membrane distribution and the role of sub-cellular compartmentalization of RasA activity in these important cellular processes are unknown. To examine the distribution of RasA, a GFP-RasA fusion was expressed in the delta-*rasA* mutant background. GFP-RasA localized primarily to the plasma membrane of actively growing hyphae and septa. Expression of GFP-RasA in the delta- *rasA* background resulted in recovery of the wild type phenotype, indicating the fusion was functional. Inhibition of protein palmitoylation using 2-bromopalmitate caused hyphal deformation and reduced growth, as well as mislocalization of the GFP-RasA protein to internal structures. To further explore the role of palmitoylation, mutations in two conserved cysteine residues, which function as palmitoylation sites, were introduced to completely block RasA palmitoylation. The palmitoylation-deficient RasA mutant (RasA-P) displayed a decreased growth rate and hyphal abnormalities similar to the delta-*rasA* strain, as well as complete mislocalization of GFP-RasA from the plasma membrane. The delta- *rasA* and RasA-P mutants displayed similarly altered glucan and chitin staining, while TEM analysis revealed similar cell wall structural differences in both strains. Virulence was decreased for both mutants in a mouse model of invasive aspergillosis. Taken together, our data reveal the importance of plasma membrane-localized RasA activity in polarized morphogenesis and virulence of *A. fumigatus*.

37) Gold nanoparticles in Aspergillus nidulans hyphae: can we study real-time physiology? Susan Kaminskyj (a), Martin Prusinkiewicz (a), Fatemeh Faraz-Khorasani (b), Merrill Isenor (b), Kathleen Gough (b) a) Dept Biology, Univ Saskatchewan, Canada; b) Dept Chemistry, Univ Manitoba, Canada.

High spatial resolution methods to analyze biochemical composition of individual hyphae can be used to assess cell physiology during growth in optimal or stressed conditions. Whole colony methods like GC-MS cannot capture all the details of physiology and organism-environment interaction. In addition to Fourier transform infrared (FTIR) spectromicroscopy, surface-enhanced Raman spectroscopy (SERS) can provide biochemical characterization of components that are in contact with gold that has been nano-patterned (Klarite substrate) or has formed nano-particles (AuNPs). SERS can potentially be used to examine biochemical processes in living cells. We have grown AuNPs within and on the surface of Aspergillus nidulans hyphae, and documented their distribution and composition using transmission electron microscopy and scanning transmission x-ray microscopy. Most AuNPs were associated with hyphal walls, both in the cytoplasm and on the wall surface. AuNPs grown in cultures treated for 2h with 1mM Au3+ appeared to be optimal for generating SERS activity. The AuNP spectra were more complex than most SERS spectra from A. nidulans hyphae grown on a Klarite substrate. Interpreting SERS spectra will be challenging, and will require validation for the diversity of molecules present on the wall and in the peripheral cytoplasm. To date we have proof in principle that it will be possible to generate SERS spectra in living hyphae.

38) The polo like kinase PLKA in *Aspergillus nidulans* is not essential, but plays important roles in vegetative growth and negatively regulates sexual development. Klarita Mogilevsky, Amandeep Glory, and Catherine Bachewich. Department of Biology, Concordia University, 7141 Sherbrooke St. West, Montreal, QC. cbachewi@alcor.concordia.ca.

The Polo-like kinases (Plks) are conserved, multi-functional cell cycle regulators that play additional roles in metazoan development. We previously identified *plkA* in *Aspergillus nidulans*, the only Plk investigated in filamentous fungi to date, and partially characterized its function through overexpression. We now report the *plkA* null phenotype. Surprisingly, *plkA* was not essential, unlike other fungal Plks. A subset of *Î"plkA* cells contained defects in spindle and chromosome organization, supporting some conservation in cell cycle function. However, septa were present, suggesting that PLKA is not a central regulator of septation like other Plks. The *Î"plkA* colonies were compact with multi-branched hyphae, implying a novel role for PLKA in hyphal morphogenesis. These defects were suppressed by high temperature or low benomyl concentrations, suggesting that PLKA functions in part through influencing microtubule dynamics. However, *Î"plkA* colonies also demonstrated benomyl and temperature- insensitive decreases in conidiation and precocious formation of Hulle cells. This represents the first example of a link between a Plk and development in fungi, and suggests that PLKA negatively regulates sexual reproduction through distinct mechanisms. Phylogenetic analyses suggest that PLKA and filamentous fungal Plks are related to the divergent metazoan PLK4, whereas yeast Plks group with metazoan PLK1-3. Thus, PLKA has some conserved functions, but may play additional novel roles in influencing morphogenesis and negatively regulating sexual development.

39) IRENI permits major advances in FTIR imaging of fungal hyphae. Kaminskyj S (a), Nasse M (b, c), Rak M (c), Gough K (d), Hirschmugl C (b, c) a) Dept Biology, Univ Saskatchewan, Canada; b) Univ Wisconsin Milwaukee; c) Synchrotron Radiation Center, Madison WI; d) Dept Chemistry, Univ

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Fourier transform infrared (FTIR) spectroscopy can characterize organic compounds including complex mixtures such as cytoplasm. Studies on rapidly frozen and dried fungal hyphae, using brilliant synchrotron IR sources, showed that fungal tips have lower content than subapical regions in the same cells, and that hyphal composition changes in response to environmental perturbation. Recently (in conjunction with other methods) we used an FTIR microscope with improved sensitivity, a globar IR source, and a 64 x 64 focal plane array (FPA) detector to document hyphal mannitol distribution. These studies were limited to ~ 6μm pixel size. Now, a unique synchrotron IR source called IRENI with 12 IR beamlines illuminating a single FPA detector permits diffraction-limited resolution. With IRENI, we can 1) collect data at 0.5μm x 0.5μm pixel definition, 2) characterize hyphal cytoplasm and exudate, 3) analyze hyphae as they grow in a moist chamber. Here, we compare wild type and single gene deletion strains of Aspergillus nidulans: A4 (used for the genome sequencing project), AAE1 (an nkuA? strain with wild type hyphal morphology), and nkuA? strains further deleted for ugmA or ugeA, key members of the galactofuranose biosynthesis pathway that have abnormal hyphal morphology and wall architecture. With this technology and these strains we are beginning to unpack the biochemical complexity of fungal tip growth.

40) DenA is a deneddylating protein involved in *A. nidulans* development Martin Christmann, Rebekka Harting, Özgür Bayram, Gerhard H. Braus, Institute for Microbiology and Genetics, Georg August University Göttingen, D-37077 Göttingen, Germany (Germany), mchrist@gwdg.de

Deneddylation is the removal of the ubiquitin (Ub)-like protein Nedd8 from cullins. Cullins are subunits of cullin-RING Ub ligases (CRL) which are controlled in their activity and assembly/reassembly by neddylation and deneddylation, respectively. The most important eukaryotic deneddylases are the COP9 signalosome (CSN) and the deneddylating enzyme 1 (DEN1). Mammalian Den1 has two functions: an isopeptidase activity removing Nedd8 from cullins and other proteins and an additional linear peptidase activity processing Nedd8 from a precursor protein. Filamentous fungi possess an eight subunit COP9 signalosome (CSN) which is reminiscent to the corresponding plant and vertebrate complex (Busch et al. 2007, Braus et al., 2010). *Aspergillus nidulans* requires CSN function to trigger development, the appropriate response of the fungus towards light, and for a coordinated secondary metabolism (Nahlik et al., 2010). We show here the characterization of the fungal Den1 ortholog DenA. The *denA* gene encodes a cysteine protease deneddylating enzyme. DenA is required for light control and the asexual fungal development whereas CSN is required for the sexual cycle. Processed Nedd8 is unable to rescue conidia formation suggesting that the lack of the DenA deneddylase isopeptidase activity is responsible for the defect. Yeast two hybrid studies suggest a physical interaction between DenA and CSN which has to be further evaluated. Busch S, Schwier EU, Nahlik K, Bayram Ö, Draht OW, Helmstaedt K, Krappmann S, Valerius O, Lipscomb WN, Braus GH (2007), PNAS, USA. 104, 8125-8130. Braus GH, Irniger S, Bayram Ö (2010), Curr. Opin. Microbiol. 13, 1-5. Nahlik K, Dumkow M, Bayram Ö, Helmstaedt K, Busch S, Valerius O, Gerke J, Hoppert M, Schwier E, Opitz L, Westermann M, Grond S, Feussner K, Goebel C, Kaever A, Meinecke P, Feussner I, Braus GH (2010), Mol. Microb. 78, 962-979.

41) A potential functional relationship between phosphorylation by mitotic kinases and protein methylation by the Set1 complex in *Aspergillus nidulans.* Meera Govindaraghavan[1], Sarah Lea McGuire[2] and Stephen A. Osmani[1] [1]Department of Molecular Genetics, The Ohio State University, Columbus, OH, [2]Department of Biology, Millsaps College, Jackson, MS, govindaraghvan.1@buckeyemail.osu.edu, osmani.2@osu.edu

The G2-M transition is regulated by the activity of two mitotic kinases, NIMA and NIMX, in Aspergillus nidulans. To gain further insight into the mechanism of NIMA function, a synthetic lethal screen was carried out utilizing the deletion of the non-essential *nimA* orthologue, *KIN3*, in Saccharomyces cerevisiae. This screen revealed a set of 11 genes involved in different cellular processes. By deletion analysis, four of these synthetic genetic interactions were found to be conserved in *A. nidulans*, one of which is between *nimA7*^{ts} and the deletion of *An- swd1*, the ortholog of a subunit of the Set1 methyl transferase complex. Moreover, the synthetic lethal interaction between *?An-swd1* and a cell cycle mutant with reduced NIMX function (*nimT23*^{ts}) suggests that lack of *An-swd1* function in combination with defects in G2-M transition is highly deleterious. These genetic interactions result from loss of protein methyl transferase activity of the Set1 complex, since the deletion of *An- set1*, which encodes the catalytic protein, also exhibits genetic interaction with *nimA7*^{ts} and *nimT23*^{ts}. Interestingly, the deletion of *An-swd1* also modifies the *nimA7* and *nimT23* phenotypes at their fully restrictive temperature, causing a drastic growth defect. Furthermore, a proportion of *nimA7+?An-swd1* cells are uninucleated yet undergo septation, a phenotype never observed in either single mutant. Collectively these results indicate an important functional relationship exists between mitotic protein phosphorylation and protein methylation.
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42) The genetic basis of conidial pigmentation in *Aspergillus niger* M. Arentshorst, T.R. Jørgensen, J. Park, A.M. van Welzen, G. Lamers, P.A. vanKuyk, R.A. Damveld and A.F.J. Ram. Institute of Biology Leiden, Leiden University, Molecular Microbiology and Biotechnology, Kluyver Centre for Genomics of Industrial Fermentation, Sylviusweg 72, 2333 BE, Leiden, The Netherlands.

A characteristic hallmark of *Aspergillus niger* is the formation of black conidiospores. To understand the genetic basis of black spore formation, we have identified four genes required for pigmentation by using a complementation approach. First, we characterized a newly isolated color mutant, *colA*, which lacked pigmentation resulting in white conidia. Pigmentation of the *colA* mutant was restored by a gene (An12g03950) which encodes the *A. niger* ortholog of the 4'-phosphopantetheinyl transferase protein (PptA). The loci giving rise to fawn, olive, and brown color phenotypes were identified by complementation. The fawn mutant was complemented by the polyketide synthase A protein (PksA, An09g05730), the *ovlA* mutant by An14g05350 (OlvA) and the *brnA* mutant by An14g05370 (BrnA), the respective homologs of *pksP/alb1, ayg1* and *abr1* in *A. fumigatus*. Targeted disruption of the four genes confirmed the complementation results. Epistasis was determined for *pksA, olvA* and *brnA* by constructing double mutants. This set of isogenic color mutants is a useful tool to do classical genetic analyses in *Aspergillus niger*.

43) Functional analysis of the AAA ATPase AipA localizing at the endocytic sites in the filamentous fungus *Aspergillus oryzae*. Yujiro Higuchi, Manabu Arioka, Katsuhiko Kitamoto. Department of Biotechnology, The University of Tokyo, Japan.

We explored novel components involved in endocytosis by the yeast two-hybrid (YTH) screening using AoAbp1 (*Aspergillus oryzae* actin binding protein) as bait. A gene named *aipA* (AoAbp1 interacting protein) which encodes a putative AAA (ATPases associated with diverse cellular activities) ATPase was obtained. Further YTH analyses showed that 346-370 aa region of AipA interacted with the two SH3 domains of AoAbp1. AipA interacted with AoAbp1 *in vitro*, and in *A. oryzae* EGFP-AipA co-localized with AoAbp1-mDsRed at the tip region, suggesting that AipA functions in endocytosis. Although *aipA* disruptants did not display any phenotypic alteration in several culture conditions, *aipA*-overexpressing strains showed defective growth and the aberrant hyphal morphology. Moreover, we generated strains which have mutations of either $aipA^{K542A}$ or $aipA^{E596Q}$. These mutations were introduced in the ATPase domain of AipA and would cause defect in the ATPase activity. In contrast to the strain overexpressing WT *aipA*, the growth of mutated strains was normal, suggesting that ATPase activity is important for the function of AipA. Furthermore, the *aipA*-overexpressing strain displayed a delay in FM4- 64 transport to Spitzenkorper, whereas the mutated *aipA*-overexpressing strains did not, suggesting that AipA negatively regulates apical endocytic recycling.

44) Investigation of the role and interaction of subunits of the Golgi vesicle tethering COG complex in *Aspergillus nidulans*. Sara Gremillion[1], Stacie Driggers[1], Darlene Loprete[2], and Terry Hill[2]. [1]Department of Biology, Armstrong Atlantic State University, 11935 Abercorn Street, Savannah, GA 31419, USA, and [2]Departments of Biology and Chemistry, Rhodes College, 2000 N. Parkway, Memphis, TN 38112, USA. Sara.Gremillion@armstrong.edu

The COG (conserved oligomeric Golgi) complex is associated with tethering of vesicles undergoing retrograde transport within the Golgi apparatus. Studies in animals and yeast revealed that the COG complex is composed of eight protein subunits which form a bi-lobed structure. In both models, the lobe containing COG1-4 is essential for proper COG function. Little is known of the COG complex in the filamentous fungi. Two temperature sensitive mutants have been discovered in *Aspergillus nidulans*, named *swoP*1 (swollen cell) and *podB*1 (polarity defective). Both mutants display abnormal spore swelling and polarity when grown at a restrictive temperature. Genes complementing the mutations of *swoP*1 and *podB*1 have sequence homology to COG4 (ANID7462.1) and a conserved hypothetical protein, likely COG2 (ANID8226.1), respectively. A GFP-tagged COG2 displayed a punctuate distribution within fungal hyphae, a pattern consistent with other Golgi protein localization. Protein over-expression studies provided evidence of inner-complex interactions between COG2 and COG4 as well as COG2 with COG3. To study the role of these proteins in the complex, an AlcA promoter replacement strategy was performed. When grown on AlcA-suppressive media, the AlcA-promoter COG4 and AlcA-promoter COG7 strains displayed wild type growth at a restrictive temperature, while the AlcA-promoter COG1 and AlcA-promoter COG2 strains displayed swollen cells and abnormal polarity similar to that observed in the *swoP*1 mutant phenotype at a restrictive temperature. This data suggests that COG1 and COG2 are necessary for proper COG function in *A. nidulans* while COG4 and COG7 are not.

45) The *snxA* gene of *Aspergillus nidulans* encodes a homolog of yeast Hrb1. S.L. McGuire Anglin[1], T. Banta[1], C. Coile[1], C. Dixit[1], S. Eastlack[1], A. Giang[1], J. Kobie[2], M. Nguyen[1], K. Shingler[2], A. Orzechowski[2], and S. James[2]. [1]Millsaps College, Jackson, MS. [2]Gettysburg College, Gettysburg, PA. mcguisl@millsaps.edu

The *snxA1* cold-sensitive mutation of *A. nidulans* was originally identified as an extragenic suppressor of the $nimX2^{F223L}$ heat sensitive mutation. Analysis of double mutants showed that *snxA1* suppresses all three heat sensitive $nimX^{CDC2}$ mutations as well as mutations in $nimE^{CYCLINB}$ and $nimT^{CDC25}$; that *snxA1* has a synthetic phenotype in combination with a deleted $ankA^{WEE1}$; and that *snxA1* is not a general cell cycle suppressor. Western blot data suggest that the levels of both NIMX and CYCLINB are not affected by the *snxA1* mutation. We have recently cloned snxA and found that it encodes a homolog of *Saccharomyces cerevisiae* Hrb1, a protein involved in mRNA transcription and export. SNXA is nonessential; however, deletion of *snxA* results in an extremely cold-sensitive phenotype similar to but more extreme than the *snxA1* mutation; deletion also suppresses $nimX^{CDC2}$ mutations. Overexpression of wild-type SNXA complements *snxA1*. These data suggest that nonmutant SNXA functions to restrain NIMX/CYCLINB activity. GFP-labelled SNXA localizes to the nucleus in 87% of germlings when grown at 29ºC; 13% of germlings have no detectable SNXA. Studies are currently underway to determine if SNXA localization correlates with CYCLINB localization. Funding provided by NIH NCRR P20RR016476 and NIH2R15GM055885

46) Defining the essential location and function of Nup 2 in *Aspergillus nidulans*. Subbulakshmi Suresh, Sarine Markossian, Aysha H. Osmani and Stephen A. Osmani, Department of Molecular Genetics, The Ohio State University, USA-43210.

Nuclear Pore Complexes (NPCs) are macromolecular assemblies spanning the nuclear envelope and they are made up of 30 different proteins called Nucleoporins (Nups). In *Aspergillus nidulans* , the NPC undergoes partial disassembly during mitosis during which peripheral Nups disperse from the NPCs throughout the cell. However, Nup2 has a unique mitotic behavior as it translocates from NPCs onto chromatin specifically during mitosis. Nup2 is targeted to NPCs and chromatin by a newly identified Nup called NupA. We aim to define if the sole role of NupA is to target Nup2 to the NPC and chromatin by creating Nup2-NupA chimeras. We also aim to characterize whether the essential role of Nup2 is at NPCs during interphase, or on chromatin at mitosis, by artificially tethering Nup2 exclusively to NPCs or to chromatin. Also, since Nup2 is a mitotic phosphoprotein, we seek to define its phosphorylation sites. To answer the above aims, we have successfully created Nup2-NupA chimeras. We have also achieved artificial tethering of Nup2 to specific cellular locations and find that constitutive tethering of Nup2 to the NPC compromises cell viability. We also find that Nup2 is highly phosphorylated in response to activation of the spindle assembly checkpoint and have begun to map the phosphorylation sites using MS analysis of purified Nup2. In conclusion, our studies shed light on the mitotic functions of Nup2 on chromatin, regulated by mitotic kinases. (Supported by National Institutes of Health grant GM042564)

47) Identification of a new transmembrane nuclear pore protein in Aspergillus nidulans using global proteomic analysis. Aysha H. Osmani, Hui-Lin Liu, Colin P. De Souza and Stephen A. Osmani. The Ohio State University, Columbus, OH, USA. (osmani.2@osu.edu)

We have previously reported the unexpected finding that in Aspergillus nidulans removal of all three fungal transmembrane nuclear pore complex (NPC) proteins (An-Ndc1, An-Pom152, and An-Pom34) does not cause lethality (Liu et al., 2009 MBC 20, 616-630). This suggests either that transmembrane proteins are not required for NPC function or that additional transmembrane NPC proteins remain to be discovered. To address this issue we have completed a global proteomic analysis of A. nidulans Nups (NPC proteins) using affinity purification and mass spectrometry analysis. This approach involved endogenously tagging >40 different proteins and completing LC/MS/MS analysis of >100 purified samples. The data set has allowed identification of numerous proteins that interact with NPCs including an orthologue of a new transmembrane Nup (Pom33) recently discovered in budding yeast (Chadrin et al., 2010 JCB 189, 795-811). An- Pom33 purified with An-Ndc1. Using GFP tagging and deletion analysis we have asked if An-Pom33 is a new transmembrane Nup in A. nidulans. As expected of a transmembrane Nup, An-Pom33-GFP locates to the nuclear envelope throughout interphase, concentrating at spindle pole bodies during mitosis in a manner similar to An-Ndc1. In addition, An-Pom33 is also present in the cytoplasm in an ER-like structure. Removal of An-Pom33 does not cause lethality but the quadruple transmembrane Nup mutant (lacking An-Ndc1, An-Pom152, An-Pom34, and An-Pom33) is inviable. Pairwise deletions revealed that lack of An-Pom33 with lack of An-Ndc1 is the only lethal combination. This study identifies An-Pom33 as a conserved transmembrane Nup that functions redundantly with An-Ndc1. (Supported by NIH grant GM042564)

48) Gle1 translocates from the nuclear pore complex to the nuclear membrane during mitosis in *Aspergillus nidulans*. Mahesh Chemudupati, Aysha Osmani and Stephen A. Osmani Department of Molecular Genetics, The Ohio State University, Columbus, OH

During mitosis in *Aspergillus nidulans*, a subset of nuclear pore complex proteins (Nups) disperses from the core pore structure. This subset of Nups includes all budding yeast orthologs classified as peripheral Nups, with one exception. The predicted peripheral Nup An-Gle1 is unique in that it remains at the nuclear envelope (NE) during mitosis, an attribute typical of core Nups (Osmani et al. *Mol. Biol. Cell*, 17, 4946–4961, 2006). Additionally, GFP labeled An- Gle1 is distinctly located at the nuclear membrane surrounding the nucleolus during mitosis (Ukil et al. *Mol. Biol. Cell*, 20, 2132–2145, 2009). Affinity purification of all known Nups in *A. nidulans* identified a protein that co- purified with An-Gle1. This protein, AN0162, has a predicted C-terminal transmembrane domain. Endogenously GFP- tagged full-length AN0162 locates to the nuclear membrane throughout mitosis in a manner identical to An-Gle1 as does the GFP-tagged C-terminal half of the protein. Further analysis showed that AN0162 is responsible for tethering An-Gle1 to the NE specifically during mitosis, but not during interphase. This is the first known instance of a protein targeted to the NE by two different mechanisms. An-Gle1 is targeted to NPCs during interphase, but during mitosis An-Gle1 is targeted to the nuclear membrane by associating with AN0162. Our findings thus agree with the categorization of An-Gle1 as a peripheral Nup. This expands our knowledge of how proteins can be targeted to the NE in a regulated cell-cycle dependent manner. Supported by National Institutes of Health grant GM042564

49) Identification of New DNA Damage Response Proteins Using a Genetic Screen. Larson, J. R. and Osmani, S. A. The Ohio State University, Columbus, Ohio. larson.315@osu.edu, osmani.2@osu.edu

The cellular response to DNA damage involves many well characterized proteins. However, there are likely still unidentified proteins that play important roles in these pathways. For example, several nuclear pore complex proteins (Nups) are required for resistance to DNA damage via unknown mechanisms. SonB is an essential Nup in *Aspergillus nidulans* and a mutant allele of this protein that confers temperature-dependent DNA damage sensitivity was previously isolated in a genetic screen for suppressors of the temperature-sensitive *nimA1* mitotic kinase mutation (De Souza et al., 2006 Genetics 174, 1881-93). Importantly, subsequent analyses showed that SonB is involved in a novel DNA damage response pathway. No other proteins have yet been linked to this pathway. To identify other proteins involved with SonB we have undertaken a genetic screen for DNA damage-sensitive *nimA1* suppressors and have isolated two previously uncharacterized proteins, AN1902 and a new Nup, AN11115. AN11115-GFP localizes to the nuclear periphery throughout the cell cycle and at mitosis forms several distinct foci similar to another Nup, Pom152. Deletion of AN11115 causes marked temperature-dependent DNA damage sensitivity, similar to SonB mutants. Affinity purification and mass spec analysis of AN11115 identified AN1902 thus linking together the genetic and biochemical data. Further studies will add to our understanding of how the nuclear pore complex and the NIMA kinase are involved in the DNA damage response and allow us to map out this novel DNA damage response pathway.

BIOCHEMISTRY AND METABOLISM

50) Targeted gene deletion and genome mining of the predicted *Aspergillus carbonarius* secondary metabolome. Michael Praseuth[1], Antonia Gallo[2], Scott E. Baker[3] , Clay C. C. Wang[1], and Kenneth S. Bruno[3] [1]Department of Pharmacology and Pharmaceutical Sciences, University of Southern California, School of Pharmacy, Los Angeles, California, USA. [3]Chemical and Biological Process Development Group, Energy and Environment Directorate, Pacific Northwest National Laboratory, Richland, Washington, USA [2]National Research Council, Institute of Science of Food Production (ISPA) Bari, Italy

Interest in natural products produced by fungi has grown substantially in recent years. Available genome sequences of filamentous fungi have revealed that these organisms contain the capacity to produce far more secondary metabolites than previously identified by conventional analysis. In large part, the reason that these compounds remain uncharacterized is due to the lack of gene expression under typical laboratory conditions. One approach to identifying novel compounds produced by fungi is heterologous expression of predicted secondary metabolite genes in a tractable model organism such as *Aspergillus nidulans*. Although this has proved successful with some compounds, an analysis in the native organism could prove more fruitful in determining all of the enzymes necessary to produce a given compound. For this reason we have developed a transformation protocol for transformation in Aspergillus carbonarious strain ITEM 5010. We have successfully deleted the kusA orthologue in this strain using the hygromycin phosphotransferase marker. In a subsequent transformation we deleted the orthologue of an *Aspergillus niger* gene responsible for naptha-pyrone production. Gene deletion and overexpression will be used to identify genes responsible for production of secondary metabolites in this species.

51) Impact of glucose metabolism during pathogenesis of *Aspergillus fumigatus* Christian B. Fleck[1], Ilse D. Jacobsen[2], Silvia Slesiona[1,2] and Matthias Brock[1] [1] Microbial Biochemistry and Physiology; [2] Microbial Pathogenicity Mechanisms Leibniz Institute for Natural Product Research and Infection Biology, -Hans Knoell Institute-, Beutenbergstr. 11a, 07745 Jena, Germany Contact: Matthias.brock@hki-jena.de

Aspergillus fumigatus is the major cause of life-threatening invasive bronchopulmonary aspergillosis. Manifestation and progression of infection in the immunocompromised host requires the efficient uptake and metabolism of nutrients. However, nutrient sources available and used by the pathogen are mainly unknown. Here, we focused on the impact of glucose metabolism during pathogenesis. Glucose is highly abundant in the bloodstream and may also be available within tissues. In order to activate glucose to glucose-6-phosphate *A. fumigatus* possesses two catalytically active hexose kinases, glucokinase and hexokinase (Fleck and Brock, 2010). Analysis of deletion mutants and biochemical characterisations of the enzymes showed that glucokinase possesses a low K_m value for the substrate glucose and is required for rapid germination of conidia. In contrast, hexokinase possesses a broader specificity for various sugars and is the dominating hexose kinase during vegetative growth. Deletion of either one enzyme caused only weak effects on glucose, but the hexokinase was essential for growth on fructose. A mutant deleted in both genes revealed normal growth on most gluconeogenic nutrient sources, but growth was abolished when glucose or fructose served as sole carbon source. When tested in a murine model for invasive aspergillosis, both single mutants caused disease comparable to the wild type. However, the double deletion mutant was strongly attenuated in virulence regardless the immunosuppression regimen. Thus, glucose seems to provide an abundant nutrient source during infection and interruption of glucose metabolism might provide a tool to inhibit growth during pathogenesis. Fleck CB, Brock M. (2010) *Aspergillus fumigatus* catalytic gluco- and hexokinase: Expression analysis, importance for germination, growth and conidiation. *Eukaryot Cell* **9**:1120-35

52) The additional D-galacturonate reductase genes in *Aspergillus niger* and *Hypocrea jecorina* Joosu Kuivanen, Satu Hilditch, Dominik Mojzita, Merja Penttilä and Peter Richard VTT Technical Research Centre of Finland, Espoo, Finland joosu.kuivanen@vtt.fi

Pectin, a cheap and abundant raw material has a huge potential in white biotechnology. D-galacturonate, the main component of pectin, is catabolised to pyruvate and glycerol through a reductive pathway in eukaryotic microorganisms like filamentous fungi. This pathway also enables the utilization of pectin for the production of more valuable compounds. An example from that is the production of *meso*-galactarate (mucic acid) in metabolically engineered fungal strains. Enzymes functioning on the reductive D-galacturonate pathway are a D-galacturonate reductase, L-galactonate dehydratase, 2-keto-3-deoxy-galactonate aldolase and glyceraldehyde reductase. The genes coding for these enzymes in *Aspergillus niger* are *gaaA*, *gaaB*, *gaaC* and *gaaD*, respectively, while in *Hypocrea jecorina* (*Trichoderma reesei*) the corresponding genes are *gar1*, *lgd1*, *lga1* and *gld1*. *A. niger* also has a homologue gene sequence for *H. jecorina gar1* whereas *H. jecorina* has a homologue gene sequence for *A. niger gaaA* respectively. Functions of these two additional D-galacturonate reductase genes have remained unclear. We have now studied the roles of these two genes.

53) Why are Aspergilli so different in their expression of secondary metabolites from section to section? Jens Christian Frisvad* and Thomas Ostenfeld Larsen. Department of Systems Biology, Technical University of Denmark, Kgs, Lyngby, Denmark, * JCF@bio.dtu.dk

Aspergillus species can produce an very large number of secondary metabolites and have a lot of these in common with Penicillium spp. related to the perfect state Eupenicillium. Some of these extrolites have exclusively been found within Aspergillus and Penicillium, including ochratoxins, aspergamides, mycophenolic acids, fumagillins, roquefortines, paxillins etc., while Aspergilli and Penicillia share secondary metabolites with widely different genera in other cases. The latter extrolites include citreoviridin, citrinin, melleins, patulin, pseurotins, penicillic acids, sterigmatocystins, xanthomegnins, simple diketopiperazines, gliotoxins, etc. Interestingly within Aspergillus sections there are major differences and few secondary metabolites in common. Aspergillus section Candidi species are known for production of terphenyllins, candidusins, xanthoascins, and chloroflavonins not found in any other section of Aspergillus. In section Circumdati ochratoxins, aspergamides, penicillic acids, ochratoxins, xanthomegnins, melleins, circumdatins are common. The aspergamides in section Circumdati are the optical antipods of aspergamides in section Versicolores, so even within Aspergillus sections, there are very interesting differences. We will suggest some explanations for these major differences between species sections.

54) The chemical heritage of *A. flavus* in *A. oryzae* RIB40. Christian Rank*, Marie Louise Klejnstrup, Lene Maj Petersen, Jens Christian Frisvad, Thomas Ostenfeld Larsen. Department of Systems Biology, Technical University of Denmark * cr@bio.dtu.dk

Aspergillus oryzae is a very important species in biotechnology and has been used for centuries in traditional Asian fermentation. The RIB40 strain is particularly interesting as it was one of the first genome sequenced aspergilli together with *A. flavus*, a prominent food and feed contaminant capable of producing aflatoxin. These species can be perceived as ecotypes. We have analyzed *A. oryzae* RIB40 and found that the chemical potential could be enhanced significantly under certain conditions. Delicate analysis of their metabolic profiles allow for chemical insight on the transcription level with indications to specific genetic changes. Several new metabolites and changes in biosynthetic routes have been found in *A. oryzae*, indicating subtle changes in the genomic heritage from *A. flavus*.

55) Mapping of polyketide biosynthesis pathways in *Aspergillus nidulans* using a genome wide PKS gene deletion library. Thomas O. Larsen*, C. Rank, M. Klejnstrup, M.L. Nielsen, J.B. Nielsen, D.M.K. Holm, K.H. Brogaard, B. Hansen, J.C. Frisvad and U.H. Mortensen. Department of Systems Biology, Technical University of Denmark *tol@bio.dtu.dk

In order to map new links between PKS genes and their products in *Aspergillus nidulans* we have systematically deleted all thirty-two individual genes predicted to encode polyketide synthases in this model organism. This number greatly exceeds the number of currently known PKs calling for new approaches for triggering "cryptic" or "silent" genes to see production of compounds not previously observed under laboratory conditions. We therefore decided to challenge our deletion library on eight different complex media, spanning a large variety of alternating carbon and nitrogen sources, vitamins and other nutrients. Comparative UHPLC-DAD analyses indeed revealed that a large number of secondary metabolites were produced by the *A. nidulans* reference strain on the different media. Careful investigation, including LC-DAD-HRMS data, has led to the linking of several known compounds to their PKS genes. For example we have found that a whole series of prenylated PKs such as arugosins and shamixanthones can be linked to the *mdpG* PK gene cluster. Previously only emodins and monodictyphenone were known gene products of *mdpG*. Further examples of new links between PK products and genes will be given illustrating the scope of using of diode array detection and electrospray in the negative mode for detection and tentative identification of non-reduced polyketides.

56) Sorbitol dehydrogenase as a part of *Aspergillus niger* D-galactose catabolism .Outi M. Koivistoinen, Peter Richard, Merja Penttilä, Dominik Mojzita VTT Technical Research Centre of Finland, Espoo, Finland outi.koivistoinen@vtt.fi

D-galactose is a hexose sugar found in hemicellulose. There are probably three different pathways for D-galactose catabolism in fungi, the Leloir, an oxidative and an oxidoreductive. The Leloir pathway has phosphorylated intermediates and galactokinase as the first enzyme. The oxidative pathway has no phosphorylated intermediates and D-galactose is oxidised to D-galactonate in the first reaction. In subsequent reactions D-galactonate is converted to pyruvate and glycerol. In addition to these two pathways an oxidoreductive pathway for D-galactose catabolism, that partly employs the enzymes of the L-arabinose pathway, was suggested to exist. In this work we studied the oxidoreductive D-galactose pathway where D-galactose is first reduced to D-galactitol by a reductase that is also active with L-arabinose. It is then oxidised to L-xylo-3-hexulose by the L-arabitol dehydrogenase. It was suggested that L-xylo-3-hexulose is then reduced to D-sorbitol which is finally oxidised to D-fructose by a sorbitol dehydrogenase. We identified a gene coding for a sorbitol dehydrogenase that is strongly induced on sorbitol and we evaluated the role of the enzyme in D-galactose catabolism.

57) Heterologous expression of *Trichoderma reesei* polyketide synthases and non-ribosomal synthases in *Aspergillus nidulans*. Jorgensen, Mikael S.[1], Hansen, Bjarne G.[1], Skovlund, Dominique A.[2], and Mortensen, Uffe. H.[1]. [1]Center for Microbial Biotechnology, Technical University of Denmark, DK-2800 Lyngby, Denmark. [2]Novozymes A/S, DK-2880 Bagsværd, Denmark.

Polyketides (PKs) and non-ribosomal peptides (NRPs) constitute two classes of diverse secondary metabolites with a wide range of activities which can be both beneficial and disadvantageous from a human perspective. On one side PKs and NRPs include many toxins and virulence factors, but they also act as one of the main natural sources of medicines for treating diseases. PKs and NRPs are synthesized by large multi-functional proteins referred to as polyketide synthases (PKSs) and non-ribosomal peptide synthases (NRPSs). PKS and NRPS genes have been identified in many organisms such as bacteria, fungi and plants which therefore constitute a substantial pool of interesting gene candidates for production of new drugs. The lack of genetic tools for many of the original producers and the difficulty of linking the complex PKSs and NRPSs to their final PK or NRP product have led to the development of a heterologous expression system in *A. nidulans*. Many genetic tools are at disposal for *A. nidulans* and its metabolome has been well studied which is of great advantage when investigating expression of heterologous metabolite genes. A range of *T. reesei* PKS and NRPS genes have been heterologous expressed in *A. nidulans*. The results from these expressions will be presented.

58) Heterologous expression of fungal polyketide synthases. Dorte K. Holm, Bjarne G. Hansen, Thomas O. Larsen, Uffe H. Mortensen Technical University of Denmark, Department of Systems Biology, Center for Microbial Biotechnology, Denmark.

Polyketides are a group of secondary metabolites that comprise molecules of very distinct structures and biological activities. In fungi, polyketides are synthesised via a multi-domain enzyme called a type I iterative polyketide synthase (PKS). As many polyketides are of medical or industrial use, various approaches have been addressed to link genes to known polyketide products e.g. deletion of genes encoding PKSs, overexpressing transcription factors, or using wide domain activators. Although these attempts have substantially increased our insights into polyketide biosynthesis, much is still to be learned especially in fungi where the full genome sequence is known, but where no efficient genetic tools exist. Based on a gene expression platform developed in our laboratory for heterologous or ectopic expression of genes in *A. nidulans*, we present an efficient approach for investigating polyketide synthesis in filamentous fungi. As a test case, 37 putative and known PKS encoding genes from the industrially relevant fungus *Aspergillus niger* were individually expressed in *A. nidulans* using *A. nidulans* promoters and terminators to ensure efficient transcription. This approach proved robust, fast, and efficient, and it is highly suitable for automating the generation of expression constructs for expression in *A. nidulans*.

59) A combined genetic and multi medium approach reveals new secondary metabolites in *Aspergillus nidulans*. Marie Louise Klejnstrup*, Morten Thrane Nielsen, Jens Christian Frisvad, Uffe Mortensen, Thomas Ostenfeld Larsen. Department of Systems Biology, Technical University of Denmark, Lyngby, Denmark, *marlk@bio.dtu.dk

Secondary metabolites are a diverse group of metabolites which serve as important natural sources of drugs for treating diseases. The availability of full genome sequences of several filamentous fungi has revealed a large genetic potential for production of secondary metabolites that are not observed under standard laboratory conditions. Genetic approaches have proven a fruitfull strategy towards the production and identification of these unknown metabolites. Examples include deletion of the *cclA*[1] and *laeA*[2] genes in *A. nidulans* which affects the expression of secondary metabolites including monodictyphenone and terrequinone A respectively. We have deleted the *cclA* gene in *A. nidulans* and grown the mutants on several complex media to provoke the production of secondary metabolites. This resulted in the production of several metabolites not previously reported from *A. nidulans*. [1] Bok, J.W. *et al*, Nat. Chem. Biol., 5, 462-464 (2009). [2] Bok J.W. *et al*, Chem. Biol., 13, 31-37 (2006).

60) Genome wide polyketide synthase gene deletion library in *Aspergillus nidulans*. Uffe H. Mortensen, Michael L. Nielsen, Jakob B. Nielsen, Christian Rank, Marie L. Klejnstrup, Dorte K. Holm, Katrine H. Brogaard, Bjarne G. Hansen, Jens C. Frisvad, Thomas O. Larsen Center for Microbial Biotechnology, Department of Systems Biology, Technical University of Denmark, Søltofts Plads B221/B223, 2800 Kgs. Lyngby, Denmark

Filamentous fungi produce a wealth of secondary metabolites that dramatically influence human life as they comprise both mycotoxins and pharmaceuticals. To forward the understanding of fungal secondary metabolism we have adopted a multidisciplinary strategy based on fungal ecology, analytical chemistry and molecular biology. Here we present an analysis of a library containing individual deletions of all known and putative PKS genes in the model fungus *Aspergillus nidulans*. The library has been challenged on a number of different media to uncover new links between genes and polyketide products. At our conditions, we detect several of the known products of *A. nidulans* including the PKS gene responsible for production of austinol. The validity of this conclusion is ensured by further mutagenesis including site directed mutagenesis of the locus as well as ectopic expression of the gene to indentify the first intermediate in austinol production. Based on our findings we call the gene *ausA*. Moreover, our results demonstrate several examples of crosstalk between different pathways in polyketide synthesis. To this end, it is important to stress that by investigating a genome wide deletion library at different conditions the chance of mis-assigning genetic links to products due to such crosstalk is dramatically reduced. Together the results and conclusions presented constitute our first step towards a systems understanding of the secondary metabolism of *A. nidulans*.

61) The complexity of polyketide synthase OrsA action in *Aspergillus nidulans*. Jakob B. Nielsen, Michael L. Nielsen, Christian Rank, Marie L. Klejnstrup, Paiman Khorsand-Jamal, Dorte M. K. Holm, Bjarne G. Hansen, Jens C. Frisvad, Thomas O. Larsen and Uffe H. Mortensen. Center for Microbial Biotechnology, Department of Systems Biology, Technical University of Denmark, Søltofts Plads B221/B223, 2800 Kgs. Lyngby, Denmark.

Polyketides (PKs) are chemically diverse molecules characterized by a broad spectrum of biological activities. Polyketide synthases (PKSs) are the main engines in the synthesis of PKs, and in fungi the PKS assembles small subunits to polymers in an iterative manner. Mapping of PKs to PKSs are not straight forward. The chemical architecture of the compounds produced depends on the primary structure and organization of the domains within the PKS molecule, and a majority of the PK products are also influenced by other gene products typically clustered in vicinity of the PKS. Moreover, activation of these clusters can be cumbersome, due to the clandestine regulation of the PK biosynthesis. One example is the gene cluster of PKS AN7909, *orsA*, in *Aspergillus nidulans*, which produces orsellinic acid and several other products. This synthesis has been provoked by several strategies. Here we report products that previously have not been linked to OrsA activity as well as cross-talk with the PKS AN7903 located approximately 20 kb downstream of *orsA*. These new observations emphasizes the challenges there are in uncovering the PK metabolome in fungi and the molecular genetics herein, which ultimately will facilitate rational engineering of the PK production apparatus.

62) Production of terpenes with *Aspergillus nidulans* Kiira Vuoristo, Kirsi Bromann, Mervi Toivari, Laura Ruohonen, Tiina Nakari-Setälä VTT Technical Research Centre of Finland Address: Tietotie 2, Espoo, P.O. Box 1000, FI-02044 VTT, Finland. Email: kiira.vuoristo@vtt.fi

Terpenes are a large and diverse group of hydrocarbons with many pharmaceutical and industrial applications including fragrances, preservatives, flavouring agents and drugs. Terpenes with rearrangements in their carbon skeleton are referred to as terpenoids. The low water solubility, high volatility and complex structure of terpenoids make them difficult to produce in industrial scale. Modification of readily available precursors of terpenoids in filamentous fungi would present an alternative way to produce value-added compounds. There are not many reports on microbial transformation of terpenoids by fungi of the *Aspergillus* genus, although their ability to transform terpenes is recognised. The biotransformation of terpenes is of interest because it allows production of enantiomerically pure compounds under mild conditions. In nature, filamentous fungi produce terpenoids as secondary metabolites; i.e. they are not required for growth or development but instead function in communication or defence. The fungal terpenoids are involved in pathogenesis, production of toxins and utilization of specific carbon sources. Filamentous fungi have been used in industrial scale production and especially *Aspergillus niger* and *Aspergillus nidulans* represent potential host organisms for the production and modification of terpenoid products. This work describes genetic engineering of *A. nidulans* for terpenoid production.

63) Characterization of an *Aspergillus oryzae* cysteinyl dipeptidase expressed in *Escherichia coli*. Ryota Hattori[1], Mayumi Matsusita-Morita[1], Sawaki Tada[1], Junichiro Marui[1], Ikuyo Furukawa[1], Satoshi Suzuki[1], Hitoshi Amano[2], Hiroki Ishida[3], Youhei Yamagata[4], Michio Takeuchi[4], Ken-Ichi Kusumoto[1] i[1]National Food Research Institute, Ibaraki, Japan, [2]Amano Enzyme Inc., Gifu, Japan, [3]Gekkeikan Sake Company Ltd., Kyoto, Japan, [4]Tokyo University of Agriculture and Technology, Tokyo, Japan j

Cysteinyl dipeptidase from *A. oryzae* (CdpA) was produced in *Escherichia coli* and purified. CdpA formed a homodimer and its molecular mass was determined as 109 kDa. CdpA-specific activity to Cys-Gly was 3.04 U/mg. The enzyme showed maximum hydrolyzing activity toward Ala-Cys, followed by Leu-Cys, Cys-Gly, Cys-Ala, and Gly-Cys among the cysteine-containing dipeptide substrates. Its substrate specificity was distinct from those of other cysteinyl dipeptidases of the M20 family. The activity of CdpA was increased by addition of Mn^{2+}, Zn^{2+}, and Co^{2+} at 0.1 mM. The activity was inhibited in the presence of Fe^{2+}. Several protease inhibitors reduced the activity. The complete inhibition of enzyme activity by EDTA indicates that CdpA is a metallopeptidase. It was optimally active at pH 7-8 and stable at pH 6-9 and at up to 40 . This study was supported by the Program for Promotion of Basic Research Activities for Innovative Biosciences (PROBRAIN).

64) Glu31, Asp142 and Asp171 of *Aspergillus oryzae* cutinase CutL1 are required for both interaction with hydrophobin RolA and consequent stimulation of polyester-degradation. Kimihide Muragaki[1], Kenji Uehara[1], Toru Takahashi[2,3], Youhei Yamagata[3] and Keietsu Abe[1,3*] [1]Grad. Sch. Agric. Sci., Tohoku Univ., Japan, [2]NRIB, Japan, [3]NICHe, Tohoku Univ., Japan [*]kabe@niche.tohoku.ac.jp

Hydrophobins are amphipathic proteins, and are ubiquitous among filamentous fungi. When the industrial fungus *Aspergillus oryzae* is cultivated in a submerged medium containing a biodegradable polyester polybutylene succinate-coadipate (PBSA), cutinase CutL1 and hydrophobin RolA are simultaneously secreted into the medium. RolA attached to the surface of PBSA particles specifically recruits CutL1, resulting in stimulation of PBSA hydrolysis[1)]. In our previous study, we studied amino acid residues involved in the RolA-CutL1 interaction by means of chemical modification and site-directed mutagenesis of RolA and CutL1. As a result, we found that His32 and Lys34 of RolA and Glu31, Asp142, Asp171 of CutL1 are involved in the RolA-CutL1 interaction. In the present study, to quantitatively elucidate the role of the three acidic amino acid residues of CutL1 in the RolA-CutL1 interaction, we characterized the interaction between CutL1 variants of the three residues an d wild type RolA by using Quartz Crystal Microbalance (QCM). The QCM analysis revealed that replacement of the three acidic amino acid residues of CutL1 to serine caused increases in *K*D values for interaction with RolA. In conclusion, Glu31, Asp142 and Asp171 of CutL1 are critically required for the RolA-CutL1 interaction by multivalent effect.
1) Takahashi et al. Mol Microbiol. 57:1780 (2005)

65) Genome mining of secondary metabolite genes in Aspergillus species. Clay, Wang2,3; Yi-Ming, Chiang1; Berl, Oakley5,6; Nancy, Keller7,8; Kenneth, Bruno4; Scott, Baker4; Kristen, Meyer4; Michael, Praseuth2; Edyta, Szewczyk5; Ashley, Davidson6; Ruth, Entwistle6; James, Sanchez2; Manmeet, Ahuja6; Jin Woo, Bok7,8; Jonathan, Palmer9 1 Graduate Institute of Pharmaceutical Science, Chia Nan University of Pharmacy and Science, Tainan 71710, Taiwan, ROC. 2 Department of Pharmacology and Pharmaceutical Sciences, 3 Department of Chemistry, University of Southern California, Los Angeles, California 90089, USA. 4 Chemical and Biological Process Development Group, Energy and Environment Directorate, Pacific Northwest National Laboratory, Richland, Washington, 99352, USA 5 Department of Molecular Genetics, Ohio State University, Columbus, Ohio 43210, USA. 6 Department of Molecular Biosciences, University of Kansas, Lawrence, KS 66045, USA. 7 Department of Medical Microbiology and Immunology, 8 Department of Bacteriology, 9 Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706, USA.

Aspergillus species are known to produce high value secondary metabolites such as lovastain. Recently the genomes of several Aspergillus species have been sequenced. One of the most important findings from these genome efforts is the realization that these organisms have the potential to produce far more secondary metabolites than have ever been isolated and identified. I will present recent updates in identifying new secondary metabolites and their corresponding biosynthesis pathways from several different Aspergillus species. Funding from the NIGMS is acknowledged.

66) Characterization of *Emericella nidulans* RodA and DewA hydrophobin mutants. Britt Guillaume Jensen*, Jakob Blæsbjerg Nielsen, Mona Højgaard Pedersen, Ib Søndergaard, Jens Christian Frisvad and Kristian Fog Nielsen. Department of Systems Biology, Technical University of Denmark, Denmark, *brgj@bio.dtu.dk

Hydrophobins are small amphiphilic proteins containing an eight cysteine pattern only found in filamentous fungi. They are involved in the attachment of hyphae to hydrophobic structures and the formation of aerial structures. Five *Emericella nidulans* mutant strains were examined to study the two hydrophobins RodA and DewA. Individual knock-out mutants *rodA?*, *dewA?* and the double deletion strain *rodA?dewA?* were constructed. Furthermore, two strains containing a point mutation in the first of the cysteines of RodA (*rodA-C57G*), where one was coupled to the *dewA* deletion, were included. The reference strain (NID1) and *dewA?* displayed green conidia. However, *rodA?* and *rodA?dewA?* showed a dark green/brown conidial pigmentation, while *rodA-C57G* and *rodA-C57G dewA?* displayed lighter brown conidia. *rodA?* and *rodA?dewA?* displayed a higher degree of hülle cells compared to the moderate amount observed for NID1 and *dewA?*, while *rodA-C57G* and *rodA-C57G dewA?* displayed a low number of hülle cells. NID1 and *dewA?* conidia were dispersed as spore chains. *rodA?*, *rodA?dewA?*, *rodA-C57G* and *rodA-C57G dewA?* spores were associated in large clumps, where the conidia seemed to adhere to one another. The largest degree of spore clustering was observed for *rodA?* and *rodA-C57G dewA?*.

67) Oxido-reductive metabolism of L-arabinose and D-galactose in filamentous fungi: Metabolic crosstalk versus specific enzymes. Dominik Mojzita, Outi M. Koivistoinen, Kiira Vuoristo, Laura Ruohonen, Merja Penttilä and Peter Richard VTT Technical Research Centre of Finland, Espoo, Finland dominik.mojzita@vtt.fi

L-arabinose, the second most abundant pentose sugar, is used as a carbon source by a variety of microorganisms living on decaying plant material. Fungal microorganisms catabolize L-arabinose through an oxido-reductive pathway. We have identified two missing links in the pathway, L-arabinose and L-xylulose reductases in *A.niger*. D-galactose is a relatively rare hexose sugar in the plant cell wall mainly found in galactoglucomannan. There are three pathways indentified in fungi for D-galactose degradation; 1) the Leloir pathway in which D-galactose is phosphorylated, 2) the oxidative pathway which starts by an extracellular galactose oxidase reaction, and 3) a recently proposed oxido-reductive pathway which resembles the pathway for L-arabinose catabolism. It has been suggested in *T. reesei* and *A. nidulans* the oxido-reductive D-galactose pathway employs the enzymes from the L-arabinose pathway. It starts with the conversion of D-galactose to D-galactitol, probably carried by the xylose/arabinose reductase. The second step is catalyzed by L-arabitol dehydrogenase and the product of the reaction is an unusual sugar L-xylo-3-hexulose. We have identified the L-xylulose reductase possesses the activity with this intermediate which is converted to D-sorbitol. Finally, D-sorbitol is oxidized to D-fructose, which enters glycolysis. We have studied the pathway in *A. niger* and uncovered a more complex picture. Apart from showing the possible involvement of the L-arabinose pathway enzymes, we identified two dehydrogenases specifically induced on D-galactose, suggesting that *A. niger* might have specific genes for catabolism of D-galactose rather than using metabolic crosstalk suggested for *T. reesei* and *A. nidulans*.

68) Sexual recombination and the possibility of cryptic heterokaryosis in *Aspergillus flavus*. Rodrigo A. Olarte[1], Bruce W. Horn[2], James T. Monacell[3], Rakhi Singh[1], Eric A. Stone[3,4], Ignazio Carbone[1]. [1]Plant Pathology, NCSU, Raleigh, NC 27695 [2]NPRL, USDA-ARS, Dawson, GA 39842 [3]BRC, NCSU, Raleigh, NC 27695 [4]Genetics, NCSU, Raleigh, NC 27695

Aspergillus flavus infects both plants and animals and is of toxicological importance due to its production of aflatoxins (AFs). Recent efforts to reduce AF concentrations have focused on the use of the biocontrols AF36 and Afla- Guard®, both of which contain nonaflatoxigenic *A. flavus* strains as an active ingredient. Biocontrols are applied to fields, where they competitively exclude native aflatoxigenic strains. Although biocontrol is effective in reducing AF contamination in crops, the extent to which these strains recombine with native strains and the overall effect on fungal populations are unknown. Here we show that the recombination breakpoints in the F1 correlate with the breakpoints inferred from population genetic studies of natural isolates. Furthermore, we demonstrate that a crossover event within the AF cluster can repair a nonsense mutation, resulting in a regained aflatoxin-producing phenotype. Finally, we observed non-Mendelian inheritance of extra-genomic AF cluster alleles in crosses with partial AF cluster parents, suggesting a possible role of cryptic heterokaryosis, in addition to sexual recombination, in modulating AF production. Collectively, these processes may contribute to increased effective population sizes and drive genetic and functional hyperdiversity in *A. flavus*.

69) Hybridization between *Aspergillus flavus* and *Aspergillus parasiticus*. Carolyn J. Worthington[1], Bruce W. Horn[2], Geromy G. Moore[1], James T. Monacell[3], Rakhi Singh[1], Eric A. Stone[3,4], Ignazio Carbone[1]. [1]Plant Pathology, NCSU, Raleigh, NC 27695 [2]NPRL, USDA-ARS, Dawson, GA 39842 [3]BRC, NCSU, Raleigh, NC 27695 [4]Genetics, NCSU, Raleigh, NC 27695

To date the sexual stages or teleomorphs have been described for three aflatoxigenic species in *Aspergillus* section *Flavi: Petromyces flavus, P. parasiticus* and *P. nomius*. In this study we examined the possibility of interspecific matings between *A. flavus* and *A. parasiticus*. These species can be distinguished morphologically and genetically, as well as functionally via the biosynthesis of specific aflatoxins and an unrelated mycotoxin, cyclopiazonic acid (CPA). *A. flavus* produces both B aflatoxins and CPA, B aflatoxins or CPA alone, or neither mycotoxin; *A. parasiticus* produces B and G aflatoxins or O-methylsterigmatocystin but not CPA. Only four out of forty-five attempted interspecific crosses between compatible mating types of *A. flavus* and *A. parasiticus* were fertile. Single ascospore strains from each cross were isolated and shown to be hybrids using array comparative genome hybridization and multilocus sequence typing. Higher mean concentrations of B_1, B_2, G_1 and G_2 aflatoxins, and CPA in the F1 progeny compared to midpoint parent toxin levels indicate high heritability of these toxins and possibly hybrid heterosis.

70) Re-sequencing shows biased SNP distribution along 8 A. fumigatus chromosomes Suman Pakala, Jessica Hostetler, Suchitra Pakala, Vinita Joardar, Paul Bowyer, David Denning, William C. Nierman, Natalie D. Fedorova. J. Craig Venter Institute, Rockville MD, USA. Email: natalief@jcvi.org

Aspergillus fumigatus is the most common causative agent of invasive aspergillosis (IA), an invasive and deadly infection that affects mostly immunocompromised individuals. To enhance genomic resources available the A. fumigatus community, this study aims to provide (i) 2 additional reference genome sequences; (ii) RNA-Seq based genome annotation upgrades; and (iii) mutational profiling of drug resistant strains. Here we report the successful completion of the pilot phase of the project. To estimate the feasibility of the NGS approach, we first calculated the Illumina sequencing error rate at the 10X cut-off, which turns out to be extremely low (2.4E-06). Sequencing two more azole-susceptible isolates, A1163 and AF210, demonstrated high genetic variability of the A. fumigatus nuclear genome. Our preliminary analysis identified 57,000 and 77,000 SNPs in A1163 and AF210, respectively, relative to AF293. With a few exceptions, SNPs were strongly enriched in the arms of the chromosomes relative to the center. Further analysis identified over 50 highly variable genes (over 15 SNPs per 1 Kb) including the FluG-like protein and general amidase GmdB, which may be useful for MLST typing. Notably, the cyp51A gene, associated with azole resistance has 10 SNPs in AF210, some previously described as not related to drug resistance. The funding for the project has been provided by NIAIAD/NIH.

OTHER TOPICS

71) *In vitro* production of neutrophil extracellular traps against *Aspergillus fumigatus* is influenced by the conidial surface protein hydrophobin RodA. Sandra Bruns[1,2], Mike Hasenberg[3], Olaf Kniemeyer[1,2], Andreas Thywißen[1,2], Vishukumar Aimanianda[4], Jean-Paul Latgé[4], Matthias Gunzer[3], Axel Brakhage[1,2] [1] Department of Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology - Hans-Knoell-Institute (HKI) - Jena, Germany [2] Department of Microbiology and Molecular Biology, Friedrich-Schiller-University-Jena, Germany [3] Institute for Molecular and Clinical Immunology, Otto-von-Guericke-University, Medical Faculty, Magdeburg, Germany [4] Unite des Aspergillus, Institue Pasteur, Paris F-75015, France

Aspergillus fumigatus is the most important airborne fungal pathogen causing life-threatening infections in immunocompromised patients. Conidia as the infectious agent infiltrate the lungs and get in contact with alveolar macrophages and neutrophil granulocytes, which repesent the first line of defense. These cells kill fungal conidia by phagocytosis. Neutrophils are also able to form neutrophil extracellular traps (NETs) against *A. fumigatus* conidia and hyphae. These sticky filaments consist of nuclear DNA decorated with histones and fungicidal proteins. Time-lapse movies of coincubations of *A. fumigatus* with neutrophils revealed that NET production was a highly dynamic process which, however, was only exhibited by a sub-population of cells. In addition the intensity of NET formation by unstimulated, human neutrophils was strain- and morphotype-dependent. The killing of *A. fumigatus* conidia was not influenced by the amount of released extracellular DNA, but metabolic activity of hyphae seemed to be reduced by NETs after longer incubation periods of 12h. Our data suggest that NETs prevent further spreading, but apparently do not represent the major factor for killing. By using fungal mutants and extracted RodA protein we demonstrate that the conidial hydrophobin RodA, a surface protein rendering conidia immunologically inert, led to reduced NET formation by neutrophils encountering *Aspergillus*. We are currently investigating NET formation against different *A. fumigatus* mutants to identify fungal components, which stimulate or reduce NET formation. Bruns et al.(2010) Plos Pathogens 6:e1000873 Brakhage, A.A., S. Bruns, A. Thywissen, P. F. Zipfel, J. Behnsen(2010) Curr Op Microbiol 13:409

72) Optimizing the RNA-Seq transcriptome discovery pipeline for *Aspergillus fumigatus* Suman Pakala, Vinita Joardar, Nikhat Zafar, Suchitra Pakala, Sean Murphy, Natalie Fedorova and William Nierman J. Craig Venter Institute, Rockville, MD, USA. spakala@jcvi.org

RNA-Seq has become invaluable to applications such as transcript expression quantification and genome annotation, including discovery of non-coding RNAs and identification of new gene models and isoforms. The continued evolution of deep sequencing technologies has resulted in active development of new data analysis tools. It has become necessary for research groups to identify the appropriate set of tools that best suit their needs. As part of the *Aspergillus fumigatus* re-annotation project, we sequenced several cDNA libraries using Illumina GA II and evaluated publicly available transcript discovery approaches. Specifically, this comparison includes (i) the de novo transcript assembly approach, (ii) the alignment followed by assembly of transcripts approach and also (iii) a hybrid approach for transcript identification. Performance of various short read aligners, splice junction mappers, and transcript assemblers that implement these approaches have been evaluated. Based on this study, we present a general framework for evaluating RNA-Seq data analysis tools and discuss our results of such an evaluation and optimization of the analysis pipeline for the *A. fumigatus* genome

73) The Aspergillus and Candida Genome Databases (AspGD and CGD), curated gene and protein information resources for the fungal research community. Martha B. Arnaud 1, Jonathan Binkley 1, Gustavo Cerqueira 2, Marcus C. Chibucos 2, Maria C. Costanzo 1, Jonathan Crabtree 2, Diane O. Inglis 1, Joshua Orvis 2, Prachi Shah 1, Marek S. Skrzypek 1, Gail Binkley 1, Stuart R. Miyasato 1, Jennifer Russo Wortman 2, and Gavin Sherlock 1 1 Department of Genetics, Stanford University, Stanford, CA; 2 Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD arnaudm@stanford.edu

The Aspergillus Genome Database (www.aspgd.org) and the Candida Genome Database (www.candidagenome.org) are web-based genomics resources for researchers studying two important groups of fungi, the Aspergillus and Candida species. We provide high-quality manual curation of the experimental scientific literature, as well as tools for exploring these data, and community resources. The curated information for each gene appears on its Locus Summary page (LSP) with links to pages that provide phenotype and GO details, sequence and annotation history and a comprehensive list of reference citations. As we add multiple species, the LSPs for orthologs are interlinked to facilitate browsing of related genes. At AspGD, we initially curated the gene-specific literature for the model organism Aspergillus nidulans, and now also for the pathogen A. fumigatus. At CGD, we have curated Candida albicans and are now curating C. glabrata. In the future, we will expand our efforts to other species, including A. niger and A. oryzae for AspGD, C. dubliniensis and C. parapsilosis for CGD. AspGD and CGD are supported by NIH RO1 AI077599 from the NIAID and R01 DE015873 from the NIDCR, respectively.

74) *Aspergillus fumigatus* conidia modulate the endocytic pathway of alveolar macrophages. Andreas Thywissen[1, 3], Thorsten Heinekamp[1], Hans-Martin Dahse[2], Peter F. Zipfel[2, 3], and Axel A. Brakhage[1, 3]. [1] Leibniz Institute for Natural Product Research and Infection Biology – Hans-Knoell-Institute (HKI), Department of Molecular and Applied Microbiology, Jena, Germany. [2] Leibniz Institute for Natural Product Research and Infection Biology – Hans-Knoell-Institute (HKI), Department of Infection Biology, Jena, Germany. [3] Friedrich Schiller University, Jena, Germany.

The mould *Aspergillus fumigatus* is the main causative agent of invasive pulmonary aspergillosis in immunocompromised patients. Infection starts with the inhalation of *A. fumigatus* conidia that germinate in the lung. Professional phagocytes like alveolar macrophages contribute to the efficient clearance of fungi from the lung by phagocytosis and degradation of conidia followed by release of chemokines and cytokines in order to trigger neutrophil migration at the site of infection. In the immunocompromized host, at least some conidia are able to evade macrophage degradation, resulting in germination and outgrowth of intracellularly residing spores. Therefore, conidia must be able to evade recognition and processing by phagocytes. The avirulent *pksP* mutant of *A. fumigatus* lacking the melanin layer present on wild-type conidia exhibited increased phagocytosis by macrophages apparently due to the loss of masking immunogenic glucan-structures. Furthermore, by analysing phagolysosome fusion and acidification we show that intracellular processing of *pksP* mutant conidia is drastically increased in comparison to wild-type conidia, suggesting that *A. fumigatus* conidia interfere with the endocytosis pathway, similar to obligate human pathogens like *Legionella sp*. or *Mycobacterium sp*.. The process by which wild-type conidia mediate endocytotic alterations seems to be connected to the surface structure of melanized conidia but is independent of the presence of a functional RodA-derived rodlet layer. Moreover, inhibition of phagolysosome acidification by macrophages is controlled by the fungal cAMP signaling pathway.

75) Fed-batch production of the hydrophobins RodA and RodB from *Aspergillus fumigatus* in host *Pichia pastoris* M.H. Pedersen, I. Borodina, J.C. Frisvad and I. Søndergaard Systems Biology, Technical University of Denmark, Lyngby, Denmark. E-mail: mohp@bio.dtu.dk

Objectives: *Aspergillus fumigatus* expresses the hydrophobins RodA and RodB on the surface of its conidia. RodA is known to be important for the pathogenesis of the fungus, but the role of RodB is unknown. The aim was to produce recombinant RodA and RodB for further characterication. Methods and materials: The genes encoding hydrophobins RodA and RodB was amplified by RT-PCR from the total RNA isolated from *A. fumigatus* (AF296 strain), and cloned into expression vectors pPICZalphaA and pPICZB while adding a C-terminal 6xHis-tag. The linearized plasmids were transformed into *P. pastoris* strain X33. The expression of the RodA and RodB genes was first studied in culture flasks in buffered complex methanol medium as protein production was dependent on the methanol- induced AOX1 promoter. Later production was scaled up to a 2 L fed-batch fermentor. Hydrophobins were purified using His-select Nickel Affinity gel. The emulsifying properties of recombinant hydrophobins were investigated using oil-water emulsions studied by light microscopy. Results: Protein bands of expected size were detected by SDS-PAGE and western blotting in the fermentation broth. Fed-batch production yielded approximately 300 mg/l. Recombinant RodB showed good emulsifying properties. Conclusion: RodA and RodB from *A. fumigatus* were successfully produced by yeast host *Pichia pastoris* with good yields.

76) Heterologous expression in *Aspergillus niger* - a case study. Nada Kraševec[1], Irena Lukanèiè[1,2], Tatjana Milunoviæ[1], Marija An ur Lasnik[2], Mojca Benèina[1], Vladka Gaberc Porekar[1], Radovan Komel[1]. [1]National Institute of Chemistry, POB 660, SI-1001 Ljubljana, Slovenia. [2]Lek Pharmaceuticals d.d., a Sandoz company, Verovškova 57, SI-1000 Ljubljana, Slovenia. nada.krasevec@ki.si.

Filamentous fungi are convenient for the commercial production of heterologous proteins because of their natural ability to secrete high amounts of variety of proteins. The species of the genus *Aspergillus*, which have obtained the GRAS status, are being used extensively for large scale production of enzymes. Although today most of the therapeutic proteins are produced in mammalian cell cultures or prokaryotic systems, depending on the nature and molecular weight of the protein, fungi still hold promises as an alternative expression system. To explore their potential, expression of model therapeutic protein, human granulocyte colony stimulating factor (GCSF) was studied in the filamentous fungus *Aspergillus niger*. GCSF has an important role in myeloid lineage of haematopoiesis. The natural human GCSF is O-glycosylated protein which exists in a shorter and more active form of 174 amino acids and a longer one with 3 additional amino acids. Molecule contains one free cysteine residue and two disulfide bonds, which stabilize a functional 4 alfa-helix tertiary structure. Hereby, we report the secretion and isolation of processed, folded and biologically active GCSF in filamentous fungi *A. niger*.

77) New Resources for Genetic Research with Aspergillus. McCluskey, Kevin, and Aric Wiest. Fungal Genetics Stock Center, University of Missouri-Kansas City.

Recent growth of the FGSC collection has been dominated by genetically engineered strains and material for genetically engineering fungi. This is as true for Aspergillus related resources as it is for any with the recent deposit of hundreds of fungal strains, plasmids, and nearly ten thousand gene disruption constructs. All of these materials have been in great demand and we have adapted techniques developed at the FGSC for managing Neurospora, Pichia, Candida, and Cryptococcus resources to our growing collection of Aspergillus mutants. Among the challenges facing the growing Aspergillus community is the division of effort among widely disparate areas. The FGSC seeks to be a bridge between the research communities and provides materials for research with model organisms, plant and medical pathogens, industrial strains as well as strains used in manufacture of household products, or foods and beverages. The tools developed in these systems including cloning vectors, expression and protein tagging vectors, find wide application, crossing species and even genus barriers to impact research and development around the world. The FGSC has distributed materials to clients in over 40 different countries on every continent in the last ten years. Having materials publicly available amplifies the impact of those materials. Publicly available receive as much as twice as many citations as do materials held privately. Amplify the impact of your research. The FGSC is supported by grant 0742713 from the US National Science Foundation.

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