5th International Aspergillus Meeting (Asperfest)
April 4-5, 2008

Edinburgh, Scotland

Organized by the
Aspergillus Genomes Research Policy Committee

Aspergillus Genomes Research Policy Group (AGRPG)

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

2007 AGRPC

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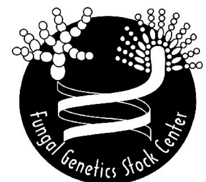

The 5th Aspergillus Meeting

April 3-4, Edinburgh, Scotland

All sessions in the Appleton Tower LT 1

Scientific Program

April 3, Thursday

7:00 Committee members: "Get-together dinner"

April 4, Friday

8:00-9:00 Registration

9:00-9:15 **Welcome, introductions and announcements: Michelle Momany/Gerhard Braus**

9:15-10:30 **Session I: Aspergillus Resources**
Chair: Scott Baker

Scott Baker (Pacific Northwest National Lab. Richland, USA):
"Aspergillus genomics and microarrays"

Kevin McCluskey (Fungal Genetics Stock Center, USA):
"Aspergillus resources"

10:30-11:00 Coffee Break

11:00- 12:30 **Session II: Genomic approach to industries/Biotechnology**
Chair: Masayuki Machida

Geoff Turner (The University of Sheffield)
"Secondary metabolic diversity in the genus Aspergillus"(25-30 min.)

Carsten Hjort (Novozymes)
"Introduction of Genomics as a powerful screening tool in biotechnology" (25-30 min.)

Mizuki Tanaka, Masafumi Tokuoka, Katsuya Gomi (Tohoku University)
"Codon optimization of heterologous protein expression in Aspergillus oryzae"(15-20 min.)

Masahiro Ogawa et al. (Noda Institute for Scientific Research)
"Construction of a disruption mutant library of the transcription regulatory genes in koji-mold Aspergillus oryzae"(15-20 min.)

12:30-01:30 Lunch

1:30-3:15 **Session III: Cell Biology**
Chair: Gerhard Braus

Steve Osmani (Ohio State, Columbus OH USA):
"Cell cycle regulation in Aspergillus nidulans" (25 min.)

Claudio Scazzocchio (Université Paris Sud, France):
"*Casein kinase I and amino acid transporter topogenesis in Aspergillus nidulans* "
(25 min.)

Takahiro Shintani (Tohoku University, Sendai, Japan):
"Cellular response to the expression of an aberrant secretory protein in Aspergillus oryzae" (15 min.)

Ronald de Vries (Leiden University, Leiden, The Netherlands):
"*Spatial differentiation of mannitol dehydrogenase and mannitol-1-phosphate dehydrogenase explains the absence of a mannitol cycle in Aspergillus niger*" (15 min.)

Terry Hill (Rhodes College, Memphis TN, USA)
"*Two GDP-mannose transporters contribute to cell wall integrity in Aspergillus nidulans"*
(15 min.)

3:15-3:45 Coffee Break

3:45-4:30 **Community Directions Discussion Moderator: Michelle Momany**

4:30-5:30

Pontecorvo Lecture
Chair: Gary Payne

Michael Hynes (Univ. of Melbourne):
"Aspergillus nidulans : the perfect fungus"

6:00-7:00 **Posters**

7:00-8:00 Free for Dinner (not included in the fees)

April 5, Saturday

7:30-9:00 Breakfast (Hotel)

9:00-9:15 Elections

9:15-10:15 **Session IV: Secondary Metabolism**
Chair: Arthur Ram/Kevin McCluskey

Jonathan M. Palmer (University of Wisconsin, MA/USA):
"Increased asperthecin production in a mutant of Aspergillus nidulans defective in ascospore production" (20 min.)

Ken Oda (Kanazawa Institute of Technology, Ishikawa/Japan):
"Aspergillus oryzae LaeA Affects Production Of Kojic Acid" (20 min.)

Özgür Bayram (Georg August University, Göttingen/Germany):
"The velvet complex coordinates light, fungal development and secondary metabolism" (20 min.)

10:15-11:15 **Session V: Selected posters**
Chair: Arthur Ram/Kevin McCluskey

Celine O'Gorman (University College, Dublin/Ireland):
"Confirmation of identification of a population of Aspergillus fumigatus isolates from Ireland using a polyphasic approach"
(10 min.)

Feng Jie Jin (Noda Institute, Japan):
"A trial of the minimization of the chromosome 7 by large-scale chromosomal deletion in A. oryzae RIB40" (10 min.)

Birte Könnecke (Georg August University, Göttingen/Germany):
"The COP9 signalosome influences cell wall degradation and development of Aspergillus nidulans" (10 min.)

Marcia Kress (Georg August University, Göttingen/Germany):
"Three Different Cullins are Neddylated in Aspergillus nidulans" (10 min)

11:15-11:45 Coffee Break

11:45-13:00 **Session VI: Comparative Aspergillus studies/evolution/systematics**
Chair: Jennifer Wortman

Herman Pel (DSM Food Specialities):
"Comparative genomics of A. niger strains"

Mikael Rørdam Andersen (Denmark Technical University):
"Comparative transcriptomics of three Aspergillus species"

Mike Cornell (University of Manchester):
"Development of the e-fungi database: problems and possibilities of data integration"

13:00 Announce election results and take any further discussion

13:15 **WRAP UP / End of Asperfest**
Michelle Momany

Poster Abstracts

BIOCHEMISTRY AND METABOLISM

1. The Aspergillus fumigatus ABC Transporter AbcB Is Involved In Excretion Of Siderophore Breakdown Product.

Claudia Kragl, Markus Schrettl, Martin Eisendle and Hubertus Haas Division of Molecular Biology, Biocenter, Innsbruck Medical University, Fritz-Pregl-Str. 3, A-6020 Innsbruck, Austria, Email: hubertus.haas@i-med.ac.at

Aspergillus fumigatus is a ubiquitous fungal saprophyte and an important opportunistic pathogen in immunocompromised patients. Siderophore biosynthesis was shown to be essential for virulence of A. fumigatus in a mouse model of invasive aspergillosis. A. fumigatus produces two major siderophores: desferri-triacetylfusarinine C (DF-TafC) to mobilize extracellular iron and desferri-ferricrocin (DF-FC) for intracellular iron storage. Subsequent to uptake, DF-TafC is hydrolyzed and the resulting fusarinines are excreted. The *A. fumigatus* B-type ABC transporter-encoding *abcB* is located within an iron-regulated gene cluster, indicating a role in iron metabolism. Enhanced green fluorescent protein (EGFP)-tagging localized AbcB in the plasma membrane. Deletion of *abcB*caused increased intracellular and decreased extracellular accumulation of fusarinines. Consistently, AbcB-deficiency impaired the growth rate during iron depleted but not iron-replete conditions. Phylogenetic analysis suggested that AbcB is a prototype of a clade of the ABC transporter superfamily that is involved in siderophore metabolism with members in siderophore- producing but not siderophore-lacking fungal species. Most ABC transporters are implicated in multidrug resistance. AbcB is one of few members of this protein family with a known physiologically relevant function in the absence of xenobiotics and the first eukaryotic ABC-transporter that is involved in siderophore metabolism. This work was supported by the Austrian Science foundation (grants FWF-P15959-B07 and FWF-P18606-B11)

CELL BIOLOGY

2. Cellular response to the expression of an aberrant secretory protein in *Aspergillus oryzae*

Takahiro Shintani, Daisuke Shiro, Jun-ichi Yokota and Katsuya Gomi Graduate School of Agricultural Science, Tohoku University, Sendai, Japan shintani@biochem.tohoku.ac.jp

To address the issues that underlie the decreased production of higher eukaryotic proteins in filamentous fungi, we analyzed the cellular responses to the overexpression of an aberrant secretory protein in *Aspergillus oryzae*. We expressed the mutant version of 1,2-alpha-mannosidase from *Aspergillus saitoi*, whose Cys residue at the position 443 was substitute to Phe (C443F MsdS). It has been reported that this amino acid residue is crucial to maintain the proper folding of MsdS and this mutation therefore decreased its production in *A. oryzae*. When expressed from a moderate *enoA* promoter, the mutant MsdS seen in intracellular and extracellular fractions were much less than the wild type. Moreover, a deletion of the *hrdA* gene, which encodes the *Aspergillus* homolog of Hrd1 implicated in the ER-associated degradation pathway, accumulated the mutant MsdS intracellularly. When expressed from a strong No.8142 promoter, the mutant MsdS was secreted at a level comparable to the wild type, but hyperglycosylated, which is rarely seen in *Aspergillus*. DNA microarray analysis revealed that the transcriptions of ER chaperones and components of the ER-Golgi transport were specifically upregulated in the strain overexpressing the mutant MsdS, suggesting that the accumulated mutant MsdS induced the unfolded protein response.

3. Two GDP-mannose transporters contribute to cell wall integrity in *Aspergillus nidulans*

Loretta Jackson-Hayes, Barry Gordon, Lauren Fay, Sonia Nkashama, Ravi Patel, Terry Hill, Darlene Loprete, Departments of Biology and Chemistry, Rhodes College, 2000 N. Parkway, Memphis TN 38112, Loprete@rhodes.edu, Hill@rhodes.edu

In order to identify novel genes affecting cell wall integrity, we have generated mutant strains of the filamentous fungus *Aspergillus nidulans*, which show hypersensitivity to the chitin-binding agent Calcofluor White. The phenotype of one of these (*calI*) also includes shortened hyphal compartments and increased density of branching. Hyphal walls also show reduced staining by the mannose-specific lectin FITC-ConA. We have identified two *A. nidulans* genes (AN8848.3 and AN9298.3, hereafter designated *GmtA* and *GmtB*, respectively) which complement all aspects of the phenotype. Both genes show strong sequence similarity to GDP-mannose transporters (GMT) of *Saccharomyces* and other yeasts. Sequencing of *GmtA* in the *calI*mutant strain reveals a point mutation, resulting in an A-to-P substitution at amino acid position 332 within a region that is highly conserved among other fungi. No mutations were observed in the mutant strain's allele of *GmtB*. Meiotic mapping demonstrated a ca. 1% recombination frequency between the calI locus and the *phenA* locus confirming that *GmtA* and *CalI* are identical. A C-terminal GFP chimera of GMTA exhibits a punctate distribution pattern, consistent with those shown by putative Golgi apparatus markers in *A. nidulans*. However, the GFP-GMTA signal did not overlap with that of the putative Golgi protein mRFP-COPA, which may be explained by a cisternal maturation model in which the various Golgi equivalent organelles of *A. nidulans* represent physically separated counterparts of the stacked cisternae of the plant and animal Golgi apparatus. These findings suggest that *GmtA* and *GmtB* play roles in cell wall metabolism in *A. nidulans*.

4. A Mutation in a COG4 Homologue Affects Polarity Establishment in *Aspergillus nidulans*

Sara Gremillion, Darlene Loprete, Terry Hill, Kaddy Camara*, Felicia Samuels, † and Sarah Mercer, Departments of Biology and Chemistry, Rhodes College, 2000 N. Parkway, Memphis TN 38112. *Department of Biology, Rust College, Holly Springs, MS 38635. †Department of Biology, Tougaloo College, Tougaloo, MS 39174, Gremillions@rhodes.edu, Loprete@rhodes.edu, Hill@rhodes.edu

We have identified a mutation in *Aspergillus nidulans*, designated *swoP*(for swollen cell phenotype), causing a temperature-sensitive morphological defect during spore germination and hyphal growth. Conidia swell to approximately 1.5 times the normal diameter and establish multiple points of polarity, which grow isotropically before arrest. Swollen cells tend to burst when observed under a coverslip. At 42C, a very small minority of cells eventually produce colonies and asexual spores. Growth at 30C is essentially normal, though hyphae are slightly wider than wild type and exhibit a minor "steering" defect. Cells grown at 42C for up to 18 hours recover normal hyphal morphology upon transfer to 30C. In hyphae transferred from 30C to 42C, both terminal and sub-apical compartments swell irregularly and may produce multiple buds. The growth defect of *swoP* is complemented by the wild type allele of AN7462, which shows strong sequence homology to COG4, a component of the Golgi tethering complex associated with retrograde transport of COPI-coated vesicles. Sequencing of the COG4 allele of the *swoP* mutant reveals a mutation at base pair 2672, introducing a stop codon at amino acid 780. Meiotic mapping produces a ca. 12% recombination frequency between the *swoP* and *AcuK* loci, which is consistent with the chromosomal location of AN7462. We are currently working to localize the putative COG4 protein of *A. nidulans* via GFP-tagging and immunolocalization, as well as to demonstrate interactions between *A. nidulans* COG4 and other proteins involved in retrograde vesicle transport in the Golgi apparatus.

5. Apical sterol-rich membranes are essential for localizing cell end markers that determine growth directionality in the filamentous fungus *Aspergillus nidulans*.

Norio Takeshita, Yuhei Higashitsuji, Sven Konzack, and Reinhard Fischer. Applied Microbiology, University of Karlsruhe, D-76187 Karlsruhe, Germany. norio.takeshita@mbio.uka.de

In filamentous fungi hyphal extension depends on the continuous delivery of vesicles to the growing tip. Here, we describe the identification of two cell-end marker proteins, TeaA and TeaR, in *Aspergillus nidulans*, corresponding to Tea1 and Mod5 in *Schizosaccharomyces pombe*. Deletion of *teaA* or *teaR* caused zig-zag-growing and meandering hyphae, respectively. The Kelch-repeat protein TeaA, the putatively prenylated TeaR protein, and the formin SepA were highly concentrated in the Spitzenkorper, a vesicle transit station at the tip, and localized along the tip membrane. TeaA localization at tips depended on microtubules and TeaA was required for microtuble convergence in the hyphal apex. The CENP-E family kinesin KipA was necessary for proper localization of TeaA and TeaR, but not for their transportation. TeaA and TeaR localization were interdependent. TeaA interacted in vivo with TeaR, and TeaA co-localized with SepA. Sterol-rich membrane domains localized at the tip in *teaA* and *teaR* mutants like in wild type, and filipin treatment caused mislocalization of both proteins. This suggests that sterol-rich membrane domains determine cell-end factor destinations and thereby polarized growth.

6. Spatial differentiation of mannitol dehydrogenase and mannitol-1-phosphate dehydrogenase explains the absence of a mannitol cycle in *Aspergillus niger*

Patricia vanKuyk[1], Guillermo Aguilar[2], Dirk Blom[2], Arman Vinck[2], Han Wösten[2], Ronald de Vries[2] [1] Molecular Microbiology, Leiden University, Leiden, The Netherlands; [2]Microbiology, Utrecht University, Padualaan 8, 3584 CH, Utrecht, The Netherlands e-mail: p.a.vankuyk@biology.leidenuniv.nl; r.p.devries@uu.nl

Mannitol is the most abundant polyol in spores from *Aspergillus niger*, and it disappears during early germination. The presence of a mannitol cycle has been suggested in several fungi in which mannitol is produced from fructose-6-phosphate by mannitol-1-phosphate dehydrogenase (MPD) and mannitol-1-phosphate phosphatase and converted back to fructose-6-phosphate by mannitol dehydrogenase (MTD) and hexokinase. Speculations into the function of such a cycle mainly focus on its role in the production of NADPH, based on the presence of all the enzymes from the pathway in fungal mycelium. However, no effects on NADPH production could be observed when either of the dehydrogenase-encoding genes was disrupted in several fungi. Here we demonstrate that spatial differentiation occurs with respect to the expression of mpdA and mtdA using GFP and dTomato fused to the promoters of these genes. While *mpdA* expression occurs in substrate hyphae, expression of *mtdA* is restricted to conidia. These data indicate that MPD is involved in mannitol synthesis and MTD in mannitol degradation, but that these processes do not occur in the same part of the colony.

7. The gene for the ubiquitin ligase regulator Cand1 is split into two genes in the filamentous fungus *Aspergillus nidulans*

Elke U. Schwier[1], Martin Christmann[1], Krystyna Nahlik[2], Silke Busch[3] and Gerhard H. Braus[1] [1]Institut fur Mikrobiologie und Genetik, Gottingen, Germany. [2]Center for Cancer Research & Cell Biology, Belfast, UK. [3]Zentrum fur Arzneimittelforschung, Entwicklung und Sicherheit, Frankfurt, Germany.

Ubiquitin dependent proteolysis plays an important role in many cellular processes in eukaryotes. Cullin containing ubiquitin ligases like the SCF (Skp1-Cullin-F-box protein) complex mark proteins for degradation by ubiquitinylation. It has been shown that the protein Cand1 binds to cullins. The Cand1 C-terminus blocks the SKP1 binding site of the SCF complex component Cul1 and affects thereby the assembly/disassembly of the ubiquitin ligase. The N-terminus of Cand1 buries the neddylation site on Cul1, which prevents its modification by Nedd8, an ubiquitin-like protein. In *Aspergilli*, the corresponding gene is split into two independent open reading frames encoding the N- and C-terminal part (*candA-N, candA-C*) of the mammalian homolog. Deletion of the single or both *candA* genes leads to mutants with identical phenotypes. They produce only few asexual spores, are blocked in early sexual development and appear dark red when grown under development inducing conditions. All defects of the *candA* deletion mutants can be complemented by a *candA-N::C* fusion construct indicating that the split is not crucial for protein functions. Only CandA-C, but not CandA-N, binds to cullins. Since both CandA proteins interact with each other, binding of CandA-N to cullin is presumably mediated by CandA-C. CandA-C is nuclear enriched and expressed in vegetative cultures but degraded at an early stage of sexual development indicating a role during onset of development. The split *candA* gene makes the fungus *A. nidulans* an attractive model organism for studying the putative different functions of the two parts of the Cand1 protein.

8. Fungal bestrophins; the rate limiting step for organic anion secretion from filamentous fungi?

Stephen K. Roberts, Biology Department, Lancaster University, UK. email: s.k.roberts@lancaster.ac.uk

A defining feature of filamentous fungi is their ability to release substantial amounts of organic anions (OAs) into their environment. OAs released by fungal cells play important roles in fungal phsiology, for example, in the acquisition of nutrients from the soil solution, chelation of toxic heavy metals and in the regulation of cytosolic pH during acid stress. Also, the ability of fungi to secrete OAs is heavily exploited by the biotechnology industry. Despite the central importance of OA release, little is known about the mechanisms mediating OA transport across the plasma membrane (PM); though, it is universally accepted that OA release will occur passively, most likely via ion channels. Consistent with this, it is established that anion efflux channels are prevalent in the PM of Aspergillus. For the first time, two genes encoding anion efflux channels have been cloned from Aspergillus nidulans. These genes are referred to as AnBEST1 and AnBEST2 on account that they show limited similarity to the recently identified bestrophin gene family. The fungal bestrophins appear to be represented in all filamentous fungal genomes but are absent in yeasts. Functional heterologous expression of AnBEST1 and AnBEST2 has established that they are; a) anion selective and mediate citrate efflux, b) gated by cytosolic Ca2+, and c) able to restore the growth of the pdr12delta yeast mutant on inhibiting concentrations of extracellular weak OAs consistent with the fungal bestrophins being permeable to a range of organic anions.

9. The COP9 signalosome influences cell wall degradation and development of *Aspergillus nidulans*

Krystyna Nahlik[1], Silke Busch[2], Elke U. Schwier[1], Birte Könnecke[1], Reiner Hitt[3] and Gerhard H. Braus[1] [1]Institut für Mikrobiologie und Genetik, Göttingen, Germany. [2]Zentrum für Arzneimittelforschung, Entwicklung und Sicherheit, Frankfurt, Germany. [3]Transkriptom Analyse Labor, Georg-August-Universität, Göttingen, Germany.

Fruit body formation in the filamentous fungus *Aspergillus nidulans*requires complex genetic regulation and mobilization of energy, stored mainly in cell wall glucans. The COP9 signalosome (CSN), a conserved eukaryotic multiprotein complex playing a role in the regulation of protein degradation by the ubiquitin system, is necessary for sexual fruit body formation and establishment of balance between sexual and asexual development in this fungus. We have analysed the mechanism of CSN action during development by genome-wide transcriptome profiling and proteome analyses of a *csnE* deletion strain. We show that the fungal CSN affects the regulation of distinct groups of genes, including oxidoreductases, genes involved in secondary metabolite biosynthesis and cell wall glucan degradation. Furthermore, CSN is required for expression of a hormone synthesis involved in regulation of developmental balance and necessary for developmentally induced beta-glucanase activity, which might be prerequisite for fruit body formation.

10. The nuclear migration protein NUDF/LIS1 forms a complex with NUDC and BNFA at spindle pole bodies

Kerstin Helmstaedt[1,3], Karen Laubinger[1,3], Katja Voßkuhl[1], Özgür Bayram[1], Silke Busch[1], Michael Hoppert[2], Oliver Valerius[1], Stephan Seiler[1,3], Gerhard H. Braus[1] [1]Molekulare Mikrobiologie und Genetik, Institut für Mikrobiologie und Genetik, Georg-August-Universität Göttingen, Grisebachstraße 8, D-37077 Göttingen, Germany, [2]Allgemeine Mikrobiologie, Institut für Mikrobiologie und Genetik, Georg-August-Universität Göttingen, Grisebachstraße 8, D-37077 Göttingen, Germany, [3]DFG Research Center for Molecular Physiology of the Brain (CMPB), Germany.

Nuclear migration depends on microtubules, the dynein motor complex and regulatory components like LIS1 or NUDC. We sought to identify new binding partners of the fungal LIS1 homolog NUDF to clarify its function in dynein regulation. Therefore, we analysed the association between NUDF and NUDC in *Aspergillus nidulans*. NUDF and NUDC directly interacted in yeast two-hybrid experiments via NUDF's WD40 domain. NUDC-GFP was localized to immobile dots in the cytoplasm and at the hyphal cortex, some of which were spindle pole bodies (SPBs). We showed by bimolecular fluorescence complementation microscopy that NUDC directly interacted with NUDF at spindle pole bodies at different stages of the cell cycle. Applying tandem affinity purification, we isolated the NUDF-associated protein BNFA (binding to NUDF). BNFA was dispensable for growth and for nuclear migration. GFP-BNFA fusions localized to SPBs at different stages of the cell cycle. This localization depended on NUDF, since loss of NUDF resulted in cytoplasmic accumulation of BNFA. BNFA did not bind to NUDC in a yeast two-hybrid assay. These results show that the conserved NUDF and NUDC proteins play a concerted role at spindle pole bodies at different stages of the cell cycle and that NUDF recruits additional proteins specifically to the dynein complex at spindle pole bodies.

COMPARATIVE AND FUNCTIONAL GENOMICS

11. A (semi) high-through-put pipeline for the functional characterization of putative sugar transporters.

M.C. Askin[1], D. Doevendans[1], A. Ram[1,2], J. Visser[3], B.M. Bakker[4],P.A. vanKuyk[1] 1 Molecular Microbiology, Leiden University, The Netherlands 2 Kluyver Centre, Delft, The Netherlands 3 FGT Consultancy, Wageningen, The Netherlands 4 Molecular Cell Physiology, Vrije Universiteit Amsterdam, The Netherlands

In order to fully understand an organisms potential a better understanding of its components is required. Although thousands of Major Facilitator (MFS) Superfamily members have been identified from genome sequence projects, a relatively small number have been functionally characterized. Sugar transport is an area of research traditionally not well studied, with the exceptions of *Saccharomyces cerevisiae* and *Arabidopsis thaliana*. To date only 5 of the 34 S. cerevisiae sugar porters remain uncharacterized. All known eukaryotic sugar transporters are members of the MFS. Genome comparisons show that MFS proteins constitute a large portion of the transporter capacity of eukaryotes. Due to the tendancy of MFS proteins of (distantly related) organisms to cluster based on organism, sequence comparison of uncharacterized MFS proteins to functionally characterized proteins is of limited assistance in indicating substrate specificity. Most MSF protein sequences from filamentous fungi, for example the Aspergilli, tend to form separate clusters from the (characterized) yeast transporters. With the goal of increasing our understanding of sugar transport we have developed a system for the rapid functional characterization of putative sugar transporter encoding genes.

12. Artificial generation of large chromosomal deletions in *Aspergillus oryzae* and *Aspergillus sojae*.

Tadashi Takahashi, Feng Jie Jin, Yukio Senou, Yasuji Koyama. Noda Institute for Scientific Research, Noda City, Japan.

By using the combination of a *ku70*-deficient strain and bidirectional markers of the koji molds *Aspergillus oryzae* and *A. sojae*, we established a fundamental technology for efficiently generating large chromosomal deletions. In this study, we investigated the limitations of the possible deletion range in one deletion cycle and the efficiency of 2 deletion methods, the loop-out (resolution-type recombination) and direct (replacement-type recombination) deletions. The large genomic deletions generated by a loop-out deletion mechanism enable us to make multiple deletions by using marker recycling. As a result, over 300- kb regions of chromosome 3 including the aflatoxin biosynthetic gene cluster and 200-kb region of chromosome 4 have been completely deleted from the koji molds. In addition, we recently discovered that the limitation of deletion range in the replacement-type recombination method was highly expanded in *ku70* mutant strains. And we have successfully generated deletion of 470 kb in chromosome 8 with high efficiency. The results obtained in this study indicate that the *ku70*mutation elevated not only the conventional gene- targeting frequency but also the frequency of recombination between 2 distant sites. The technology described here is applicable for genomic engineering and molecular breeding of industrially used filamentous fungi by artificially generating large-scale genomic deletions and marker-free strains

13. Insights in Short Term Evolution of Aspergillus niger Strains.

Peter van de Vondervoort1), Mikael Andersen2), Scott Baker3), Martin Meijer4), Noël van Peij1), Herman Pel1), Hans Roubos1), Robert Samson4), Peter Schaap5) 1) DSM Food Specialties, Delft, The Netherlands 2) Center for Microbial Biotechnology, DTU, Lyngby, Denmark 3) Pacific Northwest National Laboratory, Richland, USA 4) Centraalbureau voor schimmelcultures, Utrecht, The Netherlands 5) Laboratory of Microbiology, Wageningen University, the Netherlands

In most post-genomic studies genomes are treated as static entities describing the genomic potential of a species. To cope with the challenges of a constantly changing environment in reality genomes are dynamic systems subject to constant changes. Due to the costs involved eukaryotic species are rarely sequenced twice. This is not so for Aspergillus niger, an important fungal species used in the commercial production of various extracellular enzymes and organic acids. Currently a near complete genome sequence with a high quality annotation is publicly available of strain CBS513.88, a selected industrial enzyme producer (Pel et al., Nature Biotech. 2007) and a draft sequence of strain ATCC1015 a historic citric acid producer can be retrieved from DOE. (http://genome.jgi-psf.org/Aspni1/Aspni1.home.html). Both genome sequences have been assembled successfully into a set of large scaffolds but both sets still suffer from a reasonable number of sequence gaps. To improve annotation and to allow a detailed high quality genome comparison between the respective A. niger strains PCR-sequencing is being used for gap closure in the A. niger CBS513.88 using the ATCC1015 genome sequence as reference. A comparison of the revised CBS513.88 genome sequence with ATCC1015 genome sequence reveals that a number of the approximately 14,700 protein-coding genes in CBS513.88 is lacking in the ATCC1015 strain. Overall these strains are virtually identical at DNA level, however a number of large and small chromosomal rearrangements are observed. Some of these chromosomal recombination events and their consequences will be discussed in more detail.

14. A trial of the minimization of the chromosome 7 by large-scale chromosomal deletion in *A. oryzae* RIB40.

Feng Jie Jin[1], Toshi Furukido[1], Masafumi Tokuoka[1], Masahiro Ogawa[1], Tadashi Takahashi[1], Masayuki Machida[2], and Yasuji Koyama[1].
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Chromosome engineering is a powerful technology with effectively allowing any chromosomal rearrangement to create desired strains. We have previously developed a method to efficiently construct large-scale deletion mutants by using a *ku70*- disrupted strain (1). In this study, using this method with *pyrG*-mediated transformation system, we attempted to construct a mutant with the minimum set of genes in chromosome 7, which is the smallest chromosome (2.93 Mb) in *A. oryzae*RIB40 strain. In this way, our final goal is to breed industrially favored strains by appropriately altering regulation of metabolite production. We first constructed series of large-scale deletion mutants with a main focus on the non-syntenic regions (2). Based on these results, we extrapolated that approximately a quarter of chromosome 7, at least 740 kb sequence, was non-essential and could be deleted. We then sequentially deleted these confirmed non-essential regions using the *A. oryzae ku70* disruption strain. Until now, we have successfully constructed a mutant lacking as large as 685 kb genome sequence (23.4% of chromosome 7) by multiple deletions. (1) Takahashi *et al*. (2006) In: Abstracts of ECFG8, P. 272. (2) Tamano *et al*. (2008) Fungal Genet Biol. 45, 139-151.

15. Functional analysis of essential genes in *A. fumigatus* by co-precipitation of associated proteins using an S-tag fusion peptide.

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In the opportunistic human pathogen *A. fumigatus* essential gene products are potential drug targets, however, because *A. fumigatus*is an asexual haploid organism traditional techniques used to identify and characterise genes are not always feasible. Essential genes can be identified using the MycoBank® screening method where genes are disrupted in a diploid background and essentiality of the disrupted gene is determined by loss of a marker. Bioinformatics can sometimes indicate the functions of genes but this is not always fruitful and often functional analysis is required. Genes can be characterised by analysing the phenotypes of mutants. Disruption of an essential gene is lethal so mutant strains are difficult to generate and the phenotypes are often uninformative. Identifying previously characterised proteins that interact with uncharacterised proteins can provide clues to the function of the uncharacterised proteins. We used the S-tag fusion peptide as an affinity tag for co-precipitation experiments to identify proteins that interact with a selection of putative essential proteins. The S-tag was used because the purification procedure is simple and the tag is very small so that it will not significantly disrupt the structure or function of the tagged protein. The tagged protein must be functional otherwise strains containing S-tagged essential genes will be inviable. This approach has identified a novel component of the SWI/SNF ATP-dependent chromatin remodelling complex that is unique to fungi and conserved across many fungal species. This data correlates with data obtained from other approaches; GFP localisation had previously shown this protein to be present in the nucleus and probably closely associated with DNA.

16. Construction of a disruption mutant library of the transcription regulatory genes in *koji*-mold *Aspergillus oryzae*.

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Koji-mold *Aspergillus oryzae* is used in Japanese traditional fermented food industry such as sake, soy sauce and miso (soybean paste) production. Genomic sequencing data indicates that the genome size of *A. oryzae*is 37 Mb containing over 12,000 genes. However, more than half of these genes are left functionally unknown. It is necessary to elucidate the role of the function-unknown genes for efficient breeding of *A. oryzae*. In this study, we developed a method of high-throughput disruption mutagenesis using *ku70* mutant strain and tried to disrupt transcription regulatory genes in *A. oryzae*, and characterize the disruptants. We tried to disrupt about 400 genes for transcription regulation factors and obtained 300 disruptants. About 100 genes that we failed to disrupt may be necessary to grow on the minimal medium or essential for growth of *A. oryzae*. We gained 15 mutants with reduced conidiation. Eight of them were orthologs of the regulatory genes related to asexual development in *A. nidulans* and seven were novel genes for conidiation control. The transcriptome analysis of these disruptants will be reported.

17. Prevalence of transcriptional regulators across the fungal kingdom

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Transcriptional regulators are the major control points for all aspects of fungal life. Over the years, many transcriptional regulators have been identified and their biological function has been studied. Many of these regulators have been mainly studied in a relatively small number of model fungi, although some have also only been described for less commonly used fungal species. The availability of more than 40 fungal genomes allows an analysis on the prevalence of these regulators. We have selected 60 characterized fungal transcriptional regulators and analyzed their presence in the available fungal genomes. This demonstrated that some regulators are commonly found throughout the fungal kingdom (e.g. CreA), while others are restricted to a class (e.g. PrnA in the ascomycetes) or a genus (e.g. AraR). The presence or absence of a regulator has been linked to the natural habitat of the fungi to find correlations with respect to fungal physiology.

GENE REGULATION

18. Unravelling The Complex Transcriptional Regulation Of The *afp* Gene Encoding The Antifungal Protein AFP of *Aspergillus giganteus* A. Spielvogel[1,2], U. Stahl[1], E.A. Espeso[2],V. Meyer[1] 1) Technische Universität Berlin, Institut für Biotechnologie, Fachgebiet Mikrobiologie und Genetik, 13355 Berlin, Germany 2) Departamento de Microbiología Molecular, Centro de Investigaciones Biológicas CSIC, Madrid 28040, Spain A.Spielvogel@lb.tu-berlin.de

Aspergillus giganteus is known to secrete a small basic protein named AFP with highly selective antifungal activities against numerous plant- and human-pathogenic fungi. To study the biological function of AFP, we have among other things focussed on the identification of signals involved in regulating transcription of the corresponding *afp* gene. We have previously shown that transcription of the *afp* gene is strictly coupled to the asexual development of *A. giganteus* and strongly up-regulated under several environmental stress conditions such as alkaline pH, osmotic stress, heat shock and phosphate limitation. Interestingly, alkaline pH-induced increase in *afp* transcription is not dependent on the Pal/PacC signalling cascade, pointing to the involvement of another pH-dependent regulatory system so far not described for filamentous fungi. We here show that *afp* gene expression is also up-regulated under cell wall stress provoked by Congo Red and the presence of high external calcium concentrations, suggesting that expression of the *afp* gene is under control of multiple regulatory systems. To unravel these networks, we have screened the *afp*promoter sequence and have identified single or multiple putative binding sites for transcription factors such as StuA (asexual development), RlmA (cell wall integrity), PhoA (phosphate regulation), CrzA (calcium signalling) and SltA (salt stress). Results obtained from different approaches, such as determination of reporter activities in a heterologous system (*afp::lacZ* reporter in wild-type and different mutant strains of *A. nidulans*), phosphate uptake measurements and gel shift assays, let us postulate that StuA, PhoA, SltA and CrzA might indeed be involved in *afp* gene regulation. Furthermore, our data indicate that pH-dependent and phosphate-dependent regulation of the *afp* gene are linked to each other. The *afp* gene is thus under control of different regulatory systems some of which might even be interconnected. Hence, the *afp* promoter could serve as an excellent model promoter to study the interaction of important signalling pathways in *Aspergillus*

19. The use of the exo-inulinase promoter *(PinuE)* as a new inducible promoter for heterologous protein production in *Aspergillus niger*

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The replacement of harsh, toxic chemicals by enzymes in industrial processes related to lignin degradation is highly desirable. To produce these enzymes in the high amounts needed for industrial applications, *Aspergillus niger* is a promising host for the production of these enzymes because of its high secretion capacity (Punt et al., 2002). As an alternative for the well established Glucoamylase (GlaA) based protein expression system in *A. niger* we have used an another inducible expression system. We previously identified the *inuE* gene, encoding for the exo-inulinase protein in *A. niger*, as the most strongly induced gene in the presence of inulin and sucrose (Yuan et al., 2006; Yuan et al., 2007). To further study the expression of the inuE gene, a PinuE-eGFP reporter strain was constructed. The reporter strain confirmed previous Northern blot analyses (Yuan et al., 2006) and showed that the *inuE*gene is highly expressed on inulin and sucrose. Analyses of GFP fluorescence of the reporter strain revealed no expression from the inuE promoter on glucose, fructose and xylose, indicating a tight control of the regulation of the inuE promoter in relation to the different carbon source. Advantages of the use of the inuE expression system compared to the *glaA* expression include: (i) the *inuE* system allows production of enzymes on substrates containing sucrose and inulin as the expression of the *glaA*gene is low at these carbon sources (ii) it allows tight control of the expression of protein of interest which might be advantageous if the protein of interest hampers the growth of the fungus. We have used the *inuE* expression system for the successful expression of *Coprinus cinereus* peroxidases in *A. niger*. Punt P.J., van Biezen, N., Conesa, A., Albers, A., Mangnus, J. and van den Hondel, C.A.M.J.J., Trends in Biotechnol., 20 (5), 200- 206. 2002. Yuan, X.L., Goosen, C., Kools, H., van der Maarel, M.J.E.C., van den Hondel, C.A.M.J.J., Dijkhuizen, L. and Ram, A.F.J., Microbiol., 152, 3061-3073. 2006. Yuan, X.L., Roubos, J.A., van den Hondel, C.A.M.J.J. and Ram, A.F.J., Mol. Genet. Genomics, 2007.

20. The heme pathway in *Aspergillus niger*

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The production of enzymes by microorganisms as “biofriendly” replacements for chemical and polluting industrial processes has gained increasing attention over the last years. Due to their broad range of substrates, oxidoreductases like peroxidases are potent enzymes to be used for these purposes, but production in their natural host is far from optimal for industrial application. For the overproduction of oxidoreductases, filamentous fungi like *Aspergillus sp.* are preferred host organisms due to their high capacity of producing homologous and heterologous proteins. However, only limited success has been obtained so far with the overproduction of fungal peroxidases in *Aspergillus sp.*Incorporation of heme as a cofactor, essential for enzyme activity, is one of the limiting factors in the overproduction of fungal peroxidases which require heme. Heme is an essential molecule for the cell due to its involvement in many essential processes. On the other hand, free heme and accumulation of its intermediates is toxic when present in high concentrations. This is caused by the generation of reactive oxygen species (ROS) and photosensitivity of the porphyrins. As a consequence, in order to increase heme availability for the overproduction of peroxidases, insight in the complete heme pathway and its regulation is necessary. With the recent publication of the *Aspergillus niger* genome, a first approach to determine the heme biosynthetic pathway in *A. niger*is performed by in silicio analysis. The results show a putative conserved heme biosynthesis pathway in which all enzymes are represented although subtle differences also become apparent in comparison with various organisms. After analysis and identification of specific motifs within the enzymes belonging to the heme pathway, these enzymes appear to be more closely related to their mammalian counterparts than to *Saccharomyces cerevisiae*.

21. Investigating The Link Between TORC1 Signalling And Nitrogen Metabolism In *Aspergillus fumigatus*.

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The capacity to reprogramme metabolism in response to different nitrogen sources is a crucial virulence determinant for *Aspergillus fumigatus*. Although, the genetic response to different nitrogen sources, nitrogen catabolite repression (NCR), is very well understood in *Saccharomyces cerevisiae*, less in known in filamentous fungi, and some differences are apparent between different fungal species. A key area of interest is in determining whether the rapamycin-sensitive TORC1 complex is involved in NCR in *A. fumigatus*. We determined that rapamycin inhibited the growth of *A. fumigatus* in the same way as it did *A. nidulans*, confirming that TORC1 activity is required for growth of *A. fumigatus*. We then investigated whether *S. cerevisiae* could be used as a surrogate host to study the function of *A. fumigatus* genes. Using the *A. fumigatus* genome sequence, we identified and cloned TORC1/ NCR genes into yeast vectors to test whether they would complement yeast mutants. We showed that *AfareA, Afure2, Afgap1* and *AfprA* could complement *S. cerevisiae* mutants lacking the orthologous genes. We also used RT-PCR to look at expression of TORC1 and NCR regulated genes in *A. fumigatus* and *A. nidulans* when growing under different nitrogen conditions and when TORC1 was inhibited by rapamycin. In agreement with previous workers, we did not see a link between TORC1 and NCR in *A. nidulans*, but did find that TORC1 regulated NCR genes such as *areA, mepA* and *gap1* in *A. fumigatus*. This indicates a significant difference in genetic and metabolic wiring between the non-pathogenic and pathogenic *Aspergilli*. Currently, we are carrying out whole genome analysis using *A. fumigatus* gene arrays to determine the full-spectrum of genes regulated by the TORC1 and NCR systems.

22. The novel transcription activator AtrR regulates gene expression of ABC transporters and contributes to azole resistance in filamentous fungi

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Overexpression analyses of transcription factor genes found in *Aspergillus oryzae* genome revealed that upregulation of a zinc cluster gene, designated *atrR*, resulted in increased azole drug resistance and also induced the gene expression of ABC transporters in *A. oryzae*. Deletion of the *atrR* reduced the expression level of at least three ABC transporter genes and consequently resulted in significant increase in azole drug susceptibility, especially the *atrR* mutant was also susceptible to fluconazole. Orthologous genes of the *A. oryzae atrR* have been found widely in genomes of filamentous fungi, including *A. nidulans*, *A. fumigatus*, and *Magnaporthe grisea*. Both strains with deletion of these orthologs in *A. nidulans* and *A. fumigatus* were also hypersensitive to azole drugs. These results indicate that the novel transcription factor, AtrR, regulates gene expression of ABC transporters that would function as drug efflux pumps and contributes to the azole resistance in *Aspergillus* fungi. In addition, a deletion mutant of the *atrR* ortholog has been constructed in *M. grisea* and is now being examined for azole susceptibility.

23. Identification of *SKI* genes and their involvement in nonstop mRNA decay in *A. oryzae*.

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In the previous study, we showed that premature polyadenylation within ORF of the heterologous gene occurred but was prevented by codon optimization in *A. oryzae*. This observation suggested that low mRNA level of the heterologous gene is caused by the degradation process of mRNA lacking translation termination codon, which is called 'nonstop mRNA decay' pathway. Nonstop mRNA decay (NSD) mechanism has been studied mainly in yeast, and Ski7p-exosome-dependent 3'-to-5' degradation and Ski7p-independent 5'-to-3' degradation models have been proposed. In the *Aspergillus* genomes, there are no homologues of Ski7p that is known to be most important for NSD in yeast, suggesting that NSD mechanism in filamentous fungi differs from that in yeast. On the other hand, except for Ski7p there are other corresponding Ski protein homologs, especially Ski1p (5'-to-3' exoribonuclease), Ski2p (one of the components of Ski complex required for 3'-to-5' decay), and Ski4p (subunit of the exosome) homologs share high identity with those of yeast. In *A. oryzae*, whereas the expression level of nonstop mRNA significantly reduced compared with that of normal mRNA in wild-type and *Dski1*, this reduction was depressed clearly in *Dski4* and also slightly in *Dski2*. This suggested that nonstop mRNA is degraded by 3'-to-5' decay pathway in *A. oryzae*.

24. The MAL cluster in *A. oryzae* is involved in production of amylolytic enzymes at early stage of maltose induction
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A. oryzae produces a copious amount of amylolytic enzymes and these genes are regulated by a transcriptional activator, AmyR, in the presence of starch or malto-oligosaccharides including maltose. The amyR gene disruptant showed significantly poor growth on starch medium but showed normal growth on maltose medium, suggesting the existence of alternative maltose-utilizing enzymes whose expression might not be regulated by AmyR. We have found a gene cluster highly homologous to the yeast maltose-utilizing MAL. This cluster consists of malP (MAL61 homolog), malT (MAL62 homolog), and malR (MAL63 homolog), and thus is designated MAL cluster in *A. oryzae.* The purpose of this study is to elucidate the expression and function of MAL cluster genes. Northern analyses showed that malP and malT were expressed at high level in the presence of maltose but malR was expressed constitutively. Disruption of malR resulted in the loss of expression of malP and malT. These results indicated that MalR is required for gene expression of malP and malT at post-translational level. The malP or malR disruptant showed poor growth on maltose as well as on starch, suggesting that malP and malR are somehow involved in assimilating starch. Alpha–amylase activities of both disruptants were significantly low compared with those of wild type at early stage of maltose induction. The result suggested that maltose transported into cell mainly by maltose permease encoded by malP is required for AmyR activation and resulting induction of amylolytic enzymes.

25. The Role of *Aspergillus nidulans's* Pumilio family (PUF) RNA Binding Proteins implicated in mRNA Stability during Nitrogen Metabolism.
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The stability of any given transcript plays an important role in determining the level of the mRNA and subsequent gene expression. Highly stable transcripts are optimal for achieving high levels of gene expression. We have previously demonstrated a differential rate of decay for the areA transcript coding for the major transcription factor mediating nitrogen metabolite repression. The areA mRNA is destabilised by the presence of intracellular Gln, a signal of nitrogen sufficiency. This mechanism is also important to the expression of various structural genes involved in nitrogen metabolism, including niiA and niaD. Bioinformatics analysis of the untranslated regions of Aspergillus genes revealed the presence of a number of conserved motifs, including many likely to be involved in interactions with the Pumilio homology domain (Puf) RNA-binding proteins. In other eukaryotes, including S. cerevisiae and D. melanogaster, these proteins have been found to coordinate expression of specific groups of genes. We have identified a number of proteins whose orthologues are shown to be involved in RNA degradation. Amongst these are the five Puf proteins, PufA, PufB, PufC, PufD and PufE. All five A. nidulans's puf genes have been successfully deleted. Deletion of pufD results in a dramatically reduced response to Gln with respect to destabilisation of areA and meaA transcripts, and the areA transcript also reveals significantly reduced basal degradation. Deletion of pufA appears to disrupt the sexual cycle. The pufB, pufC and pufE deleted strains are currently being characterised

POPULATION AND EVOLUTIONARY GENETICS

26. Confirmation of identification of a population of *Aspergillus fumigatus* isolates from Ireland using a polyphasic approach.
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A collection of 94 *Aspergillus fumigatus sensu lato*isolates was cultured from air samples from four outdoor locations in Dublin, Ireland during 2005. A polyphasic approach was taken for the identification of the isolates. Phenotypic characters were examined using established protocols, and included a morphological and physiological analysis. Results confirmed that all 94 isolates were *A. fumigatus sensu stricto*. Genotypic variation within the population was analysed by RAPD-PCR which revealed that the isolates are closely related but not identical. The distribution of the two mating-type genotypes of the fungus was determined using a multiplex mating-type PCR assay. The population consisted of equal proportions of the two mating-types (49.4% *MAT1-1*, 50.6% *MAT1-2*), with no geographical clustering of mating-types evident. A representative sub-sample of 12 isolates was chosen for multi-locus sequence typing of the beta-tubulin and carboxypeptidase genes. Phylogenetic analysis clearly grouped the 12 representative isolates with known *Aspergillus fumigatus sensu stricto* isolates. The results show that *A. fumigatus* isolates of complementary mating-type can be readily found in close proximity in nature, consistent with a sexually reproducing species.

SECONDARY METABOLISM

27. Increased asperthecin production in a mutant of *Aspergillus nidulans* defective in ascospore production.

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Methylation processes in the aspergilli have many functions that range from the production of small bioactive compounds, such as the conversion of demethylsterigmatocystin to sterigmatocystin by StcP, to posttranslational modifications effecting transcription, such as the histone arginine methyltransferases RmtA – RmtC. Here we describe a putative methyltransferase with unknown function that plays a role in sexual development as well as secondary metabolite production in the model organism *Aspergillus nidulans*. This gene has been named *llmE* (LaeA-like methyltransferase E) and disruption of this locus resulted in a mutant that is drastically reduced in ascosporogenesis and showed alterations in its secondary metabolite profile. Using liquid chromatography – mass spectrometry (LC-MS), DeltallmE was shown to produce asperthecin, an anthraquinone hypothesized to be found in cleistothecia of *A. nidulans*, however, asperthecin was not detected in wild type. Based on our thin layer chromatography (TLC) profiles of this mutant, there are several other metabolites that are differentially regulated and we are currently working on identification of these compounds as well as elucidating the involvement of LlmE in sexual development.

28. *Aspergillus oryzae* LaeA Affects Production Of Kojic Acid.

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Filamentous fungi produce a large variety of secondary metabolites, and are used as medicine provider. Recently secondary metabolite regulating gene laeA was found in *Aspergillus nidulans*, and the regulation of metabolites production has begun to be clarified in filamentous fungi. It was reported that *laeA* affects the production of secondary metabolites differently depending on species. For the purpose of elucidating the regulation mechanism of laeA on secondary metabolites production in A. oryzae, we cloned *A. oryzae laeA* gene and produce a disruption mutant. We used delta*ligD* delta*pyrG* strain as a host, of which homologous recombination efficiency was increased by *ligD* gene deletion. We disrupted *laeA* by deleting putative S-adenosylmethionine binding site with *pyrG* marker. The *laeA* disruptant (delta LaeA) showed pigment decreasing phenotype similar to other species. Delta LaeA strain exhibited extreme reduction of conidia production, which did not change in *A. nidulans*. We examined the effect of *laeA* deletion on production of kojic acid, one of the most famous secondary metabolites in *A. oryzae*. In delta LaeA strain production of kojic acid was greatly reduced in kojic acid production medium. While in *laeA* complement strain (delta LaeAcomp) kojic acid production was recovered as much as control strain. These suggested that LaeA affects kojic acid production in *A. oryzae*. In addition, analysis of LaeA-regulated gene by DNA microarray will be also discussed.

29. Metabolome analysis of *Aspergillus oryzae* : Effect of environmental conditions and genetic backgrounds.

Masafumi Tokuoka[1], Masahiro Ogawa[1], Tadashi Takahashi[1], Toshi Furukido[1], Hideaki Koike[2], Yasunobu Terabayashi[2], Noriko Yamane[2], Masayuki Machida[2], Yasuji Koyama[1] [1]Noda Institute for Scientific Research, [2]AIST mtokuoka@mail.kikkoman.co.jp

We analyzed the metabolite profile of *A. oryzae* RIB40 strain in various culture conditions by LC/MS, and found that the temporal pattern of most metabolites could be classified into several groups. We also analyzed the metabolite profiles of the strains of a gene-disruption library which consists of disruptants of over 300 transcription factors. Interestingly, aberrant conidiation mutants exhibited a reduced number of metabolites, and could be separated into at least three groups based on principal component analysis of their metabolite profiles. These results suggest that production of many metabolites are associated with conidiation, and metabolite profiles may reflect the conidiation processed. In conclusion, metabolome analysis has a high potential to find out a gene function related to not only metabolism but also morphological differentiation. Further analysis is in progress, and results will be discussed.

30. The *velvet* complex coordinates light, fungal development and secondary metabolism.

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Differentiation and secondary metabolism are correlated processes in fungi and respond to light. In *Aspergillus nidulans*, light inhibits sexual reproduction as well as secondary metabolism. We identified the heterotrimeric *velvet*complex as link between light-responding developmental regulation and control of secondary metabolism. VeA, which is primarily expressed in the dark, physically interacts with VelB that is expressed during sexual development. VeA bridges VelB to the nuclear master regulator of secondary metabolism LaeA. Deletion of either *velB* or *veA* results in defects in both sexual fruiting body formation and production of secondary metabolites.

OTHER TOPICS

31. Three Different Cullins are Neddylated in *Aspergillus nidulans*

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The NEDD8/RubA protein belongs to the Ubiquitin-like protein group and its conjugating process is mechanistically similar to ubiquitination. In this conjugating process NEDD8/Rub1 is activated by E1 enzyme (Uba3/Ula1) and conjugated by E2 enzyme (Ubc12). The known substrates of the NEDD8 are the Cullin family proteins, and the activity of COP9 Signalosome Complex is required for the de-neddylation of the neddylated cullins. The neddylation pathway is essential in mammalian cells, *C.elegans* and fission yeast. In this work, the filamentous ascomycete *A. nidulans* was used as a model organism for the identification of RubA-interacting proteins and RubA-substrates. The phenotype of the delta*rubA* null allele was defined and has been confirmed by Heterokaryon rescue technique that *rubA* gene is essential in *A. nidulans*. Through the protein complex purification of TAP-tag:: *rubA* and TAP-tag::cDNA*rubA* strains of *A. nidulans*, some expected proteins were isolated and identified by Mass Spectrometry analysis. Among the detected proteins by Mass Spectrometry are all cullins described until now for *A. nidulans*(CulA, C and D), two E1 enzymes already described in the literature as partners of Nedd8/Rub1 (UlaA and UbaC), as well RubA and other proteins as ribosomal proteins, transcription factor protein, chaperonine and F-box proteins.

32. The Central *Aspergillus* Data REpository (C*A*DRE): Viewing and Analysing Sequences.

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The Central *Aspergillus* Data REpository (C*A*DRE; http://www.cadre-genomes.org.uk) is a public resource for genomic data extracted from species of Aspergillus. It arose to enable the management of the annotated sequence of *Aspergillus fumigatus* and to provide tools for searching, analysing and visualising features of fungal genomes. The resource has now expanded to encompass the assemblies and annotated sequence from *Aspergillus nidulans*, *Aspergillus oryzae* and *Aspergillus niger*. Genomic data for *Aspergillus clavatus* and *Neosartorya fisheri* are also currently available in-house and, after processing, will shortly be publicly available within C*A*DRE. In addition, during the process of handling *A. nidulans* annotation, we extracted data from the Aspergillus linkage map resource . This resource sought to improve the original assembly by (a) determining the correct mapping of contigs to supercontigs, (b) assigning supercontigs to linkage groups on the genetic map and (c) determining the correct orientation of each supercontig. In an attempt to provide the most accurate *single* assembly, we have now combined these data and made them available within C*A*DRE.

33. An improved & markerfree expression system for Aspergillus niger enzyme production

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Genetically modified industrial strains of Aspergillus niger have been used as a host to overexpress food and feed enzymes, such as xylanase. Traditionally, the genes encoding these enzymes are integrated in a random fashion in the genome of the host organism. New technological developments have enabled us to construct a new generation of A. niger CBS513-88 lineage strains according to a "design and build" concept, in which the genes of interest are targeted integrated in the host genome. A recombinant strain was derived from a glucoamylase production strain, in which the glucoamylase (glaA) genes were deleted, creating so-called ΔglaA loci. Each of these loci was designed in such a way that it individually can be detected by gel electrophoresis. Therefore, targeted integration of the gene of interest in all seven loci can be monitored, allowing selection of strains with multiple integrated expression units of the gene of interest. The homologous integration frequency was improved by disruption of the A. niger hdfA and/or hdfB genes, homologues of the human KU70 and KU80, that are essential for non-homologous end joining of DNA in double strand break repair. Deletion of hdf genes greatly reduces the frequency of non-homologous integration of transforming DNA fragments leading to dramatically improved gene targeting. The hdf knockout has contributed to a highly controlled strain construction process, with both increased targeting frequency and increased co-transformation percentage.

34. Automated screening for high-copy *Aspergillus niger* production strains.

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The filamentous fungus *Aspergillus niger* has extensively been used for production of recombinant proteins. We have modified *A. niger*CBS513-88 lineage strains in order to enable highly controlled production strain construction, in which genes of interest are integrated at defined high expression loci. A recombinant strain was derived from a glucoamylase production strain, harboring 7 glucoamylase (*gla*A) genes. The *gla*A genes were deleted stepwise; the resulting delta *gla*A- loci serve as tool for recombinant production strain construction according to the 'design and build' concept (see poster De Lange *et al*). Following integration of genes of interest at the target delta *gla*A-locus, the gene copy number is subsequently amplified via gene conversion of the different delta *gla*A-loci. This process can easily be monitored via PCR-screening and gel-electrophoresis. The throughput of this screening has significantly been increased by implementation of the LabChip 90 system (Caliper Life Sciences), thereby accelerating production strain construction.

35. Modelling the Conidial Aggregation of Filamentous Fungi by Population Dynamics,

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For coagulating filamentous fungi conidial aggregation is the first step of filamentous morphogenesis and influence subsequently the development of pelleted morphology. Conidial aggregation kinetics based on the time-dependent development of particle concentration studied with an in-line particle size analyzer (FBRM D600L, Lasentec) suggested that two separate stages of aggregation can be distinguished. The first step of conidial aggregation starts immediately after inoculation. Both the rate constants of formation and disintegration of aggregates have been determined by measuring of the concentration of conidia at the beginning of the cultivation and the concentration of particles at steady state. The second aggregation step is thought to be initiated by germination of conidia. Growing hyphae provide additional surface for the attachment of non-germinated conidia which leads to a strong decrease in particle concentration. The specific hyphal length growth rate and the ratio of particle concentration to the growing adhesion hyphal surface are decisive matters of the second aggregation step. Both aggregation steps was comprehensively characterized, described by population dynamics(the development of a particle collective over the time) and the applied population balance is validated by comparison of experimental data and simulation with the program package PARSIVAL (PARticle SIze eVALution, CiT).

36. The contribution of the *Aspergillus niger* genome sequence and annotation to the discovery of novel products.

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The filamentous fungus *Aspergillus niger* is widely exploited by the fermentation industry for the production of extracellular enzymes and organic acids, particularly citric acid. We sequenced 33.9 megabases of the genome of A. niger CBS 513.88, the ancestor of currently used enzyme production strains[1]. The genome sequencing and annotation enabled on one side the construction of a metabolic model, the development and application of proteomics and transcriptomics[2]. On the other side the sequencing yielded easy access to numerous (14,165) protein encoding genes. Some of these genes encode for valuable industrial proteins. Using our STIPT technology[3], production strains were generated expressing numerous extracellular proteins. The sequencing and annotation of the *A. niger* CBS 513.88 genome contributed to the rapid discovery of several interesting enzyme products, some of which examples will be shown. [1]Pel *et al.*, Nat Biotechnol. 2007 Feb;25(2):221-31 [2]see poster van de Vondervoort *et al* [3]see poster de Lange *et al*

37. Insights in Growth and Protein Formation of *Aspergillus niger* on a Molecular Level

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The filamentous fungus *Aspergillus niger* is known for its high secretion efficiency of homologous proteins. Its ability to carry out post-translational modifications results in an increasing usage of *A. niger*as production host for the formation of therapeutic agents. However, the obtainable yields of recombinant proteins are considerably lower than of homologous proteins. Hence, current research is focussed on the optimization of cultivation processes to increase the production of desired products. In this study, the production of a recombinant protein is analysed in submerged cultivations of a genetically modified*A. niger*strain. Its genome comprises extra copies of the homologous beta-fructofuranosidase gene regulated by a constitutive promoter. The influences of different environmental parameters on morphology as well as the resulting productivity in regard to the recombinant product are investigated. The transcription activity of genes involved in morphogenesis are quantified by *real- time* PCR. Results are related to microscopic images. Furthermore, the corresponding gene expression of beta- fructofuranosidase as model product is monitored via *real-time* PCR. The authors gratefully acknowledge funding provided by the German Research Foundation through SFB 578 “From Gene to Product”.

38. Post-genomic Research On *Aspergillus niger*

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For many decades, *A. niger* has been safely used in the commercial production of various food enzymes, such as glucose oxidase, pectinase, alpha-amylase and glucoamylase. DSM and the *A. niger* consortium published the genome of CBS 513.88, the ancestor of currently used enzyme production strains. A high level of synteny was observed with other aspergilli sequenced. Based on the sequence, Affymetrix TX chips were developed and used to study enzyme production in production strains. The knowledge obtained from this genomics effort enabled us to improve our “PluGbug” in a generic way, for example by using codon-optimization and to further develop proteomics. We will present important findings that led to a rational improvement of protein production in *Aspergillus niger*.

39. Development of high expression system for the lipase from *Cyrptococcus* sp S-2. in *Aspergillus oryzae*.

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Our aim is to develop bioremediation technology using enzymes as environmentally friendly tools. For this purpose, the efficient heterologous expression system is essential for the development of the enzymatic bioremediation. Our target enzyme for enzymatic bioremediation was Lipase (CSL) produced from yeast, *Cryptococcus*sp. S-2 (Masaki K et al., Appl. Environ. Microbiol. 2005 71(11) 7548-50). The CSL has rapid degradation ability of biodegradable plastics and high-synthetic ability of ester. In this study, to achieve a high-yield production of the CSL, we constructed high-expression system for CSL production in *Aspergillus oryzae* by using an improved enolase promoter, enoA142 (Tsuboi H et al., Biosci. Biotechnol. Biochem. 2005 69(1) 206-8). We used a disruption strain of *prtR* and *pepE* genes (*prtR pepE*), which encode a transcription factor that regulates extracellular proteolytic genes and a putative vacuolar aspartyl protease homologous to S. cerevisiae *PEP4*, respectively, and *A. oryzae* niaD300 (control) strain as expression hosts. The amounts of the CSL produced by transformants of each host were compared. Consequently, the amount of the CSL produced in the *prtR pepE* was higher than that of the control strain in liquid culture. Furthermore, the production of the CSL in solid culture using wheat bran as a substrate in the *prtR pepE* will be presented.

40. Casein kinase I and amino acid transporter localisation in *Aspergillus nidulans*

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In the genome of *Aspergillus nidulans* two Type 1 kinases can be detected, AN4563.3 (gene *aauZ,* for amino acid uptake defective, see below) and AN5757.3. AauZ is the nearest homologue in *A. nidulans* of *Hhp1* of *Schizosaccharomyces pombe*, one of a pair of redundant proteins involved in DNA repair. Deletion of both Hhp1 and Hhp2 results in a sick phenotype in *S. pombe*, while deletion of *aauZ* is lethal. AauZ has homologues in every single ascomycete genome we have checked, with an interesting characteristic: In all the Pezizomycotina the putative AauZ orthologue shows a carboxy-terminal conserved extension that is lacking in the Saccharomycotina and in *S. pombe* (Taphrinomycotina). Part of this extension, composed by eight contiguous residues, is absolutely conserved in every member of the Pezizomycotina where a sequenced genome is available.
Recessive, viable alleles mapping in *aauZ* have been identified using a number of screens aimed at obtaining strains defective simultaneously in the expression and/or function of a number of amino acid transporters. Two alleles map within the catalytic domain, while the allele with the most extreme phenotype maps in the first residue of the octapeptide sequence conserved in the Pezizomycotina. We have identified one of the targets of the AauZ mutated protein as the dicarboxylic amino acid transporter, AgtA. Strains expressing AgtA fusion proteins with both GFP and HA were constructed. Mutations in *aauZ* do not affect *agtA* transcription but result in localisation of AgtA to vacuoles rather than to the plasma membrane. In strains carrying the more extreme allele, AgtA is also degraded. The effect of AauZ on amino acid transporters is most likely indirect as AgtA lacks any kinase I consensus phosphorylation sites and we have not detected a phosphorylated form of the protein in membrane preparations. This work strongly suggests that in *aauZ* mutants AgtA (and by implication other amino acid transporters) is efficiently sorted into the multivesicular body pathway, which precludes its (their) correct expression at the plasma membrane. These results also suggest that in the Pezizomycotina, the homologues of AuuZ have acquired a carboxy-terminal motif with a function absent in the ancestral casein I kinase. This work illustrates that the filamentous habit of growth may involve specific sorting mechanisms for membrane proteins.

41. *Aspergillus nidulans* UDP-galactopyranose mutase (UgmA) plays key roles in colony growth, hyphal morphogensis, and conidiation.

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Fungal wall components provide useful targets for antifungal drug development, since they are not found in humans. However, there is growing resistance to most of the current drugs that target fungal walls. Galactofuranose (Gal*f*) is found in the walls of many fungi including *Aspergillus fumigatus*, currently the most prevalent opportunistic fungal pathogen in developed countries, and *A. nidulans,* a closely-related, tractable model system. Analysis of Gal*f* biosynthesis in prokaryotes shows that UDP-Gal*f* (the five-membered ring form) is generated from UDP-galactopyranose (UDP-Gal*p*, the six-membered ring form) by UDP-galactopyranose mutase (UGM) prior to cell wall incorporation.
UGM is essential in prokaryotes including *Mycobacterium tuberculosis*, and is important for virulence in the protozoan, *Leishmania major*, which causes Chagas' disease. Prokaryotic and eukaryotic UGMs share ~20 % sequence identity, confined to the catalytic site. The crystal structure of prokaryotic UGMs has been solved; the crystal structure of *A. fumigatus* UGM is currently resolved to 2.4 Å. Eukaryotic UGMs have four major insertions with respect to prokaryotic UGMs, which are thought to form loops that are important for regulation and interaction. We deleted the single-copy UGM sequence (AN3112.4, which we call *UgmA*) from an *A. nidulans nkuA*D strain, creating *ugmA*D. Haploid *ugmA*D strains were able to complete their asexual life cycle, showing that *UgmA* is not essential. However, *ugmA*D strains had compact colonial growth. Conidiation in *ugmA*D had 500-fold reduced abundance. In addition, *ugmA*D strains had aberrant hyphal morphology, producing wide, uneven, highly-branched hyphae. Hyphal defects were partially remediated by growth on 1 molar sucrose, or on 10 µg/mL Calcofluor. The *ugmA*D phenotype is consistent with Gal*f* residues being important in cell wall structure and/or function. Thus UGM may be a useful target for anti-fungal drug development. We are currently testing a suite of putative UGM inhibitors for their effect on *A. nidulans* growth.

Index to poster abstracts

Aguilar, Guillermo 6
Allen, Gethin 15
Andersen, Mikael 13
Apostolaki, Angeliki 40
Arst, Herbert N Jr. 40
Askin, MC 11
Attwood, Teresa 32
Baker, Scott 13
Bakhuis, Janny 33, 34
Bakker, BM 11
Battaglia, E 17
Bayram, Özgür 10, 30
Blom, Dirk 6
Bohle, Kathrin 37
Bok, Jin Woo 30
Bowyer, Paul 32
Braus, Gerhard H 9, 10, 7, 30, 31
Braus-Stromeyer, Susanna 30
Breestraat, Stefaan 38
Bromley, Mike 15
Busch, Silke 7, 9, 10
Caddick, Mark X 25
Calcagno-Pizzarelli, Ana María 40
Camara, Kaddy 4
Chooluck, Sunthorn 25
Christmann, Martin 7
Damveld, Robbert 36, 38
de Lange, Ilse 33, 34
de Weert, S 20
de Vries, Ronald 6, 17
de Weert, Sandra 19
Denning, David 32
Dobson, Alan 21
Doevendans, D 11
Dyer, Paul S 26
Eisendle, Martin 1
Espeso, EA 18
Fay, Lauren 3
Fischer, Reinhard 5
Franken, Angelique CW 19, 20
Fujii, Tsutomu 39
Fuller, Hubert T 26
Furukido, Toshi 14, 29
Giles, Peter 32
Gilsenan, Jane Mabey 32
Göcke, Yvonne 37
Gomi, Katsuya 2, 22, 23, 24, 39
Gordon, Barry 3
Gremillion, Sara 4
Grimm, LH 35
Gruben, BS 17
Haas, Hubertus 1
Harispe, Laura 40
Hasegawa, Sachiko 24
Helmstaedt , Kerstin 10, 30
Hempel, Dietmar C 35, 37
Higashitsuji, Yuhei 5
Hill, Terry 3, 4
Hitt, Reiner 9
Hoppert, Michael 10
Iefuji, Haruyuki 39
Jackson-Hayes, Loretta 3
Jacobs, Denise 38
Jin, Feng Jie 12, 14
Jones, Meriel G 25
Kakizono, Dararat 39
Keller, Nancy P 27, 30
Kiep, Katina A 37
Kobayashi, Akiko 28
Koike, Hideaki 29
Könnecke, Birte 9
Konzack, Sven 5
Koyama, Yasuji 12, 14, 16, 29
Kragl, Claudia 1
Krappmann, Sven 30
Kress, Marcia 31
Krull, Rainer 35, 37
Kwon, Nak-Jung 30
Laubinger, Karen 10
Lin, P-J 35
Lokman, B Christien 19, 20
Loprete, Darlene 3, 4
Los, Alrik 33, 34
Machida, Masayuki 14, 16, 29
Masaki, Kazuo 39
Matsushima, Ken-ichiro 16
Matsuura, Tomomi 39
Meijer, Martin 13
Mercer, Sarah 4
Meyer, V 18
Miura, Daisuke 22
Mizutani, Osamu 39
Morozov, Igor Y 25
Morrissey, John P 21
Nahlik, Krystyna 7, 9
Ni, Min 30
Nishimura, Marie 22
Nkashama, Sonia 3
O'Gorman, Celine M 26
Oda, Ken 28
Ogawa, Masahiro 14, 16, 29
Ohashi, Shinichi 28
Olsthoorn, Maurien 38
Ooba, Ayumi 22
Palmer, Jonathan M 27
Patel, Ravi 3
Pel, Herman 13, 33, 34, 36
Peñalva, Miguel Angel 40
Power, Tracy 21
Ram, Arthur FJ 11, 19, 20
Roberts, Stephen K 8
Roubos, Hans 13, 38
Samson, Robert 13
Samuels, Felicia 4
Sanchez, James F 27
Sano, Motoaki 28

Scazzocchio Claudio 40
Schaap, Peter 13
Schrettl, Markus 1
Schwier, Elke U. 7, 9
Seiler, Stephan 10
Senou, Yukio 12
Shimizu, Kiminori 22
Shintani, Takahiro 2, 22, 24, 23
Shiro, Daisuke 2
Spielvogel, A 18
Stahl, U 18
Stam, Hein 36, 38
Takahashi, Tadashi 12, 14, 16, 29
Takeshita, Norio 5
Tanaka, Mizuki 23
Terabayashi, Yasunobu 29
Toda, Tomomi 16
Todd, RB 17
Tokuoka, Masafumi 14, 16, 23, 29
Turner, Geoff 15
Valerius, Oliver 10, 30, 31
van de Vondervoort, Peter 13, 38
van Peij, Noël 13, 33, 34, 36, 38
van den Hondel, Cees AMJJ 19, 20
vanKuyk, Patricia 6, 11
Vinck, Arman 6
Visser, L 17
Visser, J 11
Voßkuhl, Katja 10
Wang, Clay 27
Wösten, Han AB 6, 17
Wulkow, M 35
Yamane, Noriko 29
Yokota, Jun-ichi 2
Yu, Jae-Hyuk 30
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